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CONTENTS / CUPRINS

Zbyšek ŠUSTEK - <i>Ad Multos Annos</i> to Museum of Oltenia Craiova / <i>Ad Multos Annos</i> Muzeului Olteniei Craiova (homage message / cuvânt omagial)	7
Mircea VARVARA - The Oltenia Museum. Department of Natural Sciences - 90 years of existence (1923-2013) / Muzeul Olteniei. Secția de Științele Naturii - 90 ani de existență (homage message / cuvânt omagial)	9
Gabriel CORNEANU - Memories at an anniversary moment / Amintiri la o dată aniversară (homage message / cuvânt omagial)	11
Cornelia CHIMIȘLIU - The Natural Sciences Department of Museum Oltenia Craiova, 90 years of hopes and accomplishments (1923-2013) / Secția de Științele Naturii a Muzeului Olteniei Craiova - 90 de ani de speranțe și împliniri (1923-2013)	15

I. MINERALOGY - PALEONTOLOGY / MINERALOGIE - PALEONTOLOGIE

Vlad CODREA, Bogdan G. RĂȚOI, Laurențiu URSACHI, Alexandru SOLOMON, Mihai BRÂNZILĂ - The Pleistocene of the Simila open pit (Scythian Platform, Romania) / Pleistocenul din cariera Simila (Platforma Scitică, România)	19
Stănilă IAMANDEI, Florina DIACONU - The coal-generating Neogene forests from the Dacian Basin / Pădurile neogene carbogeneratoare din Bazinul Dacic	30
Ștefan VASILE, Emanoil ȘTIUCĂ, Dragoș PANAITEȘCU - First find of elephantid remains from the Pleistocene of Copăcenii (Ilfov county, Romania) / Prima descoperire a unor resturi de elefantide din Pleistocenul de la Copăcenii (Județul Ilfov, România)	42
Constantin ENACHE, Constantin CÂRLAN - Pliocene from Logrești / Pliocenul de la Logrești	52

II. VEGETAL BIOLOGY / BIOLOGIE VEGETALĂ

Mihaela CORNEANU, Gabriel C. CORNEANU - The adaptogenic species: theoretical and practical importance. A review / Specii adaptogene: importanță teoretică și practică. O trecere în revistă	58
Violeta BUTNARAȘ, Maria GONCEARIUC, Svetlana MAȘCOVȚEVA - The perspective polycross hybrids of <i>Lavandula angustifolia</i> MILL. / Hibridi policross perspectivi de <i>Lavandula angustifolia</i> MILL.	66
Maria GONCEARIUC, Zinaida BALMUSH - Biodiversity of <i>Hyssopus officinalis</i> L. genotypes growing in Moldova / Biodiversitatea genotipurilor de <i>Hyssopus officinalis</i> L.	71
Desislava DANCHEVA, Nasko ILIEV, Ivan ILIEV - In vitro propagation of <i>Fraxinus excelsior</i> L. / Propagarea in vitro la <i>Fraxinus excelsior</i> L.	78
Rodica CATANĂ, Irina HOLOBIUC, Mirela MOLDOVEANU - In vitro seed germination in three rare taxa from Romanian Carpathians flora / Germinarea in vitro a semințelor la trei specii de plante amenințate din flora Carpaților din România	85
Irina HOLOBIUC, Rodica CATANĂ, Carmen VOICHIȚĂ, Florența HELEPCIUC - In vitro introduction of <i>Dianthus trifasciculatus</i> KIT ssp. <i>parviflorus</i> as ex situ preservation method / Introducerea in vitro ca metodă de conservare ex situ a taxonului <i>Dianthus trifasciculatus</i> KIT ssp. <i>parviflorus</i>	93

Ioana CIORTAN, Gavril NEGREAN - Macromycetes from the Geopark Platoul Mehedinți (Oltenia, Romania) (1 st Note) / Macromicete din Geoparcul Platoul Mehedinți (Oltenia, România) (Nota I)	101
Florența-Elena HELEPCIUC, Monica Elena MITOI, Aurelia BREZEANU, Călina Petruța CORNEA - Cellular aspects of root colonization by antagonistic bacteria and phytopathogenic fungi / Aspecte celulare ale colonizării rădăcinii plantelor cu bacterii antagoniste și fungi fitopatogeni	109
Yavuz MUSTAFA - Lichens in prescriptions of Pliny the Elder / Lichenii în scrierile lui Plinus Cel Bătrân	115
Florentina ALDEA, Florența Elena HELEPCIUC, Cristian BANCIU, Aurelia BREZEANU, Liliana-Cristina SOARE - Comparative studies between ferns gametophyte and sporophyte by bidimensional electrophoresis / Studii comparative privind gametofitul și sporofitul ferigilor prin electroforeză bidimensională	120
Nicolae ION, Luminița BUȘE-DRAGOMIR - Physiological effects of treatment with fungicides in <i>Malus domestica</i> BORKH. attacked by <i>Venturia inaequalis</i> (COOKE) WINT. / Efectele fiziologice ale tratamentului cu fungicide la <i>Malus domestica</i> BORKH. atacat de <i>Venturia inaequalis</i> (COOKE) WINT.	125
Alina ANDRONESCU, Anca AIFTIMIE-PĂUNESCU - Morpho-anatomical features of the vegetative organs of <i>Saponaria pumilio</i> (L.) FENZL ex A. BRAUN and their ecological significance / Caracteristicile morfo-anatomice ale organelor vegetative la <i>Saponaria pumilio</i> (L.) FENZL ex A. BRAUN și semnificația lor ecologică	131
Gheorghe DIHORU, Violeta BORUZ - Species to the limit of specific spreading area in Romania: <i>Zingieria pispidica</i> (BOISS.) TUTIN / Specii la limita de areal pe teritoriul României: <i>Zingieria pispidica</i> (BOISS.) TUTIN	137
Iulian BERCEA - Germination, upshot and growth of Hungarian and Turkey Oak seedlings in the woodlands of the western part of the Getic Plateau / Germinația, răsărirea și dezvoltarea plantulelor de gârniță și cer în pădurile din partea vestică a Podișului Getic	145
Daniel RĂDUȚOIU, Iulian COSTACHE, Cătălin SIMEANU - Contributions to the romanian vascular flora / Contribuții la flora vasculară a României	151

III. ANIMAL BIOLOGY / BIOLOGIE ANIMALĂ

III.a. INVERTEBRATES VARIOUS / NEVERTEBRATE DIVERSE

Yunus Ömer BOYACI, Pinar GÜLLE, Hakan DİDİNEN - Water mites of the genus <i>Unionicola</i> HALDEMAN, 1842 (Acari, Hydrachnidia, Unionicolidae) and a new species record from Turkey / Acarieni de apă din genul <i>Unionicola</i> HALDEMAN 1842 (Acari, Hydrachnidia, Unionicolidae) și o specie nouă pentru fauna Turciei	155
Nicolae LOTREAN - Data on spider fauna (Arachnida: Araneae) from the Natural Reservation Springs from Corbii Ciungi (Dâmbovița) / Contribuții la cunoașterea faunei de aranee din Rezervația Naturală Izvoarele de la Corbii Ciungi (Dâmbovița)	158

III.b. ENTOMOLOGY / ENTOMOLOGIE

Daniela BĂRBUCEANU, Liliana Vasiliu-OROMULU, Mircea BĂRBUCEANU - <i>Matricaria chamomilla</i> as trophic niche for thrips populations (Insecta: Thysanoptera) / <i>Matricaria chamomilla</i> ca nișă trofică pentru populațiile de tripsi (Insecta: Thysanoptera)	166
Gheorghe MANIC - Contribution to the knowledge of the pteromalids (Hymenoptera, Chalcidoidea, Pteromalidae) from "Codrii" Natural Rezerve / Contribuții la cunoașterea pteromalidelor (Hymenoptera, Chalcidoidea, Pteromalidae) din Rezervația naturală „Codrii”	171

Alena BRECHKA - Changes of some bioecological and phenological peculiarities of the colorado potato beetle (<i>Leptinotarsa decemlineata</i> SAY) in the agroclimatic zones in Belarus / Schimbările unor particularități bioecologice și fenologice ale gândacului de Colorado (<i>Leptinotarsa decemlineata</i> SAY) din zonele agroclimatice ale Belarusiei	174
Sanda MAICAN, Cornelia CHIMIȘLIU - Leaf beetles (Coleoptera: Chrysomeloidea, Chrysomelidae) preserved in the patrimony of the Oltenia Museum Craiova / Crisomelide (Coleoptera: Chrysomeloidea, Chrysomelidae) din patrimoniul Muzeului Olteniei Craiova	179
Dina ELISOVETSKAYA, Nastas TUDOR - Biological activity of the extract of <i>Veratrum lobelianum</i> BERNII. Against harmful species of insects and mites and its impact on entomophages / Eficacitatea biologică a extractului din specia <i>Veratrum lobelianum</i> BERNII, împotriva speciilor de insecte dăunătoare și acariene, și influența lui asupra entomofagilor	185
Lidia GAVRILIȚA - <i>Trichogramma</i> - effective solution for biological control over the <i>Laspeyresia pomonella</i> L. in the orchards / <i>Trichogramma</i> - soluție efectivă pentru protecția biologică în combaterea dăunătorului <i>Laspeyresia pomonella</i> L. în livezile de măr	193

III.c. VERTEBRATES / VERTEBRATE

Galina CURCUBET, Vasili DOMANCIUC, Nina FULGA - The hybrids carp-crucian as prospective object of freshwater aquaculture / Hibrizii crap-caras obiect de perspectivă al acvaculturii de apă dulce	197
Vasili DOMANCIUC, Galina CURCUBET - Influence of selection for increasing resistance to infectious diseases on morphological and reproductive characteristics of Moldavian carp breeds / Influența selecției pentru mărirea rezistenței la bolile infecțioase asupra caracteristicilor morfologice și reproductive ale raselor de crap din Moldova	203
Carmen Daniela BĂLESCU - New data regarding the aquatic avifauna on the territory of Craiova city (Dolj county) / Noi date referitoare la avifauna acvatică de pe teritoriul municipiului Craiova (județul Dolj)	209
Natalia SOCHIRCĂ, Victoria NISTREANU, Larisa BOGDEA, Vlad POSTOLACHI, Alina LARION, Natalia CARAMAN, Vasile CRUDU, Vlad CALDARI - Diversity and ecological peculiarities of terrestrial vertebrate fauna of Chișinău city, Republic of Moldova / Diversitatea și particularitățile ecologice ale faunei de vertebrate terestre din municipiul Chișinău, Republica Moldova	219

IV. ECOLOGY - THE ENVIRONMENT PROTECTION / ECOLOGIE - PROTECȚIA MEDIULUI

Alexandru-Ionuț PETRIȘOR - Are human settlements ecological systems? / Sunt așezările umane sisteme ecologice?	227
Andreea Floriana MARINICĂ, Cornelia CHIMIȘLIU, Ion MARINICĂ - Considerations on climatic conditions in Oltenia during the warm winter of 2012-2013 / Considerații privind condițiile climatice în Oltenia din iarna caldă 2012-2013	233
Mădălin ENACHE, Masahiro KAMEKURA - Halophilic archaea in the neogene salt massif from Slănic Prahova, Romania / Aspecte privind prezența unor microorganisme halofile arheane în zăcămintul de sare format în zona Slănic Prahova, România în perioada Neogen	245
Mirela MOLDOVEANU, Larisa FLORESCU - Long - term analysis of cyanobacterial blooms in lake Roșu (Danube Delta) / Analiza pe termen lung a înfloririlor cianobacteriene din lacul Roșu (Delta Dunării)	252

Carmen Mădălina CIȘMAȘIU - The acidophilic bacteria ability to produce metalloenzymes responsible for the starch degradation in the presence of heavy metal ions / Capacitatea bacteriilor acidofile de a produce metaloenzime responsabile cu degradarea amidonului în prezența ionilor de metale grele	260
Ioana VICOL - Chorology of the <i>Physcia</i> (SCHREB.) MICHIAUX (1803) genus in Romania / Corologia genului <i>Physcia</i> (SCHREB.) MICHIAUX (1803) în România	268
Gina Raluca KERKMANN - Some data concerning the application of Webb method to sediment samples from the Murat River (Ağrı Region-Turkey) / Câteva date privind aplicarea metodei Webb la probe de sediment provenite din Râul Murat (Regiunea Ağrı-Turcia)	283
Larisa FLORESCU, Mirela MOLDOVEANU - Multiannual variability of rotifer production in Sfântu Gheorghe branch / Variabilitatea multianuală a producției rotiferelor în brațul Sfântu Gheorghe	288
Olivia CIOBOIU - The distribution of the gastropod populations along the characteristic sectors of the Danube / Distribuția populațiilor de gastropode în sectoare caracteristice Dunării	296
Sára FERENTI, Nicoleta DIMANCEA - Some data upon the terrestrial isopod assemblage from a north-western Romanian wetland / Date asupra comunității de izopode terestre dintr-o zonă umedă din nord-vestul României	302
Minodora MANU, Marilena ONETE - Structural characteristics of soil mite populations (Acari-Mesostigmata) from the oak-hornbeam forests from southern Romania / Caracteristicile structurale ale populațiilor de acarieni de sol (Acari-Mesostigmata) din stejăreto-cărpinete din sudul României	306

V. SCIENTIFIC ESSAYS AND SCIENCE NEWS / REFERATE ȘI NOUTĂȚI ȘTIINȚIFICE

Petre NEACȘU, Olivia CIOBOIU - Ecotourism. Present and future / Ecoturismul. Prezent și viitor	313
Gheorghe BENGĂ - Aquaporinology (the study of water channel proteins - aquaporins and relatives) as a new domain of natural sciences / Aquaporinologia (studiul proteinelor canal pentru apă - aquaporine și rudele lor) ca un domeniu nou al științelor naturii	316

**AD MULTOS ANNOS
TO MUSEUM OF OLTENIA CRAIOVA**

The Museum of Oltenia has a similar history as many other museums, which were emerging in Central and Eastern European provincial towns from the second half of the 19th century to the early 20th century. They were invariably founded by circles of local intelligentsia, the priests, secondary school professors, physicians, pharmacists and jurists, who occupied in their free time with active research in nature, history or ethnography of their town or region. They worked with minimal financial means and equipment, but with a great enthusiasm, admirable self-sacrificing and often also remarkable effectiveness. They built up collections and libraries, discussed their results, popularized them and, finally, initiated founding of municipal museums or small local learned societies issuing annuals or journals of their own.

*Perhaps from the present-day view of the formalized and somewhat dehumanized science, deeply infected by the simplicistic economism and scurrilous scientometric criteria alien to the science's nature (cf. Theory of Miseducation by K. P. Liessmann, the Austrian philosopher), their activity could be seen, with contempt, as some kind of erratic naiveté. But just the opposite is true. They formed a multilateral solid background, on which the science could be developed on the nation-wide or even international level. Let us considerate just Gregor Mendel, the founder of the modern genetics. He was the typical representative of the free-time researchers of those times. He published all his results – inclusively of the greatest conceptual discovery of biology – in one of such local journals, *Verhandlungen des Naturforschenden Vereines in Brünn*, issued in a town of about 60,000 inhabitants. But not only it. Just this journal published, for example, several identification keys of European or even Palaearctical beetles elaborated by other representative of this type of scientists, Edmund Reitter. Ulterior his keys become a basis for large modern monographs, in which his original keys are always cited. In Romania, in Sibiu, a similar example is the Museum Bruckenthal and the journal *Verhandlungen und Mitteilungen des Siebenbürgischen Vereins für Naturwissenschaften zu Hermannstadt*. Such museums and the associations working around them also created conditions for searching for young talented people, attracting them by the mystery of collections and offering them help at their first steps in research and scientific activity. They also played a significant role of centers of education, social life and national cohesion.*

The Museum of Oltenia is a younger brother of such museums being founded as late as at the very end of this "Gründner" period. But, in spite of this, it became without any doubt a very dignified representative of them. Its life was not easy. It was forced to overcome very hard periods, in which it could not open its door to the public and had to execute its activities almost in internal immigration. It had to move several times from one building to other. However, like the mythic bird Phoenix, it always found a way to a revival and to a higher level.

About the last decade I had personally honor and pleasure to observe the remarkably dynamic and progressive development of the Museum. There has been completely rebuilt the permanent exposition of natural sciences. It unifies the verified traditional modes of collection presentation with the modern information technologies. The wonderful dioramas display the plants and animals in their environment and mutual interaction. This approach is particularly significant in the present day, when children loss contact with nature thinking that milk is produced by a factory or some "science coryphées or managers" declare ecology to be outmoded. The exposition rooms also offer a beautiful place with unrepeatably genius loci for the conferences.

Also other two sections of the Museum, of Ethnography and History and Archaeology, progressed considerably, in particular the former one built up a reputed center of conservatory works. Quality of the journal "Oltenia. Studii și comunicări. Științele Naturii." and its extent increased incomparably. From a regional journal publishing papers presenting the museum collections or texts helping the teachers of primary or secondary schools it

was transformed, into an international journal, with international editorial board, exceeding the Romanian borders, with increasing citation rate and attracting authors from abroad to publish results of experimental and long-termed field studies or synthetic essays.

A great, unappreciable personal merit for the recent progress of the Museum of Oltenia, in particular for the Department of Natural Sciences, and of the "Oltenia. Studii și comunicări. Științele Naturii." belongs to Ph.D. Cornelia Chimișliu. Her strategy of systematic, patient work, progressing by small, but effective steps, has brought admirable fruits. Thanks to her gentle, demanding but tolerant and empathic approach to people, she succeeded to create a wide collective of contributors, lecturers and reviewers from almost all fields of biology, geology and environmental sciences. It includes, as a kind of advisers, the outstanding representatives of other institutions from Romania. To name only some of them, I mention academician Murariu and professors Varvara, Pisică, Andreiescu or Brezeanu. This collective consists not only from the reputed researchers, whose names appear in the international journals, but also of the beginning authors offered by the editorial board by sufficient space and help in perfecting their papers. In the present day it is unusual, but just in this direction, the museum continues, in the best sense of word, the tradition and mission of the older museums mentioned above. Every participant of the conferences organized by the Museum of Oltenia sees that all this functions in full harmony like a large symphonic orchestra conducted by Mrs. Ph.D. Chimișliu. Perhaps it sounds too metaphorically, but it is so.

Of course, nothing of this could not be achieved without the enormous effort of the whole museum staff; without help of its direction, and in the last, but not last place, without the permanent institutional and financial support and a rare understanding from the part of municipal and county administration.

Wilhelm von Humbolt, distinguished three kinds of scientific institutions, academies and universities as the "living" institutions and the museums and archives as the "dead" ones. But the Museum of Oltenia belongs to the "living" ones. Long life - La mulți ani - to the Natural Sciences Department of Museum Oltenia Craiova!

*Ing. ŠUSTEK Zbyšek, CSc.
Institute of Zoology, Slovak Academy of Science*

THE OLTENIA MUSEUM, DEPARTMENT OF NATURAL SCIENCES 90 YEARS OF EXISTENCE (1923-2013)

This short exposure intends to conduct a tribute dedicated to the celebration of nine decades of existence of the Department of Natural Sciences of the Oltenia Museum, Craiova. For this purpose, I have as informative support my life experience of eight decades, as well as my professional, teaching, educational, scientific activity of half a century at the Faculty of Biology, Iași, the participation in numerous scientific congresses in Europe, the reviewer activity for some works on Entomology published in the Journal of the Museum for 7 years and information available on the Museum website.

Our life is influenced by all known or unknown, visible or invisible, physiological, ecological, natural, local, mental factors, by the will and light of knowledge, by motivated work and objectives in the context of local resources and in line with the steps of society civilization.

The concretization of the local values of the Oltenia Museum, Craiova, Department of Natural Sciences, represents the holistic transformation of the specialists' experience, builders of scientific collections over time, especially from the zoogeographical area of Oltenia.

The patrimony of the Department of Natural Sciences, under the leadership of the Head of Department, Dr. Chimișliu Cornelia, in collaboration with the Museum management team and the experts of the museum resulted in Collections, exhibitions, scientific, cultural and educational events, scientific activity.

The department has ten types of collections, grouped in two scientific fields: Mineralogy and Biology. The Entomology collections are representative and impressive comprising 53,870 specimens of insects from three sources: purchases (20.84%), donations (14%), own collections (65.15%). Their own collections are the result of the museum specialists' activity over time who presently are persons with a university degree, many of them even with a PhD title.

The educational value of the Department has resulted in the organization of exhibitions that played a documentary-scientific and cultural-educational role, inciting those people interested in, especially young people, pupils or students, but also other categories of visitors, to deepen their knowledge.

Natural objects, images play a stimulating role on memory, representation, understanding and learning. A proverb points out: "It is better to see once than to speak several times about that object." Thus, there were organized fundamental exhibitions: Physical-geographical conditions and ecosystems of Oltenia, Oltenia Terra-fossilis and the permanent exhibition The universe and our solar system, which incites to reflection on the vastness of the universe, its organization and structure, its permanent existence in balance and movement.

*The organization of the **Annual Scientific Sessions** and the publication of the works in English in its own journal, which is already included in international databases, are remarkable and thus, the Museum, as well as the Department, is among the leading institutions of Romania in the field.*

I participated in eight such sessions (2005 -2013) directly, by my own works, or indirectly, as scientific reviewer for some works of Entomology. These sessions provided the opportunity for the participation of specialists from different museums from some counties of Romania, of academics from the Faculties of Biology from Iași, Bucharest, Cluj, Sibiu, Craiova, etc., from certain Romanian research institutes, as well as specialists from nine European countries, such as Slovakia, Turkey, Moldova, Bulgaria, Italy.

The organization of scientific documentary trips, with all the participants in order to see special natural places, nature monuments, geological phenomena, etc. of Oltenia is a generous feature of the Oltenia Museum.

The prestige of the Oltenia Museum, respectively of the Department of Natural Sciences, is the cumulative prestige of the management quality, of the quality of the involved people, creators of collections, activity, of the society civilization and of the times we live.

I express my appreciation and wishes for more and more progress and achievements over many years, towards the horizon of the future!

VARVARA Mircea,
Professor PhD. Emeritus, Associate, Faculty of Biology, Iași

MUZEUL OLTENIEI, SECȚIA ȘTIINȚELE NATURII LA 90 DE ANI DE EXISTENȚĂ (1923-2013)

Conținutul acestei scurte expuneri are intenția să desfășoare un omagiu, prilejuit de împlinirea a nouă decenii de existență a Secției de Științele Naturii a Muzeului Olteniei Craiova. Pentru acest scop, am ca suport informativ experiența de viață, de opt decenii, cea profesională, didactică, educativă, științifică, de o jumătate de secol, la Facultatea de Biologie, Iași; călătoriile la Congrese științifice în Europa, activitatea de recenzor pentru unele lucrări de Entomologie la Revista Muzeului, timp de 7 ani și informațiile de pe site-ul muzeului.

Viața noastră este influențată de toți factorii cunoscuți sau necunoscuți, vizibili sau invizibili, fiziologici, ecologici, naturali, locali, psihici, de voință și de lumina cunoștințelor, a muncii cu scop și obiective în contextul resurselor locale și în consonanță cu treptele civilizației societății.

Concretizarea valorilor locale ale Muzeului Olteniei Craiova, Secția de Științele Naturii reprezintă transformarea holistă a experienței specialiștilor, făuritori de colecții științifice de-a lungul timpului, în special din zona zoogeografică a Olteniei.

Patrimoniul Secției de Științele Naturii, sub conducerea Șefului de Secție, Dr. Chimișliu Cornelia, în colaborare cu Conducerea Muzeului și cu munca experților din schema administrativă s-a concretizat în: colecții științifice, expoziții, evenimente științifice și cultural-educative, activitate științifică.

Secția posedă zece tipuri de colecții, grupate în două domenii științifice: Mineralogie și Biologie. Sunt reprezentative și impresionante colecțiile de Entomologie cuprinzând 53.870 de exemplare de insecte, provenite din trei surse: achiziții (20.84 %), donații (14 %), colecții proprii (65,15 %). Colecțiile proprii sunt rodul activității specialiștilor Secției de-a lungul timpului, iar în prezent, persoane cu studii universitare, doctorate.

Valoarea educativă a Secției s-a concretizat în organizarea de expoziții, cu rol documentar-științific și cultural-educativ, de incitare spre adâncirea cunoașterii de cei interesați, în special tineri, elevi sau studenți, precum și alte categorii de public vizitator.

Obiectele naturale, imaginile au rol stimulatv asupra memoriei, asupra reprezentării, asupra înțelegerii și învățării. Un proverb atrage atenția: „Este mai bine să vezi odată decât să vorbești de mai multe ori despre acel obiect”. Astfel, au fost organizate expozițiile de bază: „Condițiile fizico-geografice și ecosistemele Olteniei”, „Oltenia Terra-fossilis” și expoziția permanentă „Universul și Sistemul nostru solar”, care incită la reflecții despre imensitatea universului, organizarea și structura sa, existența sa în echilibru și permanentă mișcare.

Activitatea de organizare a Sesiunilor științifice anuale și publicarea lucrărilor în limba engleză într-o revistă proprie, cu circuit internațional, este remarcabilă, ierarhizând Muzeul și Secția printre Instituțiile de profil fruntașe din România.

Am fost martor la 8 asemenea Sesiuni, (2005 -2013) prin lucrări proprii, dar și prin funcția de referent științific pentru unele lucrări de Entomologie. Aceste Sesiuni au oferit prilejul de participare a specialiștilor de la Muzeele de profil din unele județe ale României, a unor cadre didactice universitare de la facultățile de Biologie din orașele Iași, București, Cluj, Sibiu, Craiova etc., institute de cercetări din România, precum și a unor specialiști din 9 țări europene, din care menționez: Slovacia, Turcia, Republica Moldova, Bulgaria, Italia.

Organizarea excursiilor documentar-științifice, cu toți participanții pentru cunoașterea unor locuri naturale, speciale, monumente ale naturii, fenomene geologice etc. din Oltenia, este o particularitate generoasă a Muzeului Olteniei.

Prestigiul Muzeului Olteniei, al Secției de Științele Naturii este prestigiul cumulat al calității conducerii, a calităților oamenilor implicați, făuritori de colecții, de activitate, al civilizației societății și a timpurilor la care s-a ajuns.

Îmi exprim aprecierea și urarea de tot mai multe progrese și realizări, în decurs de Mulți Ani, departe spre Orizontul viitorului!

VARVARA Mircea,
Profesor dr. emerit, asociat, Facultatea de Biologie, Iași

MEMORIES AT AN ANNIVERSARY MOMENT

The Department of Natural Sciences from the Oltenia Museum celebrates 90 years of existence in this period. As it depends on the historical time and country, the figure itself may seem large or small. Reported to a human life, under normal conditions of existence, this figure seems normal. During this period, the museum, founded by Professor Marin Demetrescu in Craiova in 1923, confronted with a bloody world war, recorded several social movements that took place in Romania, faced many natural disasters (two devastating earthquakes, a period of drought followed by famine, another period of great drifts of snow that paralyzed everyday life of the country for a while, floods, etc.).

In its 90 years of existence, the Museum of Natural Sciences from Craiova went through ordeals, but now, it successfully participates in cultural activities in the locality of residence, Craiova. This phenomenon was and is possible, as the administrative bodies, regardless of the political regime, nature and social hardships, has always supported this elite culture establishment from Romania.

Being born in Craiova before mid-century, and my parents and relatives paying attention to the activity of the cultural institutions of the city, I have known several aspects in the development and evolution of this place of culture over the years. In the first half of the century (1923-1973), the existence and activities of the Museum of Natural Sciences from Craiova are related to the work of three people who collaborated: **Marin Demetrescu**, **Constantin S. Nicolaescu-Plopșor** and **Ion Fîru**.

Marin Demetrescu (1881-1936) graduated the Faculty of Pharmacy in Bucharest and was a teacher at the College Carol I from Craiova. In 1923, he founded the **Natural History Museum of Craiova** (1923). He was the director of the Museum of Natural Sciences from Craiova (1923-1928) and of the Oltenia Museum (1928-1936), along with C. S. Nicolaescu-Plopșor.

C. S. Nicolaescu-Plopșor (1900-1968), archaeologist, ethnographer and folklorist, was the director of the Oltenia Museum (1946-1952), correspondent member of the Romanian Academy (1963). Together with Ion Fîru, he conducted anthropological excavations at Tetoiu (Bugiulești, Grăunceanu Valley, Vâlcea, 1960-1967), where there was discovered evidence of the conscious activity of the human ancestors dating back two million years ago, during the period called "pebble culture". Dependent on the knowledge accumulated during his paleontological and anthropological research, C.S. Nicolaescu-Plopșor oscillated in the determination of the historical timing of these relics, which were accepted by some scholars (the famous South African anthropologist, of Australian origin, Raymond Arthur Dart came especially in Romania, in 1965, to study them) or were challenged by others. At present, in the context of the new discoveries in anthropogenesis, these findings regain in importance.

Ion Fîru (1926-1984), born in Craiova, graduated the Faculty of Natural Sciences at the University of Cluj (1950); he was initially teaching assistant at the Faculty of Veterinary Medicine and Zootechnics of Arad (1950), after which, he worked for the rest of his life at the Oltenia Museum, Craiova, where he was assistant (1950), lecturer (1951), director of the Oltenia Museum Craiova (1952-1973) and secretary of the Commission of Natural Monuments, Craiova. He had multiple activities and achievements in the field of natural monuments in Oltenia. He reshaped the space of the Oltenia Museum, where he organized the permanent exhibition of Natural Sciences (1963), where there were 12 dioramas, attraction of European fame, the achievement of which belongs to him. His fields of interest were varied: palaeontology, botany, entomology, museology. Being extremely meticulous, he finished his doctoral thesis (**Entomofauna of sandy lands around Craiova, 1982**) after a long reviewing process.

After graduating the Faculty of Biology of the University of Bucharest and a stage of almost two years at the Biological, Geological and Geographical Research Station Stejarul - Pangarati, A. I. Cuza University from Iași, I was employed at the University of Craiova (May 1, 1966) until 2011, when I retired. During this time, I participated in many scientific and cultural activities of the city and its neighbourhoods ("neighbourhoods" that reached ... Japan!). I had the opportunity to know him, to listen to his conferences and talk to the eminent historian, archaeologist and palaeontologist (among other areas of reference), who was Constantin S. Nicolaescu-Plopșor, one of the mentors of this place of culture from Craiova.

My choice for Biology, I owe to Mr. Ion Fîru, who was the Director of the Oltenia Museum and a friend of my father. In the spring of 1960, the last class of high school, I did not know what studies to follow. My passion was aviation and philosophy. The first option was not accepted by the family (they called me "airplane driver"), while in case of the second

one, the excitement decreased when I saw that the main activity was to study the works of Marx, Engels, Lenin and Stalin ... In these circumstances, resigned, I oriented toward medicine, due to a family friend, Ph.D. Emilian Culescu (he studied and specialized in France), a reputed ENT doctor in Craiova (returned home to perform military service). My father told me to go to consult with the director Firu Ion. The building of the Museum (Street Madona Dudu) and the exhibits were very familiar to me because, when my father was working at the Museum settings, together with Mr. Firu Ion and his collaborators, I was scouring the exhibition halls. In the director's office, we were exposed the fields a graduate of the Faculty of Biology may activate in. By the end of the meeting, the secretary handed the correspondence to the director and, among it, there was a package. With his proverbial tact, Mr. Firu unwrapped the package and took out a book, browsed it a little and handed it to me saying: "Here are described aspects regarding the fields a biologist may activate in, if he does not want to teach at a school or faculty". It was an album of monuments from an African country. From that moment, my choice for the Faculty of Biology was sure! The speeches delivered by Professor Alexandru Buia that, at his meetings with the students from the Carol I College (ex-Nicolae Bălcescu) of Craiova, presented issues from Russia, Kazakhstan and other countries, also contributed to my choice.

Another episode, sad this time. In 1975, it was to take place a double anniversary of historical nature: 1750 years from the first representation of the settlement Pelendava (current Craiova) on *Tabula Peutingeriana* (a copy from the 12th century of a map executed in 225, rendering the public roads from the Roman Empire) and 500 years from the first historical mention of the town Craiova (initially presented in the inscription on the tomb of Vladislav I, then in a charter issued by the ruler Basarab Laiotă on the 1st of June 1475). To mark this event, it was decided that the two departments of the Oltenia Museum that worked in the same building, located in Madona Dudu street, to function in separate buildings, each having at its disposal a double exhibition space: the Department of Archaeology and History to remain in the building in Madona Dudu street and the Department of Natural Sciences in a building in Brestei street. Personally, I did not and I do not think that this action represented an "abusive dissolution" of the Department of Natural Sciences! However, there occurred the earthquake of March 4, 1977 and both buildings were badly damaged. Obtaining funding for the restoration of the building of the History Museum located in Madona Dudu street was simpler as there had already functioned a museum. As for the space for the Museum of Natural Sciences, the situation was more difficult because the building in Brestei street, although grandiose, was not an architectural monument, being in the custody of the University of Craiova. At the request of my colleagues and friends from the Museum (Ph.D. Elena Bazilescu, Ph.D. Irina Păunescu and others), who stood cramped with inventory items in different locations (one being the current notary near Dolj County Court), we found an interesting aspect: in the building from Brestei street, there have functioned administrative units since the Austrian domination in Oltenia (1718-1739), and, according to some people, here was even the Austrian governor's residence!. This information, along with others, helped in raising money to save collections, as well as in obtaining the current building located in Popa Sapca street, which is the current premises of the Museum of Natural Sciences from Craiova.

An achievement of the present staff of the Museum of Natural Sciences, is the Scientific Communications Session, become the **International Conference entitled Museum and scientific research**. Annually, at the middle of September, eminent specialists from different areas of life sciences research, from the country and from abroad, gather at Craiova. It is a prestigious event, all participants enjoying and benefitting from the existence. Its organization is due both to the efforts of the entire staff of the museum and to the financial support provided by Dolj County Council (Mr. Ion Prioteasa, president of the council, being a permanent presence) and other sponsors. I remember that at the sessions of scientific communications organized during 1965-1980, there attended celebrities in the field of biology from Romania, the sessions being organized in co-operation with the University of Craiova, the Commission of Natural Monuments of the Romanian Academy (the late Prof. Bogdan Bobârnac being the co-ordinator of the sub-commission Oltenia of Natural Monuments), SIRAR organization, etc. The difference is made by the graphic quality of the published volume (coloured plates, coated paper, etc.), as well as by the presence of numerous foreign experts, which gives an authority in the field.

I wish the scientific and administrative staff of the museum a fruitful activity and long life to this valuable cultural unit.

Prof. Ph.D. CORNEANU C. Gabriel
University of Craiova

AMINTIRI LA O DATĂ ANIVERSARĂ

Secția de Științe ale Naturii, din cadrul Muzeului Olteniei din Craiova, aniversează în această perioadă 90 ani de la înființare. Dependent de timpul istoric și țară, cifra în sine, poate părea mare sau mică. Raportată la viața unui om, în condiții normale de existență, aceasta cifră pare normală. Muzeul înființat în anul 1923 de către profesorul Marin Demetrescu în Craiova, s-a confruntat în această perioadă de timp cu un război mondial sângeros, a înregistrat câteva mișcări sociale care au avut loc în România, a înfruntat mai multe cataclisme naturale (două cutremure devastatoare, o perioadă de secetă urmată de foamete, o altă perioadă cu mari troiene de zăpadă care a paralizat pentru un timp viața cotidiană a țării, inundații, ș.a.

În cei 90 ani de existență, Muzeul de Științe Naturale din Craiova, a trecut prin încercări grele, iar în prezent participă cu brio la activitățile culturale în localitatea de reședință, municipiul Craiova. Acest fenomen a fost și este posibil, deoarece forurile administrative, indiferent de regimul politic, vitregiile naturii și sociale, au sprijinit această unitate de cultură de elită din România.

Fiind născut în Craiova, înainte de jumătatea secolului trecut, iar părinții și rudele mele fiind atenți și la activitatea instituțiilor culturale ale orașului, am cunoscut de-a lungul timpului câteva aspecte în dezvoltarea și evoluția acestui lăcaș de cultură. Prima jumătate de secol (1923-1973) din existența și activitatea Muzeului de Științele Naturii din Craiova, este legată de activitatea a trei personalități care au colaborat: **Marin Demetrescu, Constantin S. Nicolaescu-Plopșor și Ion Firu**.

Marin Demetrescu (1881-1936), a absolvit Facultatea de Farmacie din București, fiind cadru didactic la Colegiul Carol I din Craiova. În anul 1923 a fondat **Muzeul de Istorie Naturală al Craiovei** (1923). A fost director al Muzeului de Științele Naturii din Craiova (1923-1928) și al Muzeului Olteniei (1928-1936), împreună cu C. S. Nicolaescu-Plopșor.

C.S. Nicolaescu-Plopșor (1900-1968), arheolog, etnograf și folclorist, a fost director al Muzeului Olteniei din Craiova (1946-1952), membru corespondent al Academiei Române (1963). Împreună cu Ion Firu, au efectuat săpături antropologice la Tetoiu (Bugiulești, Valea lui Grăunceanu, Vâlcea; 1960-1967), unde au fost descoperite dovezi privind activitatea conștientă a strămoșilor omului actual, în urma cu două milioane de ani, în perioada "cultura de prund". Dependent de cunoștințele acumulate în cercetarea paleontologică și antropologică, C.S. Nicolaescu-Plopșor a oscilat în plasarea în timp istoric a acestor vestigii, care au fost acceptate de unii savanți (celebrul antropolog sud-african, de origine australiană, Raymond Arthur Dart a venit special în România, în anul 1965, pentru a le studia), sau au fost contestate de alții. În prezent, în contextul noilor descoperiri în domeniul antropogenezei, acestea revin în actualitate.

Ion Firu (1926-1984), originar din Craiova, a absolvit Facultatea de Științe Naturale la Universitatea din Cluj (1950), a fost inițial asistent universitar la Facultatea de Medicină Veterinară și Zootehnie din Arad (1950), după care a activat tot restul vieții la Muzeul Olteniei Craiova, unde a fost asistent (1950), șef de lucrări (1951), director la Muzeul Olteniei Craiova (1952-1973) și secretar al Comisiei Monumentelor Naturii, filiala Craiova. A avut multiple activități și realizări în domeniul monumentelor naturii din Oltenia. A reorganizat spațiul Muzeului Olteniei, unde a organizat expoziția permanentă de Științele Naturii (1963), în care se aflau 12 diorame, punct de atracție de faima europeană, a căror realizare îi aparține. Domeniile de investigație au fost variate: paleontologie, botanică, entomologie, muzeografie. Fiind o fire extrem de migăloasă, a susținut Teza de Doctorat (*Entomofauna terenurilor nisipoase din împrejurimile Craiovei, 1982*), după o lungă perioadă de verificare.

După absolvirea Facultății de Biologie de la Universitatea din București și un stadiu de aproape doi ani la Stațiunea de Cercetări Biologice, Geologice și Geografice Stejarul-Pangarati, de la Universitatea A. I. Cuza din Iași, am fost încadrat la Universitatea din Craiova (1 mai 1966), de unde am fost pensionat (2011). În acest interval am participat la numeroase activități culturale și științifice din oraș și împrejurimi ("împrejurimi" care au ajuns până în ... Japonia!). Am avut ocazia să îl cunosc, să îi audiez conferințele și să discut cu eminentul istoric, arheolog și paleontolog (printre alte domenii de referință), care a fost Constantin S. Nicolaescu-Plopșor, unul din mentorii acestui lăcaș de cultură din Craiova.

Opțiunea mea pentru Biologie, o datorez domnului Ion Firu, care era Director la Muzeul Olteniei și prieten cu tatăl meu. În primavara anului 1960, în ultima clasă de liceu, nu știam ce studii voi urma. Pasiunea mea erau aviația și filosofia. Prima opțiune nu era agreată de familie (mă numeau "birjar de avioane"), iar pentru cea de a doua se răcise

entuziasmul, când am văzut că activitatea de bază era studiul lucrărilor lui Marx, Engels, Lenin și Stalin... În aceste condiții, resemnat, mă orientasem pentru medicină, un prieten al familiei, dr. Emilian Culescu (cu studii și specializare în Franța) fiind un reputat medic ORL la Craiova (revenise în țară pentru efectuarea serviciului militar). Tatăl meu mi-a spus să mergem să ne consultăm cu domnul director Fîru Ion. Clădirea Muzeului (din strada Madona Dudu) și exponatele de aici îmi erau foarte cunoscute, deoarece când tatăl meu lucra la amenajerile din Muzeu, alături de domnul Fîru Ion și colaboratorii acestuia, eu cutreeram sălile de expoziție. În cabinetul directorului, mi s-au expus domeniile în care poate activa un absolvent al facultății de Biologie. Spre sfârșitul întrevederii, secretara a înmănat directorului corespondența, printre care se afla un colet. Cu tactul său proverbial, domnul Fîru a desfăcut coletul din care a scos o carte, a răsfoit-o puțin și mi-a lasat-o să o analizez, zicându-mi: "aici sunt aspecte cu domeniile în care activează un biolog, dacă nu vrei să predai la o școală sau facultate". Era un album cu monumente ale naturii dintr-o țară din Africa. Din acel moment opțiunea mea pentru facultatea de biologie a fost certă! La aceasta au contribuit și expunerile reputatului profesor Alexandru Buia, care la întâlnirile sale cu elevii de la Colegiul Carol I (ex-Nicolae Bălcescu) din Craiova, ne prezentă aspecte din Rusia, Kazahstan sau alte țări.

Un alt episod, trist de această dată. În anul 1975 urma să aibă loc o dublă aniversare de natură istorică: 1750 ani de la prima reprezentare pe *Tabula Peutingeriana* (o copie din sec. XII a unei hărți executată în anul 225, cu drumurile publice din Imperiul Roman) a localității Pelendava (actualul oraș Craiova) și 500 ani de la prima citare a localității Craiova (prezentă inițial în inscripția de pe mormântul lui Vladislav I, apoi într-un hrisov al domnului Laiotă Basarab emis la 1 iunie 1475). În vederea marcării acestui eveniment, s-a hotărât ca cele două secții ale Muzeului Olteniei care funcționau în aceeași clădire, situată pe strada Madona Dudu, să funcționeze în clădiri separate, fiecare având la dispoziție un spațiu expozițional dublu: Secția de Arheologie și Istorie să rămână în clădirea din strada Madona Dudu, iar Secția de Științele Naturii într-o clădire de pe strada Brestei. Personal, nu am avut și nu am impresia, că această acțiune a însemnat o "desființare abuzivă" a Secției de Științele Naturii! A venit însă cutremurul din 4 martie 1977 și ambele clădiri au fost grav avariate. Obținerea finanțării pentru refacerea clădirii Muzeului de Istorie din strada Madona Dudu a fost simplă, deoarece în ea a funcționat un Muzeu. Pentru spațiul destinat Muzeului de Științele Naturii situația era mai dificilă, deoarece clădirea din strada Brestei, deși impozantă, nu era monument arhitectonic, fiind în custodia Universității din Craiova. La solicitarea colegilor și prietenilor mei de la Muzeu (doamnele dr. Elena Bazilescu, dr. Irina Păunescu și alții), care stăteau înghesuiți cu obiectele de inventar lângă ele în diferite locații (una fiind la actualul notariat de lângă Tribunalul Județean Dolj, am aflat un aspect interesant: în clădirea din strada Brestei au funcționat unități administrative din timpul stăpânirii austriece în Oltenia (1718-1739), după unii aici fiind chiar reședința Guvernatorului austriac! Această informație, alături de altele, a ajutat la obținerea de fonduri pentru salvarea colecțiilor, precum și pentru repartizarea actualei clădiri din strada Popa Șapcă, care este actualul lăcaș al Secției de Științele Naturii a Muzeului Olteniei Craiova.

O realizare a actualului colectiv al Muzeului de Științele Naturii, o constituie Sesiunea de Comunicări Științifice, devenită **Conferința Internațională "Muzeul și cercetarea științifică"**. Anual, la mijlocul lunii septembrie, se adună la Craiova eminente specialiști din diferite domenii ale studiului științelor vieții, din țară și străinătate. Este o manifestare de prestigiu, de existență căreia se bucură și beneficiază toți participanții. Existența ei este datorată atât activității întregului colectiv al muzeului, cât și sprijinului financiar oferit de Consiliul Județean Dolj (o prezentă "de greutate" fiind domnul Ion Prioteasa, președintele acestui for administrativ), precum și alți sponsori. Imi amintesc că și în cazul sesiunilor de comunicări științifice din perioada 1965-1980, participau somități ale biologiei din România, sesiuni care erau organizate prin co-operare cu Universitatea din Craiova, Comisia Monumentelor Naturii din cadrul Academiei Române (co-ordonator al subcomisiei Oltenia a Monumentelor Naturii fiind regretatul prof. dr. Bobârnac Bogdan), organizația SIRA, s.a. Diferența în plus, constă atât în calitatea grafică a volumului în care sunt tipărite lucrările, precum și prezența a numeroși specialiști străini, care conferă o autoritate științifică în domeniu.

Doresc activitate rodnică personalului științific și administrativ și viață îndelungată acestei valoroase unități de cultură.

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THE NATURAL SCIENCES DEPARTMENT OF MUSEUM OLTENIA CRAIOVA, 90 YEARS OF HOPES AND ACCOMPLISHMENTS (1923-2013)

CHIMIȘLIU Cornelia

Abstract. This work presents the history of the Natural Science Department of the Oltenia Museum. Craiova and also mentions the goals and hopes of the management and staff for the future development of the department. There are also presented the accomplishments during nine decades of existence of the department. For the writing of this article there were also used previous articles of the author and I. Firu. This is the reason for which certain concepts are not presented in detail but the appropriate references are provided.

Keywords: Natural Science Department, nine decades, activities, accomplishments.

Rezumat. Secția de Științele Naturii a Muzeului Olteniei Craiova - 90 de ani de speranțe și împliniri (1923-2013). Materialul prezintă sintetic istoricul Secției de Științele Naturii a Muzeului Olteniei Craiova cu menționarea speranțelor conducerii și colectivelor de specialiști ale secției, de dezvoltare a secției. Sunt menționate totodată împlinirile și realizările de-a lungul a nouă decenii de existență. Pentru elaborarea acestui material au fost consultate și utilizate lucrările publicate anterior de autoare și de I. Firu. De aceea nu sunt dezvoltate anumite idei, ci se face trimitere la lucrările publicate anterior, pe aceeași temă.

Cuvinte cheie: Secția de Științele Naturii, 9 decenii, activități, realizări.

INTRODUCTION

During the nine decades of its existence, the Natural Science Department had an eventful existence. Due to the fact that the department was a part of the Oltenia Museum, the allocated funds were distributed according to priorities amongst the departments of the Museum. During the years, even if some of the goals of the department were achieved there are goals that are still to be achieved.

During the time, the specialists of the department managed to enrich the scientific patrimony, to realize new exhibitions, to organize a lot of scientific manifestations and also to publish the results of their scientific research.

The most difficult time for the department was during the years 1975-1986, when due to the lack of expository space, the main way for the specialists of the department to interact with the public stopped. This led to the exclusion of the department from the cultural-educational circuit. The students' generations of that time were deprived of the knowledge regarding flora and wild fauna they could have acquired from the department.

The fast development period of the department started after the year 2003 and reached a peak during the years 2008-2012. During this time, the building the department is based in has been completely renovated, the display furniture in the deposits has been upgraded and new permanent exhibitions have been built. We also mention that the National Conference "*Museum and the Scientific Research*" became an international scientific manifestation. The scientific journal of the department „*Oltenia. Studii și comunicări. Științele Naturii*” climbed the highest level of scientific acknowledgment at a national level and also entered in the international scientific databases.

CONTENT

The 2nd of December of the year 2013 marks the 90 years anniversary of the birth of the natural Science History Museum. The creation of the museum was the dream of the professor of natural science Marin Demetrescu from Carol I College from Craiova. His hope was the creation of a natural science museum in Craiova similar to the "Grigore Antipa" Museum in Bucharest (FIRU, 1969; CHIMIȘLIU, 1999).

The Natural Science History Museum functioned under this name until the 13th of May 1928 when it became a department of the Oltenia Regional Museum. This new museum was formed by uniting the Natural History Museum and the Regional Antiques and Ethnography Museum of Dolj County. This union was the wish of C. Negrescu, the prefect of that time, who helped the two museums very much. The newly created museum consisted of seven "Sections" and its management was entrusted to Marin Dementrescu (VINCENZ, 1928). Therefore, the year 2013 also marks the 85th anniversary of the creation of the Oltenia Museum.

At the very beginning of its existence, the Natural Science Department was based in a few rooms from the basement of the prefecture building.

The increase in size of the museum collection and also the increase of the interest of the visiting public led to a new necessity and goal for the department. This was that of obtaining a larger space for the museum in order to organize larger expositions for the visiting public. This goal was achieved in 1935 when the museum moved in Casa Baniei, the current location of the Department of Ethnography of the Oltenia Museum. Here, new expositions were organized based on the increased patrimony.

Thanks to the donations of the intellectuality from Craiova, the patrimony of the museum kept increasing so that soon the need for an even larger space arose. That is why the Natural Science Department was moved in the current location of the History and Archaeology Department of the Oltenia Museum where during 1962 the main exposition was created (CHIMIȘLIU, 1999; 2003; 2011). This modern exposition had a significant impact at that time. Even if it only functioned for 12 years (CHIMIȘLIU, 1999) the exposition has remained in the memories of the visitors ever since.

After the closing of this exhibition, the patrimony of the department was moved in several other buildings where it did not have any expositional space. So the expositional activity of the department ceased during 1974 - 1986. The hope of the department director and his staff of obtaining a new space for properly storing the patrimony of the department and organizing new exhibitions was partially fulfilled in 1984 (CHIMIȘLIU, 2003). In that year, the building form Popa Șapcă street was assigned to the Oltenia Museum for the Natural Science Department. Due to financial issues the renovation of the whole building was not possible so that, at first, only the patrimony of the department was moved to the new location. Two years later (the 13th of March 1986) after preparing the space in the ground floor of the building, the expositional activity of the department was resumed starting with two temporary exhibitions followed by several dozens of such expositions until the year 2008 (CHIMIȘLIU, 1999; 2003; 2010). Also due to financial reasons, the organisation of a new main exposition could not be achieved. The thematic for a new main exhibition was agreed upon and elaborated by: PhD. Elena Bazilescu - the chief of the department and the curators: PhD. Irina Păunescu, Adrian Nastase, Cornelia Chimișliu. There were two attempts of organizing this new exposition, two technical projects were elaborated and the scientific thematic was constantly updated. Only 22 years later, after moving to the new building on 18th of September 2008, on the first floor the main exposition "*The physical-geography conditions and the ecosystems from Oltenia*" (Fig. 1) was opened. The new revised scientific thematic of the exposition was realized by: PhD. Cornelia Chimișliu - the chief of the department and curators: PhD. Adrian Năstase, PhD. Aurelian Popescu, PhD. Mirela Ridiche, PhD. Olivia Cioboiu, PhD. student Claudia Goga (CHIMIȘLIU, 2010).

This main exposition provides the visiting public with the opportunity of getting to know the physical-geography conditions and Oltenia ecosystems through modern means of exhibitions. Along with the classical exhibition means (showcases), modern interactive informational systems were used. These systems comprise 2D/3D interactive animated multimedia presentations and Flash technology. The interactive informational systems (CHIMIȘLIU, 2011) have a pronounced didactical character and this is a main reason for teachers and professors to hold some of their classes at the exposition. The terrestrial and aquatic ecosystems from Oltenia are presented with the help of four dioramas. The cave ecosystem is represented by an artificial cave realized within the exposition (CHIMIȘLIU, 2010, 2011).

At the same time, the exposition "*The Mineral and Rock Collection*" was opened to the public. This exhibition displays the most valuable pieces from the mineral and rocks collection of the Oltenia Museum (CHIMIȘLIU, 2010). This modern exhibition turned the department into an important cultural-scientific attraction of Craiova.

The size and diversity of the patrimony, the generous space of the building, the professionalism of the staff have given birth to new hopes and goals. These are the creation of a new main palaeontology exposition on the ground floor of the building, a new cosmogony and a Planetarium on the second floor.

Some of these goals were fulfilled so that the palaeontology expositions "*Oltenia-Terra fossilis*" was opened on the 22nd of February 2012. The exhibition displays part of the palaeontology patrimony of the department (16,000 pieces) gathered over the years. There are exhibited genuine pieces (plants, invertebrates and vertebrate fossils), scientific and illustrative material.

On the 9th of December 2012 the exhibition "*The Universe and our Solar System*" and the *Planetarium* were opened to the public. They provide the visitors with knowledge regarding different aspects of the Universe and our Solar System focusing on the planet Earth and its natural satellite, the Moon.

These were goals regarding the appropriate space and the activity of taking advantage of the expositional value of the patrimony of the department. The whole staff of the department contributed to achieving these goals. An important contribution was also provided by the manager of the museum prof. PhD. Mihai Viorel Fîfor and the management of Dolj County Council, respectively Ion Prioteasa, Chairman.

Other hopes of specialist team were related to the exploit and scientific capitalization activity of the museum heritage. It was always wanted that every collection of the museum heritage to be scientifically processed by a specialist, but it was not possible.

That is why the goal of having specialists for every category of the collection forming the scientific patrimony of the department has only partially been achieved. During 1988 and 1992, the department had only two curators, the botanic specialist Adrian Nastase and entomology specialist, Cornelia Chimișliu. Starting with 1993 (CHIMIȘLIU, 1998), five new curator joined the department for the collections: Minerals and Palaeontology (Aurelian Popescu), Ornithology (Ridiche Mirela), Malacology (Olivia Cioboiu), Ichthyology (Claudia Ionelia Goga), Botany (Gima Lila) (CHIMIȘLIU, 2010). The collections of Herpetology and Mammalogy are still to be assigned their own curators.

The goal of the curators for professional development has delivered four PhD. theses. Thus, four of the curators of the department have a PhD. title. The last two curators that joined are PhD. students.

The scientific preparation of the patrimony, the elaboration of scientific papers and their publication contributes to the mediatisation of the patrimony and the introduction of these data into the national and international scientific circuit.



Figure 1. 18 September 2008 - The grand opening of the permanent exhibition on the first floor.

In order to communicate the results of the scientific activity of the department, starting with 1993, the annual scientific manifestation “*The Museum and Scientific Research*” has been organized.

Our hope that this scientific event will bring together specialists from Romania and abroad, has been fulfilled gradually. Initially, the participants were mostly curators of museums and natural sciences departments from Romania. Subsequently, the number of participants from universities and research institutes increased. The next step was to attract specialists from outside Romania. The first participants from abroad were specialists from Chișinău (Republic of Moldova) and in Bratislava (Slovakia). Next, there were Turkey, Bulgaria, Albania, Italy, Czech Republic, etc. Thus, the Scientific Conference (Fig. 2) became an international scientific manifestation. (www.sesiuneinternationalamuzeulolteniei.ro/). This year (2013) is the 20th event organized under the title “*The museum and scientific research*” and the 5th international edition.



Figure 2. Image from the opening of the Conference in 2012.

The publication of the scientific papers presented by participants was done in the scientific journal of the department “*Oltenia. Studii și comunicări. Științele Naturii*” (<http://www.olteniastudii.3x.ro/>), which starting from

1999 to the present (2013), has had annual periodicity. Our hope that the modest journal of the department will become a valuable scientific journal, was fulfilled in 2008 when, on nationally level, it was included in the scientific journal "B +" (code CNCSIS-703). It is also indexed BDI, Zoological Record link to ISI Master Journal List (<http://science.thomsonreuters.com/cgi-bin/jrnlst/jlresults.cgi?PC=MASTER&Word=oltenia>).

Another hope was fulfilled when our conference room was created, where all the cultural, educational and scientific team of specialists organized by the Division can take place.

Other hopes related to the collaboration with teachers with various degrees became a reality gradually.

CONCLUSIONS

In these nine decades of existence, the teams of specialists who worked in the department showed professionalism, patience and perseverance in the fulfilment of their hopes. The desire for better was always present no matter how working conditions were.

All hopes have been, are and will be channelled to enrich and diversify the scientific heritage, the preservation and the preparation, as well as to the its cultural and educational scientific exploitation.

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THE PLEISTOCENE OF SIMILA OPEN PIT (SCYTHIAN PLATFORM, ROMANIA)

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SOLOMON Alexandru, BRÂNZILĂ Mihai

Abstract. Pleistocene deposits recorded on the Bârlad Valley area are largely exposed, mainly on the river terraces. An illustrative outcrop can be observed on the right bank of the Bârlad River few kilometres upstream from Bârlad town, in Simila open pit. Based on detailed study, a number of characteristic sedimentary packages were outlined, referred to as lithofacies associations. There were established a total of eight lithofacies types and three architectural elements. The interpretation of these architectural elements revealed a braided river environment with low sinuosity channels. There are two kinds of fossils collected in the sands and gravels from Simila: Late Miocene ones (fossil wood, molluscs, and vertebrates), all being reworked there: Pleistocene (most probably Middle or Late Pleistocene) ones, including freshwater molluscs and vertebrates (large herbivores). Therefore, the deposits from Simila open pit are Pleistocene and in no wise, older as presumed before.

Keywords: Pleistocene, fluvial environments, sedimentology, palaeontology, Bârlad Valley.

Rezumat. Pleistocenul din cariera Simila (Platforma Scitică, România). În aria Văii Bârladului, depozite pleistocene sunt consemnate îndeosebi la nivelurile diferitelor terase. Un afloriment deosebit de ilustrativ poate fi studiat pe malul drept al râului Bârlad, la câțiva kilometri amonte de orașul Bârlad, în cariera de la Simila. Pe baza studiului detaliat efectuat, au fost conturate o serie de litofaciesuri sedimentare grupate unor elemente arhitecturale. Au fost astfel stabilite opt tipuri de litofaciesuri sedimentare și trei elemente arhitecturale. Interpretarea acestor elemente arhitecturale evidențiază un sistem fluvial împletit, cu o serie de canale cu sinuozitate mică. În nisipurile și pietrișurile fluviale pot fi întâlnite două tipuri de fosile: unele care aparțin Miocenului superior (lemn fosil, moluște și vertebrate), toate însă remaniate; altele - pleistocene (foarte probabil din Pleistocenul mediu sau superior), precum moluște de apă dulce și vertebrate (ierbivore mari). În consecință, depozitele din cariera de la Simila sunt pleistocene și nicidecum mai vechi, precum fuseseră considerate anterior.

Cuvinte cheie: Pleistocen, paleomedii fluviale, sedimentologie, paleontologie, Valea Bârladului.

INTRODUCTION

Pleistocene deposits are largely exposed in Moldova (Eastern Romania) on the Bârlad Valley, on the terraces of both this river and its tributaries. One of the most illustrative Pleistocene successions can be studied in Simila open pit, where in the last decade, large amounts of sand and gravel were extracted from for constructions. This open pit is located on the right bank of the Bârlad River, 7 km upstream from Bârlad town, on Simila commune territory near the national road 24 (Fig. 1).

Due to the mentioned works and a continue survey in the last five years, several fossils were unearthed and collected, belonging in majority to vertebrates. All are curate at Vasile Pârvan Museum in Bârlad, Natural Sciences Branch (abbreviated VPMNSB). This paper is focused on the sedimentology and palaeontology of these deposits.

GEOLOGICAL SETTING

The whole area that Simila open pit belongs to, is located from geological viewpoint, to the Scythian Platform (SÂNDULESCU, 1984). This platform recorded a long geological history. However, in spite of numerous boreholes already drilled, there still remain few unclear geological details as the ones related to the platform basement, never crossed by drillings in Romania. Therefore, there are different opinions about the age and lithology of this basement: while students as SÂNDULESCU (1984) interpreted this platform as younger compared to the East European one, others (PARASCHIV, 1970 in IONESI, 1994) presumed that it could mean nothing else but a deeper block of the Moldavian Platform (i.e. a southwestern area of the East European Platform). In his sections concerning this region (e.g. A 14), ȘTEFĂNESCU (1985) illustrated a Proterozoic basement, but he did not give additional details concerning the geological age. In such context, we can refer to the data issued from the boreholes drilled in Ukraine, as the ones from Zadunaivka and Suvorovo (BELOV et al., 1987), where the oldest rocks are Vendian black shales, while metamorphic rocks were never drilled.

In Romania, more precisely in Bârlad area, the basement is covered by four sedimentary megacycles: Devonian, Permian-Triassic, Jurassic-Cretaceous-Eocene, Middle Miocene (Late Badenian)-Pliocene (Romanian) (IONESI, 1994). On surface, only the last sedimentary megacycle rocks are exposed, these ones being the basement for the Pleistocene and Holocene deposits. Due to the monocline structure of the platform, the last megacycle rocks are differently exposed, the older ones (Late Miocene) in northwestern areas and younger (Pliocene) in southeastern areas (Fig. 2).

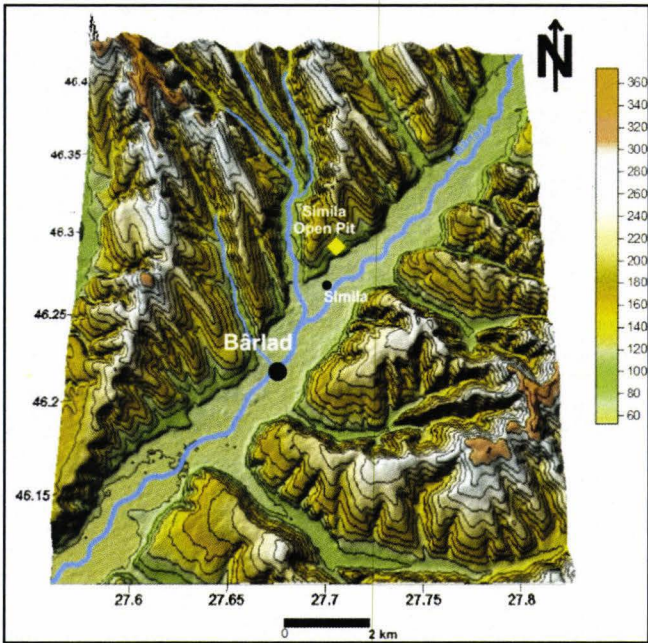


Figure 1. Location of Simila open pit (GlobalMapper13 and Surfer 8).

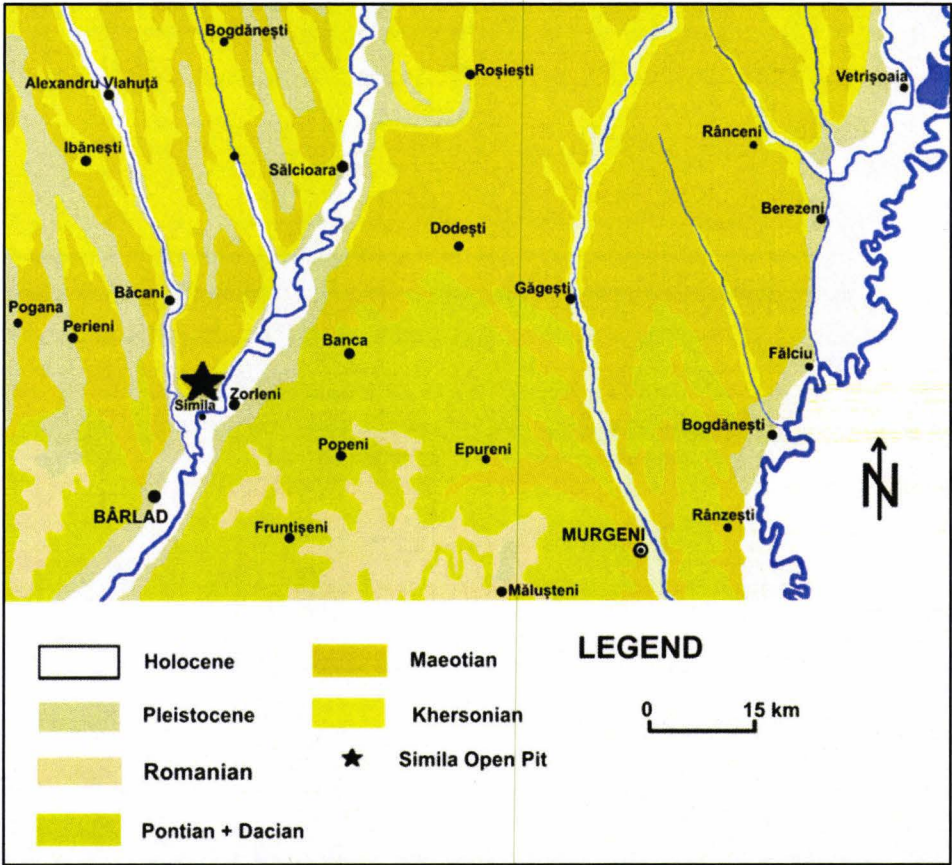


Figure 2. Geological map of the southern area of the Moldavian Platform and northern area of the Scythian Platform indicating the Miocene, Pliocene and Quaternary deposits (after GHENEA et al., 1967).

Consequence of the works carried out in Simila open pit in the last decade, a succession of clastic rocks (gravels and sands) can be now observed. All are the result of the evolution of an ancient fluvial system. The majority are channel fills, bearing also several fossils, mainly vertebrates (Fig. 3).

SEDIMENTOLOGY OF SIMILA OPEN PIT

The methods used in this preliminary study include stratigraphic section measurements, fluvial architecture and palaeocurrent analysis. The facies codes follow MIALL’s fluvial lithofacies charts (1985, 2010). The designation and definition of lithofacies are based on the correlation of a rock of certain characteristics related to a specific depositional environment (READING, 1996). The basic elements used to describe these sedimentary facies are included in Table 1 and the architectural elements in Table 2.

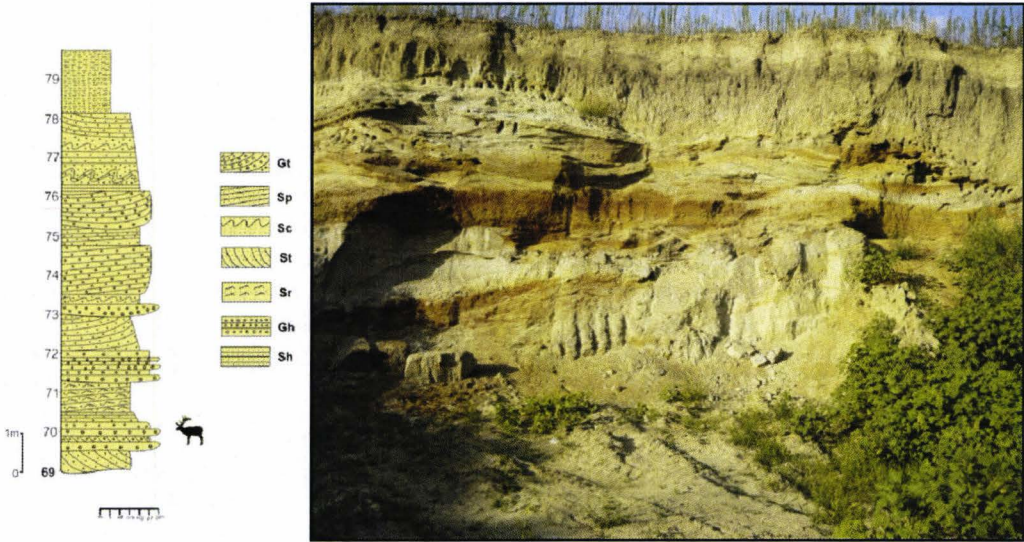


Figure 3. Lithological log of the deposits exposed in Simila open pit, indicating the level where giant deer *Megaloceros* remains were collected (also with lithofacies codes identified and described in table 1).

Table1. Lithofacies summary of codes, physical characteristics and depositional interpretations. Codes adapted from MIALL (1984, 2010).

Facies code	Facies	Sedimentary Structures	Interpretation
Gh	Clast supported crudely bedded gravel	Horizontal bedding, Imbrications	Lag deposits
Gt	Gravel stratified	Through crossbeds	Minor channel fills
St	Sand medium to coarse	Solitary or grouped through cross-stratification	Sinuuous crested and linguloid (3-D) dunes
Sr	Sand medium to coarse	Climbing ripple cross lamination	Lower flow regime
Sp	Sand medium to coarse	Solitary or grouped planar cross stratification-09	Linguloid or transverse-(2-D) dunes
Sl	Medium sand	Low angle cross beds (≤15°)	Antidunes
Sh	Sand medium to coarse	Horizontal lamination	Plane-bed flow (super critical flow)
Sc	Sand medium to coarse	Convolute lamination	Soft sediment deformation structure

Table 2. Architectural elements, their two-letter codes, and a short description of their characteristics as used in this study (following MIALL, 2010).

Element	Symbol	Brief description
Channels	CH	Basal contacts usually erosive, convex upwards lens or sheet like cross section, variable scales
Sandy bedforms	SB	Lens, sheet, minor bars, occurs like channel fills
Lateral accretion deposits	LA	Commonly wedge shaped interlayer silt and sands

CH: Channels, CH: channel fills

Channel deposits comprise lithofacies Sc, Gt, and Gh (Photos 1; 2). Few abandoned channels in Simila open pit are filled by Sl and Sh lithofacies. Channel deposits expose sharp erosion bases with relief of about 1.2 – 2 m. They often erode sand bed forms and other channels. Their geometry is of the concave-up channel shape, occasionally forming multi-storey channel geometry. Channel thicknesses are up to 2 m in most cases, and rarely up to 3.5 m.

Interpretation: channels comprising facies Sc, Gcm, and Gcc record channel deposition. The presence of coarse-grained deposits may indicate a sudden increase in the velocity of the depositional current. High lateral migration of channels is a typical feature. Most channels have a multi-storey and multi-lateral nature. Channels with simple fill were frequent, e.g. in the northern part of the section cropping out in Simila open pit.

SB: Sand bars and bed forms

Sheet-like bodies of sand bed deposits comprise facies Sh, Sr, and Sc (Photos 3-5). They have sharp bases and are often eroded by channels. The erosion relief of the sand bed forms can be up to 2 m. Sand bed forms are usually about 1.5 m thick, but occasionally exceed 2 m.

Interpretation: sand bed forms record intra-channel deposition. They were probably produced by migrating dunes within the channel. This is supported by palaeocurrent measurements, which show palaeoflow directions to the NNW, NE. Because of the SSE-NNW orientations of all exposures, where channel geometry is displayed close to the longitudinal cross section (subparallel to palaeoflow direction), the channel geometry appears sheet-like. Sand bed forms were probably deposited by migrating dunes within the channel.

LA - Lateral accretion deposits

These deposits include units with geometries of wedges and lobes or sandy sheets that are reduced to a thickness between 1.2 and 2.5 m and widths of 3-5 m, without lateral continuity (photo 10). The facies components are: St, Sp, Sl (Table 1). They represent deposits accumulated by lateral accretion, with a reactivation surface, occurring as a result of changes in the base level of the river.

Interpretation: braided rivers have many channels, separated by temporary bars and islands. Rivers in alpine and arctic areas have seasonally high discharge variations and tend to be braided (MIALL, 2010).

The palaeocurrent analysis (Fig. 4) shows a relatively low spread of palaeocurrent vectors, which is characteristic of low sinuosity streams. The major palaeoflow trends are to the SE and to the SW. Therefore, one can estimate that the main sediment supply was from the NE and NW.

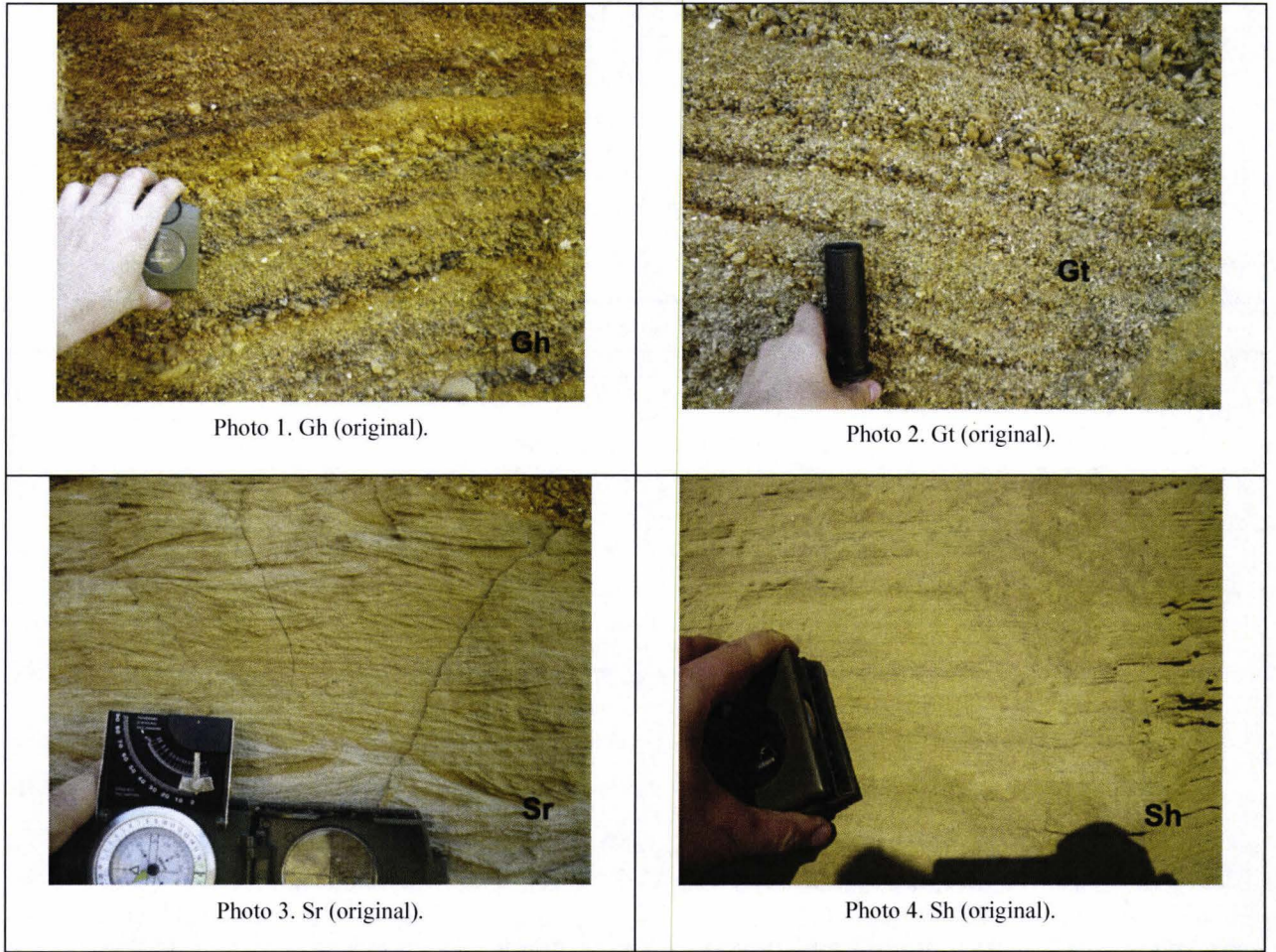




Photo 5. Sc (original).



Photo 6. St (original).



Photo 7. Sl (original).



Photo 8. Sp (original).

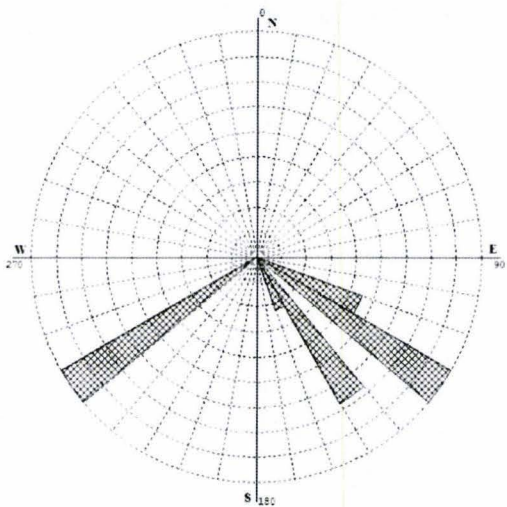


Figure 4. Rose diagram of palaeocurrent directions for Simila Open Pit.

There were collected ca. 150 pebbles to which measured axes were taken. The morphometric analysis was made after classic methods (ZINGG, 1935). Through direct measurements, by calipers, the values of the three axes (a, b, c) for each pebble issued. The distribution of the 150 pebbles in four classes (ZINGG, 1935) is: i. isometric class: 20; ii. prismatic class: 23; planar – lamellar class: 34; planar – disk class: 75 (Fig. 5). The pebbles analysed originated from jasper, sandstone and quartzite.

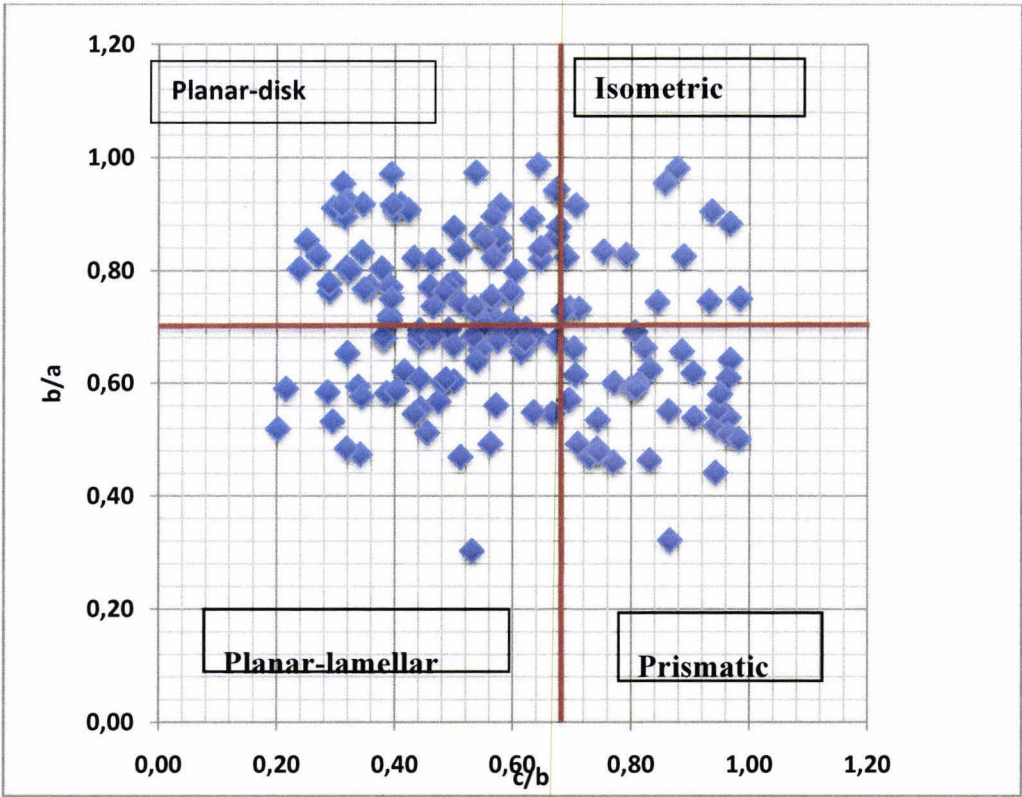
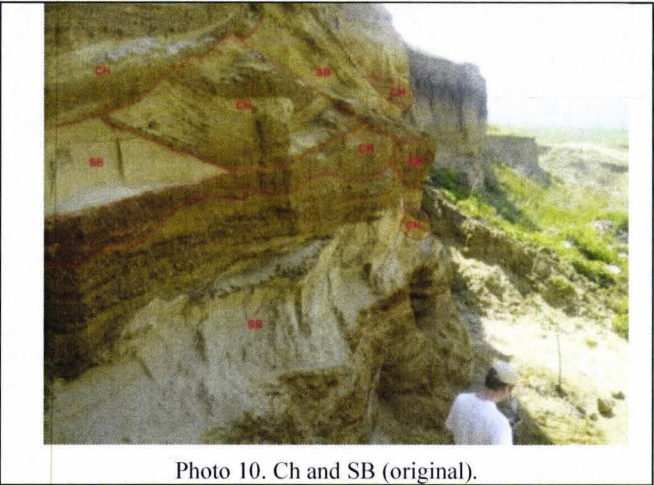
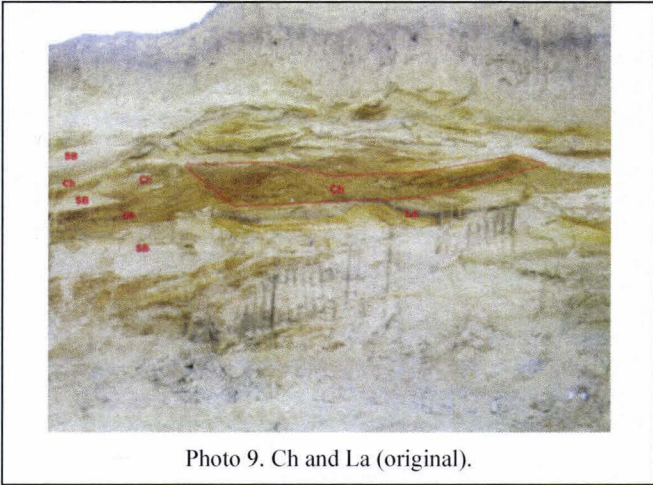


Figure 5. Zingg diagram with the distribution of the 150 pebbles in four classes.

PALAEONTOLOGY

Two kinds of fossils were collected in Simila open pit: the first are Miocene and refer to fossil wood fragments, invertebrates (molluscs) as well as to various vertebrates; the second ones are Quaternary and concern rather numerous mollusc shells belonging to Painter’s Mussel [*Unio pictorum* (LINNAEUS, 1758)] and vertebrate remains. Each of them have peculiar taphonomy: while all the Miocene fossils are in majority fragmentary, marked by a rather long transport, being reworked from older deposits, the Quaternary ones were always found as disarticulate teeth and bones, some of them being also transported by the water streams, but probably only on small distances. Our study is focused on the vertebrate fossils, the following taxa being recorded in Simila open pit.

I. Miocene vertebrates

Class Reptilia LAURENTI 1768
 Order Chelonii BRONGNIARD (LATREILLE) 1800
 Family Testudinidae BATSCH 1788
 Testudinidae indet.

Plate I, Fig. 1

Numerous carapace fragments, extremely rolled by the fluvial transport were collected at Simila. Some of them allow an assignation to Testudinidae (e.g. VPMNSB C5270).

Remains of Testudinidae were reported from the Sarmatian (Late Bessarabian) deposits at Draxeni (Moldavian Platform) by CODREA & URSACHI (2007). Although found transported and buried in brackish littoral deposits, such remains are there widespread, indicating their rather high frequency in the continental Middle Sarmatian biota. Representatives of the genus *Protestudo* CHIKHIKVAÐZE 1970 were also reported in the Middle Sarmatian-Meotian several localities in the Republic of Moldova (KHOSATZKY & REDKOZUBOV, 1989; LUNGU & RZEBIK-KOWALSKA, 2011).

Class Mammalia LINNAEUS 1758
 Order Rodentia BOWDICH 1821
 Castoridae GRAY 1821
 Castoridae indet. (cf. ?*Chalicomys jaegeri* KAUP 1832)

Plate I, Fig. 2

Only a fragment (VPMNSB C5271), belonging probably to a broken upper premolar or molar may be related to this group. The fragment is probably preserving a part of the paraflexus, mesostyle, paracone, mesoflexus and the posterior wall of the metacone. This cheek tooth could eventually belong to *C. jaegeri*, a castorid with a long Miocene history in Europe: first occurrence in MN4 unit, last occurrence in MN13, but typical for MN9 (HUGUENEY, 1999). It is the oldest Neogene castorid reported from Moldavia.

Ord. Perissodactyla OWEN 1848
 Genus *Hippotherium* VON MEYER 1829
Hippotherium sp. (cf. *Hippotherium primigenium* VON MEYER 1829)

Plate I, Fig. 3

Numerous hipparion teeth were collected at Simila open pit, but the majority is in a very poor state of preservation. A lot of this sample includes only fragments, clearly indicating a long transport by the river streams. Such fossils could be reworked either from the Upper Sarmatian (Khersonian), or from the Meotian deposits. Among these fragments, the most illustrative for instance are a heavy worn crown of a left P2 (VPMNSB C5272; crown length: 29.3 mm; crown breadth: 22.6 mm), and an upper incisor (VPMNSB C5273). The size, the presence of a double pli-caballin, as well as the high enamel plication in fossettes could suggest a representative of the *Hippotherium primigenium* group (BERNOR & ARMOUR-CHELU, 1999).

From the Republic of Moldova, such hipparions had been described as a distinct species, *Hipparion sarmaticum* LUNGU 1973 (LUNGU, 1984), largely distributed in the Middle and Late Bessarabian between the Prut and the Nester (LUNGU & RZEBIK-KOWALSKA, 2011). Same form of hipparions is reported in Moldova, starting with the Middle Bessarabian (COCHIOR & NECHITA, 1993) until the Late Sarmatian or perhaps the Early Meotian (RĂDULESCU et al., 1995; ȘTIUCĂ, 2003). In ALBERDI's (1989) viewpoint, such hipparions would be included in the group she called "morphotype 1".

Family Rhinocerotidae OWEN 1845
 Tribe Aceratherini DOLLO 1885
 Aceratherini indet.

Plate I, Figs. 4; 5

Several teeth fragments belonging to rhinoceros were collected at Simila, but only a single one is enough diagnostic for a convenient systematic assignation (VPMNSB C5274). It is a fragment of a right P2, preserving a root and a portion of the crown with the whole ectoloph. The ectoloph (length: 29.1 mm) is labially convex and at this wearing, practically unfolded, although a reminiscent metacone fold can be however, noted. A continuous, but not very strong labial cingulum is present.

The tooth size is rather small, smaller than in *Aceratherium incisivum* KAUP 1832 (GUÉRIN, 1980), a frequent rhinoceros in the Late Miocene of Moldova (CODREA, 2000). This size could correspond to *Aceratherium (Alicornops) simorreense* LARTET 1851, a small rhinoceros which stratigraphic range in Western Europe is in MN6-MN10 units time span (GUÉRIN, 1980), recorded also in the Republic of Moldova (as the subspecies *Aceratherium (Alicornops) simorreense orientalis* LUNGU 1984) in the Middle and Late Bessarabian (LUNGU, 1984; LUNGU & RZEBIK-KOWALSKA, 2011). But same sizes of such teeth is mentioned also in other rhinoceros finds, as "*Aceratherium simplex*" (KROKOS, 1914). Even if the available characters are very scarce on such a fragmented and worn premolar, we consider that it belonged to an acerathere and not to a *Chilotherium*, *Acerorhinus* or *Dihoplus* representative (KROKOS, 1917; KOROTKEVICH, 1970; GERAADS & SPASSOV, 2009) based on the estimated low hypsodonty and size.

Ord. Artiodactyla OWEN 1848

Family Cervidae GRAY 1821

Cervidae indet.

Plate I, Fig. 6

A single antler fragment (VPMNSB C5276) could document unambiguously the presence of cervids at Simila. Although broken and heavy rolled, this beam with surface marked by irregular dispersed longitudinal grooves, clearly was positioned near an antler dichotomy. The size, as well as the antler outline is very close to the ones known in *Procapreolus* SCHLOSSER 1924, a genus widely distributed in Eastern Europe in the Late Miocene (DIMITRIEVIĆ & KNEŽEVIĆ, 1988; KRAKHMALNAYA, 2008; LUNGU & RZEBIK-KOWALSKA, 2011), when it occurred in this area (VALLI, 2010).

Subfamily Antilopinae BAIRD 1857

Antilopinae indet. (cf. *Gazella* BLAINVILLE 1816)

Plate I, Fig. 7

Few horn fragments could be related to Antilopinae, but the most diagnostic is a fragment of a middle portion of a horn (VPMNSB C 5277; transverse diameter of the horn: 15.14 mm; antero-posterior diameter: 13.13 mm), marked by longitudinal narrow grooves, arched backward, document most probably a representative of *Gazella*. Such presence would not be surprising, as long as gazelles are known in neighbourhood areas both in the Republic of Moldova (LUNGU & RZEBIK-KOWALSKA, 2011) and Ukraine (KRAKHMALNAYA, 2008) in the Late Sarmatian and the Lower and Middle Meotian.

II. Pleistocene vertebrates

Order Proboscidea ILLINGER 1811

Family Elephantidae GRAY 1821

Elephantidae indet.

Plate I, Fig. 8

Only few tusk fragments (VPMNSB C5278), in a very poor preservation document the presence of mammoths at Simila. These remains could belong to *M. primigenius* (BLUMENBACH 1799), but these fossils are too damaged for a clear assignment.

Family Rhinocerotidae GRAY 1821

Genus *Stephanorhinus* KRETZOI 1942

Stephanorhinus hemitoechus (FALCONER 1868)

Plate I, Fig. 9

An isolated upper right P4 crown (VPMNSB C5279; crown length: 39.8 mm; crown breadth: 62.0 mm; high of ectoloph: 15.0 mm) extremely worn, with broken roots, can be assigned to this species. The dimensions are in accordance with the ones indicated by GUÉRIN (1980) for this species. The ectoloph is inclined and short. The occlusion surface has an oblong outline. At this wearing few morphologic details can be observed, but obviously any crista, crochet or anticrochet were present. On any side, there is no cingulum. Cement is still adhering to ectoloph.

According to GUÉRIN (1980), this rhinoceros first occurred in Western Europe since the Middle Pleistocene (GUÉRIN's Zone 22) until the Late Pleistocene (Zone 26). It was a prairie dweller, but it could occur in woody environments too (GUÉRIN, 1980; ORAIN et al., 2013). In the Middle and Late Pleistocene, the "steppe rhino" *S. hemitoechus* was gradually replaced by *Coelodonta* (KAHLKE & LACOMBAT, 2008), and certainly this process progressed from east towards western areas of Europe. In such circumstances, the Simila deposits could be even older than the Weichsel/Würm glacial. It is the first record of this Pleistocene rhinoceros in Moldova.

Ord. Artiodactyla OWEN 1848

Family Cervidae GOLDFUSS 1820

Genus *Megaloceros* BROOKES 1828

Megaloceros giganteus (BLUMENBACH 1803)

Plate I, Fig. 10

A fragment of a right antler (VPMNSB C5280) was unearthed near the base of the fluvial deposits in Simila open pit (Fig. 3). It concerns a broken beam, with the first and second tines, as well as the "palmation" broken too. The morphology of the antler resembles the one from Worms (Germany; POHLIG, 1892; LISTER, 1994). The morphology of antlers in *Megaloceros* is of low utility for the species stratigraphy, the variability being high. A similar morphology is recorded in Transylvania, in specimens as the one from Ciubanca (Cluj County; SZENTPÉTERY, 1911). CODREA & SOLOMON (2011) recently discussed the stratigraphic distribution of this species; therefore, we will not renew such a discussion. In Bârlad region, the giant deer was reported from Zorleni (CODREA et al., 2011).

CONCLUSIONS

A total of eight lithofacies types and three architectural elements were outlined. The sedimentological study of Simila deposits indicates that these rocks belong to a fluvial environment. The absence of additional outcrops in Simila

open pit area is an odd in establishing a trend of the ancient river flows and sedimentary input within the fluvial deposits. The facies analysis outlined a braided river environment with low sinuosity channels. Facies indicative for deposition from suspension or low velocity currents are absent. Palaeocurrent data indicate a prevailing SE to SW direction for the main clastic transport. The morphometric analysis on the 150 pebbles by the classical Zingg's method indicates a frequency of the planar-disk class.

We consider that the Sarmatian molluscs collected in the channel fill deposits are nothing but plain and simple reworked from older deposits largely exposed in the northeastern and northwestern areas of Vaslui County by the Pleistocene fluvial network, as the sedimentological study demonstrates now. The description of several Miocene mollusc taxa collected at Simila by BEJAN et al. (2012) was a useful paleontological study, but the deposits bearing these molluscs at Simila and Sâlcioara are certainly not Late Sarmatian (Khersonian), as their study tried to conclude. Same reworked status has the fossil wood fragments and all the Miocene vertebrate remains reported now at Simila.

The Pleistocene large herbivores give the correct geological age for the sands and gravels from this open pit. As the giant deer remains were collected near the base of the outcrop, it is obvious that all this sedimentary succession is most probably Late Pleistocene. The presence of the "steppe rhino" could argue for an older age of the Simila sands and gravels than Weischel/Würm glacial. For an advanced stratigraphy at Simila open pit, a richer sample of fossils is needed, but the promising potential of these deposits is an argument for such further results.

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Plate I : Fossil vertebrates from Simila open-pit, Vaslui County



Miocene fossils:

- Figure 1. Testudinidae indet., plate fragment (VPMNSB C5270).
Figure 2. Castoridae indet. (cf. ?*Chalicomysjaegeri*) ? upper cheek tooth fragment (VPMNSB C5271).
Figure 3. *Hippotherium* sp. (cf. *Hippotherium primigenium*), left upper P2, occlusal view (VPMNSB C5272).
Figure 4. Aceratherini indet., upper cheek tooth fragment (VPMNSB C5275).
Figure 5. Aceratherini indet., right upper P2 fragment, labial view (VPMNSB C5274).
Figure 6. Cervidae indet., antler fragment (VPMNSB C5276).

- Figure 7. Antilopinae indet. (cf. *Gazella*), horn fragment (VPMNSB C 5277).
Pleistocene fossils:
Figure 8. Elephantidae indet., damaged tusk fragment (VPMNSB C 5278).
Figure 9. *Stephanorhinus hemitoechus*, upper right P4, occlusal view (VPMNSB C 5279).
Figure 10. *Megaloceros giganteus*, right antler fragment, front view (VPMNSB C 5280).

THE COAL-GENERATING NEOGENE FORESTS FROM THE DACIAN BASIN

IAMANDEI Stănilă, DIACONU Florina

Abstract. Small or more extended Neogene intra and extra-Carpathian Coal Deposits are known in Romania, either related to a lacustrine environment or to the evolution of the Dacian Basin, one of the last remnants of the Central Paratethys. Here we made a short synthesis and a re-evaluation of all the previous research studies, part of them of the authors. As main results, beside the quantitative evaluation of coal resources in the basin, it is demonstrated that the most important Neogene coal-genesis within the Dacian Basin developed during the early Dacian to the late Romanian, and covered almost all the western part of the basin with large quantities of brown woody coal (lignite). It is also demonstrated that there can be outlined specific associations in the coal-generating vegetation living in the marsh and lake areas, and around, typical for each specific biotope and which gave specific lithotypes with specific technical properties. Also, an obvious cyclicity in the coal-genesis was observed during a sustained subsidence, the vegetal material being accumulated during four steps: fluvial, fluvio-lacustrine, telmatic and finally, lacustrine. And this was several times repeated, because more than twenty two coal seams, sometimes thicker than two meters, are known in the western part of the Dacian Basin.

Keywords: coal-generating forest, coal seam, palaeoenvironment, palaeoclimate, cyclicity.

Rezumat. Pădurile neogene carbogeneratoare din Bazinul Dacic. În România sunt cunoscute zăcămintele de cărbuni neogeni mai mici sau mai extinse în spațiul intra și extracarpatic, fie în relație cu medii lacustre, fie cu evoluția Bazinului Dacic, unul din ultimele rămășițe ale Paratethysului Central. Am făcut aici o scurtă sinteză și o revedere a tuturor cercetărilor anterioare, parte din ele și ale autorilor. Ca principal rezultat, pe lângă evaluarea cantitativă a resurselor de cărbune în bazin, se demonstrează că cea mai importantă carbogeneză din Bazinul Dacic s-a desfășurat în intervalul Dacian inferior - Romanian superior, acoperind aproape toată partea vestică a bazinului cu mari cantități de cărbune brun lemnos (lignit). Se demonstrează, de asemenea, că în cadrul vegetației carbogeneratoare se pot contura asociații specifice, trăind în arii mlăștinoase sau lacustre și împrejurul lor, tipice pentru fiecare biotop specific, și care dau litotipi specifici, cu proprietăți tehnice specifice. De asemenea, s-a observat o ciclicitate evidentă de-a lungul unei subsidențe susținute, materialul vegetal fiind acumulat în cursul a patru pași: fluvial, fluvio-lacustru, telmatic și, în sfârșit, lacustru. Iar aceasta s-a repetat de mai multe ori, deoarece mai mult de 22 straturi de cărbune, uneori mai groase de doi metri, sunt cunoscute în partea vestică a Bazinului Dacic.

Cuvinte cheie: pădure carbogeneratoare, strat de cărbune, paleomediu, paleoclimat, ciclicitate.

INTRODUCTION

During the Neogene, within the Romanian Carpathians area, coal deposits were formed within three coal-generating phases: the first phase - developed during the Early Sarmatian (Volhynian), the second phase developed during the Early-Middle Pontian and the third phase, the most important one, developed during the Pliocene (Figs. 1, 2), in most favourable conditions for the accumulation of vegetal remains created into the Dacian Basin, especially between the Danube and the Olt rivers, i.e. Oltenia region (Fig. 1).

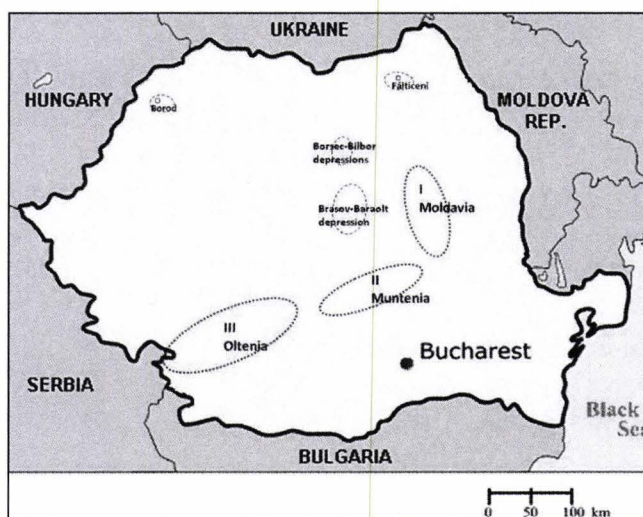


Figure 1. General location of the Neogene coals on Romania map.

Over 3 billion tons of brown woody coal (lignite) has been evaluated within this coaly region situated in the western part of the Dacian Basin. Oltenia is the most important Late Neogene coaly region (with Motru-Rovinari and Dedovița-Husnicioara-Pinoasa areas), followed by Muntenia (with Schitu-Golești area), and with numerous exploitations as mines or open pits (Figs. 1, 3).

Researches were done and numerous scientific papers were written on the Neogene coals from the Carpathian area and on their genesis by RĂILEANU (1963); PREDA et al. (1981); PAULIUC & BARUS (1982); PETRESCU et al. (1987); BARUS (1987); PAULIUC et al. (1988); ȚICLEANU (1986a, b, 1992a, b, 1995a, b, 2006); ȚICLEANU & ANDREESCU (1988); ȚICLEANU & BIȚOIANU (1988, 1989); ȚICLEANU & DIACONIȚA (1997); ȚICLEANU et al. (1982b, 1985, 1988, 1999, 2001, 2004); DIACONU (2000a, b, c, 2001, 2002b, 2004a, b, c, 2005, 2006a, b, c, 2008).

The complex processes of carbo-genesis or coal genesis, usual terms in coal science signifying all the processes of genesis of the coals – (ȚICLEANU, 1992b; BERGOSSI, 2003; ZAITSEVA et al., 2004) were well understood and explained in the Dacian Basin (ȚICLEANU, 1992b, 1995a, 2006; DIACONU, 2000a, 2002a, 2004a, 2007; DIACONU & ȚICLEANU, 2008).

Thus, Oltenia region has an extended Pliocene coal-genesis, represented by more than twenty thick lignite seams, some of them largely distributed within the Dacian Basin. They were explored and partially exploited by mining or in open pit, most of them being abandoned now.

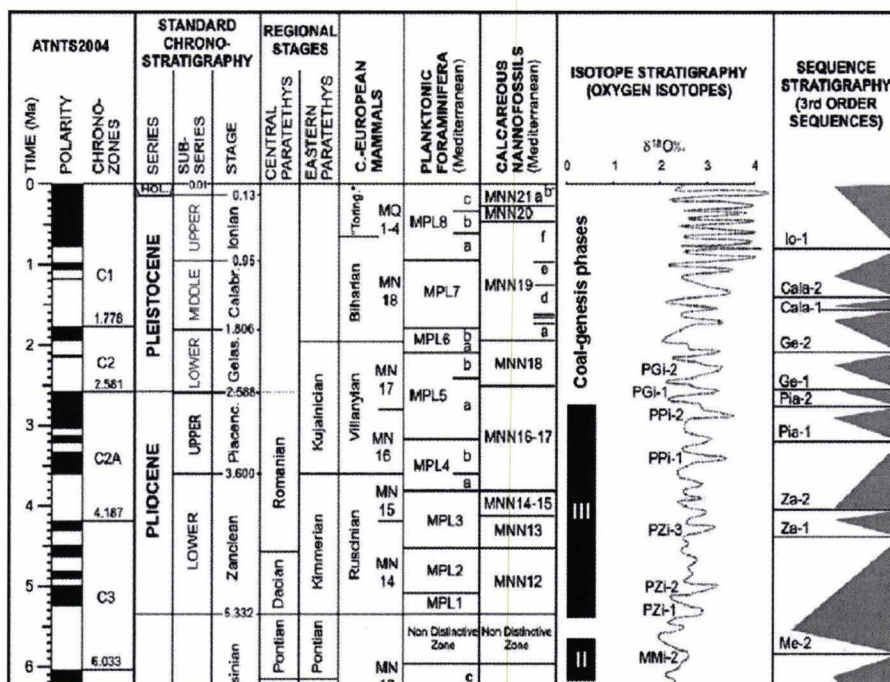
Other coal genesis phases are poorer. For example some small lenses or decimetric seams of coals were discovered within the sediments from the Meotian till the Pliocene in the Subcarpathians, in Moldavia region, between the Trotuș and the Buzău rivers (Fig. 3). Comănești area is more important – with Comănești, Asău, Dărmănești coal deposits – exploited by the mines Comănești, Lăloaia, Lapoș, Sălătruc, Larga, Leorda and Vermești. Less important is Focșani-Odobești area with some occurrences of thin levels of lignites (at Pralea-Căiuți, Gospei-Secu, Reghiu-Poenița and Milcov Valley), and Buzău area (at Ojasca, Berca, Aricești). Also, in the Southern Subcarpathians, beside the Schitu Golești, which is a better developed coal deposit, some coal seams were mined at Aninoasa, Doicești, Șotânga, Filipeștii de Pădure and Ceptura. Almost all of these coal deposits have smaller economic importance (RĂILEANU, 1963; PETRESCU et al., 1987) and even if they were locally exploited, they were not included in a distinct coal-genetic phase (ȚICLEANU, 2006).

During the Pliocene, some intramontaneous basins developed within the Carpathians, resulting in coal deposits, locally exploited, as those from Brașov-Baraolt (or Bârsei-Baraolt) depression (at Vârghiș, Doboșeni, Căpeni, Baraolt, Herculan, Bățanii Mari, Bodoș-Aita, Vlădeni, Ilienii, Arcuș-Criș Valley, Ghidfalău), and from Bilbor, Borsec and Jolotca small depressions (Fig. 1). Nearly all of these coal deposits were subject of local exploitation by mining or quarry, beginning with the 19th century, now being abandoned.

The sediments of all these coal deposits include clayey levels providing fossilized leaves, fruits, seeds and pollen coming from the plants which lived around or in the coal-generating area. Most of the Romanian palaeobotanists focused on these sediments. They realized and published a lot of specialized studies, in an attempt to reconstruct the coal-generating palaeovegetation, the palaeoenvironment, the palaeoclimate and the palaeogeography of those final stages of the Neogene: POP (1936); BARBU (1954, 1960); GIVULESCU (1967, 1992, 1996); PETRESCU & KOLOVAS (1983); PETRESCU et al. (1987, 1989a, b); ȚICLEANU (1986a, b); ȚICLEANU & ANDREESCU (1988); ȚICLEANU & BIȚOIANU (1988, 1989); ȚICLEANU & DIACONIȚA (1997); ȚICLEANU & PARASCHIV (2000); ȚICLEANU et al. (1982a, 1985, 1988, 2002, 2004); IAMANDEI (2000); IAMANDEI & IAMANDEI (2000); DIACONU (2000a, 2002a, 2004a, 2007); DIACONU & ȚICLEANU (2008); POPESCU (2001a, b); POPESCU et al. (2006). A small synthesis of their researches on the Pliocene coals will be included in this paper.

The Dacian Basin, as a remnant of the Paratethys, was an important area of sedimentation. Here, especially during the Pliocene, ideal environmental conditions were created for the development of vegetation with a great quantitative growing and replacement ratio. This way the basin of accumulation was fed for a long time with vegetal material, within a fluvial, deltaic or lacustrine environment with specific sedimentation. Huge quantities of vegetal material, often mainly woody, were accumulated. Periodic catastrophic precipitations can be assumed, at least as a logical explanation for those lignite seams looking like “log deposits”, from Motru-Rovinari area. This is very probably, since the Messinian crisis was not only a regional event within the Mediterranean area. At least within the Carpathians, this interval corresponds with the progressive closing of the Dacian Basin, a part of the Paratethys marked by a very important Pliocene coal-generating episode. Also here, during the Pliocene, the palaeoenvironment was rapidly modifying, the Carpathians being an active zone, which is still moving. The last Pliocene volcanism was still acting, and the mountains are still arising nowadays. The last small intramontaneous basins were filled by sediments, sometimes including variably sized coal levels. All the observations and the studies done till now on these coal deposits give us an idea, not only on the palaeoenvironment and the sedimentation style, but on the vegetation of those times. Unfortunately few palaeoxylotomical investigations were done in these coals in order to clarify the taxonomic composition of the xylitic coal seams (IAMANDEI & IAMANDEI, 2000).

To have a good geochronological setting for the next discussions, it must be taken into consideration that in June 30, 2009, IUGS ratifying the “ICS Recommendation on redefinition of Pleistocene and formal definition of base of Quaternary” changed the Pliocene chronostratigraphy, since Gelasian stage go representing the Pleistocene basal (Fig. 2), so the Pliocene start from 5.332 MY with Zanclean, and goes, with Piacenzian, up to 2.588 MY (WALKER & GEISSMAN, 2009). The equivalent stages within the Central Paratethys are represented by the Dacian, which would correspond to calcareous nannoplankton zone NN12, and the Romanian which would correspond to NN13 to NN17 as well. The changes should be in Fig. 2.



COAL-GENERATING PALAEOENVIRONMENTS WITHIN THE DACIAN BASIN. THE PLIOCENE MODEL

The Dacian Basin - in Romanian it is named and orthographed “Dacic”; similarly it is pronounced as in French “Dacique”, see JIPA (2006) - was a part of the late Paratethys (Fig. 3).

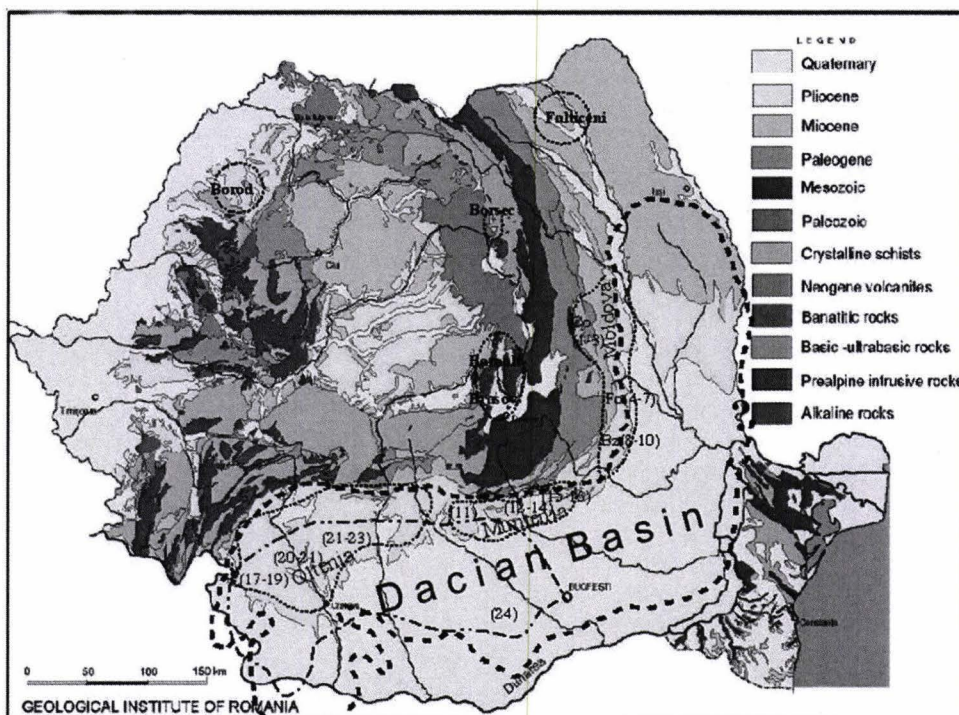


Figure 3. Areas of the main Neogene coal genesis within the Dacian Basin.

(Sketch of the Geological Map of Romania, with the extension of the Dacian Basin, modified after JIPA & OLARIU, 2009).

Moldavia region – The Sarmatian coal genesis: Fălțiceni area, Co-Comănești area (1-Comănești, 2-Asău, 3-Dărmănești); + some Meotian to Pliocene coal occurrences: FO-Focșani-Odobești (4-Pralcă-Căiuți, 5-Gospei-Secu, 6-Reghiu-Poenița, 7-Milcov Valley); Bz-Buzău (8-Ojasca, 9-Berca, 10-Aricești); *Muntenia region* – The Pontian coal genesis (11-Schitu-Golești, 12-Șotânga, 13-Doicești, 14-Aninoasa); + some Pliocene coal deposits: 15-Filipeștii de Pădure, 16-Ceptura. *Oltenia region* – The Pliocene coal genesis (17-Dedovița, 18-Husnicioara, 19-Pinoasa, 20-Motru, 21-Rovinari, 22-Plotina, 23-Mătășari, 24-Deep extension).

This basin was intensely studied from all points of view, not only because of its huge coal deposits, which were formed during the most important Neogene coal genesis within the Carpathian area, the Pliocene one. For comparison it might be useful to mention also the previous two coal genesis phases on Romania's territory, in terms of the Romanian scientific literature:

I. The Sarmatian coal genesis is represented by the early Sarmatian (Volhynian) lignite of the Borod basin (POPA, 2000; PETRESCU, 2003). Also some Volhynian occurrences were described by ȚIBULEAC (2001) in Fălticeni area (at Fălticeni, Boroaia, Bogdănești), on the Moldavian Platform (Fig. 1), where a coal-generator peat formed on the paralic plains of the Dacian Basin which had a typical peat vegetation with *Glyptostrobus*, *Myrica*?, *Alnus*, *Salix*, *Liquidambar* and also with *Phragmites* and *Typha*. Even if the biotic and climatic conditions were convenient, the tectonic and palaeogeographic conditions were not too favourable and therefore few coal seams of reduced thickness and extension were formed.

II. The Pontian coal genesis was developed especially in Muntenia region (Figs. 1, 3), in Schitu-Golești area (exploited points at: Berevoiești, Godeni, Pescăreasa, Poienari and Jugur), as well as in some other places where thinner coal intercalations within Middle Pontian appear (in PETRESCU et al. 1987). Aninoasa and Doicești coal deposits could be added (other coal deposits in the same Subcarpathian region, situated much to East: Filipeștii-de-Pădure, Șotânga and Ceptura - are more recent, of Pliocene age, and were not included in this phase of coal genesis). The Pontian coal genesis developed only in the inner part of the Carpathian Foredeep, where the palaeorivers resulted in not too extended alluvial-lacustrine accumulation plains with a continuous subsidence (e.g. between the Argeș and the Topolog rivers). The wet and warm-temperate climate was favourable to a coal-generating peat and to large forests with *Glyptostrobus europaeus*, *Alnus cecropiaefolia*, *Bytneriophyllum tiliaefolia*, a. o..., as other Pontian forests in the Carpathian area show (GIVULESCU, 1967, 1992, 1996, 1997; ȚICLEANU & PARASCHIV, 2000; ȚICLEANU et al., 2002; PETRESCU et al., 2002; DIACONU, 2004a).

III. The Pliocene coal genesis developed within the Dacian Basin, which still had a large extension during the Pliocene, especially during the Dacian stage to the middle-late Romanian (Fig. 2). Some coeval deposits were developed in the intramontaneous small depressions (Fig. 3). The Dacian Basin had a fluctuant evolution in relation with the Euxinian Basin during the Dacian stage, as well as during the Romanian stage. As a result, favourable conditions were created for an extended coal genesis on the depositional plains situated at the foot of the arising Carpathians. The type of sedimentation consisted dominantly of a fluvial facies and swampy, but into the basin – a proximal (littoral) or distal (of deeper water) sedimentation can be separated. The stratigraphic columns in this area show many lignite seams: around 22 coal seams can be counted from the Early Dacian up to the Late Romanian, in Oltenia coaly region (Fig. 4).

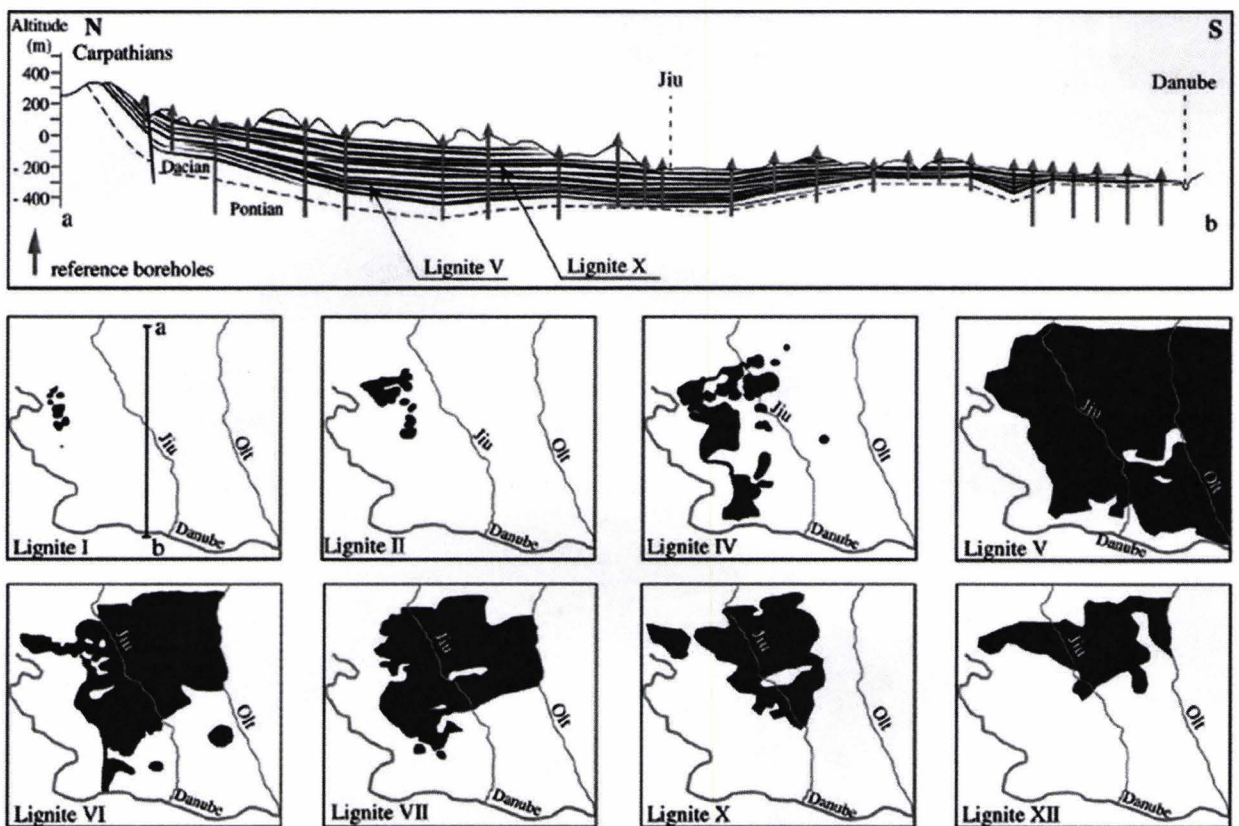


Figure 4. Extension of main coal layers within the Dacian Basin, N-S section (a-b) and maps. (reproduced from POPESCU et al., 2006, by courtesy).

Some small coal seams appear up to the Early Pleistocene, but of small economic importance (ȚICLEANU, 2003). It is interesting that the area covered by the lignite layers is variable. This was demonstrated on the basis of the drilling results. For example, the 5th coal seam, which is marked by a strange high level of radioactivity, is the most extended one (ȚICLEANU, 2006; Fig. 4).

There is a palaeogeographic and tectonic explanation: the Dacian Basin was a variably extended alluvial-lacustrine accumulation plain situated in front of the mountains. It had a longtime stable subsidence rate which was equal to the accumulation velocity of coal-generating vegetal material and this happened during 2.000 up to 18.000 years (ȚICLEANU et al., 2004; ȚICLEANU in JIPA, 2006).

Within the inner foredeep, the thickness of the coal layers or of the coal complex (coals and associated sediments) is more developed than in the outer foredeep of the Carpathians, because a role in the subsidence could be paid to the differentiated compaction of the sediments (ȚICLEANU, 2003; ȚICLEANU et al., 2004). The negative relief of the platform also had much importance in the coal accumulation and again, the tabular and the compactional subsidence could be considered.

In fact, these structural and palaeogeographic conditions favoured the coal accumulation in these polygenetic accumulation-plains of fluvial, deltaic and lacustrine origin, which is characteristic of the molassic basins situated in front of the mountains.

The Dacian Basin was also filled with sediments along the entire region arising as a result of plate movements, and the lower Danube was born. In the Dacian Basin, the most important Neogene coal genesis developed between the Early Dacian and the Middle-Late Romanian and, according to ANDREESCU et al. (1985), it is composed of three coal complexes:

- Vișenilor Valley Coaly Complex is represented by some coal seams hosted by the Early Dacian Berbești Formation (coal seams A-D and I-IV).
- Motru Coaly Complex, the most important one, is represented by the coal seams V-XIV hosted by the Jiu-Motru Formation. It was formed during the Late Dacian to the Middle-Late Romanian. The seams V-VII and X had the best qualitative characteristics.
- Bălcești Coaly Complex, hosted by Cândești Formation of the middle-late Romanian to the early Pleistocene age, developed within the central-western part of Oltenia coaly basin. It is represented by the seams XV-XVIII, having reduced economic importance.

Some coal seams are variably extended under the blanket of the Moesian Platform. As we mentioned, a coal seam of more than 4 m thickness was identified from drillings extended up to Bucharest area, at the depth 400–600 m. It is most probably synchronous with 5th coal layer from the Oltenia (ȚICLEANU, 2006; POPESCU et al., 2006a, b; Fig. 4).

Another Pliocene coal deposits resulted in a similar type of coal genesis, but at a smaller scale. They appeared in some small intramontaneous Carpathian depressions which were active during the Latest Pontian to the Dacian (GIVULESCU, 1996; 1997) as isolated lakes or fluvial corridors.

There, fluvial-lacustrine deposits with coal seams accumulated: within Brașov-Baraolt Depression, explored or mined in several points, now abandoned, and also within Bilbor, Borsec and Jolotca Depressions (BANDRABUR & CODARCEA, 1974; BANDRABUR et al., 1990).

Pliocene coal genesis related to some small intramontaneous basins associated with the Pannonian Basin also appeared within the western part of the Apuseni Mts., especially within Borod area (GIVULESCU, 1996, 1997).

However, it could be stressed that the most favourable palaeogeographic and tectonic conditions for a spatial and temporal extension of the Pliocene coal genesis were created within the Dacian Basin, a basin with large plains of sedimentation developed just in front of the Carpathians. These conditions and the coal-generating peat determined the qualitative and quantitative characteristics of coal seams.

Thus, the coal layers from Vișenilor Valley Complex were formed on upper deltaic plains, as their depositional system shows. The coal layers from Motru Complex were formed on fluvio-lacustrine accumulation plains where coal-generating peats were developed, following four distinct phases: fluvial, fluvio-lacustrine, palustrine (telmatic) and finally, lacustrine. This typical succession was also described by NEBERT (1983), within the Ebisswalder Basin, Germany, and is perfectly consistent to the theoretical model of coal-generating swamps of TEICHMÜLLER (1958, 1962) (Table 1).

Table 1. The model of a coal-generating swamps according to TEICHMÜLLER (1958, 1962).

No.	Lake areas	Type of vegetation
1.	Marginal zones	Forests with Conifers (<i>Sequoia</i> , <i>Glyptostrobus</i> , <i>Pinus</i> , <i>Sciadopitys</i>);
2.	Seasonally flooded zones	Swamps or marshes with <i>Carex</i> and then with hygrophytes like <i>Alnus cecropiaefolia</i> , <i>Myrica lignitum</i> or, latter, with <i>Salix</i> sp., <i>Byttneriophyllum tiliaefolium</i> ;
3.	Already permanently flooded zones	Swampy forests with <i>Glyptostrobus europaeus</i> and/or <i>Taxodium dubium</i> ;
4.	Permanently flooded zones	Vegetation with <i>Phragmites</i> and <i>Typha</i> ;
5.	Free water (deep water) zones	Aquatic vegetation with <i>Stratiotes dacicus</i> , <i>Trapa</i> sp., <i>Nelumbo protospeciosa</i> and others.

Such a coal-generating swamp is characterized by a palaeophytocoenosis which is controlled by the hydrological regime, the edaphic factor, the Eh, the pH, i.e. the important parameters of the depositional environment.

During the fluvial transport the vegetal remains mixed with inorganic sediments, which always appear in the ash-content of the coal. Plotted on maps it indicates the direction of the inorganic input (ȚICLEANU, 2006).

Also, evaluating the pollen assemblages and diverse other macroremains studies, PETRESCU et al. (1989b, 2002), and IAMANDEI (2000) estimated a pluvial palaeoenvironment (MAP=1000-1100 mm/year) in a Cfa climate (Köppen classification in PEEL et al., 2007), for the Motru area (at least for Lupoia lignite deposit). According to the cited authors, the swamp complex comprised a flora with *Glyptostrobus*, *Taxodium*, *Alangium*, plus *Salix*, *Acer*, *Alnus*, *Nyssa*, *Myrica* (abundant pollen), *Cyrilla*, *Ilex*, *Symplocos* - also with *Phragmites*, *Typha*, *Potamogeton*, *Stratiotes*, *Sparganium*, *Poaceae* div. If the open water included Nympheaceae, *Stratiotes*, *Salvinia*, *Botryococcus* and Zygnemataceae, etc., the dry peat bog was occupied by a forest of *Sequoia*, *Sciadopitys*, *Pinus* div. and ferns as *Lygodium*. More distantly, forests with *Abies*, *Cathaya*, *Cedrus*, *Quercus*, *Tilia*, *Ulmus*, *Juglans*, *Pterocarya*, *Betula*, *Parthenocissus*, *Liquidambar*, *Fagus*, *Castanea*, *Ilex*, *Magnolia*, *Liriodendron*, etc. were present.

Repeated flooding in the coal-generating swampy peat bog brought barren epiclastic intercalations of sands of fine to medium-sized granofacies (bearing now some secondary spherical concretions – so called “*trovants*”), as well as thin levels of small-sized pebbles or clay layers with plant remains as impressions, and oblique lamination. The coaly complex includes specific lithological successions, facieses and rhythms determined by the subsidence oscillation and the fluvial divagation. Usually, after a telmatic phase of the coal-generating peat, a lacustrine one followed (ȚICLEANU, 2006), with fauna of unionids and viviparidae (MARINESCU & PAPAIANOPOL., 1987). After advanced studies on the Pliocene coals from the Dacian Basin, ȚICLEANU (1995a) made an applied discussion on the palaeobotanical data implicated in the study of the coal deposits. Based upon this, he tried to answer many questions about the genesis of the coals and the necessary premises which achieved it, and defined those phytologic, climatic and palaeogeographic premises:

- *The phytologic premises* - imply a discussion about coal-generating and palaeophytocoenotic elements and about the frequency of some taxa in the fossil vegetal remains (FVR) which can indicate the source of the coal-generating phytomass. This discussion applied to the Pliocene coal genesis perfectly corresponds to other similar situations. For example, the frequency of *Glyptostrobus europaeus* in the Neogene coal deposits in Europe was still observed by SCHIMPER (1872), then confirmed by many researchers (e.g.: TEICHMÜLLER 1958; KNOBLOCH 1973; GIVULESCU 1992, 1996; ȚICLEANU 1986a), pointing out coal-generating vegetal communities. ȚICLEANU (1995b) concluded that, at least from the middle Miocene to the late Pliocene, about 90 taxa can be considered as furnishing coal-generating phytomass and he selected the most important ones (Table 2).

Table 2. Main taxa phytomass coal-generators (after ȚICLEANU, 1995b).

Taxa	Chronostratigraphic Units				
	Vh	Bs	Me	Po	Dc+Ro
<i>Osmunda regalis</i>					+
<i>Taxodium dubium</i>			+	+	+
<i>Glyptostrobus europaeus</i> <i>Glyptostroboxylon tenerum</i>	+	+	+	+	+
<i>Sequoia abietina</i>	+		+		+
<i>Liquidambar europaeum</i>	+		+	+	+
<i>Alnus kefersteini</i>		+		+	
<i>Alnus cecropiaefolia</i>		+		+	
<i>Betula macrophylla</i>	+	+		+	
<i>Salix</i> div.sp.	+		+	+	+
<i>Bytneriophyllum tiliaefolium</i>	+	+		+	+
<i>Myrica lignitum</i>	+	+	+		
<i>Phragmites oeningsensis</i>	+	+	+	+	+
<i>Typha latissima</i>	+	+		+	+
Vh – Vohlynian; Bs – Bessarabian; Me – Meotian; Po – Pontian; Dc – Dacian; Ro – Romanian.					

Even if many angiosperms could be considered coal-generators, it seems that especially some taxodiaceous gymnosperms with deciduous leaves (*Taxodium*, *Glyptostrobus* and *Sequoia*) had the most important contribution in coal genesis during all the Neogene, as big phytomass generators. Also, some monocots such as *Typha*, *Phragmites* may be considered phytomass generators, usually growing directly in the peat.

- *The climatic premises* - the temperature and especially the humidity – are essential to determine the quantity of phytomass. The mean annual precipitations (MAP) were evaluated by ȚICLEANU (1995b) to over 1500 mm/year, considering especially some hygrophytic indicators based on the presence of *Glyptostrobus*, *Bytneriophyllum* and *Salix*. This can be correlated with a positive temperature, which determined a big phytomass quantity. In Recent times for example, the phytomass vary from 189 t/ha within the boreal zones to 342-366 t/ha in the temperate zone, and up to 440 t/ha at the tropics (WALTER & WIESER, 1973).

- *The palaeogeographic premises* - imply large areas of low eutrophic swamps which are subject to subsidence, inclusively of compactional type, whose rate is quite equal to the accumulation rate of the vegetal material originating in the vegetal communities of the swamps. Such conditions can be achieved in the same palaeogeographic situation as in a paralic basin, or in intracontinentally accumulation plains of fluvial, lacustrine, deltaic origin or

combined (WILSON, 1976). The control factors of the variety of palaeophytocoenosis within the coal generating swamps are represented by hydrological regime, edaphic factor, Eh, pH, etc. These factors help create a good reconstruction of the depositional environment (ȚICLEANU, 1995a, b). The models of coal-generating swamps given by TEICHMÜLLER (1958) comprise marginal, seasonally flooded, almost permanently flooded, and permanently flooded and free water zones of a basin (i.e. pond, more or less extended lake, etc.). They can be recognized in many coal deposits because each zone is characterized by the presence of some taxa, or associations of taxa, adapted to the respective conditions. For example, they can be recognized in the western and north-western part of the Dacian Basin (Table 3).

Table 3. Pliocene vegetal communities in the western part of the Dacian Basin (according to DIACONU & ȚICLEANU, 2006).

Nr. crt.	Vegetal communities
1	Dry forest with <i>Sequoia</i> sp.:
2	Swamp with <i>Carex</i> div. sp. and <i>Pandanus</i> sp.:
3	Swamp with <i>Salix</i> sp. and <i>Myrica</i> sp.:
4	Swamp with deciduous hydrophytes forest with two variants (with <i>Salix</i> div. sp., <i>Liquidambar europaeum</i> , <i>Acer tricuspidatum</i> , <i>Alnus</i> sp., <i>Carya aquatica</i> , <i>Juglans barbut</i> , etc., or with <i>Byttneriophyllum tiliaefolium</i>):
5	Swamp of forest with <i>Glyptostrobus europaeus</i> associated or not with <i>Taxodium dubium</i> and/or <i>Nyssa</i> sp.:
6	Associations of <i>Phragmites</i> sp. and <i>Pandanus</i> sp. with or without <i>Typha latissima</i> , <i>Sparganium</i> sp., etc.
7	Aquatic plants associations with monocoenoses dominated by one of the taxa: <i>Stratiotes dacicus</i> , <i>Nelumbo</i> sp., <i>Nymphaea</i> sp., <i>Ceratophyllum</i> sp., etc.

These associations, typical for each specific biotope, determined specific lithotypes with specific technical properties (ȚICLEANU et al., 1999). For example, by analyzing the almost permanently flooded zones in the evolution of the Dacian Basin, the mid-Miocene association with *Taxodium dubium* evolved to a very developed one with *Glyptostrobus* during the Pliocene (Table 1). The permanently flooded zones dominated by *Phragmites* and *Typha* comprise also other aquatic taxa as *Stratiotes*, *Potamogeton*, *Ceratophyllum*, *Nelumbium* etc. (ȚICLEANU, 1995a, b). Extended areas of coal seams have been emphasized by a net of boreholes and by open cast which found all the concentric zones of TEICHMÜLLER (1958). We already specified that a special situation occurs at the 5th layer which is the most extended and comprises also the delta plain of river Lom which is coming from Bulgaria, as ŠIŠKOV & ANGELOV (1984) estimated.

According to the already known classic theoretical model of coal genesis and to his previous observations coupled with other researches (ȚICLEANU, 1992a, b; 1995a), a new model was imagined, valid at least for the Pliocene coal genesis from the western part of the Dacian Basin (ȚICLEANU & DIACONIȚĂ, 1997), as a result of the combined action of more factors:

- the tectonic factors - determining subsidence, accumulation velocity, diving velocity, results, rhythms, phase, active and compactional subsidence, plicative or ruptural tectonics;
- the palaeogeographic and depositional factors - referring to the peat bogs developing and the coal-generating basins as areas of sedimentation;
- the biotic factors - represented by the macroflora (the vegetation), plus the microflora and the microfauna of the peat bog which give the qualitative characteristics of coal seams and define the genetic model of coal accumulation (ȚICLEANU, 1995a) producing specific lithotypes with specific technical properties (ȚICLEANU et al., 1999);
- the climatic factors are very important, their analysis enabling the reconstruction of areal palaeogeography and palaeoclimate;
- the coaly facieses factors - as the vegetal communities, i.e. the coal-generating vegetation, the indicators of depositional environment (nutrients, pH, Eh, peat temperature, bacteria) determining the accumulation type;
- the physical and mechanical factors - which determine the differentiated compaction;
- the geothermal factors - which determine the coal metamorphism.

Surely, several palaeophytogeographic, ecologic and phytocoenotic considerations can be extracted from the analysis of these factors. Knowing well the action of each of these factors, the whole process of coal genesis can easily be reconstructed.

Among the scientific researches on coal genesis, firstly one must consider the sedimentological and the palaeobotanical studies which help us reconstruct the fluvial, deltaic, fluvio-lacustrine, or simply lacustrine palaeoenvironment and the coal-generating vegetation, especially the woody vegetation which is basic for a coal deposit formation. Sometimes, beside the vegetal fossils, vertebrate remains also can appear (CODREA & DIACONU, 2003, 2007; DICA et al., 2007).

Therefore, by prospection, exploitation works and scientific studies done within the Dacian Basin, more than 22 coal seams were identified. Distributed to some coaly complexes, they represent the rhythmic character of the sedimentation within the basin. Important data about the distribution and thickness of strata were studied and also on their morphology and on their petrography, defining the lithotypes. Their microscopic constituents as macerals and their qualitative characteristics were studied, and the process of coal genesis was reconstructed based on them [ȚICLEANU & BIȚOIANU (1989), ȚICLEANU & DIACONIȚĂ (1997), ȚICLEANU et al. (1999)].

Also, the researches of climatostratigraphy applied to Lupoia open pit (Motru area) showed repetitive clay-lignite alternations which were interpreted as corresponding to cyclic changes in climate (POPESCU, 2001b), forced by cycles of eccentricity, giving an age of 4.9-4.3 MY (Chronos C3n.3n-3n.1n) for the layers IV-XIII.

As already shown, in Oltenia area, the Pliocene coaly series was known from exploration and exploitation works and from systematic drillings. The stratigraphy of the region comprises successions of detrital rocks and numerous coal seams with various spatial and temporal extensions interpreted by DIACONU (2000b) and POPESCU (2001a, b) mostly as deltaic sediments.

Trying to reconstruct the coal-generating palaeoenvironment for the coal seams I-IV, in the western part of the Dacian Basin (Husnicioara area), DIACONU & ȚICLEANU (2006) analysed the palaeobiotopes and these vegetal communities, representing the terminal Pontian (probably) and the Earliest Dacian. In this way they defined various coal facieses and some palaeophytocoenoses were separated (Table 3) due to the vertical alternation of them, related to the modifications of the water depth in time and to the subsidence rate or to floods.

Otherwise DIACONU (2008) published the newly identified taxa within Husnicioara association and made a detailed analysis and reconstruction of the coal-generating Pliocene palaeoenvironment within the Dacian Basin. She identified the following forest association within Husnicioara coal deposit area: *Glyptostrobus europaeus*, *Ceratophyllum* sp. aff. *C. demersum*, *Platanus platanifolia*, *Carpinus betulus*, ?*Myrica lignitum*, *Quercus* sp., *Carya denticulata*, *Bytneriophyllum tiliaefolium*, *Salix* sp., *Pandanus austriacus*, *P. trinervis*, *P. barbui*, *Phragmites oeningensis*. She also indicated that most of these taxa belong to the dominant group of the coal swamps, in which the three mangrove species of *Pandanus* were firstly found in Romania's Pliocene. *Pandanus* was also mentioned by PALAMAREV & UZUNOVA (1969) within the Bulgarian Pliocene deposits, and they were considered as most presumably Oligo-Miocene relicts.

After MAI (1995) *Pandanus* lives in a mean annual temperature between 15° and 20°C, in tropical-subtropical climate, specifically to the SE Asia. For this reason DIACONU (2008) concluded that the Early Dacian climate was warm enough and characterized by precipitations of about 1400 mm/year, almost uniformly distributed, with a summer peak higher than 150 mm. However, interpreting the palynological content of the same interval, PETRESCU (2003) gave another resolution: MAT= 13°C, MAP=1300 mm/year, values rather consistent to other previous evaluations.

ȚICLEANU (1992) evaluated MAT (mean annual temperature) at least for the late Dacian and the Romanian as between 14° and 15°C, based on the presence of *Glyptostrobus europaeus* and *Bytneriophyllum tiliaefolium*. Once again, considering the macroflora and palynoflora described in the Motru area (from Țicleni, Roșia, Peșteana, Lupoia, a.o.) for the same interval, PETRESCU (2003) evaluated, with poor variations, more decreased values for MAT to 13-14°C and for MAP to 1000-1100 mm.

CONCLUSIONS

This paper tries to synthesize and re-evaluate the previous researches, part of them of the authors. It is focused on the reconstruction of the palaeoenvironment and of the favourable conditions of coal genesis during the Pliocene time. The research demonstrated that the most important Neogene coal genesis within the Dacian Basin developed during the Early Dacian to the Late Romanian. It covered almost all the western part of the basin with large quantities of brown woody coal (more than 3 billion tons of lignite estimated).

The Pliocene coaly series comprise in Oltenia region at least 22 coal seams which present various spatial and temporal extensions, and constitute three coaly complexes: Vișenilor Valley, Motru and Bălcești. On the basis of the systematic works of research and exploitation, the stratigraphy of the region is quite clarified comprising successions of detrital rocks and coal seams, interpreted mostly as a deltaic sedimentation. Compared with the previous phases of coal genesis from the Dacian Basin as they are known within the Romanian literature, it is obvious that the Pliocene phase (the 3rd) developed in Oltenia region was the most important. The early-middle Pontian (the 2nd) had a medium development within Muntenia region. The early Sarmatian phase (the 1st) had only local development (Fălticeni, Borod areas), and a reduced economic importance.

Evaluating the three Neogene coal genesis phases from coal resources point of view, it can generally be observed that the most important one is the Pliocene coal genesis, developed in the Western part of the Dacian Basin, in Oltenia coaly region. In the second place, Muntenia coaly region with Pontian coals can be considered. The early Sarmatian coal genesis was less developed and offered few coal resources. Few other small intramontaneous coal genesis developed small coal deposits of more reduced importance (Comănești, Baraolt, Borsec areas).

The coal genesis needs some spatial, temporal, tectonic, climatic and vegetation conditions to develop. The Dacian Basin offered the best conditions for this, during the Pliocene: alluvial-lacustrine plains of sedimentation at the foot of the Carpathians, stable and continuous rate of subsidence for a long period of time, accompanied by a compactional one locally augmented by the negative relief where important pelitic deposits were accumulated.

The contemporaneous flora shows favourable climatic conditions for a big and continuous production of phytomass, related to some cyclicity. In fact all these represent the necessary inferences for coal genesis, which are of phytologic, climatic and palaeogeographic nature.

Thus, as a result of the combined action of more factors of tectonic, palaeogeographic, biotic, climatic, faciesal, physical, mechanical and geothermal nature, a new model of coal genesis, valid in the western part of the Dacian Basin, was imagined.

It is therefore obvious that the main scientific researches on coal genesis could be both sedimentological and palaeobotanical, in order to reconstruct the palaeoenvironment and the coal-generating forestry vegetation, especially the woody vegetation, which was basic for a coal deposit genesis. The specific associations typical for each specific biotope produced specific lithotypes with specific technical properties. Knowing these, you can perform interpretations and reconstructions, extract conclusions on palaeogeography and further for economic evaluations.

From other point of view an obvious cyclicity in the coal genesis was observed during a sustained subsidence, the vegetal material being accumulated during the four known steps: fluvial, fluvio-lacustrine, telmatic and finally, lacustrine. On the other hand, researches of climatostratigraphy showed a repetitive clay-lignite alternations which were interpreted as corresponding to cyclic changes in climate, forced by cycles of eccentricity and which gave an age interval of 4.9-4.3MY (Chronos C3n.3n-3n.1n) for the layers IV-XIII.

Evaluating the flora and palynoflora identified in Oltenia region during the Pliocene coal genesis Țicleanu (1992) estimated MAT = 14-15°C and a wet weather, considering at least the presence of *Glyptostrobus europaeus* and *Byttneriophyllum tiliaefolium* within the vegetal association. However DIACONU (2008), finding some *Pandanus* species in Husnicioara area (west of the Dacian Basin) considered that, at least for the early Dacian, MAT could be of up to 15°C or more and MAP of about 1400 mm/year, with a summer peak higher than 150 mm. Interpreting the palaeobotanical and palynological content only in Motru area, PETRESCU (2003) estimated MAT=13-14°C and MAP=1300 mm/year during the early Dacian, and more decreased values for MAT, maybe under 13°C, and for MAP, to 1000 mm/year, during the late Dacian and the Romanian.

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FIRST FIND OF ELEPHANTID REMAINS FROM THE PLEISTOCENE OF COPĂCENI (ILFOV COUNTY, ROMANIA)

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Abstract. This paper describes the first fossil proboscidean remains found in the Pleistocene deposits of Copăceni (Ilfov County). A large number of both dental and postcranial fossil remains were recently discovered at this locality. On the basis of their morphometrical parameters the discovered molars are assigned to the species *Mammuthus meridionalis*, whereas two tusk fragments show features indicative of *Elephas antiquus*. The postcranial elements (fragmentary vertebrae, girdle and limb bones) present no taxonomically relevant features, but might belong to *M. meridionalis* because of their co-occurrence with the remains of other mammals that lived during the Early Pleistocene. Some specimens also allowed an estimation of the age at death of the individuals.

Keywords: proboscideans, *Mammuthus meridionalis*, *Elephas antiquus*, Pleistocene, Dacian Basin.

Rezumat. Prima descoperire a unor resturi de elefantide din Pleistocenul de la Copăceni (Județul Ilfov, România). Această lucrare descrie primele resturi fosile de proboscideni descoperite în depozitele pleistocene de la Copăceni (județul Ilfov). Un mare număr de resturi fosile, atât dentare cât și postcraniene, au fost descoperite recent în această localitate. Pe baza parametrilor morfodimensionali, molarii descoperiți sunt atribuiți speciei *Mammuthus meridionalis*, în timp ce două fragmente de defensă prezintă caracteristici care indică apartenența la *Elephas antiquus*. Elementele postcraniene (fragmente de vertebre, centuri și oase ale membrilor) nu prezintă caracteristici relevante din punct de vedere taxonomic, dar ar putea aparține speciei *M. meridionalis* pe baza co-ocurenței cu resturi ale altor mamifere care au trăit în Pleistocenul timpuriu. Unele specimene au permis și estimarea vârstei la care au murit indivizii cărora le-au aparținut.

Cuvinte cheie: proboscideni, *Mammuthus meridionalis*, *Elephas antiquus*, Pleistocen, Bazinul Dacic.

INTRODUCTION

Freshwater detritic sediments which accumulated during the whole Pleistocene are widespread in the southern part of Romania, where the Dacian Basin was an active sedimentation area during this epoch (for a lithostratigraphical review, see ANDREESCU et al., 2013). Although Pleistocene gravels and sands, locally interbedded with marls and clay lenses, were subsequently covered by loess deposits, they crop out along the multitude of rivers and brooks that cross the Romanian Plain. Sand and gravel used locally in construction have for long been extracted from the river banks. This activity, along with natural erosion of the river banks, has yielded numerous large mammal fossil remains, among which the proboscideans are best represented because of the good degree of preservation of their large and resistant bones and teeth. The elephantid *Mammuthus meridionalis* (NESTI 1825) is the most common fossil proboscidean reported from the area located between the Jiu and Ialomița rivers (the central part of the Romanian Plain), which overlaps the Central Dacian Basin (e. g. APOSTOL, 1968).

The proboscidean fossil remains described in this paper were found along the left bank of Argeș River, in the area located just east of the Childrens' Park of Copăceni, Ilfov County (about 200 m both upstream and downstream from the point that has the following coordinates: 44°15'13.5"N; 26°05'35.3"E). The geological succession described from this area comprises Pleistocene deposits assigned to the typical informal lithofacial units known from the Pleistocene of the Dacian Basin: "the Frătești beds", "the Marly complex", "the Mostiștea sands" and "the Colentina gravels", covered by loess or terrace deposits (MURGEANU et al., 1966). The deposits that crop out along the river are represented by gray-blue siltstones and gray to light brown fine sands comparable to those described for the "Copăceni beds", a unit with uncertain status (i. e. interpretable as either a separate formation or a member of the Coconi Formation) (ANDREESCU et al., 2013). The preliminary data on the fossil assemblage from Copăceni (Co-P) and Adunații-Copăceni (Co) restrict the age of the deposits to the middle to upper part of the Lower Pleistocene (around 1.45-1.35 Ma). The fossils were collected from respectively the left and the right bank of the Argeș River, which acts as boundary between the two localities, so they should in fact be considered parts of the same fossil assemblage. The assemblage reported from this section includes large mammals (proboscideans, rhinocerotids, cervids, bovids), micromammals (rodents, insectivores) (ȘTIUCĂ et al., 2012) and bivalves (*Unio apscheronicus* ALI-ZADE 1936; *Bogatschevia sturi* CEPALYGA 1972) (PETRU ENCIU, verbal communication). These data suggest that the "Copăceni beds" are less related to the geologically younger Coconi Formation, and hint at their separation as a distinct formation that belongs to the Argedavian local stage (ANDREESCU et al., 2013). This information is, however, only preliminary, which leaves the matter of the stratigraphical status of the "Copăceni beds" open to debate.

MATERIAL AND METHODS

Most specimens were found scattered along the river bank, either by villagers who later on donated them to the National Museum of Geology (NMG), or during field campaigns carried out by the NMG or the "Emil Racoviță"

Institute of Speleology (ISER). Specimens discovered so far are housed at the NMG and the ISER and were assigned provisional working numbers. A single large bone fragment (probably part of a tibia) was found *in situ* by the authors, in a fossiliferous pocket that also contained partially articulated bovid remains (cf. *Leptobos* RÜTIMEYER 1877 - skull fragment, metapodia) and a rhinocerotid maxilla fragment that preserved the anterior most premolars (cf. *Stephanorhinus* KRETZOI 1942). The pocket was overlain by a continuous bivalve layer rich in *Bogatschevia sturi*. The presence of articulated remains that show the same type of preservation suggests the proboscidean bones were part of the same megafaunal assemblage with the rhino and the bovid, with a lesser probability of having been reworked from older deposits, such as the sands of the Frătești Formation.

The systematics and taxonomical nomenclature of mammoths follow LISTER (1996), emended by LISTER & VAN ESSEN (2003) and LISTER et al. (2005), who consider a single monophyletic lineage comprising four species assigned to the genus *Mammuthus* BROOKES 1828: *M. rumanus* (ȘTEFĂNESCU 1924), *M. meridionalis*, *M. trogontherii* (POHLIG 1888), and *M. primigenius* (BLUMENBACH 1799). For an alternative opinion, which envisages a separate genus *Archidiskodon* (POHLIG 1888) that includes the species *A. meridionalis*, see, for example, BAYGUSHEVA & TITOV (2012). MAGLIO (1973) considered *Elephas antiquus* FALCONER & CAUTLEY 1847 to be a junior synonym of *E. namadicus* FALCONER & CAUTLEY 1845. However, some authors consider there are sufficient differences between the European and Asian species, so the former name was generally used to refer to the European species (e. g. MOI et al., 2007). In this paper, the European straight-tusked elephant is referred to as *E. antiquus*.

Molar parameters were measured according to MAGLIO (1973), whereas incisor and postcranial parameters follow ROS MONTOYA (2010); additional measurements, not mentioned by these two authors, are explained in detail in the text. A digital caliper was used for measurements under 15 cm, and a measuring tape for those that surpass this value. All values are given in millimeters.

Molar parameter abbreviations: L = maximum length; H = maximum height; W = maximum width including cover cement. Values in Table 1 are followed, in brackets, by the greatest width excluding the cover cement, and by the number of the plate where the value was measured. Roman numerals indicate that the plate count was made postero-anteriorly, whereas Arabic numbers indicate the count was made antero-posteriorly. Platelets and talonids are not counted as true plates; HI = hypsodonty index; P = total number of plates exclusive of talons (where present, talons are noted as t and platelets as p); F = lamellar frequency; ET = enamel thickness (average calculated for the anterior and posterior enamel crests of all available plates).

Tusk measurements: L = distance from the base to the tip of the fragment, measured along a straight line. Since both specimens are fragmentary, + is always added to account for the missing parts. MLDb = medio-lateral diameter at the base of the fragment; MLDm = medio-lateral diameter at the middle of the fragment; MLDt = medio-lateral diameter at the tip of the fragment; DVDb = dorso-ventral diameter at the base of the fragment; DVDm = dorso-ventral diameter at the middle of the fragment; DVDt = dorso-ventral diameter at the tip of the fragment.

Vertebral parameter abbreviations: Tc = maximum thickness of centrum (measured antero-posteriorly); TDc = transversal diameter of centrum; DVDc = dorso-ventral diameter of centrum.

Long bone parameter abbreviations: L = maximum length; APDp = antero-posterior diameter of proximal epiphysis; APDd = antero-posterior diameter of distal epiphysis; APDm = minimum antero-posterior diameter of diaphysis; MLDp = medio-lateral diameter of proximal epiphysis; MLDd = medio-lateral diameter of distal epiphysis; MLDm = minimum medio-lateral diameter of diaphysis; D = diaphysis lowest medio-lateral diameter.

The elephantid grinding teeth are named according to MAGLIO (1973), who separates three premolars and three molars, contra, for example, LAWS (1966), who names six consecutive molars. Incisors (defenses, tusks) are noted with I, and molars with M. The positions of upper teeth are indicated in superscript (e. g. I¹), whereas the positions of lower teeth are indicated in subscript (e. g. M₃).

RESULTS

Dentognathic remains. Although less abundant in the material discovered so far from Copăceni, dental and jaw remains are the most useful for taxonomical assessment. Parameters measured in molars, compared to previously published data (mainly to MAGLIO, 1973) are the primary criterion of taxonomical determination. Molar and tusk measurements are given in Table 1.

The most complete dentognathic specimen, NMGI, consists in a fragmentary mandible with parts of two molars on each side. The mandible was found *in situ* by Dan Petre Antonescu from Vidra (Ilfov County). Between the time of the discovery and the time its finder donated it to the NMG, the mandible had crumbled into more fragments because of the lack of preparation. On the basis of the existing fragments only the horizontal rami could be restored. The right ramus is the better preserved one. It allows the observation of its gently curving dorsal and ventral outlines (Fig. 1a). Its height, measured on the buccal side, below the middle of the functional tooth (in this case the M₃), reaches 163 mm. The M₂ and M₃ are present, still implanted in the alveoli.

In occlusal view, the M₂s have an oval outline. They preserve four worn plates plus half of the posteriormost plate (Fig. 1b); the other half and the posterior talonid were resorbed, as a result of the pressure exerted by the replacing M₃. The plates are worn laterally, and there are no enamel figures left at the occlusal surface, a condition normal in well-worn mammoth molars. In most plates the enamel crests are parallel to each other and only slightly folded. The

enamel becomes moderately wrinkled in the median part of the posterior most two preserved plates, where it also forms a median loop.

The M_3 s are not fully developed and with only seven plates in wear (Fig. 1b) the erupted parts are not extensively worn. None of the worn plates have the enamel crests continuous across the entire width of the molar. The wear figures range from subcircular islets (in the posterior plates) to larger more complicated closed loops, in which the enamel is moderately folded (in the anterior plates). The broken posterior part of the left horizontal branch allows the observation of the plates that had not erupted yet, which raises the total number of observable plates of the molar to 11. The parameters measured for the molars described above are consistent to those mentioned for *M. meridionalis* by MAGLIO (1973). They are wider and lower than the molars of *E. antiquus* and do not show the “dot-dash-dot” antiquoid wear figure.

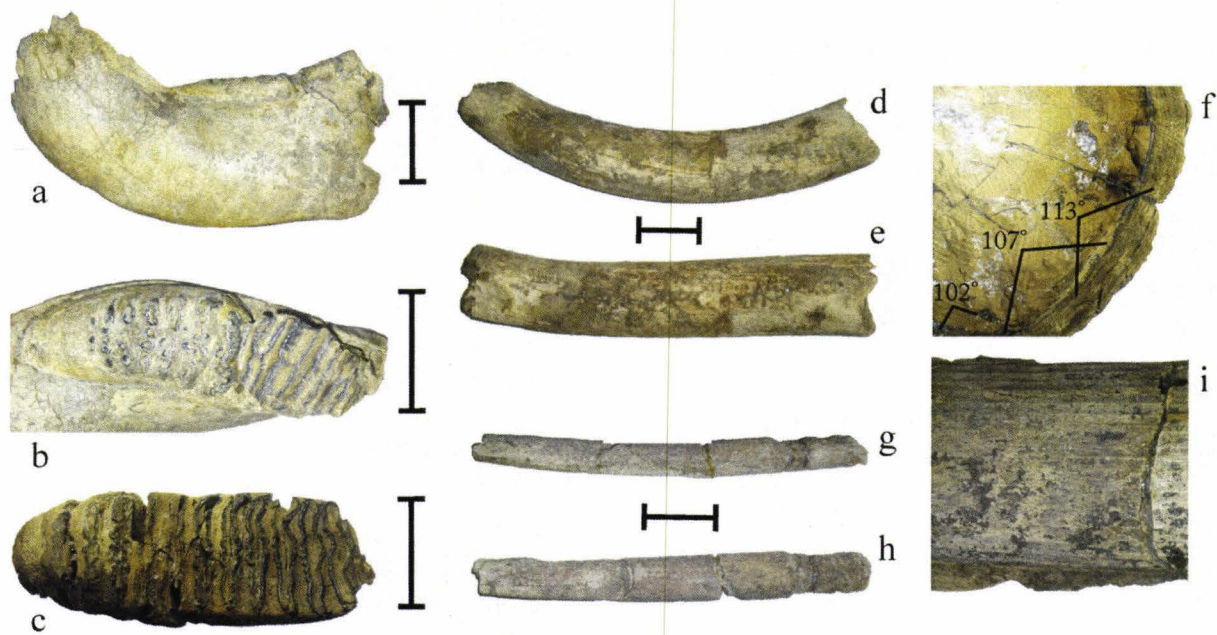


Figure 1. Proboscidean dentognathic remains from Copăceni. a - b. NMG1, right hemi-mandible bearing the M_2 and M_3 , in buccal and occlusal views; c. ISER Co-P01, left M^3 , in occlusal view; d - e. NMG2, left I^1 , in lateral and dorsal views; f. cross section of NMG2, showing selected measurements of outer Schreger angles; g - h. NMG19, juvenile I^1 , in side and dorsal views; i. ventral view of NMG19, showing the fluted outer surface of the dentine layer. Anterior is to the right for a-c and to the left for d-e and g-i. Scale bars: 10 cm.

Table 1. Dimensions of proboscidean teeth from Copăceni.

Molars								
Tooth type	Inventory number	L (mm)	H (mm)	W (mm)	HI (H/W)	P	LF (100P/L)	ET (mm)
M_2 sin	NMG1	+109.5	+30	- (70.22) (PIII)	-	+5.5	5	3.1
M_2 dex		+104	+34.8	- (71.8) (PII)	-	+5.5	5.3	3.6
M_3 sin		190+	~127	86.5 (74) (P2)	1.5 (1.7)	11+	5.8	2.8
M_3 dex		192.5	+51.4	86.53 (73.95) (P2)	-	11+	5.8	2.6
M^3 sin	ISER Co-P01	340	+116.7 (P 2)	- (120.3) (P VI)	-	14, p	4.1	3.44
Tusks								
Tooth type	Inventory number	L (mm)	MLDb (mm)	MLDm (mm)	MLDt (mm)	DVDb (mm)	DVDm (mm)	DVDt (mm)
I^1 sin	NMG2	+630	135	122.5	106.6	120	105.8	90
I^1	NMG19	+510	75	57.1	45	45	47.2	35

Another jaw fragment, NMG3, is represented by the buccal side of the left horizontal ramus. The inner side of the bone preserves a series of parallel vertical grooves separated by low ridges – imprints left by the molar plates. The imprints of six thick plates can be counted, and allow to estimate the lamellar frequency at 4.5, in most cases a value indicative of a *M. meridionalis* M_3 . The height of this preserved horizontal ramus is 119 mm, too low to accommodate the high M_3 s of *E. antiquus*.

An almost complete isolated molar, ISER Co-P01 (Fig. 1c), also provides useful information regarding the taxonomical affinities of its former bearer. The molar, reconstructed from several fragments, resembles a rounded rectangle in occlusal view. It has the anteriormost plate extremely worn and partly broken, and is only slightly tapering posteriorly. Only the posteriormost two plates are not in wear. The enamel loop is continuous along the entire width in the nine anterior plates, but is separated in islets in the posterior ones, where only the apical buds are in wear. The enamel crests are subparallel to each other, slightly folded laterally and moderately folded in the median region of the

plates. In lateral view, the occlusal surface is antero-posteriorly convex, and hence indicative of an upper molar. The slight curvature seen in occlusal view shows the molar is a left one. The low plate number, low lamellar frequency, and low hypsodonty index (Table 1) clearly show the specimen belonged to an individual of *M. meridionalis*.

A 630 mm long tusk fragment, NMG2 (Figs. 1d - 1e), shows only a moderate antero-posterior curvature in lateral view. The moderate curvature makes it difficult to assign the fragment to one of the *Mammuthus* species on one hand, and to *Elephas antiquus* on the other. The base of the recovered I¹ is slightly flattened dorso-ventrally, but the cross-section gradually turns to circular. The anteriormost preserved part also exhibits the latero-medial curvature and the twist around the long axis noticeable in elephants, which shows that the fragment comes from the middle to apical part of a left I¹. The specimen broke in two fragments during handling, which offered the opportunity to study the Schreger pattern (e. g. TRAPANI & FISHER, 2003) visible in the freshly broken cross section (Fig. 1f). The angles measured at the level of the outer layers of dentine ranged from 93° to 113°, values that fall outside the range mentioned by PALOMBO & VILLA (2001) for the different species of *Mammuthus*, but are close to the average calculated by the same authors for *E. antiquus*. Although the Schreger outer angles indicate there is a high probability that NMG2 belonged to an *E. antiquus*, this information should be regarded with caution. The values of the Schreger angles show a high variability from the axial to the cortical area, but also from the basal to the apical region of the same tusk. For example, values as high as 125° were reported in tusks of *M. primigenius* (ÁBELOVÁ, 2008).

Another tusk fragment, NMG19 (Figs. 1g - 1h), is only 510 mm long, and has an elliptical cross section, with the medio-lateral diameter greater than the ventro-dorsal one. The tusk is slightly curved ventro-dorsally. The basal part of the fragment, 123 mm long, is narrower, lacks cement and has a cavernous aspect. It probably represents the part that used to be situated within the alveolus. The small diameter of the tusk shows it belonged to a juvenile individual, which also explains the weak curvature. The cement layer is no longer present on large areas of the tusk, which exposes the outer dentine layer. The outermost dentine layer bears a series of narrow longitudinal ridges, separated by wider shallow fossae (Fig. 1i). These flutes give the dentine/cement junction the crenellated aspect considered typical for tusks of *E. antiquus* individuals (POHLIG, 1888).

The postcranial remains are numerous, yet offer no useful taxonomical information. Since most were found isolated along the river bank, no clear association can be made between them and the dental remains of any of the two species mentioned above. Although the occurrence alongside fossil remains of other vertebrates suggests the postcranial remains probably belong to *M. meridionalis* (see the Discussion section), more data are needed in order to confirm this opinion. Until such data are added, the postcranial proboscidean remains described below are assigned to an indeterminate elephantid.

Axial skeleton. The vertebrae are among the most abundant specimens recovered from Copăceni. In all but one vertebra, only the centrum is preserved. The apophyses and neural arches are completely broken off, which makes it difficult to assign the vertebrae to a precise region of the vertebral column. Vertebra measurements are given in Table 2.

A single detached neural arch, which includes the neural spine, has been discovered so far (NMG13). The anterior edge of the neural spine bears an acute ridge. The right branch of the arch preserves its base, including a small part of the centrum. The same right branch bears the posterior articular facet, but this structure is covered by a hardened sediment crust. The outline of the neural arch resembles that of an equilateral triangle. The neural spine is relatively short and the neural canal relatively wide. The size and shape of the neural spine and neural canal, similar to those seen in the 7th cervical described by MASCHENKO et al. (2011) or ATHANASSIOU (2012), hint towards a posterior position within the cervical region (5th-7th vertebra).

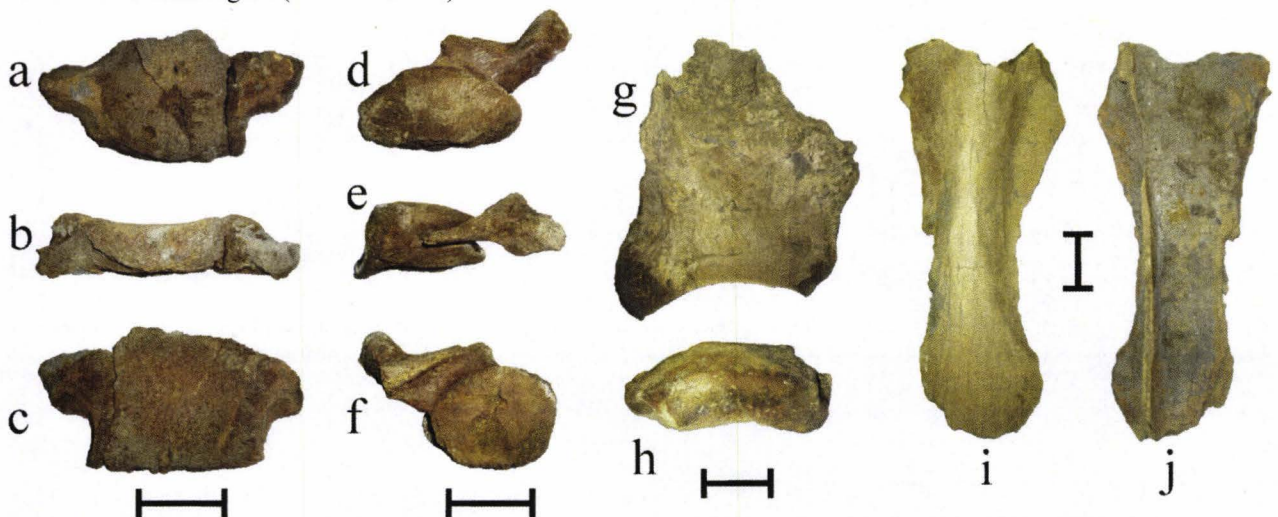


Figure 2. Proboscidean vertebra and scapula fragments from Copăceni. a - c. NMG16, cervical vertebra, in anterior, dorsal, and posterior views; d - f. NMG15, cervical vertebra, in anterior, dorso-lateral, and posterior views; g - h. NMG4, right scapula, medial and distal views; i - j. NMG11, scapula fragment, in medial and lateral views. Scale bars: 10 cm.

Two vertebrae (NMG15 and NMG16) are assigned to the cervical region, largely on the basis of the small antero-posterior length of their centrum. In anterior view, the articulation facet is wide and low, with a convex dorsal edge and a horizontal ventral margin (Figs. 2a - 2f). In posterior view, the centrum is more rounded, even if still dorso-ventrally flattened. Only NMG15 preserves the left base of the neural arch.

A rather poorly preserved centrum, NMG14, is antero-posteriorly longer and has a flat ventral side. A difference in size between the larger anterior articulation facet and the smaller posterior one is also visible. On the basis of the above features, this centrum is assigned to the sacral region of the vertebral column.

Another specimen, NMG17, including two fused fragments, shows no relevant features that could help assess its position along the vertebral column.

Table 2. Dimensions of vertebrae from Copăcenii.

Assumed vertebral region	Inventory number	TDc (mm)	DVDc (mm)	Tc (mm)
Cervical	NMG15	+127	91.2	81.6
Cervical	NMG16	155	145	61.7
Sacral	NMG14	153	95.1	99.2

Scapular girdle and front limb. Various scapular fragments and front limb partial long bones are the most abundant proboscidean remains found at Copăcenii. Among these are the larger parts of a left radius and ulna. A near-complete left pyramidal is the least damaged bone so far discovered at this site. Measurements of the ulna and radius are given in Table 3, whereas those of the pyramidal are given in Table 4.

The largest scapula fragment, NMG11, is 740 mm long and represents the anterior part of the bone (Figs. 2i - 2j). In lateral view, the base of the spine can be seen along the entire length of the fragment. Of the articulation facets or the postero-dorsal shoulder blade nothing is preserved.

The distal part of a right scapula, NMG4, preserves the glenoid cavity almost entirely; only a small part of its anterior tip is broken off (Figs. 2g - 2h). Dorsally, only a small part of the bone is preserved. It includes the distal most part of the spine. The glenoid cavity is 310 mm long and 150 mm wide.

Two other scapula fragments, NMG5 and NMG10, are poorly preserved. They include smaller parts of the distal end, among which parts of the glenoid cavity.

Table 3. Dimensions of long bones from Copăcenii.

Type of bone	Inventory number	L (mm)	APDp (mm)	APDd (mm)	APDm (mm)	MLDp (mm)	MLDd (mm)	MLDm (mm)
Ulna	NMG20	850+	300+	-	150	283	-	130
Radius	NMG9	740	109	-	-	122.4	-	-
Tibia	NMG6	850	200	150	119.8	290	205	130.8

One left ulna, NMG20, is almost complete and only misses the olecranon, which was broken off recently. Signs of erosion are visible around the distal epiphysis (Fig. 3e). The bone is robust, and appears antero-posteriorly widened because of the crests that run down from the olecranon (posteriorly) and from the medial and lateral coronoid processes (antero-medially and antero-laterally). The presence of these three crests effectuates the triangular cross section of the proximal part of the ulnar shaft. The distal epiphysis is completely fused to the diaphysis. In proximal view (Fig. 3f), the medial coronoid process is larger than the lateral one, but the difference in size is not as significant as in some specimens of *M. trogontherii* (e. g. TONG, 2012). The two coronoid processes are separated anteriorly by a U-shaped notch, which accommodated the proximal head of the radius. This notch is shallower and more rounded than the one seen in the *M. trogontherii* ulna described by TONG (2012).

Table 4. Dimensions of the pyramidal and distal femur from Copăcenii.

Distal femur, NMG7	Pyramidal, NMG12
Maximum antero-posterior diameter of the distal epiphysis: 270 mm	Maximum medio-lateral diameter: 149.4 mm
Medio-lateral diameter of the lateral condyle: 113.4 mm	Maximum antero-posterior diameter: 149.8 mm
Antero-posterior diameter of the lateral condyle: 116.1 mm	Greatest height: 68.6 mm
Medio-lateral diameter of the medial condyle: 103.5 mm	Medio-lateral diameter of the ulnar articulation facet: 146.7 mm
Antero-posterior diameter of the medial condyle: 94 mm	Antero-posterior diameter of the ulnar articulation facet: 112.2 mm
Distance between the trochlear sulcus and the intercondylar fossa: 145.5 mm	Medio-lateral diameter of the unciform articulation facet: 120.2 mm
Maximum medio-lateral diameter of the distal epiphysis: 240 mm	Antero-posterior diameter of the unciform articulation facet: 135.9 mm
	Medio-lateral diameter of the lunar articulation facet: 82.3 mm
	Antero-posterior diameter of the lunar articulation facet: 28.3 mm
	Medio-lateral diameter of the pisiform articulation facet: 81.8 mm
	Antero-posterior diameter of the pisiform articulation facet: 47.3 mm

Much of a left radius, NMG9, including the proximal epiphysis and more than half of the diaphysis (Fig. 3b), was glued together from recently broken fragments. The proximal head has a triangular articulation facet (Fig. 3c), and is continued distally by a thinner diaphysis, which is curved and twists before expanding more distally. The curvature of the shaft runs from the front of the proximal end to the medial side of the distal shaft and end. The proximal epiphysis is completely fused to the diaphysis. More than that, the posterior side of the shaft, just below the proximal end, shows the

area where the radius and ulna were becoming fused to one another (Fig. 3d). The two bones show the same type of fossilization (i. e. in colour and bone texture), are comparable in size, and are partially covered by the same type of crust. These features might suggest that they belonged to the same individual, but there is no direct evidence to support this. If the two bones did indeed belong to the same animal, they were separated prior to burial, since the medial side of the ulna was encrusted, which masks the presumed fusion area.

A left pyramidal, NMG12 (Figs. 3i - 3k), is the least damaged and best preserved of all postcranial elements discovered so far. All articulation facets are fused to the bone, evidence it belonged to an adult. In proximal view, the bone has a triangular outline, which includes the concave and flat ulnar articulation facet and the characteristic medio-proximal beak-like process. The distal facet resembles a rounded triangle, and serves for the articulation with the unciform and the 5th metacarpal. The lateral side is subrectangular, with an antero-posterior groove, below which lies the elongated and narrow articulation facet for the magnum. The pisiform articulation facet is placed on the distal part of the lateral side, where it appears as a triangular depression.



Figure 3. Proboscidean limb bones from Copăceni. a. NMG7, right femur, in distal view. b - d. NMG9, left radius, in anterior, proximal, and posterior views. The bulge on the posterior side near the proximal end detailed in d represents the fusion area between the radius and ulna. e - f. NMG20, left ulna, in medial and proximal views; g - h. NMG6, left tibia, in anterior and proximal views; i - k. NMG12, left pyramidal, in proximal, distal, and anterior views. Scale bars: 10 cm.

Hind limb. Only two bones of the posterior member have so far been discovered at Copăceni: a left tibia, almost complete, and a distal right femur, not that well preserved. Measurements of the two specimens are given in Tables 3 and 4 respectively.

Only a distal end of a femur was found: NMG7 (Fig. 3a). The lateral and the medial condyle are both in a decent state of preservation, which allows the measurement of the distal articulation facet parameters (Table 4). The fragment suffered from the transport that occurred after it was unearthed by erosion. The shaft of the femur is broken just above the condyles, with no clues left regarding the degree of fusion between the distal epiphysis and the shaft.

The tibia, NMG6 (Fig. 3g), although found broken in two fragments and showing signs of weathering and transport, is fairly complete. Only a fragment from the proximal part of the anterior side is broken, whereas the distal end is eroded around the edges of the articulation facet. Both epiphyses are completely fused to the shaft, to such a degree that the suture lines cannot be recognized anymore. In proximal view, the medial articulation facet is larger than the lateral one, and is placed at a slightly higher level. The lateral facet is subcircular, whereas the medial one is oval, more developed in antero-posterior direction. The two slightly concave facets are separated by a distinct antero-posterior crest, blunted by recent transport and weathering of the bone.

DISCUSSIONS

So far, only vertebrates that co-existed during the Early Pleistocene have been reported from Copăceni and Adunații-Copăceni (ȘTIUCĂ et al., 2012). Their fossil remains were found both *in situ* (especially the microvertebrates, but also several bovid, elephantid, and rhinocerotid bones) and *ex situ* (recently unearthed and transported by the Argeș). The age of the deposits containing the fossil assemblage mentioned by ȘTIUCĂ et al. (2012) is restricted by the biostratigraphical range of the identified taxa to the Lower Pleistocene (1.45-1.35 Ma). The oldest European occurrence of *E. antiquus* is reported from Spain, from around the Early/Middle Pleistocene boundary (0.8 Ma) (MAZO, 1989). This makes it extremely unlikely for the *E. antiquus* remains to come from the same beds as the rest of the Early Pleistocene taxa, and more plausible for the postcranial remains to belong to individuals of *M. meridionalis*, the other elephantid known from Copăceni. Since only the two tusk fragments assigned to *E. antiquus* fall outside the time range indicated by all the other taxa, it seems more probable that they were recently reworked from geologically younger strata located upstream from the site, and transported by the Argeș.

If the postcranial remains found at Copăceni belong to *M. meridionalis*, this will place this collection among the richest of such type described so far from Romania. Only two other sites yielded comparable samples: Dealul Viilor (Oradea, Bihor County) and Leu (Dolj County). Even if the sample from Dealul Viilor is very rich and contains elements that belong to the same individual (JURCSÁK, 1983), the taxonomical assignment to *M. meridionalis* is not supported by any kind of argument. On the other hand, even if the postcranial remains described from Leu only include carpal and tarsal bones (POPESCU, 2008, 2011), the taxonomical assignment is well supported, since these were collected from the same layer that yielded molars positively assigned to *M. meridionalis* (POPESCU, 2004).

Estimates of the ontogenetic development stage of the individuals at the time of death can be based on the wear and replacement stages of the grinding teeth, as well as on the degree of synostosis in long bones.

The wear stage of grinding teeth and their consecutive replacement during the life span of elephantids are useful in estimating the age at death of mammoths, by comparison to the stages seen in extant elephants. LAWS (1966) separated 30 age groups for the African elephant. Given that *Loxodonta africana* (BLUMENBACH 1797) and *M. meridionalis* have similar plate numbers, the use of the age identification system proposed by LAWS (1966) serves as a fairly good approximation for the individual age of mammoths. Differences obviously exist, induced by the different feeding habits of the two species, as well as by the particular development of each individual. The situation seen in the mandible NMG1, where the last plates of M_2 are present, and the M_3 is not completely erupted, is similar to age group XXII described by LAWS (1966). This leads to the estimation of 39 ± 2 African Equivalent Years (AEY) for the mandibular *M. meridionalis* molars from Copăceni. A similar system was developed by ROTH & SHOSHANI (1988), based on data collected from *Elephas maximus* LINNAEUS, 1758. They mentioned a couple of individuals which still had some plates of the M_2 in wear along with plates of the erupting M_3 , at the ages of 35-37 years. ATHANASSIOU (2012) argues that using African or Indian elephant equivalent years underestimates the age of the male *M. trogontherii* from Loussiká, since the latter species was larger than extant elephants, and must have had a greater lifespan. Since *M. meridionalis* was similar in size to *M. trogontherii*, the same line of reasoning should also apply to the former. Comparable to the Loussiká mammoth in dental eruption and wear stage, the NMG1 mandible from Copăceni probably belonged to an individual older than 45. The ISER Co-P01 M^3 is completely erupted and almost all plates are in wear, with the anterior most ones almost lost. This wear stage is similar to age group XXV as described by LAWS (1966), and corresponds to 47 ± 2 AEY. Given the large size of the molar, and if one takes into account the issue of the larger size and lifespan of *M. meridionalis*, it is possible that the M^3 belonged to an individual older than 50.

An estimate of the age at death based on the degree of synostosis in limb bones can be made after the relevant information regarding the African elephant as gathered by HAYNES (1993). The larger fragments of long bones (ulna, radius, tibia) all have their epiphyses completely fused to the shaft, showing they all come from adult individuals. The estimates are less precise than the dental development stages, since there are greater differences in body development between males and females of the same age. Males are known to keep on growing until late in life, so their bones fuse at

older ages than in females. The tibia is less useful for age estimates, since both ends fuse to the diaphysis earlier in life than the epiphyses in most of the other limb bones do. In extant African elephants, both epiphyses of the tibia become completely fused with the shaft around the age of 20-24 AEY in females, and around 32 AEY in males. The proximal epiphyses of the radius and ulna fuse to the shaft around 19 AEY in females and earlier than 32 AEY in males. The distal epiphysis of the ulna, however, fuses later than 24 AEY in females and during the late 40s in males. If the radius and ulna from Copăceni belonged to the same individual (as the similar state of preservation suggests), the age of the individual might be even older than the age the distal epiphysis of the ulna becomes fused at. An older age is suggested by the presence on the posterior side of the radius of the area where the fusion with the ulna takes place, which occurs later in life than epiphyseal fusion. The considerable size of the left pyramidal likewise suggests that this bone belonged to an old individual. The specimen is larger than similar bones reported from other sites (POPESCU, 2008; ROS MONTOYA, 2010).

It is a difficult task to taxonomically assess postcranial remains of elephantids because morphology and proportions of these bones are similar in different species. The size ratios drawn from the length of limb bones of partial adult mammoth skeletons proved successful only in separating *M. primigenius* from the larger *M. trogontherii* and *M. meridionalis* (LISTER & STUART, 2010; LISTER et al., 2012). A distinction between the latter two species could not be made, because of their similar size.

An attempt to find some metrical data useful for separating mammoth long bones was made by plotting the measurements taken from the specimens from Copăceni alongside information collected from the literature. The data used in this attempt only include measurements taken from specimens that belong to adult individuals and were compiled from: FELIX (1912); VÖRÖS (1975); AZZAROLI (1977); BAYGUSHEVA & GARUTT (1987); AOUADI (2001); ZIEGLER (2001); MASCHENKO (2002); VASILIEV (2007); LISTER (2009); MASCHENKO et al. (2011); ATHANASSIOU (2012); BAYGUSHEVA et al. (2012); KIRILLOVA et al. (2012); HARINGTON et al. (2012); TONG (2012).

Plotting the transverse diameter against the length of the ulna proved irrelevant because of the superposition of *M. trogontherii* and *M. meridionalis* measurements. The same result was obtained when the length of the glenoid was plotted against its width. A better result was obtained when the length of the tibia was plotted against the medio-lateral diameter of its proximal end (Fig. 4). In the latter case, the values plotted for *M. meridionalis* have the tendency to group towards the right side of the graph, which reflects a wider proximal head of the tibia, whereas the tibia length is similar in *M. meridionalis* and *M. trogontherii*. This result is, however, only preliminary, since it only includes data from a small number of *M. meridionalis* specimens.

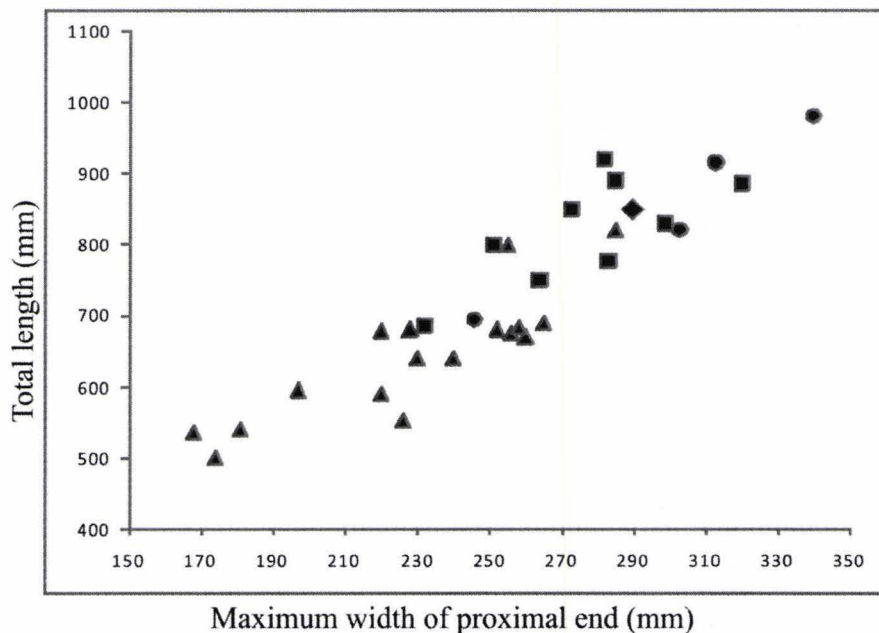


Figure 4. Relationship between the maximum length and the maximum proximal medio-lateral diameter (width) of mammoth tibia. Data for *M. primigenius* are represented by triangles, for *M. trogontherii* by squares, and for *M. meridionalis* by circles. Measurements of the tibia from Copăceni are represented by a rhombus.

CONCLUSIONS

The proboscidean remains from Copăceni include molar and tusk fragments assigned to *M. meridionalis* and *E. antiquus*. A large collection of postcranial remains cannot be clearly assigned to one of the two species, but the other vertebrate taxa mentioned from the same site by ȘTIUCĂ et al. (2012) suggest there is a higher possibility these remains belong to *M. meridionalis*. If this interpretation is correct, the collection of *M. meridionalis* postcranial remains from Copăceni is one of the most abundant samples found in Romania assigned to this species.

With the exception of a single tusk fragment the small diameter of which shows it stems from a juvenile, all the other remains from Copăceni belong to individuals that died during adulthood. Some bones, such as the left pyramidal, are very large, which suggests they belonged to older males.

The results of the attempt to separate mammoth species on the basis of limb bone measurements remains are uncertain for most parameters. However, plotting the length of the tibia against the transverse diameter of its proximal epiphysis offers encouraging results, and supports further study of this approach, but based on a more numerous sample.

The specimens described here add to the material already mentioned from Copăceni and Adunații-Copăceni (ȘTIUCĂ et al., 2012), and underline the fossiliferous potential of this section, the study of which will also be useful in assessing the status of the "Copăceni beds".

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THE PLIOCENE FROM LOGREȘTI

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Abstract. At Logrești, Frunza village, in two hydrogeological boreholes, from which we have collected fragments of bivalve and gastropod fossils, we have identified the Getian, Parsecovian and Pelendavian, for the first time, setting a lithostratigraphic column for this sector.

Keywords: Pliocene stratigraphy, fossils, lithostratigraphic section Frumușei-Frunza (Gorj county).

Rezumat. Pliocenul de la Logrești. În comuna Logrești, satul Frunza, am colectat din probele de sită de la două foraje hidrogeologice, fragmente de bivalve și gastropode fosile, pe baza cărora am identificat Gețianul, Parsecovianul și Pelendavianul, alcătuind coloana litostratigrafică pentru acest sector.

Cuvinte cheie: stratigrafia Pliocenului, fosile, secțiunea litostratigrafică Frumușei-Frunza (județul Gorj).

INTRODUCTION

The fossil fauna has not been examined in no one of the boreholes from Logrești area. This is the first attempt of establishing a paleontological stratigraphic column.

MATERIAL AND METHODS

In Logrești settlement, Frunza village, two hydrogeological drillings have been executed, F1 and F2, each reaching a depth of 260 m. Since in the area there are no boreholes to provide knowledge of the Pliocene sequence based on encountered fossil fauna, we have given special attention to sieve samples and their content because, out of hydrogeological needs, the wells were performed in continuous drilling with reverse circulation.

The fauna collected from the sieve samples, although numerous but triturated, have allowed us, through a careful examination of significant fragments, to identify levels that contain with certainty: Romanian, Parsecovian and Getian.

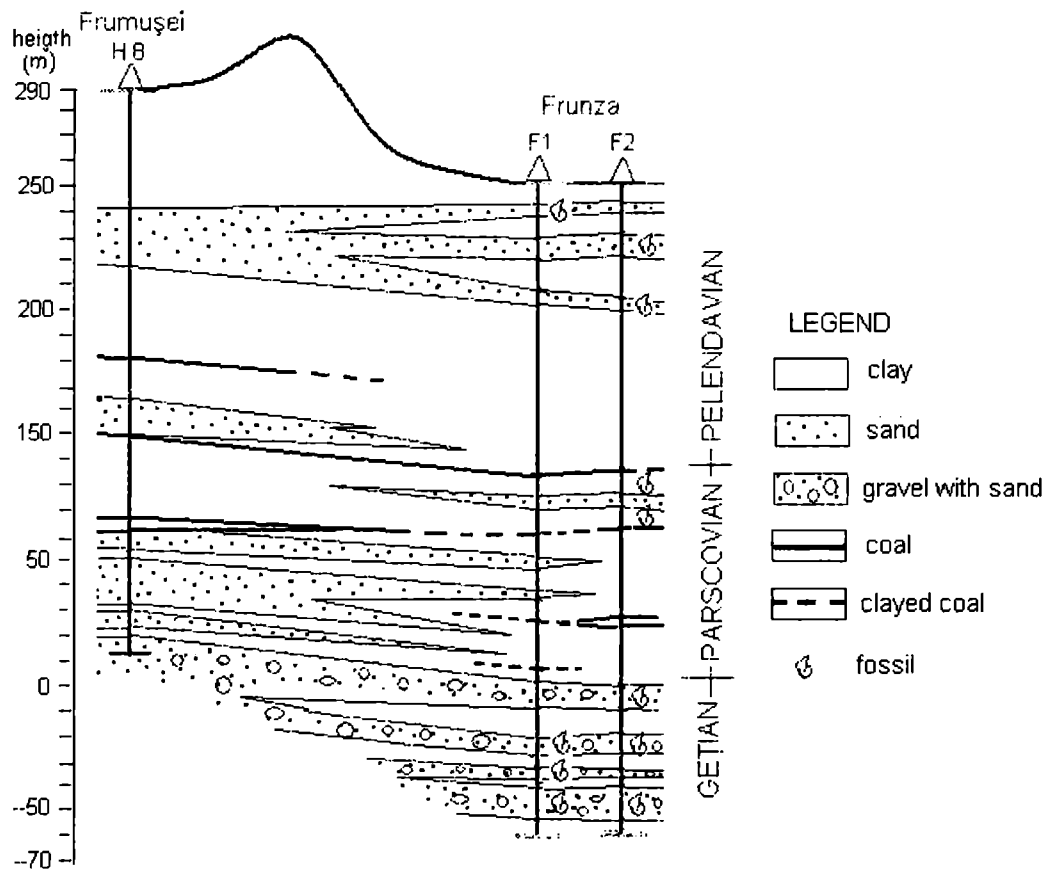


Figure 1. Section Frumușei - Frunza.

RESULTS AND DISCUSSIONS

The lithologic columns obtained from sieve samples based on the levels of the determined fauna, correlated with geophysical investigation of the borehole and the nearby columns of the wells, as well as the lithological sections previously prepared, in which the correlation was made only by lithology and in particular taking as benchmark the coal strata encountered, allowed us to establish strata packets belonging to three levels mentioned above (Fig. 1) in the area between the Gilort and the Amaradia, south of the outcrop area of the Târgu Cărbunești - Seciuri - Copăcenii line.

The following fossil forms have been identified:

Description of the fossil fauna found in drillings

Pachyprionopleura haueri haueri (SABBA) 1896

Figs. 1a, 1b, 1c, 1d, 1e, 1f, 1g.

1907 *Prosodacna haueri*, TEISSEYRE, p. 300-301, Pl. IX, Figs. 4-5.

1942 *Prosodacna (Prosodacna) munieri*, WENZ, p. 122, Pl. 62, Figs. 653b, 654a, 655.

1973 *Prosodacna (Psilodon) haueri porumbarui*, MOTĂȘ et al., p. 65, Pl. III, Figs. 11, 16.

1981 *Pachyprionopleura haueri haueri*, PANĂ et al., p. 86-87, Pl. 50, Fig. 5a.

Fragments with the outer face (1a, 1f) show large ribs from the lower part of the valve separated by narrow intercostal spaces. Fragments with the internal face (1b, 1c, 1d, 1e, 1g) show flattened ribs (1e, 1g), which on the lower edge of the shell tend to widen and are crossed by a string shaped hollow in the middle, finished at the top of the lower board in a lance shape, separated by narrow spaces.

Drilling F2 Frunza, depth 234-251 m.

Dacian.

Dreissena rostriformis (DESHAYES) 1838

Figs. 2a, 2b.

1918 *Dreissena corniculata*, IONESCU-ARGETOAIA, p. 421, Pl. 15, Figs. 7, 7a.

1942 *Dreissena rostriformis*, WENZ, p. 118, pl. 60, Figs. 626, 630, 631.

1962 *Dreissena rostriformis*, PANĂ, Pl. V, Figs. 70-73.

1981 *Pachyprionopleura munieri*, PANĂ et al., p. 64-65, Pl. 43, Fig. 10a.

Oval, convex valves, with a terminal umbo recurved as a rostrum. Strongly curved dorsal edge, with a convex ventral edge. The outer surface has under the umbo a thick growing streak (2a, 2b).

Drilling F1 Frunza, depth 243-254 m.

Drilling F2 Frunza, depth 251-256 m.

Lower Dacian (Getian).

Dreissena rimestiensis (FONTANNES) 1886

Figs. 3a, 3b, 3c.

1942 *Dreissena rimestiensis*, WENZ, p. 119, Pl. 61, Figs. 634, 636, 641b.

1962 *Dreissena rimestiensis*, PANĂ, Pl. V, Figs. 55-69.

1976 *Dreissena rimestiensis*, PAPAIONOPOL, p. 121, Pl. 11, Figs. 5-6.

1981 *Dreissena rimestiensis*, PANĂ et al., p. 64, Pl. 43, Figs. 12-14.

Ovate-oblong valves, slightly curved, with small umbo situated anterior terminally. Rounded rear part. On the inside (3a, 3b), it has dysodont teeth, represented by a ligament moat. Integripallial shell impressions. The dorsal (c) has more developed growth streaks.

Drilling F1 Frunza, depth 243-254 m.

Drilling F2 Frunza, depth 228-234 m, 234-240 m, 251-256 m.

Lower Dacian (Getian).

Pristinunio cf. pristinus (BIELZ) 1864

Figs. 4a, 4b, 4c.

1942 *Pristinunio pristinus*, WENZ, p. 284, Pl. 52, Figs. 563a, 563b, 566.

1981 *Pristinunio pristinus*, PANĂ et al., p. 55, Pl. 1, Figs. 1b, 2a, 2b.

Fragment (4a) shows an internal lower paleal visible line. This excerpt reveals the elongated shape of the valve. Fragment (4b) shows the internal face of the umbo of the left valve with 2 small cardinal teeth and the fossa of the right valve from the strong cardinal tooth. Fragment (4c) displays the external face of the valve from the front, with prominent growth lines (which is why fragments have been attributed to this species).

Drilling F2 Frunza, depth 21-27 m, 44-48 m.

Romanian (Pelendavian).

***Stylodacna heberti* (COBĂLCESCU) 1883**

Figs. 5a, 5b, 5c, 5d.

1942 *Prosodacna (Stylodacna) heberti*, WENZ., p. 128, Pl. 65, Figs. 688a-d; Pl. 66, Fig. 689.1976 *Prosodacna heberti*, PAPAIANOPOL., p. 107, Pl. 11, Figs. 5-6.1977 *Prosodacna heberti*, ANDREESCU, p. 51-53, Pl. XVI, Figs. 1-4.1981 *Prosodacna heberti*, PANĂ et al., p. 79-80, Pl. 49, Figs. 7, 8; Pl. 50, Fig. 1.

Large shell, with rounded oval shape. The outer surface shows flatted ribs, relatively high, which widen towards the bottom of the valve, separated by linear grooves (fragments A, B, C). Internal surface covered by strong ribs, tall, flattened, appropriate with external grooves, separated by wide intercostal spaces (fragment 5d).

Drilling F1 Frunza, depth 244-254 m.

Drilling F2 Frunza, depth 221-225 m, 228-231 m, 237-243 m, 251-256 m.

Lower Dacian (Getian).

***Sulcopotomida cymatoides* (BRUSINA) 1874**

Fig. 6.

1918 *Unio clivosus*, IONESCU-ARGETOAIA, p. 385, Figs. 6, Pl. 1.1918 *Unio Gorjensis*, IONESCU-ARGETOAIA, p. 410-411, Pl. XI, Figs. 9-10.1918 *Unio subclivosus*, IONESCU-ARGETOAIA, p. 388-389, Pl. III, Figs. 1-2.1942 *Psilunio (psilunio) cymatoides*, WENZ., p. 97, Pl. 38, Figs. 535a, 536b.1981 *Sulcopotomida cymatoides*, PANĂ et al., p. 50-51, Pl. 4, Figs. 1-9.

Fragment of thick valve, with numerous concentric "ribs", wave shaped, characteristic for this species.

Frunza Drilling F2, depth 21-27 m.

Romanian (Pelendavian).

***Prosodacnomys sturi sabbae* ANDREESCU 1975**

Figs. 7a, 7b, 7c.

1942 *Prosodacna (Stylodacna) sturi*, WENZ., p. 125, Pl. 64, Fig. 675.1977 *Prosodacnomys sturi sabbae*, ANDREESCU, p. 25-29, Pl. III, Figs. 17, 18; Pl. IV, Fig. 15; Pl. V, Fig. 18.1981 *Prosodacnomys sturi sabbae*, PANĂ et al., p. 77, Pl. 46, Fig. 12; Pl. 47, Figs. 18, 20.

Fragments of small valves with strong anterior twisted umbo (7a), with the external surface covered by dense ribs, softly flattened, curved, radiating from the umbo towards the inferior edge of the valve, separated by linear moats.

Drilling F2 Frunza, depth 237-243 m, 251-256 m.

Upper Pontian (Bosphorian) - Lower Dacian (Getian).

***Hydrobia grandis* COBĂLCESCU 1883**

Figs. 8a, 8b.

1942 *Hydrobia grandis*, WENZ., p. 46, Pl. 14, Figs. 177a-b.1981 *Hydrobia grandis*, PANĂ et al., p. 108, Pl. 65, Figs. 1-4.

Small shell, conical oblong, turriculated, with 8 spiral laps of which usually the last are not kept, relatively flat. Deep oblique sutures facing anterior-posterior diameter. The shell surface is smooth. The aperture is suboval-subrhombical with arched labrum, slightly flared and also curved labium.

Drilling F2 Frunza, depth 204-207 m.

Higher Dacian (Parscovian).

***Hydrobia gorjensis* nov. sp.**

Figs. 9a, 9b.

Small shell, conical, elongated, turriculate with flattened spiral turns, with the last ones usually not preserved. Deep oblique sutures facing anterior-posterior diameter. On both sides of the suture shows one weak burelet characteristic of this new species. Shell surface without ornamentation. The aperture is suboval-subrhombical with arched labrum, slightly flared and also curved labium.

Drilling F2 Frunza, depth 251-256 m.

Higher Dacian (Parscovian).

***Valvata crusitensis* FONTANNES 1886**

Fig. 10.

1942 *Valvata (Cincina) crusitensis*, WENZ., p. 41, Pl. 10, Figs. 122-126.1981 *Valvata (Cincina) crusitensis*, PANĂ et al., p. 106-107, Pl. 67, Figs. 14-18.

Shell triangular – rounded shaped, small, with 3.5 spiral turns, with short shift, less prominent, the last spiral round being highly developed, globular, accounting for over two thirds of the height of the shell. The first shifts are

slightly bulging, separated by superficial sutures. Last winding turn is separated by a deep suture. Subcircular aperture, very sharp peristome holostome. Prominent navel.

Drilling F2 Frunza, depth 21-27 m.

Romanian (Pelendavian).

***Lithoglyphus amplus* BRUSINA 1878**

Fig. 11.

1942 *Lithoglyphus amplus*, WENZ, p. 49, Pl. 15, Fig. 206.

Shell stuffed with very short winding. It shows 3-4 turns, with deep sutures and quite a blunt navel. Last turn is very broad, covering almost all previous windings. Large aperture, straight callous rim.

Drilling F2 Frunza, depth 204-207 m.

Upper Dacian (Parscovian).

***Theodoxus licherdopoli scriptus* (SABBA) 1896**

Figs. 12a, 12b.

1942 *Theodoxus (Calvertia) licherdopoli scriptus*, WENZ, p. 32, Pl. 3, Figs. 44b; Pl. 4, Figs. 47a-b, 51b.

1981 *Theodoxus licherdopoli scriptus*, PANĂ et al., p. 95, Pl. 55, Figs. 4, 4a.

The shell is very small, oval spiral, very short and very little prominent, consisting of 2.5 spiral turns, of which the first ones are flattened and the last one is strongly bulged and wide. The ornamentation is removed from the collected specimens. Crescent aperture, columellar oblique edge, with strong callousness.

Drilling F2, depth 251-256 m.

Dacian-Romanian.

***Viviparus cf. turgidus turgidus* (BIELZ) 1864**

Fig. 13.

1942 *Viviparus turgidus turgidus*, WENZ, p. 36, Pl. 6, Fig. 72.

1981 *Viviparus turgidus turgidus*, PANĂ et al., p. 104, Pl. 60, Figs. 10-10a.

The shell is robust, conical-globular. Halfway through the last spiral turn, which is two times larger than the others, has a blunt, rounded hull. Rhomboid-rounded aperture with rolled labrum covering the navel. These characters have made us attribute this shell fragment to its species.

Drilling F2 depth 56-62 m.

***Pachyprionopleura cf. munieri* (SABBA) 1896**

Fig. 14.

1907 *Prosodacna Munieri*, TEISSEYRE, p. 304, Pl. X, Fig. 6A.

1942 *Prosodacna (Prosodacna) munieri*, WENZ, p. 122, Pl. 62, Figs. 653a, 655.

1973 *Prosodacna (Prosodacna) munieri*, MOTAŞ et al., p. 60-61, Pl. I, Figs. 2-4.

1981 *Pachyprionopleura munieri*, PANĂ et al., p. 86-87, Pl. 50, Figs. 1-2.

Fragment of the outside part of the valve, with rounded ribs separated by string linear grooves. On the ribs surface we observe two bold growth striations, prominent that makes us attach it to this species.

Drilling F2, depth 251-256 m.

Lower Dacian (Getian).

CONCLUSIONS

The following fossil forms have been identified:

Romanian (Pelendavian): *Pristinunio cf. pristinus* (BIELZ), *Sulcopotomida cymatoides* (BRUSINA), *Valvata crusitensis* FONTANNES, *Viviparus turgidus turgidus* (BIELZ).

Upper Dacian (Parscovian): *Hydrobia grandis* (COBĂLCESCU), *Hydrobia gorjensis* nov. sp., *Lithoglyphus amplus* BRUSINA.

Lower Dacian (Getian): *Pachyprionopleura munieri* (SABBA), *Dreissena rimestiensis* (FONTANNES), *Dreissena rostriformis* (DESHAYES), *Stylodacna heberti* (COBĂLCESCU), *Prosodacnomya sturi sabbae* ANDREESCU, *Theodoxus licherdopoli scriptus* (SABBA), *Pachyprionopleura haueri haueri* (SABBA).

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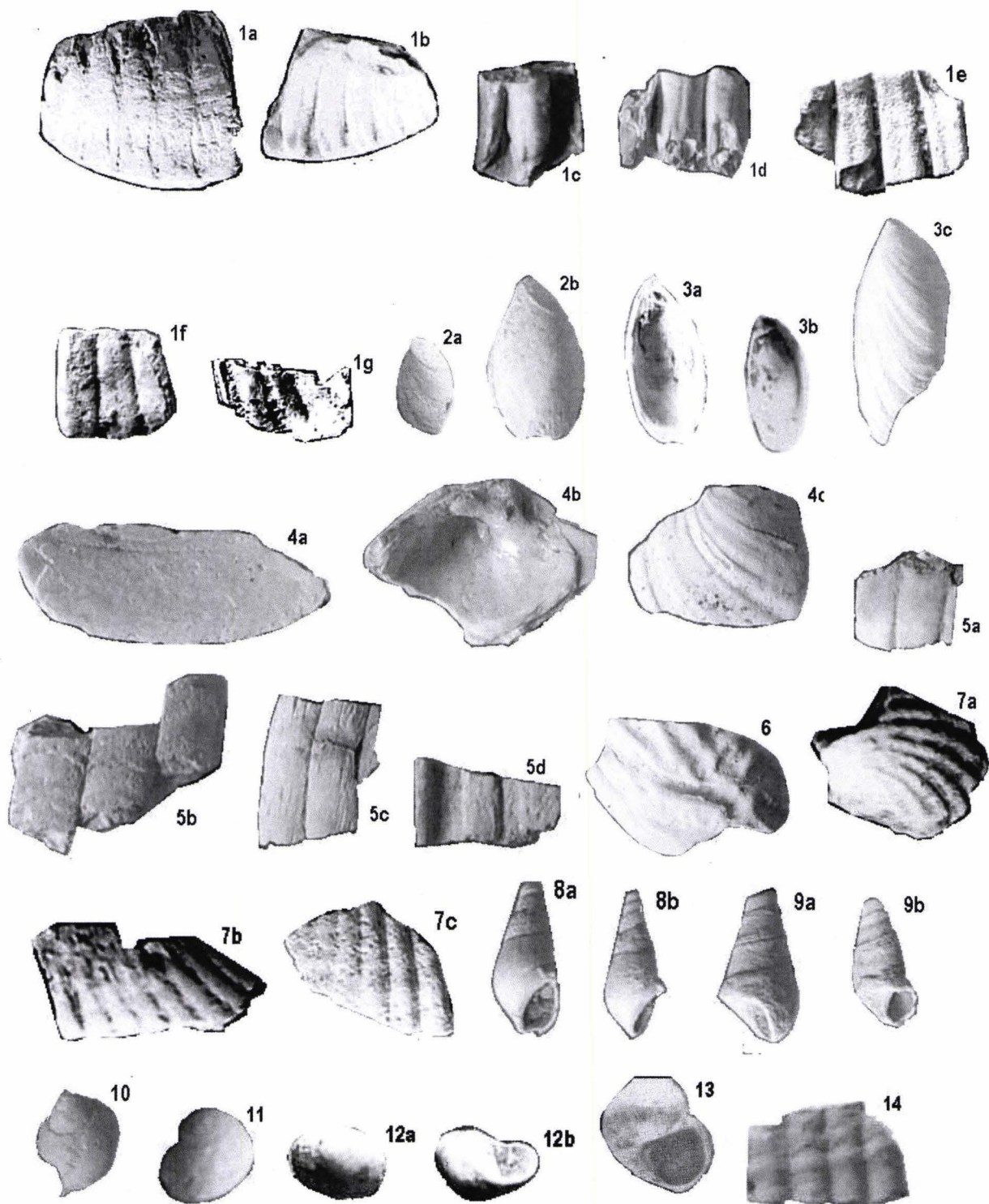
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INDEX PLATE I

- Figure 1. *Pachyprionopleura haueri haueri* (COBĂLCESCU). The external face parts (a), interior face (b) x2.
- Figure 2. *Dreissena rostriformis* (DESHAYES) a, b x2.
- Figure 3. *Dreissena rimestiensis* (FONTANNES). The internal face (a, b) x3, the outer surface (c) x2.
- Figure 4. *Pristinunio* cf. *pristinus*. (BIELTZ). Fragment with paleal line (4a), fragment with posterior lateral tooth-fossa (4b), fragment with the external surface showing prominent growth striations (Fig. 4c), fragment with the external surface with prominent growth lines (4c) x2.
- Figure 5. *Stylodacna heberti* (COBĂLCESCU) fragments 5a, 5b, 5c, 5h the outer face and 5d, 5e, 5f, 5g, the internal face x2.
- Figure 6. *Sulcopotomida cymatoides* (BRUSINA) fragment external face x2.
- Figure 7. *Prosodacnomya sturi sabbae*. ANDREESCU, fragments external face (a, b, c) x3,5.
- Figure 8. *Hydrobia grandis*, COBĂLCESCU, 1883 a, b x3.
- Figure 9. *Hydrobia gorjensis*. nov. sp. a, b x3.
- Figure 10. *Valvata crusitensis*, FONTANNES x13,5.
- Figure 11. *Lithoglyphus amplus*, BRUSINA x13,5.
- Figure 12. *Theodoxus licherdopoli scriptus* (SABBA). The dorsal face (a) x2, the underside (b) x2.
- Figure 13. *Unio* cf. *turgidus pilari* (BRUSINA). Fragment representing the last spiral turn x1.
- Figure 14. *Prosodacna munieri* (SABBA). Fragment representing the external face with 4 ribs x3.

PLATE I



THE ADAPTOGENIC SPECIES: THEORETICAL AND PRACTICAL IMPORTANCE. A REVIEW.

CORNEANU Mihaela, CORNEANU C. Gabriel

Abstract. Rediscovered at the middle of the last century, the adaptogens are a miraculous plant group. They permit the optimal-maximal expression of the physical and mental capacities of man, being used for the same purpose at some animals (thoroughbred horses). A summary insight in the past indicates they were known and used in different civilizations and philosophical theories. The actual characterization of the adaptogens is found in Ibn Sina's (Avicenna's) concepts. On the basis of recent information, the concept of adaptogen, hypotheses regarding the realization of the non-specific resistance character, the features of the three classes of adaptogens admitted at present: primary, secondary and associated adaptogens, the content of bioactive substances, and others, were defined. The analysis of the relation between the centres of origin of the species and the concomitant presence of several prehuman genotypes in these areas suggest the prehuman population's implication in the selection of species with adaptogenic features. Some commercial products with adaptogenic features are also presented.

Keywords: bioactive substances; species origin centers, adaptogens, humanoid populations, commercial products.

Rezumat. Specii adaptogene: importanță teoretică și practică. O trecere în revistă. Redescoperiți la mijlocul secolului trecut, adaptogenii sunt o clasă miraculoasă de plante. Ei permit exprimarea optimal-maximală a capacităților fizice și mentale ale omului, fiind utilizați în același scop la unele animale (cai de rasă). O sumară incursiune în trecut, arată cunoașterea și utilizarea lor în diferite civilizații și teorii filosofice. Caracterizarea actuală a adaptogenilor se regăsește în conceptele lui Ibn Sina (Avicenna). Pe baza informațiilor recente este definit conceptul de adaptogen, ipoteze privind modul de realizare a caracterului de rezistență non-specifică pe care o induc, caracteristicile celor trei categorii de adaptogeni admise în prezent: adaptogeni primari, secundari și asociați, substanțele bioactive conținute ș.a. Analiza relației dintre centrele de origine a speciilor și prezența concomitentă a mai multor genotipuri preumane în aceste regiuni, sugerează implicarea populațiilor preumane în selecția speciilor având caracter adaptogen. De asemenea, sunt prezentate unele produse comerciale având caracter adaptogen.

Cuvinte cheie: substanțe bioactive, centre de origine a speciilor, adaptogeni, populații humanoide, produse comerciale.

INTRODUCTION

The adaptogens are the most beneficial nutrients of vegetal origin, represented only by a few very rare species. They help the organism to reach optimal mental and physical performances. In actual conception, the adaptogen is a "new class of metabolic regulators which increase the ability of an organism to adapt to environmental factors and to avoid damage from such factors" (PANOSSIAN et al., 1999; SAMUELSON & BLOHIN, 2009). They induce a non-specific resistance of the organism to stress factors. The adaptogens were (re)discovered and named by the Russian scientist LAZAREV in 1946, which described the novel effect of dibazol 12-benzyl benzimidazol, an arterial dilator, which produced a "state of nonspecific resistance" (PANOSSIAN & WAGNER 1999; PANOSSIAN, 2003).

The first studies in the adaptogen field were considered the studies performed by Brekhman on the *Panax ginseng* species, which also elaborated the first definitions: (1) "the adaptogen is a substance which presents a non-specific effect, leading to an increase of the body resistance to physical, chemical or biological stress factors"; (2) "the adaptogen has a normalizing influence on a pathological state, regardless of its nature"; (3) "an adaptogen is a compound which can be inoculated and does not affect the normal function of the body" (BREKHMAN, 1968). Their existence has been signalled since Antiquity, the remedies with an adaptogenic effect being mentioned in traditional Chinese medicine (tonic Qi), African (Manyasi), Tibetan, Ayurvedic (Ramayana) and American Native medicines. Ample studies on some plant species with adaptogenic effect, met in the Russian Far East, have been performed since the middle 20th century in the ex-Soviet Union, in the Primorye region, after the elaboration of the Order No. 4654-p/04.03.1043, issued by the Council of the People's Commissaries of the SSSR (LEBEDEV, 1967). Information about the adaptogen utility was diffused in the seventh decade of the last century. After the study of the flora of the Russian Far East (Primorye region), the adaptogenic effects of seven plant species, used in single or combined administration, the last variant being more beneficial, were described and analyzed. After the ample research performed both in the Russian Far East and in other regions, the number of species with adaptogenic properties increased (MAMEDOV, 2005). The seven species constituting the initial nucleus of adaptogens were:

- *Eleutherooccus senticosus* (Acanthi Root): Called the King of Adaptogens. Counteracts stress, increases productivity and endurance and normalizes body systems.
- *Schizandra chinensis* (Chinese Magnolia Vine): Counteracts stress, increases productivity, and combats fatigue.
- *Aralia elata* var. *mandshurica* (Manchurian Thorn Tree): Increases mental acuity.
- *Rhaponticum carthimoides* (Maral Root): Increases muscle development, improves performance, endurance and circulation.
- *Rhodiola rosea* (Russian Rhodiola): Increases mental and physical performance, normalizes heart rate, improves nervous system function and stabilizes mood.

- *Glycyrrhiza uralensis* (Licorice Root): Neutralizes toxins and balances blood sugar levels.
- *Avena sativa* (Fresh Wild Oat Seed): Strengthens the entire nervous system.

The knowledge about adaptogens was initially applied only in the ex-Soviet Union, remarkable successes in sport, spatial and military programs being obtained. After 1960, the information about adaptogens was also disseminated in other countries (Germany, Sweden, Korea, Japan and the USA). The research performed on a global level led to a higher number of adaptogenic species, the elaboration of many hypotheses about their pharmacological action, the establishment of the active principles which determine the adaptogenic effect, the elaboration of some commercial products with an adaptogenic effect. a.o. BREKHMAN (1968), established the following features of adaptogens:

- adaptogens are safe with no significant side effects or contraindications;
- adaptogens have a general, nonspecific action to improve resistance to stress;
- adaptogens have a balancing, normalizing effect on body functions, regardless of the origin of disruption or the direction of the homeostatic disturbance.

Thus, the adaptogens are nontoxic in normal doses, produce a nonspecific defensive response to stress, and have a normalizing influence on the body. They normalize the hypothalamic-pituitary-adrenal axis (HPA axis). As defined, adaptogens constitute a new class of natural, homeostatic metabolic regulators (BREKHMAN (1968).

The main pharmacological properties of adaptogenic products (particularly extracts of bioactive substances of vegetal origin) were formulated in 1969 by BREKHMAN & DARDYMOV:

- the adaptogen is relatively atoxic for the recipient, regardless of the usage period;
- an adaptogen has a "non-specific" activity and determines the increase of the body resistance to a wide spectrum of physical, chemical and biological noxious agents;
- the adaptogens help the body to adjust or normalize its organ and system functions;
- adaptogens make the stress response less damaging;
- adaptogens help to maintain homeostasis in the face of stress by regulating the body's adaptive reactions;
- adaptogens reduce most signs of the alarm stage of the stress response and delay or promote avoidance of the exhaustion stage;
- the use of adaptogens eliminates or significantly decreases the classical signs of the prolonged stress reaction described by Hans Selye as "the Stress triad" (SELYE, 1973);
- adaptogens help the body utilize fuel more efficiently, with fewer toxic or waste by-products (like lactic acid), which can contribute to fatigue and reduced function;
- efficient use of energy means greater reserves that are more readily available.

The practical utilities of adaptogens are:

- **obtaining pharmaceutical products**, for different usages: tonic and stimulating substances for sports medicine; in general medicine, preparations protecting against different stress factors, organism exposure to cold in arctic regions, to the action of different pollutants produced by different industrial activities (mining and energy industries, chemical industry, mechanical vibrations, and others);
- **prevention and treatment** of some lesions or under other abnormal conditions;
- **curative agents** in the treatment of some neurological and mental diseases, such as: asthenia, neurosis, depression, alcoholism, and others, as well as adjuvant in other diseases, such as tuberculosis or in cancer therapy;
- **anti-stress substances**, inducing the increase of tolerance and resistance to physical and mental stress, as well as the reduction of the incidence of its injurious effects on the body;
- **anti-toxic action**, increasing the body tolerance to more chemicals, biological toxins or toxins produced by radiations;
- **normalizing agents**, having the effect of regulating the body functions, if they have the tendency to be fluctuant. In this group there are: the regulation of some endocrine gland activities (hypophysis, thyroid, suprarenal), the regulation of some biochemical parameters (blood pressure, cholesterol and blood glucose level, and others); the changes at the adaptogen level induced a stimulation of the nervous, endocrine and immune systems;
- **prophylactic role**, through the increase of the body resistance to many dysfunctions, having also a therapeutic action: influenza, acute respiratory diseases, atherosclerosis, hypertension, diabetes, obesity, skin diseases, cancer, and others;
- **enhancement of the performance of some thoroughbred animals**, such as horses (KING, 2006).

Pharmacological characterization of adaptogens

The tests involving exposure to heat or cold, atmospheric pressure and oxygen content different from normal, radiations, toxic substances, different storage conditions, psychical stress state, fear and chronic diseases, pointed out that the most important characteristic of adaptogens is the increase of the body resistance capacity, both to the physical stress and to the psychical-emotional stress. The same property resulted from specific biochemical tests (the NO [nitric oxide] content of the blood, spittle and expired air; cortisone level in blood; ACTH [adrenocorticotrophic hormone] and other hormones and substances), as well as some cell functions (phagocytosis, *in vivo* and *in vitro* cytokine production). It was also established that the NO donors increase and NO synthesis inhibitors diminish the swimming length time of the rats, affect their survival and their longevity under hypoxia conditions, and increase the induced immobilization of the gastric ulcer in rats.

At present, PAWAR VINOD & HUGAR (2012) considered that the adaptogens represent a new class of bioactive substances, with the following features:

- they reduce the lesions and negative effects induced by stress factors, through the activation of the nervous and endocrine systems;
- they induce the increase of attention and resistance in the situation of diminished performances, determined by extenuation and/or feebleness sensation.

Hypotheses regarding the action mode of adaptogens

Several hypotheses were elaborated to explain the protecting and stimulating effects of the adaptogenic products. Dardymov and Kirkorian (quoted by PANOSSIAN & WAGNER, 1999) consider that the main function of the adaptogens is determined by their antioxidant action, as they are a trap for free radicals. But this conception cannot explain all their medical effects. In the Panossian’s postulates (PANOSSIAN & WAGNER, 2003), adaptogens are considered to act mainly on the Hypothalamic/Pituitary/Adrenal (HPA) axis and on the Sympathoadrenal System (SAS). Thus, the adaptogens can fashion the organism response to the stress factor action (physical, environmental or emotional factors), achieving the regulation and interconnection of the endocrine, immune and nervous systems. This (re)regulation of systems disturbed or troubled by stress factors is realized through the action of some chemical mediators (cytokines, catecholamine’s, glucocorticoids, cortisol, serotonin, nitric oxide, cholecystokinin, corticotrophin-releasing factor (CRF), or sexual hormones. This theory explains the different effects of adaptogens: anti-inflammatory, antioxidant, anxiolytic, antidepressant, nerving and amphoteric. According to BREKHMAN & DARDYMOV (1969), there are many plants with an amphoteric function, but very few are adaptogens. All adaptogens act as amphoterics with a wide spectrum over the living organisms, but they rarely have a pronounced effect over an organ or specific system. The main differences between stimulating and adaptogenic plants are presented in Table 1.

Table 1. The differences between species with stimulating features and adaptogenic species (after PAWAR VINOD & HUGAR, 2012).

Feature	Stimulating species	Adaptogenic species
Recovery after exhausting physical solicitation	Low	High
Energetic exhaustion	Yes	No
Performance under stress conditions	Low	High
Survival under stress conditions	Low	High
Response quality	Slight	Good
Insomnia	Yes	No
Secondary effects	Yes	No
DNA/RNA and protein syntheses	Low	Increased

The investigations performed by PANOSSIAN & WIKMAN 2009b and PANOSSIAN et al. (2009a, 2010, 2011, 2012) established that the stress-protective activity of adaptogens is associated on the cellular level, via activation molecular chaperones Hsp70, and other key mediators of the stress response such as cortisol, nitric oxide, stress-activated protein kinase JNK (PANOSSIAN et al., 2012) and DAF-16 (PANOSSIAN et al., 2007). Studies have demonstrated that heat-shock factor 1 (HSF1) and Neuropeptide Y might be primary upstream molecular targets of adaptogens in neuroglia cells, but the results were only suggestive, not conclusive (PANOSSIAN et al., 2011, 2012).

As a result of research performed both in the Russian Far East and in the whole world, the number of species with adaptogenic properties increased (MAMEDOV, 2005). In the living world, except the higher plants, there are numerous species, from different organization levels, which present bioactive substances, with antistress or adaptogenic action. Among these: prokaryotes (Cyanophyceae, *Spirulina platensis* [GOMONT] GEITLER; DEMIREL et al., 2012), lower plants (mushroom, *Inonotus obliquus* [ACH. EX PERS.] PILÁT, chaga mushroom; BHAKUNI & RAWAT, 2005), or animals which contain bioactive substances with adaptogenic or anti-stress action (*Mytilus galloprovincialis*, bivalve, KUDRYASHOV & GONCHARENKO, 2004; some amphipods (TIMOFEYEV & STEINBERG, 2006).

CUI et al. (2005) evaluated the protecting potential of *Inonotus obliquus* against the oxidative damage to DNA in a human keratinocyte cell line. The study found the polyphenolic extract from *I. obliquus* protected these cells against hydrogen peroxide-induced oxidative stress. Another study pointed out that the endopolysaccharide of chaga produced indirect anticancer effects via immunostimulation.

TYMOFEYEV et al. (2007) consider that the natural organic matter (NOM) can modify the multixenobiotic resistance (MXR) of an organism. They consider that the MXR system is a general first non-specific line of defence against environmental contaminants. Subsequent research will establish that these are stimulating or adaptogenic species.

Different scientists (MAMEDOV, 2005; TIWARI et al., 2011; YANCE, 2013) distinguish three main categories of adaptogens and use herbs from each of these categories in all formulations to achieve the best possible results.

Primary adaptogens: meet the classical definition of adaptogens.

Secondary adaptogens: meet most of the traditional criteria or have met all of the criteria but lack sufficient scientific validation.

Adaptogen companions: may not meet all of the traditional criteria but play a supporting role by enhancing the HPA axis and anabolic metabolism.

A. Primary adaptogens are represented through the initial nucleus from Primorye region from Far East Siberia, as well as through other species, with similar properties, described in other Terra planet regions. The features of the primary adaptogens are:

- ample scientific research confirmed their adaptogenic character;
- they ensure a “general resistance” in the whole body;
- they have a non-specific action, with a “normalizing” effect toward the action of different stress factors;
- they have the capacity of maintaining or restoring the homeostasis;
- their use is safe and they do not have secondary effects, even after prolonged use.

The activity of primary adaptogens is focused on metabolic regulation through their proven effects on the hypothalamic-pituitary-adrenal (HPA) axis during stress-adaptation responses. They have an ability to maintain or restore homeostasis and allostasis and encourage anabolic restoration. Primary adaptogens enable better response and recovery because they help to smooth out the highs and lows of the neuroendocrine stress response by regulating and normalizing the hormones involved. Primary adaptogens strengthen all systems, promote optimal response and hasten recovery of function, and help to regulate energy use by enhancing cellular energy transfer. Adaptogens enable us to make more efficient use of oxygen, glucose, lipids, and proteins (YANCE, 2013). Arguments for the HPA regulation by primary adaptogens are represented through:

- increasing and modulating the flow of energy throughout the day;
- decreasing feelings of stress;
- increasing endurance;
- supporting mental alertness;
- promoting deep, restful sleep.

The primary adaptogens are represented through the initial nucleus from Primorye region from Far East Siberia, as well as through other species, with similar properties, described in other Terra planet regions. Other adaptogen species, are: *Eleutherococcus sessiliflorus* S. Y. Hu (tincture from root a/o rhizome), *Olopanax elatus* (NAKAI) NAKAI (tincture from root, a/o rhizome); *Panax ginseng* C. A. Mey (tincture from root) (MAMEDOV, 2005), *P. quinquefolius* (standardized root extract), *Withania somnifera* (aqueous ethanol root extract), *Cordiceps sinensis*, *Codonopsis pilosula*, *Glycyrrhiza glabra*, *Ganoderma lucidum*, *Schisandra splenathera* (plant, standardized extract), *Embllica officinalis*, *Aralia schmidtii*, *Bryonia alba*, *Tinospora cordifolia* (plant), *Ocimum sanctum* (leaves), *O. gratissimum* (leaves), *Gymnostemma pentaphylla*, *Rhaponticum carthamoides* (plant), and others (WINSTON & MAIMES, 2007). Recent information about the species with adaptogenic properties were presented by TIWARI et al. (2011), PAWAR VINOD & HUGAR (2012), MAHAJAN & GAJARE (2012), YANCE (2013).

As a general remark, the adaptogenic species origin is near or in the centre of origin described by VAVILOV. In these centres, the humanoid populations, which had a role in their evolution, were present. In severe climatic conditions, in some species it took place a genetics amplification process, which confers resistance for plants toward extreme environmental conditions. In this resistance, many biochemical compounds, which pass in the consumer bodies, different animal and humanoid populations from the area, are involved. The humanoid populations present in these areas contributed to the evolution of adaptogenic populations through empirical selection of the productive plants, resistant and with a big area. The knowledge about their properties was transmitted in human descend populations, being used in alimentation and for their properties. This hypothesis is supported by some findings. Thus, in the area of Denisova cave (Altai Krai, Russia), 41,000 years ago, three different humanoid populations inhabited: *Homo devisovan*, *H. neanderthalensis* and *H. sapiens sapiens* (KRAUSE et al., 2010), relatively sedentary. In the proximity of this region, there are the **Central Asiatic Centre** of domesticated species, in which there are included Northwest India (Punjab, Northwest Frontier Provinces and Kashmir), Afghanistan, Tajikistan, Uzbekistan, and western Tian-Shan (VAVILOV, 1961). 43 domesticated plants: common wheat, peas, lentil, sesame, cotton, onion, garlic, spinach (three adaptogenic species), carrot, pear, grape, apple, and others. Probably this centre was the largest, in its constitution being also included South Siberia with some classical adaptogenic species (CORNEANU & CORNEANU, 2011). In the **Mediterranean Centre**, which includes the borders of the Mediterranean Sea, there were also domesticated 84 plant species: durum wheat, emmer, oats, pea, rape, olive, cabbage, peppermint, black mustard, Baraka, and others. Baraka (*Nigella sativa* L.) is an adaptogenic species, used since Antiquity. This area was a centre of human origin, being met many human populations. Thus, in Qesem cave, situated near Tel Aviv (Israel), naturally closed 200,000 years ago, eight teeth belonging to *Homo neanderthalensis* and *H. sapiens* were discovered. They belong to some fossils with two distinct ages: (a) 300,000 – 400,000 years and (b) 200,000 - 300,000 years. As an explanation, many hypotheses were issued: (a) they belong to an archaic population from *Homo* genus; (b) fossils of *H. neanderthalensis* which evolved differently; (c) these teeth belong to a new human species; (d) *H. sapiens* appeared earlier, and thus his migration from Africa in Asia and Europe; (e) the origin sites of the human species is the Middle East, not Africa (CORNEANU & CORNEANU, 2011).

One action path of adaptogens consists, on the one hand, of support of the neural-endocrine system, maintenance of all neural-endocrine hormonal systems and of the vital organs of the organism, and on the other hand, of a stronger vital communication. They can induce a normalizing effect of the system functions, inducing either their hypo- or hyper function. The adaptogens can induce an optimal homeostasis, preventing or delaying any injurious effect

determined by stress factors or by the aging process. Thus, there is a higher probability of disease prevention and treatment, together with a qualitative and quantitative increase of health and life. They are the essential element of vitality, because they assure an equilibrium state of the whole body.

B. Secondary adaptogens

After YANCE (2013), the secondary adaptogens present most of the criteria of primary adaptogens, but not all. Also, although secondary adaptogens demonstrate some normalizing activity, especially of the immune, nervous, and hormonal systems, they may not directly support the HPA axis. The protective effects of secondary adaptogens come with regular use when combined with primary adaptogens. The secondary adaptogens manifest some features (YANCE, 2013):

- they are more numerous than the primary adaptogens;
- they manifest some normalizing activities, especially on the immune, nervous and hormonal systems;
- they were extensively studied for their adaptogenic qualities, but cannot confirm the adrenaline system (thus their activity may not directly support the HPA axis);
- while they may meet some, or most, of the qualifications of primary adaptogens, they have yet to be studied extensively;
- many of these plants are rich in fatty acids, sterols, and phenol compounds;
- many of these plants enhance anabolic metabolism;
- they can offer non-specific protecting effects for all systems and organs, thus completing the benefits induced by primary adaptogens.

Many plant species present the features of secondary adaptogens, toward different stress factors or different affections of organs and/or systems, being used in their treatment. Thus, the roots from *Angelica atropurpurea* are used as a natural remedy for cardiovascular affections, chronic inflammations (especially in rheumatism), lung and urinary systems, and female reproductive affections. *Ginkgo biloba* leaves are a secondary adaptogen for adrenal, pancreatic and cardiovascular diseases, nervous system and as great neurovasodilator. *Hypericum perforatum* (herbs) is used for its adaptogenic features in cardiovascular diseases, for nervous and digestive tract. Recent research (CORNEANU et al., 2012, CORNEANU & CORNEANU, 2013) recommended the use of volatile oils extracted from *Nigella sativa* seeds for their adaptogenic features at the liver level.

C. Associated adaptogens (herbal adaptogen companions)

The medicinal plants from this group offered enormous general benefits, such as tonicity of the entire body, protection of the vital organs and participation in the protection of the entire organism from several stress factors. Their general action can equilibrate or synergize the effects of primary and secondary adaptogens. They are not toxic and their beneficial effects are cumulative for a long period of time. The companion (associated) adaptogens present antioxidant and anti-inflammatory properties.

Many culinary and aromatic plants used in traditional cuisine (officinal rosemary, Indian saffron, green tea, turmeric, ginger, and others), as well as the nutritional agents, are associated adaptogens.

Because the species from this group manifest enormous general health benefits similar to those of primary and secondary adaptogens, but some scientists (YANCE, 2013) consider that they do not meet the criteria to be officially termed adaptogens. YANCE (2013) affirms: "I call them adaptogen companions, because their actions enhance or synergize the effects of primary and secondary adaptogens". This elite group of herbs and nutritional compounds is used in a supporting role to potentiate primary herbs, harmonize formulations, and, most often, to add high nutritive value. When combined with primary and secondary adaptogens they will significantly increase life span and quality of life.

Important Actions of Adaptogens

- **as anti-stressors:** To increase tolerance and endurance to stress, both physical and mental, as well as decrease the incidence of the harmful side effects of stress on the body;
- **as a normalizing agent:** To provide a regulatory effect on body functions, which tend to fluctuate. It has been shown that adaptogens normalize abnormalities of functions including adrenal, thyroid, blood pressure, cholesterol and blood glucose. Scientific data indicates that changes produced by adaptogens are the result of stimulation of the nervous system, endocrine system and immune system;
- **as a prophylactic:** To enhance a wide range of therapeutic action: influenza, acute respiratory disease, atherosclerosis, hypertension, diabetes, obesity, skin disorders and cancer;
- **as an anti-toxic:** To increase the body's tolerance of many chemical, biological and radioactive toxins.

Constituents of adaptogenic extracts. After PANOSSIAN (2003), depending on the bioactive substance, there are three adaptogen types.

(1) Adaptogens with phenol compounds, as phenylpropanoids, phenyl ethane derivatives, and lignans, whose structural resemblance to catecholamines could suggest an effect on the sympathoadrenal system and possibly imply an effect in the early stages of the stress response. In this group, there are included roots and rhizome of *E. senticosus* and *R. rosea*, as well as extracts of *S. chinensis* fruits.

(2) Adaptogens with tetracyclic triterpens similar to cucurbitacin-R-diglucoside, which structurally resemble the specific corticosteroids that inactivate the stress system to protect against overreaction toward stressors. This group of adaptogenic substances is in extracts of *B. alba* and *W. somnifera*.

(3) Adaptogens with oxylipins (an unsaturated trihydroxy) or epoxy fatty acids (structurally similar to leukotrienes and lipoxines). These adaptogenic compounds have been found in *B. alba* and *G. glabra*.

Commercial products with adaptogens

The **oxyfresh Primorye tonic** was the first anti-stress tonic with revigorant properties, prepared by specialists from Russia, United States and Canada. It is consumed daily by a wide people range, from professional athletes and policeman, military, to pensioners. This produce assures mental clarity, enhanced resistance and recovery of organism.

LERA is a product obtained from ten adaptogenic plant species, which vegetate in the virgin forests from East Siberia. It is produced as a liquid elixir, daily administered. The main constituent is *Eleutherococcus senticosus* (Siberian ginseng, the king of adaptogens), together with *Aralia elata* var. *mandshurica* and eight other species. LERA is efficient on the immune system, physical and mental effort capacity, performances, normalizing effect after stress, resistance toward environmental toxins, antioxidant activity, antia-aging effect, effect on the cardio-vascular system, effects on some analyzers (auditory and vestibular, visual and colour perception) and recovery processes.

Ultima Adaptogens contains the three universal adaptogens, having a maximal efficiency (ANONYMOUS, 2013):

- *Eleutherococcus senticosus*, a medicinal plant which prevents the liberation of an excessive amount of corticosteroid hormones, as a result of the presence of some stress factors;
- *Schisandra chinensis*, an adaptogenic plant native from China which increases the amount of energy liberated and tempers fatigue;
- *Rhodiola rosea*, a perennial plant which tempers stress and anxiety;
- Moreover, Ultima Adaptogen contains other three medicinal plants with synergic activity:
- *Angelica* (a biennial or perennial plant, which protects the liver);
- *Liquorice* (a common plant with antioxidant properties, which counteracts the stress effects);
- *Cinnamon* (a plant which reduces the blood pressure, the glycaemia level, cholesterol together with other beneficial activities).

Recommendations for the use of Ultima Adaptogens:

- to enhance the general health and protect from the dangers of stress;
- to protect some vital organs, such as: liver, heart, immune system and brain;
- for athletes, the working at night or partake in physical activity;
- recovery from an illness or injury.

The Ultima Adaptogens assures some advantages:

- no known adverse effects;
- no prohibited and stimulating substances;
- this product is standardized, stable and tested through scientific research;
- can restore the balance of organism in any situation.

Other important benefits adaptogens provide include:

- increasing mental and physical stamina;
- speeding recovery from physical training;
- protecting against illness and injury;
- protecting the heart and liver;
- anti-oxidant, anti-ageing, and anti-cancer properties.

CONCLUSIONS

Adaptogen is a metabolic regulator which increases the ability of an organism to adapt to environmental factors, and to avoid damage from such factors. Environmental factors can be of different nature: physical, chemical, and physiological (external), different injury, aging, anxiety, mental exhaustion, and others.

An adaptogen manifests a normalizing effect, i.e., counteracting or preventing disturbances to homeostasis brought about by stressors. Also, an adaptogen must be innocuous with a broad range of therapeutic effects without causing any major side effects.

The adaptogens act mainly on the hypothalamic/pituitary/adrenal (HPA) axis and on the sympathoadrenal system (SAS). Thus, the adaptogens can fashion the organism response to the stress factor action (physical, environmental or emotional factors), achieving the regulation and interconnection of the endocrine, immune and nervous systems.

This (re)regulation of systems disturbed or troubled by stress factors is realized through the action of some chemical mediators (cytokines, catecholamines, glucocorticoids, cortisol, serotonin, nitric oxide, cholecystokinin, corticotrophin-releasing factor (CRF), or sexual hormones. This theory explains the different effects of adaptogens: anti-inflammatory, antioxidant, anxiolytic, antidepressant, nerving and amphoteric.

The recent investigations established that the stress-protective activity of adaptogens is associated on the cellular level, via activation molecular chaperones Hsp70, and other key mediators of the stress response such as cortisol, nitric oxide, stress-activated protein kinase JNK and DAF-16. Subsequent investigations can evidence also other aspects, regarding the implication of other mediators.

Depending on the bioactive substance, there are three groups of adaptogens: adaptogenic compounds with phenol compounds, tetracyclic terpene and adaptogens with oxylipis or epoxy fatty acids. The presence of different bioactive substances explains the different action of the adaptogens.

Regarding their actions, the adaptogens can be divided into three classes: *primary adaptogens* (manifest the classical features of adaptogens), *secondary adaptogens* (manifest some normalizing activities, especially on the immune, nervous and hormonal systems but lack sufficient scientific validation), *associated adaptogens* or *herbal adaptogen companions* (present antioxidant and anti-inflammatory properties and can equilibrate or synergize the effects of primary and secondary adaptogens).

The adaptogens can present three types of bioactive substances: phenol compounds (phenylpropanoids, phenylethane derivatives, and lignans), tetracyclic triterpens, and oxylipins or epoxy fatty acids.

Many species of prokaryotes, lower plants or animals, present organic substances which confer resistance toward exobiotic factors. The subsequent research will established if these are stimulant species or adaptogenic species.

Some commercial products with adaptogenic features, such as: Oxyfresh Primorye tonic; LERA, Ultima Adaptogens, were also presented.

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THE PERSPECTIVE POLYCROSS HYBRIDS OF *Lavandula angustifolia* MILL.

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Abstract. One hundred forty F_1 heterotic polycross hybrids of *Lavandula angustifolia* MILL. were studied and evaluated in the third year of vegetation. From these, ten perspective hybrids were selected that differ in the value of quantitative characters: the vegetation period, content of essential oil, the colour of corolla etc. and the effect of heterosis manifested in these characters. The plant height of the perspective polycross hybrids is 48-67 cm, the length of inflorescence is from 24.0 to 35.3 cm, and the length of floral ear is from 4.5 to 7.4 cm, content of essential oil is 4.032%-5.165% (dry matter). The effect of heterosis manifested by the F_1 polycross hybrids on the characters "plant height" recorded the values from +1.0% to +36.1%; "the number of floral stems" from +11.4 to +109.8%, and "the length of inflorescence" from 1.3% to 82.0%. These results were evaluated by comparison with the maternal forms. The manifestation of heterosis effect on the character "the content of essential oil" of the lavender hybrids is the highest and it varied in limits of +64.3 and +110.5%. The most performing late hybrid is Fr. 8-5-34V, with the biggest indices of heterosis that has the highest contents of essential oil -5.098% (dry matter).

Keywords: *L. angustifolia*, polycross hybrid, heterosis, essential oil.

Rezumat. Hibrizi policross perspectivi de *Lavandula angustifolia* MILL. Au fost evaluați 140 hibrizi policross F_1 de *L. angustifolia* în anul al treilea de vegetație și s-au selectat 10 hibrizi de perspectivă, care se deosebesc prin valorile caracterelor cantitative: perioada de vegetație, culoarea corolei florilor etc. și efectul heterozisului manifestat la aceste caractere. Hibrizii policross perspectivi au talia plantelor de 48-67 cm, lungimea inflorescenței de la 24.0 până la 35.3 cm și lungimea spicului floral de la 4.5 până la 7.4 cm. Conținutul de ulei esențial la hibrizii perspectivi variază în limitele 4.032 % - 5.165 % (s.u.). Efectul heterozisului manifestat de hibrizii policross F_1 în raport cu forma maternă la caracterul „taliei plantei” a înregistrat valori de la +1.0 % până la +36.1 %; la numărul de tulpini florale - de la +11.4 până la +109.8 %, iar la lungimea inflorescenței de la 1.3 % până la 82.0 %. Efectul heterozisului manifestat de hibrizii de levănțică la conținutul de ulei esențial este cel mai înalt și variază în limitele +64.3 - +110.5 %. Mai performant este hibridul tardiv Fr. 8-5-34V, cu cei mai ridicați indici ai heterozisului, la care și conținutul de ulei esențial este mai ridicat - 5.098 % (s.u.).

Cuvinte cheie: *L. angustifolia*, hibrid policross, heterozis, ulei esențial.

INTRODUCTION

In order to create varieties and hybrids with increased productivity, in plant breeding, the phenomenon of heterosis is used. The term "heterosis" was introduced in 1914 by G. Shull and it means the state of hybrid, characterized by higher values of some parameters such as adaptability, productivity, disease and critical temperature resistance etc. compared with the parental forms (GONCEARIUC et al., 2011). Heterosis is manifested not only by the morphological characters, but by the physiological too. The practical importance of heterosis is very high because through this phenomenon is possible to achieve the significant increase of production, namely 30-40% higher than the parental forms (GONCEARIUC, 2008). *L. angustifolia* (Lavender) is an important aromatic and medicinal plant for agriculture and economy of the Republic of Moldova and other countries (BUIUCLI, 1969; GONCEARIUC, 2005). In our country the cultivation of lavender started in 1946 and the first industrial plantation were developed in 1948 (GONCEARIUC, 2005; MUSTEAȚĂ, 1980). In the pedoclimatic aspect, Moldova is a favourable area for lavender cultivation as it ensures a high production of raw material and essential oil with high quality. At present in our country industrial plantations of lavender occupy over a thousand hectares and continue to expand and the produced essential oil is destined for export.

The objective of this investigation is the evaluation of perspective hybrid genotypes of *L. angustifolia* and the selection of those which will combine harmoniously the remarkable indices of the main biological and crop characters such as the yield of raw materials and essential oil, resistance to winter frost and drought, disease and pests resistance, the increased content of essential oil in raw material, confirmed by the heterosis expression in quantitative characters.

MATERIALS AND METHODS

The used plant materials are represented by 140 F_1 polycross hybrids of *Lavandula angustifolia* MILL. in the third year of vegetation. The origin of the maternal form of the studied hybrids is from France. For hybridization, the varieties-clone created in Ukraine, Republic of Moldova and French genotypes were used as parental form. The research was carried out in the experimental fields of the Institute of Genetics and Plant Physiology, Academy of Sciences of Moldova. Hybrids were planted in the autumn of 2007 on the nutrition area 1m x 0.5 m. The evaluation of the studied hybrids was carried out according to the existing methods (BUIUCLI, 1969; GONCEARIUC, 2004; SHULL, 1952; DOSPELOV et al., 1990). In this context, some quantitative characters that directly influence the productivity such as plant height, length of inflorescence, length of flower stalk, and floral ear, number of verticiles per inflorescence, number of inflorescences per

plant, the vegetation period, the content and composition of essential oil were evaluated. Statistical analysis of the data was performed by DOSPEHOV (1985). The essential oil was separated from fresh flowers during the complete flowering through the hydrodistillation in the Ginsberg apparatus. Essential oil content was determined in percentage and was recalculated to the dry matter. The effect of heterosis was calculated in percentage in relation to the maternal form.

Qualitative and quantitative composition of essential oil was determined by gas-chromatographic analysis in tandem with the mass spectrometry (GC-MS). The analysis equipment included: gas-chromatograph Technologies Agilent 7890 equipped with Selective Mass Detector with Quadruple MSD Agilent Technologies 5975C, capillary column (30 m/0.25 mm/0.25 µm) with non-polar stationary phase HP-5ms. Analysis was performed at a temperature of 250°C injector and detector - 280 ° C, using a temperature gradient from T1 = 70 ° (2 min), T2 = 200 ° C (5 ° C/min), T3 = 300°C (20°C/min, 5 min). Mobile phase: Helium 1ml/min, injected volume - 0.03 ml essential oil, split rate - 1:100. Identification of chromatographic peaks was performed using the software package AMDIS™, coupled with the NIST database.

RESULTS AND DISCUSSION

During the vegetation period there were evaluated 140 F1 polycross hybrids of lavender. The biodiversity of hybrids genotypes is very large. Hybrids vary by the vegetation period, plant height, length of inflorescence, number of floral stem, colour of flower corolla and calyx. Thus, there were identified the hybrids with various periods of vegetation: early, middle ripening, and late that are distinctive by several quantitative characters, as well as colour of flower corolla that vary from blue to purple with different nuances (Figs. 1, 2, 3). The perspective polycross hybrids from the early group are: Fr.1-3-23V and Fr.1-3-29V. The semi early groups include 6 hybrids: Fr.8-5-8V; Fr.8-5-10V; Fr.8-5-33V; Fr.8-5-40V; Fr.8-5-28V and Fr.1-3-12V. The group with late ripening vegetation period consists of 2 hybrids: Fr.8-5-34V and Fr.1-3-5V (Table 1).

Table 1. Heterosis effect in the *L. angustifolia* F₁ polycross hybrids for the same quantitative characters, compared with the maternal form.

Maternal forms, Polycross hybrids F ₁	Plant height, cm	Heterosis effect, %	Inflorescence length, cm	Heterosis effect, %	No. flower stems per plant	Heterosis effect, %
Fr.1, maternal form	50.0	-	23.7	-	305	-
Fr.8, maternal form	49.6	-	19.4	-	325	-
early hybrids						
Fr.1-3-23V	50.5	+ 1.0	30.7	+ 29.5	385	+ 26.2
Fr.1-3-29V	52.5	+ 5.0	24.7	+ 4.2	352	+ 15.4
middle ripening hybrids						
Fr.8-5-8V	55.5	+ 11.9	32.5	+ 67.5	540	+ 66.2
Fr.8-5-10V	58.0	+ 16.9	26.0	+ 34.0	520	+ 60.0
Fr.8-5-33V	62.0	+ 25.0	30.7	+ 58.2	565	+ 73.8
Fr.8-5-40V	57.0	+ 15.0	29.4	+ 51.5	420	+ 29.2
Fr.8-5-28V	59.5	+ 20.0	27.0	+ 39.1	484	+ 48.9
Fr.1-3-12V	58.5	+ 17.0	24.5	+ 3.4	340	+ 11.4
late ripening hybrids						
Fr.8-5-34V	67.5	+ 36.1	35.3	+ 82.0	682	+ 109.8
Fr.1-3-5V	48.0	- 4.0	24.0	+ 1.3	485	+ 59.0

The result of F₁ lavender polycross hybrids evaluation showed that they are valuable by some quantitative characters such as: plant height, length of inflorescence, number of floral stem (Table 1).



Fig. 1. Lavender early polycross hybrid, Fr.1-3-29V with dark violet corolla (original).

Fig. 2. Lavender medium repining polycross hybrid Fr.8-5-10V, with light violet corolla (original).

Fig. 3. Lavender late polycross hybrid Fr.8-5-34V with blue corolla (original).

For these characters, there were determined the heterosis effect, compared with the maternal form. The examination of hybrids by the character “plant height”, that ranges from 48.0 cm to 62.0 cm allowed identifying eight hybrids where the heterosis was manifested, recording the indices from +1.0% to +36.1% . The effect of heterosis with negative value (-4%) was attested in the hybrid Fr.1-3-5V.

Regarding the character “length of inflorescence” all evaluated hybrids recorded positive effects of heterosis, most of them having indices with value ranges from +29.5% to 82.0%. The studied F₁ polycross hybrids of lavender are perspective also due to the possibility of each plant to form a large number of floral stems. This character determines the production of raw material. It is important to mention that in the hybrids Fr.8-5-34V the effect of heterosis for this character is the highest, registering the value of +109.8%. The quantitative characters of the inflorescence such as length of flower ear, number of verticiles per flower ear, content of essential oil were studied (Table 2).

For the selected hybrids, the length of floral ear ranges from 4.5 to 7.0 cm, and the highest index of heterosis for this character was +23.3% recorded in the late hybrid Fr. 8-5-34V. The number of verticiles per flower ear directly influences the content and lavender essential oil production. From all evaluated hybrids, only six manifested the heterosis with positive indices.

Table 2. The heterosis effect in the F₁ polycross hybrids of *L. angustifolia* for the quantitative characters of inflorescence, compared with the maternal form.

Maternal form, polycross hybrid F ₁	Floral ear length, cm	Heterosis effect, %	No. verticiles per floral ear	Heterosis effect, %	Essential oil content, % (s.u.)	Heterosis effect, %
Fr.1. maternal form	5.8	-	5.5	-	2.601	-
Fr.8. maternal form	6.0	-	5.5	-	2.421	-
early hybrids						
Fr.1-3-23V	6.9	+ 18.9	6.5	+ 18.1	5.165	+ 98.5
Fr.1-3-29V	4.7	- 18.9	4.6	- 16.3	4.453	+ 74.2
middle ripening hybrids						
Fr.8-5-8V	7.2	+ 20.0	6.3	+ 14.5	4.613	+ 90.5
Fr.8-5-10V	6.8	+ 13.3	6.0	+ 9.1	4.350	+ 79.6
Fr.8-5-33V	6.0	+ 0.0	5.8	+ 5.4	4.125	+ 70.3
Fr.8-5-40V	7.0	+ 16.6	6.5	+ 18.1	4.172	+ 72.3
Fr.8-5-28V	5.0	- 16.6	4.9	- 10.5	4.120	+ 70.1
Fr.1-3-12V	5.7	- 1.7	4.7	- 14.5	4.032	+ 82.3
late ripening hybrids						
Fr.8-5-34V	7.4	+ 23.0	6.6	+ 16.3	5.098	+ 110.5
Fr.1-3-5V	4.5	- 22.4	4.5	- 18.0	4.274	+ 64.3

Regarding the content of essential oil, all evaluated hybrids exceed the maternal form; they registered the value of 4.032 - 5.165% (dry matter). For this character, the heterosis effect is from +64.3% to 110.5%. The highest indices were recorded in the hybrids:

- Fr.8-5-34V F₁ – 5.098% (dry matter) of essential oil, heterosis effect + 110.5%;
- Fr.1-3-23V F₁ – 5.165 % (dry matter) of essential oil, heterosis effect + 98.5 %;
- Fr. 8-5-8V F₁ – 4.613 % (dry matter) of essential oil, heterosis effect + 90.5 %.

From the above mentioned results it can be seen that it was created a vast initial material to obtain the lavender varieties-clones, represented by polycross hybrids and characterized by high indices of quantitative characters and first of all by the increased content of essential oil. GC-MS analysis of essential oil separated from the fresh flowers of the hybrids with the highest oil content showed a significant variability of qualitative and quantitative composition (Table 3). The biodiversity of hybrids is expressed only by the concentration of major and minor constituents that consists of the same number - 31. It is known that the quality of lavender oil depends on the concentration of linalyl acetate. If the concentration of this component is higher, then the essential oil quality is higher.

The evaluated *L. angustifolia* genotypes contain the same three major components of essential oil: linalool, linalyl acetate and terpinen-4-ol. Thus, these genotypes take part in a single chemotype – linalool/linalyl acetate/terpinen-4-ol. The biodiversity of hybrids and maternal forms is expressed by the concentration of each component and the number of minor components. The maternal forms differ through a small number of components (17-30), in particular, the form N3 Fr. 1V the concentration of linalyl acetate ranges from 32.377% in the hybrid Fr.1-3-23V to 64.146% in the hybrid Fr.8-5-34V. For the second major component – linalyl acetate, the concentration range from 21.209 in the hybrid Fr.8-5-34V to 25.444% in the hybrid Fr.1-3-23V.

The same diversity is observed in the concentration of major component terpinen-4-ol where the high concentration was registered in the hybrid Fr.1-3-23V (22.523%), for whom the maternal form was N3.1V.

Table 3. The essential oil components of *L. angustifolia* polycross hybrids F₁, compared with the maternal form.

№	Compound, %	Area (%)			
		maternal forms		polycross hybrids F ₁	
		N3 Fr. IV	N5 Fr. 8V	Fr.1- 3-23V	Fr. 8-5-34V
1	α -pinene			0.055	
2	<i>n</i> -octen-1-ol		0.028	0.043	0.256
3	3-octenonă	1.107	0.492	0.087	0.897
4	β -myrcene		0.264	0.280	0.127
5	3-octanol		0.107		0.257
6	3-carene		0.350	0.102	0.124
7	<i>o</i> -cymene			0.169	
8	limonene		0.177	0.205	0.076
9	eucalyptol	0.914	0.417	2.623	0.632
10	<i>trans</i> -ocimene	1.829	0.999	3.117	0.498
11	<i>cis</i> -ocimene	1.183	0.591	0.516	0.180
12	γ -terpinene		0.035	0.276	
13	hexyl acetate			0.359	0.044
14	<i>cis</i> -linalool oxo		0.142		0.409
15	α -Terpinene		0.236	0.165	0.438
16	linalool	42.960	34.928	32.377	64.146
17	<i>n</i> -octen-1-yl- acetate	1.302	0.417	1.239	0.227
18	3-octanol acetate		0.116		
19	camphor		0.259	0.279	0.337
20	borneol	0.843	0.650	0.754	0.784
21	4-terpineol	8.541	0.606	22.523	1.345
22	carvacrol			0.092	0.056
23	α -terpineol	4.742	5.432	2.838	2.784
24	nerol	0.687	0.797	0.385	0.366
25	geraniol				0.049
26	linalyl acetate	27.282	43.829	25.444	21.209
27	bornyl acetate		0.182	0.180	0.053
28	lavandulol acetate	3.253	0.487	0.266	0.297
29	thymol		0.614		
30	nerol acetate	0.958	1.138	0.483	0.436
31	geranyl acetate	1.769	2.106	0.888	0.743
32	β -caryophyllene	1.601	2.027	2.451	1.363
33	germacren D		0.074	0.121	0.056
34	γ -cadinen		0.2	0.09	0.065
35	caryophyllene oxide	0.516	0.412	0.554	0.563
36	β -cadinen	0.512		0.405	0.309
Identified compounds		17	30	31	31
Total, %		99.999	98.052	99.366	99.398

The concentration of terpinen-4-ol component is also high (8.541%). The essential oil of the hybrid Fr.8-5-34V contains 1.345% terpinen-4-ol, but its maternal form (N5 Fr.8V) – 0.606%. It can be concluded that the hybrids inherit the concentration of terpinen-4-ol component in the essential oil and manifested the heterosis for this character. However, we cannot affirm the same situation regarding the concentration of linalool and linalyl acetate. On the contrary, the concentration of linalyl acetate in essential oil separated from the hybrids inflorescences is lower than in the oil of their maternal form.

CONCLUSION

One hundred forty F₁ heterotic polycross hybrids of *L. angustifolia* were studied and evaluated in the third year of vegetation. From these, ten perspective hybrids were selected. The quantitative characters of these hybrids, such as the vegetation period, content of essential oil, the colour of corolla, etc. and the effect of heterosis manifested in these characters are different.

The plant height of the perspective polycross hybrids is 48-67 cm, the length of inflorescence is from 24.0 to 35.3 cm, the length of floral ear is from 4.5 to 7.4 cm and content of essential oil is 4.032%-5.165% (dry matter).

The effect of heterosis manifested by the F₁ polycross hybrids on the characters “plant height” recorded values from +1.0% to +36.1%; “the number of floral stems” from +11.4 to +10-9.8%, and “the length of inflorescence” from 1.3% to 82.0%. These results were evaluated in comparison with the maternal forms.

The manifestation of heterosis effect on the character “the content of essential oil” of the lavender hybrids is the highest and varied in limits of +64.3 and +110.5%.

The most performing late hybrid is Fr.8-5-34V, with the biggest indices of heterosis that has the highest content of essential oil - 5.098% (dry matter).

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DIVERSITY OF THE ESSENTIAL OIL CONTENT AND CHEMICAL COMPOSITION OF *Hyssopus officinalis* L. GENOTYPES

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Abstract. The genotypes of *Hyssopus officinalis* L. *cyaneus*, *ruber* and *albus* forms of Moldova were evaluated. In the drought conditions the indices of quantitative traits which directly influence productivity have the higher value for *f. ruber* and *f. cyaneus*. The content of essential oil is different for the above mentioned genotypes and very high: *f. ruber* - 2.531, *f. cyaneus* - 1.877, and *f. albus* - 1.434% (dry matter). GC-MS analysis of essential oil identified 30-38 compounds for different genotypes. The major compounds are pinocamphone in trans (-) iso and cis forms: for *f. cyaneus*, cis (-) pinocamphone - 51.77%, trans (-) iso pinocamphone - 6.70%; for *f. ruber* - 66.94% pinocamphone, 33.31% trans (-) iso pinocamphone and 33.63% -cis(-) pinocamphone; *f. albus* - 61.1% trans (-) iso- and 2.15% cis (-) pinocamphone, for all genotypes followed by the β -pinene (*cyaneus* form, 8.49%, *albus*, 7.38%, *ruber*, 4.15%) and β -felandren, from 3.64% for *f. ruber* genotype to 6.79% for *f. albus* genotype. The minor compounds of essential oil have different concentration, some of them being present only in one or two genotypes.

Keywords: *Hyssopus officinalis*, genotype, essential oil, composition.

Rezumat. Biodiversitatea genotipurilor de *Hyssopus officinalis* L. Genotipuri de *H. officinalis* L., *f. cyaneus*, *f. ruber* și *f. albus* din Moldova au fost evaluate. În condiții de secetă indicii caracterelor cantitative, ce influențează direct productivitatea au valori mai ridicate la genotipurile ce aparțin *f. ruber* și *f. cyaneus*. Genotipurile au un conținut diferit, dar foarte înalt de ulei esențial: *f. ruber* - 2.531, *f. cyaneus* - 1.877, iar *f. albus* - 1.434% (s.u.). Analiza GC-MS a uleiului esențial a identificat 30-38 componenți la diferite genotipuri, componenții majori fiind pinocamfona în formele trans (-) iso și cis. La *f. cyaneus* cis (-) pinocamfona conține 51.77%, trans (-) iso pinocamfona - 6.70%; la *f. ruber* - 66.94% pinocamfona, 33.31% trans (-) iso pinocamfona și 33.63% -cis (-) pinocamfona; *f. albus* - 61.1% trans(-) iso - și 2.15% cis (-) pinocamfona, urmate la toate genotipurile de β -pinen (*f. cyaneus*, 8.49%, *f. albus*, 7.38%, *f. ruber*, 4.15%) și β -felandren, de la 3.64% la genotipul *f. ruber* până la 6.79% la genotipul *f. albus*. Componenții minori au concentrații diferite, unii fiind prezenți în uleiul numai al unuia sau a două genotipuri.

Cuvinte cheie: *H. officinalis*, genotip, ulei esențial, compoziție.

INTRODUCTION

Hyssop (*Hyssopus officinalis* L.) is a perennial herb of the Lamiaceae family, an evergreen sub shrub native to countries surrounding the Mediterranean Sea, and can be found in the wild flora in south-eastern Europe and western Asia. Presently, it is grown in Spain, France, Italy, Russia, Ukraine, and Balkans.

The Greek name "Hyssopus" is derived from the Arabic "azzof" (sacred grass). Today it is a well-known species, and used as a medicinal, aromatic spices and honey plant in ancient times (CUCU et al., 1982; GONCEARIUC & ROȘCA, 1997; MITIC & DORDEVIC, 2002; VOITKEVICI, 1999; WOLSKI et al., 2006). Hippocrates recommended hyssop for pleurisy, and Dioscorides used it to treat asthma and catarrh (FISCHER-RIZZI, 1990). *H. officinalis* is appreciated for its qualities to fix the eroded land and mobile sands, but also as decorative species. In Moldova hyssop is grown as aromatic plant, and its essential oils are intended for export. This species is important for our country because it is very resistant to drought, frost and winter.

Hyssop has the antitussive, expectorant, carminative, digestive, anticatarrhal, antispasmodic and sedatives, bronchodilators, diuretics (CUCU et al., 1982), antibacterial (BURT, 2004; KIZIL et al., 2010; MAZZANTI et al., 1998; RENZI et al., 1999), antiviral (CUCU et al., 1982; GONCEARIUC & ROȘCA, 1997), antioxidant (BAJ et al., 2010), antifungal (FRATERNALÉ et al., 2004; GLAMOCLJIA et al., 2005; LETESSIER et al., 2001) and spasmolytic properties (BAJ et al., 2010; LU et al., 2002) successfully used it to treat various diseases, primarily the respiratory disease, chronic bronchitis, various injuries and ecchymoses (VOITKEVICI, 1999), and also as digestive stimulant due to the bitter substances that it contains (HOFFMAN, 2010). Mostly, these properties are due to the content of highly aromatic essential oil in the leaves, stems and flowers of hyssop (CUCU et al., 1982; HOFFMAN, 2010) in concentrations of 0.3-1.0% (dry matter) (VOITKEVICI, 1999), successfully used in aromatherapy (LIS-BALCHAN, 2006) as pure essential oil or in combination with other essential oil. Essential oil is obtained from the non lignified part of the plant in the flowering stage by the hydrodistillation used the fresh raw material. It is important because in the stage of wilting and drying it is possible to lose up to 40% of the essential oil. Hyssop essential oil is a light yellow liquid with a bitter taste. Technical characteristics are: d₂₀/20: 0.917-0.965, n_D 1.473-1.486, α _D 6-250. The concentration of 4% in petroleum ether for 48 hours does not cause any skin irritation or sensitizing effect. The phototoxic effect is missing. However, hyssop essential oil should be used with caution and only in recommended doses (VOITKEVICI, 1999).

Various subspecies, varieties of hyssop have been described such as *H. officinalis* subsp. *officinalis* BRIQ; var. *vulgaris* BENTH. - flowers with blue-violet corolla (*f. cyaneus* ALEF, pink, carmine-red color (*f. ruber* (MILL.) ALEF, or white (*f. albus* ALEF.) (CHALCHAT et al., 2001; CUCU et al., 1982; GONCEARIUC & ROȘCA, 1997; SHIBKO & AKSENIY,

2011) var. *decussatus* PERS.; var. *angustifolius* (BIEB.) BENTH. and ssp. *canescens* (DC.) BRIQ (CUCU et al., 1982). According to some authors the form with blue corolla is richer in essential oil than the form with white or red corolla. According to others, on the contrary, the form with red corolla is richer than the form with blue corolla (CUCU et al., 1982). Our previous research showed that the genotypes with blue corolla (*cyaneus* form) are poorer in essential oil: 0.137-0.680% (dry matter). For more than 66% of the evaluated genotypes with white corolla (*albus* form) the content of essential oil is from 0.600 to 1.161% (dry matter), while those with pink corolla (*ruber* form) accumulated essential oil from 0.345% to 1.101% (dry matter). Thus, the highest content of essential oil was attested in the genotypes with white corolla (GONCEARIUC & ROȘCA, 1997). This paper is intended to study quantitative characters, including essential oil content and quantitative and qualitative analysis of essential oil of *H. officinalis* the *ruber*, *cyaneus* and *albus* form, selected for creation of new varieties.

MATERIALS AND METHODS

In this study, the biological material is represent by the three genotypes of *H. officinalis*, is about *f. cyaneus* with blue-violet corolla, *f. ruber* with pink corolla and *f. albus* with white flowers, selected by the higher content of essential oil in the previous research. Biometric evaluations of the quantitative characters that influence the content and production of essential oil were performed in accordance with the existing methods. In order to determine the content of essential oil, the samples of fresh herbs in the flowering stage were collected in the morning hours. The essential oil was separated by hydrodistillation for 60 minutes, using the Ginsberg apparatus: 100g of fresh aerial part into 200 ml of water. The content of essential oil was recalculated per dry matter. After distillation essential oil was dried over Na₂SO₄ and kept in the freezer. Qualitative and quantitative analysis of the essential oil was determined by Gas Chromatography coupled with Mass Spectrometry (GC-MS): gas chromatograph - Agilent Technologies 7890; mass selective detector 5975C Agilent Technologies with quadruple, capillary column (30m x 0.25mm i.d., film thickness 0.25 μm) with HP-5ms non-polar stationary phase. The injector and detector temperature were 250°C and 280°C respectively, using a temperature gradient from T₁ = 70°C (2 min), T₂ = 200°C (5°C/min) to T₃ = 300°C (20°C/min, 5 min). Mobile phase: helium 1ml/min, injected volume of essential oil - 0.03 μl, split rate - 1:100. The identification of chromatographic peaks was performed using the software package AMDIS™, coupled with NIST database.

RESULTS AND DISCUSSIONS

The evaluation of three genotypes belonging to three different forms of *H. officinalis* L. - *f. cyaneus* with blue corolla (Fig. 1), *f. ruber* with pink corolla (Fig. 2) and *f. albus* with white corolla (Fig. 3), in the drought and heat conditions in 2012 demonstrated that the first and second forms are more vigorous; the plant size (height) is 75-80 cm and we noticed a higher number of floral stems - 79-82 per plant (Table 1). The ear of inflorescence consists of a relatively larger number of verticiles, respectively, flowers especially for *ruber* form. It is known that the *H. officinalis* flowers are richer in essential oil than leaves or stems (CUCU et al., 1982). This fact was confirmed by our evaluation. Thus, plants genotypes belonging to *cyaneus* and *ruber* forms have the highest number of flower stems with longer inflorescences, which formed a larger number of verticiles and flowers. For these forms the content of essential oil is higher - 1.877% (dry matter) for *cyaneus* form, but the highest - 2.531% (dry matter) was attested to *f. ruber* (Table 1). For *f. albus*, characterized by small plant size (63.0 cm), lower number of flower stems per plant (68.0), shorter inflorescences and lower verticiles (respectively, flowers) on the spike-like inflorescence, the content of essential oil was the lowest - 1.434% (dry matter).

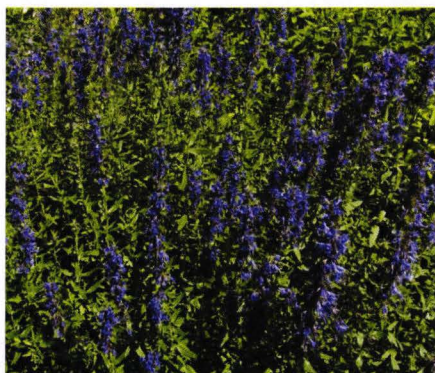


Figure 1. *H. officinalis*, *f. cyaneus* (original).



Figure 2. *H. officinalis*, *f. ruber* (original).

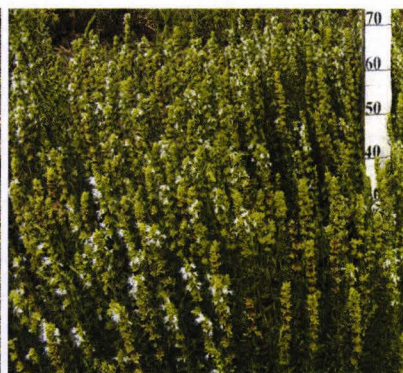


Figure 3. *H. officinalis*, *f. albus* (original).

Comparing the results obtained by other researchers who described genotypes with low content of essential oil 0.5-0.75% (CHALCHAT et al., 2001; GORINOVIC et al., 1995; MITIC & DORDEVIC, 2002) or 0.18% (dry matter) (BAJ et al., 2010; GARG et al., 1999) to the genotypes evaluated by us, it was noticed a higher content of essential oil. The cause of this difference may be the separation of essential oil from dried plants that can lead to the loss of more essential oil, especially during the drying period, mentioned in some scientific papers (CHALCHAT et al., 2001; GORINOVIC et al., 1995; MITIC & DORDEVIC, 2002).

Table 1. The indices of some quantitative traits in the genotypes of *H. officinalis*. 2012.

Genotype, Form	Plant height, cm	Inflorescence length, cm	flower stems per plant	Verticiles on the inflorescence	Content of essential oil, % (dry matter)
<i>cyaneus</i>	75.0	25.1	82.0	11.8	1.877
<i>ruber</i>	80.0	24.9	79.0	12.7	2.531
<i>albus</i>	63.0	23.6	68.0	11.0	1.434

Another reason is the growth and development in the drought conditions of 2012, year when the content of essential oil was the highest in recent years. In our opinion, the high content of essential oil is due to breeding and selection of drought resistant genotypes with high content of essential oil. This fact was confirmed by the comparison of this index while the breeding work started in 1997, when the content of hyssop essential oil ranged from 0.137-0.680 % (dry matter) for *f. cyaneus* and over 0.600-1.161% (dry matter) for *f. ruber* and *f. albus*. In those studies it was found that the highest content of essential oil is characteristic for *f. albus*, followed by the *f. ruber*, and the lowest is for *f. cyaneus* (GONCEARIUC & ROȘCA, 1997) that differs from the results presented in this paper, the highest content of essential oil being registered by *f. ruber*.

Quantitative and qualitative analysis showed that the essential oil of the evaluated genotypes contains a different number of components, and their concentration is also different. Thus, in the essential oil of *f. cyaneus* there were identified 34 components, *f. ruber* - 38 and *f. albus* - 30 components that represent 97.94%, 98.31% and 97.14% respectively of the total weight (Table 2).

Table 2. Composition of the essential oil from the genotypes of *H. officinalis*. 2012.

№	Compound / concentration	Genotypes		
		<i>f. cyaneus</i>	<i>f. ruber</i>	<i>f. albus</i>
1	o-xylene	0.16	0.12	0.12
2	α-pinene	0.36	0.17	0.29
3	camphene	-	0.05	0.06
4	sabinene	1.31	0.97	1.45
5	β-pinene	8.49	4.15	7.38
6	β-thujone	1.48	1.08	1.73
7	p-ocimene	-	0.11	0.07
8	β-felandren	4.83	3.64	6.79
9	1,8-cineole	0.22	0.27	0.24
10	α-terpinene	-	0.06	-
11	trans-β-ocimene	0.17	0.09	-
12	cis-β-ocimene	0.60	0.19	-
13	γ-terpinene	0.11	0.38	0.31
14	δ-terpinene	-	0.14	0.08
15	(+)-4-carene	-	0.08	0.06
16	Δ ³ -carene	3.20	1.27	1.51
17	β-thujone	0.16	0.10	-
18	α-thujone	0.10	0.24	0.35
19	mirtenat de myrtenil	2.33	3.17	2.59
20	Trans (-) iso pinocamphone	6.70	33.31	61.1
21	Cis (-)pinocamphone	51.77	33.63	2.15
22	α-terpineol	0.46	0.54	0.43
23	(+) pinocampeol	1.52	2.48	2.62
24	geraniol	0.51	0.04	0.07
25	borneol	0.09	-	-
26	(z)-citral	0.42	-	-
27	mirtenol	-	0.76	0.27
28	α E- citral	0.63	0.06	0.09
29	Carvacrol	0.34	3.31	5.49
30	myrtenil acetate	1.24	0.15	0.08
31	neryl acetate	0.17	0.06	-
32	geranyl acetate	1.18	0.05	-
33	(+)β-Burbonen	0.72	0.51	0.59
34	(-)α-gurjunen	0.16	0.17	0.07
35	β cariophilene	0.75	0.67	0.83
36	Allo-aromadendrene	0.63	0.62	0.23
37	D-germacren	2.55	2.88	1.80
38	β-Elemene	1.02	0.51	-
39	caryophyllene oxide	0.13	0.13	-
40	bicyclogermacrene	3.43	2.20	0.64
No. of identified components		34	38	30
Total identified components, %		97.94	98.31	97.14

In the essential oil that was separated from the evaluated hyssop genotypes, monoterpene ketones are the major components: pinocamphone in the form trans (-) iso and cis (Table 2; Figs. 4; 5; 6). For the genotype with blue corolla belonging to the *f. cyaneus*, the major component of the essential oil is cis (-) pinocamphone, with concentration of 51.77% and trans (-) iso pinocamphone with 6.70%.

The essential oil separated from the genotype with pink corolla of *ruber* form contains 66.94% of pinocamphone: 33.31% - trans (-) iso pinocamphone and 33.63% - cis (-) pinocamphone, and in the separated essential oil from the genotype with white corolla, the *albus* form, the main component is trans (-) iso pinocamphone-61.1%, the concentration of cis (-) pinocamphone was only 2.15%. Thus, the richest in iso-and cis- pinocamphone is the genotype with white corolla. In all three genotypes pinocamphone is followed by β -pinene, being the highest concentration of this component in the essential oil of *cyaneus* form - 8.49% and *albus* form - 7.38%. The *ruber* form contains 4.15% of β -pinene. The concentrations of the third component - β -felandren is from 3.64% for *ruber* form to 6.79% for *albus* form. These components are followed by the carvacrol in different concentrations (3.31%, 5.49%) in the genotypes with pink (*ruber* form) and white (*albus* form) corolla, but in case of the genotype with blue corolla (*cyaneus* form) by the 2.55% d-germacren, and 3.43% bicyclogermacrene. *H. officinalis* genotypes that were selected and described above are distinguished both by the essential oil content and composition of the hyssop and they were studied by other authors in other countries. It is known that the essential oil that accumulates in the herba of *H. officinalis* var. *decumbens* is distinguished by a lower content of ketones. The major component of this variety is not pinocamphone but linalool, 1.8-cineole, limonene, or other components. For example, the hyssop from the spontaneous flora of Montenegro has a lower content of essential oil and the number of identified components in the oil is high – 68, with the major components methyl eugenol (38.3%), limonene (37.4%) and β -pinene (9.6%) (GORUNOVIC et al., 1995).

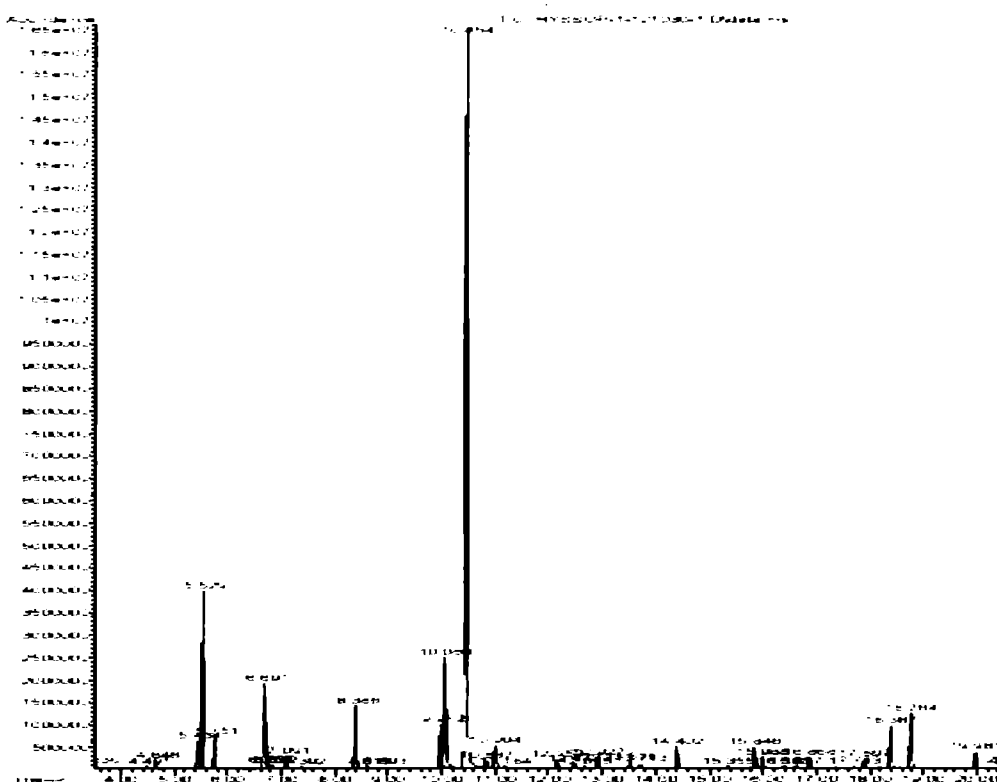


Figure 4. Chromatography of *H. officinalis*, *f. cyaneus* essential oil.

In the hyssop essential oil, *cyaneus*, *ruber* and *whites* forms from the Yugoslavian spontaneous flora the main component is cis-and trans-pinocamphone (CHALCHAT et al., 2001), as well as the genotypes evaluated by us, but only the major component in these genotypes is followed by the pinocarvone and not by β -pinene as in case of the Moldavian genotypes. In other forms of hyssop, coming from Italy, the essential oil, pinocamphone is followed by the β -pinene in concentrations higher than in our genotypes, the difference being the significant content of linalool and camphor (FRATERNALE et al., 2004) that in the essential oil content of our genotypes was not identified.

Obviously, the genotype of hyssop with white corolla (Table 2) selected by us, and *H. officinalis* from Turkey are comparable displaying similar concentrations of iso-pinocamphone (57.27%) and β -pinene (7.23%) in the essential oil (KIZIL et al., 2010). The difference is given by the concentration of the other components except β -pinene, difference highlighted by the chromatogram.

In the essential oil separated from the hyssop coming from the Turkish flora, the first two major components are followed by terpinen-4-ol and trans-pinocarvone (KIZIL et al., 2010), but in case of the Moldavian genotype with white corolla, by the β -felandren and carvacrol. There are significant differences between qualitative and quantitative composition of the essential oil separately from *H. officinalis* L. ssp. *officinalis* from Lublin, Poland and our genotypes, although the major component is the same - cis-pinocamphone (BAJ et al., 2010) as well as *f. cyaneus* from Moldova.

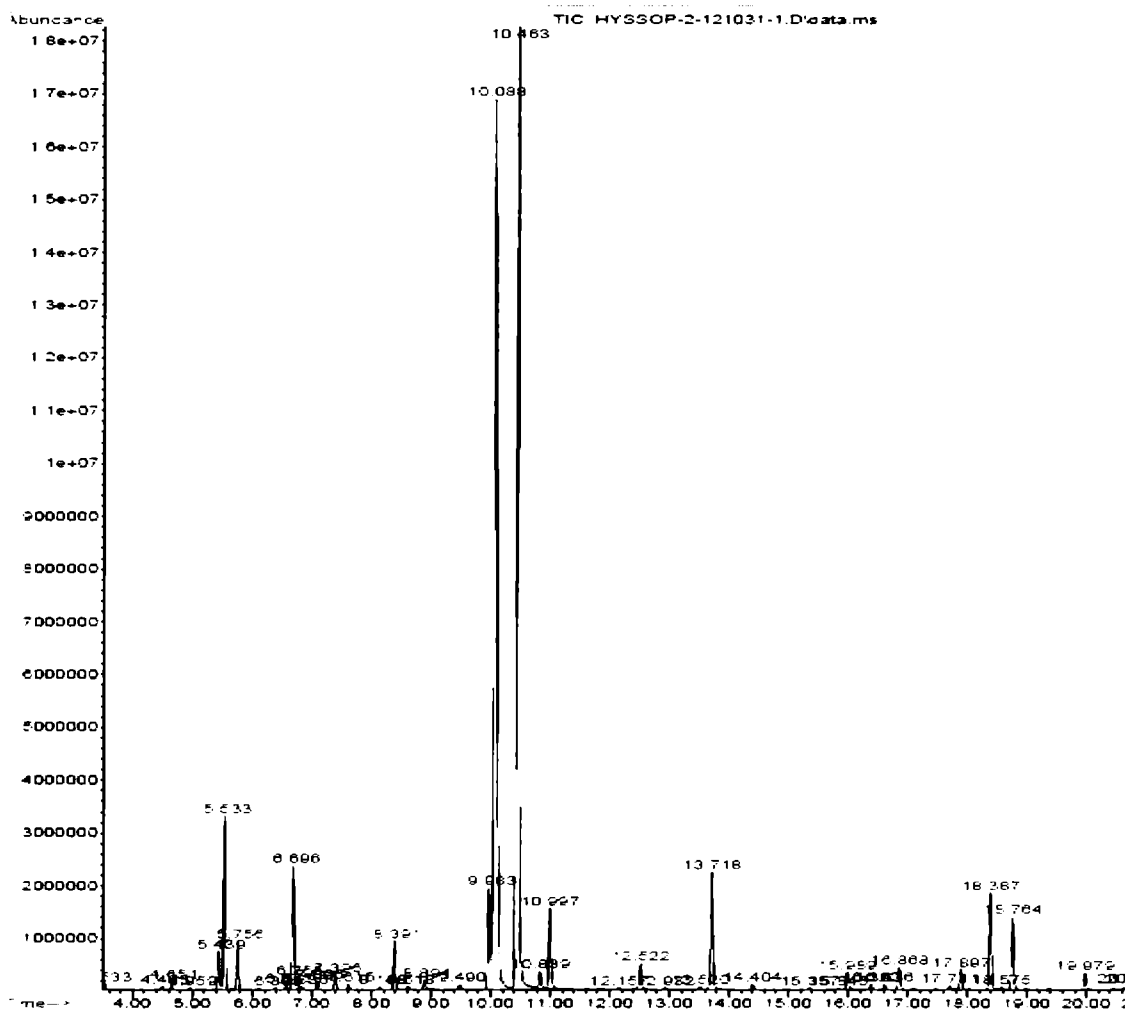
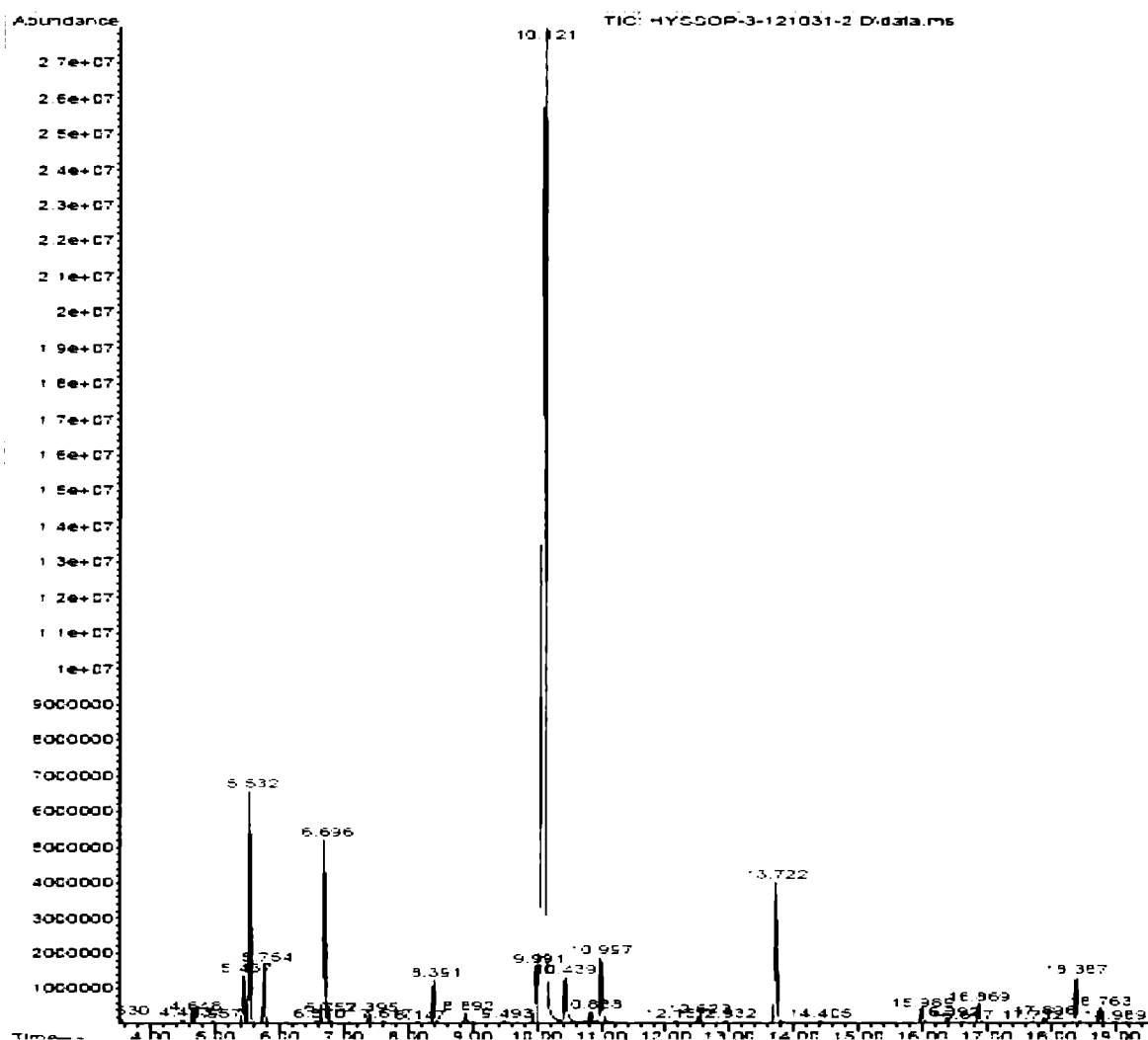


Figure 5. Chromatography of *H. officinalis* *f. ruber* essential oil.

The chemotypes described by other researchers also differ in terms of concentrations of linalool, 1, 8-cineole, methyl eugenol, limonene, β -pinene, cis and trans pinocamphone, β -cariophyllene, d-germacren etc. (CHALCHAT et al., 2001; GORUNOVIC et al., 1995; OZER et al., 2005; SALVATORE et al., 1998).

It is obvious that the *H. officinalis* genotypes the *f. cyaneus*, *f. ruber* and *f. albus* selected by us are distinguished from other genotypes, forms, varieties by the qualitative and quantitative chemical composition of the essential oil. Qualitative and quantitative chemical composition of hyssop essential oil could be strictly related to its antibacterial and healing characteristics. Thus, KIZIL et al. (2010) showed that oil rich in iso-pinocamphone has antimicrobial action against *S. aureus*, *C. albicans* and *E. coli* but has no similar action on *P. aeruginosa*.

The different individual antimicrobial, antifungal action depending on the concentration of certain components, that is also reported by several researchers (FRATERNALE et al., 1982; LETESSIER et al., 2001; MAZZANTI et al., 1998; RENZI et al., 1999). Chemical composition and, especially, the concentration of cis-pinocamphone depends on the property of hyssop essential oil to be used to treat epilepsy, especially for children (BAJ et al., 2010), other characteristics and properties (antioxidant, relaxing cytotoxic, etc.) (BAJ et al., 2010; KIZIL et al., 2010; LU et al., 2002; SALVATORE et al., 1998).



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IN VITRO PROPAGATION OF *Fraxinus excelsior* L.**DANCHEVA Desislava, ILIEV Nasko, ILIEV Ivan**

Abstract. Epicotyls, having at least one internode and with their bottom leaves removed and hypocotyls with cotyledons, isolated from *in vitro* 30-day-old seedlings were used as explants. For induction of axillary shoot formation, the explants were cultivated on WPM and MS media supplemented with 0.5 or 1.0 mg l⁻¹ TDZ plus 0.1 mg l⁻¹ IBA. The highest multiplication rate was found after cultivation for 8 weeks on MS medium supplemented with 1.0 mg l⁻¹ TDZ plus 0.1 mg l⁻¹ IBA. Also, in comparison with epicotyls, the multiplication rate of hypocotyls was lower (9.36 ± 1.94 and 4.12 ± 0.98 , resp.). However, opposite tendency was found for the length of shoots. It was significantly higher in shoots, originated from hypocotyls on MS medium supplemented with 0.5 mg l⁻¹ TDZ plus 0.1 mg l⁻¹ IBA (15.04 ± 2.58 mm). Adventitious roots formation was studied on half-strength WPM, supplemented with 2.5 mg l⁻¹ IBA plus 2.5 mg l⁻¹ NAA. After 24, 48 or 72 h on this inductive medium, the shoots were transferred on half-strength, auxin-free WPM (expressive medium). The highest rate of rooting ($73.33 \pm 3.33\%$) was achieved on an inductive medium applied for 24 h, and then transferred to an expressive medium.

Keywords: common ash, epicotyls, hypocotyls, rooting, thidiazuron.

Rezumat. Propagarea *in vitro* a *Fraxinus excelsior* L. Epicotilele, având cel puțin un internodul și cu frunzele îndepărtate de la bază, și hipocotilele cu cotiledoane, izolate din răsaduri de 30 de zile *in vitro* au fost folosite ca explante. Pentru inducerea formării lăstarilor auxiliari, explantele sunt cultivate pe medii WPM și MS suplimentate cu 0.5 sau 1.0 mg l⁻¹ TDZ plus 0.1 mg l⁻¹ IBA. Cea mai mare rată de multiplicare a fost identificată după cultivarea timp de 8 săptămâni pe mediul MS suplimentat cu 1.0 mg l⁻¹ TDZ plus 0.1 mg l⁻¹ IBA. De asemenea, în comparație cu epicotilele, rata de multiplicare a hipocotilelor a fost mai mică (9.36 ± 1.94 și respectiv 4.12 ± 0.98). Cu toate acestea, pentru lungimea lăstarilor tendința a fost opusă. Aceasta a fost semnificativ mai mare în mugurii proveniți din hipocotile pe mediul MS suplimentat cu 0.5 mg l⁻¹ TDZ plus 0.1 mg l⁻¹ IBA (15.04 ± 2.58 mm). Formarea de rădăcini adventive a fost studiată pe WPM redus la jumătate, suplimentat cu 2.5 mg l⁻¹ IBA plus 2.5 mg l⁻¹ NAA. După 24, 48 sau 72 de ore, pe acest mediu inductiv, lăstarii au fost transferați pe un mediu WPM redus la jumătate și fără auxină (mediu expresiv). Cea mai mare rată de înrădăcinare ($73.33 \pm 3.33\%$) a fost realizată pe un suport inductiv aplicat timp de 24 h și apoi transferat pe un mediu expresiv.

Cuvinte cheie: frasin, epicotile, hipocotile, înrădăcinare, tidiazuron.

INTRODUCTION

Common ash (*Fraxinus excelsior* L.) is one of the most abundant and useful of the Bulgarian native ash species, providing both ecological and forest benefits. Also, it is famous for its diversity of ornamental cultivars (KRÜSMANN 1984; DIRR & HEUSER 1987; DIRR 1998), making it suitable for use in urban areas. It is known as a tree species with a rapid growth in the first decade after establishment (HEIN 2004), and there is some evidence that it is very productive when is mixed together with *Acer pseudoplatanus* (KERR & CAHALAN, 2004). Moreover, common ash was found to have positive effects on the soil, particularly on humus type and topsoil chemistry (WEBER et al., 1993; HEITZ 1998; HAGEN-THORN et al., 2004). The wood is economically important because of its hardness, beautiful texture and use in furniture. However, cloning of economically important genotypes and ornamental cultivars in a generative way is impossible because of the heterozygosity of this species. *F. excelsior* cuttings are generally considered difficult to root (GOOD et al. 1978; DIRR 1998). The rooting has been found to be possible only in juvenile material (CORNU et al., 1977; SPETHMANN 1982; JINKS 1995; DOUGLAS 2001; THOMPSON et al., 2001; DANCHEVA 2005). Limited success is reported after annual pruning of the adult trees (GOOD et al., 1978) or using of stump sprouts (STUTZ et al., 1983). Furthermore, the production of large quantities of grafts is limited by the season and the period of rootstock production and the success depends on the method of grafting (BODZAKOV 1962; KOHNERT 1991; KRÜSMANN 1964; DOUGLAS et al., 1996; THOMPSON et al., 2001; DANCHEVA 2009).

Several promising protocols for *in vitro* propagation of some economically important ash species were summarized by VAN SAMBEEK & PREECE (2007). The success of *in vitro* cloning of woody plants depends on the age of stock plant, explants used, and culture conditions. However, *in vitro* propagation could greatly increase the number of produced plants and may provide rejuvenated plants with high rooting capacity (BONGA & VON ADERKAS 1992; HACKETT & MURRAY 1993; HARTMANN et al., 2002).

It has been reported that *in vitro* propagation of several ash species is possible by somatic embryogenesis (PREECE et al., 1989; BATES et al., 1992; PREECE & BATES 1995), axillary shoot formation (PREECE et al., 1987; NAVARRETE et al., 1989), and adventitious shoots induction (NAVARRETE et al., 1989; BATES et al., 1992; TABRETT & HAMMATT 1992; TONON et al., 2001; VAN SAMBEEK et al., 2001; DU & PIJUT 2008; PALLA & PIJUT 2011; STEVENS & PIJUT 2012). In *F. excelsior* axillary shoots has been induced by using nodal and apical segments from 13 month to 16-year-old plants (SILVEIRA & COTTIGNIES 1994; NOUGARÈDE et al., 1996; SCHOENWEISS & MEIER-DINKEL 2005), buds from mature or grafted trees (HAMMATT 1994; SILVEIRA & NOUGARÈDE 1995; NOUGARÈDE et al., 1996; PIERIK & SPRENKELS 1997; THOMPSON et al., 2001; SCHOENWEISS & MEIER-DINKEL 2005), cotyledonary nodes (HAMMATT &

RIDOUT 1992), and epicotyls (MITRAS et al., 2009). However, some of the publications did not report for the age of stock plant, type of the induced shoots, and did not present data from experiments (CHALUPA 1983, 1987a,b, 1990).

It was reported that BAP support the axillary and adventitious shoot formation from hypocotyls and epicotyls from *F. americana* (NOUGARÉDE et al., 1996, PALLA & PIJUT, 2011) and *F. excelsior* (TABRETT & HAMMATT, 1992; HAMMATT & RIDOUT, 1992). However, it was found that TDZ is more effective than BAP for the induction of axillary shoots from epicotyls and hypocotyls from *F. americana* (BATES et al., 1992, NOUGARÉDE et al., 1996) and *F. excelsior* (TABRETT & HAMMATT, 1992; MITRAS et al., 2009).

Depending on the juvenility and auxin used and its concentration, different authors achieved 0 to 100% rooting in common ash (CHALUPA 1983, 1987a,b, 1990; HAMMATT & RIDOUT 1992; TABRETT & HAMMATT 1992; HAMMATT 1994, 1996; SILVEIRA & COTTIGNIES 1994; NOUGARÉDE et al. 1996; THOMPSON et al., 2001; SCHOENWEISS & MEIER-DINKEL 2005; MITRAS et al., 2009).

The goal of this work aimed at identifying successful explants, type of nutritive medium, type, and concentration of plant growth regulators (PGRs) for *in vitro* propagation of common ash.

MATERIALS AND METHODS

Plant material.

Fruits of *F. excelsior* were sampled from a single tree growing in a park in Sofia at altitude about 600 m. The samples were taken by the end of October 2011. They were preserved at 4°C till the end of August next year. Before the establishment of the cultures, the pericarp was removed from the seeds and they were soaked for 72 h in sterile distilled water. After the soaking, they were surface was disinfected for 8 min in 0.2% HgCl₂ followed by three times for 3 min rinses in sterile distilled water.

The embryos were isolated under sterile conditions and cultured on half-strength MS medium (MURASHIGE & SKOOG 1962).

Effect of the medium, concentration of TDZ and type of explant on axillary shoot induction.

Epicotyls, having at least one internode and with their bottom leaves removed and hypocotyls with cotyledons (Fig. 1), isolated from *in vitro* 30-day-old seedlings were used as explants. Their length was higher than 15 mm. Three replications, each containing seven explants were used per treatment.

For inducing adventitious shoot formation, the MS and WPM (LLOYD & MCCAWN 1980) media were used in the following treatments: 0.5 or 1.0 mg l⁻¹ Thidiazuron (TDZ) plus 0.1 mg l⁻¹ indole-3-butyric acid (IBA) (treatments MS1, MS2, WPM1, and WPM2, respectively). Every two weeks, the explants were subcultured on the same fresh medium.

Effect of the inductive medium on adventitious root formation.

After 8 weeks, shoots that were longer than 20 mm and having one or two internodes were transferred to an inductive, half-strength WPM rooting medium, supplemented with 2.5 mg l⁻¹ IBA plus 2.5 mg l⁻¹ α-naphthaleneacetic acid (NAA) for 24, 48 or 72 h., respectively and then on an expressive rooting medium (half-strength WPM without auxins). For comparison of the results, the shoots were cultivated on the same expressive medium (control). Three replications, each containing ten explants, were cultured in each variant. After 30 days, the percentage of rooted plants, as well as the number and length of induced roots were determined.

Conditions of the cultivation.

Each variant of the media contained 7 g l⁻¹ agar (Sigma) and pH was adjusted to 5.6 - 5.7 before autoclaving (under pressure of 118 kPa and 120°C for 20 min). The cultures were grown in a cultivation chamber at 25 ± 0.5°C with 16 hrs of cool white fluorescent light at a photosynthetic photon flux density of 40 μmol m⁻² s⁻¹, daily.

The results were analysed by ANOVA (post hoc LSD test) using SPSS 10.0 (SPSS for Windows 1999).

RESULTS

The first signs of callus formation were detected on the base of the explants in all variants of the media 14 days after culture establishment. One week later, the callus developed into nodule clusters that were often found in contact with the culture medium. These nodule clusters were globular in form, greenish in colour and compact in texture. The transferring of the explants to fresh medium gave rise to enlargement of the callus and also to new nodules formation. Simultaneously, axillary shoot formation was observed on the explants (Fig. 1). It was demonstrated that thidiazuron (TDZ) promoted significantly higher levels of multiplication than BAP. However, multiplication rates of the axillary shoots were low after 8 weeks of cultivation (MITRAS et al., 2009).

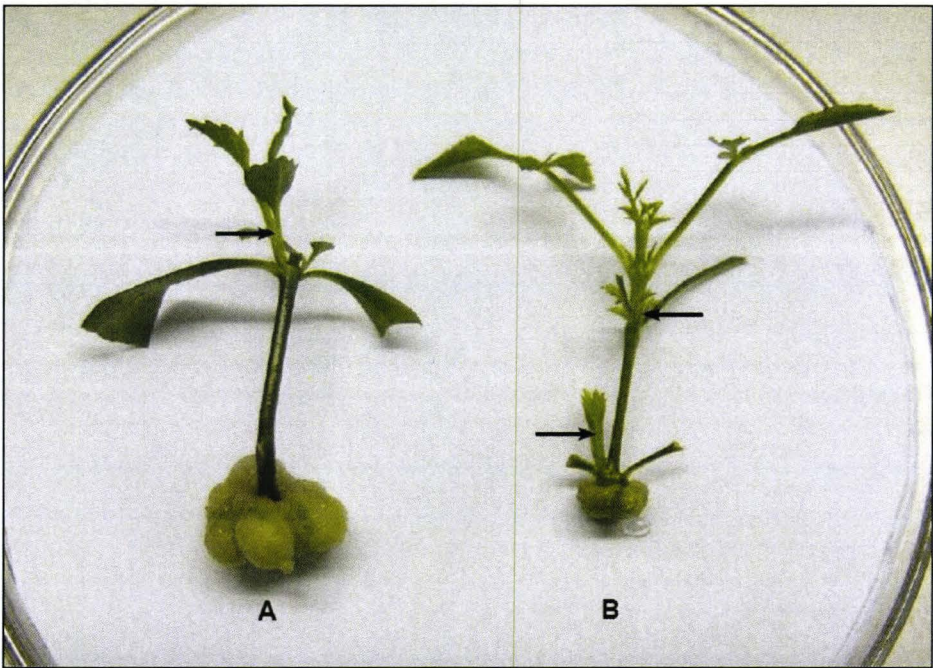


Figure 1. Callus formation on the base of hypocotyls (A) and epicotyl (B) and axillary shoots formation (arrows) on medium MS2 (original photograph).

After 4 weeks, regeneration of axillary shoots was observed. The highest multiplication rate (9.36 ± 1.94) was found on MS medium supplemented with 1.0 mg l^{-1} TDZ. The multiplication rate from the epicotyls was higher on MS medium in comparison with WPM. However, there were no significant differences among multiplication rates obtained using TDZ in different concentrations on each medium. Similar tendency was not noticed for the axillary shoots formation from hypocotyls. The highest multiplication rate (4.12 ± 0.98) was achieved on MS medium supplemented with 1.0 mg l^{-1} TDZ but there was not found any statistical difference compared with other treatments. Also, in comparison with epicotyls, their multiplication rate was lower on the variants of MS medium, but there were not differences between the variants of WPM and MS medium (Table 1).

Table 1. Mean number of the induced axillary shoots, induced from epicotyls and hypocotyls.

Medium	Epicotyls	Hypocotyls
MS1	$9.00 \pm 1.52 \text{ a}$	$2.55 \pm 0.79 \text{ bc}$
MS2	$9.36 \pm 1.94 \text{ a}$	$4.12 \pm 0.98 \text{ b}$
WPM1	$3.35 \pm 0.40 \text{ bc}$	$2.09 \pm 0.28 \text{ bc}$
WPM2	$2.95 \pm 0.19 \text{ bc}$	$1.75 \pm 0.33 \text{ c}$

Legend: Values are mean (M) \pm standard error (SE). Means followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p < 0.05$.

After 8 weeks of cultivation, the length was significantly higher in shoots, originated from hypocotyls on MS medium supplemented with 0.5 mg l^{-1} TDZ ($15.04 \pm 2.58 \text{ mm}$) (Fig. 2) and the increased concentration of TDZ induced depressive effect. However, similar reaction was not observed on the variants of WPM. The highest length of the shoots originated from epicotyls was observed on the variants of MS medium, but there were no differences in the shoot length between the used treatments (Table 2).

Table 2. Mean length (mm) of the axillary shoots, induced from epicotyls and hypocotyls.

Medium	Epicotyls	Hypocotyls
MS1	$8.28 \pm 0.74 \text{ bcd}$	$15.04 \pm 2.58 \text{ a}$
MS2	$8.32 \pm 0.69 \text{ bc}$	$9.99 \pm 1.40 \text{ b}$
WPM1	$5.79 \pm 0.49 \text{ d}$	$9.57 \pm 1.93 \text{ bc}$
WPM2	$5.91 \pm 0.30 \text{ cd}$	$7.38 \pm 1.15 \text{ bcd}$

Legend: Values are mean (M) \pm standard error (SE). Means followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p < 0.05$.



Figure 2. Multiplication and elongation of axillary shoots on medium MS2 (Original photograph).



Figure 3. Rooted shoots after application of inductive medium for 24 h and transfer of the shoots to expressive medium (Original photograph).

The number of the formed shoots depended on the medium ($F = 36.003, p < 0.05$), the type of explant ($F = 32.643, p < 0.05$), and the interaction between these two factors ($F = 13.868, p < 0.05$). Root length depended on all investigated factors ($F = 14.217, 4.228, 15.754$, resp.; $p < 0.05$) and the interaction between concentration of TDZ and type of explant ($F = 4.605, p < 0.05$) (Table 3).

Table 3. Significance of the studied factors and their combinations on the axillary shoots formation estimated by a post hoc LSD test.

Factors	Number of the shoots		Length of the shoots (mm)	
	F	Level of significance	F	Level of significance
M	36.003	0.000	14.217	0.000
C	0.233	0.630	4.228	0.040
E	32.643	0.000	15.754	0.000
M × C	1.164	0.283	0.733	0.392
M × E	13.868	0.000	0.847	0.358
C × E	0.261	0.611	4.605	0.032
M × C × E	0.215	0.643	0.654	0.419

Legend: a R Squared = 0.078 (Adjusted R Squared = 0.063), M = Medium, C = Concentration of the TDZ, E = Explant, $p < 0.05$.

Effect of the inductive medium on adventitious root formation.

Rooting of the shoots was observed in all rooting variants of the medium, and very small amount of callus was observed on the base of the shoots. The roots were thin and some of them appeared from the stem and above the callus (Fig. 3, left). The rooting percentage was significantly higher on the inductive medium applied for 24 h. The rate of rooting decreased significantly after longer periods of cultivation (48 and 72 h) on inductive medium. Also, these results were lower in the comparison with the control (Table 4).

The root number was significantly lower when the shoots were cultured on control medium i.e. half-strength and auxin free WPM. When different durations of inductive phase were applied, the opposite tendency was observed but statistical differences were not observed between the applied treatments. However, the roots reached the highest length after the cultivation of the shoots on half-strength WPM without auxins i.e. on control medium (Table 4).

Table 4. Effect of the duration of inductive phase on the rooting of axillary shoots.

Duration of the inductive phase (h)	Rooted plants (%)	Mean number of the roots	Mean length of the roots (mm)
Control	60.74 ± 3.23 b	2.1 ± 0.2 b	32.4 ± 2.1 a
24	73.33 ± 3.33 a	3.9 ± 0.6 a	25.7 ± 1.9 b
48	42.73 ± 2.72 c	3.2 ± 0.5 ab	28.5 ± 2.9 ab
72	50.00 ± 2.62 c	3.8 ± 0.6 a	24.9 ± 2.0 b

Legend: Values are mean (M) ± standard error (SE). Means in the column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p < 0.05$.

DISCUSSIONS

TDZ was found to be potential cytokinin for plant tissue cultures (HUETTEMANN & PREECE 1993; LU 1993; MURTHY et al., 1998; KHURANA et al., 2005). In previous studies on *Fraxinus excelsior* it has been reported that TDZ is effective in inducing shoot regeneration from embryo hypocotyls, cotyledons, whole leaves, and petioles (TABRETT & HAMMATT 1992; HAMMATT 1994, 1996; THOMPSON et al., 2001). To date, regeneration in common ash by adventitious shoots has only been reported from leaves (HAMMATT 1994) and embryo hypocotyls (TABRETT & HAMMATT, 1992). It was found that TDZ in concentrations 0.01-5.0 mg l⁻¹ is the best for shoots induction (TABRETT & HAMMATT, 1992; HAMMATT 1994; SCHOENWEISS & MEIER-DINKEL, 2005). Our results (data not shown) are in agreement with the findings of TABRETT & HAMMATT (1992) that some of the initial explants died without producing of shoots. It was reported that WPM is more suitable than MS medium for the survival of the explants and mean number of the induced axillary shoots (HAMMATT & RIDOUT 1992; SILVEIRA & COTTIGNIES 1994; SILVEIRA & NOUGRÉDE 1995; NOUGARÉDE et al., 1996). However, we found that MS medium is more suitable for the formation of a greater mean number of axillary shoots from epicotyls but is not in significance for the induction of the axillary shoots from hypocotyls. It could be due to the different explants and TDZ used instead of BAP in our experiments. Also, our results demonstrated that the used concentrations of TDZ did not affect the mean number of shoots induced from epicotyls and hypocotyls.

It was shown that the length of axillary shoots is higher on WPM in comparison with MS medium (HAMMATT & RIDOUT 1992) and depends on the used cytokinin and its concentration (SILVEIRA & COTTIGNIES 1994). Our results showed that the length of the axillary shoots was higher when they were induced from hypocotyls on MS medium. The high concentration of TDZ (1.0 mg l⁻¹) inhibited the elongation. However, the medium type and TDZ concentration did not affect the length of axillary shoots originated from epicotyls.

It has been shown that the type and concentration of auxin has a central role in the induction of adventitious roots (DE KLERK 2001; DE KLERK et al., 1997, 1999; KUREPIN et al., 2011). Also, it was demonstrated that auxin pulse treatment is critical for the rooting of plants *in vitro* and depends on the duration of inductive phase (DE KLERK 1996, 2001, 2002; KLERK et al. 1999; MITRAS et al., 2009). After the inductive phase, once the cells have been determined to root formation, auxin is no longer required for the rooting of *Fraxinus excelsior* shoots (HAMMATT 1996; HAMMATT & RIDOUT 1992; MITRAS et al., 2009). It was found that the rooting of common ash varies from 0 to 100% (HAMMATT & RIDOUT 1992; SILVEIRA & COTTIGNIES 1994; SCHOENWEISS & MEIER-DINKEL 2005; MITRAS et al., 2009) and depends on the age of the initial explants (SCHOENWEISS & MEIER-DINKEL 2005), genotype (TABRETT & HAMMATT 1992), type and concentration of the auxin used (HAMMATT & RIDOUT 1992; SCHOENWEISS & MEIER-DINKEL 2005; MITRAS et al., 2009). In comparison with our previous results (MITRAS et al., 2009), the pulse treatment of shoots with high concentration of auxin for shorter period lead to significantly smaller amount of callus formation. Our results showed that the percentage of rooting and number of roots depends on the duration of cultivation on inductive medium. The cultivation of shoots to a root induction medium for shorter period improved the rooting and number of the formed roots. These and our previous results (MITRAS et al., 2009) demonstrated that the balance between the concentration of the used auxin and the duration of inductive phase are critical for the rooting rate and quality of the root system.

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IN VITRO SEED GERMINATION IN THREE RARE TAXA FROM THE ROMANIAN CARPATHIANS FLORA

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Abstract. Germination of the seeds is the primary step for plant development *in vivo* and also *in vitro* conditions. The seed germination rates vary between different species, altitudes of the habitats, site locations and years, but without clear trends. In the case of endangered plant species the number of seeds may be limited or they have problems concerning their germinability. In this context, an evaluation of the seed germination under *in vitro* (using different temperatures, media variants and pH values) and *ex vitro* conditions in three rare plant species belonging to different families was made. This aspect is important for conservative purpose. Rare plant species are considered of interest because of their ability to adapt to extreme or limitative environmental conditions. The studied species were *Erigeron nanus*, *Dianthus callizonus*, *Papaver alpinum* ssp. *corona-sancti-stefani*. In *ex vitro* conditions, only *E. nanus* and *D. callizonus* germinated. In *P. corona-sancti-stefani*, seed germination was induced only in *in vitro* conditions with low germination rate (10%) at 4°C. In the case of *E. nanus*, only media variant added with sucrose (30g/l) allowed a good germination rate (66.66%). The seeds of *D. callizonus* germinated better on the media variant with sucrose (30g/l) and higher pH value (6.8).

Keywords: rare plants, seed germination, *in vitro*, *ex vitro* culture.

Rezumat. Germinarea in vitro a semințelor la trei specii de plante amenințate din flora Carpaților din România.

Germinarea semințelor reprezintă prima etapă în dezvoltarea plantelor atât *in vivo* cât și *in vitro*. Rata de germinare a semințelor variază între specii, în funcție de altitudine, de locul și anul recoltării. Întrucât la speciile de plante rare pot apărea probleme privind viabilitatea și germinarea semințelor, a fost realizată evaluarea germinării semințelor în condiții *in vitro* (diferite temperaturi, variante de medii de cultură și pH) și *ex vitro*, la trei specii de plante din familii diferite. Acest aspect prezintă importanță conservativă. Aceste specii de plante amenințate sunt considerate de interes datorită capacității lor de a se adapta la condiții de mediu extreme sau limitante. Speciile luate în studiu au fost *E. nanus*, *D. callizonus*, *P. alpinum* ssp. *corona-sancti-stefani*. În condiții *ex vitro*, au germinat numai semințele de *E. nanus* și *D. callizonus*. La specia *P. corona-sancti-stefani* a fost indusă germinarea semințelor menținute la 4°C, în condiții aseptice însă cu rate mici de germinare (10%). În cazul semințelor de *E. nanus* o rată bună de germinare (66.66%) a semințelor în condiții *in vitro* a fost obținută pe mediu adiționat cu 30g/l și pH 5.8, fără factori de creștere. Semințele de *D. callizonus* au germinat mai bine pe varianta de mediu adiționată cu 30g/l, fără factori de creștere și pH 6.8.

Cuvinte cheie: plante rare, germinarea semințelor, condiții *in vitro*, *ex vitro*.

INTRODUCTION

The seed represents the main way of plant reproduction and is one of the key factors that determine the species maintenance in time and space (VENABLE & LAWLOR, 1980). Its storage may cause the loss of its germinative capacity (AMORIM et al., 1997).

In the case of threatened, rare and endemic plant species, knowing the seed germination requirements is useful for their conservation and management. Seed germination is important for the preservation, especially for *ex situ* strategies and reproduction of rare and endangered plant species (PORTEOUS, 1993; CLEMENTE & HERNÁNDEZ, 1995; BURMEIER & JENSEN, 2008; KAGAYA et al., 2008). It is recommended to use seeds for propagation of the rare and endangered plant species (VAN WYK & SMITH, 1996). The success of rare plant conservation programs depends on the knowledge of the seed germination behaviour, being considered important in developing effective protocols for promoting *ex situ* conservation (FENNER & THOMPSON, 2005). Seed germination is also used as initial explant for obtaining *in vitro* tissue culture for secondary metabolites (VERPOORTE, 2000).

The rare plant species are that species whose taxa have small populations worldwide, which are currently not threatened but are at risk (due to their restricted area) (OLTEAN et al., 1994). Because the rare taxa are exposed to possible extinction (MACE & LANDE, 1991), studies carried on them are considered important both of practical point of view concerning biodiversity conservation (MYERS et al., 2000) and also of theoretical value due to their ability to adapt to extreme conditions.

In the case of rare plant species, some problems concerning seed viability and germination can occur.

The *ex situ* conservation means the preservation of plant species out of their natural habitats and it is ensured by *ex situ* collections (seed banks, gene banks, botanical gardens and the use of *in vitro* techniques) (IPGRI / FAO, 1996). The *in vitro* techniques can be used to optimize the culture conditions to enhance the seed germination.

Papaver alpinum L. ssp. *corona-sancti-stefani* (ZAPAL.) BORZA (Papaveraceae family) (Fig. 1) is a rare (OLTEAN et al., 1994; OPREA, 2005; CIOCĂRLAN, 2009) and endemic plant species for South Eastern Carpathian Mountains (CIOCĂRLAN, 2009). The species has a scientific importance being the only representative of the genus in the alpine Flora. Also, the species plays an important role as pioneer plant fixing the detritus on the rocky substrate. The limitative factors for this species are tourism and detritus crumbling (DIHORU & PÂRVU, 1987).

Erigeron nanus SCHUR (Asteraceae family) (Fig. 1) is considered as vulnerable /rare species (OLTEAN et al.,1994; OPREA, 2005) and endemic plant species (CIOCÂRLAN, 2009; WITKOWSKI et al., 2003). Also, it was considered as a species of European concern (OZINGA et al., 2005).

Dianthus callizonus SCHOTT & KOTSCHY (Caryophyllaceae family) (Fig. 1) is an endemic rare alpine flower native in a small area of South Eastern Carpathians Mountains. It is valuable from a scientific raison because of its genetics and taxonomy and because of the possibility to form hybrids with *D. spiculifolius* and *D. tenuifolius* (DIHORU & PĂRVU, 1987).



Figure 1. *P. corona sancti stefani* (photo: Catană Rodica), *E. nanus* (source net), *D. callizonus* (photo: Holobiuc Irina) in natural habitats.

Our aim was to evaluate the seed germination in different conditions in the case of three rare plant species belonging to different families in order to improve the seed germination.

MATERIAL AND METHODS

The plant material was represented by mature seeds. The plant material was collected in 2006 and 2008 from the natural habitats. The germination rate was tested in 2009 for all three species.

In the case of *E. nanus* the seeds were collected from Piatra Craiului Massif in 2006.

The evaluation of the seed germination was realized on the media variants added with different growth factors (Table 1), in two different temperatures and light/dark regime.

In the case of *P. corona-sancti-stefani*, part of the seeds was obtained from the Botanical Garden Cluj-Napoca “Al. Borza” in 2003 and also some of them were collected from Piatra Craiului Massif in 2008.

In the case of *D. callizonus*, *in vitro* germination of the seeds collected from two sites, in two years and on media variants with different pH values.

The TTC test was made in accordance with ISTA international standards. The TTC is used to differentiate metabolic active and inactive tissues and to check the seeds viability. 1% tetrazolium chloride (2,3,5-triphenyl-2H-tetrazolium chloride) was prepared in distilled water with pH 6.5. The seeds were immersed in TTC solution and kept at room temperature in the dark for 24 hours.

In vitro seed sterilization. The seeds were first washed for 2 hours in running tap water, followed by HgCl₂ 0.1 % treatment for 10 minutes and finally, three washing in sterile distilled water were done.

The **germination rate** (%) represents the number of germinated seeds/ the number of total seeds x100. The germination rate was tested **in vitro** (at different temperatures, media variants, pH value) and **ex vitro** conditions.

For in vitro germination, the sterilized seeds were inoculated on the Murashige and Skoog basal media variant (MURASHIGE & SKOOG, 1962) without plant growth factors at a 16/8 photoperiod 4°C and 25°C for *P. corona-sancti-stefani*.

In *E. nanus*, four media variants (Table 1) were tested at 4°C and 25°C.

Table 1. Media variants tested for *E. nanus* seed germination.

Media variants	Basal media	Sucrose (g/l)	Growth factors (mg/L)
S1	MS	30	BAP 1, NAA 0.1
S2	MS	30	-
S3	MS	-	-
S4	MS	30	GA ₃ 100

Legend: MS - Murashige & Skoog medium, (MURASHIGE & SKOOG, 1962); BAP - benzylaminopurine; NAA - alfa-naphtyl acetic acid; GA₃ - gibberellic acid.

In the case of *D. callizonus*, MS without growth factors and three pH values was used.

For ex vitro germination, the seeds were placed in Petri dishes on the filter paper in distilled water. The Petri were kept in 25°C and a 16/8 photoperiod.

A number of 60 seeds per plant species was tested for the evaluation of the seed germination using three repetitions / treatment variant.

A seed was considered to be germinated when its radicle was emerged.

Statistics. The results were assessed using the 1-way ANOVA created by Daniel's XL Toolbox version 5.04. A Bonferroni-Holm posthoc test was used.

RESULTS AND DISCUSSIONS

Knowing the seed germination rate is relevant for plant ecology. In the case of alpine plant species, the asynchronous germination rate may be considered as an adaptation to the environment, where medium controlled germination can be crucial to seedling survival (PELTON, 1956; AMEN, 1966; BLISS, 1971).

Previous studies concerning seed germination of the threatened plant species were done, but a specific methodology for germination of seeds belonging to the alpine taxa was not found (KÖRNER, 1999). The germination behaviour can vary within a single species from one population to another, from year to year and among individuals (URBANSKA & SCHUTZ, 1986).

A lot of studies concerning the evaluation of seed germination of rare plant species in non-sterile and sterile conditions were performed (CUEVAS & FIGUEROA, 2007; KANDARI et al., 2008; KADIS et al., 2010; SAYANIKA DEVI et al., 2012; ABDOLLAHI et al., 2012).

The storage conditions of the seeds are one of the many other factors which can influence the seed quality expressed as seed viability and vigour. It is known that there are differences between species concerning seed longevity (how long seeds can be stored under given conditions). This aspect is crucial for an effective management of seed conservation collections (PROBERT et al., 2009).

The TTC test is an important rapid seed viability test based on the activity of dehydrogenases that catalyse mitochondrial respiration. In the living tissues of the seeds, dehydrogenases convert the TTC to formazan (NETO et al., 1999) an insoluble red compound that stains in red the living tissues. The presence of formazan allows the differentiation between living and inactive metabolic tissues (FILHO, 1999).

The TTC test results in the case of the three threatened plant taxa are shown in Table 2.

Table 2. TTC test performed on the seeds of three different plant taxa.

Species	Collected data	Sources	TTC reaction
<i>P. corona-sancti-stefani</i>	2003.	Botanical Garden Cluj.	-
	2008	Piatra Craiului	+++
<i>E. nanus</i>	2006	Piatra Craiului	++
<i>D. callizonus</i>	2004.	Piatra Craiului	+
	2006		+++

Legend: +++ good reaction, ++ low reaction, + very low reaction, - no reaction.

Germination of the seeds is a complex physiological process determined by the imbibitions of the tissues with water, after the dormancy mechanisms were delayed. The seed germination depends on the internal (endogenous plant hormones) and external conditions (temperature, water level, oxygen content and light or darkness regime) (RAVEN et al., 2005; SEN, 2010). Also, it is already known that there are differences concerning the germination requirements connected to the geographical distribution (PROBERT, 2000).

In our case, the seed germination in sterile conditions (*in vitro*) was higher than in non-sterile conditions (*ex vitro*) for the all three plant taxa (Table 3).

In *ex vitro* conditions, only *E. nanus* and *D. callizonus* have germinated.

In the case of *in vitro* conditions, the seed germination was induced at low rate in *P. corona-sancti-stefani* and improved in *E. nanus* and *D. callizonus*.

Table 3. Formation of seedlings and % of seed germination in the three threatened taxa.

Species	Conditions	No. of seeds	Mean no. of seedlings	% of seed germination	P value	F Critical
<i>P. corona-sancti-stefani</i>	<i>In vitro</i>	30	4	13.33*	0.01613	16
	<i>Ex vitro</i>	30	0	0		
<i>E. nanus</i>	<i>In vitro</i>	30	21	70*	0.03759	9.375
	<i>Ex vitro</i>	30	6	20		
<i>D. callizonus</i>	<i>In vitro</i>	30	24	80*	0.047421	8
	<i>Ex vitro</i>	30	14	46.66		

Legend: Values marked by * are significantly different between *in vitro* and *ex vitro* conditions at $p < 0.05$ using the posthoc test Bonferroni-Holm.

Some *in vitro* studies were done concerning regeneration in *Papaver* species starting from seeds (TISSERAT & BERHOW, 2009; ZAKARIE et al., 2011). Also, a comparative study of seed germination ecology in four *Papaver* taxa was made (KARLSSON & MILBERG, 2007).

In the case of *P. corona-sancti-stefani*, the seeds collected in 2003 showed no TTC positive reaction and no germination rate neither in *ex vitro* nor in sterile (*in vitro*) conditions (Table 2). The seeds collected in 2008 germinated only in sterile conditions. There are some studies concerning the germination requirements which differ from year to year (KAYE et al., 1999). The variation in germination characteristics could be interpreted as one of the most important survival strategies for species growing under unpredictable environmental conditions (GUTTERMAN, 1994; KIGIEL, 1995).

Two different temperatures (25°C and 4°C) were tested for the seeds collected in 2008 to induce the germination in vitro. After 45 days, the germination rate of the seeds was low, with non-significant differences registered between the tested temperatures (Fig. 2).

Despite of the data which proved that the seeds belonging to *Papaver* genus germinate and growth at 20-25°C (TISSE RAT & BERTHOW, 2009; GORGOROV et al., 2011), in our case, *P. corona-sancti-stefani* seeds germinated better at 4°C.

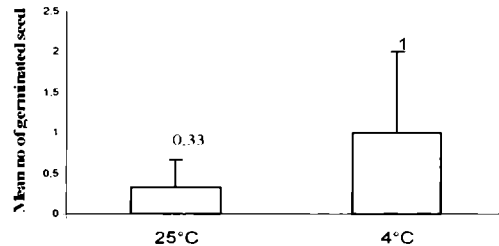


Figure 2. Mean number of *P. corona-sancti-stefani* seeds germinated at 25°C and 4°C.

Some studies in *Erigeron* species concerning seed germination in laboratory conditions were made (CUEVAS & FIGUEROA, 2007).

In the case of *E. namus* SCHUR, four media variants, two different temperatures and light/dark regime were tested to increase the germination rate. A high percentage of seed germination of *E. namus* (66.66%) was obtained at 25°C in the presence of light, while at 4°C in the dark, the seed germination rate was inhibited (Fig. 3). According to the literature, more than 86% of the species require light to germinate without requiring cold treatment. The data obtained are in concordance with studies on seed germination ability in other species of rare plants (KOORNNEEF et al., 2002).

The seed germination is genetically determined and affected by endogenous plant hormones (BENTSINK & KOORNNEEF, 2002). The applications of exogenous plant growth factors (PGR) have been extensively used for enhancing the seed germination and the development of seedlings in the laboratory. There are several studies regarding the influence of cytokines on the germination capacity of seeds in different species (NIKAM & BARMUKII, 2009; NIKOLIĆ et al., 2006). The gibberellins are most prominent growth regulator used (SHUE-LOCK, 1968; CERABOLINI et al., 2004).

In our case, the addition of BAP (1mg/l) in one medium variant had no significant effect on the seed germination, this being lower than the rate obtained in the case of S2 medium variant (MS medium without PGR).

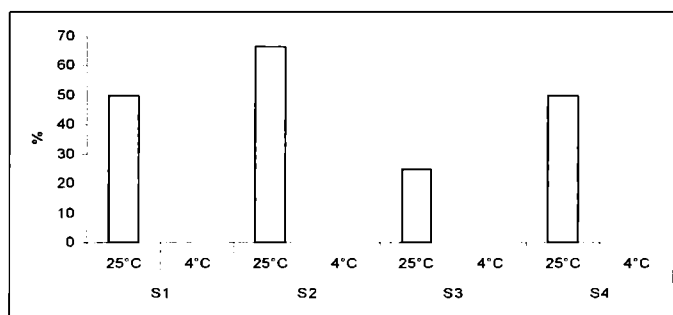


Figure 3. The seed germination rate of *E. namus* at 25°C and 4°C and light/dark regime.

S4 variant added with 100mg/l GA₃ allowed about 20% of the seeds to germinate (Fig. 4). Between the four media variants tested for seed germination there are statistically significant differences ($p < 0.05$).

The four tested media variants (S1-S4) (Table 2) allowed the seeds to germinate with different rates, with good results on the S2 media variant added with 30g/l sucrose (Fig. 4).

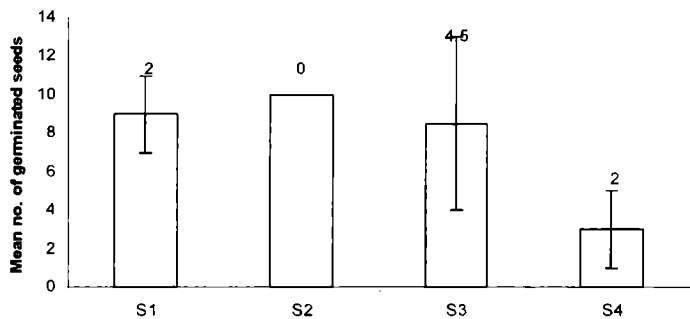


Figure 4. The mean number of *E. nanus* seeds germinated on S1-S4 media variants.

There are some studies concerning the seed germination in Caryophyllaceae family. A comparative research concerning germination of the seeds collected from different side of Europe was done (THOMPSON, 1970). Other studies of the seed size variation in the populations in relation to habitat conditions were performed in *Silene dioica* (L.) CLAIRV (THOMPSON, 1981). Studies concerning *in vitro* seed germination in *Dianthus* species were made in *D. nardiformis* JANKA (HOLOBIUC et al., 2009), in *D. ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus* (RADOJEVIĆ et al., 2010), in *D. henteri* (CRISTEA et al., 2010), in *D. barbatus* (LĂPĂDĂTESCU et al., 2012).

In our case, the *D. callizonus* seeds tested in *ex vitro* conditions showed over 40% germination rate. These data are in accordance to those obtained by MICLE in 1967, who reported that the germination of *D. callizonus* seeds in natural habitat was around 35-50% (MICLE, 1967).

Comparing the germination rate of the seeds collected in 2004 and 2006, the seeds collected in 2004 lost their germinability (Fig. 5). These data are in accordance with the data of KAYE et al., 1999.

The seeds collected in 2006, are originated from two different sites of Piatra Craiului Massif: Piatra Craiului Mică (1811 m altitude) and Padina Popii (2018 m altitude). The distance between the two sites is ~ 20 km. In our case, non-significant differences were obtained concerning the seed germination rate from the two sites (Fig. 4). The same results were reported by GIMÉNEZ-BENAVIDES et al., 2005 in 20 endemics species of the Iberian Peninsula who did not find a consistent pattern in the germination rate related to altitude variation.

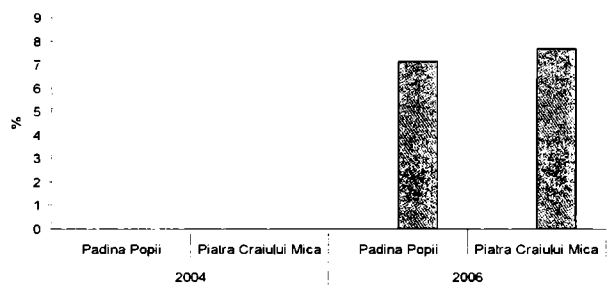


Figure 5. The germination rate of *D. callizonus* seeds collected in 2004 and 2006 from two different sites.

Other important factor that can influence the seed germination is the pH value. Three different pH values (4.8; 5.8 and 6.8) were tested. The higher number of germinated seeds was obtained on the medium variant with 6.8 pH (Fig. 6). The differences between the three used pH values were statistically non-significant ($p < 0.005$). These results are in accordance with the data recorded in the natural habitats, *D. callizonus* being an alpine species growing on calcareous substrate with 6-7.5 pH (ONETIE, 2011). Our data in *D. callizonus* are similarly to the experiment made in *Salvia* sp. where the 5.0 to 6.5 pH values increased seed germination (SHOEMAKER & CARLSON, 1990).

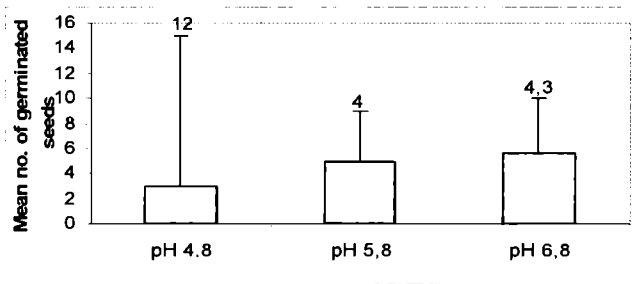


Figure 6. The mean number of germinated seed of *D. callizonus* at different pH values.

The plants obtained through *in vitro* seed germination were used for the establishment of short-term preservation protocol in *E. nanus* and *D. callizonus* (HOLOBIUC et al., 2005; HOLOBIUC & BLÎNDU, 2006; BLÎNDU & HOLOBIUC, 2007), for *in vitro* conservation under slow-growth conditions during medium-term (HOLOBIUC & BLÎNDU, 2006; CATANĂ et al., 2010a). In *E. nanus*, *in vitro* regenerants obtained from one germinated seed was used to assay the genetic stability (CATANĂ et al., 2010b).

CONCLUSIONS

The seed germination rates were improved in the case of *in vitro* conditions in all three taxa tested.

Seeds of *P. corona-sancti-stefani* germinated better at 4°C.

Seeds of *E. nanus* germinated at 25°C in the presence of light on the medium variant added with 30g/l sucrose, without plant growth factors and 5.8 pH value.

For *D. callizonus* seeds germination, it is suitable a medium added with 30g/l sucrose without plant growth factors, at 6.8 pH.

In vitro germinated seedlings were used for further studies concerning *in vitro* preservation during short and medium-term preservation.

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IN VITRO INTRODUCTION OF *Dianthus trifasciculatus* KIT ssp. *parviflorus* AS EX SITU PRESERVATION METHOD

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Abstract. Taking into account the strong decrease of plant diversity owing to the anthropically modified environment and climatic changes, it is necessary to adopt integrative measures performed both *in situ* and *ex situ* to ensure plant preservation. Among *ex situ* methods, besides classical ones, plant biotechnologies bring an important contribution for biodiversity preservation. *In vitro* cultures can provide plant material for natural habitats repopulation and restoration of wild populations, and also for *ex situ* conservation during different period. *D. trifasciculatus* KIT ssp. *parviflorus* is a critically endangered taxon and it is common to the South of Romania and the North of Bulgaria. This taxon is endangered owing to anthropically influences and invasive species. *In vitro* cultures were started from explants collected from a plant from the Botanical Garden "Al. Borza" Cluj Napoca. Different media variants added with growth factors (cytokinins and auxins) were tested for primary cultures induction, for optimization of the regeneration and for rooting. An efficient multiplication protocol was established for this taxon useful for conservation purpose, which can be extended for the multiplication of several genotypes from natural populations.

Keywords: *in vitro*, conservation, direct morphogenesis, *Dianthus trifasciculatus* KIT ssp. *parviflorus*.

Rezumat. Introducerea *in vitro* ca metodă de conservare *ex situ* a taxonului *Dianthus trifasciculatus* KIT ssp. *parviflorus*. Ținând cont de diminuarea dramatică a biodiversității vegetale, în condițiile antropizării mediului, a schimbărilor climatice globale, este necesară adoptarea de măsuri integrate care să asigure conservarea speciilor de plante atât *in situ* cât și *ex situ*. Între metodele *ex situ*, pe lângă cele clasice, biotehnologiile vegetale aduc un aport important la conservarea biodiversității plantelor. Culturile *in vitro* pot asigura material biologic pentru repopularea habitatelor și refacerea populațiilor naturale, cât și pentru conservarea *ex situ* pe diferite durate de timp. *D. trifasciculatus* KIT ssp. *parviflorus* este un taxon critic periclitat, comun regiunii sudice a României și nordului Bulgariei. Acesta este amenințat ca urmare a antropizării habitatelor și a pătrunderii speciilor invazive. Culturile *in vitro* au fost inițiate de la un individ existent în colecția Grădinii Botanice „Al. Borza” Cluj Napoca. Diferite variante de medii adiționale cu factori de creștere (citokinine și auxine) au fost testate pentru inducerea culturilor primare, pentru optimizarea regenerării și pentru înrădăcinare. A fost elaborat un protocol eficient de multiplicare, util din punct de vedere conservativ care poate fi extins pentru multiplicarea mai multor genotipuri din populațiile naturale.

Cuvinte cheie: *in vitro*, conservare, morfogeneză directă, *D. trifasciculatus* KIT ssp. *parviflorus*.

INTRODUCTION

In Romanian Flora, from 3795 *Thracheophyta* species and subspecies, ~30 % are threatened (DIHORU & NEGREAN, 2009). 110 taxa are considered European or/and Globally threatened (OPREA, 2005). The causes of the disappearance of the species could be anthropic and/or natural. The increased rhythm of disappearance of plant species owing to anthropic pressures and global climatic changes imposes integrative measures of conservation performed both *in situ* and also *ex situ*. Among *ex situ* conservation measures, besides the traditional approaches (in seeds banks, field collection or botanical gardens), the biotechnological approaches can significantly contribute to the preservation of plant biodiversity.

The **Global Strategy for Plant Conservation** (2011-2020) claimed that at least 75% of the threatened plant species to be preserved in *ex situ* collections, preferably in the country of origin, and at least 20% to be available for recovery and restoration programs (Target 8).

In Romania, the **National Strategy and the Action Plan for Biodiversity** (SNPAB) refers to measures concerning the biodiversity conservation between 2011 and 2020, with 10 objectives. Among these, *ex situ* preservation has an important place sustained by scientific research and elaboration of technologies applicable in the case of threatened and/or endemic plants.

In vitro cultures can ensure in short time, in reduced space-and with relative low labour cost, plant material for recovery of wild populations or for preservation during different period of time. Another important advantage is that it can provide healthy plant material non-exposed to biotic and abiotic stress factors.

Taking into account the duration and methods used *in vitro*, the preservation can be done during short-term - through active cultures multiplication during 1-3 months or medium-term, using growth retardation protocols, during several months or years. On the other hand, long-term preservation involves the storage in liquid nitrogen of apexes, meristems or somatic embryos, which suffered different treatments to counteract the formation of the ice inside the cells.

In fact, short-term and medium term preservation can supply plant material both for *in situ* reintroduction and also for long-term preservation of threatened plant taxa.

At international level, several authors sustained the role of biotechnology in plant biodiversity conservation (BENSON, 1999; ENGELMAN, 2010; REED et al., 2011; GONZALEZ-BENITO & MARTIN, 2011; BUNN et al., 2011).

The taxon taken into study, *D. trifasciculatus* ssp. *parviflorus* is cited in the Red Book of Vascular Plants from Romania, being common to the South of Romania and the North of Bulgaria. It is a perennial, amphimictic, allogame

taxon, growing on neutral, poor substrate. It is a critically endangered taxon (DIHORU & NEGREAN, 2009), fact that justifies the *in vitro* conservation studies. The reduction of the plant areal owing to agricultural practices and invasive species determines this taxon to become threatened (DIHORU & NEGREAN, 2009).

MATERIAL AND METHODS

For *in vitro* cultures initiation, we used explants collected from a plant cultivated in the “Al. Borza” Botanical Garden, Cluj-Napoca.

In vitro primary culture was started from single node stem fragments. Sterilization was made in four steps:

I. washing in running tap water II. short immersion in 70°C ethylic alcohol for 30 second III. sterilization with mercuric chloride 0.1% for 2 times x 5 minutes; IV. three times washing with sterile distilled water. The rate of sterilized explants was recorded after one week.

As the first plant material used to start the *in vitro* cultures was limited, at the beginning, just few media variants were tested for the primary cultures induction (Table 1).

The culture media tested were based on Murashige-Skoog formula (MURASHIGE & SKOOG, 1962) modified by adding 30 g/l sucrose and B5 vitamins (GAMBORG et al., 1968) and growth factors in different combinations for the regeneration process in primary and secondary cultures (Table 1 and 2).

To stimulate rhizogenesis, there were used media based on MS formula modified through the reduction of macro- and microelements (R1-R5) and sucrose content - R5 variant (Table 3).

The media were solidified with 0.8% Plant agar and pH was adjusted at 5.75-5.8 using 1N KOH before autoclaving.

Different growth factors were added into the regeneration media to sustain the *in vitro* developmental processes: cytokinins as benzyl aminopurine - BA, kinetin - K, adenine - Ad, zeatin - Zea and auxins as alpha-naphthyl acetic acid - NAA, 2,4 - dichlorophenoxyacetic acid - 2,4-D. Glutamine was also used as supplement.

Table 1. The media cultures tested for the primary in vitro cultures.

Components		Media variants				
		M1	M2	M3	M4	M5
Macroelements		MS	MS	MS	MS	MS
Microelements		MS	MS	MS	MS	MS
Growth factors (mg/l)	BAP	1	1	1	1	-
	Kin	1	1	1	1	-
	Zea					2
	Ad	-	-	50	-	-
	NAA	0.2	0.2	0.2	-	0.2
	2,4- D	-	-	-	0.2	-
Others Compounds (g/l)		Glut	-	0.2	-	-

Table 2. The media used in the second cultures for improving the regeneration rate.

Components		Variants								
		M1	M2	M3	M4	M5	M6	M7	M8	M9
Macroelements		MS	MS 1/2	MS 1/2	MS	MS	MS	MS	MS	MS
Microelements		MS	MS 1/2	MS 1/2	MS	MS	MS	MS	MS	MS
Vitamins		B5	B5	B5	B5	B5	B5	B5	B5	B5
Growth factors (mg/l)	BAP	0.1	0.1	0.1	0.1	-	-	1	1	-
	Kin	-	-	-	-	0.1	0.1	1	1	-
	Zea				-				-	2
	GA ₃	-	-	-	-	-	-	-	0.5	-
	NAA	-	0.01	0.01		0.01	-	-	0.2	0.2
	2,4- D	-	-		0.01	-	0.01	0.2	-	-
Other compounds (g/l)		Glut	-	0.2	-	-	-	-	-	-

Legend: MS - Murashige & Skoog medium (MURASHIGE & SKOOG, 1962); B5 Gamborg - vitamins (GAMBORG et al., 1968); BAP - benzyl aminopurine; Kin- kinetin; Zea - zeatin, Ad - adenine; NAA - α-naphthyl acetic acid; 2,4-D - 2,4 - dichlorophenoxyacetic acid; Glut - glutamine, GA₃, gibberellic acid.

The evaluation of the regeneration in the primary culture was done through registering the number of developed shoots/inoculum, without statistical repetitions because of the small quantity of initial explants. For every medium variant, there were cultured 2 Petri dishes with 3 stem fragments as explants.

In the second culture cycle, for improving the regeneration rate, double node shoots fragments collected from the primary regenerative cultures were used as explants and cultured on nine media variants. Five explants in 3 repetitions were cultured for every medium.

The mean number of regenerated shoots was registered after 30 days and statistically analysed using ANOVA test.

The media with the best results in the primary cultures, containing normal levels of growth factors (M7-M9) were compared concerning the efficiency of the regeneration process with variants containing reduced levels of growth factors (M1-M6) and low content of macro- and micro-elements (M2-M3) (Table 2).

The cultures were maintained at 2000 lux illumination and 16/8 photoperiod and 25°C temperature.

For rooting, several minimal R1-R5 media were tested (Table 3). Medium MS added with 30 g/l sucrose was considered as control (R6). R1-R5 variants consisted in modified MS formula by reducing salts at ½ and ¼ and sucrose used at 20 g/l and 10 g/l. Additional shoots formation and roots development were evaluated as mean number/initial explant after 40 days of culture.

Table 3. Minimal media tested for rooting.

Components	Media variants					
	R1	R2	R3	R4	R5	R6
Macroelements	MS 1/2	MS 1/2	MS 1/2	MS 1/4	MS 1/4	MS
Microelements	MS 1/2	MS 1/2	MS 1/2	MS 1/4	MS 1/4	MS
NAA	-	-	0.01	-	-	-
AC	-	500	-	-	-	-
Sucrose (g/l)	20	20	20	20	10	30

Legend: MS- Murashige & Skoog medium. AC-active charcoal. NAA- alpha naphthyl acetic acid.

RESULTS AND DISCUSSIONS

There were already made several studies concerning *in vitro* multiplication for conservative purpose in different *Dianthus* threatened taxa (ZĂPĂRȚAN, 1995; BUTIUC-KEUL & DELIU, 2000; PĂUNESCU & HOLOBIUC, 2003; CRISTEA et al., 2002, 2004, 2006, 2010; MICLĂUȘ et al., 2003; HOLOBIUC et al. 2004-2005; MARCU et al., 2006; HOLOBIUC & BÎNDU, 2006; HOLOBIUC et al., 2009; HOLOBIUC et al., 2010; JARDA et al., 2010; RADOJEVIĆ et al., 2010, POP & PAMEIL, 2011). In almost all studied taxa, the main way of *in vitro* regeneration was direct morphogenesis.

The sterilization rate of the explants consisting in single node stem fragments was 80%.

D. trifasciculatus ssp. *parviflorus* showed a positive *in vitro* reactivity in the primary cultures (Table 4).

The cytokinin dominance in the culture media allowed a good regeneration through direct morphogenesis occurred at the level of lateral meristems.

The best responses in the primary culture was registered after one month of culture on the variants M2 (supplemented with BAP/K/NAA and glutamine), M4 (supplemented with BAP/K/2,4-D) (Fig. 3b) and M5 (supplemented with zeatin and NAA) (Fig. 3a).

On the M2 and M4 variants rooting of the shoots occurred, while in the case of M5, no roots formed, but callus structure arose at the end of the shoots.

Table 4. *In vitro* response in primary cultures.

Media variants used	Cytokinins/ auxins combination	The response after one month of culture	The response after 2 months
		The mean number of shoots/inoculum	The number of shoots/ initial explant
M1	BAP/K /NAA	4	15-20
M2	BAP/K/ANA +glutamine	3.3	15-40
M3	BAP/K/ Adenine/NAA	6	20-25
M4	BAP/K/2.4-D	9	20-30
M5	Zeatin/ NAA	15	20-25

Despite of good regeneration rate, in the first 30 days on medium supplemented with zeatin, the efficiency of the regeneration was not better than those registered on other variants added with usual cytokinins such us BAP alone or associated with kinetin. Besides this, the callus induction at the end of the shoots and no rooting process is unfavourable for conservative and multiplication purpose.

After two months of primary cultures, the regenerative response was significantly improved. The number of shoots were increased reaching a maximum of 40/ initial explant (M2) (Table 4). Medium variant supplemented both with BAP and kinetin at 1mg/l and 2.4-D at 0.2 mg/l had also a beneficial effect concerning the regeneration (Fig. 3c).

Similar results concerning *in vitro* regeneration was reported by us in other related taxa belonging to *Dianthus* genus (HOLOBIUC et al., 2006, 2009, 2010), the ratio cytokinins/ auxins 10:1 being most beneficial for a majority of *Dianthus* studied.

In the case o f *D. giganteus* ssp. *banaticus*, JARDA et al. (2010) reported that the combination of BAP at 1mg/l and NAA 1 mg/l (1/1 ratio) and sucrose at 20 g/l was suitable for primary culture establishment. For the second multiplication cycle, the level of NAA was decreased at 0.1 mg/l.

In the ratio 10/1 between BAP and NAA was also found to be optimally for shoots regeneration.

RADOJEVIĆ et al., (2010) reported in *D. giganteus* ssp. *croaticus* and *D. ciliatus* ssp. *dalmaticus* that medium MS supplemented with BAP 1 mg/l, NAA 1 mg/l and IBA 1.5 mg/l was favourable for multiplication starting from stem segment cultures. In the case of media added with high level of auxins (5mg/l 2.4-D) combined with others growth factors, callus formation was described, but this indirect way of regeneration is not preferable for threatened or endemic taxa because it can generate variability.

In this experiment, the conversion into plants of possible proembryogenic structures developed from callus did not occurred in the case of *D. giganteus* ssp. *croaticus* and *D. ciliatus* ssp. *dalmaticus*.

POP & PAMFIL (2011) also studied *in vitro* regeneration in *D. spiculifolius*, *D. henteri* and *D. giganteus* ssp. *banaticus*, the cultures being started from aseptic germinated seeds. They concluded that the ratio 10/1 of BAP/ NAA determined the best results concerning multiple shoots formation after 30 and 120 days of culture in all studied taxa, but the authors faced with some problems with the rooting of the regenerated shoots on the media tested, the results being not satisfactory enough. Due to this aspect, the acclimatization of the plants was not efficient.

In our study, in the second culture cycle, starting from shoots derived from primary cultures as inocula, it was compared the efficiency of the regeneration using culture media with low content of salts and growth factors (diluted ten times) with complex media with normal levels of cytokinins and auxins (Fig. 1).

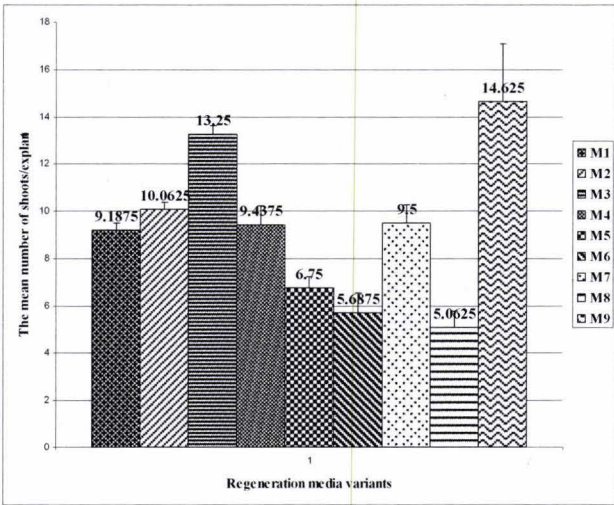


Figure 1. The mean number of regenerants/ explant in the second experiment in *D. trifasciculatus* ssp. *parviflorus* after 30 days of culture.

In this experiment, *D. trifasciculatus* ssp. *parviflorus* proved to regenerate in good conditions both on normal levels and also on reduced concentrations of growth factors.

The simpler variants with low content of growth factors (M1-M6) also proved to sustain the regeneration process through direct shoot formation with positive results.

M5 medium added with kinetin and NAA at low levels and keeping 10/1 ratio determined the development of white callus at the end of explants similar to zeatin/NAA added medium at normal concentrations.

Among the variants with reduced growth factors content, M3 variant characterized by the presence of glutamine as supplementary source of nitrogen, favoured the best results concerning regeneration (Fig. 3b). The mean number of shoots/ explants was very close to the result obtained in the presence of Zeatin (an expensive growth factor), the last one conducting to vitrification of the shoots (Fig. 3a).

Table 5. Observations concerning the *in vitro* response in the second culture cycle on optimised regeneration variants (M1-M9).

Variants	Observation
M1	Good regenerative response, shoots of 1-2 nodes, roots formation
M2	Good regeneration, reduced growth at one node, without roots, slow callusing process
M3	Very good regeneration, without rooting
M4	Good regeneration, shoots elongation at 2-3 nodes, some shoots necrosis
M5	Medium regeneration rate, absent rhizogenesis, white callus formation at the end of the shoots
M6	Good regeneration, shoots elongation at 2-3 nodes, good vigour, without roots
M7	Good regeneration, shoots elongation at 2-3 nodes, good vigour, without roots
M8	Medium regeneration rate, shoots elongation at 2-3 nodes, good vigour, without roots
M9	Very good regeneration rate, vitrified shoots without roots

For rhizogenesis process, all tested minimal media (R1-R5) sustained root formation (Fig. 2a).

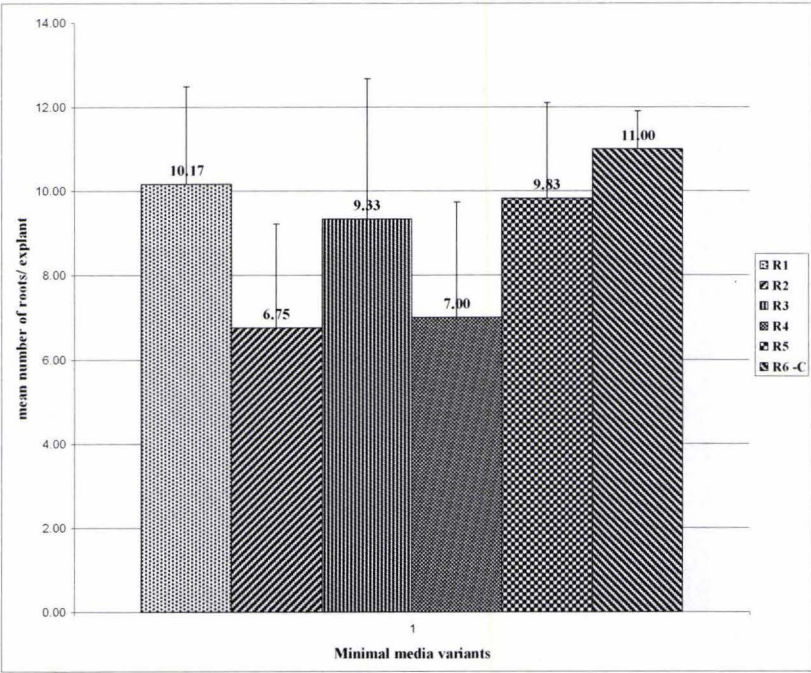


Figure 2a. Mean number of roots/ inoculum on minimal culture media after 40 days.

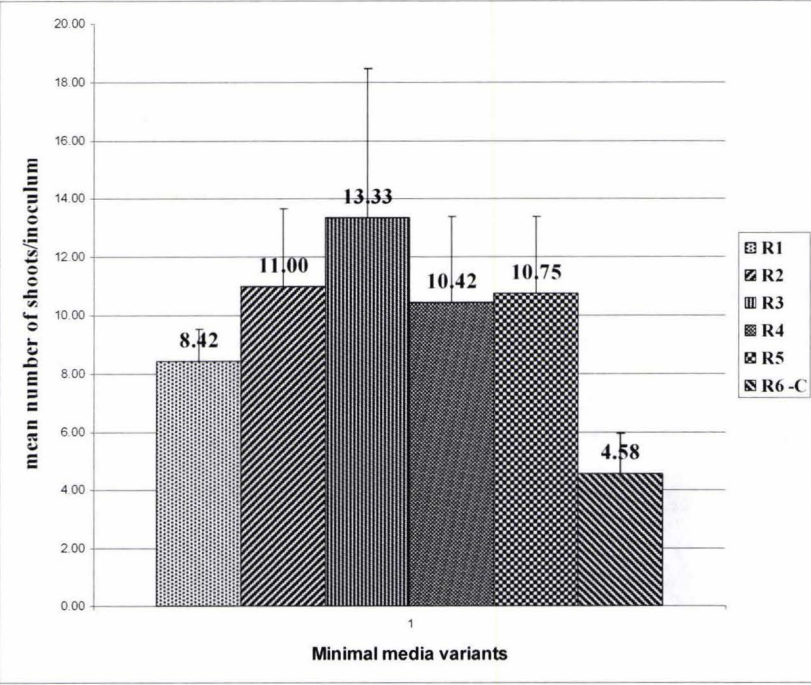


Figure 2b. Mean number of shoots/ inoculum on minimal culture media after 40 days.



Figure 3a. Shoots regeneration after 1 month on Zeatin and NAA supplemented medium in primary culture (original photo).



Figure 3b. Direct morphogenesis on variant supplemented with BAP 0.1 mg/l, NAA 0.01 mg/l 1 mg/l after 2 months of culture (original photo).



Figure 3c. Shoots formation on variant supplemented with BAP, Kinetin 1 mg/land 2.4-D 0.1 mg/l after 2 months (original photo).



Figure 3d. Explants rooting and supplementary shoots formation on minimal R1 medium (original photo).



Figure 3e. Explants rooting and supplementary shoots formation on minimal R5 medium (original photo).



Figure 3f. *Ex vitro* acclimatization of regenerated plants on pot (original photo).

R1, R3, R5 variants showed results comparable with the control R6 (MS medium added with 30 g/l sucrose), concerning the rooting, the first ones being cheaper to use.

Besides root formation, on these minimal culture media, lateral shooting occurred with different rates (Fig. 2b). The number of developed shoots was comparable to those obtained in the case of optimized culture media (Fig. 3d, e).

The best result concerning lateral shoots formation on minimal media, was registered on R3 variant with macro- and microelements reduced at half, 20 g/l sucrose and 0.1 mg/l NAA.

For economic reason, it is recommended to use for primary culture establishment, regeneration media added with cytokinins and auxins at 10/1 ration, being preferred BAP and NAA or BAP, Kinetin and NAA or 2,4-D and subsequently the transfer on minimal culture media for rooting and supplementary shooting.

The *ex vitro* acclimatization of the well-grown and rooted plants involved:

- the cultivation in pots using sterilized substrate made from ground/ Perlite in 1:1 ratio;
- the maintenance of the humidity at 80% through coverage with plastic bags during the first 4 days and subsequently the gradual opening for 30 minutes daily during two weeks.

The survival rate of acclimatized plants (Fig. 3f) was about 80% using this protocol.

CONCLUSIONS

The studied taxon showed a good *in vitro* reaction, a regeneration protocol being established for conservative purpose.

This method can be useful for the multiplication and *ex situ* preservation of several individuals (genotypes) to extent the variability of the preserved germplasm.

It is recommended to use for the primary culture induction a complex medium variant based on normal MS formula, added with 30/l sucrose and as cytokinin BAP associated with NAA and glutamine 0.2 g/l, keeping 10/1 ratio between the growth factors.

For the secondary multiplication and rooting, it can be used minimal variants based on MS formula reduced at half and added with 0.01 mg/l NAA, medium which sustains both rooting and development of supplementary lateral shoots.

In two months of culture, it can be regenerated a lot of clonally plants which can be used for further studies, acclimatized and cultured in outdoor *ex situ* collections.

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MACROMYCETES FROM THE GEOPARK PLATOUL MEHEDINȚI (OLTENIA, ROMANIA) (1st NOTE)

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Abstract. The purpose of the research conducted was the inventory of the flora and mycobiota of the Geopark Platoul Mehedinți territory. In this preliminary paper we present a total of 122 macromycetes species: 12 species of *Ascomycota* and 110 of *Basidiomycota*, mentioned for the first time in Mehedinți County, within the territory of the Geopark Platoul Mehedinți territory. With one exception, all listed species are new to the Geopark.

Keywords: macromycetes, chorology, new species, Mehedinți County, Oltenia.

Rezumat. Macromicete din Geoparcul Platoul Mehedinți (Oltenia, România) (Nota I). Scopul cercetărilor întreprinse a fost inventarierea florei și microbiotei teritoriului Geoparcului Platoul Mehedinți. Lucrarea prezintă un număr de 122 specii de macromicete: 12 specii din *Ascomycota* și 110 din *Basidiomycota*, citate pentru prima dată din județul Mehedinți, de pe teritoriul Geoparcului Platoul Mehedinți. Cu o singură excepție, toate speciile sunt noi pentru Geoparc.

Cuvinte cheie: macromicete, corologie, specii noi, județul Mehedinți, Oltenia.

INTRODUCTION

The Geopark Platoul Mehedinți is the newest protected area (the category V - IUCN) of Mehedinți County, according to the County Council (HG. no. 2151/30.11.2004). It includes the Geopark Platoul Mehedinți SCI, which represents 50% of the total area (53,892 ha). The Geopark Platoul Mehedinți is located in the south-west of Romania, to the north of Drobeta-Turnu Severin, and has a surface area of 106,000 ha, in the alternation area of the beech and oak forests. Conifers are found in the North (sporadic *Abies alba*) and *Picea excelsa*, *Pinus sylvestris* and *P. nigra* are only cultivated. The intersection of a temperate continental climate with a sub-Mediterranean climate, and even an oceanic climate, and also the lithological constitution of the Mehedinți Mountains have had an important role in the current structure of the flora and coenotic configuration of vegetation from the territory. Milder winters alternating with summers, generally warm accompanied by precipitation, with two maximum records, one in late spring and the second in late fall, in November, creates favorable conditions for the development of a large number of macromycetes with higher taxonomic diversity. The information from literature regarding the flora of the Geopark are summaries (SĂVULESCU et al., 1952-1976; TUTIN et al., 1964-1980; TUTIN et al., 1993), and regarding the macromycetes, with one exception - *Collybia longipes* (FR. ex BULL.) QUÉL. – Baia de Aramă (I. COMES in ELIADE, 1965) all the listed species are new to the Geopark.

MATERIAL AND METHODS

Observations were made in many sites from the Geopark, in different seasons, from April to November. The identified species were determined on fresh material, using special macromycetes papers (BON, 1988, 1990, 1991, 1993, 1999; CANDUSSO & LANZONI, 1990; KÜHNER & ROMAGNESI, 1978; MOSER & JÜLICIL, 1990; PALAZÓN LOZANO, 2006; SALĂGEANU & SALĂGEANU, 1985) and the materials were conditioned with a special Dorrex drier. The lists were realized from our own research. The presentation of the species is made in alphabetical order, with indication of the scientific name, ecology, choronyms, listed from North to South and West to East and the herbarium where all these are deposited, abbreviation follow THIERS, 2013. The nomenclature used – after INDEX FUNGORUM. For the species that were photographed text indications are made: Ioana Ciortan, Sorin Păunescu and Virgil Marinescu.

RESULTS AND DISCUSSIONS

Agaricus sylvicola (VITTAD.) PECK, on soil – Jidoștița NNW, Matorăț Hill, in forest, 44°43'59.438"N, 22°34'10.262"E, alt. c. 460 m, April 14, 2011, Ioana Ciortan & G. Negrean. Cireșu E, Țiganilor Valley, in *Fagetum*, 44°49'34.564"N, 22°33'43.315"E, alt. c. 395 m, August 3, 2012, Ioana Ciortan & G. Negrean.

Albatrellus ovinus (SCHAEFF.) KOTL. & POUZAR, on soil – Motrul Sec, Lupșa Valley, in *Fagetum*, 45°03'16"N, 22°47'48"E, alt. 460 m, October 9, 1984, G. Negrean [BUCM 86.281].

Aleuria aurantia (PERS.) FÜCKEL, on soil – Motrul Sec S, Lupșa Valley, in *Willdflisch*, 45°03'18"N, 22°47'47"E, alt. 440 m, October 9, 1984, G. Negrean [BUCM 86.290].

Amanita caesarea (SCOP.) PERS., on soil – Cireșu N, in *Quercetum dalechampii*, 44°48'22.1"N, 22°33'11.1"E, alt. 410 m, June 15-16, 2011, det. Ioana Ciortan & G. Negrean. Cireșu E, Pețimea fosilă Valley, in *Quercetum dalechampii*, 44°48'43.63"N, 22°33'20.25"E, alt. 411 m, August 21, 2011, Ioana Ciortan & G. Negrean [CL]. Bunoaica SE, Știubei Valley, in *Quercetum dalechampii*, 44°47'45.445"N, 22°33'22.603"E, alt. c. 460 m, June 13, 2010, G. Negrean [CL]. Cireșu E, in *Q. dalechampii*, August 8, 2012, Ioana Ciortan & G. Negrean.

- Amanita citrina* (SCHAEFF.) PERS. var. *citrina*, on soil – Motrul Sec S, Lupșa Valley, *Pinus sylvestris* (plantation), 45°03'18"N, 22°47'47"E, alt. 440 m, October 9, 1984, G. Negrean [BUCM 86.276]. Bunoaica E. above the Topolnița Valley, under *Pinus* sp. cultivated, 44°48'03.26"N, 22°32'31.16"E, alt. c. 385 m, August 5, 2012, Ioana Ciortan & G. Negrean.
- Amanita muscaria* (L.) LAM. var. *muscaria*, on soil – Motrul Sec, Lupșa (Motrișorul) Valley, near pinetum (cultivated), 45°03'18"N, 22°47'47"E, alt. 440 m, October 9, 1984, G. Negrean [BUCM 86.268].
- Amanita pantherina* (DC.) KROMBIL., on soil – Motrul Sec S, Lupșa Valley, 45°03'25"N, 22°47'48"E, alt. 360 m, June 18, 1987, G. Negrean [BUCM 103.700].
- Amanita rubescens* PERS. var. *rubescens*, on soil – Motrul Sec W, Sohodoarele Mici Valley, in *Fagetum*, 45°04'08"N, 22°45'39"E, alt. 470 m, July 25, 1986, G. Negrean [BUCM 99.984]. Motrul Sec W, Vârful Muchii ridge, in *Fagetum*, 45°04'02"N, 22°45'25"E, alt. 560 m, July 25, 1986, G. Negrean [BUCM 99.991]. Motrul Sec S, Lupșa Valley, in *Fagetum*, 45°03'18"N, 22°47'47"E, alt. 440 m, October 9, 1984, G. Negrean [BUCM 86.270]. Obârșia Cloșani N, in *Fagetum*, 45°02'52.657"N, 22°39'45.349"E, alt. 1025 m, August 18, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Seliște, Măguricea Mountain, May 30, 2010, G. Negrean. Cireșu E, in forest, June 2, 2010, G. Negrean. Cireșu E, Topolnița Valley, in forest, 44°49'21.438"N, 22°34'27.541"E, alt. c. 390 m, June 12, 2010, G. Negrean. Bunoaica SE, Știubei Valley, in *Quercetum dalechampii*, 44°47'45.445"N, 22°33'22.603"E, alt. c. 460 m, June 13, 2010, G. Negrean [CL]; Bunoaica E, above the Topolnița Valley, under *Pinus* sp. cultivated, 44°48'03.26"N, 22°32'31.16"E, alt. c. 385 m, August 5, 2012, Ioana Ciortan & G. Negrean. Cireșu NE, Polia Cireșu, in forest, 44°49'52.459"N, 22°33'03.293"E, alt. c. 390 m, June 12, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Jidoștița N, Plaiul Matorățului, in forest, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].
- Amanita vaginata* (BULL.) LAM. on soil – Motrul Sec S, Drumul Rujetului Hill, 45°03'08"N, 22°48'00"E, alt. 500 m, July 25, 1986, G. Negrean [BUCM 99.990]. Motrul Sec S, Drumul Rujetului Hill, in *Fagetum*, 45°03'13"N, 22°47'50"E, alt. 480 m, July 26, 1986, G. Negrean [BUCM 100.045]. The Motrul Sec W, Gorganul Mountain, northern slope, Plopilor Valley, in *Fagetum*, 45°05'21"N, 22°42'43"E, July 28, 1986, G. Negrean [BUCM 100.215]. Motrul Sec, Sohodoale Valley, in *Fagetum*, July 3, 2004, G. Negrean [HGN]. Obârșia Cloșani NE, Vârtoapele Mountain, in *Abieto-Fraxinetum*, 45°03'22"N, 22°42'30"E, alt. 960 m, July 28, 1986, G. Negrean [BUCM 100.188]. Isverna W, „Potcoava”, in *Quercetum petraeae*, August 26, 2007, G. Negrean [BUC].
- Armillaria mellea* (VAHL.) P. KUMM. s. l., on the roots – the Motrul Sec NW, Motrul Sec Valley, in *Alno-Padion*, 45°04'25"N, 22°45'22"E, alt. 390 m, October 9, 1984, G. Negrean [BUCM 86.244]. Motrul Sec S, Motrișorul (Lupșa) Valley, 45°03'22"N, 22°47'46"E, alt. 400 m, September 28, 1983, G. Negrean [BUCM 80.098]. Dâlbocița NNW, Firizu N, Glaceul Mare Valley, in *Fagetum*, 44°51'14.635"N, 22°43'32.510"E, alt. c. 360 m, November 2, 2010, G. Negrean [CL]. Gornenți WNW, under Montes Ciolanul Mare, La Funduri, in *Fagetum*, 44°55'30.35"N, 22°30'34.39"E, alt. c. 829 m, October 26, 2012, Ioana Ciortan & G. Negrean.
- Ascocoryne sarcoides* (JACQ.) J.W. GROVES & D.E. WILSON, matrix:
- Populus nigra* L. fallen – Bahna Valley, prope hidromola, November 5, 2010, Sorin Păunescu comm. & det. G. Negrean [CL].
- Astraeus hygrometricus* (PERS.) MORGAN, on soil – Motrul Sec NW, Motrul Sec Valley, in *Fagetum*, 45°04'20"N, 22°46'00"E, alt. 400 m, July 17, 1985, G. Negrean [BUCM 89.472]. Baia-de-Aramă N, Mănăstirii Hill, Cornetul Băii, in *Fagetum*, 45°00'25.70"N, 22°47'44.39"E, alt. 326 m, November 17, 2011, Ioana Ciortan & G. Negrean [CL]. Jidoștița N, Matorăț Hill, 44°44'46.9"N, 22°33'08.1"E, alt. c. 542 m, in the grass, June 9, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Schitul Topolniței, Schitul de Sus, 44°46'06.180"N, 22°36'04.411"E, alt. c. 500 m, June 10, 2011, Ioana Ciortan & G. Negrean.
- Auricularia mesenterica* (DICKS.) PERS., matrix:
- Juglans regia* L. – Valea Verde, the water mill, 44°59'04.67"N, 22°39'02.29"E, alt. circa 468 m, November 3, 2010, G. Negrean [CL]. Jupânești, Ponorel Valley, near the Epuran Cave, in forest, 44°49'54.36"N, 22°34'13.89"E, alt. c. 430 m, August 4, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].
- Basidioradulum radula* (FR.) NOBLES, matrix:
- On wood – Cireșul E, Valea Țiganilor, in *Fagetum*, 44°49'34.564"N, 22°33'43.315"E, alt. C. 395 m, June 13, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].
- Boletus aereus* BULL., on soil – Jupânești, Ponorel Valley, near the Epuran Cave, in forest, 44°49'54.36"N, 22°34'13.89"E, alt. c. 430 m, August 4, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].
- Boletus edulis* BULL., on soil – Obârșia Cloșani NE, Vârtoapele Mountain, Fundul Lupșei, in *Quercetum petraeae*, 45°02'55"N, 22°43'00"E, alt. 800 m, July 28, 1986, G. Negrean [BUCM 100.193]. Motrul Sec, Lupșa Valley, in *Fagetum*, 45°03'16"N, 22°47'49"E, alt. 460 m, October 9, 1984, G. Negrean [BUCM 86.286]. The Motrul Sec NW, Sohodoale Valley, in *Fagetum*, 45°04'09"N, 22°45'39"E, alt. 480 m, June 18, 1987, G. Negrean [BUCM 103.686]. Bunoaica SE, Știubei Valley, in *Quercetum dalechampii*, 44°47'45.445"N, 22°33'22.603"E, alt. c. 460 m, June 13, 2010, G. Negrean. Bunoaica E, Topolniței Valley, in talweg, 44°47'52.994"N, 22°33'55.113"E, alt. c. 290 m, June 13, 2010, G. Negrean. Schitul Topolniței, Schitul de Sus, Topolnița River, 44°46'13.712"N, 22°35'26.559"E, alt. c. 440 m, June 10, 2011, Ioana Ciortan & G. Negrean.
- Boletus erythropus* PERS. var. *erythropus*, on soil – Obârșia Cloșani N, Poiana Mare, in *Fagetum*, 45°02'51.81"N, 22°39'46.48"E, alt. 1041 m, August 18, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Schitul Topolniței, Schitul de Sus, 44°46'06.180"N, 22°36'04.411"E, alt. c. 500 m, June 10, 2011, Ioana Ciortan & G. Negrean.

Boletus luridus SCHAEFF. var. *luridus*, on soil – Schitul Topolniței, Schitul de Sus, 44°46'06.180"N, 22°36'04.411"E, alt. c. 500 m, June 10, 2011, Ioana Ciortan & G. Negrean.

Boletus queletii SCHULZER, on soil – Cireșul E, above Pețimea River, in *Fagetum*, 44°49'25"N, 22°33'32"E, alt. c. 400 m, August 21, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Bunoaica E, above Topolnița Valley, under *Pinus* sp. cult., 44°48'03.26"N, 22°32'31.16"E, alt. c. 385 m, August 5, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Boletus reticulatus SCHAEFF., on soil – Cireșul W, in *Quercetum petraeae*, June 16, 2007, G. Negrean (10.187) [BUC].

Boletus rhodoxanthus (KROMBIL.) KALLENB., on soil – Mehedinți Mountains, Gornenți NW, ad pedem Ciolanul Mare, in *Fagetum*, 44°55'43.155"N, 22°31'00.264"E, alt. c. 900 m, August 19, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Mehedinți Mountains, Gornenți NW, Râpa Verde, in *Fagetum*, 44°55'36.30"N, 22°30'46.84"E, alt. c. 857 m, August 19, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Boletus satanas LENZ – Cireșu E, in *Quercetum dalechampii*, August 8, 2012, G. Negrean & Ioana Ciortan [CL; CRAF].

Boletus subtomentosus L., on soil – Obârșia Cloșani N, Poiana Mare, in *Fagetum*, 45°02'51.81"N, 22°39'46.48"E, alt. 1041 m, August 18, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Calocera cornea (BATSON) FR., matrix:

Fagus sylvatica L. subsp. *sylvatica* – Motrul Sec, Dealul Drumul Rujetului, in *Fagetum*, 45°03'13"N, 22°47'50"E, alt. 480 m, June 26, 1986, G. Negrean [BUCM 100.046]. Baia-de-Aramă N, Mănăstirii Hill, Cornetul Băii, in *Fagetum*, 45°00'25.70"N, 22°47'44.39"E, alt. 326 m, November 17, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Calocera viscosa (PERS.) FR., matrix:

On buried wood – Obârșia Cloșani NE, Vârtoapele Mountain, 45°03'02"N, 22°42'30"E, alt. 960 m, July 28, 1986, G. Negrean [BUCM 100.186]. Obârșia Cloșani N, Poiana Mică, in *Fagetum*, 45°03'30.11"N, 22°39'58.29"E, alt. 1070 m, August 18, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Cerrena unicolor (BULL.) MURRILL, on the decaying wood – Podeni NW, Muntele Camena, northern versant, in *Fagetum*, 44°54'43.444"N, 22°30'03.048"E, alt. 650 m, May 5, 2011, Ioana Ciortan & G. Negrean [CL; CRAI].

Chalciporus piperatus (BULL.) BATAILLE, on soil – Motrul Sec NW, Motrul Sec Valley, 45°04'25"N, 22°47'22"E, alt. 390 m, October 9, 1984, G. Negrean [BUCM 86.236]. Motrul Sec, Lupșa Valley, in *Fagetum*, 45°03'18"N, 22°47'49"E, alt. 450 m, October 9, 1984, G. Negrean [BUCM 86.285].

Chlorophyllum rhacodes (VITTAD.) VELLINGA, on soil – Obârșia Cloșani NE, Vârtoapele Mountain, in *Abieto-Fraxinetum*, 45°03'22"N, 22°42'30"E, alt. 960 m, July 28, 1986, G. Negrean [BUCM 100.415]. Marga, on the roadside, June 1, 2010, G. Negrean.

Clavaria fragilis HOLMSK., on dead branches – Gornenți NNW, in forest, above „Mlaștina cu *Menyanthes trifoliata*” 44°55'4308.88"N, 22°30'44.05"E, alt. c. 826 m, June 9, 2011, Ioana Ciortan & G. Negrean.

Clitocybe gibba (PERS.) P. KUMM. – Cireșul E, in forest, 44°49'20.26"N, 23°33'07.62"E, alt. c. 455 m, August 6, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Clavariadelphus pistillaris (L.) DONK., on soil – Podeni, October 10, 2010, photo Sorin Păunescu, det. Ioana Ciortan.

Coltricia perennis (L.) MURRILL, on soil – Motrul Sec, Dealul Drumul Rujetului, in *Fagetum*, 45°03'13"N, 22°47'50"E, alt. 480 m, July 26, 1986, G. Negrean [BUCM 100.049]. Gornenți NW, in *Fagetum*, 44°55'47.07"N, 22°30'59.86"E, alt. c. 919 m, June 9, 2011, Ioana Ciortan & G. Negrean.

Conocybe tenera (SCHAEFF.) FAYOD, on soil – Cireșul E, 44°49'20.26"N, 23°33'07.62"E, alt. c. 455 m, August 6, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Coprinellus domesticus (BOLTON) VILGALYS, HOPPLE & JACQ. JOHNSON, on soil – Cireșul NE, Polia Cireșul, in deciduous, 44°49'52.459"N, 22°33'03.293"E, alt. c. 390 m, June 12, 2011, Ioana Ciortan & G. Negrean. Jidoștița N, Plaiul Matorâului, in forest, *Pinus sylvestris* (plantation) & *Carpinus betulus*, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Coprinopsis picacea (BULL.) REDHEAD, VILGALYS & MONCALVO, on soil – Cireșul SW, Valea Bahnei, talveg, 44°48'41.836"N, 22°31'05.379"E, alt. c. 230 m, November 5, 2010, G. Negrean [CL].

Craterellus cornucopioides (L.) PERS., on soil – Motrul Sec S, Lupșa Valley, 11 X 1984, leg. Violeta Iavorschi, det. G. Negrean [BUCM 86.388].

Cyathus striatus (HUDS.) WILLD., on soil – Motrul Sec, Lupșa Valley, 45°03'18"N, 22°47'47"E, alt. 440 m, October 9, 1984, G. Negrean [BUCM 86.271].

Daedaleopsis confragosa (BOLTON) J. SCHRÖT., matrix:

Salix sp. – Păunești, Borovăț Valley, 19 IV 2007, G. Negrean [BUC].

On wood – Obârșia Cloșani N, Poiana Mică, in *Fagetum*, 45°03'30.11"N, 22°39'58.29"E, alt. 1070 m, August 18, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Inter Crăgăști et Bobaița, in *Fagetum*, November 3, 2010, G. Negrean. Jidoștița N, Plaiul Matorâului, in forest, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Jupânești, Ponorel Valley, nearly Epuran Cave, in forest, 44°49'54.36"N, 22°34'13.89"E, alt. c. 430 m, August 4, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Entoloma hirtipes (SCHUMACHER) M.M. MOSER on soil – Bunoaica E, above Topolnița Valley, under *Pinus* sp. cultivated, 44°48'03.26"N, 22°32'31.16"E, alt. c. 385 m, August 5, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Fomitopsis rosea (ALB. & SCHWEIN.) P. KARST., on wood – Obârșia Cloșani NE, Vârtoapele Mountain, in *Abieto-Fraxinetum*, 45°03'22"N, 22°42'30"E, alt. 960 m, July 28, 1986, G. Negrean [BUCM 100.171].

Fuscoporia ferrea (PERS.) G. CUNN., on deciduous trunks – Cireșul E, Țiganilor Valley, in *Fagetum*, 44°49'34.80"N, 22°33'46.19"E, alt. c. 418 m, November 14, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Galerina sphagnetorum (PERS.) KÜHNER, in *Sphagnetum* – Gornenți NNW, „Mlaștina cu *Menyanthes trifoliata*”, 44°55'26.48"N, 22°30'44.56"E, alt. c. 808 m, June 9, 2011, Ioana Ciortan & G. Negrean.

Ganoderma applanatum (PERS.) PAT., on cut trunks – Obârșia Cloșani N, Poiana Mare, in *Fagetum*, 45°02'51.81"N, 22°39'46.48"E, alt. 1041 m, August 18, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Baia de Aramă, 44°59'48.781"N, 22°48'56.735"E, alt. 370 m, June 2, 2010, G. Negrean. Podeni W, Mehedinți Mountains Ridge, in *Fagetum*, near the road, 44°51'10.42"N, 22°27'29.59"E, alt. c. 898 m, August 18, 2011, Ioana Ciortan & G. Negrean.

Ganoderma australe (FR.) PAT., on wood – Obârșia Cloșani N, Poiana Mare, above the “Mlaștina fără Fund”, in *Fagetum*, 45°02'53.137"N, 22°39'45.986"E, alt. c. 1020 m, July 18, 2011, Ioana Ciortan & G. Negrean.

Ganoderma lucidum (CURTIS) P. KARST., on roots – Jidoștița N, Plaiul Matorățului, in *Pinus sylvestris* et *Carpinus betulus* plantation, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Ganoderma resinaceum BOUD., on wood – Baia-de-Aramă N, Mănăstirii Hill, Cornetul Băii, in *Fagetum*, 45°00'14.15"N, 22°48'20.00"E, alt. 380 m, November 17, 2011, Ioana Ciortan & G. Negrean. Cireșu S, to Bunoaica, 44°48'54.08"N, 22°32'25.93"E, alt. c. 400 m, August 5, 2012, Ioana Ciortan & G. Negrean.

Gloeophyllum abietinum (BULL.) P. KARST., on wood – Gornenți NNW, above the „Mlaștina cu *Menyanthes trifoliata*”, in forest, 44°55'4308.88"N, 22°30'44.05"E, alt. c. 826 m, August 19, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Gloeoporus taxicola (PERS.) GILB. & RYVARDEN, matrix:

Pinus sylvestris L. (cultivated), fallen branches – Gornenți NNW, above the „Mlaștina cu *Menyanthes trifoliata*”, in forest, 44°55'4308.88"N, 22°30'44.05"E, alt. c. 826 m, August 19, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Gymnopus dryophilus (BULL.) MURRILL, on soil – Podeni NW, above Izbucul Camena, in *Fagetum*, 44°54'19.20"N, 22°29'56.49"E, alt. c. 716 m, May 5, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Podeni W, Motrul Sec W, Sohodoarele Mici Valley, in *Fagetum*, 45°04'08"N, 22°45'39"E, alt. 520 m, July 25 1986, G. Negrean [BUCM 100.032]. Jupânești, Valea Ponorel, near the Epuran Cave, in forest, 44°49'54.36"N, 22°34'13.89"E, alt. c. 430 m, August 4, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Gomphidius maculatus (SCOP.) FR., on soil – Obârșia Cloșani N, Poiana Mică, in *Fagetum*, 45°03'30.11"N, 22°39'58.29"E, alt. 1070 m, August 18, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Gyromitra esculenta (PERS.) FR., on soil – Podeni NW, under Izbucul Camena, in *Fagetum*, 44°54'19.20"N, 22°29'56.49"E, alt. c. 716 m, May 5, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Gyroporus castaneus (BULL.) QUÉL., on soil – Obârșia Cloșani N, Poiana Mare, near „Mlaștina fără Fund”, in *Fagetum*, 45°02'53.137"N, 22°39'45.986"E, alt. c. 1020 m, July 17, 2011, Ioana Ciortan & G. Negrean. Podeni NW, under Izbucul Camena, in *Fagetum*, 44°54'19.20"N, 22°29'56.49"E, alt. c. 716 m, May 5, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Gyroporus cyanescens (BULL.) QUÉL. (*Boletus cyanescens* Bull.), on soil – Obârșia Cloșani N, Vârtoapele Mountain, in *Abieto-Fraxinetum*, 45°03'22"N, 22°42'32"E, alt. 940 m, July 28, 1986, G. Negrean [BUCM 100.200].

Hapalopilus nidulans (FR.) P. KARST., on wood – Schitul Topolniței, Schitul de Sus, 44°46'06,180"N, 22°36'04,411"E, alt. c. 500 m, June 10, 2011, Ioana Ciortan & G. Negrean.

Helvella elastica BULL., on soil – Podeni NW, under Izbucul Camenei, in *Fagetum*, 44°54'19.20"N, 22°29'56.49"E, alt. c. 716 m, May 5, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Jidoștița N, Plaiul Matorățului, in forest, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL].

Hemipholiota populnea (PERS.) BON, matrix:

Populus nigra L. – between Crăgăști and Bobaița, in *Fagetum*, November 3, 2010, G. Negrean.

Populus sp. cult. – Bahna Valley ut Cireșul, on the roadside, November 16, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Cireșu S, ut Bunoaica, 44°48'54.08"N, 22°32'25.93"E, alt. c. 400 m, August 5, 2012, Ioana Ciortan & G. Negrean.

Herichium coralloides (SCOP.) PERS., on wood – Obârșia Cloșani N, Poiana Mare, prope “Mlaștina fără Fund”, in *Fagetum*, 45°02'53.137"N, 22°39'45.986"E, alt. c. 1020 m, August 18, 2011, Ioana Ciortan & G. Negrean. Schitul Topolniței, Schitul de Sus, 44°46'06,180"N, 22°36'04,411"E, alt. c. 500 m, June 10, 2011, Ioana Ciortan & G. Negrean.

Herichium erinaceus (BULL.) PERS., on wood – Baia-de-Aramă N, Mănăstirii Hill, Cornetul Băii, in *Fagetum*, 45°00'25.70"N, 22°47'44.39"E, alt. 326 m, November 17, 2011, Ioana Ciortan & G. Negrean [CL].

Hygrocybe coccineocrenata (P.D. ORTON) M.M. MOSER, in *Sphagnetum* – Gornenți NNW, „Mlaștina cu *Menyanthes trifoliata*”, 44°55'26.48"N, 22°30'44.56"E, alt. c. 808 m, June 9, 2011, Ioana Ciortan & G. Negrean.

Hygrocybe miniata (FR.) P. KUMM., on soil – Gornenți WNW, La Funduri, serpentines, 44°55'48,888"N, 22°30'57,732"E, alt. c. 900 m, June 9, 2011, Ioana Ciortan & G. Negrean.

Hygrocybe psittacina (SCHAEFF.) P. KUMM. var. *psittacina*, on soil – Motrul Sec NW, Motrul Sec Valley, the meadow, 45°04'26"N, 22°45'12"E, alt. 380 m, October 9, 1984, G. Negrean [BUCM 86.250].

Hygrophorus eburneus (BULL.) FR., on soil – Dălbocița NNW, Firizu N, Glaceul Mare Valley, in *Fagetum*, 44°51'14.635"N, 22°43'32.510"E, alt. c. 360 m, November 2, 2010, G. Negrean [CL].

- Hymenochaete rubiginosa* (DICKS.) LÉV., on wood – Jidoștița N, Plaiul Matorâțului, in forest, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL: CRAF]. Cireșul E, Țiganilor Valley, in *Fagetum*, 44°49'34.564"N, 22°33'43.315"E, alt. c. 395 m, August 3, 2012, Ioana Ciortan & G. Negrean [CL: CRAF].
- Hypholoma capnoides* (FR.) P. KUMM. on wood – Motrul Sec NW, Motrul Sec Valley, in *Alno-Padion*, 45°04'25"N, 22°45'22"E, alt. 390 m, October 9, 1984, G. Negrean [BUCM 86.256].
- Hypholoma lateritium* (SCHAEFF.) P. KUMM., on wood-rotting – Obârșia Cloșani N, Poiana Mare, prope „Mlaștina fără Fund”, in *Fagetum*, 45°02'53.137"N, 22°39'45.986"E, alt. c. 1020 m, August 18, 2011, Ioana Ciortan & G. Negrean.
- Hypoxylon howeanum* PECK, on trunks of deciduous – Cireșul NE, Polia Cireșul, in forest, 44°49'52.459"N, 22°33'03.293"E, alt. c. 390 m, June 12, 2011, Ioana Ciortan & G. Negrean.
- Inonotus hispidus* (BULL.) P. KARST., on wood – Dâlbocița NNW, Firizu N, Valea Glaceul Mare, in *Fagetum*, 44°51'14.635"N, 22°43'32.510"E, alt. c. 360 m, November 2, 2010, G. Negrean [CL]. Schitul Topolniței, Schitul de Sus, Cracul cu Drum Hill, gnaiss, in forest, 44°45'55.115"N, 22°34'25.855"E, alt. c. 420 m, June 10, 2011, Ioana Ciortan & G. Negrean. Cireșul E, Pețimea Valley, August 3, 2012, Ioana Ciortan & G. Negrean [CL: CRAF]. Cireșul E, Țiganilor Valley, in *Fagetum*, 44°49'34.564"N, 22°33'43.315"E, alt. c. 395 m, August 3, 2012, Ioana Ciortan & G. Negrean [CL: CRAF].
- Laetiporus sulphureus* (BULL.) MURRILL, matrix:
- Alnus glutinosa* (L.) GAERTNER – Seliște, ut Măguricea, May 30, 2010, G. Negrean. Cireșul NNE, Pețimea Valley, in herbosis, 44°50'18.124"N, 22°33'09.541"E, alt. c. 390 m, June 12, 2011, Ioana Ciortan & G. Negrean [CL: CRAF].
- Fagus sylvatica* L. subsp. *sylvatica* – Giurgiani, July 23, 2007, G. Negrean.
- Malus domestica* BORKH. – Isverna, 44°58'47.307"N, 22°37'17.679"E, alt. 460 m, May 13, 2009, G. Negrean.
- Populus canadensis* MOENCH – Seliște, Valea Verde, near school, May 16, 2010, G. Negrean.
- Populus* sp. – Isverna SW, Coșuștea Valley, in *Alnetum glutinosae*, August 23, 2007, G. Negrean (10.080) [BUC].
- Prunus cerasifera* EHRH. – Gheorghești, ad marginem W, 44°58'17.776"N, 22°42'48.028"E, alt. 460 m, September 16, 2009, G. Negrean.
- Prunus avium* L. – Șiroca S, 44°49'26.014"N, 22°36'23.512"E, alt. 650 m, September 19, 2009, G. Negrean. Marga, near the church, 44°48'45.493"N, 22°34'23.920"E, alt. c. 530 m, June 11, 2011, Ioana Ciortan & G. Negrean [CL: CRAF].
- Leccinum aurantiacum* (BULL.) GRAY, on soil – Jidoștița N, Plaiul Matorâțului, in forest, September 22, 2010; photo Sorin Păunescu det. Ioana Ciortan.
- Leccinum pseudoscabrum* (KALLENB.) ȘUTARA, on soil, under *Carpinus betulus* – Jidoștița N, Plaiul Matorâțului, in *Carpinetum*, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL: CRAF].
- Leccinum scabrum* (BULL.) GRAY, on soil – under *Populus tremula*, Motrul Sec S, Drumul Rujetului Hill, in *Fagetum*, 45°03'13"N, 22°47'50"E, alt. 480 m, July 26, 1986, G. Negrean [BUCM 100.080]. Ponoarele, in *Fagetum*, fam. Eliodor Popescu, July 4, 2007, G. Negrean. In *Fagetum* with *Aremonia agrimonoides*, Giurgiani E – ‘La Polom’, 44°57'409"N, 22°34'304"E, alt. c. 496 m, August 23, 2007, G. Negrean [BUC]. Cireșul E, Țiganilor Valley, in *Fagetum*, 44°49'34.564"N, 22°33'43.315"E, alt. c. 395 m, June 13, 2011, Ioana Ciortan & G. Negrean [CL: CRAF].
- Leccinum versipelle* (FR. & HÖK) SNELL, on soil – Motrul Sec, Lupșa Valley, in *Fagetum*, 45°03'16"N, 22°47'49"E, alt. 465 m, 9 X 1984, G. Negrean [BUCM 86.287]. Motrul Sec W, Gorganul Mountain, northern slope, Plopilor Valley, under *Betula pendula*, 45°05'21"N, 22°42'43"E, alt. 700 m, July 28, 1986, G. Negrean [BUCM 100.211]. Cireșul NE, Topolnița Valley, thalweg, 44°50'06.183"N, 22°35'22.164"E, alt. c. 395 m, June 12, 2010, G. Negrean.
- Lentinellus ursinus* (FR.) KÜHNER, on wood – Schitul Topolniței, Schitul de Sus, 44°46'06.180"N, 22°36'04.411"E, alt. c. 500 m, June 10, 2011, Ioana Ciortan & G. Negrean.
- Lentinus strigosus* FR., matrix:
- Fagus sylvatica* L. subsp. *sylvatica* – Motrul Sec S, Lupșa Valley, 45°03'23"N, 22°47'50"E, alt. 400 m, May 13, 1984, G. Negrean [BUCM 82.333]. Motrul Sec W, 45°03'34"N, 22°45'50"E, alt. 600 m, July 28, 1986, G. Negrean [BUCM 100.114]. Mehedinți Mountains, Colțul Pietrei Mountain, 44°53'43"N, 22°29'01"E, alt. 980 m, November 4, 1986, G. Negrean [BUCM 102.029]. Cireșu E, in *Quercetum dalechampii*, August 8, 2012, Ioana Ciortan & G. Negrean.
- Lycoperdon echinatum* PERS., on soil – Jidoștița N, Plaiul Matorâțului, in forest, *Pinus sylvestris* (plantation) & *Carpinus betulus*, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL: CRAF].
- Macrolepiota excoriata* (SCHAEFF.) WASSER, on soil – Mehedinți Mountains: Motrul Sec, Motrișorul (Lupșa) Valley, 45°03'22"N, 22°47'46"E, alt. 400 m, November 28, 1983, G. Negrean [BUCM 80.090]. Cireșul E, in meadow, 44°49'20.26"N, 22°33'07.62"E, alt. c. 455 m, August 3, 2012, Ioana Ciortan & G. Negrean [CL: CRAF]. Gornenți WNW, at the foothills Montes Ciolanul Mare, La Funduri, in meadow, 44°55'30.35"N, 22°30'34.39"E, alt. c. 829 m, October 26, 2012, Ioana Ciortan & G. Negrean.
- Marasmiellus ramealis* (BULL.) SINGER, on wood – Bunoaica E, supra vallis Topolnița, under *Pinus* sp. cult., 44°48'03.26"N, 22°32'31.16"E, alt. c. 385 m, August 5, 2012, Ioana Ciortan & G. Negrean [CL: CRAF].
- Marasmiellus tricolor* (ALB. & SCHWEIN.) SINGER – on soil, Cireșul E, Pețimea Valley, in forest, 44°49'16.96"N, 23°33'20.79"E, alt. c. 417 m, August, 2012, Ioana Ciortan & G. Negrean [CL: CRAF].
- Marasmius rotula* (SCOP.) FR. – on the branches in the putrefaction state – Jupânești NNW, in *Fagetum*, 44°50'34.20"N, 23°34'16.39"E, alt. c. 485 m, August 4, 2012, Ioana Ciortan & G. Negrean [CL: CRAF].

Meripilus giganteus (PERS.) P. KARST., on wood – Obârșia Cloșani N, Poiana Mare, prope „Mlaștina fără Fund”, in *Fagetum*, 45°02'53.137"N, 22°39'45.986"E, alt. c. 1020 m, August 18, 2011, Ioana Ciortan & G. Negrean, Podeni W, Mountain ridge Mehedinți, „La Sulița”, in *Fagetum*, 44°51'58.573"N, 22°28'17.124"E, alt. 845 m, August 24, 2010, G. Negrean [CL; CRAF]. Mehedinți Mountains: Mountain ridge Mehedinți, in *Fagetum* with *Galium rotundifolium*, 44°51'58.573"N, 22°28'17.124"E, alt. 845 m, August 22, 2010, Ioana Ciortan & G. Negrean [CL; CRAF].

Merulius tremellosus SCHRAD., on wood – the Coșuștei hydrographical basin, Ișfani, in *Fagetum*, 44°56'54.664"N, 22°32'27.909"E, alt. 900 m, July 14, 2011, G. Negrean & Ioana Ciortan [CL; CRAF].

Morchella esculenta (L.) PERS., on soil – Podeni NW, Camena Mountain, Izbucul Camenei, in *Fagetum*, 44°54'19.131"N, 22°29'59.392"E, alt. c. 660 m, May 59, 2011, Ioana Ciortan & G. Negrean [CL].

Mutinus caninus (HUDS.) FR., on soil – Jidoștița N, Plaiul Matorățului, in forest, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [BUC; CL; CRAF].

Nemania serpens (PERS.) GRAY var. *serpens*, on wood – Podeni NW, under Izbucul Camena, in *Fagetum*, 44°54'19.20"N, 22°29'56.49"E, alt. c. 716 m, May 5, 2011, Ioana Ciortan & G. Negrean.

Panellus stipticus (BULL.) P. KARST., matrix:

Alnus glutinosa GAERTNER – Seliște S. Lunca Coșuștei, May 16, 2010, G. Negrean.

Fagus sylvatica L. subsp. *sylvatica* – Motrul Sec W, Sohodoarele Mici, 45°04'08"N, 22°45'38"E, alt. 470 m, July 25, 1986, G. Negrean [BUCM 100.052]. Baia-de-Aramă N, Cornetul Mănăstirii, in *Fagetum*, November 17, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Fagus sylvatica L. (cut trunk) – Godeanu NW, Șaua Mazdroane, November 3, 2010, G. Negrean [CL].

On wood – Obârșia Cloșani NE, Vârtoapele Mountain, in *Abieto-Fraxinetum*, 45°03'22"N, 22°42'30"E, alt. 960 m, July 28, 1986, G. Negrean [BUCM 100.169]. Baia-de-Aramă N, Mănăstirii Hill, Cornetul Băii, in *Fagetum*, 45°00'25.70"N, 22°47'44.39"E, alt. 326 m, November 17, 2011, Ioana Ciortan & G. Negrean [CL].

Panaeolus papilionaceus (BULL.) QUÉL. var. *papilionaceus* – on animal excrements – Jupânești NW, in forest, 44°50'34.20"N, 23°34'16.39"E, alt. c. 485 m, August 4, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Panus conchatus (BULL.) FR., on wood – Schitul Topolniței, Schitul de Sus, „Cracul cu Drum” Hill, gneiss, 44°45'55.115"N, 22°34'25.855"E, alt. c. 420 m, June 10, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Cireșul E, Pețimea Valley, in forest, 44°49'16.96"N, 23°33'20.79"E, alt. c. 417 m, August 3, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Paxillus filamentosus FR., on soil – the Motrul Sec W, Valea Capra, in *Alnetum*, 45°05'38"N, 22°43'17"E, alt. 490 m, July 28, 1986, G. Negrean [BUCM 100.222]. Cireșul E, Topolnița Valley, in *Fagetum*, 44°49'05.14"N, 22°34'03.83"E, alt. c. 386 m, June 13, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Paxillus involutus (BATSCH) FR., on soil – Bunoaica E, Topolniței Valley, in thalweg, 44°47'52.994"N, 22°33'55.113"E, alt. c. 290 m, June 13, 2010, G. Negrean.

Phallus impudicus L. var. *impudicus*, on soil – Cireșul South-Wasth, Bahna Valley, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, G. Negrean (photo S. Păunescu). Jidoștița North, Plaiul Matorățului, in forest, *Pinus sylvestris* (plantation) & *Carpinus betulus*, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean.

Phellinus conchatus (PERS.) QUÉL., on wood – Cireșul NE, Polia Cireșul, 44°49'52.459"N, 22°33'03.293"E, alt. c. 390 m, June 12, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Phellinus igniarius (L.) QUÉL., matrix:

Salix sp., Cireșu S, ut Bunoaica, 44°48'54.08"N, 22°32'25.93"E, alt. c. 400 m, August 5, 2012, Ioana Ciortan & G. Negrean.

Phellinus pomaceus (Pers.) Maire, matrix:

Prunus cerasifera – Isverna SW, Cănișa, 44°58'21.077"N, 22°37'00.944"E, alt. 445 m, September 10, 2009, G. Negrean.

Pholiota tuberculosa (SCHAEFF.) P. KUMM., on wood – Jidoștița N, Plaiul Matorățului, in forest, *Pinus sylvestris* (plantation) and *Carpinus betulus*, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Phylloporus pelletieri (LÉV.) QUÉLET, on soil – Jidoștița, Plaiul Matorățului, November 25, 2010, comm. and photo S. Păunescu, det. Ioana Ciortan.

Pleurotus pulmonarius (FR.) QUÉL., on deciduous trunks – Podeni W, Mountains ridge Mehedinți, August 17, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Polyporus squamosus (HUDS.) FR., matrix:

Juglans regia L. – Dolina Poiana Crovului, August 25, 2007, G. Negrean.

Fagus sylvatica L. subsp. *sylvatica* – Motrul Sec, Sohodoale Valley, July 3, 2004, G. Negrean [HGN], Motrul Sec, Lupșa Valley, 45°03'23"N, 22°47'50"E, alt. 400 m, May 13, 1984, G. Negrean [BUCM 82.338]. Mehedinți Mountains, Camena Mountain, southern slope 44°53'52.320"N, 22°29'28.051"E, alt. c. 920 m, June 15, 2010, G. Negrean [CL]. Jupânești, Ponorel Valley, above Epuran Cave, in forest, 44°49'54.36"N, 22°34'13.89"E, alt. c. 430 m, August 4, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Pseudochaete tabacina (SOWERBY) T. WAGNER & M. FISCH., on wood – Baia-de-Aramă N, Dealul Mănăstirii, Cornetul Băii, in *Fagetum*, 45°00'14.15"N, 22°48'20.00"E, alt. 380 m, November 17, 2011, Ioana Ciortan & G. Negrean.

Pycnoporus cinnabarinus (JACQ.) P. KARST., matrix:

Prunus avium L. – Obârșia Cloșani N, Poiana Mică, in *Fagetum*, 45°03'30.11"N, 22°39'58.29"E, alt. 1070 m, August 18, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Isverna N, 44°59'22.822"N, 22°37'22.019"E, alt. c. 640 m, November 13, 2009, G. Negrean. Isverna SW, Coșuștea Valley, Giurgiani W, above Piatra Pinilor, August 24, 2007, G. Negrean (10.082) [BUC]. Gornenți NNW, near the *Menyanthes trifoliata* lake, in forest, 44°55'4308.88"N, 22°30'44.05"E, alt. c. 826 m, August 19, 2011, Ioana Ciortan & G. Negrean.

Pyronema omphalodes (BULL.) FÜCKEL, on a hearth of fire – Cireșul NNW, Pețimea Valley, 44°50'18.124"N, 22°33'09.541"E, alt. c. 390 m, June 12, 2011, Ioana Ciortan & G. Negrean.

Ramaria formosa (PERS.) QUEL., on soil – Podeni NW, small village Camena, 44°54'38.05"N, 22°30'20.29"E, alt. c. 645 m, June 9, 2011, Ioana Ciortan & G. Negrean.

Russula emetica (SCHAEFF.) PERS., on soil – Obârșia Cloșani N, Poiana Mare, above „Mlaștina fără Fund”, in *Fagetum*, 45°02'53.137"N, 22°39'45.986"E, alt. c. 1020 m, August 18, 2011, Ioana Ciortan & G. Negrean.

Russula farinipes ROMELL., on soil – Bazinul Coșuștei, Ișfani, in *Fagetum*, 44°56'54.664"N, 22°32'27.909"E, alt. 900 m, July 14, 2011, G. Negrean & Ioana Ciortan [CL; CRAF].

Russula fragrans ROMAGN., on soil – Jidoștița N, Plaiul Matorățului, in forest, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Russula maculata QUEL. & ROZE, on soil – Bazinul Coșuștei, Ișfani, in *Fagetum*, 44°56'54.664"N, 22°32'27.909"E, alt. 900 m, July 14, 2011, G. Negrean & Ioana Ciortan [CL; CRAF].

Russula olivacea (SCHAEFF.) FR., on soil – Gornenți NNW, ad pedem Montes Ciolanul Mare, in *Fagetum*, 44°55'46.75"N, 22°30'59.97"E, alt. c. 920 m, August 19, 2011, Ioana Ciortan & G. Negrean.

Sarcoscypha coccinea (JACQ.) BOUD., on the fallen branches – in *Fagetum*, Coșuștea Valley 44°57'09.445"N, 22°32'37.839"E, alt. c. 700 m, April 4, 2010, leg. Virgil Marinescu, det. G. Negrean.

Steccherinum ochraceum (PERS.) GRAY, on the fallen branches – Cireșul E, Țiganilor Valley, in *Fagetum*, 44°49'34.564"N, 22°33'43.315"E, alt. c. 395 m, June 13, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Suillus granulatus (L.) ROUSSEL on soil – Bunoaica E, above Topolnița Valley, under *Pinus* sp. cult., 44°48'03.26"N, 22°32'31.16"E, alt. c. 385 m, August 5, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Suillus grevillei (KLOTZSCH) SINGER, on soil – Obârșia Cloșani N, Poiana Mică, in *Fagetum*, 45°03'30.11"N, 22°39'58.29"E, alt. 1070 m, August 18, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Obârșia Cloșani N, Poiana Mare, above „Mlaștina fără Fund”, in *Fagetum*, 45°02'53.137"N, 22°39'45.986"E, alt. c. 1020 m, August 18, 2011, Ioana Ciortan & G. Negrean.

Tarzetta catinus (HOLMSK.) KORE & J.K. ROGERS, on soil – Podeni NW, small village Camena, 44°54'38.05"N, 22°30'20.29"E, alt. c. 645 m, May 5, 2011, Ioana Ciortan & G. Negrean.

Tephrocye palustris (PECK) DONK, in *Sphagnetum* – Gornenți NNW, „Mlaștina cu *Menyanthes trifoliata*”, 44°55'26.48"N, 22°30'44.56"E, alt. c. 808 m, August 19, 2011, Ioana Ciortan & G. Negrean.

Trametes ochracea (PERS.) GILB. & RYVARDEN, on wood – Gornenți NNW, under Montes Ciolanul Mare, in *Fagetum*, 44°55'46.75"N, 22°30'59.97"E, alt. c. 920 m, August 19, 2011, Ioana Ciortan & G. Negrean.

Trametes trogii BERK., matrix:

Salix alba L. – Cireșul S, Bahna Valley, in *Alnetum glutinosae*, 44°48'29.25"N, 22°30'50.38"E, alt. c. 244 m, August 20, 2011, Ioana Ciortan & G. Negrean.

Trichaptum bifforme (FR.) RYVARDEN, on trunk deciduous – Podeni W, Mountains ridge Mehedinți, August 17, 2011, Ioana Ciortan & G. Negrean [CL; CRAF]. Jidoștița N, Plaiul Matorățului, in forest, 44°44'56.743"N, 22°32'51.614"E, alt. c. 560 m, June 9, 2011, Ioana Ciortan & G. Negrean [CL; CRAF], idem, June 17, 2011, Ioana Ciortan & G. Negrean. Jupânești, Ponorel Valley, near Epuran Cave, in forest, 44°49'54.36"N, 22°34'13.89"E, alt. c. 430 m, August 4, 2012, Ioana Ciortan & G. Negrean [CL; CRAF].

Tricholoma terreum (SCHAEFF.) P. KUMM., on soil – Cireșul E, under spelunca Topolnița Cave, in forest, 44°49'07.953"N, 22°33'54.366"E, alt. c. 380 m, June 12, 2011, Ioana Ciortan & G. Negrean [CL].

Tylopilus felleus (BULL.) P. KARST., on soil – Obârșia Cloșani N, Poiana Mare, near „Mlaștina fără Fund”, in *Fagetum*, 45°02'53.137"N, 22°39'45.986"E, alt. c. 1020 m, August 18, 2011, Ioana Ciortan & G. Negrean. Cireșul E, Țiganilor Valley, in *Fagetum*, 44°49'34.564"N, 22°33'43.315"E, alt. c. 395 m, June 13, 2011, Ioana Ciortan & G. Negrean [CL; CRAF].

Volvariella bombycina (SCHAEFF.) SINGER, matrix:

Fagus sylvatica L. subsp. *sylvatica* – Isverna, Geant (Potcoava), under the left slope, 44°59'19.235"N, 22°36'48.146"E, alt. c. 750 m, November 13, 2009, G. Negrean [CL].

On the rotten wood – Motrul Sec S, Lupșa Valley, 45°03'20"N, 22°47'50"E, alt. 460 m, July 17, 1985, G. Negrean [BUCM 89.388]. Cireșul E, Topolnița Cave, Pețimea fosilă Valley, 44°48'52.666"N, 22°33'35.915"E, alt. c. 340 m, August 21, 2011, Ioana Ciortan & G. Negrean.

Xerocomellus rubellus (KROMBH.) ȘUTARA, on soil – Coșuștea Valley (right), Ișfani SE, in *Fagetum*, 44°57'50"N, 22°34'28"E, alt. 685 m, July 13, 2011, Ioana Ciortan & G. Negrean.

Xerula pudens (PERS.) SINGER, on soil – Obârșia Cloșani NE, Vârtoapele Mountain, in *Abieto-Fraxinetum*, 45°03'22"N, 22°42'30"E, alt. 960 m, July 28, 1986, G. Negrean [BUCM 100.201]. Obârșia Cloșani N, Poiana Mică, in *Fagetum*, 45°03'30.11"N, 22°39'58.29"E, alt. 1070 m, August 18, 2011, Ioana Ciortan & G. Negrean.

Xylaria filiformis (ALB. & SCHWEIN.) FR., matrix:

Quercus spp. – Bahna Valley, 44°43'30" 22°28' N, 17"E, alt. 120 m, November 18, 1987, G. Negrean [BUCM 107.048].

Xylaria longipes NITSCHKE, on wood – Obârșia Cloșani NE, Vârtoapele Mountain, in *Fagetum*, 45°03'02"N, 22°42'30"E, alt. 960 m, July 28, 1986, G. Negrean, rev. Françoise Candousseau, 1997 [BUCM 100.157; BUCM 100.164]. Țiganilor Valley, in *Fagetum*, 44°49'36.45"N, 22°33'44.17"E, alt. c. 420 m, August 3, 2012, Ioana Ciortan, G. Negrean [BUC; CL; CRAF].

In the researched territory we observed a growth of the number of species from South to North, in direct relation with the altitude. From 122 macromycetes species 50 is lignicolous species.

The information from this note, the first of this type from the Geopark Platoul Mehedinți., to be supplemented with data gained from the future movements in the territory.

CONCLUSIONS

In this first note 122 species of macromycetes were listed. With only one exception (*Collybia longipes*), all the listed species are new to the Geopark Platoul Mehedinți.

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We thank to the staff of the Geopark Platoul Mehedinți. (Mr. Meilescu Cornel, Mrs. Monica Păunescu, Mr. Păunescu Sorin, Mr. Isverceanu Emilian and Mr. Chiliban Marius) for the support they offered in field activity and beyond.

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CELLULAR ASPECTS OF ROOT COLONIZATION BY ANTAGONISTIC BACTERIA AND PHYTOPATHOGENIC FUNGI

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BREZEANU Aurelia, CORNEA Călina Petruța

Abstract. In this study, the capacity of root colonization and plant defense activation by some *Pseudomonas aeruginosa* and *Bacillus licheniformis* strains against *Pythium debaryanum* HESSE was evaluated. There were tested a series of experimental variants in which *Cucumis sativus* L. (Wisconsin SMR58 cultivar) plantlets were pre-treated with bacterial suspensions of P7, P14, P18 (*P. aeruginosa*) and B40 (*B. licheniformis*) strains, and a series in which pre-bacterized plants were infected with *P. debaryanum* HESSE. The interactions at the site of infection, was observed by light and electron microscopy. The results showed that the bacterial strains colonized intercellular spaces of cucumber root and hypocotyl. The colonization was correlated with defense-associated reactions in cucumber, like formation of cell wall appositions, also known as papillae, and obturation of intercellular areas.

Keywords: antagonist-phytopathogen-plant interaction, root colonization, light and electron microscopy.

Rezumat. Aspecte celulare ale colonizării rădăcinii plantelor cu bacterii antagoniste și fungi fitopatogeni. În acest studiu, a fost evaluată capacitatea de colonizare a rădăcinii de unele tulpini de *Pseudomonas aeruginosa* și *Bacillus licheniformis* cu activitate inhibitorie asupra unei game largi de fitopatogeni. Au fost testate o serie de variante experimentale, în care plantule de *Cucumis sativus* L. (soiul Wisconsin SMR58) au fost pre-tratate cu suspensii bacteriene ale P7, P14, P18 (*P. aeruginosa*) și B40 (*B. licheniformis*) tulpini, precum și o serie în care plantele tratate cu suspensii bacteriene au fost infectate cu *P. debaryanum* HESSE. Interacțiunile de la site-ul de infecție au fost observate prin microscopie optică și electronică. Rezultatele au arătat că tulpinile bacteriene au colonizat spațiile intercelulare ale rădăcinilor și hipocotilului de castravete. Colonizarea a fost corelată cu reacții de apărare, cum ar fi formarea de apoziții la nivelul peretelui celular și obturarea spațiilor intercelulare.

Cuvinte cheie: interacțiune pathogen-antagonist-plantă, colonizarea rădăcinii, microscopie optică și electronică.

INTRODUCTION

Successful control of soil phytopathogens using microorganisms presumes several characteristics of the biocontrol agent, like rhizosphere competence, antagonistic activity, plant growth enhancement and activation of plant defense mechanisms. Root colonization represents the primary step in almost all types of interaction between plants and soil microorganisms. Previous studies have shown a nonuniform distribution of bacteria on the root, in case of *Pseudomonas* the junctions between epidermal root cells, indented parts of the epidermal surface, or sites of root appearance, being the most populated (LUGTENBERG et al., 2001). Among the traits involved in efficient root colonization, bacterial motility and attachment to the roots are definitory (ZHENG & SINCLAIR, 2000; LUGTENBERG & BLOEMBERG, 2004). Thus, *Pseudomonas* is considered one of the best root colonizers, being used as a model of root colonizer (LUGTENBERG et al., 2001).

There are various plant responses that appear during plant-microbe interactions, like rapid generation of reactive oxygen species, synthesis of antimicrobial compounds, pathogenesis-related proteins, and phytoalexins, but also structural changes, like reinforcement of the cell walls and formation of cytoplasmic aggregates (SCHMIDT & PANSTRUGA, 2008). Defense mechanisms induced by microorganisms can be observed locally, at the ingression site of microorganism, but also systemically, in untreated plant parts. Locally, defense reactions like strengthening the cell walls through cell wall appositions, occlusion of intercellular spaces or formation of multivesicular bodies were previously described (BENHAMOU et al., 2002; AN et al., 2006). In this study, we have focused on plant cell structural modifications in plant-microbe interaction using light and electron microscopy techniques. Among the bacterial strains used in experiments, pseudomonads are new Romanian isolates from oil - polluted areas (CORNEA et al., 2006).

MATERIAL AND METHODS

Biological material used

The bacterial strains used in this study were *Pseudomonas aeruginosa* (strains P7, P14, P18) and *Bacillus licheniformis* (B40) and were provided by the Faculty of Biotechnology, USAVM, (Bucharest, Romania). As fungal pathogen, we used *Pythium debaryanum*, obtained from the Institute of Plant Protection (Bucharest, Romania). Cucumber plants were obtained by germination of cucumber seeds (Wisconsin SMR58 cultivar).

Plant treatment with antagonistic bacteria and fungal pathogen

Cucumber seeds were sterilized for one hour in 1% sodium hypochlorite solution and then washed five times with sterile distilled water. The bacterial strains were grown for 4 days on CPM medium (1% mannitol, 0.1% casamino acids, 1% peptone, 0.5% calcium chloride, pH 7) at 28°C. Cucumber seeds were immersed for 12 hours in bacterial cultures and then germinated in sterile conditions. Subsequently, the seeds were grown in sterile perlite supplemented with Knop nutritive

solution, for two weeks. The fungal pathogen *P. debaryanum* was grown for seven days on PDA medium at 28°C. For the infection of plantlets, slices of 5 mm PDA medium with grown mycelium were placed at the basis of the plant, as near as possible to the plant root. After five days, the roots of treated plants were analysed by light and electron microscopy.

Squash analysis

Plant rootlets were used for squash preparations, which were visualized by phase contrast microscopy or contrasted with 4 % (w/v) methylene-blue solution prior to examination using an MC 1 light microscope.

Electron microscopy

Five days after fungal contamination plant roots were processed using **the method of Mascorro and Bozzola (2007)**. Samples were subjected to a pre-fixation in a solution of 3% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2 at 4°C, overnight, and rinsed with the same buffer. The biological material was washed several times with 0.05 M sodium cacodylate buffer, for 2-3 hours and then fixed in 1% (w/v) osmium tetroxide solution in the same buffer, at 4°C, overnight. After washing for 2 h with distilled water, the samples were dehydrated in a graded series of 10-100% (v/v) ethanol. The samples were washed twice with propylene oxide and finally embedded in Epon 812 resin.

The samples were ultrasectioned at ultramicrotome (LKB, Sweden) with diamond knife and ultrathin sections were stained according to Reynold's double coloration (REYNOLDS, 1963), before examination with an EM-125 (Selemi, Ukraine) transmission electron microscope at 50 kV. For light microscopy semithin sections 1–2 µm thick were stained with a solution of 1% toluidine blue in 1% borax (PICKETT-HEAPS, 1966).

RESULTS AND DISCUSSIONS

Squash analyses showed the presence of fungal mycelium on plant roots in variants subjected to pathogen infection (Fig. 1a). On plant roots treated with both antagonistic bacteria and phytopathogenic fungi hyphae were not observed (Figs. 1b,c).

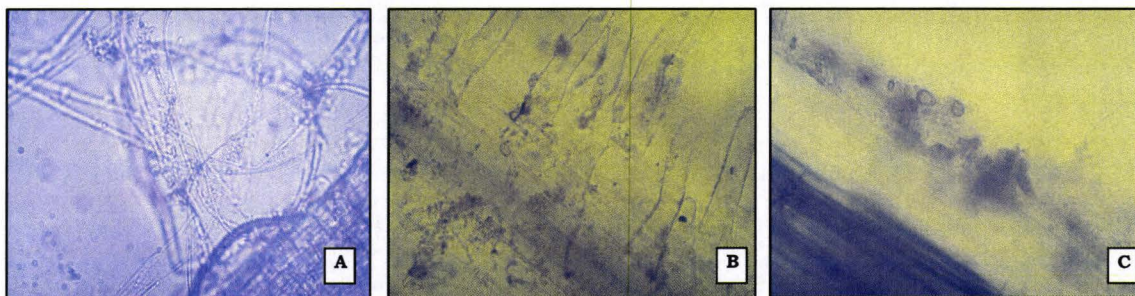


Figure 1. Microscopic aspects of squash prepares of plants treated with fungal pathogen (A) and plants treated with both antagonistic bacteria and fungal pathogen (B- P14-Py; C – B40-Py). Direct magnification 100X (A), 400X (B) and 200X (C).

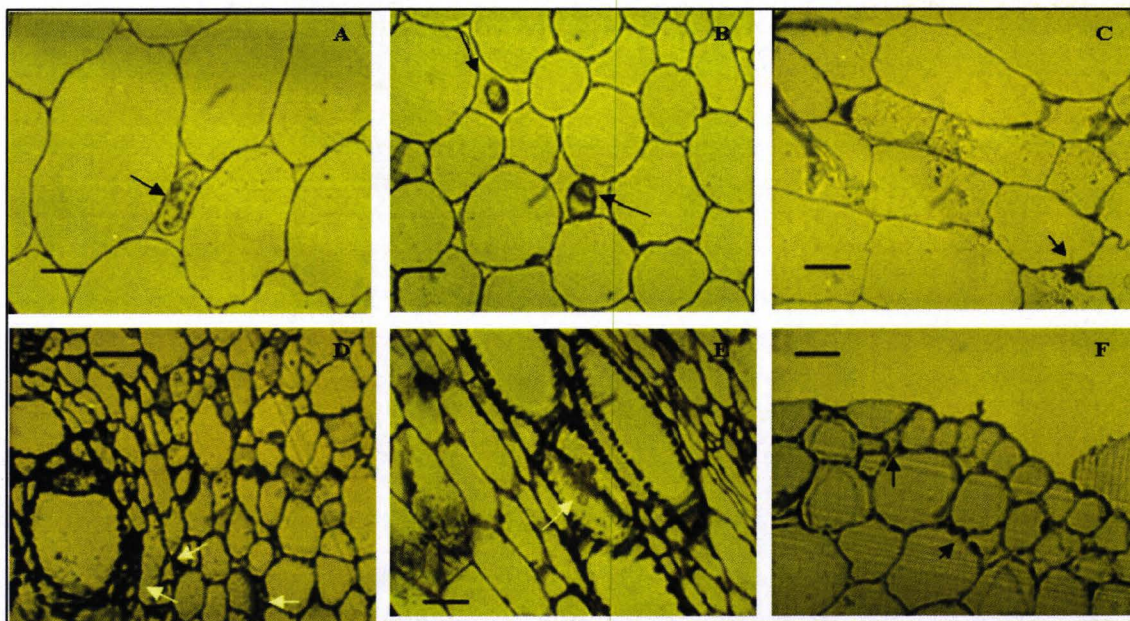


Figure 2. Aspects of cucumber plant infection with *P. debaryanum* (A, B), P14 strain (C, D), B40 strain and *P. debaryanum* (E) and P18 strain with *P. debaryanum* (F) visualized by optic microscopy on semifine sections stained with toluidine blue: intracellular proliferation of hyphae (A, B), deposits of fibrillar material (C), intercellular space occlusion (C, F), deposition of amorphous material (D), disorganized fungal cell in young xylematic vessels (E). Scale bars = 10µm.

Semithin sections through the hypocotyl of variants treated only with fungal pathogen showed the presence of fungal hyphae in intercellular spaces (Figs. 2a,b). In samples treated with bacterial suspensions, a thickening of the cell wall was observed (Fig. 2d). However, bacteria succeeded to penetrate the root tissues and invaded the intercellular spaces. In some cases, occlusion of intercellular spaces by cell wall appositions was observed (Figs. 2c,d). Sections of variants treated with antagonistic bacteria and phytopathogenic fungi showed the presence of amorphous material aggregations densely stained material toluidine blue in cortical tissue (Fig. 2f). Also, disorganized fungal cells were found in young xylematic vessels (Fig. 2e).

Electron microscopy

Transmission electron-micrographs of *P. debaryanum*-treated variant highlighted the penetration of plant cells by phytopathogen hyphae. Most of the sections showed the hyphae inside plant cells (Figs. 3a,b,c) and the pathogen abundantly invaded the intracellular area.



Figure 3. Ultrastructural aspects of invading cucumber plant cells by fungal pathogen (A, B, C). Scale bars = 1 μ m.

Ultrathin sections of cucumber plants treated with antagonistic bacteria showed the presence of bacteria in the intercellular spaces, B40 strain colonizing abundantly these areas (Fig. 4b). The presence of bacteria correlates with the formation of irregular papillae or cell wall appositions (CWA) (Figs. 4b,d). Also, in the presence of B40 bacterial strain the excretion of vesicles and electron-opaque material from the invaded tissue cell walls was observed (Figs. 4a,c). Similar to semithin sections observations, ultrathin sections showed the obturation of intercellular spaces, probably to limit further spread of bacterial cells through the plant tissue (Fig. 4e). It was previously mentioned the fill of intercellular spaces with pectic substances in plant-arthropods interaction (POLITO et al., 2002) and with dense material likely enriched in phenolics in plant interaction with pathogenic and beneficial microorganisms (BENHAMOU et al., 2000; BENHAMOU et al., 2002; HIBAR et al., 2007). It is well known that intercellular spaces are preferred sites for pathogen ingress (BENHAMOU et al., 2002), this supporting our observation that generally these areas were filled with different materials that would restrict potential pathogen entrance in plant cells. Moreover, the fact that a fungitoxic role was attributed to these substances (BENHAMOU et al., 2002), strengthens the idea that intercellular space obturation plays an important role in plant defense against pathogens.

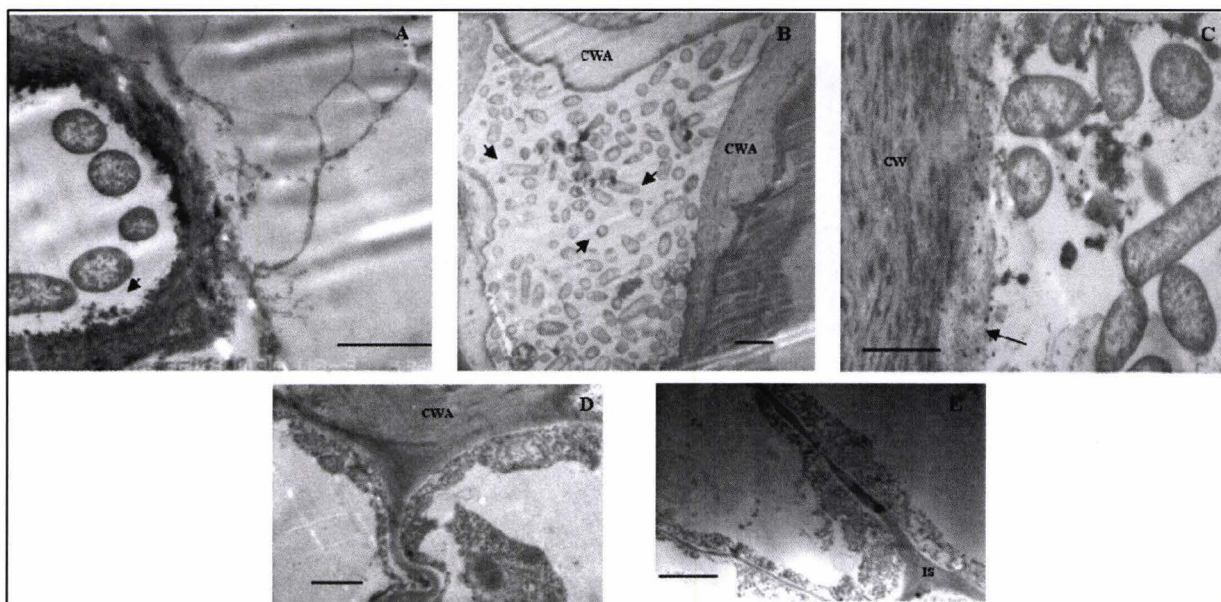


Figure 4. Some aspects regarding the interaction of the bacterial strains (A, B, C - B40 strain; D, E - P14 strain) with plant cells: the presence of bacteria near cell wall (A, C), colonization of plant intercellular territories by bacteria (B, C) cell wall appositions (CWA) in bacterial-colonized areas (B, D), fibrillar material deposition and thickness of plant cell walls (A, C), intercellular space (IS) occlusion (E). Scale bars = 1 μ m.

In contrast with the major part of the sections from the plant infected only with *P. debaryanum*, the variants treated both with pathogenic fungi and antagonistic bacteria, highlighted that fungi adhere to plant cell walls and are found in intercellular spaces, but very rare in intracellular spaces (Fig.5b,d,e,f). Most of the observed fungal hyphae presented disorganized cell content, were highly vacuolated, and had an abnormal shape. Moreover, fungi are trapped in an osmiophilic material, which prevents the penetration of host cell wall and further spreading through the plant (Figs. 5b,d,f).

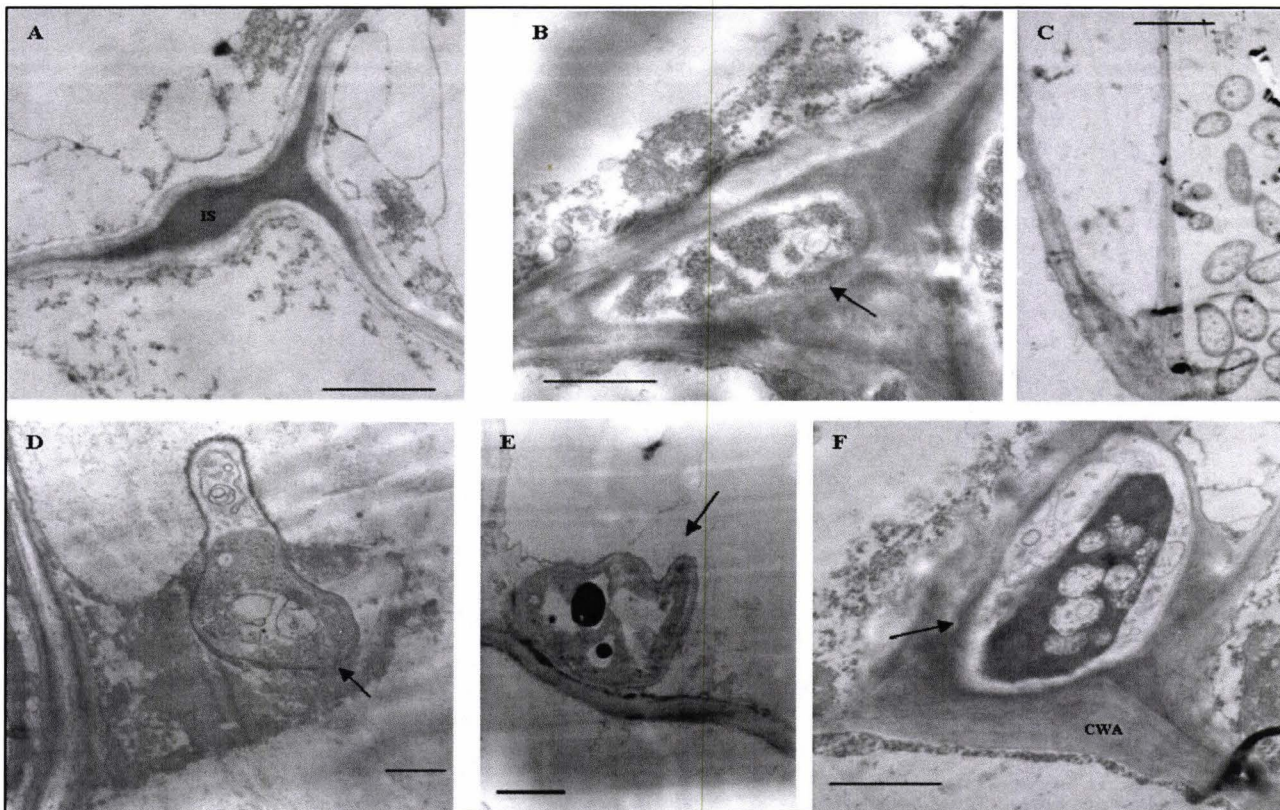


Figure 5. Ultrastructural aspects of cucumber plants treated with both bacteria and fungi (A, B – B40-Py; C – P18-Py; D, E – P7-Py; F – P14-Py): occlusion of intercellular space by osmiophilic material (A), the presence of fungi in intercellular space (IS) (B, D, E, F), fungal cell trapped in electronodense material (B, F), the presence of bacterial cells in areas other than those occupied by fungi (C), osmiophilic polymorphic material trapped the fungus near the primary host cell wall and hypha with severe alteration (D), distorted fungal cells near plant cell wall (E), the hyphae trapped by fibrillar material and vesicles accumulate in the paramural space of invading hypha and cell wall appositions (CWA) (F). Scale bars = 1µm.

Papilla formation and thickening of the plant cell walls (Fig. 5f) were correlated with fungal cell presence in plant intercellular spaces, this contributing to an increased resistance of plant to fungal pathogen. Examination of the variants that received mixed treatment allowed us to observe that fungal cells are not present in areas colonized by beneficial bacteria, this indicating the protective role of the strains used against pathogen infection.

Examination of both semithin and ultrathin sections showed papilla formation at plant cell wall level. Papillae are very complex structures (COLLINGE, 2009) and although the specific biochemical constituents of papillae vary between plant species, there are classes of compounds associated with papillae: callose, phenolics including lignin, phenolic conjugates such as phenolic-polyamines, reactive oxygen species (ROS), peroxidases, cell wall structural proteins such as arabinogalactan proteins and hydroxyproline-rich lycoproteins, cell wall polymers including pectin and xyloglucans (UNDERWOOD, 2012). The biochemical composition of papillae assigns them a double role in plant protection against pathogens. On the one hand, callose, lignin, cell wall polymers constitute a structural barrier which physically limits the pathogen entrance. On the other hand, phenolics, proteins and reactive oxygen species act as fungicide or fungistatic components, interacting with fungal cells and inducing cellular destruction in different degrees. Consequently, papillae are considered important elements and an integral part of the response of plants to microbial challenge (LEROUX et al., 2011).

CONCLUSIONS

The selected bacterial strains colonized root surface and also the intercellular areas. Plant treatments with bacteria induced structural modifications at the cellular level associated with plant resistance like the presence of cell wall appositions and thickened cell walls. Also, the occlusion of intercellular spaces was observed, and in areas colonized by bacteria no fungi presence was observed. The obtained results indicate that the selected bacterial strains had rhizosphere competence and induced structural defense responses in plants.

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LICHENS IN THE PRESCRIPTIONS OF PLINY THE ELDER

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Abstract. Caius Plinius Secundus, better known as Pliny the Elder, was a Roman author, naturalist, commander and a contemporary of Pedanius Dioscorides. He wrote an encyclopaedic work, **Naturalis Historiae**, consisting of thirty-seven books. In this study, two Latin codices and an English translation of **Naturalis Historiae** have been cross-compared and evaluated in order to investigate medicinal uses of lichens (the fungo-algal symbiotic organisms) in the antiquity. It is found that, Caius Plinius Secundus prescribes some botanical herbs -probably lichens- as remedies of dermatological diseases.

Keywords: Plinius, ethnomedicine, lichens, Naturalis Historiae, remedy.

Rezumat. Lichenii în prescripțiile lui Pliniu Cel Bătrân. Caius Plinius Secundus, mai bine cunoscut sub numele de Pliniu cel Bătrân, a fost un autor roman, naturalist, comandant și un contemporan al lui Dioscoride Pedanius. El a scris o enciclopedie **Naturalis Historiae**, constând în treizeci și șapte cărți. În acest studiu, două codice în limba latină și traducerea în limba engleză a **Naturalis Historiae** au fost confruntate și evaluate în scopul de a investiga utilizarea medicamentelor din licheni (organisme simbiotice fungo-algale) în antichitate. Se constată că, Caius Plinius Secundus prescrie unele remedii naturiste din plante medicinale - probabil licheni - pentru boli dermatologice.

Cuvinte cheie: Plinius, etnomedicină, licheni, Naturalis Historiae, remedii.

INTRODUCTION

Caius Plinius Secundus (23-79 AD), better known as Pliny the Elder, was a Roman author, naturalist, and natural philosopher, as well as naval and army commander of the early Roman Empire. He was contemporary of Pedanius Dioscorides (40-90 AD) and personal friend of the emperor Vespasian. He died on August 25th, 79 AD, while attempting the rescue by ship of a friend and his family from the eruption of Mount Vesuvius that had just destroyed the cities of Pompeii and Herculaneum (Web 01). Spending most of his spare time studying, writing or investigating natural and geographic phenomena in the field, he wrote an encyclopaedic work, "**Naturalis Historiae**", 77-79 AD consisting of thirty-seven books, which became a model for all such works written subsequently (Web 02).

Lichens are by definition symbiotic organisms, usually composed of a fungal partner, the mycobiont and one or more photosynthetic partners, the photobiont, which is most often either a green alga or a cyanobacterium (NASH, 2008). There are three major morphological kinds of thalli, namely crustose, foliose and fruticose. Crustose types adhere closely to their substrates, foliose thalli are leafy and attach more loosely, while fruticose thalli may be pendulous strands of hollow upright stalks (AHMADJIAN, 1993). According to the substrates, where they grow on, lichens are considered in three divisions as saxicolous, terricolous and epiphytic. Currently, lichens are included in the classification system of fungi under Ascomycota (NASH, 2008). Lichens have been used in medicine, pharmacy and industry from antiquity to present day in the treatment of various diseases like alopecia, arthritis, constipation, infection, kidney diseases, leprosy, pharyngitis rabies, worm and infestation (RICHARDSON, 1991; MALHOTRA et al., 2008).

Lichens produce a wide range of bioactive secondary metabolites, referred to as "Lichen Substances", which exert a wide variety of biological actions including analgesic, antibiotic, anti-inflammatory, antimicrobial, antimycobacterial, antiproliferative, antipyretic, antitumor, antiviral, cytotoxic and immunomodulator effects (HUNECK & YOSHIMURA, 1996; MÜLLER, 2002; MALHOTRA et al., 2008). The word *Lichen* is derived from the Greek word *λεικην* (*Leikhen*)- *Leprous* and refers to the use of lichens in treating skin diseases due to their peeling-skin appearance (COBANOĞLU & YAVUZ, 2003; MALHOTRA et al., 2008). In this study, Pliny the Elder has been investigated and evaluated in terms of use of lichens in medicinal prescriptions.

METHODS

In this study, a number of documents, digital resources, two Latin codices (PORTILIA, 1481; MAYHOFF, 1906) and an English translation (BOSTOCK & RILEY, 1855) of **Naturalis Historiae** have been cross-compared and evaluated in order to investigate medicinal uses of lichens in the antiquity. Relevant biochemical data from the literature on lichens are given in order to provide knowledge prior to lichens' uses in the history of medicine from antiquity to the present day.

RESULTS

It is found in **Naturalis Historiae** that, Caius Plinius Secundus prescribes some botanical herbs - probably lichens - as drugs in Book XXVI Major Medicinal Herbs, chapter 10 "The Lichen", as remedy of a skin disease called *Lichen* or *Lichenas* (Lichen planus, the tetter or eruption on skin) and in Book XXVII Minor Medicinal Herbs chapter

75. "Stone Moss", as remedy of a contagious bacterial skin infection called *impetigo*. It must be taken into consideration that, the number of books or chapters may differ among several translations or codices of *Naturalis Historiae*.

XXVI.10: *Sed in lichenis remediis atque tam foedo malo plura undique acervabimus, quamquam non paucis iam demonstratis. Medetur ergo plantago trita, quinquefolium, radix albi ex aceto, ficulni caules ex aceto decocti, hibisci radix cum glutino et aceto acri decocta ad quartas. Defricant etiam pumice, et rumicis radix trita ex aceto illinitur et flos visci cum calce subactus laudatur et tithymalli cum resina decoctum.*

Lichen vero herba omnibus suis praefertur, inde nomine invento. Nascitur in saxis, folio uno ad radicem lato, caule uno parvo, longis foliis dependentibus. Haec delet et stigmata. Teritur cum melle. Est aliud genus lichenis, petris totum adhaerens ut muscus, qui et ipse inlinitur. Hic et sanguinem sistit vulneribus instillatus et collectiones inlitus. Morbum quoque regium cum melle sanat ore inlito et lingua. Qui ita curentur, aqua salsa lavari iubentur, ungui oleo amygdalino, hortensis abstinere. Ad lichenas et thapsiae radice utuntur trita cum melle.

English Translation **XXVI.10:** But for the treatment of tetter, as it is both ugly and even more a bad disease, here is a number of additional remedies we amassed, although I have already described. Heals, and then pounded *Plantago* sp., *Potentilla* sp., root of *Asphodelus albus* Willd. in vinegar, the young stems of *Ficus* sp. tree boiled in vinegar, roots of *Althaea officinalis* L. with glue, hard vinegar boiled down to one-fourth. The sores are rubbed with pumice, and then fomented with root of *Rumex acetosa* L. bruised in vinegar, or with flowers of *Viscum album* L. kneaded up with lime, a decoction of *Euphorbia characias* L. with resin is highly esteemed for the same purpose.

Lichen is the herb preferred to all, since the name is found. It grows among rocks, and has a single broad leaf near the root, a single long stem, with long leaves hanging down. This deletes the marks (when) pounded with honey. There is another kind of lichen also adhering to the rocks so much, like moss, which is applied. Dropt into wounds, or applied to abscesses, has the property of arresting haemorrhage. Mixed with honey, it is curative of jaundice, the face and tongue being rubbed with it. Under this mode of treatment, the patient is recommended to wash in salt water, to anoint himself / herself with oil of *Amygdalus communis* L., and to abstain from garden vegetables. For the cure of tetter, root of *Thapsia garganica* L. is also used, bruised in honey.

XXVII.75: *Lapis vulgaris iuxta flumina fert muscum siccum, canum. Hic fricatur altero lapide addita hominis saliva; illo lapide tangitur impetigo. Qui tangit, dicit: φεύγετε κανθαρίδες, λύκος άγριος αίμα διώχγει.*

English Translation **XXVII.75:** There (it) grows near running streams, a dry, white moss, upon ordinary stones. One of these stones, with the addition of human saliva, is rubbed against another; after which the first stone is used for touching impetigo, the party so doing uttering these words: "*Pheugete kantharides lukos agrios aima diokhnei* - *Cantharides begone, a wild wolf seeks your blood*".

DISCUSSIONS

A previous article investigates uses of lichens in Dioscorides' *De Materia Medica* (YAVUZ, 2012). This paper is modestly focused on Lichens mentioned by Plinius. After a study on Latin codices and the English translation of *Naturalis Historiae*, it is found that Plinius mentions about some herbs "Lichens" - in chapter XXVI.10 - which are classified as "Lichenized Fungi" under the Kingdom Fungi in today's botanical taxonomy (NASII, 2008). In this chapter, in order to cure tetter, Plinius first mentions *Marchantia polymorpha* L. species with this description: "*has a single broad leaf near the root, a single long stem, with long leaves hanging down*". In as much as lichens lack of parts or organs named "roots", "stems" or "leaves", this herb is supposed to be a species of liverworts. Plinius mentions a second kind of lichen with the description: "*adhering to the rocks so much, like mosses*". This phrase is the basic definition of saxicolous-crustose lichens growing with the mosses. In chapter XXVI.10, Plinius prescribes that this kind of lichen "*is dropt into wounds*", "*applied to abscesses*", "*has the property of arresting haemorrhage*" and "*is curative of jaundice*".

In chapter XXVII.75, Plinius mentions a "dry" and "white" moss upon "ordinary stones", "near streams". Lichens can easily grow under humid microclimate effects in rocky habitats and since they have a wide range of thallus colour, one may describe them white, gray, black, yellow, orange, green, brown, etc. In this chapter, Plinius prescribes that lichens "*cure impetigo*" after rubbing with stones with the addition of human saliva. He also states an interesting expression of a ritual or spell: "*Cantharides begone, a wild wolf seeks your blood*".

ROMAGNI & DAYAN (2002) report a number of studies pertaining to the isolation, characterization, potential biological activities and / or uses of lichen extracts. In another excellent study, COCCHIETTO et al. (2002) review physiological aspects of usnic acid, which is one of the most common and abundant lichen metabolites. Medicinal uses of *Usnea* mentioned in *Liber Almansoris* (Kitab el Mansuri), the famous work of Rhazes (854-925 AD) was reported by YAVUZ & ÇOBANOĞLU (2010).

This paper is an attempt to elucidate medicinal uses of lichens mentioned in *Naturalis Historiae*. Below Plinius' prescriptions are given with potential uses, lichen metabolites, the genus / species derived and the reference cited (Table 1).

Dropt into wounds & applied to abscesses: MARX (2001) states that several lichen metabolites like atranorin, boninic, diffractaic, gyrophoric, lobaric, obstusatic, sekikaic and thamnolic acids exhibited interesting activity as inhibitors of leukotriene biosynthesis. Repression of leukotrienes has beneficial anti-inflammatory effects. ROMAGNI &

DAYAN (2002) report alectoronic, galbinic, leprarinic and protocetraric acids also show analgesic and anti-inflammatory effects. The analgesic and antipyretic effects of usnic acid (and diffractaic acid) were evaluated in orally treated mice by OKUYAMA et al. (1995); usnic acid was effective against acetic acid-induced writhing. The anti-inflammatory activity tests of usnic acid indicate a dose-dependent, significant effectiveness comparable to that of the standard reference drug ibuprofen.

Hypothetically, one can indicate that, lichens of Plinius showed anti-inflammatory and analgesic effects due to lichen acids mentioned above, thus he prescribed lichens to drop into wounds and apply on abscesses.

Table 1: Uses of Lichens and Lichen Metabolites.

Prescription	Potential use	Lichen Metabolite	Genus / Species	Reference
Drop into wounds & Applied to abscesses	Analgesic, Anti-inflammatory	Atranorin	Many species	MARN (2001)
		Alectoronic Acid	<i>Alectoria</i> spp.	ROMAGNI & DAYAN (2002)
			<i>Cetrelia</i> spp.	
			<i>Parmelia</i> spp.	
		Boninic Acid	<i>Ramalina boninensis</i>	MARX (2001)
		Diffractaic Acid	<i>Parmelia</i> spp..	
			<i>Usnea</i> spp.	
		Galbinic Acid	<i>Parmelia</i> spp.	ROMAGNI & DAYAN (2002)
		Gyrophoric Acid	Many species	MARX (2001)
			<i>Rinodia orcina</i>	
		Leprarinic Acid	<i>Lecidea lurida</i>	ROMAGNI & DAYAN (2002)
			<i>Lepraria citrina</i>	
		Lobaric Acid	Many species	MARX (2001)
		Obstusatic Acid	<i>Ramalina</i> spp.	
		Protocetraric Acid	<i>Parmelia</i> spp.	ROMAGNI & DAYAN (2002)
			<i>Usnea</i> spp.	
Arresting haemorrhage	hyphal texture of thallus	Usnic Acid	<i>Anzia</i> spp.	MARX (2001)
			<i>Ramalina</i> spp.	
			<i>Cladonia</i> spp.	
			<i>Thamnolia</i> spp.	
			<i>Thamnolia</i> spp.	
Curative of jaundice	Doctrine of Signatures	Parietinic Acid	<i>Alectoria</i> spp.	ROMAGNI & DAYAN (2002)
			<i>Cetraria</i> spp.	
			<i>Cladonia</i> spp..	
			<i>Parmelia</i> spp.	
			<i>Usnea</i> spp.	
Tongue rubbed with	Oral treatments. Toothpaste	Usnic Acid	<i>Xanthoria parietina</i>	FERRARI et al. (1988). COCCHIETTO et al. (2002). ROMAGNI & DAYAN (2002)
			<i>Alectoria</i> spp.	
			<i>Cetraria</i> spp.	
			<i>Cladonia</i> spp..	
			<i>Parmelia</i> spp.	
			<i>Usnea</i> spp.	
			<i>Alectoria</i> spp.	
			<i>Cetraria</i> spp.	
			<i>Cladonia</i> spp..	
			<i>Parmelia</i> spp.	
Face rubbed with & Cures impetigo	Acne protection & Antibacterial agent	Usnic Acid	<i>Usnea</i> spp.	ROMAGNI & DAYAN (2002)
			<i>Alectoria</i> spp.	
			<i>Cetraria</i> spp.	
			<i>Cladonia</i> spp..	
			<i>Parmelia</i> spp.	
Dermatology	Dermatology	Sitosterol	<i>Lecanora dispersa</i>	ROMAGNI & DAYAN (2002)
			<i>Sticta</i> spp.	
			<i>Sticta</i> spp.	
Hair & Skin care	Hair & Skin care	Pulvinic acid	<i>Sticta</i> spp.	ROMAGNI & DAYAN (2002)
			<i>Sticta</i> spp.	

Arresting haemorrhage: In the relevant literature of modern biology and medicine, there is not any reference to cite about lichens used to stop haemorrhage. However, as mentioned in YAVUZ (2012) study, lichens have a hyphal texture of thallus like a fibrous network, which may show an anti-haemorrhage property when applied on wounds.

Curative of jaundice: Almost every known culture has employed naturally occurring substances: animals, plants, and minerals as medicines to treat symptoms, ward off diseases, or bring physic to particular body organs. The guiding principle was that the substance used be linked to the symptoms of ailment by some shared aspect of similarity or resemblance, usually in terms of colour, shape, behaviour, or appellation. The second principle was the use of a substance that might produce symptoms of a particular disease in a healthy person to remedy those same symptoms in one who is sick (LEV, 2002). The union of these principles was so called **Doctrine of Signatures**. LLANO (1950) reported excellent samples of orange-yellowish lichen *Xanthoria parietina* - due to its colour - have been used to treat jaundice in traditional medicine from antiquity till today. There is not any biochemical evidence to support uses of lichens against jaundice.

Tongue rubbed with: *Usnea* spp. find use for mild inflammation of the oral and pharyngeal mucosa (MALHOTRA et al., 2008). Preliminary trials on human volunteers using a toothpaste containing usnic acid showed the suitability for the prevention of plaque and caries formation via inhibiting growth of *Streptococcus mutans*, the primary

pathogenic microorganism causing dental and oral diseases (FERRARI et al., 1988). COCCHIETTO et al., 2002 mention oral care-oriented commercial preparations containing usnic acid, on the market. Usnic acid containing species of *Alectoria*, *Cladonia* and *Cetraria* are reported to be used in toothpastes (ROMAGNI & DAYAN, 2002).

Face rubbed with & cures impetigo: There is much evidence from natural medicine that lichen derivatives are suitable for topical treatment against a large number of skin lesions and affections e.g.: wounds, sores, blisters, infections, furunculosis, burns and scalds (COCCHIETTO et al., 2002). Usnic acid containing *Alectoria* spp., *Cetraria stacheyi* are reported to be effective of acne protection, sitosterol producing *Lecanora dispersa* is used in cosmetics and dermatology, while calycin and pulvinic acid releasing *Sticta* spp. are consumed in hair and skin care. Recently, facial acne control creams containing lichen antibacterial agents such as Usnic Acid or Lichesterinic Acid have been developed (ROMAGNI & DAYAN, 2002). Usnic acid has been used throughout Europe as an antibacterial cream sold under the names of "Usno" and "Evosin" (ROMAGNI & DAYAN, 2002). Along the same lines, other lichen products have been used by the cosmetic industry. For example, sterols such as ergosterol and its derivatives though not unique to lichens, have been shown to stimulate the proliferation of skin cells (LUBRANO et al., 1999).

CONCLUSION

Uses of lichens are linked with history of ethno-medicine. Ethno-medicinal use of lichens can be traced back to antiquity. For instances, LLANO (1950) reports that *Pseudevernia furfuracea* has been found in an Egyptian vase from the eighteenth dynasty and was used to preserve the odour of spices employed in embalming mummies where LAUNERT (1981) states the same species was used as a drug in the period of the same dynasty. It is obvious that Plinius prescribes in his medicinal recipes and denotes "lichens" growing among mosses on rocks (saxicolous thalli) since he mentions the mosses as separate chapters in XII.50 Sphagnos and in XXIV.17 Sphagnos or Bryon. Thus, it is assumed that Plinius had mentioned true lichens in his prescriptions although there are not enough morphological statements so that one could taxonomically identify the lichen species mentioned in Naturalis Historiae.

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COMPARATIVE STUDIES BETWEEN FERNS GAMETOPHYTE AND SPOROPHYTE BY BIDIMENSIONAL ELECTROPHORESIS

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BREZEANU Aurelia, SOARE Liliana-Cristina

Abstract. *Athyrium filix-femina* (L.) ROTH, *Polypodium vulgare* L. and *Asplenium trichomanes* L. are three valuable ferns species due to their potential as ornamental plants and secondary metabolite production and they originate from Valsan Valley protected area. Our experiments were focused on developing an optimal *in vitro* multiplication method and medium term conservation for these species. The reactivity of each species has been evaluated on the basis of biometric (morphometric and gravimetric) and biochemical determinations. The current study showed the differences between gametophytes and sporophytes of the mentioned species by comparing the proteomic profiles. We used gametophytes and sporophytes from *A. filix-femina*, *P. vulgare* and *A. trichomanes* obtained by *in vitro* culture and multiplied on MS 1/2 medium. Proteins were isolated according to a phenol extraction procedure and the protein content was determined by Bradford assay. The protein samples were separated in two dimensional technique and stained with Coomassie. Although using PAGE technique we have not observed significant differences between gametophyte and sporophyte, the patterns obtained by two-dimensional gel electrophoresis showed the existence of distinct spots for both gametophyte and sporophyte extracts.

Keywords: *Athyrium filix-femina*, *Polypodium vulgare*, *Asplenium trichomanes*, two-dimensional gel electrophoresis.

Rezumat. Studii comparative privind gametofitul și sporofitul ferigilor prin electroforeză bidimensională. *A. filix-femina* (L.) ROTH, *P. vulgare* L. și *A. trichomanes* L. sunt trei specii de ferigi valoroase datorită potențialului lor ca plante ornamentale și sintezei de metaboliți secundari, fiind originare din zona protejată Valea Vâlsanului. Experimentele noastre s-au axat pe dezvoltarea unei metode optime de multiplicare *in vitro* și conservarea pe termen mediu pentru aceste specii. Reactivitatea fiecărei specii a fost evaluată pe baza determinărilor biometrice (morfoметриce și gravimetrice) și biochimice. Studiul actual arată diferențele dintre gametofiti și sporofiti speciilor menționate prin compararea profilurilor proteomice. Am folosit gametofiti și sporofiti de la *A. filix-femina*, *P. vulgare* și *A. trichomanes* obținuți prin cultura *in vitro* și multiplicați pe mediu MS 1/2. Proteinele au fost izolate în conformitate cu procedura de extracție cu fenol iar concentrația de proteine a fost determinată prin testul Bradford. Proteinele au fost separate prin electroforeză bidimensională și colorate cu Coomassie. Deși folosind electroforeza în gel de poliacrilamidă nu am observat diferențe semnificative între gametofit și sporofit, modelele obținute prin electroforeză bidimensională au arătat existența unor spoturi distincte pentru ambele extracte din gametofit și sporofit.

Cuvinte cheie: *A. filix-femina*, *P. vulgare*, *A. trichomanes*, electroforeză bidimensională.

INTRODUCTION

Peridophytes represent a very important group of vascular plants. They have been known for more than 300 million years, and present an enormous diversity of forms, growing in many different habitats around the world. During the Carboniferous Period, they dominated the vegetation. While most of them became extinct, some evolved and their currently number is now around 12.000 species all over the world (FERNANDEZ & REVILLA, 2003).

In time, the ferns stimulated the interest of many research teams. After *in vitro* techniques developed, numerous interesting approaches from scientific as well as from biotechnological point of view appeared.

Using *in vitro* culture system, the fern life cycle was analysed in detail, representing a powerful tool for studying the mechanisms underlying plant development. In the same time many fern species present a great biotechnological interest because of their economic value for ornamental purposes (*A. filix-femina*) as well as for the bioactive components produced. Among the metabolites synthesized by ferns there are triterpenoids and flavonoids (ecdysone-20E, abutasterone, polypodine B, inokosterone, 24-hydroxyecdysone, catechin, saponin, osladin) isolated from *P. vulgare* and phenolic compounds (4-vinyl-phenol, 4-vinyl-phenol-1-O-[α -L-rhamnopyranosyl-(1 \rightarrow 6) β -D-glucopyranose], arctigenin) extracted from *A. trichomanes* (HO et al., 2010).

Proteins execute and control essentially all functions in living organisms (FRAUENFELDER & MCMAHON, 1998). Proteomics allows to obtain a quantitative description of protein expression and its changes under the influences of biological perturbations, the occurrence of post-translational modifications and the distributions of specific proteins within the cell (LOPEZ, 2007). Two-dimensional gel electrophoresis is one of the most widely used techniques for resolving complex protein extracts (SHEORAN et al., 2009).

MATERIAL AND METHODS

The biological material was represented by gametophytes and sporophytes from *A. filix-femina*, *P. vulgare* and *A. trichomanes* obtained by *in vitro* culture on MS 1/2 medium (MURASHIGE & SKOOG, 1962). Plants grew under the conditions of 20 ± 2 °C and a photoperiod of 16 hours of light and 8 hours of darkness.

Proteins were isolated using a phenol-based extraction procedure (HURKMAN & TANAKA, 1986). The protein content was determined by BRADFORD (1976) assay. The protein samples were focused using 3-10 nonlinear IPG strips for the 1st dimension separated on 12.5% (v/v) acrylamide gel and stained with Colloidal Coomassie Brilliant Blue 250.

For molecular mass determination, there were used protein molecular weight markers in the size range of 14.4–116 k Da from Fermentas. Gels were scanned and calibrated with labscan 6 software (GE Healthcare). Image analysis was performed with Image Master 2D Platinum 6.0 (GE Healthcare).

For SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) extraction of total proteins from cytosol was performed by grinding the tissue in 0.1 M phosphate buffer, pH 7 at 4°C. The supernatant obtained after centrifugation at 15,000 rpm for 10 minutes, was used for electrophoretic analysis. Analyses were based on the principle of electrophoretic migration of samples in the electrical field at 20 mA for 2 hours in a batch system. The samples were run in a 10% concentration polyacrylamide gel (SDS-PAGE), a 4% polyacrylamide stacking gel and a 0.05M Tris-Gly buffer, pH 8.3. Samples were loaded in the wells of stacking gel and subjected to electrophoretic migration process at a voltage of 10 mA through the stacking gel for 30 minutes and then at a voltage of 20 mA through the separating gel for 90 minutes, at 4°C. As a marker highlighting the front of migration, bromphenol blue was used. It was used a Biometra electrophoresis apparatus. The spectra of total proteins extracted from each sample were showed by gel staining with Coomassie Brilliant Blue solution. The gels were photographed and interpreted by comparison of the similar bands.

RESULTS AND DISCUSSIONS

Spore-derived gametophytes maintained on MS medium were homogenized in aseptic conditions to obtain a high number of sporophytes (Fig. 1).

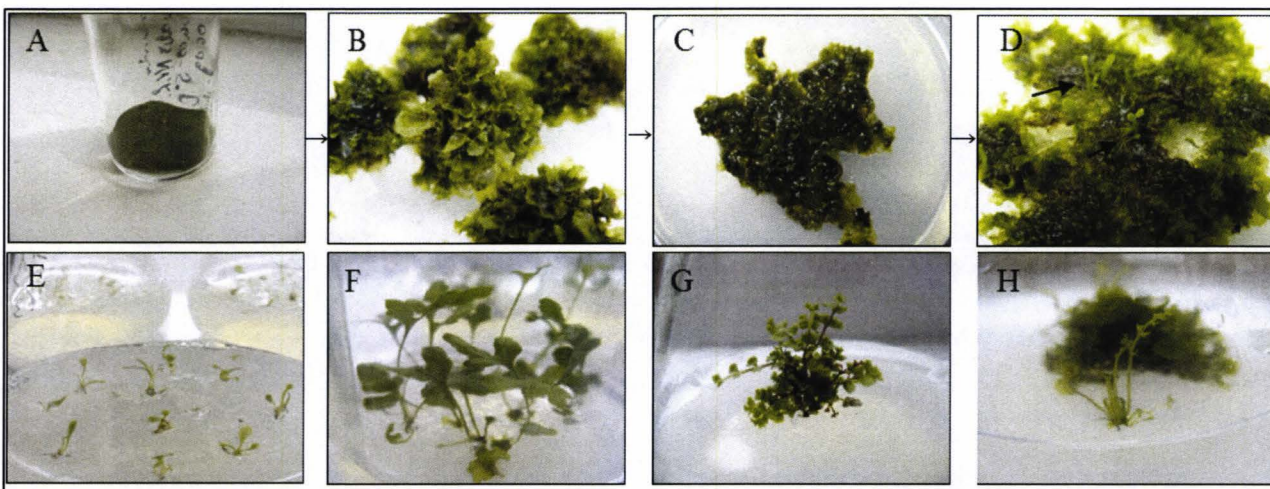


Figure 1. Sporophytes of ferns arising from homogenates of gametophytes:

A, spores; B, gametophytes; C, cultures homogenates of gametophytes; D, formation of sporophytes; E, sporophytes; F, *P. vulgare* (sporophytes); G, *A. trichomanes* (sporophyte); H, *A. filix-femina* (sporophyte).

In case of *A. trichomanes* sporophyte, the development was slower and generating of enough biological material took a long time (seven months), for these reasons we used them only for two-dimensional electrophoresis. We could not observe evident differences between gametophytes and sporophytes of some species using SDS-PAGE analysis (Figs. 2; 3). However, in case of *A. filix-femina* intensity differences were observed (Fig. 2).

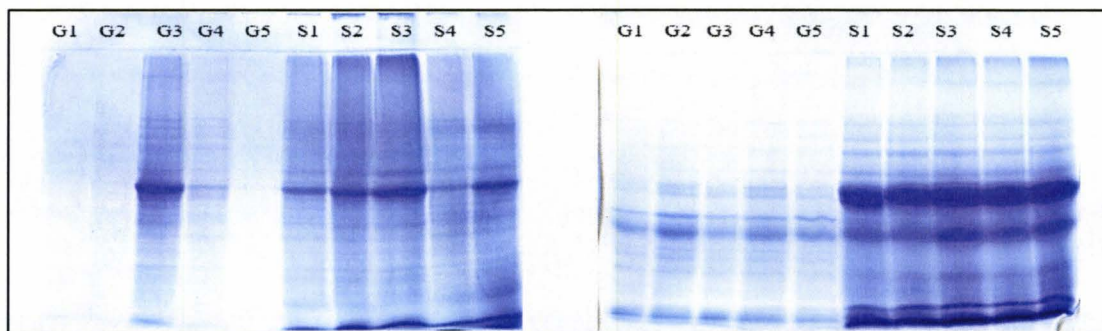


Figure 2. Protein patterns after SDS-PAGE *A. filix-femina* (left, G1-G5 gametophytes; right, S1-S5 sporophytes)

Figure 3. Protein patterns after SDS-PAGE *P. vulgare* (left, G1-G5 gametophytes; right, S1-S5 sporophytes)

SDS-PAGE is a simple method used to estimate the molecular weight of proteins, but it cannot resolve more than 80-100 different protein components.

Although using PAGE technique we have not observed significant differences between gametophyte and sporophyte, the patterns obtained by two-dimensional gel electrophoresis showed the existence of distinct spots for both gametophyte and sporophyte extracts (Figs. 4; 5; 6).

Two-dimensional gel electrophoresis separates proteins according to two independent parameters, isoelectric point (pI) in the first dimension and molecular mass in the second dimension by coupling isoelectric focusing (IEF) and SDS-PAGE (LOPEZ, 2007). Two dimensional electrophoresis is capable of resolving over 2,000 proteins in a single gel being the primary tool for proteomics research where multiple proteins must be separated for parallel analysis.

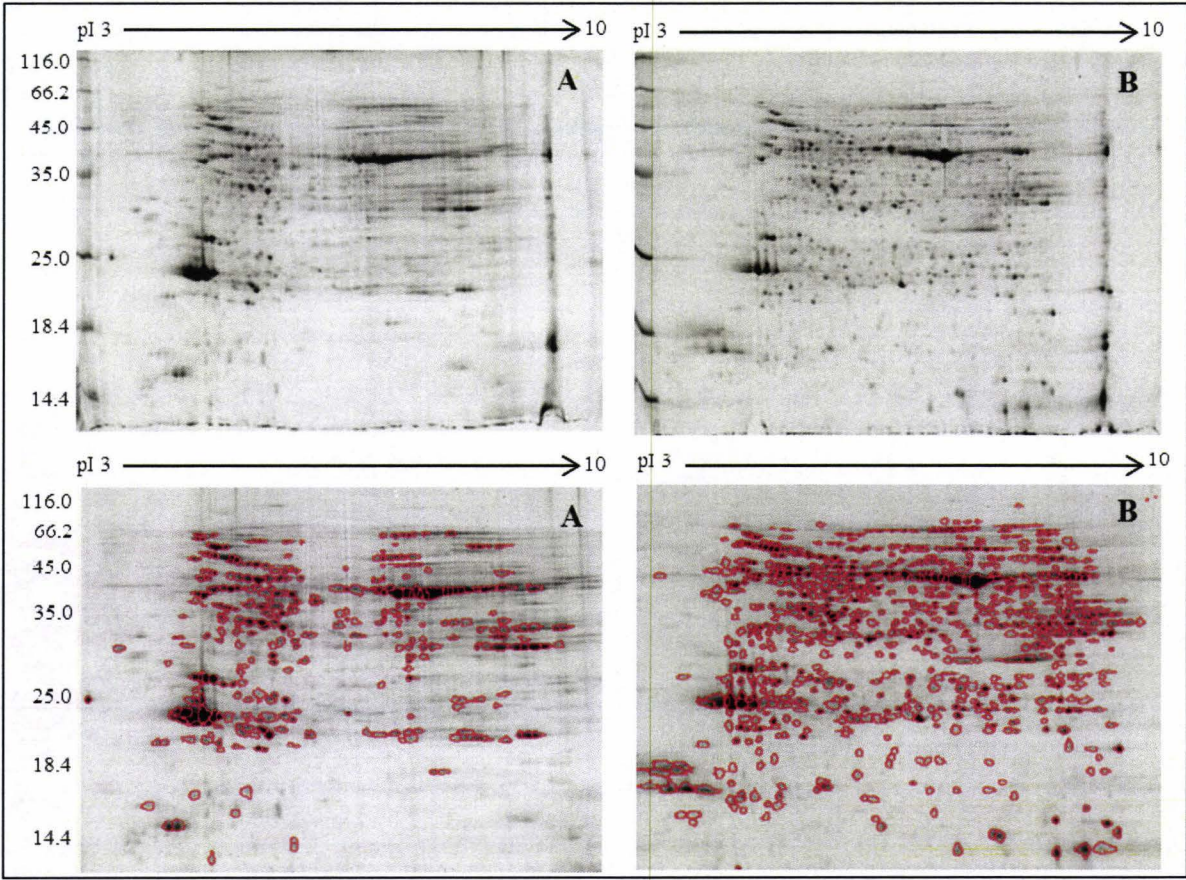


Figure 4. The comparison between Coomassie blue-stained 2-D protein maps. Protein was extracted from *A. filix-femina* (A) gametophyte and (B) sporophyte separated on 24 cm IPG strip (pH 3-10 linear gradient) through isoelectric focusing (IEF) in the first dimension, followed by 12,5% SDS-PAGE gels in the second dimension.

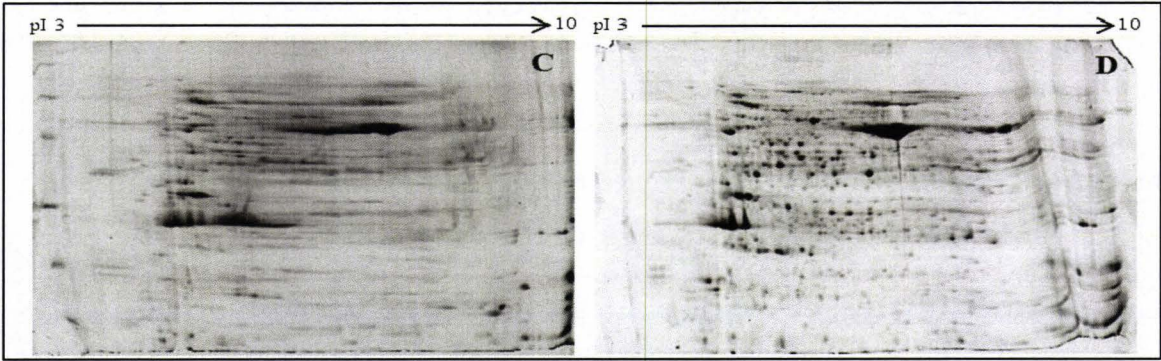


Figure 5. The comparison between Coomassie blue-stained 2-D protein maps. Protein was extracted from *P. vulgare* (C) gametophyte and (D) sporophyte separated on 24 cm IPG strip (pH 3-10 linear gradient) through isoelectric focusing (IEF) in the first dimension, followed by 12,5% SDS-PAGE gels in the second dimension.

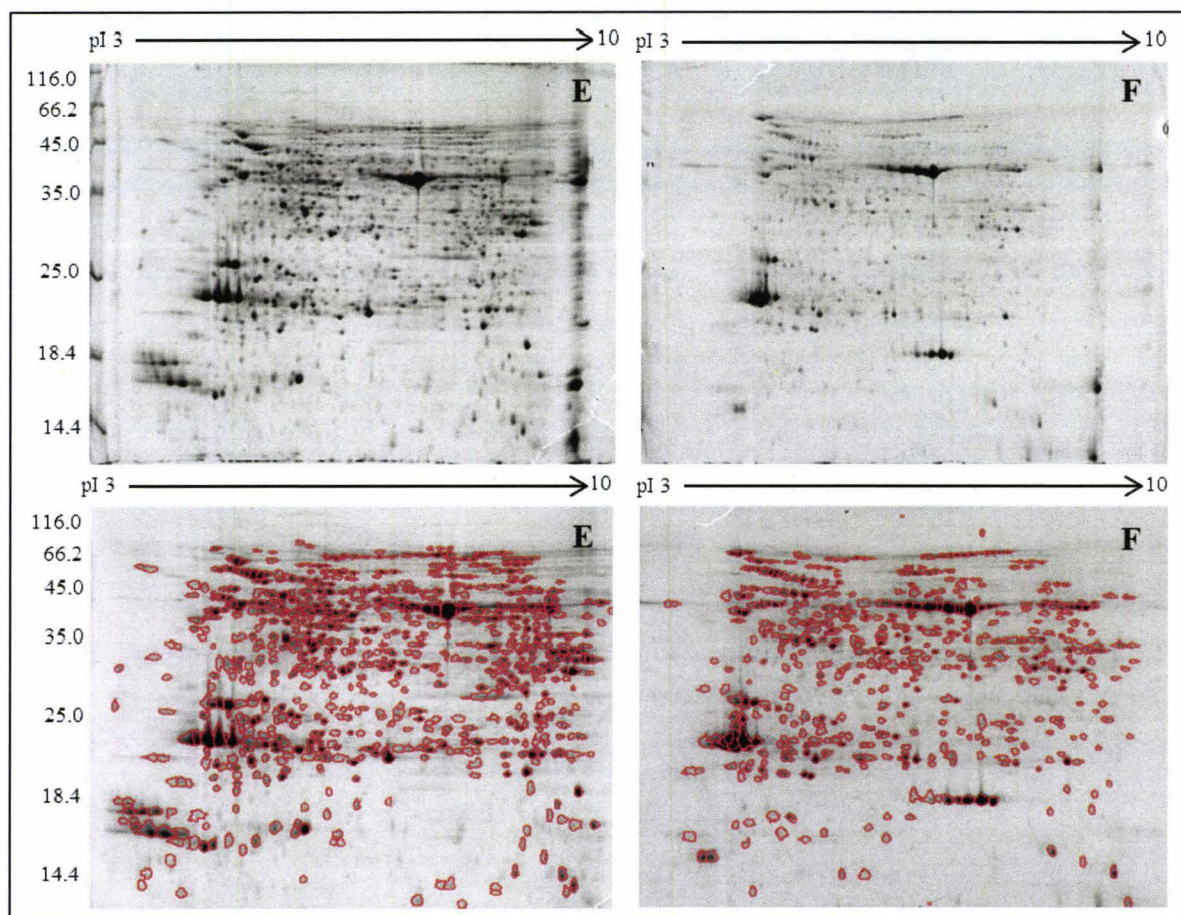


Figure 6. The comparison between Coomassie blue-stained 2-D protein maps.

Protein was extracted from *A. trichomanes* (E) gametophyte and (F) sporophyte separated on 24 cm IPG strip (pH 3-10 linear gradient) through isoelectric focusing (IEF) in the first dimension, followed by 12,5% SDS-PAGE gels in the second dimension.

For all of the three studied species, two-dimensional electrophoresis allowed the separation of a high number of proteins.

The highest number of spots was detected in *A. trichomanes* gametophyte (approximately 950 spots), while *A. filix-femina* had the smallest number of spots (approximately 400 spots).

The molecular mass of the proteins detected was in the range of 18,4-66,2 kDa. The gametophyte of *A. filix-femina* had a very low number of spots (approximately 20 spots) in the 14,4-18,4 molecular mass interval (Fig. 4A). For all the analyzed species, the highest number of spots was found in 5-7 pH range.

In case *P. vulgare* and *A. trichomanes* we could observe that the gametophyte had generally an increased number of protein spots comparing with the sporophyte. It is known that different important compounds are synthesized in greater amounts in gametophyte comparing with the sporophyte (LAFONT et al., 2010).

In *A. filix-femina*, which was not associated until now with valuable compounds production, the gametophyte had the lowest number of spots. It is possible that the presence of a higher number of spots to be correlated with increased synthesis of valuable secondary metabolites, as in case of ecdysteroids produced by *P. vulgare* gametophyte (LAFONT et al., 2010).

CONCLUSIONS

From the analysed data we concluded that 2D gel electrophoresis technique allowed a better separation of the proteins from *A. trichomanes*, *P. vulgare* and *A. filix-femina* comparing with PAGE method.

Proteomic profiles showed much clearer differences between sporophyte and gametophyte of each species.

Also, the higher number of protein spots in *A. trichomanes* and *P. vulgare* gametophyte could be explained by an increased synthesis of secondary metabolites.

This study presents a preliminary analysis of proteomic profiles for further detection of biochemical changes that may occur during conservation (*in vitro* culture, cryopreservation).

For protein identification interesting spots must be excised from preparative gels then identified using mass spectrometry and database mining.

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PHYSIOLOGICAL EFFECTS OF TREATMENT WITH FUNGICIDES IN *Malus domestica* BORKH. ATTACKED BY *Venturia inaequalis* (COOKE) WINT

NICOLAE Ion, BUȘE-DRAGOMIR Luminița

Abstract. Research regarding the physiological effects of treatment with fungicides were carried out on apple **Jonagold** variety cultivated in the region of Oltenia (Banu Mărăciine, Dolj). There have been three treatments with contact fungicide **Dithane M 45-0.2%** after the appearance of the symptoms of the attack produced by the pathogen on leaves. The first treatment has been carried out at the beginning of fruit formation (May 10th 2011) and the following treatments were applied to the period of 10 days. The physiological analyses were conducted on the attacked leaves by pathogen, before the application of treatments with fungicide and then after the last treatment with fungicide. It was found that the intensity of photosynthesis and the intensity of transpiration vary depending on the degree of the attack and the climatic conditions. Intensity of photosynthesis in attacked leaves is lower, correlated with a low chlorophyll content, and intensity of transpiration has higher values compared to the leaves of the analysed plant after the treatment with fungicides. There has been a lower water content and a higher of dry substance content in the leaves of plants attacked by pathogen.

Keywords: apple tree, attacked leaves, fungicide, healthy leaves, pathogen.

Rezumat. Efectele fiziologice ale tratamentului cu fungicide la *Malus domestica* BORKH. atacat de *Venturia inaequalis* (COOKE) WINT. Cercetările privind efectele fiziologice ale tratamentului cu fungicide s-au efectuat la soiul de măr **Jonagold** cultivat în regiunea Olteniei (Banu Mărăciine, Dolj). S-au realizat trei tratamente cu fungicidul de contact **Dithane M 45-0.2 %**, după apariția simptomelor atacului produs de patogen pe frunze. Primul tratament s-a efectuat la începutul formării fructelor (10 mai 2011) și următoarele tratamente s-au efectuat la interval de 10 zile. Analizele fiziologice s-au realizat la frunzele atacate de patogen, înainte de aplicarea tratamentelor cu fungicide și apoi după ultimul tratament cu fungicide. S-a constatat că intensitatea fotosintezei și intensitatea transpirației variază în funcție de gradul de atac și condițiile climatice. În frunzele atacate intensitatea fotosintezei este mai scăzută, fapt corelat cu conținutul scăzut în clorofilă, iar intensitatea transpirației are valori mai mari, în comparație cu frunzele plantelor analizate după tratamentul cu fungicide. S-a înregistrat un conținut mai scăzut de apă și un conținut mai mare de substanță uscată, în frunzele plantelor atacate de patogen.

Cuvinte cheie: măr, frunze atacate, fungicid, frunze sănătoase, patogen.

INTRODUCTION

Original from Central Asia, the apple is frequently attacked by disease and pests, and this is why a large number of chemical treatments have been carried out.

Apple scab produced by *Venturia inaequalis* (COOKE) WINT, represents a major problem in apple production and to ensure high yields and fruit quality requires the application of fungicides.

Application of fungicides may affect crop physiology by various disruptions such as growth reduction, perturbation in the development of reproductive organs, alteration of nitrogen, and/or carbon metabolism leading to a lower nutrient availability for plant growth. The sensitivity of some plant species may depend on the developmental stage (more sensitive to the treatments at young stages or during critical events such as reproduction) or the type of pesticides used (PETIT et al., 2012).

The net photosynthetic activity is subjected to seasonal changes and to diurnal changes, which are mainly influenced by the stage of shoot development, leaf ageing, hormones and carbohydrates accumulation in leaves, as well as by light intensity fluctuations, leaf temperature, air temperature and humidity (LAKSO, 1985).

Other research conducted on apple leaves shows there was a slight decrease in the intensity of photosynthesis at midday (LANDSBERG et al., 1975).

The intensity of the photosynthesis process is higher in the case of the apple leaves located at a height of 1.8 m compared with those located at 1.0 m above the ground (CORELLI & SANSVINI, 1989).

The young leaves have the highest intensity of the transpiration process and as they get older, the transpiration intensity decreases, the lower values being recorded at senescent leaves (BURZO et al., 1999).

The intensity of transpiration process proportionally increases with that of photosynthesis, both processes being dependent on solar radiation intensity (BIGNAMI & NATALI, 1992).

The intensity of photosynthetic active radiations is higher near the edge of the crown and close to the stem axis and decreases from higher to lower levels (MARINI & MARINI, 1983).

Positive correlations were established between the intensity of the physiological processes and the photosynthetic active radiation, the leaf temperature and stomatal conductance of CO₂ (NICOLAE, 2010).

The chlorophyllian pigment content was higher in plant leaves analysed after treatments done with fungicide, compared with the leaves attacked by the pathogen, there being a positive correlation between the chlorophyllian pigment content and the photosynthesis intensity (NICOLAE & BUȘE-DRAGOMIR, 2012).

MATERIAL AND METHODS

The research regarding of the physiological changes produced by the *V. inaequalis* were carried out in the *M. domestica* (**Jonagold** variety) cultivated in the climatic conditions specific to the region of Oltenia (Banu Mărăcine, Dolj).

M. domestica grows in all countries with temperate, warm climates and rain during winter, but also in the vegetation period. This fruit tree has a thick trunk, the leaves have oval or elliptical form, have serrate edge and are petiolated. The flowers are hermaphrodite, arranged in corymb inflorescence. The apple is the fruit of the *M. domestica* and it differs in shape, size, colour, texture of peel and time of maturity.

The apple **Jonagold** variety comes from the crossing of the species *Jonathan* x *Golden*. It is a variety with vigorous growth, the fruit is spherical, with a sea of red colour, smooth, ripen in the second half of September.

The physiological processes were established with the ultra-compact photosynthesis measurement system - Lci. The results obtained were graphically represented and statistically interpreted. The water contents and dry substance were determined by the gravimetric method. The chlorophyll content was estimates by Minolta SPAD 502.

The estimate of the attack was made using the calculation formulae by SĂVESCU & RAFAILĂ, 1978.

The treatment with the fungicide **Dithane** M 45 (0.2%) was applied in three stages on the leaves, after the appearance of the symptoms on the attacked leaves by the pathogen. The first treatment has been carried out at the beginning of the formation of fruit and other treatments have been carried every 10 days. The physiological analyses were conducted on the attacked leaves by pathogen before the application of treatments and then two weeks later after the last treatment with fungicides.

Dithane M 45 is a contact fungicide with a very broad spectrum in combating pathogens agent in vegetables, flowers, fruit trees, vines, and treating seeds.

RESULTS AND DISCUSSIONS

Apple scab appears in orchards from all regions, being considered one of the most damaging diseases due to high both quantitative and qualitative crop losses.

The symptoms are generally most noticeable and serious on leaves and fruit. On the leaves spots more or less circular in shape, undefined, light gray are being observed at the beginning of the attack. Later, with the formation of conidiophores with conidia, the spots acquire an olive brown colour and velvety appearance (Figs. 1; 2).

The fruit attack manifests itself through the appearance of gray-olive spots and cracks in the tissues corresponding to spots.

V. inaequalis contains mycelium which is developing under cuticle, is brownish-olive, is septal, branched and forms brown stroma that appear on conidiophores and conidia. Conidiophores are short, cylindrical, brown, and on these at each end a brown conidia is formed, initially unicellular, and then bicellular (Fig. 3).

The autumn in fallen leaves form ascogonium and antheridium and then form asci with ascospores, closed in the perithecia (MITREA, 2006).



Figure 1. The *M. domestica* (Jonagold variety) attacked by *V. inaequalis* (original).



Figure 2. Detail of the leaf in *M. domestica* (Jonagold variety) attacked by *V. inaequalis* (original).

The *V. inaequalis* is usually controlled with fungicides applied in the period between leaf fall and bud break, or during the period of vegetation. After treatments with **Dithane** M 45 fungicide of the leaves with specific symptoms (light gray spots or the olive brown spots) the attack was stopped and the symptoms produced on leaves due to the pathogen have gradually disappeared (Fig. 4).

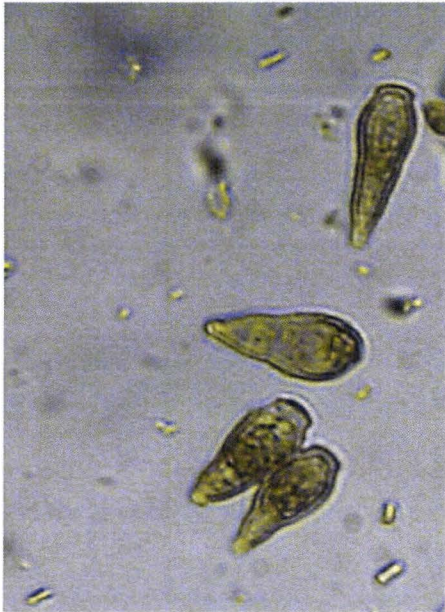


Figure 3. *V. inaequalis* - brown conidia (oc. 10 x ob. 20) (original).



Figure 4. The *M. domestica* (Jonagold variety) after treatment with fungicide (original).

The physiological analyses were performed on the leaves of the plants attacked by *V. inaequalis* before the application of three treatments with fungicide (May 10th 2011) and then two weeks after the last treatments.

The estimation of the attack (frequency, intensity and degree of attack) caused by *V. inaequalis* in apple **Jonagold** variety, before the application of treatments with fungicide, is presented in Fig. 5.

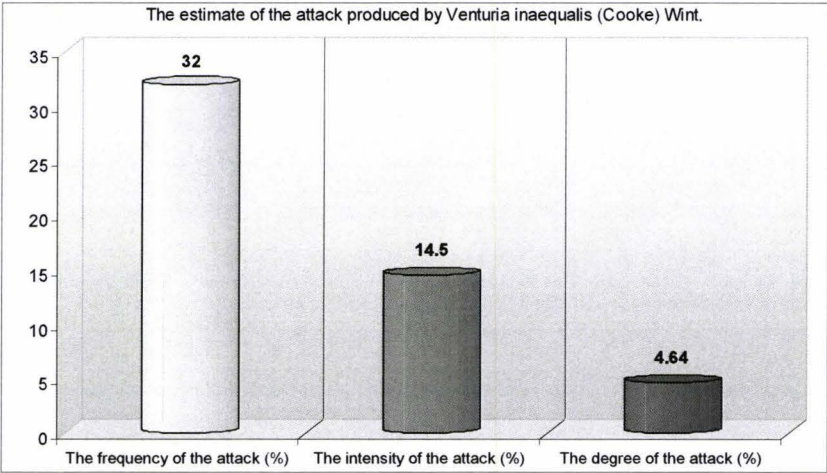


Figure 5. The estimate of the attack produced by *V. inaequalis* (COOKE.) WINT. in *M. domestica* BORKH.

The diurnal dynamics of photosynthesis and transpiration in the attacked leaves plants is similar to that in the plant leaves analysed after treatments, but the recorded values are lower in the attacked leaves (Figs. 6; 7).

Physiological processes intensity is lower in the leaves attacked by the pathogen as a result of the reduction of the assimilation surface due to the reaction of plants to pathogen, reduction of leaf surface due to the formation of light gray spots or the olive brown spots, deterioration of the chlorophyll pigments and stomata coverage of the pathogen.

The intensity of the physiological processes (photosynthesis and transpiration intensity) at the apple leaves depends on the photosynthetic active radiation received by leaves, the leaf temperature, the stomatal conductance for CO₂, etc.

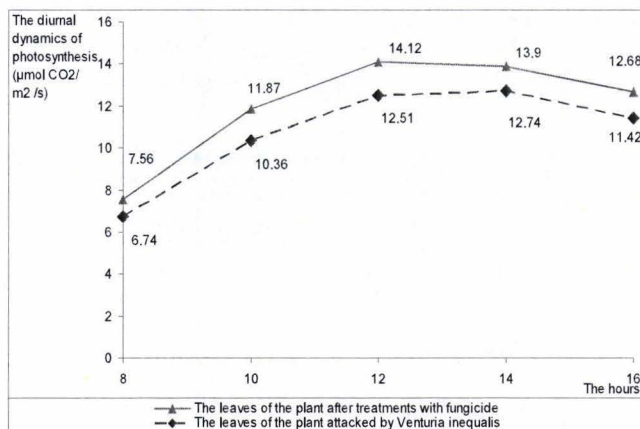


Figure 6. The intensity of photosynthesis in leaves of *M. domestica* - Jonagold variety.

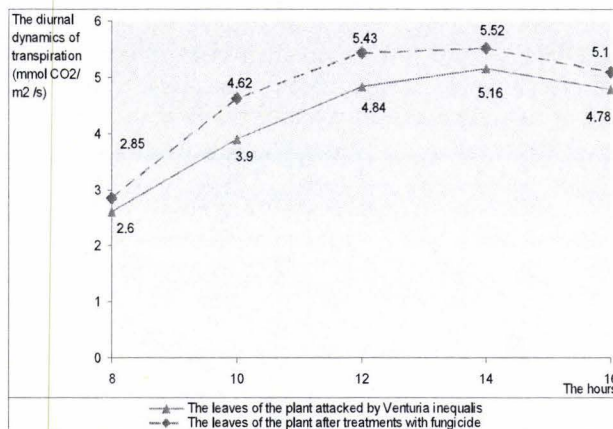


Figure 7. The intensity of transpiration in leaves of *M. domestica* - Jonagold variety.

At the analysed plant, one can observe an increase of the **photosynthetic active radiations** present on the surface of the leaves beginning in the morning (8 a.m.), when it records the values of 1292 $\mu\text{mol} / \text{m}^2 / \text{s}$ in the leaves attacked by *V. inaequalis* and of 1150 $\mu\text{mol} / \text{m}^2 / \text{s}$ in the plant leaves after treatments with fungicide, their growth after lunch (12 a.m.), when it records the values of 1460 $\mu\text{mol} / \text{m}^2 / \text{s}$ in the attacked leaves and of 1415 $\mu\text{mol} / \text{m}^2 / \text{s}$ in the leaves after treatments with fungicide, and decreases in intensity towards evening (4 p.m.), when it records the values of 1465 $\mu\text{mol} / \text{m}^2 / \text{s}$ in the attacked leaves and of 1410 $\mu\text{mol} / \text{m}^2 / \text{s}$ after treatments with fungicide.

Linear regression performed between the photosynthesis intensity and photosynthetic active radiations shows a good positive correlation between these; the coefficient of determination (R^2) is 0.94 for the attacked leaves and 0.96 for the plant leaves after treatments with fungicide. Linear regression made between the transpiration intensity and photosynthetic active radiations shows a good positive correlation between these; the coefficient of determination (R^2) is 0.96 for the attacked leaves and 0.98 for the plant leaves after treatments with fungicide (Figs. 8; 9).

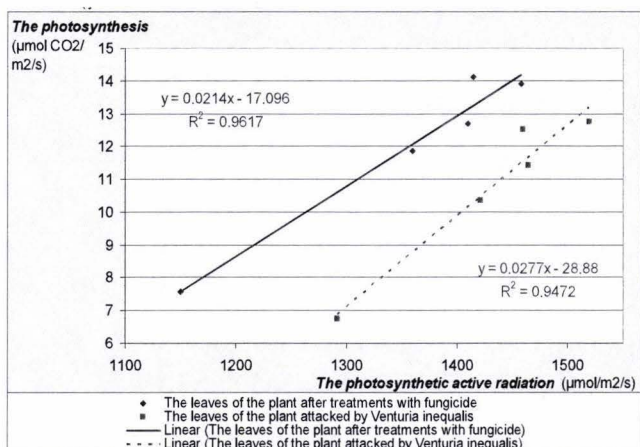


Figure 8. The correlation between the intensity of photosynthesis and the photosynthetic active radiation in *M. domestica* - Jonagold variety.

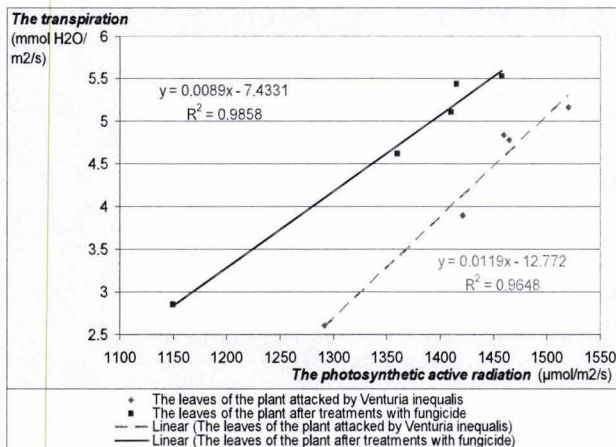


Figure 9. The correlation between the intensity of transpiration and the photosynthetic active radiation in *M. domestica* - Jonagold variety.

The *leaf temperature* increases beginning in the morning (8 a.m.), when it records the values of 27.9 $^{\circ}\text{C}$ in the attacked leaves and of 28.4 $^{\circ}\text{C}$ in the plant leaves after treatments, their growth after lunch (12 a.m.), when it records the values of 32.5 $^{\circ}\text{C}$ in the attacked leaves and 33.4 $^{\circ}\text{C}$ in the leaves after treatments and decreases towards the evening (4 p.m.), when it records the values of 31.6 $^{\circ}\text{C}$ in the attacked leaves and of 32.5 $^{\circ}\text{C}$ in the plant leaves after treatments.

Linear regression performed between the photosynthesis intensity and leaf temperature show a good positive correlation between these; the coefficient of determination (R^2) is 0.92 for the attacked leaves and 0.97 for the plant leaves after treatments with fungicide. Linear regression made between the transpiration intensity and leaf temperature show a good positive correlation between these; the coefficient of determination (R^2) is 0.95 for the attacked leaves and 0.97 for the leaves plant after treatments with fungicide (Figs. 10; 11).

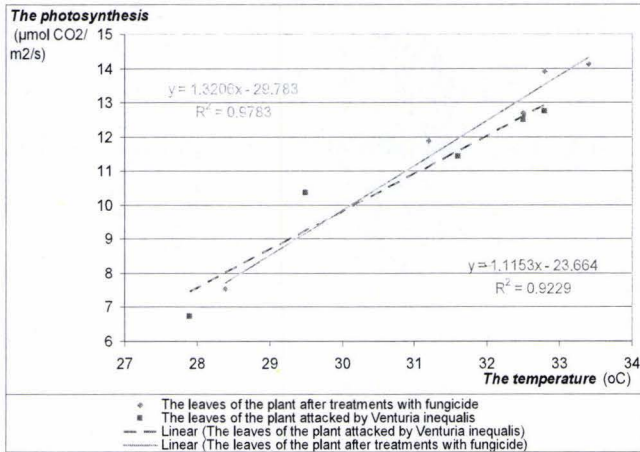


Figure 10. The correlation between the intensity of photosynthesis and the leaf temperature in *M. domestica* - Jonagold variety.

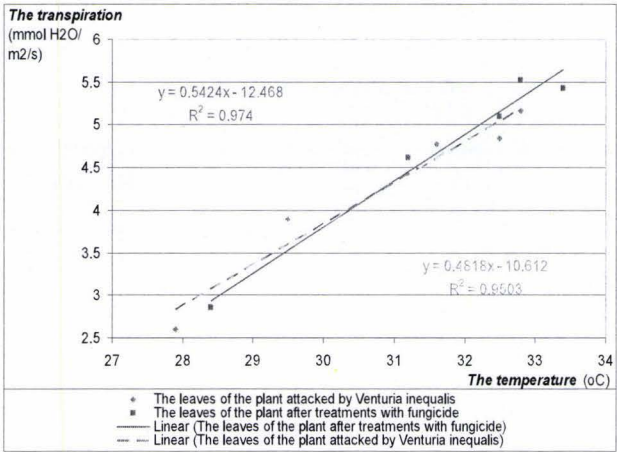


Figure 11. The correlation between the intensity of transpiration and the leaf temperature in *M. domestica* - Jonagold variety.

The **stomatal conductance** of CO_2 increases beginning in the morning (8 a.m.), when it records the values of $0.12 \text{ mol} / \text{m}^2 / \text{s}$ in the attacked leaves and $0.2 \text{ mol} / \text{m}^2 / \text{s}$ in the plant leaves after treatments with fungicide, their growth after lunch (12 a.m.), when it records the values of $0.32 \text{ mol} / \text{m}^2 / \text{s}$ in the attacked leaves and $0.45 \text{ mol} / \text{m}^2 / \text{s}$ in the leaves after treatments and decreases towards the evening (4 p.m.), when it records the values of $0.23 \text{ mol} / \text{m}^2 / \text{s}$ in the attacked leaves and $0.31 \text{ mol} / \text{m}^2 / \text{s}$ in the plant leaves after treatments.

Linear regression performed between the photosynthesis intensity and stomatal conductance of CO_2 shows a good positive correlation between these; the coefficient of determination (R^2) is 0.84 for the attacked leaves and 0.86 for the plant leaves after treatments with fungicide. Linear regression made between the transpiration intensity and stomatal conductance of CO_2 shows a good positive correlation between these; the coefficient of determination (R^2) is 0.73 for the attacked leaves and 0.79 for the leaves after treatments with fungicide (Figs. 12; 13).

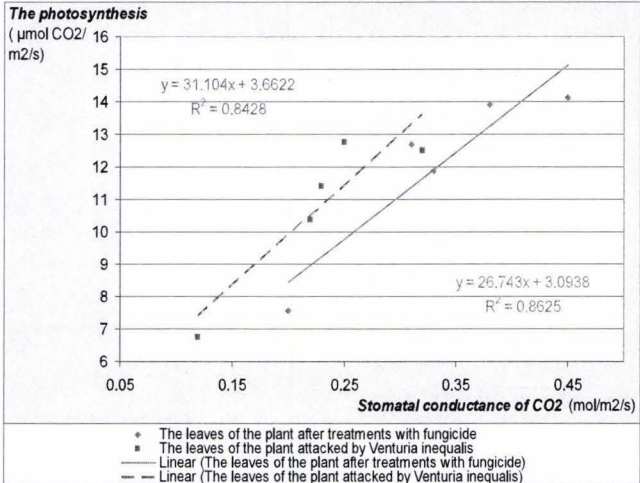


Figure 12. The correlation between the intensity of photosynthesis and the stomatal conductance in *M. domestica* - Jonagold variety.

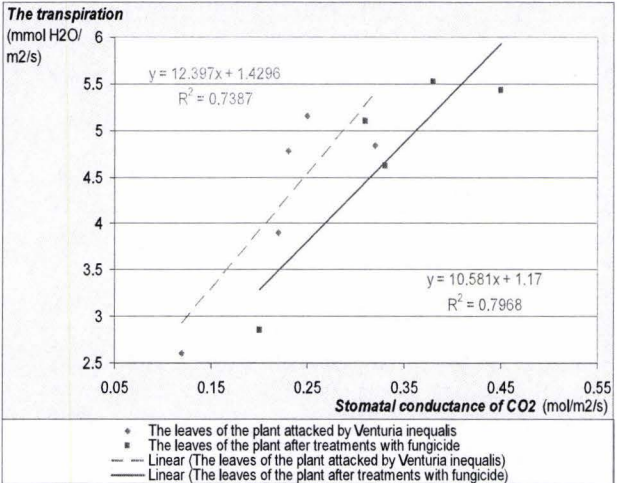


Figure 13. The correlation between the intensity of transpiration and the stomatal conductance in *M. domestica* - Jonagold variety.

In the leaves plant attacked by pathogen, in comparison with the leaves analysed after treatments, it is noticed a lower chlorophyll content by 11.80 % as a result of the reductions of biosynthesis chlorophyll and the deterioration of the chlorophyllian pigments (Fig. 14).

The leaves attacked by *V. inaequalis* present a lower water content by 2.08 % and a higher dry substance content by 4.23 % in comparison with the leaves analysed after the treatments with fungicide (Fig. 15).

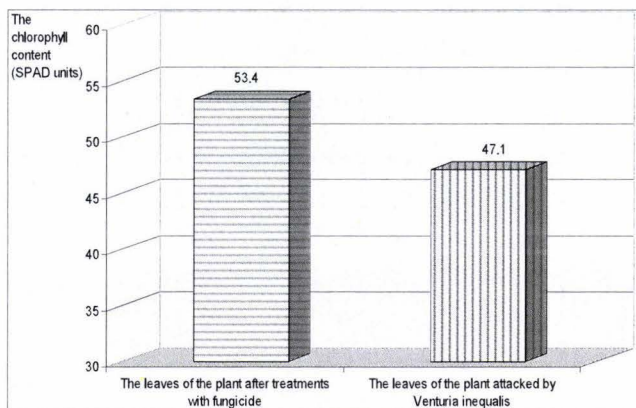


Figure 14. The chlorophyll content in leaves of *M. domestica* - Jonagold variety.

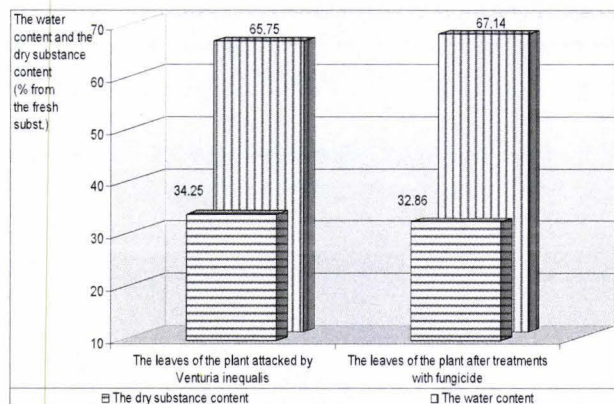


Figure 15. The water content and the dry substance content in leaves of *M. domestica* - Jonagold variety.

CONCLUSIONS

In *M. domestica* - Jonagold variety, it has been noticed that the dynamics of photosynthesis and transpiration, during the day, in the leaves attacked by *V. inaequalis* is similar to that in plant leaves analysed after treatments with fungicide **Dithane** M 45, but the recorded values are lower in the attacked plant leaves.

In the leaves attacked by the pathogen it was noticed that physiological processes intensity is lower as a result of the reduction leaf surface due to the formation of light gray spots or the olive brown spots, deterioration of the chlorophyll pigments and stomata coverage of the pathogen.

The chlorophyll content presents a lower value in attacked leaves, in comparison with the leaves analysed after treatments, as a result of the pathogen action. The attacked leaves present a lower water content and a higher dry substance content and this causes the hydric and metabolic imbalances.

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MORPHO-ANATOMICAL FEATURES OF THE VEGETATIVE ORGANS OF *Saponaria pumilio* (L.) FENZL EX A. BRAUN AND THEIR ECOLOGICAL SIGNIFICANCE

ANDRONESCU Alina, AIFTIMIE-PĂUNESCU Anca

Abstract. For a better understanding of the levels of plant adaptations to alpine environment a study on *Saponaria pumilio* root, stem and leaf morpho-anatomy was performed. Among the morphological adaptations we underline the small size of the plant, the horizontally extended root system, and the leaves arrangement in a acute angle. The structural described adaptations comprise thick cuticle, compact and homogenous mesophyll, and Krantz specific anatomy of leaf. This research recommends *S. pumilio* as an excellent model plant in studies of plant adaptations to alpine environment.

Keywords: *Saponaria pumilio*, morpho-anatomy, adaptation, ecology, alpine environment.

Rezumat. Caracteristicile morfo-anatomice ale organelor vegetative la *Saponaria pumilio* (L.) și semnificația lor ecologică. Pentru o mai bună înțelegere a nivelurilor de adaptare a plantelor la condițiile mediului alpin au fost efectuate studii privind morfo-anatomia rădăcinii, tulpinii și frunzei la *Saponaria pumilio*. Dintre adaptările morfologice subliniem dimensiunile reduse ale plantei, sistemul radicular extins pe orizontală și aranjarea frunzelor în unghi ascuțit. Adaptările structurale descrise se referă la cuticula groasă, mezofilul compact și omogen și la anatomia specifică de tip Krantz a frunzei. Acest studiu recomandă specia *S. pumilio* ca fiind un excelent model de studiu al adaptărilor plantelor la mediul alpin.

Cuvinte cheie: *S. pumilio*, morfo-anatomie, adaptare, ecologie, mediu alpin.

INTRODUCTION

Saponaria pumilio (L.) FENZL EX A. BRAUN, the pygmy pink, is a perennial species belonging to the Caryophyllaceae family. It is a caespitose plant forming hummocks on rocky slopes and screes on acid soils. The pygmy pink is known to be an endemic plant species to Europe (JALAS & SUOMINEN, 1988), being native to Eastern Alps and South Carpathian Mountains, Italy, Austria and Romania (TUTIN, 1996). Being mistaken for another taxon, *Saponaria pulvinaris* BOISS. (synonym with *S. pumilio* BOISS.), (BOISSIER, 1843, 1849), followed by an incorrect synonymization of this with *S. pumilio* (named *pumilio* by A. Braun after a description of E. Fenzl), the species is also reported from Turkey, Lebanon and Syria. Furthermore, it was mentioned, from North America on the Amanoosuc Ravine Trail of Mount Washington (MAGEE & AHLES, 1999) where it is believed to have been intentionally planted (introduced and naturalized). The species' occurrence in Romania refers to Făgăraș and Iezer Mountains (BELDIE, 1977).

Not only species distribution, but also taxonomic position was the subject of many controversial changes. The species was assigned first to *Cucubalus* genus as *C. pumilio* L., and after to other different genera like *Lychnis* as *L. pumilio* (L.) SCOP. or *Silene* as *S. pumilio* (L.) WULFEN., being finally transferred to *Saponaria* as *Saponaria pumilio* (L.) FENZL EX A. BRAUN, which is presently accepted as the valid botanical name (TUTIN, 1996).

One of the first biologists who systematically study variation in plant morphology along with environmental gradients was Gaston Bonnier. Since the late nineteenth century, Bonnier experimentally showed that alpine climate strongly modified the morphology and the structure of well adapted plant species (BONNIER, 1895). His pioneering research was an open door for many further studies.

The species subject of this study is very well adapted to specific climate condition as proved by the well established and numerous populations. Moreover the plant is successfully cultivated in alpine gardens. These considerations recommend *S. pumilio* as a model plant to investigate the morphological and structural levels of adaptation of herbaceous plant to alpine environments.

Studies concerning morpho-anatomical features of *Saponaria* genus are very few and refer to other species (ATAŞLAR, 2004; ÇINBINGEL et al., 2007) none of them from alpine environment. In Romania there are some references but from other related genera like *Silene* (TOMA, 1969; AIFTIMIE-PĂUNESCU, 2000).

MATERIAL AND METHODS

Plant samples were collected in late July 2012 from populations in flowering stage, naturally growing in Iezer Mountains (N 45° 27' 36.354" E 24° 57' 41.011"), at 2161 m high.

Fresh samples from the vegetative organs were fixed in a mixture (3:1) of ethanol and glacial acetic acid for at least 7 days and after in ethanol 70° until processed. Transverse cuttings from root, stem and leaves were made using a hand microtome and a botanical razor blade. Sections between 10-15 µm thick were double stained with 1 % w/v light green and 1 % w/v safranin and finally mounted in synthetic resin (Entellan). For surface view of the epidermis, peelings from the adaxial and abaxial surface of the leaf were coloured with 1 % w/v safranin and also mounted in Entellan. The used double coloration is applied for general plant tissues where lignified and cutinized walls appear bright red, cellulose walls green, and meristems light blue.

Histological observations were made with bright field microscopy, under a Nikon Eclipse E200 microscope, and micrographs were recorded with a Nikon Coolpix 5400 digital camera.

RESULTS AND DISCUSSIONS

As general morphology the plant is very dwarf forming hummocks up to 40 cm across but only a few (1-8) centimetres high (Figs. 1a, b).



Figure 1. *S. pumilio* habitus - a. general view, b. detail. (photo original)

Root

S. pumilo has a perennial and well developed root system, with slender and branched tap roots ranging from 0.5 to 4mm thick. In cross section the distal end of the root (Fig. 2a) shows an early secondary structure being covered by a thin periderm originated from pericycle and made by 3-4 layers of cells with suberized walls, whereas rhizodermis (epiblem), cortical parenchyma and endodermis are exfoliated. Under the periderm the ring of pericycle with bundles of primary phloem are visible.

The central part of the root is filled by primary xylem and in peripheral position a few secondary xylem vessels. At this level the rays are not distinguishable. The cross-section through the middle part of the root shows an advanced secondary structure with more peridermal layers, obvious secondary phloem and xylem, and parenchymatous rays with starch deposits (Fig. 2b).

Stem

The stem is short, measuring 5-7 mm (the sterile shoots), with very shorts (0.5-1.5 mm) internodes. Flowering stems are up to 20 mm long and bear a solitary flower. In cross section the stem has a typically caryophyllaceous structure (METCALFE & CHALK, 1965): epidermis with almost isodiametric cells with cutinized external walls (Fig. 3a), cortical assimilatory parenchyma (2-3 layers of cells at the basal level, and up to 5 layers in flowering stems), and central cylinder with a partially sclerified pericycle, vascular tissues (external phloem and internal xylem) and a central parenchymatous pith, complete not disorganized, even in fertile shoots.

The pith, mostly in sterile shoots, has big cells with numerous crystals of calcium oxalate and also smaller parenchymatous cells with starch deposits. (Figs. 3b, c). The structure of the basal node is more complex showing the leaf sheath of mature leaves, leaf primordia, and stem primary tissues (Figs. 4a, b, c).

Leaves

The leaves are opposite to each node and with grown together sheaths (Fig. 4a), linear, small, up to 2 mm wide, 3-10 mm long onto floral stem and 10-20 mm onto sterile shoots. Leaf margins shows some papillose cells (Figs. 5b,c) and the sheaths at node level bear trichomes (Fig. 5a). In cross section through the lamina (Figs. 6a, b), the upper and the lower epidermis comprise slightly tangentially elongated, to isodiametric cells with external walls covered by a thick cuticle.

The mesophyll appears relatively homogenous with palisade tissue towards both epidermis but more compact and with more elongated cells towards adaxial surface. A cross section of the midrib exhibits one main vascular bundle accompanied by 3-4 smaller ones. Vascular bundles are collateral made by adaxial xylem and abaxial phloem and

surrounded by a one-layered sheath of enlarged parenchymatous cells with chloroplasts. The cells from palisade extend radially from the bundle sheath cells. This distinctive leaf structure is known as **Kranz anatomy** and characterizes the C4 plants. Some cells from the mesophyll contain big crystals of calcium oxalate. The crystals are big enough to be visible thorough leaves transparency (Figs. 5b, c).

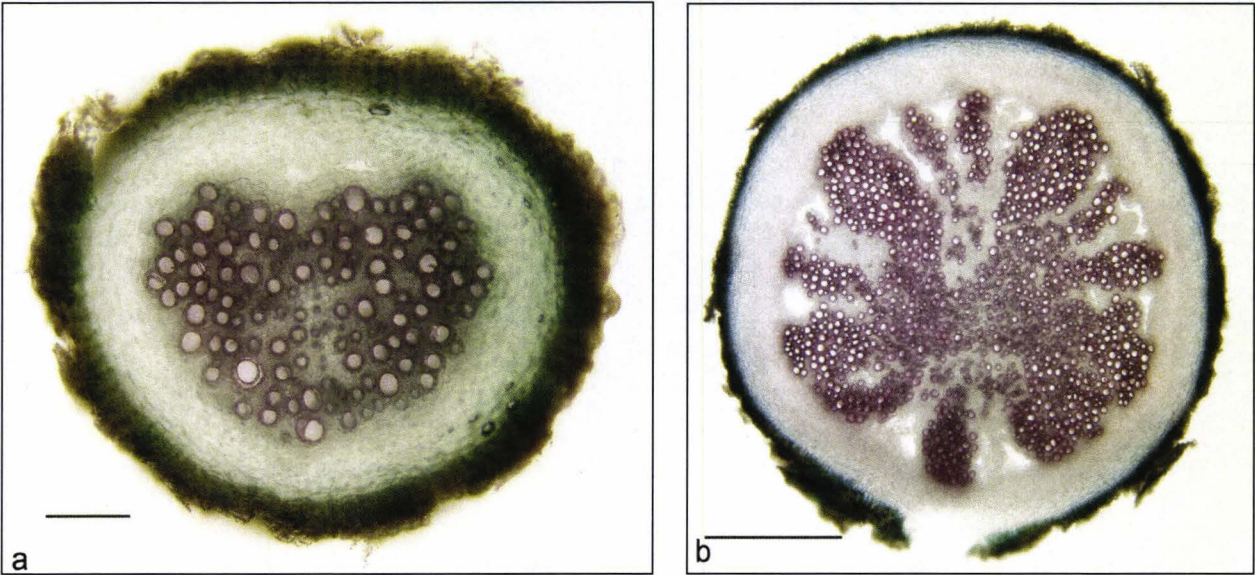


Figure 2. Root cross section. a - distal end (bar: 100μm);
b - middle part (bar: 500μm) (photo original).

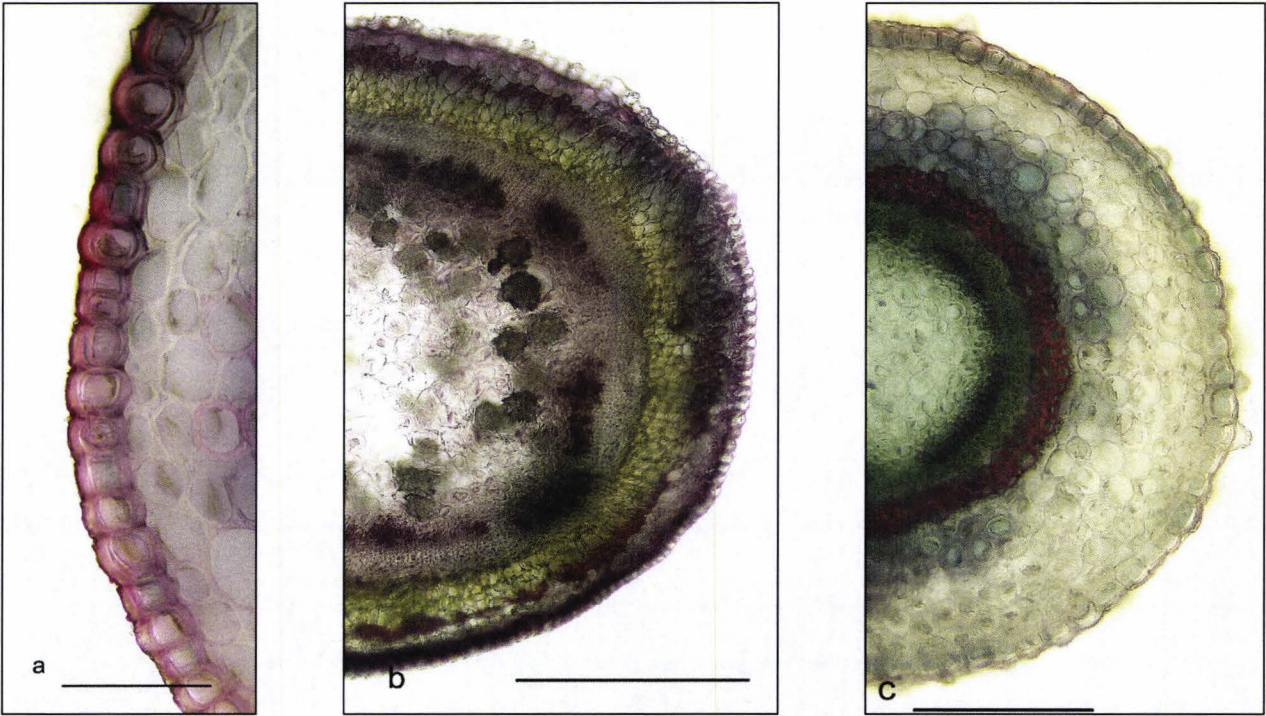


Figure 3. Stem cross section (internode). a – epidermis detail (bar: 50 μm);
b - sterile stem (bar: 250μm);
c - flowering stem (bar: 250μm) (photo original).

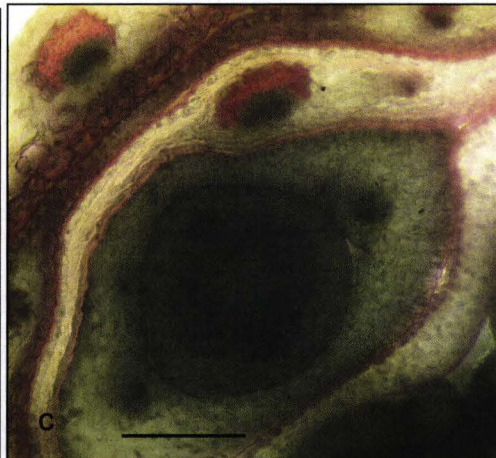
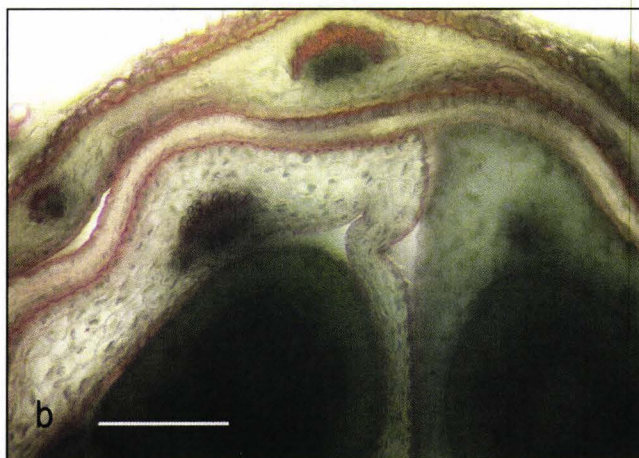


Figure 4. Stem cross section (basal node). a - general view (bar: 600 μ m); b , c - details (bar: 150 μ m) (photo original).

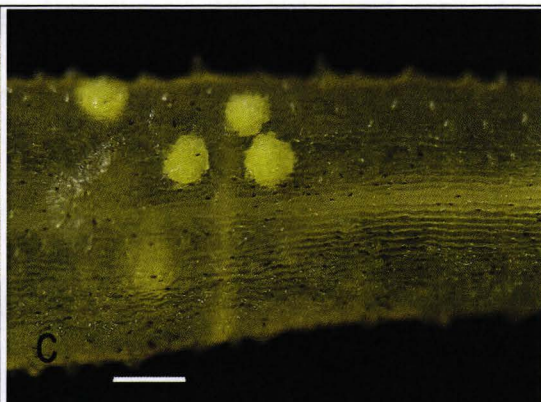
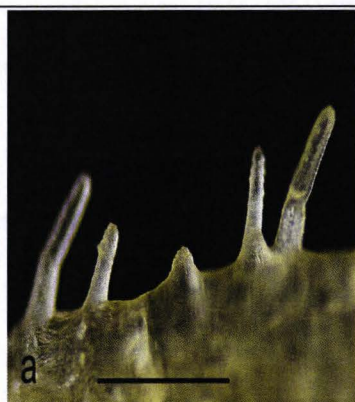


Figure 5. Leaf: a - trichomes on sheaths (bar: 400 μ m);
b , c - lamina with crystals of calcium oxalate (250 μ m) (photo original).

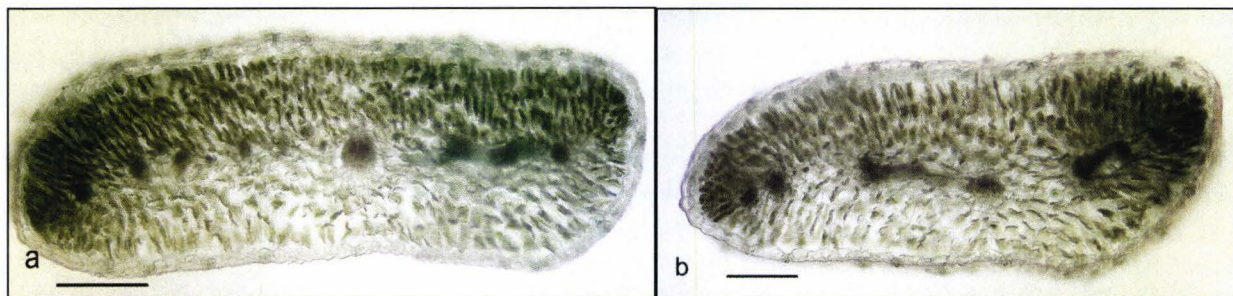


Figure 6. Leaf cross section: a - middle lamina (bar: 150 µm); b - base of lamina (bar: 150 µm) (photo original).

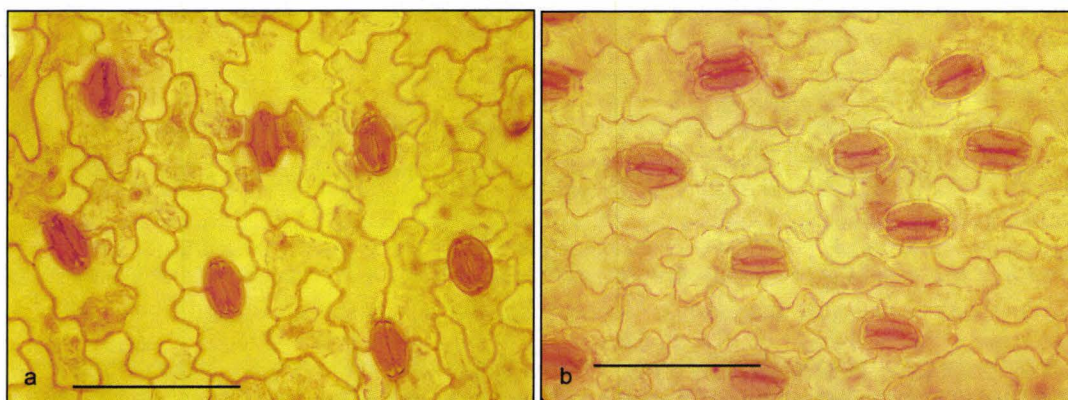


Figure 7. Leaf - surface view of epidermis: a - adaxial (bar: 100 µm); b - abaxial (bar: 100 µm) (photo original).

The transverse sections of different levels of lamina (middle, Fig. 6a and base, Fig. 6b) do not reveal significant structural modifications. In surface view, the epidermis is composed of cells with undulating walls with stomata mostly of anomocytic type and rare typically diacytic (caryophyllaceous). There are no notable differences in the epidermal cells between abaxial and adaxial surfaces (Figs. 7a, b).

CONCLUSIONS

The study on morpho-anatomical features of *S. pumilio* vegetative organs showed a series of adaptations specific to alpine plants. From the morphological ones the most obvious are: shorter stems, shorter internodes, and smaller leaves compare with other *Saponaria* species from lower altitude. Also, the well developed mainly horizontally extended root system that ensures plant fixation and an optimal water supply (especially pluvial). Leaves are orientated in a acute angle with the stem and this arrangement prevents heatstroke.

From the structural adaptation, at root and stem level we have noted the starch deposits which will ensure plant survival under harsh conditions.

At stem and leaves level there are also some notable adaptations as follows:

- thick cuticle that reduces water loss and plays a role in controlling surface temperatures by reflecting the incoming radiation;
- the arrangement of palisade tissue maximizes the efficiency rate of photosynthesis by placing cells at the optimum angle to the incoming sun rays;
- the relative homogenous and compact mesophyll is an adaptive response to high levels of incident light;
- leaf specific anatomy (**Krantz anatomy**) confers a greater photosynthetic efficiency by using the double-carbon fixation pathway.

Another structural adaptation to local conditions (substrate) is calcium deposits in form of calcium oxalate crystals (mainly in stem pith and leaf mesophyll) *S. pumilio* being a calcifuge species.

All these complex adaptation make *S. pumilio* an excellent candidate for a model plant in researches of mechanisms of plant adaptation to alpine climate.

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SPECIES TO THE LIMIT OF SPECIFIC SPREADING AREA IN ROMANIA:

Zingeria pisidica (BOISS.) TUTIN

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Abstract. *Zingeria pisidica*, of an uncertain origin in the Romanian flora, is considered to be an allochthonous plant, according to the occupied habitats and the increase of its spreading area in our flora, both in altitude and northward, in the last decades. From the eco-coenological point of view, it is a helophilic plant, sometimes found in undersalty micro-depressions, but which becomes dominant in habitats strongly influenced by human activity. It is present in different coenoses where the water factor is more or less in excess; therefore, the authors consider it to belong to *Potentillion anserinae*. The numerous and outstanding cohabitants are extracted from the phytocoenological tables in the specialty literature.

Keywords: *Zingeria pisidica*, chorology, eco-coenology, area, Romania.

Rezumat. Specii la limita de areal pe teritoriul României: *Zingeria pisidica* (BOISS.) TUTIN. *Z. pisidica*, de origine incertă în flora României, este apreciată a fi o specie alohtonă, după habitatele ocupate și după extinderea ariei de răspândire spre nord și altitudinal în flora noastră, în ultimele decenii. Ecocenologic este o specie helofilă de microdepresiuni, uneori subsăraturate, dar devine dominantă în habitate influențate puternic de activitatea umană. Este prezentă în diferite cenoze în care factorul hidric este ± în exces, de aceea autorii consideră că aparține de *Potentillion anserinae*. Coabitantele, numeroase și deosebite, sunt extrase din tabelele fitocenologice publicate în literatura de specialitate.

Cuvinte cheie: *Z. pisidica*, corologie, ecocenologie, areal, România.

INTRODUCTION

Zingeria pisidica (BOISS.) TUTIN (Poaceae) is a special plant in the flora of Romania, so this is maybe the reason why, at its characterization, there has been invoked more than once the term *endemit* and it has already been the object of detailed research (SORAN, 1951). The existence of this plant in the flora of the country reminds us of *Nitraria schoberi* L., as it occurs as an isolated island, only in Romania, at great distance westward from its original area, placed in Anatolia and the Caucasus. Until recently, it could be considered a *vulnerable plant* because it was present in a relatively small area in the southwestern part of the country, on the wet pasture land, including the Danube river meadow, so where human activity is intense. On the other hand, the plant has been recently reported in Moldova (RĂVĂRUȚ et al., 1960; MITTELU & BARABAȘ, 1975), in Dobrogea (ȘERBĂNESCU, 1965) and in Apuseni (RAȚIU, 1964). The fact that it has been recently noticed in the Romanian Flora, in areas more distant as compared to those of 90 years ago, when it was known only in Dâmbovița County (GRECESCU, 1898; PRODAN, 1923), makes us think that it could be an *adventive* plant, which expands its area, as it is the case of the recently discovered *Tragopogon graminifolius* DC. and some others, though it is not included in this category (SÎRBU & OPREA, 2011).

Its closest relative, *Zingeria biebersteiniana* (CLAUS) P. A. SMIRN. develops in Russia, on the lower courses of the Volga and the Don, and uncertain in Crimea (TZVELEV, 1976; RUBTOV, 1972).

People in Oltenia would call it "short field grass" (PĂUN, 1967a, 1967b). GRECESCU (1898) used for this plant the vernacular name "small grass".

MATERIAL AND METHODS

From the list of species with a limited spreading area in Romania, one of them, *Zingeria pisidica*, raised a lot of questions; we do not know for sure what geographic element it is, what ecological preferences it has or what kind of coenologic alliance it fits in. In the specialty literature, it is indicated the legitimate binomial, but the eco-coenology, which is variable, is to be deduced after reviewing several situations, particularly from synthetic tables where it can be found alongside species ranging from slightly halophilic to swamp ones. The chorology, within counties, comes from the literature (including the phytocoenological tables) and from the major collections in the country. The counties are alphabetically ordered. For the localities transposition on the map, where the presence of the plant was indicated, the network system UTM (Universal Transverse Mercator) was used, with UTM indexes (LEHRER & LEHRER, 1990). The herbariums were abbreviated according to Index herbariorum (THIERS, 2011), the nomenclature follows the CIOCĂRLAN (2009), TUTIN (1980), TUTIN et al., (1964-1976), and other works of taxonomy.

RESULTS AND DISCUSSIONS

Taxonomy

Zingeria pisidica (BOISS.) TUTIN 1978, Bot. J. Linn. Soc. 76: 365.

Syn.: *Agrostis pisidica* BOISS. 1854, Ann. Sci. Nat. ser 4(2): 255; Tarnavski 1947, Bul. Grăd. Bot. Cluj, 27: 16; Soran 1951, Stud. Cerc. Biol. Cluj, 2(1-2): 152; Zahar. 1955, in Herb.; - *A. densior* HACK. 1898 ex GRECESCU, Consp. Fl. Rom.: 603; - *A. trichoclada* GRISEB. var. *pisidica* (BOISS.) BOISS. 1884, Fl. Or. 5: 516; - *Milium trichopodium* BOISS. var. *poaeforme* BOISS. 1884, Fl. Or. 5: 511 (BOISSIER, 1884); - *Zingeria pisidica* (BOISS.) TUTIN subsp. *poaeforme* (BOISS.) M. DOĞAN 1982, Notes R. B. G. Edinb. 40: 86 (DOĞAN, 1982); - *Z. densior* (HACK.) CHRTEK 1963, Novit. Bot. Delect. Seminum Horti Bot. Univ. Carol. Prag.: 3.

Icon.: Nyárady 1931, Bul. Grăd. Bot. Cluj, 10: 197; Prodan 1939, Fl. Rom., 2: tab. 87; Beldie 1972, Fl. Rep. Soc. Rom., 12: Pl. 31.

Exs.: Fl. Rom. Exs. no. 924, 924b; Fl. Olt. Exs. no. 241.

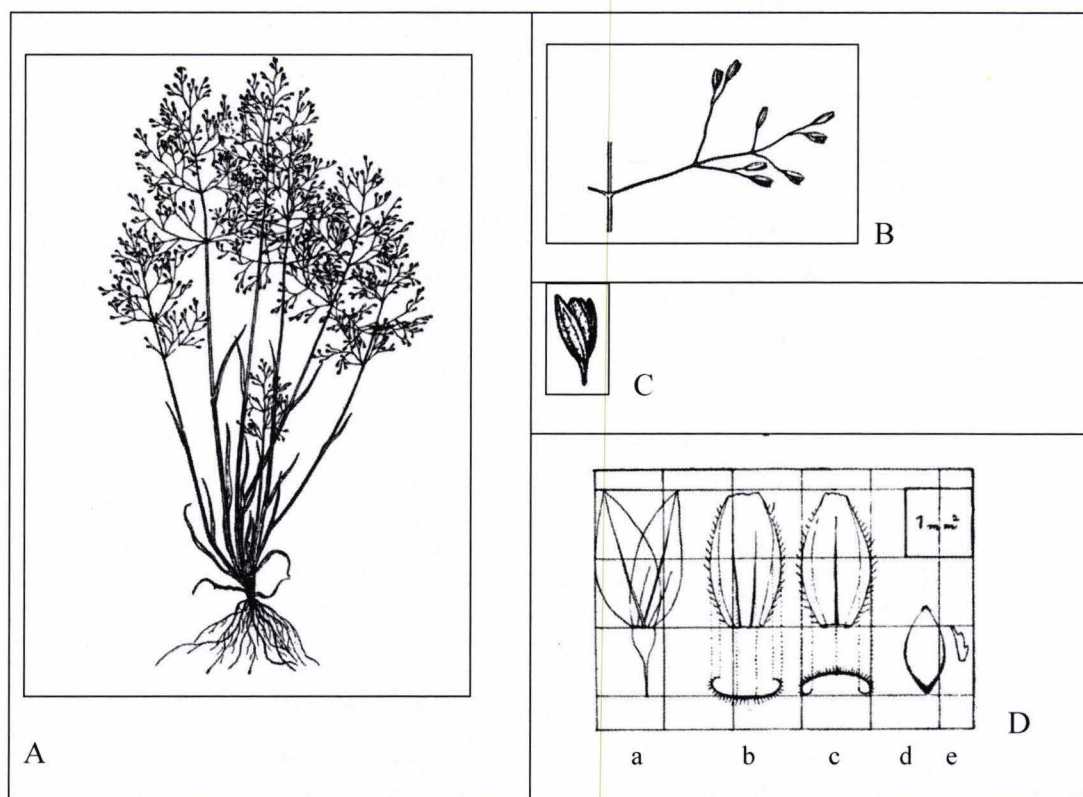


Figure 1. *Z. pisidica*: A - Habit, B - Tricuspid branch of panicle, C - Spikelet (after PRODAN, 1939), D - Spikelet analysis: a - clavate pedicel and glumes, b - palea, c - lemma, d - fruit, e - lodicule (after NYÁRADY, 1931).

Although GRECESCU (1898) was surprised to find about the presence of this plant, he correctly framed it for that time, but temporary, to *Agrostis* (*A. divaricatissima*) and appealed to A. Degen (Budapest), who did not decide upon the identity of the plant and sent it in Austria, to E. Hackel, who named it *A. biebersteiniana* CLAUS var. *densior* HACK. GRECESCU was not totally satisfied and considered it an endemic plant of the Romanian Plain, as *A. densior* (HACK.) GRECESCU. He was right in doing this because *A. biebersteiniana* has convolute leaves (not flat), the branches of the panicle are under an acute angle (not horizontal), spikelets are under 1.5 mm (not 1.8 mm) and it displays only hairy dorsally lemma (not hairy lemma and palea).

For a long time, the plant was classified under the genus *Agrostis*, according to uniflorous spikelets and the appearance of the panicle, although some characteristics differentiate it from the other species of the genus. First of all, it is an annual plant, with all the fertile shoots at flowering (the others are perennial), the glumes are acute (not acuminate), the palea is subequal with the lemma (not obvious shorter or more rudimentary), it has short hairy mature lemma and becomes stiff and bright as it is in *Milium*, separated by the quality of being hairy (not glabrous, herbaceous and dull), the branches of the panicle are smooth and regularly trifurcate, obviously clavate to the end (not \pm scabrous, irregularly divided and weak clavate) (Fig. 1). For these reasons, it was recently transferred to *Zingeria* (CHRTEK, 1963) with the specific epithet known in the Romanian literature and then unified with the one in Western Asia (TUTIN, 1978). However we should point out that TARNAVSKI (1947) was the first Romanian botanist who assigned the material from Romania to that of Asia and the Caucasus, then SORAN (1951) and ZAHARIADI (1955, in herbarium, with some uncertainty).

Area

The first one who dealt with the presence of the species *Z. pisidica* in the Romanian flora was, as I said, GRECESCU (1898), if we refer to its identity, not to its origin, so maybe that is why PRODAN (1923) and later BUIA (1959) consider it *endemit*, SĂVULESCU (1933), *very rare endemit*, and BORZA (1947), *local sub-Charpatian endemit* in

Muntenia, since they did not connected it to its basic area. NYÁRÁDY (1931) argues the same thing, that "our plant is one of the most interesting and important *endemisms*". At that time, it was indeed very rare. Other authors (SORAN, 1951) believe that it is an error to characterize this plant as an endemit.

We should emphasize that the plant lacks from the flora of the neighbouring countries. The fact that an annual plant was initially identified in the ditches, then in the cereal crops, so, in \pm anthropogenic habitats, that it appears only in Muntenia and then in Oltenia and that its basic area is in the Western Asia, convinces us to suspect it as being an **adventive** plant. The geographical type indicated in Turkish Flora (DOĞAN & MILLI, 1985) is Irano-Turanian. If the plant had been for a long time in our country, it could have spread, but it has spread only in the last 80-90 years. It might have been brought by the Turkish or by the birds, no one knows. We do not have solid evidences that the plant is adventitious, but an autochthonous plant does not spread so fast. This species is present in anthropogenic habitats, even if at first it was discovered in ditches. We are not sure about its mentioning in Moldova (RĂVĂRUȚ et al., 1960), if we do not have material for the herbarium, but it has been noticed again after 15 years (MITTELU & BARABAȘ, 1975), it is the same thing with the reference from Dobrogea (ȘERBĂNESCU, 1965) and especially from Stâna de Vale (RĂȚIU, 1964). Nevertheless, we consider them to be valid information, as they come out in favour of the idea of being an adventive plant, which is gradually expanding nowadays in front of us in various eco-coenological conditions, which is not too notable to endemic species.

The habitat characterization of the species in Romanian literature is varied: **Pontic-Mediterranean, Therophyte, Beckmannion, Agrostion** (POPESCU & SANDA, 1998), **Romania, Anatolia, Caucasus, Therophyte** (CIOCĂRLAN, 2009), **Romania** (ALEXIU, 2011), **Dacian-Anatolia** (POPESCU et al., 2001). Therefore we launch the idea that *Z. pisidica* could be **adventive** in the flora of Romania, taking into account the reasons indicated in the previous paragraph.

Ecology

"In damp places that retain rain water, ditches, pits, near earth roads" (GRECESCU, 1898) or "Ditches which hold rain water" (PRODAN, 1923). It has been more recently noticed in the "micro-depressions in rye fields and on the road between parcels" (POPESCU, 1981), then in the marshy meadow of the Prut River (MITTELU & BARABAȘ, 1975). It is hardly found in the mountain lawns, 1100-1600 m altitude (RĂȚIU, 1964, 1973). Therefore, we should conclude that it is a species that can be found in marshy places within meadows, on depression podzols, so a Helophilic Therophyte in expansion; being an annual plant, it develops predominantly in \pm anthropogenic habitats or with weak competition. Many samples in the herbarium are collected in the month of May, so one can draw the conclusion that the plant is vernal and does not seem to be a thermophilic one, as we assumed according to the old chorology in the southwestern part of Romania. For its extension, Transylvania seems to be the only place left, corresponding to its water requirements, but with slightly lower temperatures, taking into account its sub-thermophilic characteristic.

Coenology

First of all, we have to record the opinion of PRODAN (1939), "a particularity of the Danubian Plain can be considered the *Agrostis densior* gramineae, which has been found, so far, in the ditches that retain rainwater and in damp places: between Ionești, Morteni and Vultureanca (Dâmbovița County) and at Tâmburești in Dolj County". He includes this plant within the steppe class.

From many other studies (SANDA et al., 2001; DOLTU et al., 1984; ȘERBĂNESCU, 1965, 1971, and others), we could extract numerous cohabitant species and some associations *Z. pisidica* is part of. This is an annual species, with habitats of excessive humidity, where it becomes dominant, being also present in various meadows of **Agrostion** (*Alopecuretum pratensis*, *Poetum sylvicolae*, *Agrostetum stoloniferae*) as well as in the light salty one of **Beckmannion** (*Beckmannietum eruciformis*, *Trifolietum angulati*) and more rarely in the acute salty ones of **Scorzonero-Juncion gerardi** (*Caricetum divisa*) and in the mountain meadows (**Cynosurion**) as a random plant.

Z. pisidica, in the association of *Beckmannia eruciformis*, cohabits with:

Agrostis stolonifera, *Aster tripolium*, *Carex melanostachya*, *Juncus gerardi*, *Lepidium latifolium*, *Puccinellia distans*, *Ranunculus sardous*, *Trifolium fragiferum* (POPESCU, 2003), although we do not consider it a typical halophilic plant, tolerant at the most. According to this author, one concludes that *Zingeria pisidica* would grow at Frasinu and Spătaru (Buzău County), which is not true, because no botanist who studied these forests has ever recorded that species (Dihoru, mns. 2012).

In the associations of *Poa sylvicola*, *Agrostis stolonifera*, *Alopecurus pratensis* and *Pholiurus pannonicus* (BUIA, 1959; BUIA et al., 1960; PUȘCARU-SOROCEANU et al., 1963) it cohabits with: *Agrostis stolonifera*, *Allium vineale*, *Alopecurus pratensis*, *Bromus commutatus*, *Carex spicata*, *C. distans*, *C. divisa*, *C. hirta*, *C. melanostachya*, *C. ovalis*, *C. vulpina*, *Centaurea calcitrapa*, *Cynodon dactylon*, *Daucus carota*, *Eleocharis palustris*, *Festuca arundinacea*, *F. pratensis*, *Holosteum umbellatum*, *Hordeum bulbosum*, *H. geniculatum*, *Lepidium ruderalis*, *Lolium perenne*, *Lotus corniculatus*, *Matricaria recutita*, *Medicago arabica*, *M. hispida*, *M. lupulina*, *M. rigidula*, *Melilotus officinalis*, *Mentha pulegium*, *Myosurus minimus*, *Oenanthe banatica*, *Pholiurus pannonicus*, *Plantago lanceolata*, *P. major*, *P. media*, *Poa bulbosa*, *P. pratensis*, *P. sylvicola*, *Potentilla reptans*, *Ranunculus bulbosus*, *R. repens*, *R. sardous*, *R. sceleratus*, *Rorippa austriaca*, *R. sylvestris* subsp. *kernerii*, *Rumex crispus*, *Salvia nemorosa*, *Taraxacum officinale*, *Trifolium angulatum*, *T. campestre*, *T. fragiferum*, *T. hybridum*, *T. incarnatum* subsp. *molinerii*, *T. michelianum*, *T. montanum*, *T. pallidum*, *T. retusum*, *T. pratense*, *T. repens*, *T. resupinatum*, *T. squamosum*, *T. striatum*, *Ventenata dubia*.

Also present in the meadows of Oltenia, *Zingeria pisidica* is recorded as appearing in the company of the following species, without knowing whether they cohabit: *Agrostis capillaris*, *Alopecurus pratensis*, *Artemisia santonicum*, *Cyperus longus*, *C. serotinus*, *Goniolimon besserianum*, *Dasyphyrum villosum*, *Crypsis alopecuroides*, *Hordeum geniculatum*, *Medicago arabica*, *M. hispida*, *Orchis elegans*, *O. militaris*, *Scorzonera laciniata*, *Polygala comosa*, *Trifolium dubium*, *T. incarnatum* subsp. *molinierii*, *T. ochroleucon*, *T. ornithopodioides*, *T. resupinatum*, *Ventenata dubia* (BUIA et al., 1961).

One can read about the detailed and actual coenotic relationships of the species *Zingeria pisidica* in the vast synthetic tables published by ȘERBĂNESCU (1965), from which we learn that it participates in the associations of *Beckmannia eruciformis*, *Pholiurus pannonicus*, *Trifolium angulatum* and *Carex divisa*, that is in light salty communities, with excessive humidity. We could have the following synthesis of the relationships of the species with the main cohabitants in these salty associations: *Agrostis stolonifera*, *Alopecurus pratensis*, *Artemisia santonicum*, *Eleocharis palustris*, *Hordeum marinum*, *Mentha pulegium*, *Poa sylvicola*, *Ranunculus repens*, *Rorippa sylvestris*, *Trifolium angulatum*, *T. fragiferum*, *Zingeria pisidica* and **more rarely**, with *Beckmannia eruciformis*, *Bromus commutatus*, *Carex divisa*, *C. melanostachya*, *Lolium perenne*, *Matricaria recutita*, *Myosurus minimus*, *Oenanthe fistulosa*, *Pholiurus pannonicus*, *Plantago lanceolata*, *P. major*, *P. tenuiflora*, *Poa bulbosa*, *P. pratensis*, *Potentilla reptans*, *Puccinellia distans*, *Ranunculus repens*.

If we confine to *Zingerietum pisidicae*, described according to the dominance of the species, at Tâmburești, Filași, Desa, Nebuna (BUIA et al., 1960), Breasta (BUIA et al., 1960; CÎRTU, 1971) and Calopăr, Radovan, Podari, Radovan - Podari (CÎRTU, 1971) the cohabitants are also helophilic plants: *Alopecurus aequalis*, *Alisma gramineum*, *Lolium perenne*, *Lotus corniculatus*, *Lythrum hyssopifolia*, *Mentha pulegium*, *Pholiurus pannonicus*, *Plantago lanceolata*, *Poa sylvicola*, *P. pratensis*, *Ranunculus lateriflorus*, *Rorippa austriaca*, *R. sylvestris* subsp. *kernerii*, *Trifolium fragiferum*.

From the coenotic point of view, the species would belong, according to some authors, to *Al. Beckmannion*, to others to **Agrostion**, but as an annual species, growing on lands with temporarily excessive humidity; according to the presence of some non-halophilic cohabitants, one could frame it within **Nanocyperion** (possibly equivalent of *Myosuretum minimi* or of *Pholiuro-Plantaginetum tenuiflorae*). However, the cohabitants *Agrostis stolonifera*, *Carex hirta*, *Juncus tenuis*, *Plantago major*, *Poa annua*, *Potentilla reptans*, *Ranunculus repens*, *R. sardous*, *Rorippa sylvestris* subsp. *kernerii*, *Rumex crispus*, *Trifolium resupinatum*, *T. fragiferum* lead us to **Agropyro-Rumicion**, thus belonging to associations of perennial, hygrophilic plants, within light salty lands (*Beckmannion*) or not (*Agrostion*). The transfer made by POPESCU (1974, 1981) might seem successful but this alliance is no longer used, so in our view, this could have the following framing: **Potentillion anserinae** Tx. 1937 (*Potentillo-Polygonetalia* Tx. 1947, *Molinio-Arrhenatheretea* Tx. 1937). The closest view to ours belongs to CÂRTU (1979) who includes *Zingerietum pisidicae* in *Molinio-Arrhenatheretea*, *Molinietalia*, *Agrostidion albae* and mentions the species in helophilic associations (*Poetum sylvicolae*, *Alopecuretum pratensis* and *Agrostetum caninae*) where there is a high frequency of the species: *Agrostis canina*, *A. stolonifera*, *Alopecurus aequalis*, *A. pratensis*, *Festuca pratensis*, *Galium palustre*, *Lotus corniculatus*, *Phleum pratense*, *Poa sylvicola*, *Ranunculus repens*, *Trifolium incarnatum*, *T. repens* (CÂRTU, 1979).

A special situation is given by the Prut Meadow (MITITELU & BARABAȘ, 1975). The main co-dominants, *Alisma plantago-aquatica*, *Epilobium parviflorum*, *Galium palustre*, *Glyceria notata*, *Lycopus europaeus*, *Lythrum salicaria*, *Myosotis scorpioides*, *Berula erecta*, *Veronica anagallis-aquatica*, *V. beccabunga*, suggest other coenologic framing: **Sparganio-Glycerion fluitantis** BR.-BL. & SISS. 1942 (*Nasturtio-Glycerietalia* PIGN. 1953, *Phragmiti-Magnocaricetea* KLIKA 1941).

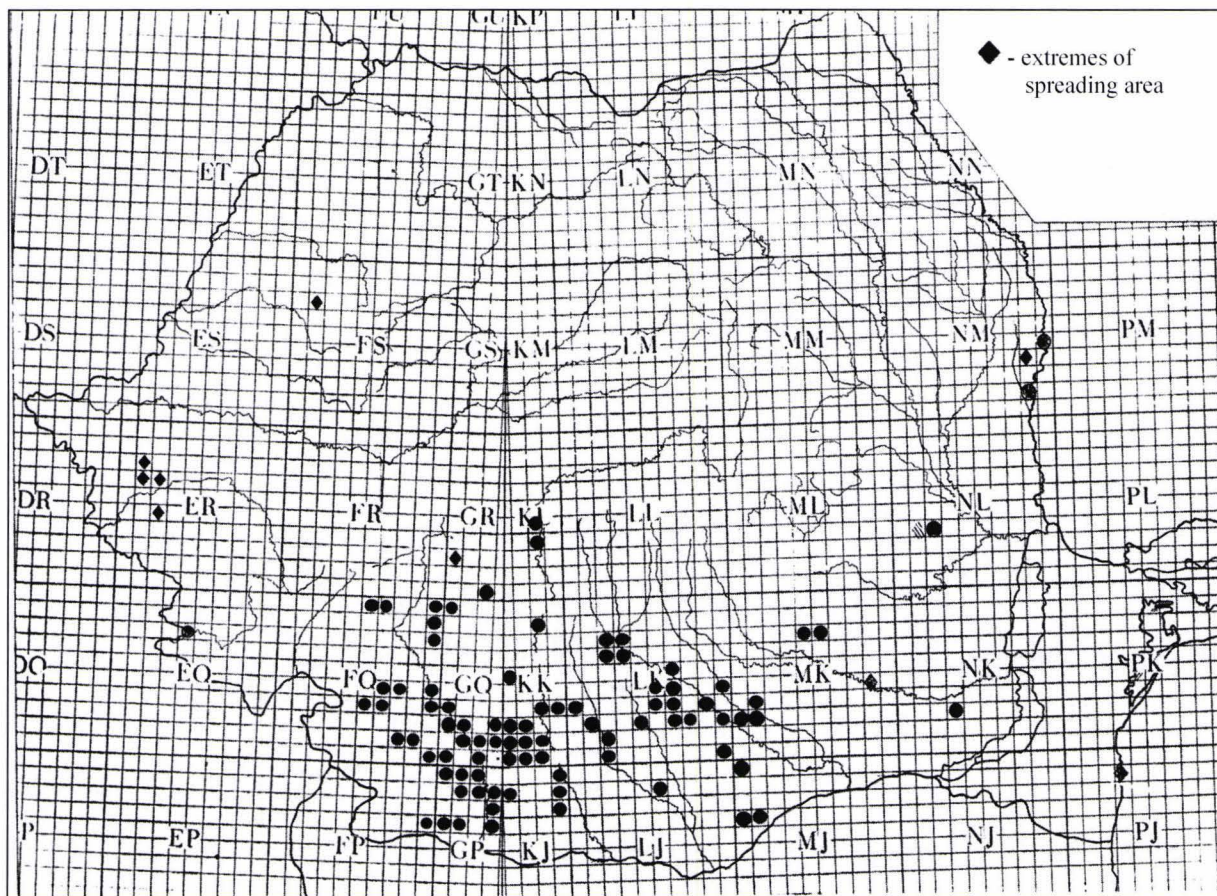
The most bizarre chorological and coenological state is to be found in *Festuco rubrae* - *Agrostetum capillaris* HORV. 1951 from Stâna de Vale, where it cohabits with *Festuca rubra*, *Deschampsia cespitosa*, *Alchemilla glaucescens*, *Potentilla erecta*, *Campanula abietina*, *Nardus stricta*, *Arnica montana*, *Achillea distans*, *Vaccinium myrtillus*, *Cynosurus cristatus* (RĂȚIU, 1964), so **Cynosurion** Tx. 1947 (*Arrhenatherethalia* PAWL. 1928, *Molinio-Arrhenatheretea* Tx. 1937). This point is reinforced by the herbarium material collected in Parâng Mountains, at 1600 m alt. (Leg. M. Păun 1956) [CRAI].

The name was updated in *Zingerietum pisidicae* BUIA et al., 1959 em. Cîrtu 1971 from the *As. Agrostis densior* BUIA et al., 1959.

Distribution in Romania

The administrative reorganization changed the counties of many places given in the literature.

It was originally identified in Ionești-Morteni-Vultureanca, in Dâmbovița County (GRECESCU, 1898), a place also mentioned by different botanists, as in "Distr. Dâmbovița Rarity" (PRODAN, 1923) or "very rare" (SĂVULESCU, 1933). Much later, it received the same qualifier for Oltenia, "great rarity" (BUIA et al., 1961). In 1929, it was so rare that E. NYÁRÁDY hardly found at Tâmburești enough copies for exsiccata, and SORAN (1951) mentioned it in 4-5 places. Over the years, it has been identified throughout the southern part of the country, from Timiș up to Constanța, including two remote counties, but those choronyms do not appear anywhere, not even in the so-called Addenda (NEGREAN, 2011). *Z. pisidica* is now known in the flora of Romania in 115 places, transposed on the map (Fig. 2):

Figure 2. Distribution of *Z. pisidica* in Romania (original).

Argeș County: Mărgineni “At Canton” (M. Toma, June 10, 1966) [IAGB 2418]; Bucovu (BELDIE, 1972; ALEXIU, 2011); Dâmbovicului Meadow at Negrași (NEGREAN, 1968); Albota in the rivulet meadow with the same name (June 7, 1955) [I 65197]; Pitești, edge of a swamp (June 11, 1955) [I 65198]; Ștefan cel Mare (Leg. P. Diaconu, Det. D. Parascan) [BVS].

Bihor County: Stâna de Vale (RĂȚIU, 1964, 1973).

Caraș-Severin County: Petriloa (“Petrila”) (SORAN, 1951; BELDIE, 1972).

Constanța County: West Eforie (ȘERBĂNESCU, 1965).

Dâmbovița County: between Ionești, Morteni and Vultureanca (SĂVULESCU, 1933; SORAN, 1951; ALEXIU, 2011); Conțești de Sus, Ionești (BELDIE, 1972); Cacova, pond in the Neajlov Meadow (June 19, 1963), [I 87372-3]; Corbii Mari at Corbii Ciungi (Leg. G. Dihoru, May 24, 1981) [BUCA 138359].

Dolj County: Obedin (BELDIE, 1972; CĂRȚU, 1973); Apele Vii, Bistrețu Nou, Cârcea, Raeți, Toceni (BELDIE, 1972); Mofleni (BUIA, 1959; BELDIE, 1972); Dobrești (BUIA, 1959; BELDIE, 1972); Sadova (BUIA, 1959; BELDIE, 1972; ȘERBĂNESCU, 1965); Preajba (BELDIE, 1972); the pond Craiovița, Breasta in the village Marioara, Coțofenii din Față (BUIA, 1959; BUIA et al., 1960, 1961; BELDIE, 1972); Filiași, Glod (BUIA et al., 1960, 1961); Lascăr Catargiu - Craiova (Leg. M. Răvărut, May 15, 1958) [IASI 7842]; Craiova (SORAN, 1951); Livezi, Malu Mare (BUIA et al., 1959; BUIA et al., 1960, 1961; BELDIE, 1972); Murta (BUIA et al., 1960, 1961; BELDIE, 1972); Lișteava, Măceșul de Sus, Popânzălești (BUIA et al., 1960, 1961); Popoveni 80 m (Leg. M. Păun, M. Olaru, June 10, 1961, FOE 281) [IASI 7839; IAGB 6645; I 46625; BVS 1195; BUCA 60982, 156328] (BUIA, 1959; BELDIE, 1972); Răcari (BUIA, 1959; BUIA et al., 1960, 1961); Rojiște (BUIA et al., 1960, 1961; ȘERBĂNESCU, 1965); Tâmburești near the Jiu, 40 m (Leg. E. Nyárády, June 2, 1929, FRE 924) [I 46623; IASI 7843; BVS 1180; SIB 129362; BUCA 42990, 67657], (SĂVULESCU, 1933; SORAN, 1951; BUIA, 1959; BUIA et al., 1960; ȘERBĂNESCU, 1965; BELDIE, 1972; CĂRȚU, 1973); Tâmburești – Piscul Sadovei, Segarcea on Valea Rea (BUIA et al., 1961); Tântăreni, Vârâți (BUIA et al., 1960, 1961); Căciulești (ȘERBĂNESCU, 1965); Calopăr, Radovan, Radovan - Podari, Breasta (CÎRȚU, 1971); Podari (BUIA, 1959; BUIA et al., 1960, 1961; CÎRȚU, 1971; BELDIE, 1972); Bojoiu, Robănești and Drăgotești in the meadow of the Teslui (PĂUN, 1967a); the area between the Jiu-the Desnățui-Craiova and the Danube (CĂRȚU, 1979); Ciutura (Leg. M. Păun 1957) [CRAI]; Țuglui (Leg. A. Buia, V. Năzdrăvan 1955) [CRAI]; Almăj (Leg. A. Buia, M. Păun 1956) [CRAI]; Pielești (Leg. I. Crețu 1969) [I 21095]; Sâlcuța, Panaghia, Valea Rea (CĂRȚU, 1973); Bratovoiești (G. Dihoru!).

Gorj County: Săcelu, Gornovița, Arcanu (BELDIE, 1972); the Gilort Basin (ZAHARIA, 1972); Bengești-Ciocadia (G. Popescu, ined); Parâng Mountains (Peaks Cioara, Bălescu, Mușetoiu, 1600 m) (Leg. M. Păun 1956)

[CRAI]: Câmpul Mare close to Cărbunești (Leg. M. Păun, C. Maloș 1962) [CRAI]: the Sadu Valley, 600 m alt. (Leg. M. Păun & al. 1963) [CRAI].

Ialomița County: T. Vladimirescu, saucer in the Forest Stejeret (Leg. G. Babaca, May 22, 1963) [I 67143].

Ifov County: Bălănoaia, Milcovățul, Stănești, Trestieni (BELDIE, 1972); Cartojani, southwest from the forest with the same name (Leg. N. & Ș. Roman, May 16, 1963) [I 39736-38]; Chiajna, in the Dâmbovița meadow (May 20, 1957) [I 65200]; Crevedia (Leg. E. Nyárády, June 1, 1929) [SIB 129361]; the forest Malu Spart (Leg. G. Negrean, July 19, 1971) [BUCA 139280].

Mehedinți County: between Ciochiuța (Strehaia) and Tâmba (BUIA et al., 1961); Gvardinița, depression in a wheat crop (June 29, 1956) [I 65203]; Croica, in the Olt Meadow (Leg. N. & Ș. Roman, May 18, 1956) [I 66771-2 and 66793-95].

Olt County: Baldovinești, Pietrișu in the Geamărtălu Meadow (PĂUN, 1967a, 1967b; BELDIE, 1972); Bobicești in the Olteț meadow (PĂUN, 1967a); Bălți, Fărcașu, Găvănești, Ghimpași, Hotărani, Icoana in the Călugărească Forest, Olari, Romula, Valea Satului, Vlădila (BELDIE, 1972); Pârșcoveni (G. Popescu, ined.; BELDIE, 1972); Criva de Jos, in the Olt Meadow (May 16, 1958) [I 65204]; Piatra Olt, in meadows (Leg. V. Soran, May 20, 1958) [I 66777]; Văleni, northeast of Ciuperceanca Forest (Leg. G. Turcu, May 26, 1960) [I 66786-66788-91]; Slatina to Curtișoara (Leg. A. Popescu, V. Sanda, May 29, 1974) [BUCA 126191]; Romula (Reșca) (Leg. M. Păun, M. Pop 1955) [CRAI]; Saru forest (Leg. D. Răduțoiu, D. Dumitriu 2006) [CRAI].

Prahova County: Valea Tolii 3 km southwest of Fulga; Parepa (ȘERBĂNESCU, 1965).

Teleorman County: Rădoiești Station, low salty places near the railway (Leg. C. Zahariadi, June 10, 1955, under *Agrostis pisidica* an *A. densior* ?) [BUCA 36437]; Tecuci (BELDIE, 1972; Leg. G. Turcu, May 26, 1960, [I 65201, 66787]).

Timiș County: Liebling, at "Pusta", 94-97 m (Leg. V. Soran, May 1, 1951) [I 46624; IAGB 6644; BVS 1194; SIB 103759; 119462, 122292] (SORAN, 1951; BELDIE, 1972); Timișoara (SORAN, 1951; BELDIE, 1972).

Vaslui County: Berezeni in the Prut Meadow (RĂVĂRUȚ et al., 1960; BELDIE, 1972), Cârja or more exactly Vetrișoia (MITITELU & BARABAȘ, 1975). These authors (MITITELU & BARABAȘ, 1975) indicate four phytocoenosis where *Zingeria pisidica* could grow (Cârja, Vetrișoia, Cristești and Trifești); we focused on the first two, more closely to Berezeni, where it was mentioned for the first time.

Vâlcea County: Slătioara (ȘERBĂNESCU, 1965); Băbeni in Romani Village (POPESCU, 1981); the lake Robești (June 11, 1954) [I 65202].

Vrancea County: - The forest Proca Mare - Ciorăști (ȘERBĂNESCU, 1965).

CONCLUSIONS

Z. pisidica, in the Romanian flora, is considered to be an allochthonous plant. Being an annual plant, it develops predominantly in \pm anthropogenic habitats or with weak competition. It is present in different coenoses where the water factor is more or less in excess; therefore, the authors consider it to belong to *As. Potentillion anserinae* Tx. 1937.

In this paper, there are also presented the chorology information from literature, collections, and personal observations; they are included in a map. *Z. pisidica* is now known in the flora of Romania in 115 places.

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GERMINATION, UPSHOT AND GROWTH OF HUNGARIAN AND TURKEY OAK SEEDLINGS IN THE WOODLANDS OF THE WESTERN PART OF THE GETIC PLATEAU

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Abstract. The climatic changes of the last decades have affected the forest ecosystems. Among these species, the most affected have been the Hungarian oak (*Quercus frainetto* TEN) and the Turkey oak (*Quercus cerris* L.), which are widespread (76%) in the sample area (BERCEA, 2007; BERCEA & COJOACĂ, 2012). In addition to the mass drying of these two species, the natural regeneration process underlying forest sustainability and perpetuation of species in the areas occupied was also affected. In order to adjust the regeneration process to the climatic changes, intensive research was carried out on the germination, upshot and growth of seedlings in the envisaged areas. The Hungarian oak and the Turkey oak are the only forest species to take full advantage of the fertile clay, heavy, compact soils (Preluvissols, Luvisols -74.5%) prevailing in the Getic Plateau (BERCEA, 2007; BERCEA & COJOACĂ, 2012). Research has shown that Hungarian oak acorns germinate much faster than Turkey oak acorns, even from the autumn when fructification occurs. As a rule, the germination of the Turkey oak takes place in the spring following the fructification year and starts by a 2-week gap as compared to the Hungarian oak acorn since it is less affected by the late freezing, which justifies the existence of the Turkey oak seedlings on the northern shadowy slopes and at the foot of the plateau. The higher multiannual average temperatures and the poorer multiannual average rainfall in the area enhance germination with the first sufficient humidity values. The enhanced germination of the Hungarian oak acorn makes it possible to have seedlings during years of low fructification as such acorns also spring up from *Balaninus glandium*. The enabling of natural regeneration, the establishment of the optimal period for the land work in the microproduction units will take place according to the germination period and the germination characteristics in the case of the Hungarian oak and the Turkey oak. Likewise, there is need to determine the moment to cease the exploitation of the fully-grown oak trees and to clean the woodlands, before the upshot of stems, and, exceptionally, to postpone the cut works for a year when the germination has extensively taken place in the fructification year.

Keywords: *Quercus frainetto* TEN, *Q. cerris* L., germination, upshot, seedlings.

Rezumat. Germinația, răsărirea și dezvoltarea plantulelor de gărnită și cer în pădurile din partea vestică a Podișului Getic. Schimbările climatice la care asistăm în ultimele decenii au influențat și ecosistemele forestiere. Dintre toate speciile forestiere cele mai afectate au fost gărnita (*Q. frainetto* TEN) și cerul (*Q. cerris* L.), specii ce au o răspândire foarte mare (76%) în teritoriul luat în studiu (BERCEA, 2007; BERCEA & COJOACĂ, 2012). Pe lângă uscarea în masă a exemplarelor acestor specii, a fost afectat procesul de regenerare naturală care stă la baza continuității pădurilor și perpetuarea speciilor în teritoriile ocupate dintotdeauna. Pentru a se adapta procesul de regenerare la schimbările climatice produse s-au inițiat numeroase cercetări și cu privire la germinația, răsărirea și dezvoltarea plantulelor celor două specii în teritoriile ocupate. Gărnita și cerul sunt singurele specii forestiere care valorifică superior potențialul productiv al solurilor argiloase, grele, compacte (prelivosoluri, luvosoluri -74.5%) predominante în Podișul Getic (BERCEA, 2007; BERCEA & COJOACĂ, 2012). Din cercetări rezultă că ghinda de gărnită germinează mult mai repede decât a cerului, chiar din toamna anului cu fructificație. Germinația la cer se produce, de regulă, primăvara următoare anului de fructificație și începe cu un decalaj de circa două săptămâni, mai târziu față de ghinda de gărnită, fiind mai puțin afectată de înghețurile târzii, fapt ce explică instalarea semințurilor de cer cu mai mare ușurință pe versanții nordici, umbriți, sau la baza versanților. Temperaturile medii multianuale mai mari și precipitațiile medii multianuale mai reduse din acest areal, determină începerea germinației de la primele semne de umiditate suficientă pentru declanșarea procesului. Germinația mai ușoară a ghindei de gărnită favorizează instalarea semințurilor și în anii cu fructificații slabe, prin răsărirea puieților și din ghinda cu atacuri slabe de *B. glandium*. Efectuarea lucrărilor de ajutorare a regenerării naturale, stabilirea perioadei optime de execuție a lucrărilor pe microstațiuni se vor face luând în calcul perioada de germinare și modul cum decurge aceasta diferențiat pentru gărnită și cer. De asemenea, se va stabili concret momentul opririi exploatarei arborilor maturi și al efectuării lucrărilor de curățire a parchetelor, înainte de începerea apariției tulpinilor, iar în mod excepțional, amânarea tăierilor cu un an în cazul în care germinarea semințelor s-a produs în proporție foarte mare în toamna anului de fructificație.

Cuvinte cheie: *Q. frainetto* TEN, *Q. cerris* L., germinare, răsărire, plantule.

INTRODUCTION

Across Romania, the climatic changes manifest in the long-term dry periods and high temperatures succeeding at short intervals followed, generally, by years of heavy rainfall over short periods of time. The Getic Plateau is situated in the south of the Carpathians, characterised by a warm climate and lack of rainfall, which triggers even poorer rainfall that is not uniformly distributed over the year. In summertime, the warm and dry air advections are more numerous and longer, coming from North Africa, which lead to higher evapotranspiration, impeding on the adjustment of species to the dry atmospheric and edaphic conditions. The climatic pressure put on the forest species caused the mass wilting of trees and, more particularly, the low fructification and high frequency fructification, compromising the natural regeneration process. The most affected species was the Hungarian oak, out of which more than 45% trees were affected during the dry period of 1988-2004 (BADEA & TĂNASE, 2002). The woodlands of the Hungarian oak and the Turkey oak cover a surface area of 72,151 ha (43%) out of the total area of 167,248 ha of the woodland in the Jiu river

basin to the south of the Carpathians (BERCEA, 2007). The poor rainfall for several consecutive years resulted in severe edaphic dry weather negatively impacting on the Hungarian oak, which depends on the suction force of the roots as a physiological mechanism to adjust to the dry conditions, unlike the Turkey oak, which depends on the earlier closure of stomata in daytime, reducing transpiration. The high frequency of fructification in the case of the Hungarian oak caused the imbalance of the regeneration process that should be compensated for through forestry measures of monitoring and regeneration of seedlings containing the Hungarian oak. To this end, research was carried out to study and manage the natural regeneration process, as well as the germination, upshot and growth of the seedlings of the two species.

MATERIALS AND RESEARCH METHODS

The sample area covers the western part of the Getic Plateau in 6 woodlands of pure and mixed seedlings of the Hungarian oak and Turkey oak in the hydrographic basin of the Jiu river.

To determine the distance and the number of the seedlings at the end of the plateau and in the group cuts, both for the Hungarian oak and for the Turkey oak, sample areas were chosen, where analyses of the 1-year-old seeds, not heavily influenced by the microclimatic conditions at the extremity of the plateau and in group cuts of various sizes, were made. The research identified both similarities and dissimilarities of the two species depending on the weather conditions, the forest type, the woodland and the seedlings condition. In 2003, 2004, 2005 and 2006, periodic observation and inventory targeted the sample areas in the woodlands of Craiova, Filiași, Strehaia, Motru, Turceni and Cărbunești.

RESULTS AND DISCUSSION

Characteristics of the Hungarian oak. The observation and research highlighted the following:

In early November 2003, which was a high fructification year for the Hungarian oak, 33% of the healthy disseminated acorns had seedlings (Table 1), and the length of the roots ranged between 0.5 cm and 10 cm.

In early winter, on December 6, 2003, 92 % of the healthy disseminated acorns had seedlings (Table 1), and the average length of the radicle was 11 cm (0.5 - 22 cm).

Seedlings of the Hungarian oak acorns were found before dissemination.

The very high percentage of the acorns germinating in dissemination autumn was due to weather conditions. In September and October 2003, there was heavy rainfall in the sample area – between 91.1 - 174 mm at the three weather forecast stations of Craiova, Băcleș and Târgu Jiu, and the average monthly temperatures in September, October and November ranged between 6.2 and 16.3° C (Table 2).

The germination of the Hungarian oak acorn was faster in the sample areas with no grass and bedding plants, or with a thin 2-3-year-old bedding plants rotting layer, where the seeds had contact with the wet soil on more than 20% of the surface of the acorn.

Table 1. Percentage of germinated acorns in the dissemination autumn.

Species	Sample area u.a.	Number of healthy acorns (items/m²)				
		Total (items/m²)	out of which, seedlings at ...		percentage at	
			01.11.2003 (items /m²)	06.12.2003 (items /m²)	01.11. 2003 (%)	06.12. 2003 (%)
1	2	3	4	5	6	7
Hungarian oak	124 A	142	39	128	27	90
	82 M	180	67	170	37	94
	112 B	206	81	193	39	94
	112 C	196	52	175	26	89
Average		181	60	167	32	92

Table 2. Variation of rainfall and autumn temperatures.

Weather forecast station	Year	Average monthly rainfall					Average monthly temperatures				
		08	09	10	11	12	08	09	10	11	12
1 Craiova	2003	9.2	121.0	132.1	39.0	51.8	24.9	16.3	9.2	6.7	-0.4
	2004	27.6	55.6	17.5	99.2	35.7	21.9	17.1	12.6	6.5	1.3
Băcleș	2003	4.3	91.1	114.5	43.6	23.4	24.3	15.3	8.0	6.4	-0.6
	2004	60.8	39.2	33.5	90.4	11.6	21.1	16.0	11.9	5.8	1.0
Târgu Jiu	2003	26.6	128.8	174.1	49.7	33.2	23.6	16.0	8.9	6.2	0.1
	2004	85.9	67.5	40.4	131.8	35.2	20.8	15.7	11.7	6.2	1.3

The smallest number of germinated acorns (11%) with a radicle up to 0.5 cm, were found in the sample areas of dry hard soil. In early December in 2003, the radicles of some germinated acorns fallen on the grassy land did not touch the soil that should have fixed them, they entered a decay process during the dry period through the dehydration of the cotyledons, of the radicle and its drying, thus, interrupting the germination process.

The germination of the Hungarian oak acorn attacked by *Balaninus glandium* takes place only when the embryo is not destroyed or when the hypocotyl is not cut in the early growth stage. With 80% of the attacked acorns, by a maximum of two larvae, only the cotyledons are eaten up, the embryo being eaten up only in the cases of heavy attacks.

In the early spring of 2004, germination was activated in the second decade of March following the heavy rainfall of 9–10 March and the subsequent high temperatures. Thus, on April 10, 2004, the healthy acorn and not heavily attacked by *B. glandium*, germinated in a very (high) percentage - 99%. The average length of the radicle was of 1.0 - 22 cm.

Germination can be compromised only when the acorn is dehydrated before the beginning of the process. Research showed that with 70% of the acorns where the germination process had reached the emergence stage, the radicle having penetrated the skin and being less than 0.5 cm long, water scarcity or lack of water during the growth of the radicle stopped the process and resulted in the death of the embryo. The phenomenon is readily noticeable in the sample areas on the dry hard soil, where the acorns had the necessary water to start the germination process. The vegetation process of the plants changed the acorns initial stage enabling the process; practically, they were lifted, thus, reducing the contact area with the soil and eliminating the contact with the wet soil and the water supply necessary for the on-going germination. In 30% of the ceased germination cases, the radicle reaches about 3.0 cm, not being able to fix in the soil, either because of the grass or of the thick dry plant bedding. The thick and partly wet plant bedding enhances the horizontal growth of the radicle due to the need for a wet area allowing its fixation in the soil.

The alternation of wet and dry seasons at extremely large intervals causes the acorn germination to stop at different stages; consequently, it compromises their germination. The constant sufficient humidity of the soil hosting the germinating acorns is crucial to the Hungarian oak seedlings. This is secured only in the woodlands with no grassy soil, with thin rotting plant bedding, which keeps wet and which is covered by a plant bedding layer dating from the fructification year, partly covering the acorns, preventing the rapid evaporation of the humidity of the soil and maintaining a wet microclimate in the acorn layer.

The penetration of the radicle in the soil takes place only in the sufficiently wet areas and it is a prerequisite for the successful germination process and the emergence of seedlings, through the rapid growth of the root and its deep fixation in the soil in a short period of time, as well as through the development of a fascicle of side roots absorbing the water and securing the physiological processes triggered by germination.

The high temperatures in late February 2004, followed by a wet and warm period starting from the second decade of March, favoured the emergence of the stems. It happened after the penetration of the roots in the soil and the extension of the hypocotyl axis, followed by the unfolding of cotyledons in two symmetrical parts and the emergence of the epicotyl axis in between, giving rise to the main stem and the first leaves (protophylls) which grow very fast.

The growth of the stems also started in the second decade of March, with reference to the acorns having germinated in the previous autumn and having their roots already fixed in the soil.

Temperatures above 10⁰ C in daytime for more than 5 days at the level of the soil causes the upshot of the stem and coincides with the next period of vegetation of the vernal plants (*Viola silvestris*, *Corydalis cava*).

On 31 March, 60 % of the stems had already sprung, and on 15 April - 98 %.

The growth of the stems is fast during the first 10 days, depending on the temperature in daytime and being enhanced by high temperatures at the level of the soil in the woodland. Nevertheless, on July 3, 2004, newly sprouted stems were found in u.a. 99 F, U.P. II Argetoaia under a very thick layer of plant bedding made up of last year leaves, gathered in a pool. The upshot of stems was due to the rainfall that took place two days before drawing up the inventory list, resulting in a pool where the 11 acorns were found, and the stems had already reached 4 cm and become wilted because of darkness, striving to penetrate the thick plant bedding (Figure 1). The soil temperature reached 28 °C. Hence, the upshot of the stem depends not only on temperature, but also on the soil humidity.

The vertical growth of the stems is fast in the first weeks, and the growth of leaves is very slow; after stems stop growing, leaves grow fast. Each seedling has 3-5 leaves (80 % have 5 leaves, 10 % 4 leaves and 10 % 3 leaves) and the best developed leaves go to the top of the seedlings.

On May 30, the seedlings stopped growing, the average height was of 11 cm (3 cm - 18 cm), the average length of the root was of 19 cm (12 cm - 36 cm), and the average diameter of the root crown is of 3 mm (1 - 5 mm).

On rare occasions (in less than 0.1% of the cases), two stems sprang out of the same root and grew similarly, having the same height when they stopped growing.

The mechanically affected stems are able to recover through drying, even if this mechanical action took place in the first days of the stem growth.

During the first vegetation year, a second growth was extremely rare in the sample areas - only in 0.3% of the total number of seedlings, irrespective of the position of the sample areas.

Research on upshot of the Hungarian oak seedlings was also carried in U.P. II Argetoaia, u.a. 99 F, on flat area of low inclination, with sample areas of 1 m², at 1 m from one another, to the north, south, east and west. One year after the high fructification of the Hungarian oak in the autumn of 2003, i.e. in November 2004, the seeds belonging to the sample areas in 4 group cuts of 1/2 of the average height of the seedlings (0.5 H), to 3 group cuts of the average height of the seedlings (1.0 H) and 4 group cuts of 1.5 of the average height of the seedlings (1.5 H) were examined – see table 3.



Figure 1. Upshot of Hungarian oak seedlings in July 2004 (BERCEA, 2004).

Table 3 indicates that the fixation of the Hungarian oak seedlings in a flat area or in an area of low inclination took place up to 5 m from the end to the centre of the group cut, and outwards, uniformly below the regeneration trees, their number increasing from the end of the group cut to the centre.

In the small-sized group cuts, of $\frac{1}{2}$ of the average height of the regeneration trees (9.5 m), the fixation of the seedlings covered the whole surface area, the average number of seedlings/m² ranging between 12.5 and 14.7.

Table 3. Emergence of Hungarian oak seedlings at the end of the woodland.

Size of group cuts vs. the height of seedlings (H)	Maximum emergence distance to the centre of the group cuts	Average no. of 1-year seedlings/m ² , sprung at a distance of... m										
		1	2	3	4	5	6	1	2	3	4	5
		From the end to the centre of the group cut						From the end of the group cut outwards				
1	2	3	4	5	6	7	8	9	10	11	12	13
0.5 H	5	14.7	15.1	13.1	12.9	12.5	0	9.5	12.8	16.5	19.7	20.1
1.0 H	5	14.3	14.5	12.3	9.8	4.5	0	7.5	12.5	16.3	19.5	20.1
1.5 H	5	13.6	13.8	11.7	9.1	3.9	0	7.3	12.4	16.4	19.7	19.9

In the group cuts of the same size of the height equal to the height of the regeneration trees (18.5 m), the average number of seedlings/m² ranged between 4.5 – 14.3, whereas there were no seedlings in the centre of the group cut.

It is also the case of the group cuts of 1.5 H, where the average number of seedlings/m² ranged between 3.9 – 13.6, and where there were no seedlings in the centre of the group cut.

Table 3 also shows that the highest number of seedlings/year was in the group cuts of small diameter. Admittedly, in the group cuts with the diameter equal to $\frac{1}{2}$ of the height of the seedlings, the average number of seedlings/m² ranged between 14.7 – 12.5, almost uniformly distributed in the centre of the group cut and outside the group cut, recording only a slight decrease in the first m² towards the centre of the regeneration trees area. In the group cuts with the diameter equal to the height of the seedling, the number of seedlings/m² at the end of the group cut to the centre of the group cut decreases from 14.3 to 4.5, whereas outside the group cut it increases from the end of the regeneration group cut to the centre, from 7.5 to 20.1 seedlings/m².

Furthermore, the average number of seedlings in the group cuts of 1.5 H of the seedlings is smaller, decreasing from the end of the group cut to the centre, while it increases outwards from 7.3 to 19.9 as in the case of the group cut equal to the height of the seedlings.

The research carried out in the sample areas belonging to the group cuts with central regeneration trees or with regeneration in u.a. 153 B, U.P. II Argetoaia, Ocolul Filași and in u.a. 50 D, U.P. IV Șușița, Ocolul Strehaia indicates that the Hungarian oak acorns were sufficiently disseminated in the centre of the group cuts.

Characteristics of the Turkey oak. 2002 and 2004 were good fructification years for the Turkey oak, while 2005 was extremely good in this respect. Periodic observation, inventory lists and growth measurements targeted the very good and good fructification years as well as some other years. The research carried out with Turkey oak group cuts, with Turkey oak and Hungarian group cuts and with Sessile oak, Turkey oak and Hungarian oak group cuts showed that:

- the germination of the healthy Turkey oak acorns reached 8% up to December 5 in the fructification year, and it ranged between 5.7% in 2005 and 9.4 % in 2002, according to the weather conditions, condition of the seedlings, the size of the group cut and the spread of the grass on the soil (Tables 4, 5);
- the radicle of the germinated acorns is of 1.0 cm (0.3 – 1.5 cm);
- in the early spring of 2003 and 2006, germination was activated gradually starting from March 20 in 2003 and March 25 in 2006, respectively, according to the weather conditions, exposure, thickness of the plant bedding in the fructification year, condition of the seedlings, and the spread of the grass on the soil;
- germination may be considered complete on April 30 in 2002 and on May 5 in 2006;
- the radicle penetrates the skin slower as compared to the Hungarian oak acorn, requiring a longer period of water absorption;

Table 4. Percentage of germinated Turkey oak acorns in the autumn of dissemination in 2002.

Species	Sample area u.a.	Number of healthy acorns (items/ m ²)				
		Total items/m ²	out of which, seedlings at ...		percentage at	
			31.10.2002	02.12.2002	31.10. 2002	02.12. 2002
1	2	3	4	5	6	7
Turkey oak	80 II	3.9	0	0	0	0
	81 D	12.7	1	2	7.9	15.7
	82 A	11.8	1	1	8.5	8.5
	153 B	15.0	1	2	6.7	13.3
Average		10.9	0.8	1.3	7.5	9.4

Table 5. Proportion of germinated Turkey oak acorns in the autumn of dissemination in 2005.

Species	Sample area u.a.	Number of healthy acorns (items/ m ²)				
		Total items/m ²	out of which, seedlings at ...		percentage at	
			09.11.2005	05.12.2005	09. 11. 2005	05.12. 2005
1	2	3	4	5	6	7
Turkey oak	153 B	84	3	5	3.6	6.0
	124 A	53	2	3	3.8	5.7
	81 D	141	4	7	2.8	5.0
	44 B	67	2	4	3.0	6.0
Average		86.3	2.8	4.8	3.3	5.7

- the radicle penetrates the soil fast and deeply, developing fascicular side roots through which it absorbs the water and maintains the cotyledons alive;
- the average growth of the root in the first year reaches 17 cm (10 - 34 cm);
- the hypocotyl gets longer, it splits up into two symmetrical parts, and the stem springs from its end;
- the stem grows fast, reaching an average height of 15 cm (5 - 26 cm), followed by leaves growing - from 3 to 5 (in number);
- like the Hungarian oak, the Turkey oak rarely witnesses 2 stems from the same acorn;
- the 1-year seedlings rarely undergo a second growing process.

The germination of the two oak species indicates similarities and dissimilarities. Thus, the Hungarian oak acorn germinates faster than the Turkey oak one, from the very autumn of the fructification year, although the research could not be conducted in parallel because of the lack of fructification of the two species in the same year. The germination of the Turkey oak generally takes place in the spring following the fructification year and is marked by a 2-week gap as compared to the Hungarian oak acorn, being less affected by the late frost, which justifies the easier upshot of the Turkey oak seedlings on the north shadowy slopes or at the foot of the slopes. The early germination of the Hungarian oak acorn, as well as the high germination rate in the autumn of the fructification year justifies the spread of this species to the south. The higher multiannual average temperatures and the poorer multiannual average rainfall in this area cause the germination to start as soon as humidity allows it.

The easier germination of the Hungarian oak acorn favours the dissemination of seeds in the years of low fructification through the upshot of seedlings even from the acorns attacked by *B. glandium*.

The enhancement of natural regeneration, the establishment of the optimal period for microproduction units will take into consideration the germination period and the characteristics of the process with the Hungarian oak and the Turkey oak.

Also, there is need to establish when to stop the exploitation of the fully grown trees and to clean the woodlands, before the upshots of stems, and, exceptionally, to postpone the cuts for the next year when the germination was high in the fructification year. Losses can be severe when the acorns are moved away from the germination area (as it happened in 2003).

CONCLUSIONS

1. The Hungarian oak acorn germinates several times starting from the autumn of dissemination year in proportions of more than 90% of the number of healthy acorns, according to the amount of rainfall and the temperature during the whole period, while roots can be up to 22 long. A small part may germinate before dissemination.

2. The germination of the Hungarian oak acorn is faster in the areas where the soil is not grassy or covered by a plant bedding or by a 2-3 year-old rotting plant bedding, where seeds come into contact with the wet soil, the contact area being larger than 20% of the total surface of the acorn.

3. The smallest number of acorns where germination involves the growth of the radicle up to 0.5 cm are found in the dry hard soil. In the case of some acorns germinating on the grassy soil, the radicles do not come into contact with the soil to be able to fix, they start decaying in the dry season and due to the dehydration of the cotyledons, of the radicles and through its drying, germination is interrupted.

4. The germination of the Hungarian oak acorn attacked by *Balaninus glandium* takes place only when the embryo has not been eaten up or the hypocotyl was not cut in the early growth stage. In 80% of the cases, when the acorns are attacked by a maximum of 2 larvae, only the cotyledons are eaten up, the embryo being eaten up in heavier attacks.

5. In early spring, germination is activated starting from the second decade of March, following the heavy rainfall and high temperature. The average length of the radicle reaches 11 cm (1.0 to 22 cm).

6. Germination can be compromised only when the acorns become dehydrated after germination has started. The constant soil humidity that the acorns need after germination has started depends on the fixation of the Hungarian oak seedlings.

7. The penetration of the roots in the soil and the extension of the hypocotyl axis are followed by the unfolding of cotyledons in two symmetrical parts and the emergence of the epicotyl axis in between, giving rise to the main stem and the first leaves (protophylls) which grow very fast.

8. The growth of stems begins with the acorns having germinated in the previous autumn and having the roots already fixed in the soil in the second decade of March.

9. Warmth triggers the growth of the stems. Temperatures above 10 °C in the soil in daytime for more than five consecutive days cause germination to start.

10. The growth of the stem is fast during the first 10 days since its upshot, according to the temperature values in daytime, being enhanced by high temperatures of the soil in the woodland and by the soil humidity.

11. With the Turkey oak, germination takes place up 8% of the healthy acorns until December 5 in the fructification year, and, according to the weather conditions, condition of the seedlings, size of the group cut and spread of grass in the soil, the radicle of the germinated acorns is 1.0 cm long (0.3 – 1.5 cm).

12. In early spring, germination is activated gradually starting from the third decade of March, according to the weather conditions, exposure, thickness of the plant bedding in the fructification year, condition of the seedlings, spread of grass in the soil.

13. The radicle of the Turkey oak penetrates the skin slower than that of the Hungarian oak, requiring a longer period of water absorption.

14. The radicle penetrates the soil fast and deeply and it develops side roots through which water is absorbed and cotyledons are kept alive.

15. The root can reach 17 cm in the first year (10 - 34 cm).

16. The hypocotyl gets longer, cotyledons split up into two symmetrical parts, and the stem springs from its end. The stem grows fast, reaching an average height of 15 cm (5 - 26 cm), followed by leaves growing - from 3 to 5 (in number).

17. Like the Hungarian oak, the Turkey oak rarely witnesses 2 stems from the same acorn.

18. The 1-year seedlings rarely undergo a second growing process.

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CONTRIBUTIONS TO THE ROMANIAN VASCULAR FLORA

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Abstract. In the present paper there are presented 2 new species for the spontaneous Romanian flora: *Urtica pilulifera* L. and *Lagurus ovatus* L. For each taxon a short botanical description is presented, general distribution and few general aspects referring to biology, ecology and their utility (where is necessary).

Keywords: flora news, *Lagurus ovatus*, Romania, *Urtica pilulifera*.

Rezumat. Contribuții la flora vasculară a României. În lucrarea de față se prezintă 2 specii noi pentru flora spontană a României: *Urtica pilulifera* L. și *Lagurus ovatus* L. La fiecare taxon se prezintă o scurtă descriere botanică, distribuția generală și câteva aspecte generale referitoare la biologia, ecologia și utilitatea acestora (acolo unde este cazul).

Cuvinte cheie: noutăți floristice, *L. ovatus*, România, *U. pilulifera*.

INTRODUCTION

In the Romanian specialty literature there are not known any data referring to the presence of these two taxa in the Romanian vascular flora. There is no herbarium material to certify the existence of these species in the spontaneous or subspontaneous Romanian flora.

BUIA (in SĂVULESCU, 1952) says that *Urtica pilulifera* represents a southern element, that is present to us only as cultivated plant, and OPREA (2005), mentions that *Lagurus ovatus*, could be subspontaneous in the south part of Romania, but without giving any location.

MATERIAL AND METHODS

The material with the two species was collected by the authors of this paper after a trip in Costinești (Constanța County). It has been identified in ruderalised meadows. Plants are found as herbarium samples and included in the herbarium from the University of Craiova (CRA). Subsequently, *Lagurus ovatus* has been found in “Valea Stanciului” locality (Dolj County).

The identification was made according to Flora Europaea, in the Romanian flora not being included in the dichotomous keys for the identification of these species.

The authors' abbreviations are according to BRUMMITT & POWELL (1992).

RESULTS AND DISCUSSIONS

In Flora Europaea (TUTIN et al., 1964) *Urtica* genus is represented by 8 species. Among these, so far, in Romania were known only *Urtica dioica* L., *U. kioviensis* ROGOW. and *U. urens* L. (CIOCĂRLAN 2000, 2009).

BELDIE (1977) mentions to *Urtica* genus only *U. dioica* as species with two subspecies (*dioica* and *kioviensis* (ROGOW.) BUJA) and *U. urens* L.

Urtica pilulifera has been mentioned by BRÂNDZĂ (1898) from Constanța, along railways lines. Although it was mentioned, there is no herbarium material certifying its presence in the Romanian spontaneous flora (fact mentioned by CIOCĂRLAN (2000, 2009), too).

Because *U. pilulifera* is a new taxon for the Romanian flora, we present the description of these (based on herbarium material), starting from the existing description in the specialty foreign literature.

Urtica pilulifera L., Sp. Pl. 983. (1753).

It is an herbaceous plant, annual or biennial with sparse stinging hairs. Stem is quadrangular, high of 20-100 cm; monoecious. Leaves with 2-10 cm long petiole, lamina to 2-10 cm, ovate, truncate to subcordate at base, serrate or entire, acuminate apex; 4 stipules to each node, free lateral, triangular-ovate to lanceolate, 3-6 mm long, membranous. Racemes unisexual; female long pedunculated with flowers in globose heads, long peduncle head, c. 1 cm in diameter; male flowers spiked. Female lowers with inflated perianth. Achenes broadly ovate, c. 2.5 mm long, c. 2 mm broad, dark brown, enclosed by enlarged dorsal sepals.

Thus, *U. pilulifera* is easily distinguished from the other species of *Urtica*, which are found nowadays in the Romanian flora (CIOCĂRLAN, 2000; 2009).

- 1a. Female flowers in long-pedunculated, globose heads *Urtica pilulifera*
 1b. All flowers in spike-like racemes 2
 2a. Annual plant *Urtica urens*
 2b. Perennial plant 3
 3a. Dioecious plant, with erect stem, pubescent. External tepals from the female flowers shorter than $t \frac{1}{2}$ of the length of the internal ones..... *U. dioica*
 3b. Monoecious plant, with creeping stem in the inferior side, hairless. External tepals from the female flowers longer than $t \frac{1}{2}$ of the length of the internal ones *U. kioviensis*

General distribution. This is a native species in the south of Europe (Fig. 1) or occasionally naturalized in Austria, Belgium, England, Czech, Germany, Hungary, Switzerland (BALL in TUTIN et al., 1964) and Bulgaria, Slovakia and Ukraine (in SÎRBU & OPREA, 2011, according to DAISIE).

Biology and ecology. *U. pilulifera* is a diploid species ($2n = 26$) (LUQUE & DIAZ LIFANTE, 1991) that is vegetating in ruderalised places, garbage area sometimes. It prefers sunny soils. It blooms from June to September, and the seeds ripen from July to October.

Uses. The leaves of this plant can be used as those of *U. dioica* in folk medicine as a tea, because of its tonic and purifying effect on blood.

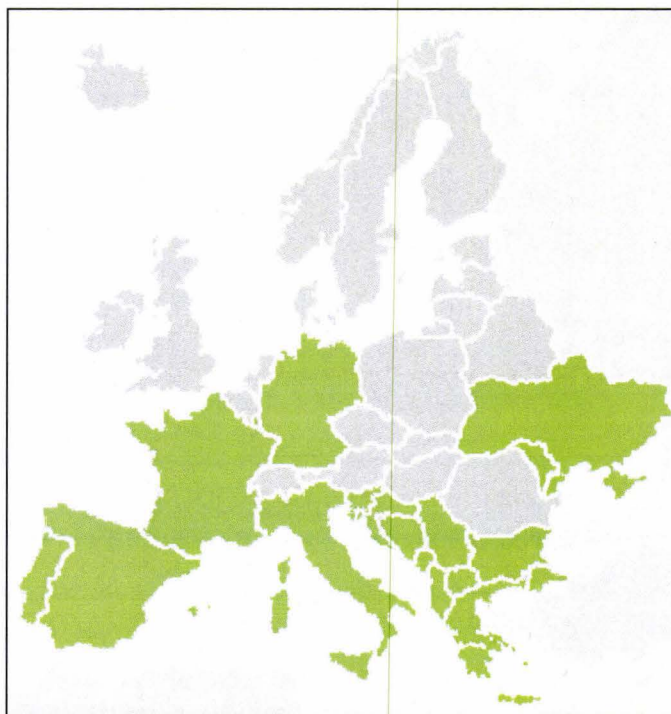


Figure 1. *U. pilulifera* distribution in Europe (source net).

***Lagurus ovatus* L., Sp. Pl. 81 (1753)**

It is the only species of this genus from Europe Flora (TUTIN in TUTIN et al., 1980). SCHUR (1866) mentioned it from Transylvania, according to KOTSCHY, but with doubts about this fact. Also, SIMONKAI (1886) said that *Lagurus ovatus* is found in Transylvania only in cultivated places.

It is an herbaceous plant, annual, with erect or ascendant stem (Fig. 2), that can reach up to 60 cm. It can be simple or slightly branched at the basal nodes. It grows in small narrow tuft. The leaves are flat, width up to 1 cm. These present an obvious ligula, up to 3 mm, obtuse or truncated, villous and often broken. Vagina and lamina are soft and densely hairy.

The inflorescence is ovoid or subcilindrical to subglobulous (Fig. 3). The spikelets are between 7-10 mm in length and present villous glumes. Lemma is 3 mm in length (excluding awn and apical setae), is lanceolate, with 5 nervures, awn 8-20 mm; apical setae is 2-6 mm in length. Palea is shorter than lemma. Anthers are 1.5 mm.

General distribution. It is a Mediterranean species, met on the marine sands from southern Europe (TUTIN, in TUTIN et al., 1980), rarely in ruderal dry places, (Fig. 5). Adventive (occasional), it is known from Austria, Belgium, Czech Republic, Germany (SÎRBU & OPREA, 2011).

Biology and ecology. *Lagurus ovatus* is a diploid ($2n = 14$) therophyte (TUTIN in TUTIN et al., 1980) flowering from March to May. It prefers sandy soils. It grows in medium to lit places, moderately fertile soil, in a warm, sunny site.



Figure 2. Herbarium material with collected *L. ovatus* (original). Figure 3. Inflorescence detail of *L. ovatus* (original).

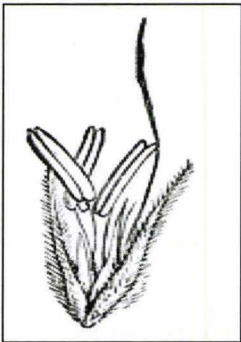


Fig. 4. *L. ovatus* spikelets - scheme (original).



Figure 5. *L. ovatus* distribution in Europe (source net).

CONCLUSIONS

From the presentation of this paper we can draw few conclusions:

- the paper indicates the certain existence of the species *L. ovatus* and *U. pilulifera* in Romania.
- the Romanian floristic inventory becomes richer with two more species.
- the altitude where the two species were found is between 0-10 m.s.m.
- these two species are not affected by the influence of the zooanthropogenic factor, that is why we consider

that in the future, the area of these taxa could be larger.

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WATER MITES OF THE GENUS *Unionicola* HALDEMAN 1842 (ACARI, HYDRACHNIDIA, UNIONICOLIDAE) AND A NEW SPECIES FOR TURKEY

BOYACI Yunus Ömer, GÜLLE Pınar, DİDİNEN Hakan

Abstract. The paper contains the list of species of the genus *Unionicola* HALDEMAN 1842 known in Turkey. A new species, *Unionicola* (*Unionicola*) *gracilipalpis* (VIETS 1908), for the fauna of Turkey and 6 new localities from 4 provinces for two previously recorded species, *Unionicola* (*Unionicola*) *crassipes* (MÜLLER 1776) and *Unionicola* (*Unionicola*) *minor* (SOAR 1900) are given. In total, up to the present, 5 species of the genus *Unionicola* are known for Turkey.

Keywords: water mites, Acari, *Unionicola*, a new species for Turkey.

Rezumat. Acarieni de apă din genul *Unionicola* HALDEMAN 1842 (Acari, Hydrachnidia, Unionicolidae) și o specie nouă pentru fauna Turciei. Este prezentată lista speciilor ce aparțin genului *Unionicola* HALDEMAN 1842 cunoscute în Turcia. S-a înregistrat o nouă specie pentru fauna de acarieni de apă din Turcia, *Unionicola* (*Unionicola*) *gracilipalpis* (VIETS 1908), și șase locații noi, în patru provincii diferite, pentru alte două specii identificate anterior. În total, până în prezent, în Turcia, sunt cunoscute cinci specii ce aparțin genului *Unionicola*.

Cuvinte cheie: acarieni de apă, Acari, *Unionicola*, specie nouă pentru Turcia.

INTRODUCTION

The subfamily Unionicolinae OUDEMANS 1909 includes a single genus, *Unionicola* HALDEMAN 1842. Within the cosmopolitan genus *Unionicola*, distributed in fresh water habitats in all continents except Antarctica, 56 subgenera and more than 200 species are present (SMIT, 2008). Diverse relationships with other freshwater organisms are shown. Although many free living species are known, more than half of all species are parasitic on freshwater mollusks (especially inside mantle cavities of Unionidae species) (VIDRINE 1992; EDWARDS & VIDRINE, 2006).

There are 4 species of the genus *Unionicola* recorded so far in Turkey. These are: *U. (Majumderatax) hankoi* (SZALAY 1927), *U. (Pentatax) bonzi* (CLAPAREDE 1869), *U. (Unionicola) crassipes* (MÜLLER 1776) and *U. (Unionicola) minor* (SOAR, 1900) (ÖZKAN, 1982; ÖZKAN et al., 1996; ESEN et al., 2010).

With the new record of the species *U. gracilipalpis* Çapalı Lake from Afyonkarahisar Province, the species of the genus now reaches 5 in the fauna of Turkey. Description, zoogeographical distributions, drawings and measurements of certain organs of *U. gracilipalpis* and new localities to previously recorded species are given in the present paper.

MATERIALS AND METHODS

Water mites were collected by hand netting and sorted on the spot from the living material, preserved in Koenike's fluid (50% glycerine, 20% acetic acid, 30% aqua dest.). The composition of the material is given as males/females. All measurements are given in μm . The following abbreviations are used: L = length, H = height, W = width, Cx-I = first coxae, P-I = palp segment I.

RESULTS

Family Unionicolidae OUDEMANS 1909

Genus *Unionicola* HALDEMAN 1842

1. *Unionicola* (*Majumderatax*) *hankoi* (SZALAY 1927)

Records in Turkey: Bingöl province (ESEN et al., 2010).

Habitat: Pools and ponds in running waters.

Distribution: Hungary, Italy, Iberian Peninsula, Poland, Iran, Turkey (VIETS, 1956; ESEN et al., 2010).

2. *Unionicola* (*Pentatax*) *bonzi* (CLAPAREDE 1909)

Records in Turkey: Erzurum and Muş provinces (ÖZKAN, 1982).

Habitat: Parasitic on freshwater mussels.

Distribution: Europe, Japan and Turkey (VIETS, 1956; ÖZKAN, 1982).

3. *Unionicola* (*Unionicola*) *crassipes* (MÜLLER 1776)

New records: **Afyonkarahisar Province:** 38°04' N, 30°16' E, 21.04.2008, 10/3; 23.05.2008, 9/13; 26.06.2008, 8/9; 22.07.2008, 8/3; 23.08.2008, 5/0; 30.09.2008, 0/7; 28.10.2009, 2/0, 1–2 m deep lake area covered by aquatic plants.

Çapalı Lake. **Isparta Province:** 37°45' N, 31°02' E, 04.06.2008, 3/3, Bağılı reservoir channel, Bağılı, Eğirdir. **Burdur Province:** 37°04' N, 29°30' E, 12.06.2008, 7/5, Evciler pond, Gölhisar. **Konya Province:** 38°23' N, 31°53' E, 14.06.2008, 0/2, Ilgın lake, Ilgın, collected by Y. Ö. BOYACI.

Records in Turkey: Kayseri Province (ÖZKAN et al., 1995, 1996); Afyonkarahisar Province (ÖZKAN et al., 2003; BOYACI & ÖZKAN, 2003, 2004; AŞÇI et al., 2006–2007).

Habitat: All types of stagnant waters, mainly larger water bodies like lakes and channels.

Distribution: The species is among the most widespread and tolerant species in the Holarctic Region. Especially common in Europe, also known in Turkey, Palestine, Iran, Turkestan, Yakutia, Mongolia, India and China in Asia, Kapland in Africa, USA (including Alaska), Canada and Guatemala in America (VIETS, 1956; ÖZKAN et al., 1996).

4. *Unionicola (Unionicola) minor* (SOAR 1900)

New records: **Afyonkarahisar Province:** 38°04' N, 30°16' E, 21.04.2008, 7/3; 23.05.2008, 6/10; 26.06.2008, 5/8; 22.07.2008, 8/3; 23.08.2008, 2/0; 30.09.2008, 0/4, 28.10.2009, 2/0, 1–2 m deep lake area covered by aquatic plants, Çapalı lake. **Isparta Province:** 37°45' N, 31°02' E, 04.06.2008, 5/3, Eğirdir. **Burdur Province:** 37°25' N, 29°49' E, 02.02.2008, 2/3, Stream feeding dam lake, collected by Y. Ö. BOYACI.

Records in Turkey: Adana Province (SMIT, 1995); Kayseri Province (ÖZKAN et al., 1995, 1996); Afyonkarahisar Province (ÖZKAN et al., 2003; BOYACI & ÖZKAN 2003, 2004; AŞÇI et al., 2006–2007).

Habitat: All types of stagnant waters.

Distribution: Common in Europe. Also known from Japan and Turkey in Asia (VIETS, 1956; ÖZKAN et al., 1996).

5. *Unionicola (Unionicola) gracilipalpis* (VIETS 1908)

Male. Idiosoma L/W 856/64. Capitulum short nosed, L 120. Chelicerae 206, claw 62. P–2 slender, ventrally concave and almost equal in size with P–4. Ventral of P–4 with typical projections, at distal end also a narrow and truncated third projection without seta. P–5 longer than ½ of P–4. Forward projected second group coxae forming pointed tips at anteriointerior edges, posterior margin smooth, anterior margin concave. Lengths of palp segments: 15–143–70–160–128=516, heights; 38–51–40–30–28 (Fig. 1a). Cx– I–IV, 257, 232, 341, 254. L/W of genital field 205/86. Lunar shaped genital flaps narrowed anteriorly and enlarged posteriorly (Fig. 1a). 12 acetabulum grouped into two pairs of triplets are present; the first pair is concentrated more to anterior section and posterior triplets are shifted medially.

Habitats of studied material: 38°04' N, 30°16' E, 21.04.2009, 3/0; 23.08.2009, 2/0; 28.10.2009, 4/0, 1–2 m deep lake area covered by aquatic plants, Çapalı Lake, Afyonkarahisar.

Distribution: Common in Europe (VIETS, 1956).

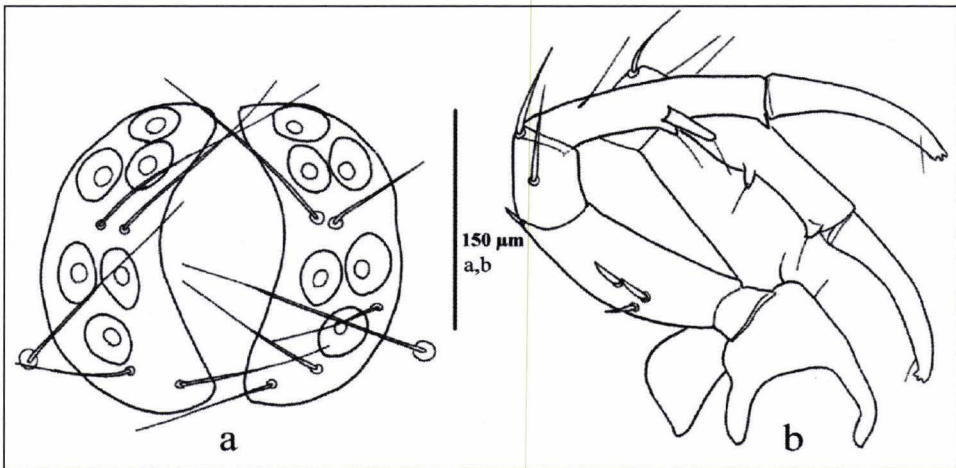


Figure 1. *U. gracilipalpis* Male, a) genital field; b) gnathosoma.

DISCUSSIONS

The species of the genus *Unionicola* have been recorded from stagnant and slow flowing waters like ponds, pools and deep channels. *U. gracilipalpis*, on the other hand, is considered an indicator of clean and clear waters rich in aquatic vegetation (SMIT & HAMMEN, 2000). Although, this species is generally common throughout Europe, the number of collected individuals is low in general (ERMAN et. al, 2010). From *U. (Unionicola) crassipes* it is identified by relatively long and concave P–2, shorter projections on P–4 and much longer P–5 being longer than ½ P–4.

Four species of the genus *Unionicola* are recorded in Turkey (ERMAN et. al, 2010). With the *U. gracilipalpis* this number reaches 5. Together with *U. gracilipalpis* (VIETS 1908), the following species were found as accompanying species at the locality: *Hydrachna piersigi* (KOENIKE 1897), *H. skorikowi* (PIERSIG 1900), *H. globosa* (GEER 1778), *Eylais megalostoma* KOENIKE 1897, *E. hamata* KOENIKE 1897, *E. degenerata* KOENIKE 1897, *E. extendens* (MÜLLER 1776), *E. setosa* (KOENIKE

1897). *Hydrodroma despicens* (MÜLLER 1776), *Hydryphantes crassipalpis* KOENIKE 1914, *Georgella fimbriata* WALTER 1925, *Limnesia undulata* (MÜLLER 1776), *Tiphys ornatus* (KOCH 1836), *Piona variabilis* (KOCH 1836), *P. alpicola* (PIERSIG 1896), *P. carnea* (KOCH 1836), *Hydrochoreutes krameri* PIERSIG 1896, *Hygrobates quanticola* SCHWOERBEL VE SEPASGOZARIAN 1976, *U. minor* (SOAR 1900), *Neumania deltoides* PIERSIG 1894, *Oxus longisetus* (BERLESE 1885), *Arrenurus affinis* KOENIKE 1887, *A. afyonensis* ERMAN & ÖZKAN 1997, *A. furcillatus* VIETS 1930, *A. batillifer* KOENIKE 1894, *A. claviger* KOENIKE 1882, *A. rodrigensis* LUNDBLAD 1954, *A. truncatellus* (MÜLLER 1776), *A. globator* (MÜLLER 1776), *A. fimbriatus* KOENIKE 1885, *A. walconoffi* VIETS 1926 and *A. novus* (GEORGE 1884).

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DATA ON SPIDER FAUNA (ARACHNIDA: ARANEAE) FROM THE NATURE RESERVE SPRINGS FROM CORBII CIUNGI (DÂMBOVIȚA)

LOTREAN Nicolae

Abstract. The paper presents the results of the research carried out on the spider fauna of the Nature Reserve Springs from Corbii Ciungi, Dâmbovița County, during April-December 2012. There were identified 74 species of spider belonging to 19 families, of which only four species: *Ceratinella major* KULCZYŃSKI, 1894, *Tetragnatha dearmata* (THORELL, 1873), *Zelotes gracilis* (CANESTRINI, 1868) and *Mendoza canestrini* (CANESTRINI & PAVESI, 1868) can be considered relatively rare for the Romanian fauna. The families Lycosidae, as number of specimens and Linyphiidae, as number of species and genera, were the dominant families. The spider fauna of the reserve was dominated by four species: *Trochosa ruricola* (DE GEER, 1778) (20.55 %), *Pardosa prativaga* (L. KOCH, 1870) (18.00%), *Pachygnatha degeeri* SUNDEVALL, 1830 (9.20%) and *Piratula hygrophila* (THORELL, 1872) (8.71%). The sex ratio was relatively balanced. Diversity and equitability values were comprised between 1.00 and 1.30, respectively between 0.66 and 0.77. From biogeographical point of view we found the presence of a large number of Palearctic species. The estimation of the conservation status of the Nature Reserve Springs from Corbii Ciungi, according to the spider fauna, emphasizes an unfavourable conservation status.

Keywords: spider, fauna, abundance, dominance, diversity, reserve, conservation.

Rezumat. Date asupra faunei de aranee din Rezervația Naturală Izvoarele de la Corbii Ciungi. Lucrarea prezintă rezultatele cercetărilor întreprinse asupra faunei de aranee din Rezervația Naturală Izvoarele de la Corbii Ciungi, județul Dâmbovița, în perioada aprilie-decembrie 2012. Au fost identificate 74 de specii de aranee încadrate în 19 familii, dintre care patru specii: *Ceratinella major* KULCZYŃSKI, 1894, *Tetragnatha dearmata* (THORELL, 1873), *Zelotes gracilis* (CANESTRINI, 1868) și *Mendoza canestrini* (CANESTRINI & PAVESI, 1868) pot fi considerate relative rare pentru fauna României. Familiile Lycosidae, ca număr de exemplare și Linyphiidae, ca număr de specii și genuri, au fost familiile dominante. Fauna de aranee din rezervație a fost dominată de patru specii: *Trochosa ruricola* (DE GEER, 1778) (20.55%), *Pardosa prativaga* (L. KOCH, 1870) (18.00%), *Pachygnatha degeeri* SUNDEVALL, 1830 (9.20%) și *Piratula hygrophila* (THORELL, 1872) (8.71%). Valorile diversității și echitabilității au fost cuprinse între 1.00 și 1.30, respectiv între 0.66 și 0.77. Din punct de vedere biogeografic am constatat prezența unui număr mare de specii Palearctice. Estimarea stării de conservare a Rezervației Naturale Izvoarele de la Corbii Ciungi, prin prisma faunei de aranee, a arătat o stare de conservare necorespunzătoare a habitatelor investigate.

Cuvinte cheie: aranee, faună, abundență, dominanță, diversitate, rezervație, conservare.

INTRODUCTION

The paper aims to provide an inventory of spider species of the Nature Reserve Springs from Corbii Ciungi (Dâmbovița County) and some of the structural features of spider fauna of this area.

Beginning with year 1959, L. Botoșăneanu and Șt. Negrea performed a systematic study on springs and groundwater fauna of the Romanian Plain. The most interesting discovery, made during this research, was the finding at Neajlov Valley, near the village Corbii Ciungi, of a complex of springs, marshes and creeks collectors, with cold water, relatively stenothermal all year and neutral pH.

The rich biological material collected in two years (14 May 1959 - 5 April 1961) from this area, formed the basis of publication of the first faunal lists, which highlighted extraordinary diversity of the springs complex and relict character of many species found here (BOTOȘĂNEANU & NEGREA, 1961; BOTOȘĂNEANU & NEGREA, 1962). Initial data were then supplemented and included in a monograph paper, which presents the results of a research conducted on the 70 springs or complex of springs from the Romanian Plain (MOTAȘ et al., 1962). For the complex of springs from Corbii Ciungi 73 over specific taxa were mentioned, most hydrobionts (aquatic macroinvertebrates).

Based on these faunal data, completed by the floristic data, the authorities made the decision to declare the complex of springs from Corbii Ciungi reserve, on the 24th of June 1966, through the decision of People's Council of Argeș Region, under the title: *Nature Reserve Springs from Corbii Ciungi*, with an area of about 8 hectares.

In the period 2005-2007, under the coordination of C. Ciubuc (CIUBUC, 2007), it was made the last study on the fauna of the complex of springs from Corbii Ciungi. This was the first time when such captures were made in the riparian area, adjacent springs and creeks collectors, but with this occasion, there were inventoried beetles only.

MATERIALS AND METHODS

The complex of springs is located in the Romanian Plain, in the lower basin of the Argeș River, on the left bank of the Neajlov Valley, about 800 meters from the Neajlov River and about 2 km from the exit of the village Corbii Mari, to village Izvoru (the former village Corbii Ciungi), on the right side of the National Road 61, (about 200 meters from it), average altitude of 110 meters. The complex consists of a large number of springs: reocrene, limnocrene and helocrene, performed on an arc of circle with a length of about 600 meters; these are grouped into two complexes

(fountains), separated from a marshy area which substitutes the “watershed” (NEGREA & NEGREA, 1999): Fountain of Lisandru Vlăduț in the west and Fountain of Cacaletilor in the east. These form two creeks of about 800-1000 m of length each; they are close to their front sections then they divide in a divergent path and thus an area of about 90,000 m² is created between them; then they reunite for shedding the River Neajlov (Fig. 1).

For the catching of spiders three collecting stations were established (Fig. 1). **Station 1 (SR1)** was located near the creek Lisandru Vlăduț, in open area, herbaceous vegetation and shrub layer poorly individualized, represented by the isolated specimens: *Rosa canina* (LINNAEUS, 1753), *Crataegus monogyna* (JACQ., 1775) and *Euonymus europaeus* (LINNAEUS, 1753). **Station 2 (SR2)** was set in the proximity of the creek Cacaletilor, at the edge of a selva formed of very young specimens of: *Salix* sp. and *Alnus glutinosa* (LINNAEUS, 1754), accompanied by *Cornus sanguinea* (LINNAEUS, 1753). **Station 3 (SR3)** was placed in a tree vegetation area, formed of young specimens of *A. glutinosa* (LINNAEUS, 1754), around a spring.

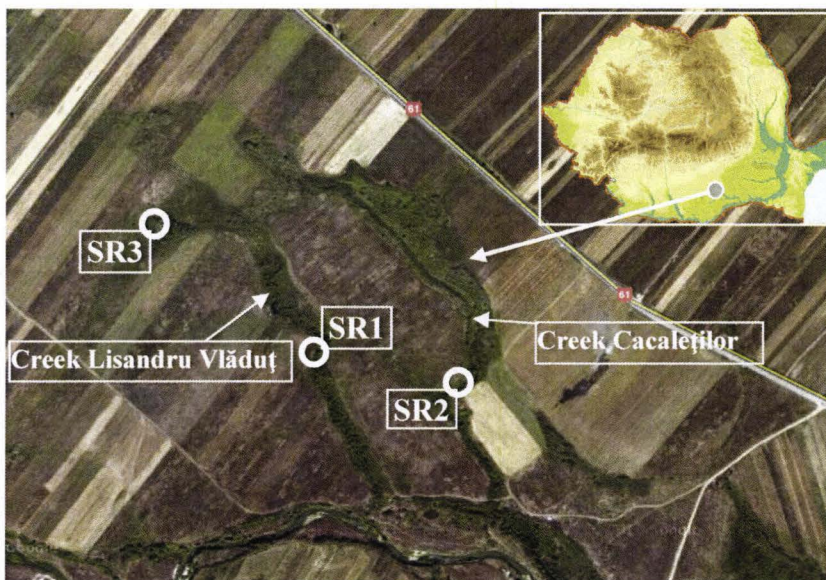


Figure 1. Location of the wetland complex Springs from Corbii Ciungi and of the collecting stations (<http://maps.google.ro>).

The capture of the spiders was done using wet pitfall traps (interception traps), used for collecting the invertebrates active at ground level. There were used plastic tumblers (buried in the ground), with a capacity of 500 cm³, opening diameter of 9 cm, the height of 12 cm and opening surface area of 63.58 cm². In each trap, there were placed 150 cm³ formaldehyde solutions (4%), approximately 1/3 of the vessel volume. In each station 5 pitfalls were installed. They were set in line, 5 m distant one from another, perpendicular to the thread/surface water; the first trap was installed very close to the water limit. The material was monthly collected, from April to December (2012). These pitfalls have operated in the field for 255 days.

RESULTS AND DISCUSSIONS

After the collecting, sorting and determination of the spiders there were obtained 1,094 specimens, of whom 1,021 specimens were determined down to species level; the remaining 73 specimens were identified down to genus or family, due to the impossibility of establishing exactly the species for the immature specimens. There were 1,012 specimens identified to species level: 577 were males (56.51%) and 444 females (43.49%). In terms of systematics, the material was classified in: 19 families, 57 genera and 74 species. The full list of the spider species collected in the Nature Reserve Springs from Corbii Ciungi, with data on the number of males and females collected from each station, relative abundance, class dominance, spread and originality of habitat is presented in Table 1.

Table 1. List of spider species identified in the Nature Reserve Springs from Corbii Ciungi.

No.	Taxa	SR1	SR2	SR3	Sum	Ar %	Class	Spread	Originality of habitat
	Ord. ARANEAE								
	Fam. Dysderidae								
1	<i>Dysdera crocata</i> C. L. KOCH, 1838	4♂, 1♀	21♂, 3♀		29 ex.	2.84	D3	COS	A, SN
	Fam. Mimetidae								
2	<i>Ero furcata</i> (VILLERS, 1789)	1♂			1 ex.	0.10	D1	PAL	CL, SN
	Fam. Theridiidae								
3	<i>Enoplognatha ovata</i> (CLERCK, 1757)			1♀	1 ex.	0.10	D1	HOL	CL, SN, DI
	Fam. Linyphiidae								
4	<i>Bathyphanes gracilis</i> (BLACKWALL, 1841)			1♂, 1♀	2 ex.	0.20	D1	HOL	CL, SN, DI

5	<i>Centromerus sylvaticus</i> (BLACKWALL, 1841)	1♂, 2♀			3 ex.	0.29	D1	HOL	CL, SN, DI
6	<i>Ceratinella major</i> KULCZYŃSKI, 1894			2♀	2 ex.	0.20	D1	PAL	CL
7	<i>Dicymbium nigrum</i> (BLACKWALL, 1834)		2♂		2 ex.	0.20	D1	PAL	CL, SN, DI
8	<i>Diplostyla concolor</i> (WIDER, 1834)	6♂, 7♀	3♂, 8♀	1♂	25 ex.	2.45	D3	HOL	CL, SN
9	<i>Meioneta rurestris</i> (C.L.KOCH, 1836)	1♂	2♂, 1♀		4 ex.	0.39	D1	PAL	CL, SN, DI
10	<i>Nerene clathrata</i> (SUNDEVALL, 1830)		1♀	1♀	2 ex.	0.20	D1	HOL	CL, SN
11	<i>Oedothorax apicatus</i> (BLACKWALL, 1841)			4♂, 2♀	6 ex.	0.59	D1	PAL	CL, SN, DI
12	<i>O. fuscus</i> (BLACKWALL, 1841)	6♂			6 ex.	0.59	D1	PAL	CL, SN, DI
13	<i>O. retusus</i> (WESTRING, 1851)	7♂, 3♀			10 ex.	0.98	D1	PAL	CL, SN, DI
14	<i>Pocadicnemis pumila</i> (BLACKWALL, 1841)			1♂, 1♀	2 ex.	0.20	D1	HOL	CL, SN
15	<i>Tenuiphantes flavipes</i> (BLACKWALL 1854)	2♂, 1♀			3 ex.	0.29	D1	PAL	CL, SN
16	<i>T. tenuis</i> (BLACKWALL, 1852)			1♂	1 ex.	0.10	D1	PAL	CL, SN, DI
17	<i>Troxochrus scabriculus</i> (WESTRING, 1851)	1♂, 1♀			2 ex.	0.20	D1	PAL	CL, SN, DI
18	<i>Walckenaeria antica</i> (WIDER, 1834)		1♀		1 ex.	0.10	D1	PAL	CL, SN
Fam. Tetragnathidae									
19	<i>Pachygnatha degeeri</i> SUNDEVALL, 1830	30♂, 31♀	8♂, 5♀	12♂, 8♀	94 ex.	9.20	D4	PAL	CL, SN, DI
20	<i>Tetragnatha dearmata</i> (THORELL, 1873)	1♀			1 ex.	0.10	D1	HOL	CL, SN
21	<i>T. extensa</i> (LINNAEUS, 1758)	1♂		2♀	3 ex.	0.29	D1	HOL	CL, SN
22	<i>T. montana</i> SIMON, 1874	2♂, 2♀	1♂, 2♀	1♀	8 ex.	0.78	D1	PAL	CL, SN
Fam. Araneidae									
23	<i>Araneus quadratus</i> CLERCK, 1757	1♂			1 ex.	0.10	D1	PAL	CL, SN
24	<i>Argiope bruennichi</i> (SCOPOLI, 1772)	3♂, 4♀	5♀		12 ex.	1.17	D2	PAL	CL, SN, DI
25	<i>Hyposinga sanguinea</i> (C. L. KOCH, 1844)	1♀			1 ex.	0.10	D1	PAL	CL, SN
26	<i>Larinioides cornutus</i> (CLERCK, 1757)	1♂, 2♀		3♂	6 ex.	0.59	D1	HOL	CL, SN
27	<i>Nuctenea umbratica</i> (CLERCK, 1757)		1♀	1♂, 1♀	3 ex.	0.29	D1	ETU	CL, SN, A
28	<i>Singa nitidula</i> C. L. KOCH, 1844		1♀	1♀	2 ex.	0.20	D1	PAL	CL, SN
Fam. Lycosidae									
29	<i>Alopecosa pulverulenta</i> (CLERCK, 1757)	4♂, 2♀	4♂, 3♀		13 ex.	1.27	D2	PAL	CL, SN, DI
30	<i>Arctosa leopardus</i> (SUNDEVALL, 1833)	1♀	1♂, 2♀	1♂, 1♀	6 ex.	0.59	D1	PAL	CL, SN
31	<i>Aulonia albimana</i> (WALCKENAER, 1805)	12♂, 6♀	24♂, 5♀	1♀	48 ex.	4.70	D3	PAL	CL, SN
32	<i>Hogna radiata</i> (LATREILLE, 1817)	2♂, 1♀			3 ex.	0.29	D1	MCA	
33	<i>Pardosa agrestis</i> (WESTRING, 1861)	1♂, 3♀	3♀		7 ex.	0.68	D1	PAL	SN, DI
34	<i>P. amentata</i> (CLERCK, 1757)	1♀			1 ex.	0.10	D1	EUS	CL, SN, DI
35	<i>P. hortensis</i> (THORELL, 1872)	1♂			1 ex.	0.10	D1	PAL	CL, SN, DI
36	<i>P. paludicola</i> (CLERCK, 1757)	1♀	4♂, 2♀	2♀	9 ex.	0.88	D1	PAL	CL, SN, DI
37	<i>P. prativaga</i> (L. KOCH, 1870)	28♂, 29♀	46♂, 40♀	7♂, 34♀	184 ex.	18.00	D5	EUS	CL, SN, DI
38	<i>Piratula hygrophila</i> (THORELL, 1872)	13♂, 2♀	14♂, 5♀	37♂, 18♀	89 ex.	8.71	D4	PAL	CL, SN
39	<i>P. latitans</i> (BLACKWALL, 1841)	23♂, 6♀	2♂, 4♀	1♂, 2♀	38 ex.	3.72	D3	ETU	CL, SN
40	<i>Trochosa robusta</i> (SIMON, 1876)	2♂, 5♀	3♂, 3♀	1♂, 2♀	16 ex.	1.57	D2	PAL	CL, SN
41	<i>T. ruricola</i> (DE GEER, 1778)	40♂, 25♀	38♂, 31♀	43♂, 33♀	210 ex.	20.55	D5	HOL	CL, SN, DI
Fam. Pisauridae									
42	<i>Pisaura mirabilis</i> (CLERCK, 1757)	1♂, 1♀			2 ex.	0.20	D1	PAL	CL, SN, DI
Fam. Oxyopidae									
43	<i>Oxyopes ramosus</i> (MARTINI & GOEZE, 1778)	2♀			2 ex.	0.20	D1	PAL	CL, SN
Fam. Zoridae									
44	<i>Zora spinimana</i> (SUNDEVALL, 1833)	1♂	1♀		2 ex.	0.20	D1	PAL	CL, SN, DI
Fam. Agelenidae									
45	<i>Allagelena gracilens</i> KOCH 1841			1♂	1 ex.	0.10	D1	MCA	CL, SN, A
Fam. Hahniidae									
46	<i>Antistea elegans</i> (BLACKWALL, 1841)			4♂, 4♀	8 ex.	0.78	D1	PAL	CL, SN
47	<i>Hahnia nava</i> (BLACKWALL, 1841)	2♂			2 ex.	0.20	D1	PAL	CL, SN
Fam. Liocranidae									
48	<i>Agroeca cuprea</i> MENGE, 1873		1♂, 1♀	1♂	3 ex.	0.29	D1	ECA	CL
Fam. Clubionidae									
49	<i>Clubiona diversa</i> O.P.-CAMBRIDGE, 1862	1♀			1 ex.	0.10	D1	PAL	CL
50	<i>C. lutescens</i> WESTRING, 1851			1♀	1 ex.	0.10	D1	HOL	CL, SN, DI
Fam. Corinnidae									
51	<i>Phrurolithus festivus</i> (C. L. KOCH, 1835)	1♂	1♀		2 ex.	0.20	D1	PAL	CL, SN
Fam. Gnaphosidae									
52	<i>Drassodes pubescens</i> (THORELL, 1856)	1♂, 1♀			2 ex.	0.20	D1	PAL	CL, SN
53	<i>Drassyllus pusillus</i> (C. L.KOCH, 1833)	2♂, 1♀	2♂, 1♀		6 ex.	0.59	D1	PAL	CL, SN, DI
54	<i>Micaria pulicaria</i> (SUNDEVALL, 1831)	2♂, 1♀	1♀		4 ex.	0.39	D1	HOL	CL, SN
55	<i>Trachyzelotes pedestris</i> (C. L. KOCH, 1837)	3♂	9♂, 2♀	4♂, 2♀	20 ex.	1.96	D2	ETU	CL, SN
56	<i>Zelotes apricorum</i> (L. KOCH, 1876)	1♂		1♀	2 ex.	0.20	D1	ETU	CL, SN
57	<i>Z. gracilis</i> (CANESTRINI, 1868)	2♂			2 ex.	0.20	D1	EUS	CL, SN
58	<i>Z. latreillei</i> (SIMON, 1878)	2♂, 3♀	2♂, 5♀	3♂, 2♀	17 ex.	1.66	D2	PAL	CL, SN, DI
Fam. Philodromidae									
59	<i>Thanatus arenarius</i> L. KOCH, 1872	1♂			1 ex.	0.10	D1	ETU	CL
60	<i>T. atratus</i> SIMON, 1875	1♀	3♂		4 ex.	0.39	D1	PAL	CL
61	<i>Tibellus oblongus</i> (WALCKENAER, 1802)			1♀	1 ex.	0.10	D1	HOL	CL, SN
Fam. Thomisidae									
62	<i>Ebrechtella tricuspidata</i> (FABRICIUS, 1775)	1♂			1 ex.	0.10	D1	PAL	CL, SN

63	<i>Ozyptila praticola</i> (C. L. KOCH, 1837)	3♂, 4♀	19♂, 3♀	2♂, 1♀	32 ex.	3.13	D3	HOL	CL, SN
64	<i>Xysticus acerbus</i> THORELL, 1872		1♀		1 ex.	0.10	D1	ECA	CL
65	<i>X. kochi</i> THORELL, 1872		1♂		1 ex.	0.10	D1	ECA	CL, SN, DI
	Fam. Salticidae								
66	<i>Euophrys frontalis</i> (WALCKENAER, 1802)	2♂, 1♀	3♂, 1♀	1♂	8 ex.	0.78	D1	PAL	CL, SN
67	<i>Evarcha arcuata</i> (CLERCK, 1757)	1♀	3♀		5 ex.	0.49	D1	PAL	CL, SN
68	<i>E. falcata</i> (CLERCK, 1757)		1♂, 2♀		3 ex.	0.29	D1	PAL	CL, SN
69	<i>Heliophanus cupreus</i> (WALCKENAER, 1802)	2♂			2 ex.	0.20	D1	PAL	CL, SN
70	<i>Mendoza canestrini</i> (CANESTRINI & PAVESI, 1868), (syn. <i>Marpissa canestrinii</i>)	1♀			1 ex.	0.10	D1	PAL	CL
71	<i>Myrmarachne formicaria</i> (DE GEER, 1778)	1♂, 1♀	2♀		4 ex.	0.39	D1	PAL	CL
72	<i>Neon levis</i> (SIMON, 1871)	1♂, 5♀	2♂, 2♀	1♂	11 ex.	1.08	D1	PAL	CL
73	<i>Pseudeuophrys erratica</i> (WALCKENAER, 1826)		3♂, 3♀		6 ex.	0.59	D1	PAL	CL, SN
74	<i>Talavera aequipes</i> (O. P.- CAMBRIDGE, 1871)		3♂, 2♀		5 ex.	0.49	D1	PAL	CL, SN
	Total	224♂, 162♀	222♂, 157♀	131♂, 125♀	577♂, 444♀	100			
		386 ex.	379 ex.	256 ex.	1,021 ex.				
	No. species	53	39	33					

Legend: ♂-male, ♀-female. CL - climax, habitats unchanged or very slightly affected by human action; SN - semi-natural habitats; DI – strong disturbed habitats, degraded; A - artificial habitats).

From the fauna point of view, spider species collected from the Nature Reserve Springs from Corbii Ciungi are generally common species, not cited in any of the categories: species of community interest, IUCN species, endemic species and species mentioned in the Annex OUG 57/2007 or species present on red lists in Romania. However, we want to draw attention on some species of spider we can consider relatively rare for the Romanian fauna:

Ceratinella major KULCZYŃSKI, 1894, from the family Linyphiidae, is a relatively rare species found in scree forests, with moderate humidity, in semi-open or partially shaded places, between 200 meters and 500 meters altitude. It is active at the ground level, under rocks, rarely found in the litter. It is an endangered species in Poland and near threatened in Germany.

Tetragnatha dearmata (THORELL, 1873) from the family Tetragnathidae. It is a relatively rare species, present in different types of forests and open areas, mostly on bushes near waters, at altitudes between 100 meters and 400 meters. It prefers semi-open, partly shaded and shaded places. Adults are present from May until June/July. The species is considered endangered in Slovakia and near threatened in Germany.

Zelotes gracilis (CANESTRINI, 1868), from the family Gnaphosidae, is a rare species, which prefer steppes, sandy zones with scrubby patch; found at ground level, in open or partially shaded areas, in dry places. On altitude, the species can be found from 100 meters to 500 meters altitude. Adults are active from May to August. In the Czech Republic, it is considered an endangered species and near threatened in Slovakia. Collected personally on Lecsoare hill (near Ștefănești city, Argeș), from a sunny slope with south-western exhibition, about 400 altitude (LOTREAN, 2010).

Mendoza canestrini (CANESTRINI & PAVESI, 1868), from the family Salticidae. It is a relatively rare species, present in wetlands, among the reeds near water, retreat in spun reed tips, at altitudes between 100 meters and 500 meters. It prefers semi-open places. The species is considered critically endangered in the Czech Republic and vulnerable in Slovakia.

In terms of the sex ratio, in the collected material, 56.51% were male and 43.49% were females. The sex ratio, for the 36 spider species for which both sexes were collected, in 21 cases (28.37%), it was favourable for males, for 15 species (20.27%) it was favourable to females and for 11 species (14.86%) it was relatively balanced, being very close to the theoretical value of 1:1. For the rest of the species, 27 species (36.49%), there were collected either males, in most cases, or females. The analysis of data on sex ratio showed that it was relatively balanced; overall ratio is to 1.3:1.

From the quantitative point of view, most of the collected specimens belonged to the family Lycosidae (61.15%), followed by the families: Tetragnathidae (10.37%), Linyphiidae (6.95%), Gnaphosidae (5.19%), and Salticidae (4.40%). The rest of the spider families had weights less than 4%.

The hierarchy changes if we consider the number of genera and species. From this point of view, most genera and species belonged to the family Linyphiidae (21.05%, respectively 20.00%), followed by the families: Lycosidae (12.28% for genera and 17.33% for species), Salticidae (14.04% for genera, 12.00% for species), Araneidae (10.53% for genera and 8.00% for species), Gnaphosidae (8.77% for genera and 9.33% for species) and Thomisidae (5.26%, respectively 5.33%). The rest of the spider families had weights below 5%, as well as the number of genera and/or the number of species.

The values of the relative abundance calculated for each species in all three stationeries indicated that two species, i.e. *Trochosa ruricola* (DE GEER, 1778) and *Pardosa prativaga* (L. KOCH, 1870) were eudominant species. They were followed by two dominant species, *Pachygnatha degeeri* SUNDEVALL, 1830 and *Piratula hygrophila* (THORELL, 1872), and five subdominant species: *Aulonia albimana* (WALCKENAER, 1805), *Piratula latitans* (BLACKWALL, 1841), *Ozyptila praticola* (C. L. KOCH, 1837), *Dysdera crocata* C. L. KOCH, 1838, and *Diplostyla concolor* (WIDER, 1834). The rest of the spiders species were recedent (4 species) and under-recedent (61 species).

Eudominant species for SR1 station were: *Trochosa ruricola* (DE GEER, 1778), *Pachygnatha degeeri* SUNDEVALL, 1830 and *Pardosa prativaga* (L. KOCH, 1870), followed by *Piratula latitans* (BLACKWALL, 1841)

as dominant species and *Aulonia albimana* (WALCKENAER, 1805), *Piratula hygrophila* (THORELL, 1872), *Diplostyla concolor* (WIDER, 1834) and *Oedothorax retusus* (WESTRING, 1851) as subdominant species.

Two species were eudominant in SR2, *Pardosa prativaga* (L. KOCH, 1870) and *Trochosa ruricola* (DE GEER, 1778). In this station four species had the dominant species status: *Aulonia albimana* (WALCKENAER, 1805), *Dysdera crocata* C. L. KOCH, 1838, *Ozyptila praticola* (C. L. KOCH, 1837) and *Piratula hygrophila* (THORELL, 1872). *Pachygnatha degeeri* SUNDEVALL, 1830, *Diplostyla concolor* (WIDER, 1834) and *Trachyzelotes pedestris* (C. L. KOCH, 1837) represented subdominant species.

Three eudominant species were recorded in SR3: *Trochosa ruricola* (DE GEER, 1778), *Piratula hygrophila* (THORELL, 1872) and *Pardosa prativaga* (L. KOCH, 1870) followed by one dominant species, *Pachygnatha degeeri* SUNDEVALL, 1830, and three subdominant species: *Antistea elegans* (BLACKWALL, 1841), *Oedothorax apicatus* (BLACKWALL, 1841) and *Trachyzelotes pedestris* (C. L. KOCH, 1837).

Arranging spider species in descending order according to relative abundance values (Fig. 2) allowed the identification of species forming the core of the spider fauna from the Nature Reserve Springs from Corbii Ciungi. There are differences from one habitat to another, but the same species of spider are eudominant or dominant in all three collecting station. It is amended depending on the particularities of the habitat and position of the dominant species and species group which accompany them. *Trochosa ruricola* (DE GEER, 1778) was an eudominant species in all three collecting stations and also for all the reserve level. Optimal humidity of this species is over 95%, while optimum temperature is about 24°C (FHUN & NICULESCU-BURLACU, 1971). It is a mesophilic, photophilous, eurytherme and hygrophilous species, which prefer open areas, wetlands, grassy areas, near water. In the collecting station SR3, previous species was accompanied by *Piratula hygrophila* (THORELL, 1872). It is a species with a very small ecological valence, hygrophilous, which prefer shady places, under trees and shrubs, where the soil is covered with *Sphagnum* sp. Most specimens were collected in the pitfalls traps installed near a spring (SR3), in a marshy area covered with young specimens of *Alnus glutinosa* (LINNAEUS, 1754). The species is characteristic for this type of habitat, moist and shady, which in the past probably occupied wider areas of the reserve surface. In SR1 and SR2 station, the species were collected in close proximity of water in shady areas, covered with shrubs. *Pardosa prativaga* (L. KOCH, 1870) is a hygrophilous and thermophilous species, found in open grassy places. This species had eudominant species status in all three habitats investigated. The eudominant and dominant species group is completed by three species frequently found in open areas: *Piratula latitans* (BLACKWALL, 1841), *Pachygnatha degeeri* SUNDEVALL, 1830 and *Aulonia albimana* (WALCKENAER, 1805). Of the three, the first is a species with a small ecological valence, hygrophilous and photophilous, characteristic for open wetlands. It was the dominant species only in the SR1. The other two species, *Dysdera crocata* C. L. KOCH, 1838 and *Ozyptila praticola* (C. L. KOCH, 1837), dominated in the SR2 station, cannot be considered riparian species, because they were frequently encountered in different types of habitats in lowlands. Of the species listed above only *Piratula hygrophila* (THORELL, 1872) is considered an enlightening species for oligotrophic bogs (URÁK, 2008), being a typical element of peat lands.

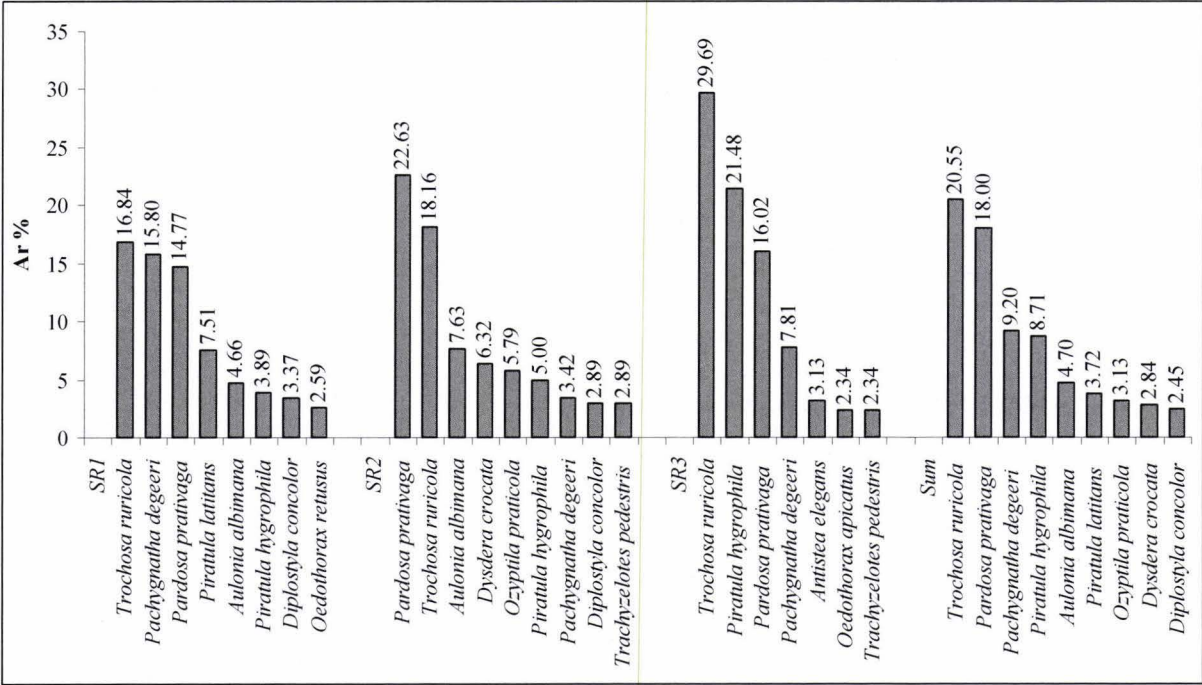


Figure 2. The hierarchy of the values of the relative abundance for the spiders species identified in the Nature Reserve Springs from Corbii Ciungi (species with Ar > 2%).

The hierarchy of the stations depending on the diversity and equitability values was established using the Shannon index. The Shannon diversity index ranged from 1.00 to 1.30 and equitability from 0.66 to 0.77 (Fig. 3). The highest values of the diversity index were recorded in the SR1 station, where the majority of the species were identified. The lowest values of both parameters were observed in the sites where only one species was highly predominant, i.e. SR3 station, where there were identified the fewest species. For SR2 the values obtained were very close to those calculated for SR1. In SR3, the small values calculated for the index of diversity Shannon could be explained because the investigated habitat is of “insular” type, having clear limits and characteristic biotope conditions, different from those of the adjacent areas. The other stationeries became “open” as a result of the clearings and consequently they “continue” in the neighbourhood areas. This fact permitted the increasing of the number of species as a result of the invasion of the riparian habitats by characteristic species for open areas, frequently met in ruderal and agricultural areas, expansive and tolerant open landscape species.

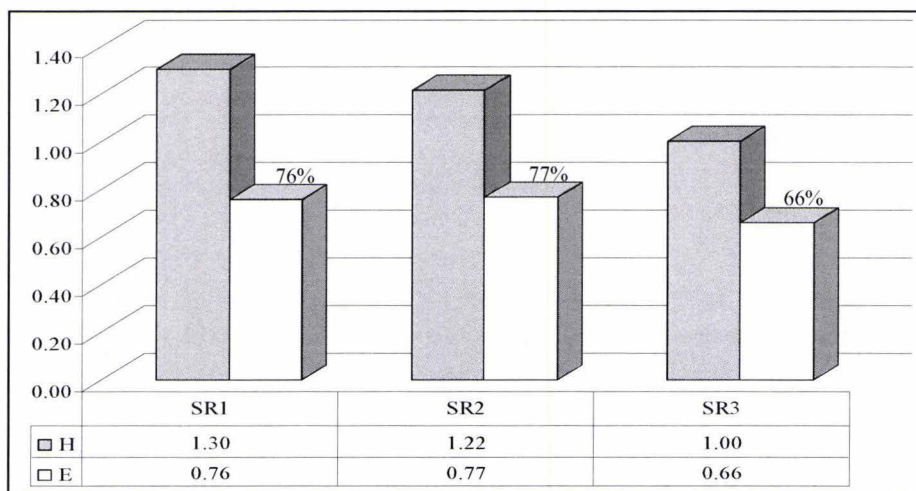


Figure 3. The values of the diversity (H) and the equitability (E) for the spiders fauna in the three collecting stations from the Nature Reserve Springs from Corbii Ciungi.

Generally, the values of this index vary between 1.5 and 3.5 (VARVARA & ZUGRAVU, 2006; URÁK, 2008), results that for all the investigated habitats the Shannon index had small values. These low values of diversity are characteristic of relatively homogeneous habitats, pioneer or degraded, in which one, two, rarely three species, well adapted to the environmental conditions of that habitat dominates the other species from studied group.

In accordance to their current spreading, the 74 species of spider identified in the Nature Reserve Springs from Corbii Ciungi, were classified into 7 zoogeographical groups (DELTSHEV, 2005). In terms of number of spider species for each zoogeographical groups, I found the presence of large numbers of Palearctic species, more than half (62.00%) of the identified species belonging to this category. These were followed by: the Holarctic species (19%), European-Turanian species (7.00%), European-Siberian and European Central-Asian species (4.00%, each). The rest of the zoogeographical elements had less than 4% of weight (Fig. 4). It is noted that, from the zoogeographical structure of the spider fauna, the species with the small areas of distribution are missing (“continental and subcontinental” species).

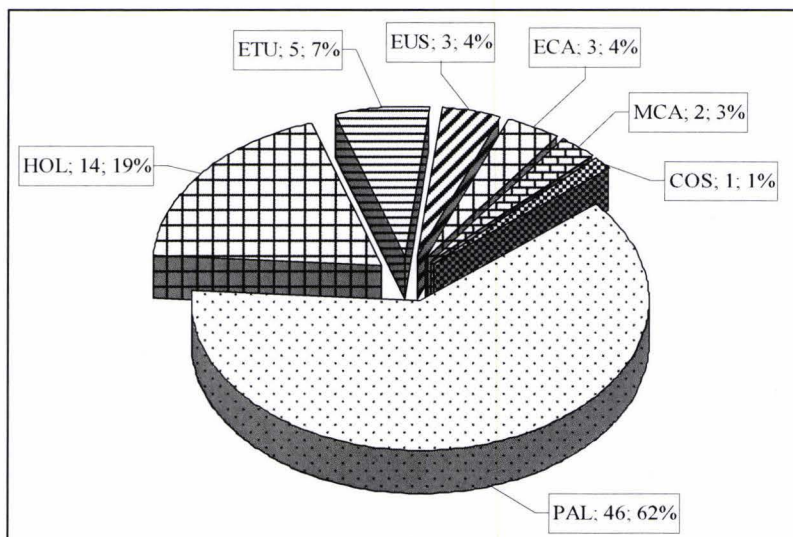


Figure 4. Distribution of the spiders species on zoogeographical groups (PAL – Palearctic, HOL – Holarctic, ETU – European-Turanian, ECA – European Central-Asian, EUS – European-Siberian, MCA – Mediterranean-Central Asian, COS – Cosmopolitan).

For grouping the habitats, according to the spider fauna, I used the Jaccard index, based on the presence/absence of the species (Fig. 5). From this viewpoint the SR3 station clearly detaches from the other investigated habitats. The similarity to the others habitats, SR1 and SR2, was 38.26%. Low similarity with the rest of habitats was determined by the presence of young trees that form a compact cluster (a grove), probably the result of natural regeneration. This habitat is damp and dark, fact that is reflected in the structure of the spider fauna. Between SR1 and SR2 the similarity was 43.63%. The difference between the two habitats is determined by the presence, in the case of SR2 station, of thin selvedge shrubs which partially cover a very wet area, but also by the existence of ruderal and cultivated land very close to the water limit. This explains the presence in the samples of species with different ecological requirements: *Trochosa ruricola* (DE GEER, 1778), *Piratula hygrophila* (THORELL, 1872), *Pardosa prativaga* (L. KOCH, 1870) and *Diplostyla concolor* (WIDER, 1834), hygrophilous species, alongside xerophytes species such as *Trachyzelotes pedestris* (C. L. KOCH, 1837) and *Dysdera crocata* C. L. KOCH, 1838.

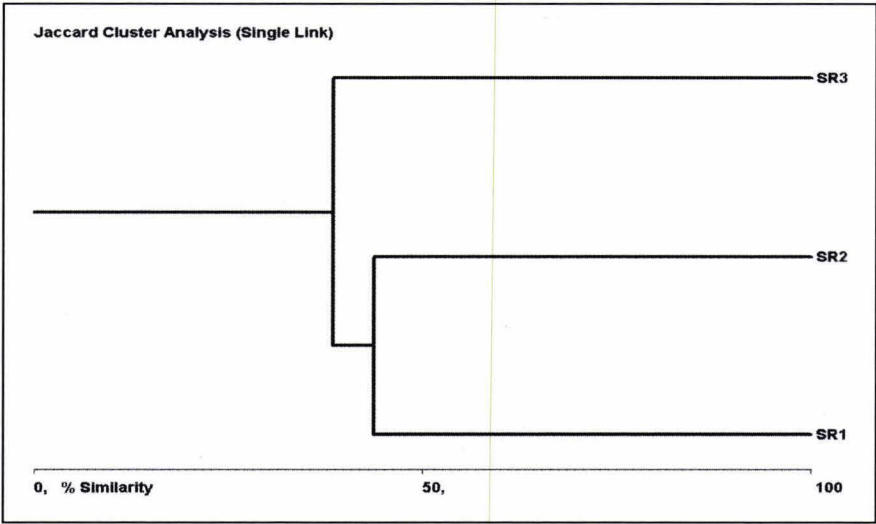


Figure 5. The similarity of studied habitats according to the specific composition of spider fauna.

In order to estimate the degree of conservation of the studied area, I considered useful grouping the identified spider species depending on their tolerance to the originality of habitat (degree of degradation of the habitats) that they populate, according to the classification proposed by Buchar and Růžicka (BUCHAR & RŮŽIČKA, 2002). The species from the well preserved or merely disturbed (semi-natural) habitats represents only 62.16% of all identified species (Fig. 6). This was observed for all three investigated habitats. The percentage of the cumulative values for the two categories (CL + CL, SN) ranged between 64.15% (SR1) and 61.54% (SR2). The presented value indicates an inadequate conservation status of the investigated habitats.

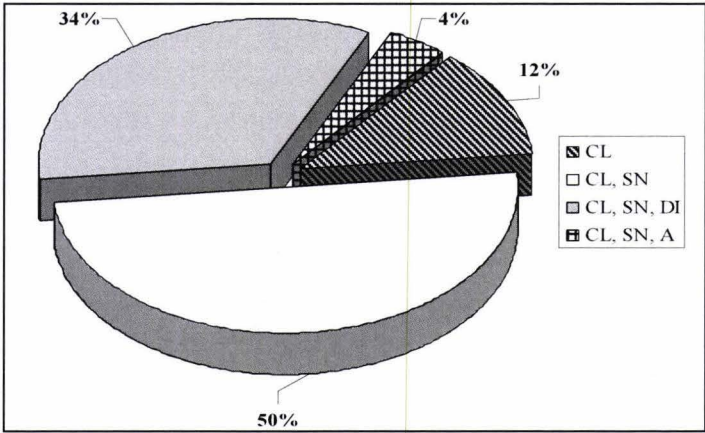


Figure 6. Grouping the spider species, collected from the Nature Reserve Springs from Corbii Ciungi, according to their preferences to the originality of habitat (CL - climax, habitats unchanged or very slightly affected by human action; SN - semi-natural habitats; DI - strong disturbed habitats, degraded; A - artificial habitats).

This feature is a result of the anthropogenic activities (agriculture, grazing and fires) which determined the fragmentation, the diminution and even the disappearance of the original riparian habitats that covered the complex of springs and creeks collectors down to the shedding in the Neajlov River. At present (2012), the surface of the reserve decreased to about 5 ha, because of the expansion of crops and grazing. It was grubbed up “riverside coppice-looking jungle” (NEGREA & NEGREA, 1999) that covered the complex of springs and creeks collectors down to the shedding in the Neajlov River. This

type of habitat had a great importance in maintaining low water temperatures and thus the protection of cold water aquatic species, some of these species being relict species. Some of them have disappeared due to the degradation of their habitats; they have not been found during the last research (year 2012). Others are in a strong numerical decline.

CONCLUSIONS

This is the first study of the spider fauna from the Nature Reserve Springs from Corbii Ciungi; the study led to the identification of 74 species of spider, grouped in 57 genera and 19 families. All species are at the first citation for this area. From the point of view of fauna only four species: *Ceratinella major* KULCZYŃSKI, 1894, *Tetragnatha dearmata* (THORELL, 1873), *Zelotes gracilis* (CANESTRINI, 1868) and *Mendoza canestrini* (CANESTRINI & PAVESI, 1868) can be considered relatively rare for the Romanian fauna.

The families Linyphiidae (20.0%) and Lycosidae (17.33%) were the best represented, as species. As individuals, the hierarchy is reversed ascertaining the numerical dominance of the species from the family Lycosidae (61.15%), followed by families Tetragnathidae (10.37%) and Linyphiidae (6.95%).

The small biodiversity and equitability values, as well as the way the domination exercising, show the existence of some spider associations characteristic for the pioneer or degraded habitats.

Grouping species of spiders according to their distribution area showed the net dominance of widely spread elements, Palearctic and Holarctic species, which totalized nearly 80% of the identified species.

The values less than 50% of the Jaccard similarity index reflect a low similarity of the studied habitats through the spider fauna, which indicates a high heterogeneity of the studied area.

According to the originality of the habitat only 12.16% of the spider species are considered climax species. These indicate that all the types of investigated habitats are not well-preserved and classify this area among those which need urgent protection measures. From a conservative viewpoint we remarked the spiders association from the station SR3, which differs from the others. This association characterizes a type of habitat that resembles the most with the original riparian habitats of the reserve. From this viewpoint such a habitat can be considered a regeneration centre for "riverside coppice-looking jungle", which must be protected.

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***Matricaria chamomilla* L. AS TROPHIC NICHE FOR THRIPS POPULATIONS (INSECTA: THYSANOPTERA)**

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Abstract. The collections carried out in May-June 2010 regarding the Thysanoptera fauna on *Matricaria chamomilla* L. from a grassland ecosystem from Scornicești, Olt County, show a rich specific diversity, namely 11 species. The number of species, the composition of the species as well as the number of individuals depend on the time of sampling. The Thysanoptera coenosis has been constantly composed of a characteristic species, *Haplothrips leucanthemi* and *Thrips tabaci*, so we can consider chamomile as a host plant for these species. *H. leucanthemi* presents the highest values of structural indicators and a relative abundance value of 70.96%, followed by *T. tabaci* with 22.26%. The Shannon-Wiener diversity index presented low values in the studied site. The geographical distribution of Thysanoptera indicates a dominance of the Euro-Siberian species, situation encountered in Thysanoptera coenoses from other types of ecosystems. Such studies reveal the importance of flowering plants, as a trophic niche in ensuring and preserving the biodiversity of species of this small order of insects.

Keywords: *Matricaria chamomilla*, Thysanoptera, specific diversity, ecological indices.

Rezumat. *Matricaria chamomilla* L. ca nișă trofică pentru populațiile de tripsi (Insecta: Thysanoptera). Colectările efectuate în perioada mai-iunie 2010 privind fauna de tisanoptere de pe plantele de mușetel dintr-un ecosistem de pășuni din Scornicești, județul Olt, relevă o diversitate specifică mare, de 11 specii. Numărul speciilor, compoziția precum și numărul indivizilor depind de perioada de colectare. Cenoza de tisanoptere a fost constant alcătuită din speciile *Haplothrips leucanthemi* și *Thrips tabaci*, astfel încât putem considera mușetelul ca plantă gazdă pentru aceste două specii. *H. leucanthemi* prezintă cele mai ridicate valori ale indicatorilor structurali și o valoare a abundenței relative de 70.96%, urmat de *T. tabaci* cu 22.26%. Indicele de diversitate Shannon-Wiener a avut valori mici în situl studiat. Distribuția geografică a tisanopterelor de pe mușetel indică o pondere mare a speciilor euro-siberiene, situație întâlnită în cenozele de Thysanoptera din alte tipuri de ecosisteme. Astfel de studii relevă importanța plantelor cu flori, ca nișă trofică în asigurarea și conservarea biodiversității speciilor din acest mic ordin de insecte.

Cuvinte cheie: *M. chamomilla*, Thysanoptera, diversitate specifică, indicatori ecologici.

INTRODUCTION

Matricaria chamomilla L. can be found all over Europe, temperate Asia, in North America and Australia, growing near roads, cereal crops and landfills. This Asteraceae species offers optimum life conditions to a group of delicate insects, less known – the Thysanoptera order. Over time, there have been several taxonomic studies on *Thrips* fauna inhabiting the organs of different host plants. KNECHTEL (1951), LEWIS (1973), SCHLIEPHAKE & KLIMT (1979), RASPUK et al. (2009) mention in their works thrips species on *M. chamomilla*, most often collected being the *Haplothrips leucanthemi* species.

The present study completes the list of host plants for Thysanoptera in Romania, as in the work of VASILIU OROMULU (2002) on the distribution of thrips species on different host plants *M. chamomilla* is not found.

This study is probably the first worldwide ecological research of Thysanoptera that have *M. chamomilla* as a host plant.

MATERIAL AND METHODS

The observations were carried out during May-June 2010 on inflorescences of *Matricaria chamomilla* in a lowland grassland ecosystem from Scornicești (Olt County). Collections were made during the flowering of chamomile, other herbaceous species being in bloom at the same time, so that not to influence the relationship between thrips species and host plants. In this way it can be established objectively the attachment of thrips species to the preferred plant. Ten (10) samples were collected every two weeks, and a sample consisted of 10 inflorescences. Adult individuals and larvae were collected from inflorescences and preserved in vials with AGA, a mixture of 60% ethyl alcohol (10 parts), glycerine (1 part) and glacial acetic acid (1 part). The thrips species was identified with the following keys of determination: KNECHTEL (1951), SCHLIEPHAKE & KLIMT (1979), STRASSEN (2003).

In order to assess the diversity of the ecosystem, the Shannon-Weaver diversity index was calculated, using the formula improved by LLOYD and GHILARDI:

$$H(S) = \frac{K}{N} (N \log_{10} N - \sum_{r=1}^S N_r \log_{10} N_r) \text{ where:}$$

H = index; S = total number of species; K = 3, 321928; N = total number of individuals; N_r = total number of individuals in species r. (SIMIONESCU 1984; ȘCIROPU 1997). Immature thrips were not used in the count.

RESULTS AND DISCUSSIONS

In the study on thrips populations inhabiting the inflorescences of *Matricaria chamomilla* there were tracked the following aspects: specific diversity, sex ratio, geographical distribution, ecological indicators, and predators.

a. Specific diversity

The numerical abundance reveals a total of 1 698 adults and 87 larvae, which belong to 11 species (Table 1). Three of them, *Frankliniella intonsa*, *Haplothrips leucanthemi* and *H. setiger* are mentioned by RASPUDIC et al., 2009 on *M. chamomilla*, in Croatia.

Of these species, most are primary consumers and polyphagous. Only one species belongs to the secondary consumers, the polyphagous *Aeolothrips intermedius*. Ecologically, the taxonomic spectrum of Thysanoptera on *Matricaria chamomilla* is dominated by typical floricolous forms. Thus, in chamomile inflorescences, *Haplothrips leucanthemi*, *Thrips tabaci*, *Haplothrips angusticornis*, *H. reuteri*, *H. setiger*, *Thrips pillichii*, *T. validus*, *Frankliniella intonsa* find optimal living conditions. One species is a gramineous form - *Limothrips denticornis* that preferentially grow on Poaceae. Moreover, in terms of hydric needs, most species are mesophilous, situation explicable by the collections performed in May and June, characterized by high humidity and moderate temperatures; except for the xerophilous species, *Frankliniella intonsa*.

The presence of numerous Terebrantia and Tubulifera larvae expresses the high level of renewal of thrips populations. Corresponding to the higher number of adults, Tubulifera larvae are much more numerous than the Terebrantia ones (Table 1).

Table 1. Specific diversity of Thysanoptera fauna on *M. chamomilla*.

Suborder	Family	Species	No. ind.	A (%)	Geographical distribution
Terebrantia	Aeolothripidae	<i>Aeolothrips intermedius</i> BAGNALL 1934	4♀♀	0.24	PAL
	Thripidae	<i>Frankliniella intonsa</i> (TRYBOM 1895)	1♀; 1♂	0.12	EUS
		<i>Limothrips denticornis</i> HALIDAY, 1836	1♀	0.06	EUS
		<i>Thrips pillichii</i> (PRIESNER 1924)	22 ♀♀; 1♂	1.35	WPAL
		<i>T. tabaci</i> (LINDEMAN, 1888)	378♀♀	22.26	COS
		<i>T. validus</i> UZEL, 1895	1♀	0.06	EUS
		<i>Thrips</i> sp.	1♀	0.06	-
		larve Terebrantia	33	-	-
Tubulifera	Phlaeothripidae	<i>Haplothrips angusticornis</i> (PRIESNER, 1921)	28/♀♀; 22♂♂	2.94	WPAL
		<i>H. leucanthemi</i> (SCHRANK 1781)	779♀♀; 426♂♂	70.96	EUS
		<i>H. reuteri</i> KARNY, 1907	12♀♀; 6♂♂	1.06	PON-MED
		<i>H. setiger</i> (PRIESNER, 1921)	13♀♀; 2♂♂	0.88	WPAL
		larve Tubulifera	54	-	-

Legend: COS=Cosmopolite; EUS=Euro-Siberian; PAL= Palearctic; WPAL= West -Palearctic; PON-MED = Ponto-Mediterranean.

b. Sex ratio

Table 1 notes the dominance of females in most thrips species, common situation for this group of insects.

We notice a more balanced sex ratio for the *Haplothrips leucanthemi* species, with an overall percentage of females of 64.6% and a sex-ratio value of 0.54. As shown in Fig. 1, sex ratio has close values at each collection date.

Moreover, LEWIS (1973) mentions that in many species with equal number of both species, females seem to prevail because of their much higher longevity.

Instead, in some cosmopolitan species, sex ratio varies in different regions depending on the temperature. For example, *Thrips tabaci* is represented only by parthenogenetic females in the temperate zone, this situation being found in all collections so far in our country. In *Frankliniella occidentalis*, pest and in Romanian greenhouses, higher temperatures in greenhouses favour the occurrence of a much larger number of females, sex ratio value being of 0.11 (BĂRBUCEANU & VASILIU-OROMULU, 2012).

c. Geographical distribution

The geographical distribution of thrips species on *Matricaria chamomilla* presents 4 Euro-Siberian, 3 W-Palearctic, 1 cosmopolite, 1 Palearctic, and 1 Ponto-Mediterranean species. We can notice a higher proportion of the Euro-Siberian species, a situation encountered, for example, in the vineyard ecosystem (VASILIU-OROMULU, 1998; VASILIU-OROMULU & BĂRBUCEANU, 2010) (Table 1).

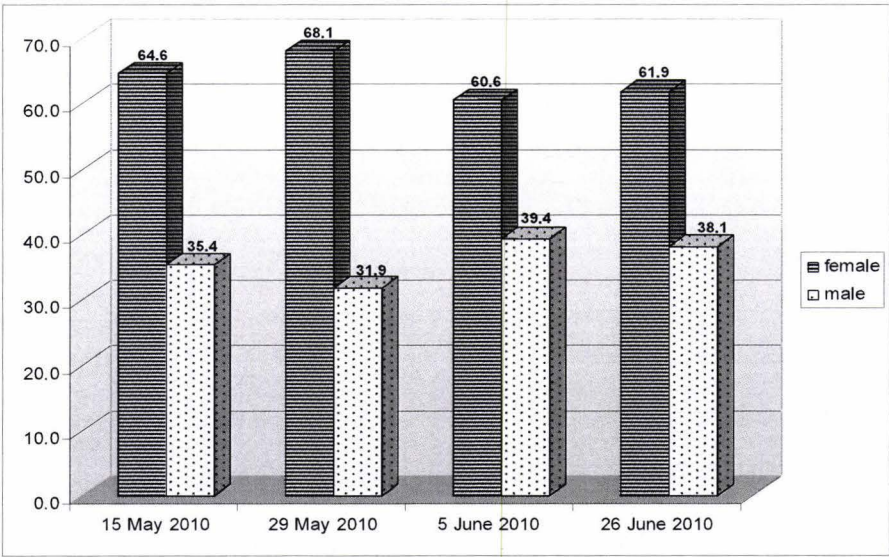


Figure 1. Sex ratio on *H. leucanthemi* species.

d. Ecological indices

At all four collection dates, the “specific nucleus” of the Thysanoptera association was formed by two species: *Haplothrips leucanthemi* and *Thrips tabaci* (Fig. 2; Table 2).

The highest specific diversity, 9 species, is found in the collection of 15 May, when the chamomile flowering is maximum. The trophic generous offer attracts many Terebrantia and Tubulifera species, thus the zoophagous *Aeolothrips intermedius* being also favoured. It is the collection with the highest values of thrips species numerical density: 757 individuals/inflorescence, situation also reflected in the values of dry biomass. Also, numerical density values prove negative binomial distribution of this group of insects. The species with an important role in establishing coenosis structure are *H. leucanthemi* and *T. tabaci*, with maximum values of frequency in samples. *Thrips pillichi* and *Haplothrips reuteri*, although with frequency values of 60% and 70%, respectively, have low values of relative abundance, thus they do not go beyond the status of accompanying species. The value of Shannon-Wiener diversity index of only 1.42 is influenced by low equitability. Thus, although the number of species involved in the coenosis structure is large, their numerical abundance is low, the two dominant species totalling 91.3% of the collected individuals.

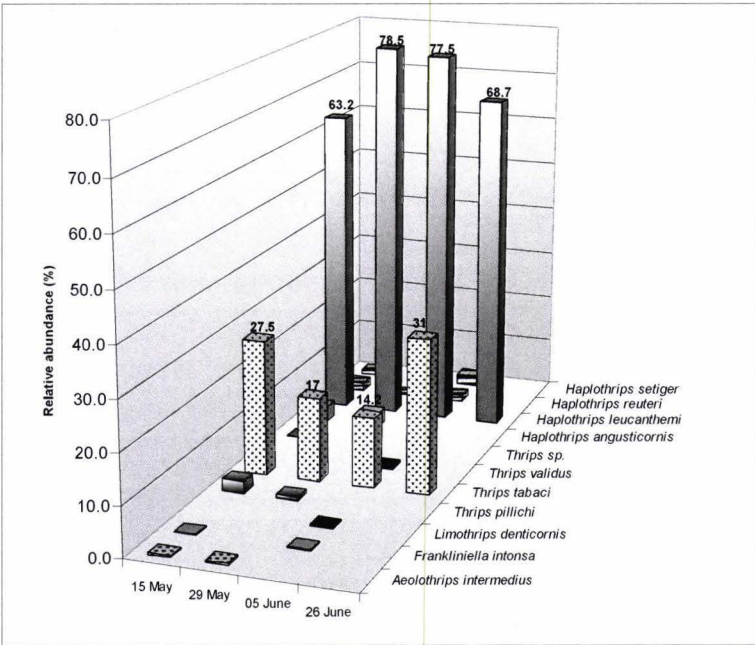


Figure 2. Relative abundance (%) of thrips species on *M. chamomilla*.

On 29 May, the association of Thysanoptera was composed of six species, *Haplothrips leucanthemi* and *Thrips tabaci*, as eudominant and euconstant species, having the largest weight in using trophic resources. *Aeolothrips*

intermedius, *Thrips pillichi*, *Haplothrips angusticornis*, *H. reuteri*, *H. setiger* species are accompanying species. Low equitability value, 28.44, has repercussions upon the diversity index - 0.98, the lowest during all collection period.

On 5 June collection, the populations of Thysanoptera association belonged to 8 species, indicating a high specific diversity, with a specific nucleus formed by *Haplothrips leucanthemi* and *Thrips tabaci* species; only the first species has a maximum sample frequency, while *T. tabaci* proved attachment to this trophic substrate only in 60% of the samples. The other species have sporadic presence, recording frequency values below 40%. Equitability and diversity index have low values.

At the end of chamomile flowering season, namely the collection of 26 June, the Thysanoptera coenosis was composed only of the characteristic species of *H. leucanthemi* and *T. tabaci*, with the lowest values of numerical abundance of all collections and a low diversity index value.

The xerophilous *Frankliniella intonsa*, known as “the thrips of inflorescences” due to its presence in considerable number in the inflorescences of several species, has a sporadic presence, the species being disadvantaged by the wet microclimate during the two collection months.

Haplothrips leucanthemi is a mesophilous floricultural polyphagous species, known in the literature as having a special attachment to the inflorescences of *Leucanthemum vulgare*, from where its name is derived. KNECITEL (1951) mentions its presence on the inflorescences of *M. chamomilla*. This study highlights large populations of this species in chamomile inflorescences; thus, *M. chamomilla* can be considered a host plant. As shown in Figure 2 and Table 2, at all collection dates, “daisy thrips” overwhelmingly dominate the other species of the Thysanoptera association.

Table 2. The structural indices of the thrips populations on *M. chamomilla*.

15 May 2010	Σ	x	No.ind./ inflorescence	s ²	STDEV	s'	mg.dry matter/m ²	A%	C%	P _i log p _i
<i>Aeolothrips intermedius</i>	2	0.2	2	0.4	0.6	0.06	0.20	0.3	10	-0.007
<i>Frankliniella intonsa</i>	1	0.1	1	0.1	0.3	0.03	0.10	0.1	10	-0.004
<i>Thrips</i> sp.	1	0.1	1	0.1	0.3	0.03	0.10	0.1	10	-0.004
<i>T. pillichi</i>	19	1.9	19	4.3	2.1	0.21	1.90	2.5	60	-0.040
<i>T. tabaci</i>	208	20.8	208	184.4	13.6	1.36	20.80	27.5	100	-0.154
<i>Haplothrips angusticornis</i>	23	2.3	23	13.3	3.7	0.37	2.30	3.0	40	-0.046
<i>H. leucanthemi</i>	483	48.3	483	863.8	29.4	2.94	48.30	63.8	100	-0.125
<i>H. reuteri</i>	13	1.3	13	2.5	1.6	0.16	1.30	1.7	70	-0.030
<i>H. setiger</i>	7	0.7	7	0.7	0.8	0.08	0.70	0.9	50	-0.019
Σ	757	76	757	1436.9	37.9	3.79	75.70	100.0		-0.428
29 May 2010		H(S)	=1.42		Hmax	=3.46	E%	=41.14		
<i>Aeolothrips intermedius</i>	2	0.2	2	0.4	0.6	0.06	0.20	0.4	10	-0.010
<i>Thrips pillichi</i>	4	0.4	4	0.7	0.8	0.08	0.40	0.8	20	-0.017
<i>T. tabaci</i>	82	8.2	82	12.0	3.5	0.35	8.20	17.0	100	-0.131
<i>Haplothrips angusticornis</i>	14	1.4	14	9.6	3.1	0.31	1.40	2.9	40	-0.045
<i>H. leucanthemi</i>	379	37.9	379	76.8	8.8	0.88	37.90	78.5	100	-0.083
<i>H. reuteri</i>	1	0.1	1	0.1	0.3	0.03	0.10	0.2	10	-0.006
<i>H. setiger</i>	1	0.1	1	0.1	0.3	0.03	0.10	0.2	10	-0.006
Σ	483	48	483	120.2	11.0	1.10	48.30	100.0		-0.266
5 June 2010		H(S)	=0.98		Hmax	=3.46	E%	=28.44		
<i>Frankliniella intonsa</i>	1	0.1	1	0.1	0.3	0.03	0.10	0.3	10	-0.008
<i>Limothrips denticornis</i>	1	0.1	1	0.1	0.3	0.03	0.10	0.3	10	-0.008
<i>Thrips tabaci</i>	46	4.6	46	81.2	9.0	0.90	4.60	14.2	60	-0.120
<i>T. validus</i>	1	0.1	1	0.1	0.3	0.03	0.10	0.3	10	-0.008
<i>Haplothrips angusticornis</i>	13	1.3	13	9.6	3.1	0.31	1.30	4.0	40	-0.056
<i>H. leucanthemi</i>	251	25.1	251	355.9	18.9	1.89	25.10	77.5	100	-0.086
<i>H. reuteri</i>	4	0.4	4	0.7	0.8	0.08	0.40	1.2	20	-0.024
<i>H. setiger</i>	7	0.7	7	1.1	1.1	0.11	0.70	2.2	40	-0.036
Σ	324	32	324	278.9	16.7	1.67	32.40	100.0		-0.345
26 June 2010		H(S)	=1.15		Hmax	=3.46	E%	=33.14		
<i>Thrips tabaci</i>	42	4.2	42	39.3	6.3	0.63	4.20	31.3	50	-0.158
<i>Haplothrips leucanthemi</i>	92	9.2	92	66.8	8.2	0.82	9.20	68.7	80	-0.112
Σ	134	13	134	31.2	5.6	0.56	13.40	100.0		-0.270
		H(S)	=0.90		Hmax	=3.46	E%	=25.93		

e. Predators

Thrips are attacked by predators and parasitoids that contribute to the maintenance of the balance between species in natural ecosystems. The collection of 29 May highlighted the presence of two young predatory larvae of *Chrysopa*. According to LEWIS (1973), chrysopidae larvae are amongst the most voracious predators of thrips, hence their importance in controlling thrips populations.

CONCLUSIONS

The component populations of the Thysanoptera association on *M. chamomilla* belong to 11 species, which denotes a rich diversity of the coenosis inhabiting a single host plant.

The species constantly participating in the coenosis structure are *Haplothrips leucanthemi* and *Thrips tabaci*, with the highest values of ecological indicators and a relative abundance of 70.96% and 22.26%, respectively.

Sex ratio of *H. leucanthemi* species is an expression of female dominance, situation typical to Thysanoptera.

The highest densities of individuals/inflorescence for *H. leucanthemi* species were recorded in May, during the period of chamomile maximum blooming.

Such studies reveal the importance of flowering plants as a trophic niche in ensuring and preserving the biodiversity of species of this small order of insects.

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CONTRIBUTION TO THE KNOWLEDGE OF THE PTEROMALIDS (HYMENOPTERA, CHALCIDOIDEA, PTEROMALIDAE) FROM “CODRII” NATURAL RESERVE

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Abstract. After having made the investigation for the first time in this reserve there were found 128 species of pteromalids (Hymenoptera, Pteromalidae) belonging to 12 subfamilies and 58 genera, of which 2 genera (*Hyperimerus* GIRAULT, *Toxuma* WALKER) and 9 species (*Spalangia irregularis* BOUCEK, *Hyperimerus pusillus* WALKER, *Platygermus affinis* WALKER, *Trigonoderus pulcher* WALKER, *Toxuma fuscicornis* WALKER, *Semiotellus diversus* WALKER, *Dibrachys fuscicornis* WALKER, *Trichomalus flagellaris* GRAHAM, *Pteromalus procerus* GRAHAM) are new for the fauna of the Republic of Moldova.

Keywords: Hymenoptera, Pteromalidae, “Codrii” Natural Reserve, new genera and species for the Republic of Moldova, geographical distribution.

Rezumat. Contribuții la cunoașterea pteromalidelor (Hymenoptera, Chalcidoidea, Pteromalidae) din Rezervația naturală „Codrii”. Ca rezultat al investigațiilor efectuate în această rezervație, au fost identificate 128 specii de pteromalide (Hymenoptera, Pteromalidae) incluse în 12 subfamiliile și 58 genuri dintre care 2 genuri (*Hyperimerus* GIRAULT, *Toxuma* WALKER), 9 specii (*S. irregularis* BOUCEK, *H. pusillus* WALKER, *P. affinis* WALKER, *T. pulcher* WALKER, *T. fuscicornis* WALKER, *S. diversus* WALKER, *D. fuscicornis* WALKER, *T. flagellaris* GRAHAM, *P. procerus* GRAHAM) sunt noi pentru fauna Republicii Moldova.

Cuvinte cheie: Hymenoptera, Pteromalidae, Rezervația Naturală „Codrii”, genuri și specii noi pentru Moldova, răspândire geografică.

INTRODUCTION

“Codrii” Reserve was founded by the Decision of the Council of Ministers of the S. S. R. of Moldova no. 310 from September. 27th, 1971 in order to preserve (conserve) the most representative areas of the forests specific to the Central Plateau. It is situated at the 49th km, southeast of the capital of the Republic of Moldova and has the following geographical coordinates: 47°06' - Northern latitude; 28°21' - Eastern longitude. The Reserve is subordinated to the forestry Agency “Moldsilva” and works according to special Regulations adopted by the Government. The total surface of the reserve is of 5,170 ha among which 5,040 ha is covered by forest.

MATERIAL AND METHODS

All the individuals were collected using an entomological net, between 2005 and 2012, mostly by the author. The majority of the identified species were collected from different biotopes situated within the territory of the reserve.

RESULTS AND DISCUSSIONS

Family PTEROMALIDAE

Subfamily Spalangiinae

I. Genus *Spalangia* LATREILLE, 1805

1. *Spalangia irregularis* BOUCEK, 1963

Material examined: “Codrii” Reserve, Lozova (District Strășeni): 1 ♀ collected on July 16, 2011, orchard; (Manic leg.).

Geographical distribution: Israel, Cyprus, Romania (ANDRIESCU & MITROIU, 2008; DZHANOKMEN, 1987).

Biology: unknown (ANDRIESCU & MITROIU, 2008; DZHANOKMEN, 1987).

The species is new to fauna of the Republic of Moldova.

Subfamily Asaphinae

II. Genus *Hyperimerus* GIRAULT, 1917

2. *Hyperimerus pusillus* (WALKER, 1833)

Material examined: “Codrii” Reserve Lozova (District Strășeni): 1 ♀, June 14, 2012, floodable meadow; (Manic leg.).

Geographical distribution: Great Britain, ex Czechoslovakia, Romania, Sweden.

Biology: attacking various Pseudococcidae and Psyllidae (Homoptera). (BOUCEK & RASPLUS, 1991; DZHANOKMEN, 1987; MITROIU, 2006).

The genus and the species are new to fauna of the Republic of Moldova.

Subfamily Miscogasterinae
 III. Genus *Platygerrius* THOMSON, 1878

3. *Platygerrius affinis* (WALKER, 1836)

Material examined: "Codrii" Reserve: 1 ♂, July 8, 2012, meadow; (Manic leg.).

Geographical distribution: Sweden, Great Britain, ex Czechoslovakia, Poland, Romania. (DZHANOKMEN, 1987; MITROIU, 2005).

Biology: primary parasitoid of wood-boring Coleoptera, e. g. *Anobium punctatum* (Anobiidae), *Leripus punctulatus*, *Stenostola ferrea* (Cerambycidae), *Ernoporus tiliae*, *Ips curvidens* (Scolytidae), Cucujidae, but also of some Diptera e. g. *Agromyza*. (BOUCEK & RASPLUS, 1991; DZHANOKMEN, 1987; MITROIU, 2005).

The species is new to fauna of the Republic of Moldova.

IV. Genus *Trigonoderus* WESTWOOD, 1832

4. *Trigonoderus pulcher* (WALKER, 1836)

Material examined: "Codrii" Reserve: 1 ♀, June 5, 2011, orchard; (Manic leg.).

Geographical distribution: Great Britain, ex Czechoslovakia, Romania, Sweden.

Biology: the hosts are unknown, but they are probably xylophagous beetles (Cerambycidae, Scolytidae). (BOUCEK & RASPLUS, 1991; MITROIU et al., 2007).

The species is new to fauna of the Republic of Moldova.

V. Genus *Toxeuma* WALKER, 1833

5. *Toxeuma fuscicorne* (WALKER, 1833)

Material examined: "Codrii" Reserve: July 7, 2012, meadow; (Manic leg.).

Geographical distribution: Germany, Hungary, Ireland, Romania, Sweden, Switzerland (DZHANOKMEN, 1987).

Biology: The flying period is from May to July. Parasitoid of some Agromyzidae (Diptera) developing in seeds of various Poaceae. (BOUCEK & RASPLUS, 1991; DZHANOKMEN, 1987).

The genus and the species are new to fauna of the Republic of Moldova.

VI. Genus *Semiotellus* WESTWOOD, 1840

6. *Semiotellus diversus* (WALKER, 1834)

Material examined: "Codrii" Reserve: 1 ♀, June 4, 2011, orchard; 1 ♂, May 25, 2012, meadow, (Manic leg.).

Geographical distribution: England, Romania, Sweden (DZHANOKMEN, 1987).

Biology: Reared from pupae of Cecidomyiidae. (BOUCEK & RASPLUS, 1991).

The species is new to fauna of the Republic of Moldova.

Subfamily Pteromalinae
 VII. Genus *Dibrachys* FORSTER, 1856

7. *Dibrachys fuscicornis* (WALKER, 1836)

Material examined: "Codrii" Reserve: 1 ♀, June 8, 22, 2012, orchard; (Manic leg.).

Geographical distribution: England, Romania (DZHANOKMEN, 1987; MITROIU et al., 2007).

Biology: it is a parasitoid of various species of Tenthredinidae (Hymenoptera), Lymantriidae and Yponomeutidae (Lepidoptera) (DZHANOKMEN, 1987; MITROIU et al., 2007).

The species is new to fauna of the Republic of Moldova.

VIII. Genus *Trichomalus* THOMSON, 1878

8. *Trichomalus flagellaris* (GRAHAM, 1969)

Material examined: "Codrii" Reserve: 2 ♂♂, July 16, 2011, orchard, meadow; (Manic leg.).

Geographical distribution: England, Romania. (DZHANOKMEN, 1987).

Biology: unknown (DZHANOKMEN, 1987).

The species is new to fauna of the Republic of Moldova.

IX. Genus *Pteromalus* SWEDERUS, 1795

9. *Pteromalus procerus* (GRAHAM, 1969)

Material examined: "Codrii" Reserve: 1 ♀, August 11, 2011, floodable meadow; (Manic leg.).

Geographical distribution: England, Ireland, Romania. (DZHANOKMEN, 1987).

Biology: unknown (DZHANOKMEN, 1987).

The species is new to fauna of the Republic of Moldova.

CONCLUSIONS

Two genera, *Hyperimerus* GIRAULT, *Toxeuma* WALKER, and nine species, *Spalangia irregularis* BOUCEK, *Hyperimerus pusillus* WALKER, *Platygerhus affinis* WALKER, *Trigonoderus pulcher* WALKER, *Toxeuma fuscicorne* WALKER, *Semiotellus diversus* WALKER, *Dibrachys fuscicornis* WALKER, *Trichomalus flagellaris* GRAHAM, *Pteromalus procerus* GRAHAM were recorded for the first time in the Republic of Moldova. All the species were obtained from reared material. The species: *Platygerhus affinis* and *Trigonoderus pulcher* are associated with species of Cerambycidae, Scolytidae. *Toxeuma fuscicorne* - parasitoid on some Agromyzidae (Diptera) developing in seeds of various Poaceae. One species (*Semiotellus diversus*) is reared from pupae of Cecidomyiidae. The species (*Hyperimerus pusillus*) attacks many Pseudococcidae and Psyllidae (Homoptera). The biology of three species (*Spalangia irregularis*, *Trichomalus flagellaris*, *Pteromalus procerus*) is unknown.

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CHANGES OF SOME BIOECOLOGICAL AND PHENOLOGICAL PECULIARITIES OF THE COLORADO POTATO BEETLE (*Leptinotarsa decemlineata* SAY) IN THE AGROCLIMATIC ZONES IN BELARUS

BRECHKA Alena

Abstract. In the present paper, there are rendered the results on studying *Leptinotarsa decemlineata* SAY occurrence and development in different agroclimatic zones in Belarus. Abiotic and trophic factors effect on the pest phenology, biology, ecology changes in time and space are determined. It is determined that the bioecological peculiarities of the Colorado potato beetle populations in different regions of the republic have a significant zone differentiation. Association between potato crops development and phytophage stages, which are different according to the precocity varieties, in different ecological conditions is also studied.

Keywords: Belarus, Colorado potato beetle, biology, ecology, phenology, variety.

Rezumat. Schimbările unor particularități bioecologice și fenologice ale gândacului de Colorado (*Leptinotarsa decemlineata* SAY) din zonele agroclimatice ale Belarusiei. Lucrarea redă rezultatele studiului asupra apariției și dezvoltării *Leptinotarsa decemlineata* SAY în diferite zone agroclimatice din Belarus. Sunt determinate influența factorilor abiotici și trofici asupra fenologiei dăunătorului, biologia, schimbările ecologice în timp și spațiu. Se stabilește că particularitățile bioecologice ale populațiilor gândacului de Colorado în diferite regiuni ale republicii înregistrează diferențieri zonale semnificative. Este de asemenea studiată legătura dintre dezvoltarea culturilor de cartof și stadiile de dezvoltare ale dăunătorului, care diferă în funcție de precocitatea varietății cartofului, în condiții ecologice diferite.

Cuvinte cheie: Belarusia, gândacul de Colorado, biologie, ecologie, fenologie, varietate.

INTRODUCTION

Colorado potato beetle is widely spread and met in different states all over the world. At present, Colorado potato beetle area includes 4 geographically isolated regions: American, European-Siberian, Central Asian, Far East located on two continents (except the islands) (BULLETIN OEPP, 2012; PAVLUSHIN et al., 2009). For the last 20 years a significant progression of the Colorado potato beetle to the north and east in its second, Eurasian area, is marked; as a result, in the north it occurred in many regions of Arkhangelsk region, Kareliya and Komi republics. In the east, it has reached Yenisei and Khakassia, in the south – Krasnoyarski region, it is found on Buryatiya and Primorski territory (PAVLUSHIN et al., 2009; FASULATI, 2004), and has settled in foreign Asia in Iran and appeared in China.

Ecological plasticity and polymorphism promote the pest adaptation to soil-climatic conditions. Thus, the pest quickly adapts to the environmental abiotic factors changes, preserving a high viability, which is shown in its bioecological peculiarities changes (USHATINSKAYA, 1981; POTATO BEETLE, 2013).

On the territory of Belarus (Brest and Grodno regions), the Colorado potato beetle appeared in 1956. Until 1958, the focuses were of isolated character: small and quickly liquidated. Nevertheless, a mass arrival on the territory of the western regions was registered in 1959 from Poland. In a year the pest was found in Gomel and Minsk regions of the republic. In spite of the applied every year quarantine measures, the pest area has expanded and its field colonization has increased (DROZDOV et al., 1960).

In the sixties - seventies of the last century, the Belarusian scientists carried out the extensive researches on biology, ecology, phenology. But on some questions the inconsistent data are obtained. Thus, in literature different opinions in relation to the pest generations number are met. According to the data of L. S. Drozdov, A. F. Markovets, M. M. Pilko one generation is developed. F. N. Irodova and V. I. Kurilova have revealed that in the southern regions, the pest develops in 3 generations, in the northern ones – in one (IRODOVA & KURILOV, 1976). The results obtained by L. I. Arapova testify that in the republic, characterized on the whole by temperate climate, in the northern zone, the Colorado potato beetle develops mainly in one generation, in the southern zone the second full generation is possible (ARAPOVA, 1976).

Colorado potato beetle phenological terms calculation, done about 40 years ago, has shown, that in the European part of Russia not more than 3 generations of the Colorado beetle can develop (ARAPOVA, 1972). However, in the last years the pests generations number increased.

At present, in spite of regularly carried out protective measures in the republic, a high density of natural populations has formed, the Colorado potato beetle harmfulness increasing in potato agrocoenosis, under some circumstances. On the one hand, it is caused by the variable ecological plasticity, genetic polymorphism, the pest ability to an intensive adaptation in different conditions. On the other hand, the change of the agroclimatic zones borders (Fig. 1a, SHKLYAR, 1973; Fig. 1b, MELNIK, 2004) provided favourable conditions for the Colorado potato beetle development.

Based on the aforementioned ideas, the research aim was studying the changes and occurrence specification, some eco-biological peculiarities, phenology, number dynamics, association in potato crops development and the phytophage stages by precocity varieties for anthropogenic factors use substantiation while regulating Colorado beetle number taking into account zonal distribution.

MATERIAL AND METHODS

The pest occurrence and records were carried out by itinerary inspections of potato crops according to the general entomology methods, by recorded plants (areas) methods consisting in 5-20 adjoining to each other plants (VOLOVIK et al., 1995).

The researches were carried out in the northern, central, the southern and new agroclimatic zones of Belarus (Fig. 1b, MELNIK, 2004).

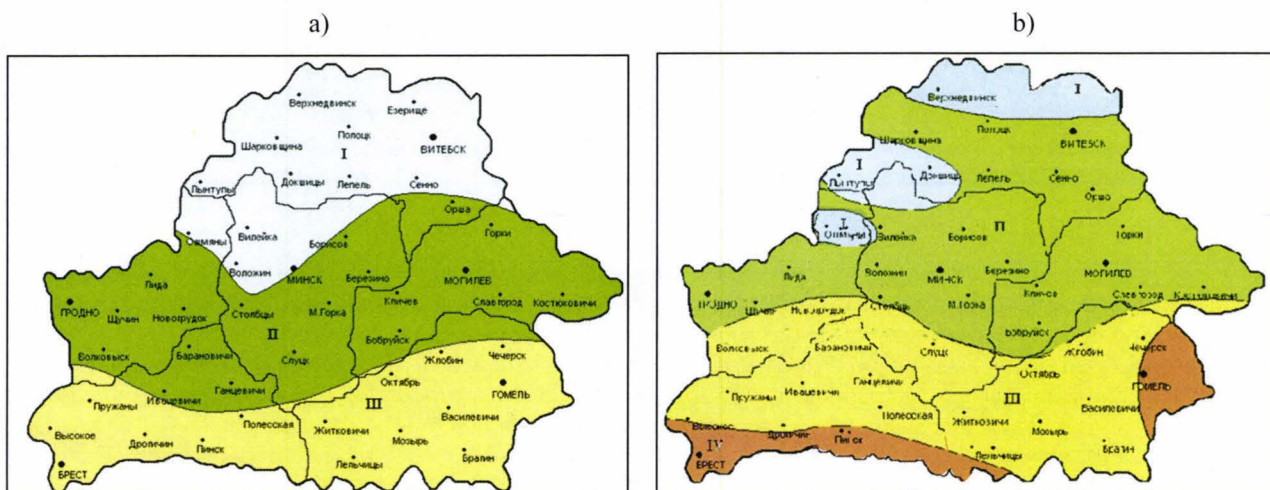


Figure 1. Change of agroclimatic regions borders in Belarus.

Legend:

a) borders of agroclimatic regions acc. to A.K. Shklyar (1973).

b) borders of agroclimatic regions acc. to V.I. Melnik in 1989-1999 Agroclimatic regions: I – The Northern zone, II – Central zone, III – the Southern zone, IV – New zone (active air temperatures sum is higher 10°C in zone: I – less than 2200, II – 2200 – 2400, III – 2400 – 2600, IV – more than 2600).

The population dynamics study, the pest bio-ecological peculiarities specification were carried out in 2000-2002, 2006-2009 on the base of the Republican Unitary Enterprise “Institute of Plant Protection” and in potato-cultivating agricultural enterprises in different agroclimatic zones of the republic by field trial carrying out.

The researches were done on different ripeness groups of Belarusian selection varieties: early – Lazurit, Delfin; medium-early – Arkhideya, Yavar, Sante; medium-ripe – Skarb, Krinita; medium-late – Lasunak, zhuravinka; late – Orbita, Atlant, Temp.

Weather conditions during potato vegetation were significantly differed: 2000, 2007 – warm and moderate-humid, 2002 – dry and hot, 2001, 2006, 2008, 2009 – humid and warm, what enabled us to study the dynamics of the Colorado potato beetle population.

The obtained data are treated by mathematical statistics method using the programs Microsoft Excel, Oda.

RESULTS AND DISCUSSIONS

Phytosanitary situation monitoring has shown that the Colorado potato beetle colonizes 98-100% of the potato crops every year. It is revealed that in comparison with the results obtained about 20 years ago, the character of the phytophage distribution in the republic has changed. At present, the area, colonized by the pest, has increased. Thus, if earlier a gradation of the potato colonized area in the northern part was from 2 to 30%, in 2007-2008 the pest colonized 100%. In the central zone an increase of the colonized area from 10-50% to 92-100% is marked, in the southern and new zones – from 60 to 94-100%.

In different climatic conditions during the years of researches a positive dynamics of the plants colonization increase and the pest number is marked in the southern and new zones in comparison with the northern and central ones. Thus, the plants colonization by the Colorado potato beetle in 2007–2008 during a period of larvae mass hatching in a new zone has reached 63.8–98.0%, which is 2.9 and 1.3 times higher in comparison with the northern (33.3–49.6%) part. A similar tendency in number is marked: in a new zone – 19.6–56.2 indiv./plant, 2 and 7 times higher than the parameters of the northern zone (8.1–9.8 indiv./plant) (Table 1).

It is determined that the potato and pest seasonal development phenology register zonal differences. In the southern regions, potato phenophases are registered 10-20 days earlier in comparison with the central and the northern regions, accordingly the phytophagous colonization takes place 1-2, in some years 3 weeks earlier in comparison with the central and 2-3 weeks and more, with the northern agroclimatic zone. This situation is caused by more favourable conditions for the pest development in the southern and new zones, providing the phytophage intensive development and the generations number increase.

Table 1. Dynamics of the potato plants colonization and the Colorado potato beetle number in different agroclimatic zones (according to the results of itinerary inspections).

Agroclimatic zone	Plants colonization, %	Number, indiv./plant			
		In all	Including		
			imago	eggs	larvae
2007					
The Northern zone	33.3	8.1	0.1	6.5	1.5
Central zone	81.4	45.3	0.3	32.1	12.9
The Southern zone	78.0	60.8	0.5	50.5	9.8
New zone	98.0	56.2	0.5	41.0	14.7
2008					
The Northern zone	49.6	9.8	0.2	4.0	5.6
Central zone	42.4	10.1	0.1	6.0	4.0
The Southern zone	68.0	23.2	0.2	17.2	5.8
New zone	63.8	19.6	0.2	10.3	9.1
2009					
Central zone	88.8	16.4	2.6	0.7	13.1
The Southern zone	86.4	26.9	0.1	1.7	25.1
New zone	74.8	27.6	0.9	17.3	9.4

In connection with the pest zonal distribution a differentiation of protective measures carrying out tactics is possible; for this, a specification of the pest biological and phenological peculiarities in different ecological conditions is necessary. Thus, we have analysed certain bio-ecological Colorado potato beetle peculiarities in the agroclimatic zones of the republic.

It is determined that the pest number control parameter is soil temperature both during winter and spring-summer periods. It is revealed that at soil temperature decrease in winter period to -9-12°C at the depth of 1-3 cm, the insect depressive development is marked during potato vegetation. The phytophage vital activity takes place at soil temperature +13°C, while according to L. I. Arapova's data (1976) – at +14°C. Zonal differences are marked in terms of the pest going out of wintering places, what later on influences the population dynamics. In the southern and new zones such temperature is observed at the beginning of May, in the central and the northern – in the 3rd decade of May – the 2nd decade of June. Mass living of wintering places by the beetles takes place in the 2nd half of May (the southern and new zones) – June (the northern and central) when the average air temperature is +14-15°C (earlier – at +16°C). Air temperature fall delays the imago leaving for 7-10 days.

In the years of researches the oviposition started during mass wintered beetles' leaving. The intensive egg laying is observed at an air temperature +16-21°C. In the central zone it is marked in the 2nd - 3rd decades of June, at low air temperature – in the 1st decade of July, at the increased one – in the 1st decade of June. In the southern zone – mainly in the 2nd - 3rd decades of June, at the increased temperature – in the 3rd decade of May.

The larvae hatching is noticed at air temperature increase for more than +15.5°C, which often takes place in the central zone in the 1st – 3rd decades of June, in the southern zone it starts 5-15 days earlier and takes place in the 3rd decade of May 2nd decade of June. At an air temperature within +16-22°C the larvae mass hatching and development fluctuate from 17 to 32 days. A mass pupation is noticed at an air temperature +16-24°C and lasts 10-14 days. In the central zone young beetles appeared in the 2nd decade of July, at an air temperature decrease – at the beginning of August, in the southern zone – in the 1st and 3rd decades of July, accordingly.

The Colorado potato beetle is a polycyclic species, which has a different number of generations in a year, forming under abiotic and biotic factors influence. As a result of carrying out the phenological researches it is revealed, that in the southern and new zones the phytophage developed in two generations with its incomplete and complete development cycle (Fig. 2). During the years with the increased air temperatures in the southern agroclimatic zone (for 0.8-7.1°C) the 3rd generation development is observed (Fig. 3), while ARAPOVA (1976) revealed that in the south only two generations developed. In the northern and central zones during all years of researches the 1st complete and the 2nd incomplete generations were noticed.

Potato crops and the pest development association study has allowed us to determine the periods of its maximum harmfulness. It is determined that they differed depending on the meteorological conditions, varietal peculiarities and agroclimatic zone of potato cultivation. Thus, during the years with air temperature close to the average perennial parameters during the pest going out of wintering places in the central agroclimatic zone, this period is observed on early and mid-early varieties at budding-blossoming stage, on mid-ripe, mid-late and late varieties – budding beginning-blossoming; in the southern agroclimatic zone accordingly – blossoming and budding-blossoming, accordingly.

In the years with the air temperature higher than the average perennial parameters during the pest going out of wintering places in the central (for 4.0-7.6°C) and in the southern (for 0.8-7.1°C) zones the phytophage harmfulness period coincided: on early and mid-early varieties with the complete seedlings-budding stage, mid-ripe, mid-late and late – budding beginning-budding. The pest number dynamics research taking into account the population age structure has shown that at that time the 2nd age larvae represented more than 40-50% from total number.

Analysing the data revealing the regularities of potato colonization by the pest depending on potato variety, it is determined, that early and mid-early varieties are colonized 5-7 days earlier in comparison with mid-ripe, mid-late and late ones. The larvae maximum number on early and mid-early varieties fluctuated within 23.0-65.1 indiv./plant, what

significantly increased the parameters on mid-ripe, mid-late and late – 12.0–45.3 indiv./plant. At a comparative estimation of the pest number dynamics in different agroclimatic zones on mid-ripe, mid-late and late potato varieties it is revealed that in the research years during the larvae mass hatching their density in the southern zone in comparison with the central one was 1.4-3.5 times higher.

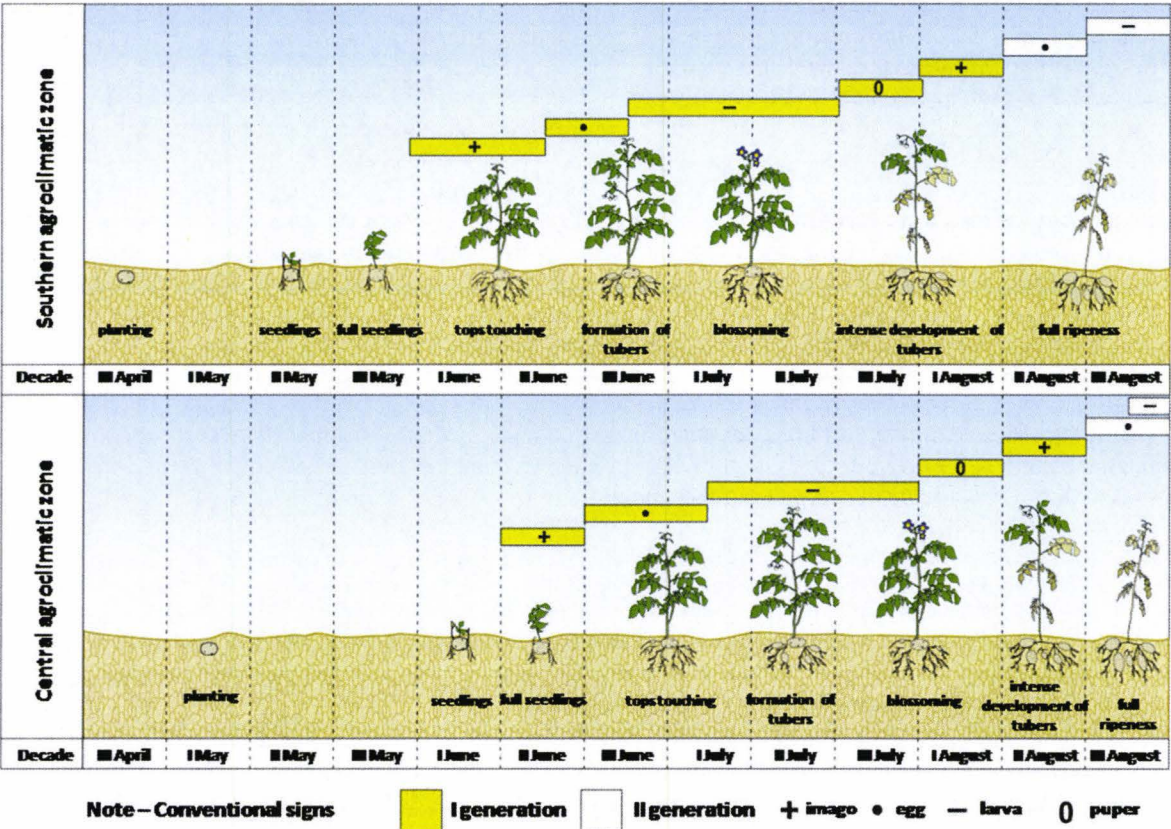


Figure 2. Phenological calendar of the Colorado potato beetle development on middle-ripening, middle-late and late potato varieties, 2009.

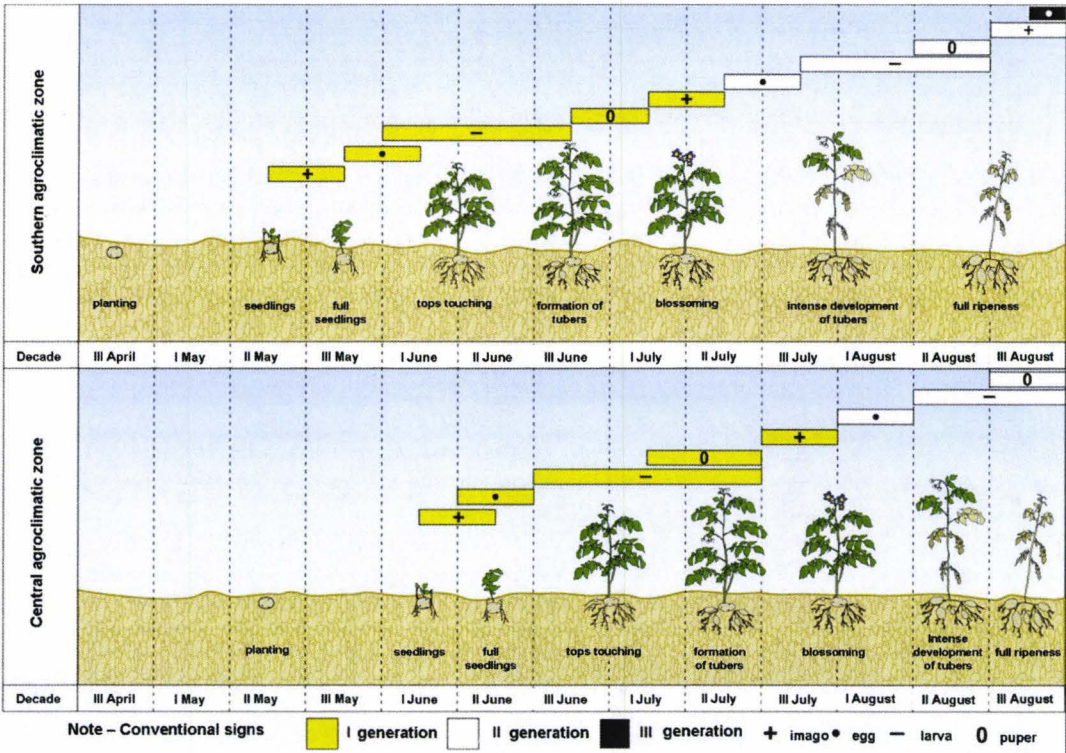


Figure 3. Phenological calendar of the Colorado potato beetle development on middle-ripening, middle-late and late potato varieties, 2007.

CONCLUSIONS

The results of the potato agrocoenosis itinerary inspections on the Colorado potato beetle incidence in Belarus have shown changes in the pest zonal distribution both on colonization and number. It is revealed that, every year, the Colorado potato beetle colonizes 98–100% of potato crops. The crops colonization and the pest number significantly differ while cultivating potato in different agroclimatic zones. During the larvae mass hatching the crops colonization by the Colorado potato beetle in a new agroclimatic zone was 1.3–2.9 times higher, the number – 2–7 times in comparison with the northern one.

Significant differences in biological peculiarities, the phytophage and potato phenology in different agroclimatic zones are revealed. In the southern and new zones the crops colonization by the phytophage takes place 1–2, in some years – 3 weeks earlier in comparison with the central zone and 2–3 weeks and more – in comparison with the northern agroclimatic zone. In the central agroclimatic zone the 1st complete and 2nd incomplete generations develop. In the southern zone during the years with early colonization of potato crops and the Colorado potato beetle intensive development, the 2nd complete and the 3rd generation beginning are noticed.

The pest larvae maximum harmfulness depending on meteorological conditions, potato varietal peculiarities cultivated in different agroclimatic zones is dated to the period of full sprouting-blossoming.

Thus, the diversity of the republican natural conditions creates the phytophage zonal occurrence, what causes a differentiated approach for carrying out protective measures. The Colorado potato beetle number regulation can be carried out using insecticides by different mechanism action and method of application (before-planting tubers treatment and potato vegetative plants spraying) taking into account the pest phenological terms and biological peculiarities in different agroclimatic zones of the republic.

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LEAF BEETLES (COLEOPTERA: CHRYSOMELOIDEA, CHRYSOMELIDAE) PRESERVED IN THE PATRIMONY OF THE OLTENIA MUSEUM CRAIOVA

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Abstract. The paper presents new data referring to the presence of leaf beetles species (Chrysomelidae family) in the Oltenia region, based on the study of the material preserved in the entomological collections of the Department of Natural Sciences from Oltenia Museum Craiova. 53 species of 23 genera, belonging to 6 subfamilies are listed. The material examined was collected from 37 sites. 18 species are new mentions for these collections.

Keywords: Coleoptera, Chrysomelidae, collections, Oltenia Museum Craiova.

Rezumat. Crisomelide (Coleoptera: Chrysomeloidea, Chrysomelidae) conservate în patrimoniul Muzeului Olteniei Craiova. Lucrarea prezintă date noi referitoare la prezența speciilor de crisomelide în regiunea Oltenia, pe baza studiului materialului conservat în colecțiile entomologice ale Secției de Științe Naturale din cadrul Muzeului Olteniei Craiova. Sunt prezentate 53 de specii din 23 genuri, aparținând la 6 subfamilii. Materialul studiat provine din 37 de situri. 18 specii sunt menționate pentru prima dată în aceste colecții.

Cuvinte cheie: Coleoptera, Chrysomelidae, colecții, Muzeul Olteniei Craiova.

INTRODUCTION

The Chrysomelidae family (known as leaf beetles) together with Cerambycidae, Megalopodidae and Orsodacnidae constitute the polyphagous superfamily Chrysomeloidea. According to the recent classification, this family comprises 12 subfamilies (LÖBL & SMETANA, 2010). About 1780 leaf beetle species described until the end of 2000 have been recorded in Europe and the Mediterranean area (WARCHALOWSKI, 2003). In Romania, 571 species from 82 genera and 13 subfamilies have been recorded (MAICAN, 2005).

The entomological collections of the Oltenia Museum comprise over 54.000 specimens, especially from the Coleoptera order. It contains material collected mainly from the Oltenia region. Information on the material from Chrysomelidae family preserved in these collections are found in some papers published by: CHIMIȘLIU, 1990-1993 (15 species, 9 genera and 5 subfamilies), BOBÎRNAC et al., 1999 (19 species, 15 genera and 6 subfamilies), ILIE & CHIMIȘLIU, 1999 (71 species, 28 genera and 8 subfamilies), CHIMIȘLIU & MOGOȘEANU, 2009 (35 species, 15 genera and 5 subfamilies).

The objective of this study consists in scientific harnessing of data from the collections preserved in the Museum of Oltenia Craiova, the results contributing to the completion of the information on leaf beetles diversity and distribution in the Romanian fauna.

MATERIAL AND METHODS

The material examined (531 specimens) was collected between 1969 and 2008 by the specialists (Elena Bazilescu, Cornelia Chimișliu, Adrian Năstase, Aneta Vișan, Gheorghița Filcu, Gima Mogoșeanu, Loredana Durău) from the Department of Natural Sciences from the Oltenia Museum. Most collection sites are located in the Oltenia region.

For each recorded species, the sites and collection data, the number of examined specimens, previous records from the patrimony of the museum and the bibliographical sources are given.

The identification of specimens was made by Sanda Maican, according to the external morphology and to the genitalia (aedeagus), based on the monograph of WARCHALOWSKI (2003). The arrangement of the taxa and the nomenclature are presented in accordance with LÖBL & SMETANA (2010).

Abbreviations:

County names: AG - Argeș; CT - Constanța; DB - Dâmbovița; DJ - Dolj; GJ - Gorj; MH - Mehedinți; OT - Olt; VL - Vâlcea.

Other abbreviations: spec./s. – specimen/s.

* - species mentioned for the first time in the museum patrimony.

Collecting sites:

Bala Băi (MH); Balta Verde (DJ); Bratovoiești (DJ); Bucovăț forest (DJ); Bugiulești (VL); Canaraua Fetiș (CT); Cheile Bistriței (GJ); Cheile Galbenului (GJ); Cheile Sohodol (GJ); Cireșu (MH); Ciuperceni (DJ); Colonești (OT); Craiova (DJ); Craiova (Romanescu Park) (DJ); Craiova (the Botanical Garden) (DJ); Crihala forest (MH); Cula Cerneți (MH); Drobeta Turnu-Severin (MH); Găioara. Iron Gates Natural Park (MH); Gura Văii (DJ); Iezer Mountains (AG); Ișalnița (DJ); Izverna (MH); Melinești (DJ); Negoi (DJ); Orșova (Cula Tricule) (MH); Pietrele Albe (GJ); Răcari (DJ); Râpa Roșie (DJ); Secui (DJ); Schela Cladovei (MH); Svinița (MH); Șimnic (DJ); Tărtășești (DB); Valea Bahnei (MH); Valea Cheii (VL); Valea Oglănic (MH).

RESULTS

The examined material included 53 species from 23 genera and 6 subfamilies, as it follows: Criocerinae - 3 species (5.66%), Cassidinae - 4 species (7.54%), Chrysomelinae - 20 species (37.73%), Galerucinae - 3 species (5.66%), Cryptocephalinae - 22 species (41.50%) and Eumolpinae - one species (1.88%).

Family **CHRYSOMELIDAE** LATREILLE, 1802I. Subfamily **CRIO CERINAE** LATREILLE, 18041. *Crioceris duodecimpunctata* (LINNAEUS, 1758)

Material examined - 1 spec.: Craiova (the Botanical Garden), May 4, 2004.

Records from the patrimony of museum: Craiova (ILIE & CHIMIȘLIU, 1999).

2. *Lilioceris lilii* (SCOPOLI, 1763)

Material examined - 26 specs.: Craiova (the Botanical Garden) - 2 specs., June 15-20, 2005; Șimnic - 5 specs., March 29, 2005; 6 specs., April 22, 2005; 9 specs., April 2007; 2 specs., June 1, 2007; 2 specs., April 12, 2008.

Records from the patrimony of the museum: Cheile Sohodol, Craiova (ILIE & CHIMIȘLIU, 1999); Craiova (Romanescu Park), Șimnic (CHIMIȘLIU & MOGOȘEANU, 2009).

*3. *Oulema gallaeciana* (HEYDEN, 1870)

Material examined - 1 spec.: Cula Cemeți, May 5, 1982.

II. Subfamily **CASSIDINAE** GYLLENHAL, 18131. *Cassida murraea* LINNAEUS, 1767

Material examined - 1 spec. (without collecting data).

Records from the patrimony of the museum: Cărbunești, Băniei forest, Orodul, Plenița, Poiana Mare, Racovița, Slănic Prahova (ILIE & CHIMIȘLIU, 1999).

2. *Cassida nobilis* LINNAEUS, 1758

Material examined - 1 spec.: Valea Oglănic, May 8, 1981.

Records from the patrimony of the museum: Lunca Jiului, Craiova (Romanescu Park) (ILIE & CHIMIȘLIU, 1999).

3. *Cassida viridis* LINNAEUS, 1758

Material examined - 2 specs.: Valea Oglănic - 1 spec., June 5, 1985; Cheile Bistriței - 1 spec., August 10, 2005.

Records from the patrimony of the museum: Bucovăț, Cheile Sohodol, Negoii, Poiana Mare, Târgu Logrești (ILIE & CHIMIȘLIU, 1999).

*4. *Pilemostoma fastuosum* (SCHALLER, 1783)

Material examined - 2 specs.: Craiova (Romanescu Park) - 1 spec., May 3, 2004; Craiova (the Botanical Garden) - 1 spec., May 4, 2004.

III. Subfamily **CHRYSOMELINAE** LATREILLE, 18021. *Chrysomela populi* LINNAEUS, 1758

Material examined - 19 specs.: Bucovăț forest - 4 specs., July 5, 2005; Bugiulești - 1 spec., July 25, 2004; Cheile Sohodol - 1 spec., August 12, 2005; Ciuperceni - 1 spec., May 22, 2004; Craiova (the Botanical Garden) - 1 spec., June 20, 2005; Negoii - 7 specs., May 3, 2005; 3 specs., May 2, 2008; Secui - 1 spec., May 26, 2007.

Records from the patrimony of the museum: Baia de Fier (BOBÎRNAC *et al.*, 1999); Coțofenii din Față, Craiova, Desa (ILIE & CHIMIȘLIU, 1999); Craiova (the Botanical Garden), Zvorsca (CHIMIȘLIU & MOGOȘEANU, 2009).

*2. *Chrysomela saliceti* SUFFRIAN, 1849

Material examined - 1 spec.: Cheile Bistriței, August 10, 2005.

3. *Chrysomela tremula* FABRICIUS, 1787

Material examined - 1 spec.: Craiova, June 14, 2007.

Records from the patrimony of the museum: Cheile Sohodol, Desa, Radovan (ILIE & CHIMIȘLIU, 1999).

*4. *Linnaeidea aenea* (LINNAEUS, 1758)

Material examined - 20 specs.: Valea Cheii, May 30, 2008.

5. *Gastrophysa polygoni* (LINNAEUS, 1758)

Material examined - 12 specs.: Canarua Fetii - 2 specs., June 7, 2008; Colonești - 1 spec., April 22, 2004; Râpa Roșie - 3 specs., May 12, 2007; Șimnic - 1 spec., June 23, 2004; 1 spec., June 23, 2007; 3 specs., April 12, 2008, 1 spec., June 12, 2008.

Records from the patrimony of the museum: Cheile Sohodol, Ciuperceni, Gura Motrului, Șimnic (ILIE & CHIMIȘLIU, 1999).

6. *Gastrophysa viridula* (DEGEER, 1775)

Material examined - 4 specs.: Negoii, June 21, 2008.

Records from the patrimony of the museum: Craiova, Secui, Sibiu (ILIE & CHIMIȘLIU, 1999).

*7. *Neophaedon pyritosus* (ROSSI, 1792)

Material examined - 1 spec.: Craiova (the Botanical Garden), May 23, 2006.

8. *Chrysolina (Colaphodes) haemoptera* (LINNAEUS, 1758)

Material examined - 2 specs.: Șimnic, May 20, 2008.

Records from the patrimony of the museum: Dănciulești, Răcari (ILIE & CHIMIȘLIU, 1999).

9. *Chrysolina (Colaphosoma) sturmi* (WESTHOFF, 1882)

Material examined - 2 specs.: Bratovoiești, June 17, 2008.

Records from the patrimony of the museum: Boișoara, Bratovoiești, Cetate, Căineni, Leamna, Ocnele Mari, Olănești, Radovan (CHIMIȘLIU & MOGOȘEANU, 2009).

10. *Chrysolina (Erythrochrysa) polita* (LINNAEUS, 1758)

Material examined - 32 specs.: Craiova (the Botanical Garden) - 2 specs., May 20, 2008; Secui - 5 specs., April 18, 2007, July 23, 2007; Secui - 22 specs., April 14, June 21, 2008.

Records from the patrimony of the museum: Bistreț, Cheile Sohodol, Craiova, Delta Dunării, Filiași, Novaci, Rotunda, Tismana (ILIE & CHIMIȘLIU, 1999); Cheile Sohodol, Craiova (the Botanical Garden, Romanescu Park), Tărtășești (DB), Leamna (CHIMIȘLIU & MOGOȘEANU, 2009).

11. *Chrysolina (Fastuolina) fastuosa* (SCOPOLI, 1763)

Material examined - 46 specs.: Balta Verde - 3 specs., June 14, 2007; Bucovăț forest - 4 spec., May 29, 2004; Cheile Bistriței - 1 spec., August 10, 2005; Cheile Galbenului - 1 spec., August 14, 2003; Craiova (the Botanical Garden) - 10 specs., May 4, 2004, May 20, 2008; Craiova (Romanescu Park) - 10 specs., May 3, 2004; Cula Cerneți - 3 specs., July 4, 1985; Drobeta Turnu-Severin - 2 specs., June 2, 1982; Negoii - 4 specs., May 3, 2005, May 2, 2008; Secui - 3 specs., May 26, 2007; Șimnic - 1 spec., June 1, 2007; Tărtășești - 3 specs., June 20, 2005; Valea Oglănic - 1 spec., May 8, 1981.

Records from the patrimony of the museum: Cheile Sohodol, Novaci, Tismana (BOBÎRNAC et al., 1999); Breasta, Craiova, Pădurea Sarului, Rezervația Retezat, Rîca, Sibiu (ILIE & CHIMIȘLIU, 1999); Bociu, Cheile Sohodol, Coșoveni, Craiova (the Botanical Garden, Romanescu Park), Leamna, Plenița, Preajba, Segarcea, Zăval (CHIMIȘLIU & MOGOȘEANU, 2009).

*12. *Chrysolina (Sphaeromela) varians* (SCHALLER, 1783)

Material examined - 10 specs.: Cheile Bistriței - 5 specs., August 10, 2005; Cheile Sohodol - 4 spec., August 12, 2005; Valea Cheii - 1 spec., May 30, 2008.

*13. *Chrysolina (Stichoptera) rossia* (ILLIGER, 1802)

Material examined - 22 specs.: Pietrele Albe - 1 spec., June 18, 1999; Ciuperceni - 6 specs., May 1-22, 2004; Craiova (Romanescu Park) - 5 specs., June 8, 2004; Bucovăț forest - 4 specs., June 13, 2004; Craiova (Romanescu Park) - 1 spec., June 8, 2004; 1 spec., June 2, 2005; Șimnic - 1 spec., June 15, 2008; Canaraua Fetei - 1 spec., June 7, 2008; Negoii - 1 spec., June 21, 2008; Secui - 1 spec., May 26, 2007, 1 spec., July 12, 2008.

14. *Chrysolina (Synerga) coerulans* (L. G. SCRIBA, 1791)

Material examined - 1 spec.: Bratovoiești, June 17, 2008.

Records from the patrimony of the museum: Baia de Fier, Cheile Sohodol, Craiova (ILIE & CHIMIȘLIU, 1999).

15. *Chrysolina (Synerga) herbacea* (DUFTSCHMID, 1825)

Material examined - 136 specs.: Canaraua Fetei - 1 spec., June 7, 2008; Cheile Bistriței - 2 specs., August 10, 2005; Cheile Sohodol - 5 specs., August 12, 2005; Craiova - 5 specs., May 3, 2004; 17 specs., May 27, 2005; 8 specs., June 25, 2005; 2 specs., July 8, 2008; Craiova (the Botanical Garden) - 14 specs., May 4, 2004; 2 specs., June 17, 2004; 4 specs., June 20, 2005; 4 specs., June 21, 2005; 15 specs., May 20, 2008; Craiova (Romanescu Park) - 7 specs., May 3, 2004; Iezer Mountains - 1 spec., July 27, 2008; Melinești - 1 spec., May 25, 2008; Negoii - 2 specs., May 3, 2005; 3 specs., June 28, 2005; Râpa Roșie - 6 specs., May 12, 2007; Secui - 2 specs., April 18, 2007; 7 specs., May 27, 2008; 9 specs., June 21, 2008; Șimnic - 3 specs., May 13, 2004; 1 spec., June 20, 2004; 6 specs., May 29, 2005; 4 specs., June 15, 2005; 2 specs., June 20, 2007; 3 specs., June 10, 2008.

Records from the patrimony of the museum: Bistreț, Cheile Sohodol, Olănești (ILIE & CHIMIȘLIU, 1999); Cheile Sohodol, Craiova (the Botanical Garden), Leamna, Negoii, Preajba (CHIMIȘLIU & MOGOȘEANU, 2009).

16. *Chrysolina (Zeugotaenia) limbata* (FABRICIUS, 1775)

Material examined - 2 specs.: Secui - 1 spec., July 12, 2008; Șimnic - 1 spec., June 23, 2007.

Records from the patrimony of the museum: Craiova, Secui (ILIE & CHIMIȘLIU, 1999); Craiova (Romanescu Park) (CHIMIȘLIU & MOGOȘEANU, 2009).

17. *Leptinotarsa decemlineata* (SAY, 1824)

Material examined - 7 specs.: Melinești - 2 specs., May 25, 2008; Negoii - 2 specs., May 1, 2004; Secui - 1 spec., June 20, 2007; 2 specs., July 23, 2007.

Records from the patrimony of the museum: Baia de Fier, Budieni (BOBÎRNAC et al., 1999); Bistreț, Craiova, Preajba, Racovița (ILIE & CHIMIȘLIU, 1999).

18. *Colaphellus sophiae* (SCHALLER, 1783)

Material examined - 8 specs.: Ciuperceni - 2 specs., May 22, 2004; Craiova (Romanescu Park) - 1 spec., June 8, 2004; Izverna - 1 spec., August 17, 1984; Negoii - 4 specs., May 2, 2008.

Records from the patrimony of the museum: Bistreț, Corabia, Craiova, Negoii, Răcari (ILIE & CHIMIȘLIU, 1999); Bucovăț, Dunăreni (CHIMIȘLIU & MOGOȘEANU, 2009).

19. *Entomoscelis adonidis* (PALLAS, 1771)

Material examined - 2 specs.: Craiova (the Botanical Garden) - 1 spec., May 19, 2004; 1 spec., June 17, 2004.

Records from the patrimony of the museum: Craiova, Predești, Poiana Mare (ILIE & CHIMIȘLIU, 1999); Bucovăț, Zvorsa (CHIMIȘLIU & MOGOȘEANU, 2009).

20. *Gonioctena fornicata* BRÜGGEMANN, 1873

Material examined - 21 specs.: Craiova (Romanescu Park) - 7 specs., June 8, 2004; Drobeta Turnu-Severin - 2 specs., June 6, 2005; Șimnic - 1 spec., June 20, 2004; 1 spec., April 12, 2008; Tărtășești - 2 specs., June 20, 2005; Negoii - 8 specs., April 27, 2007, May 2, 2008.

Records from the patrimony of the museum: Bugiulești, Olănești (BOBÎRNAC et al., 1999); Craiova, Ghercești, Slănic Prahova (ILIE & CHIMIȘLIU, 1999); Șimnic, Zvorsa (CHIMIȘLIU & MOGOȘEANU, 2009).

IV. Subfamily GALERUCINAE LATREILLE, 1802

1. *Galeruca pomonae* (SCOPOLI, 1763)

Material examined - 3 specs.: Șimnic - 1 spec., June 23, 2004; 1 spec., June 10, 2007; Orșova (Cula Tricule) 1 spec., June 11, 1970

Records from the patrimony of the museum: Craiova, Melinești, Poiana Mare, Segarcea, Tismana (ILIE & CHIMIȘLIU, 1999).

2. *Galeruca tanacetii* (LINNAEUS, 1758)

Material examined - 48 specs.: Bratovoiești - 3 specs., May 27, .05.2001; Canarua Fetii - 1 spec., June 7, 2008; Ciuperceni - 4 specs., May 1, 2004; Craiova - 2 specs., June 2, 2005; Craiova (the Botanical Garden) - 2 spec., June 20, 2005; 1 spec., June 21, 2005; 1 spec., May 20, 2008; Craiova (Romanescu Park) - 6 specs., June 8, 2004; Drobeta Turnu-Severin - 3 specs. June 6, 2005; Râpa Roșie - 4 specs. May 12, 2007; Secui - 2 specs., May 27, 2008; Șimnic - 5 specs., June 13, 2004; 6 specs., July 7, 2004; 8 specs., June 15, 2005.

Records from the patrimony of the museum: Tismana (BOBÎRNAC et al., 1999); Bistreț, Berbești, Bârza-Balș, Craiova, Racovița, Tismana (ILIE & CHIMIȘLIU, 1999); Bratovoiești, Bucovăț, Craiova, Craiova (the Botanical Garden), Craiova (Romanescu Park), Craiova (Valea Gangului), Mischii, Negoii, Preajba, Răcari, Segarcea, Șimnic (CHIMIȘLIU & MOGOȘEANU, 2009).

3. *Galeruca rufa* GERMAR, 1824

Material examined - 6 specs.: Crihala Forest - 1 spec., July 18, .07.1984; Cula Cernetești - 1 spec., July 4, 1985; Craiova - 1 spec., May 16, 1981; Găioara, Iron Gates Natural Park - 1 spec., March 27, 1985; Gura Văii, Valea Oglănic - 1 spec., April 26, 1985; Valea Bahnei - 1 spec., April 20, 1984.

Records from the patrimony of the museum: Baia de Aramă, Negoii, Plenița, Racovița, Șimnic, Vâlcom (ILIE & CHIMIȘLIU, 1999); Bratovoiești, Coțofenii din Față, Mischii, Negoii, Șimnic (CHIMIȘLIU & MOGOȘEANU, 2009).

V. Subfamily CRYPTOCEPHALINAE GYLLENHAL, 1813

1. *Clytra (Clytra) laeviuscula* (RATZBURG, 1837)

Material examined - 24 specs.: Bratovoiești - 1 spec., June 17, 2008; Bucovăț forest - 6 specs., July 5, 2005; Craiova - 1 spec., June 9, 2008; Craiova (the Botanical Garden) - 9 specs., June 20-21, 2005; 5 specs., May 20-25, 2008; Drobeta Turnu-Severin - 1 spec., June 6, 2005; Răcari - 1 spec., June 9, 1999.

Records from the patrimony of the museum: Cheile Sohodol, Ponoare (BOBÎRNAC et al., 1999); Baia de Aramă, Bucovăț, Caracal, Craiova, Negoii, Țințăreni (ILIE & CHIMIȘLIU, 1999); Craiova (the Botanical Garden), Craiova (Romanescu Park), Răcari (CHIMIȘLIU & MOGOȘEANU, 2009).

2. *Labidostomis (Labidostomis) longimana* (LINNAEUS, 1760)

Material examined - 1 spec.: Craiova (the Botanical Garden), May 20, 2008.

Records from the patrimony of the museum: Bistreț, Bucovăț, Craiova Gura Motrului, Mofleni, Predești, Turceni (ILIE & CHIMIȘLIU, 1999); Cheile Sohodol (CHIMIȘLIU & MOGOȘEANU, 2009).

*3. *Labidostomis (Labidostomis) pallidipennis* (GEBLER, 1830)

Material examined - 2 specs.: Șimnic - 1 spec., June 23, 2004; 1 spec., June 15, 2008.

4. *Lachnaia (Lachnaia) sexpunctata* (SCOPOLI, 1763)

Material examined - 4 specs.: Bucovăț forest - 1 spec., June 13, 2004; Craiova (the Botanical Garden) - 1 spec., May 19, 2004; Craiova (Romanescu Park) - 2 specs., June 8, 2004.

Records from the patrimony of the museum: Baia de Fier (BOBÎRNAC et al., 1999); Bucovăț, Craiova, Leamna, Orodol, Segarcea (ILIE & CHIMIȘLIU, 1999); Bratovoiești, Bucovăț, Craiova (Romanescu Park), Răcari, Segarcea (CHIMIȘLIU & MOGOȘEANU, 2009).

5. *Smaragdina affinis* (ILLIGER, 1794)

Material examined - 1 spec.: Craiova (Romanescu Park), May 24, 2006.

Records from the patrimony of the museum: Bucovăț (CHIMIȘLIU & MOGOȘEANU, 2009).

6. *Smaragdina aurita* (LINNAEUS, 1767)

Material examined - 1 spec.: Bucovăț forest, May 17, 2000.

Records from the patrimony of the museum: Ghercești, Negoii, Radovan, Segarcea, Turceni (ILIE & CHIMIȘLIU, 1999).

*7. *Smaragdina limbata* (STÉVEN, 1806)

Material examined - 1 spec.: Negoii, June 21, 2008; 1 spec. (without collection data).

8. *Smaragdina salicina* (SCOPOLI, 1763)

Material examined - 6 specs.: Craiova - 2 specs., May 3, 2004; Craiova (the Botanical Garden) - 3 specs., 17.06.2004; Negoii - 1 spec., May 2, 2008.

Records from the patrimony of the museum: Bratovoiești, Bucovăț, Craiova (the Botanical Garden), Craiova (Romanescu Park) (CHIMIȘLIU & MOGOȘEANU, 2009).

9. *Smaragdina xanthaspis* (GERMAR, 1824)

Material examined - 4 specs.: Drobeta Turnu-Severin - 2 specs., June 6, 2005; Șimnic - 2 specs., June 15, 2008.

Records from the patrimony of the museum: Mischii (ILIE & CHIMIȘLIU, 1999); Craiova (the Botanical Garden) (CHIMIȘLIU & MOGOȘEANU, 2009).

*10. *Tituboea macropus* (ILLIGER, 1800)

Material examined - 2 specs.: Schela Cladovei, July 11, 1971; May 5, 1980.

*11. *Cryptocephalus (Asionus) apicalis* GEBLER, 1830

Material examined - 1 spec.: Svinița, January 16, 1969.

*12. *Cryptocephalus (Burlinius) connexus* OLIVIER, 1808

Material examined - 2 specs.: Valea Bahnei, August 5, 1984.

*13. *Cryptocephalus (Cryptocephalus) androgyne* MARSEUL, 1875

Material examined - 1 spec.: Răcari, May 10, 1999.

14. *Cryptocephalus (Cryptocephalus) octacosmus* BEDEL, 1891

(syn: *anticus* SUFFRIAN, 1848)

Material examined - 7 specs.: Bratovoiești - 1 spec., June 17, 2008; Craiova (the Botanical Garden) - 1 spec., May 20, 2008; Gura Văii - 1 spec., May 28, 1980; Secui - 4 specs., May 27, 2008.

Records from the patrimony of the museum: Craiova, Gura Motrului, Leamna, Logrești (ILIE & CHIMIȘLIU, 1999).

15. *Cryptocephalus (Cryptocephalus) bipunctatus* (LINNAEUS, 1758)

Material examined - 3 specs.: Bucovăț forest - 2 specs., June 9, 1999; Drobeta Turnu-Severin - 1 spec., June 6, 2005.

Records from the patrimony of the museum: Bucovăț, Craiova, Bistreț (ILIE & CHIMIȘLIU, 1999), Șimnic (CHIMIȘLIU & MOGOȘEANU, 2009).

*16. *Cryptocephalus (Cryptocephalus) flavipes* FABRICIUS, 1781

Material examined: 8 specs.: Gura Văii - 3 specs., May 15, 1978; Valea Oglănic - 4 specs., May 8, 1981; Cireșu - 1 spec., June 12, 1981.

17. *Cryptocephalus (Cryptocephalus) moraei* (LINNAEUS, 1758)

Material examined - 9 specs.: Bala-Băi - 5 specs., June 14-15, 1985; Craiova (the Botanical Garden) - 1 spec., May 20, 2008; 3 specs. (without collection data).

Records from the patrimony of the museum: Cheile Sohodol (BOBÎRNAC et al., 1999); Leamna, Logrești, Negoii, Tismana (ILIE & CHIMIȘLIU, 1999).

*18. *Cryptocephalus (Cryptocephalus) nitidus* (LINNAEUS, 1758)

Material examined - 1 spec.: Valea Oglănic, May 8, 1981.

*19. *Cryptocephalus (Cryptocephalus) sexpunctatus* (LINNAEUS, 1758)

Material examined - 1 spec.: Valea Cheii, May 30, 2008.

20. *Cryptocephalus (Cryptocephalus) violaceus* LAICHARTING, 1781

Material examined - 5 specs.: Bucovăț forest - 2 specs., May 17, 2000; Ișalnița - 1 spec., May 26, 1999; Răcari - 1 spec., June 6, 1999; Râpa Roșie - 1 spec., May 12, 2007.

Records from the patrimony of the museum: Cheile Sohodol (ILIE & CHIMIȘLIU, 1999).

21. *Pachybrachis (Pachybrachis) hippophaes* (SUFFRIAN, 1848)

Material examine - 1 spec. (without collection data).

Records from the patrimony of the museum: Racovița (ILIE & CHIMIȘLIU, 1999).

*22. *Pachybrachis (Pachybrachis) sinuatus* (MULSANT & REY, 1859)

Material examined - 1 spec.: Gura Văii, May 27, 1977.

VI. Subfamily EUMOLPINAE HOPE, 1840

*1. *Eupales ulema* (GERMAR, 1813)

Material examined - 4 specs.: Cula Cerneți - 1 spec., June 21, 1976; 3 specs., May 7, 1984.

DISCUSSIONS

Most of the identified species are common in the Romanian fauna (e.g.: *Clytra laeviuscula*, *Cassida viridis*, *Chrysomela populi*, *Chrysolina herbacea*, *C. fastuosa*, *C. varians*, *Labidostomis longimana*, *Galeruca pomonae*, *G. tanacetii*, *Cryptocephalus moraei*, *Pachybrachis sinuatus*).

The following 18 species are mentioned for the first time in the collections of the Oltenia Museum: *Oulema gallaeciana*, *Pilemostoma fastuosum*, *Chrysomela saliceti*, *Linnaeidea aenea*, *Neophaedon pyritosus*, *Chrysolina rossia*, *C. varians*, *Labidostomis pallidipennis*, *Smaragdina limbata*, *Tituboea macropus*, *Cryptocephalus apicalis*, *C. connexus*, *C. androgyne*, *C. flavipes*, *C. nitidus*, *C. sexpunctatus*, *Pachybrachis sinuatus* and *Eupales ulema*. At the 90

species previously recorded from the museum patrimony (CHIMIȘLIU & MOGOȘEANU, 2009), there are now added 18 species. Thus, the number of leaf beetle species preserved in the collections of the Oltenia Museum currently amounts to 108 (18.91%) of the 571 species recorded in the Romanian fauna (MAICAN, 2005).

Among the valuable species preserved in these collections, we mention *Tituboea macropus*, a Ponto-Turanian species, distributed in Austria, Hungary, Moravia, Balkans, Turkmenistan and Uzbekistan. It occurs especially in sandy areas, with steppe specific vegetation (MAICAN, 2006).

Also, *Smaragdina limbata* (east-Mediterranean species, distributed in the Balkan Peninsula, Asia Minor, Caucasian countries, Middle East and northern Iran), *Eupales ulema* (distributed in the Balkan Peninsula, Hungary, Turkey) and *Colaphellus sophiae* (Central and Southeastern Europe, Turkey) are rare species in the Romanian fauna.

CONCLUSIONS

This paper presents data on 53 leaf beetle-species (belonging to 23 genera and 6 subfamilies) preserved in the entomological collections of the Oltenia Museum Craiova.

Most of the localities and sites represent new records for the presence of Chrysomelidae in the Romanian fauna. Among the valuable species preserved in these collections, the following should be mentioned: *Tituboea macropus*, *Smaragdina limbata* and *Eupales ulema*.

By identifying the 18 new species for the collection of the museum, the total number of leaf beetle species preserved in the patrimony of the museum stands at 108. It is necessary to further explore the data from the collections of natural sciences museums in order to increase knowledge of the entomofauna diversity in Romania.

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BIOLOGICAL ACTIVITY OF THE EXTRACT OF *Veratrum lobelianum* BERNH. AGAINST HARMFUL SPECIES OF INSECTS AND MITES AND ITS IMPACT ON ENTOMOPHAGES

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Abstract. It has been experimentally proved the existence of high insecticidal activity of the extract of *Veratrum lobelianum* BERNH. (0.1 to 0.5% of active substance) against imagoes and larvae of *Leptinotarsa decemlineata* SAY, larvae of the age I-III of *Heliothis armigera* HBN., *Mamestra brassicae* L., as well as against *Acyrtosiphon pisum* HARR. The extract shows high ovicidal (61.5 to 100%) and antifeedant (1-2 points) activity against *L. decemlineata*. It has been experimentally confirmed that the extract shows repellent activity against the larvae of *G. mellonella*. It has been proved that carrying out a single treatment with 0.1% extract of *V. lobelianum* (water-alcohol solution, 7.5 l/ha, 40 g/l of active substance) is enough to reduce the numerosity of *L. decemlineata* of the first generation. On the 21st day after being treated, the efficiency remained at reference level (92.0%), and plant damage did not exceed 5% (1 point). It has been determined that the treatment of potato plants with the extract of *V. lobelianum* has no negative impact on entomophages of *Chrysopa carnea* STEPH., *Coccinella septempunctata* L. and *Zicrona caerulea* L.; their numerosity remained at reference level and significantly exceeded chemical reference (by 1.5-4.0 times).

Keywords: biological activity, extract, *Veratrum lobelianum*, entomophages.

Rezumat. Eficacitatea biologică a extractului din specia *Veratrum lobelianum* BERNH. împotriva speciilor de insecte dăunătoare și acarlene, și influența lui asupra entomofagilor. Experimental s-a demonstrat că extractul din specia *Veratrum lobelianum* BERNH. (0.1-0.5% s.a.) posedă proprietăți înalte insecticide asupra fazelor imago și larvelor *Leptinotarsa decemlineata* SAY, larvelor de vârstele a II-III *Heliothis armigera* HBN., *Mamestra brassicae* L., precum și asupra *Acyrtosiphon pisum* HARR. Extractul posedă o activitate înaltă ovicidă (61.5-100%) și antifidantă (1-2 puncte) contra *L. decemlineata*. Experimental s-a confirmat faptul că extractul manifestă o activitate repelentă asupra larvelor de *G. mellonella*. S-a demonstrat că efectuarea unui tratament la cartof cu extractul de 0.1% din *V. lobelianum* (soluție alcoolică apoasă, 7.5 l/ha, 40 g/l s.a.) este suficientă pentru diminuarea densității populației *L. decemlineata* de prima generație. Eficacitatea la 21 de zile după tratament a fost menținută la nivelul standardului (92.0%), precum și defolierea tufelor de cartofi nu a depășit nivelul 5% (1 punct). A fost stabilit că tratarea cu extractul din *V. lobelianum* nu provoacă efecte negative asupra entomofagilor *Chrysopa carnea* STEPH., *C. septempunctata* și *Z. caerulea* L.; densitatea populației lor se menține la nivelul variantei martor și depășește esențial pe cel al etalonului chimic (1.5-4.0 ori).

Cuvinte cheie: eficacitatea biologică, extract, *V. lobelianum*, entomofagi.

INTRODUCTION

One of the most dangerous pests on solanaceous crops in all parts of constantly expanding wide area is *Leptinotarsa decemlineata* SAY (Coleoptera, Chrysomelidae). Potato beetle inflicts the largest harm to plants at the first development stages, starting from seedling stage till flowering phase. At bud stage, the nutrition of 10 pest larvae on a potato plant during a week results in a decrease of harvest quality by 40-45% – reduced plant size and changed biochemical parameters (KOVALI, 2005). At a density of 25 larvae per plant during flowering phase (tuber formation), the degree of leave damage can reach 50-80% with the harvest decreased by 25-52%. In areas where pest management is not held, the losses reach 70-80% (KOVALI, 2005). At present, there is a practical necessity to control resistant populations of potato beetle selected as a result of many years of pyrethroid products application (MOTA-SANCHEZ et al., 2006). One of the efficient ways to reduce pest numerosity is to use specialized entomophages. However, optimal combination of using specialized entomophages against *L. decemlineata* has proved to be problematic due to complex interspecific correlations, various adaptability degree and ecological valence of entomophage species and potato beetle acclimatized to conditions of immigration areas, as well as due to mismatched development phases of *L. decemlineata* and some of its predators in new areas (FILIPPOV et al., 1985). That is why it is so important to preserve natural populations of native entomophage species, such as ladybirds (Coleoptera, Coccinellidae), golden-eyes (Neuroptera, Chrysopidae), bugs (Heteroptera - Pentatomidae, Miridae, Nabidae), etc. Despite the fact that these predators do not influence on the reduction of pest population density significantly, however they are capable to restrain the numerosity of other harmful phytophagous species, contributing to the preservation of healthy phytosanitary environment of the agrocoenosis.

In this regard, the tendency of studying and applying herbal substances to reduce the density of pest populations is of current interest at present stage associated with the development of environmental systems for plant protection and ecological agriculture. Products based on the substances extracted from *Pyrethrum cinerariaefolium* TREV., *Nicotiana tabacum* L., *Quassia amara* L., *Anabasis aphylla* L., *Derris elliptica* (WALL.) BENTH., *Lonchocarpus nicou* (AUBLET) DC., *Tephrosia vogelii* HOOKF. and *Azadirachta indica* A. JUSS. are the most studied and used in plant protection (HUMMEL, 2007; ISMAN, 2006; KHAMBAJ& JEWESS, 2000). At this stage, some tests of plant extracts against the larvae of *L. decemlineata* are carried out in Germany, Spain, Turkey, Canada, USA, Japan, China, India and many

other countries (KUMAR & POEHLING, 2007; KOUL et al., 2004; ZABEI et al., 2002). Some researches of secondary plant metabolites with insecticidal activity are carried out in Russia and Belarus as well (LITVINOVA et al., 2004; ZOLOTAR et al., 2001).

We have also carried out a research to identify the substances of plant origin that show biological activity against various types of the phytophagous species – insects and mites. About 200 plant species from 50 families have been tested against 14 species of pests. As a result, a number of plants with extracts showing high insecticidal, antifeedant, deterrent, repellent, or ovicidal properties have been selected. The purpose of the present research is to determine biological activity of the extract of *V. lobelianum* against harmful insects and mites, as well as to study its impact on some species of entomophages.

MATERIAL AND METHODS

The extract of *Veratrum lobelianum* BERNH. (Liliaceae) has served as an object for the research. Collecting, drying, grinding, extraction of active substances from roots of *V. lobelianum*, as well as determination of the sum of active substances has been carried out according to standard methods (MURAVIOVA, 2007).

Experiments have been carried out using egg clutches, larvae and imagoes of potato beetle - *Leptinotarsa decemlineata* (SAY 1824) (Coleoptera, Chrysomelidae) due to its greatest damage in conditions of Moldova and quick development of the resistance to existing protection methods. As a feeding plant, it has been used "Amerikanka" potato variety with a medium ripening period (*Solanum tuberosum* L., Solanaceae), which is damaged by the potato beetle significantly. The extract has been also tested against the following types of phytophagous species: Insecta - *Heliothis armigera* (HÜBNER, 1808) (Lepidoptera, Noctuidae), *Mamestra brassicae* (LINNAEUS 1758) (Lepidoptera, Noctuidae), *Galleria mellonella* (LINNAEUS 1758) (Lepidoptera, Pyralidae), *Acyrtosiphon pisum* (HARRIS, 1776). (Homoptera, Aphididae), Arachnida - *Tetranychus viennensis* (ZACHER 1920) and *Tetranychus urticae* (KOCII 1836)(Acariformes, Tetranychidae).

Laboratory testing of the extract has been carried in a climate cell with adjustable conditions at set temperatures of +22...+24°C, air humidity of 70-80% and 16-hour photoperiod. For *L. decemlineata*, insects of a natural population have been used. Other types of phytophagous species have been bred using an artificial nutrient medium or plants grown under laboratory conditions. Young leaves of potatoes and tomatoes with standard sizes have served as a substrate for feeding *L. decemlineata* and *H. armigera*. Young cabbage leaves cut in rectangles with a size of 5 x 5 cm have served as a substrate for feeding *M. brassicae*. Leaves have been treated using a method of immersing into the extract and then kept in an exhaust hood for 1 hour until full evaporation of the solvent, and then they have been placed into Petri dishes with insects. Cut pea shoots have served as a substrate for feeding *A. pisum*. Shoots have been treated together with colonies of aphids using a standard manual sprayer at a rate of 5 ml per shoot. Variants of treating leaves with 12% alcoholic solution have served as the reference. An artificial nutrient medium (ANM) has served as a food for *G. mellonella* with the addition of 2 ml of 0.1% extract. Variants of feeding with the addition of 2 ml of 12% alcohol solution have been used as the reference. Insecticidal activity (efficiency) has been determined using the number of dead insects for three days in comparison with the reference according to standard methodology (DOLJENCO, 2004).

Young leaves of the plum and the soy with standard sizes has served as a substrate for feeding *T. viennensis* and *T. urticae*. They have been treated using a method of immersing into 0.1% extract for several seconds and then kept for 1 hour until full evaporation of the solvent. Treated leaves have been placed into Petri dishes with cotton mattresses wetted with distilled water according to standard methodology. Then female mites have been placed thereinto. Records of dead individuals have been made in a day. Variants of treating leaves with 12% alcoholic solution have served as the reference. Acaricidal activity has been determined on the basis of the number of dead mites in comparison with the reference according to standard methodology.

Under laboratory conditions antifeedant activity of the extract against potato beetle has been determined on the third day after the beginning of the experiment. Antifeedant activity level has been assessed according to a standard scale in points (DOLJENCO, 2004).

Ovicidal activity of extracts of *V. lobelianum* has been tested using egg clutches of *L. decemlineata*, collected from untreated potato plants. Each variant consists of three replications, three egg clutches each. Leaves with egg clutches have been treated using a method of immersing into the extract for several seconds and then kept in an exhaust hood for 1 hour until full evaporation of the solvent. Then they have been placed into double dishes. Leaves with egg clutches treated with 12.0% alcoholic solution have served as the reference. Ovicidal activity has been determined on the 10th day according to standard methodology (DOLJENCO, 2004).

Tests of the extract of *V. lobelianum* were carried out in 2012 on an experimental field of the Institute of Plant Protection and Ecological Agriculture of the ASM using 'Amerikanka' potato variety with a medium ripening period. The experiment has been made in three variants. Each variant included four replications, and the area of a single replication was 50 m². Experimental plots were positioned using randomization method. During vegetation period agrotechnical measures of weed protection were carried out on this potato field.

The extract of *V. Lobelianum* has been tested at a concentration of 0.1% (water-alcohol solution, active substances – sum of alkaloids, in equivalent of protoveratrine 40 g/l) with a dose of 7.5 l/ha. 2.5% alcohol solution has been used as the reference. 'Confidor Maxi 70 WG', an insecticide with systemic and contact action (water-soluble

granules. 70%. active substance – imidacloprid 700 g/kg), has been used as a chemical standard with a dose of 0.08 kg/ha. Treatment operations have been carried out using “KWAZAR COR” knapsack sprayer with a capacity of 12 l. The consumption of working solution was 300 l/ha.

Biological efficiency of the extract of *V. lobelianum* has been determined using a method of recording 15 plants for each replication. Pest population density has been recorded before treating plants and on the 1st, 3rd, 7th, 14th, 21st day after being treated. Biological efficiency has been determined according to standard methodology. Antifeedant activity (damage degree of potato plants) has been determined on the 1st, 3rd, 7th, 14th, 21st day according to standard methodology (DOLJENCO, 2004).

The impact of the extract of *V. lobelianum* on Chrysopacamea [Chrysoperlacamea] (STEPHENS, 1836) (Neuroptera: Chrysopidae), *Coccinella septempunctata* (LINNAEUS, 1758) (Coleoptera: Coccinellidae) and *Zicronacaerulea*(LINNAEUS 1758) (Heteroptera: Pentatomidae) has been recorded simultaneously. The population density of areas treated with the extract has been compared with the reference and chemical standard.

Mathematical processing of obtained data has been carried out according to the method of unifactor variance analysis (DOSPEHOV, 1979).

RESULTS AND DISCUSSIONS

As a result of laboratory testing against imagoes of *L. decemlineata*, it has been found that at low concentrations of active substances of 0.05 to 0.025% the extract of *V. lobelianum* mostly suppresses the nutrition of insects, slightly reducing their numerosity from 6.7 to 20.0%. When raising the concentration of active substances up to 0.3-0.5%, the efficiency of the extract against imagoes of potato beetle increases and ranges from 66.7 to 73.3% (Table 1).

Table 1. Insecticid alandantifeedant activity of the extract of *V. lobelianum* against larvae and imagoes of *L. decemlineata* under laboratory conditions depending on the concentration, n= 5x4.

Variant	Concentration of active substances, %	Insecticidal activity, %				Antifeedant activity, points			
		Larvae (by ages)			Imagoes	Larvae (by ages)			Imagoes
		I-II	II-III	III-IV		I-II	II-III	III-IV	
Reference	-	0	0	0	0	5	5	5	5
Extract of <i>V. lobelianum</i>	0.5	100	100	100	73.3	1	1	1	1
	0.4	100	93.3	80.0	73.3	1	1	1	1
	0.3	100	93.3	80.0	66.7	1	1	1	1
	0.2	100	93.3	73.3	53.3	1	1	2	2
	0.1	100	86.7	60.0	40.0	1	1	2	2
	0.05	93.3	80.0	53.3	20.0	1	1	2	2
	0.025	86.7	80.0	26.7	6.7	1	1	2	2
HSD _{0.05} = 14.1									

It has been determined that the larvae of *L. decemlineata* of younger ages (I-III) are the most sensitive to the action of the extract. Even at the lowest concentrations of 0.025%, their death has reached 80.0%-86.7%. At the same time, older larvae (III-IV) show a high resistance degree. At concentrations of the extract of 0.025%, only a quarter (26.7%) of insects died, and only after increasing the concentrations of the extract up to 0.1% the death has increased up to 60.0%.

Antifeedant activity of the extract against older larvae and imagoes of *L. decemlineata* depends on the concentration of active substances and slightly decreases with reduced concentration. Thus, at a concentration of 0.3-0.5% antifeedant activity against imagoes and larvae of the age III-IV has reached 1 point (browsing of leaf surface up to 5%). When lowering the concentration up to 0.025-0.2%, leaf damage has reached 15% that equals to 2 points. For younger larvae, antifeedant properties of the extract are not dependent on the concentration of active substances (within the range of tested concentrations), as leaf damage has not exceeded 5% – 1 point (Table 1).

As a result of observing the behaviour of insects during laboratory tests, it has been noticed that after being placed into Petri dishes with treated food both larvae and imagoes did not immediately approach to potato leaves, but travelled across the perimeter of dishes for 10-15 minutes and more at the most remote distance. The nutrition was of a short duration, and individuals left leaves repeatedly and nervously moved across the perimeter of Petri dishes again. Insects eating treated potato leaves show signs of metabolic disturbance and diarrhoea symptoms. Insects became limp and stiff, and many individuals have shown complete or partial paralysis. Subsequently all paralyzed younger larvae died. After the death their bodies become very wrinkly due to the hydration, and coverings lose the elasticity. Our observations have revealed that imagoes are able to recover, as after 2-day paralysis with obvious signs of metabolic disturbance many individuals return to normal state on the third day, being ready to coupling and egg laying. After placing such insects (on the fourth day of the experiment) onto fresh untreated food and monitoring them for 3 more days, we have observed no death of any individual. Thus, it has been revealed that active substances contained in the extract of *V. lobelianum* come out with metabolism products of insects and do not accumulate in the body.

When determining the nature of the action of 0.1% extract of *V. lobelianum*, it has been noticed that it shows mostly intestinal action against insects. At the same time, the extract also shows contact action, as the death of insects has been recorded as a result of topical application on dorsal area. The highest death percentage as a result of topical method of treating insects has been recorded for larvae of the age I-III (80.0 to 100%) and the smallest one – for imagoes (up to 26.7%).

We have also determined ovicidal activity of the extract against potato beetle. As a result, it has been found that the treatment with the extract sets back terms of hatching out of eggs by 1-2 days compared to the reference. In addition, it has been noticed that depending on the concentration of active substances the extract suppresses hatching out of eggs by 5.5%-25.5%. Corrected to the number of dead eggs in the reference - 1.5%, the number of dead eggs in the experiment ranges from 4.0% to 24.0%. At the same time, it has been found that larvae of *L. decemlineata* die during hatching period when gnawing through the chorion. At high concentrations of the extract from 0.4 to 0.5%, most of larvae die before being able to completely break free out of chorions. At lower concentrations, larvae die later, in a range from 1 to 24 hours. As a result, it has been found that depending on the concentration of active substances under laboratory conditions total ovicidal activity ranges from 61.5 to 100% (Fig. 1). Thus, we have proved that ovicidal action of extracts of *V. lobelianum* comprises the ovicidity itself and the death of embryos at the time of hatching. It has been noticed that at concentrations of the extract from 0.05 to 0.5% major number of eggs in an egg clutch (83.5-100%) die.

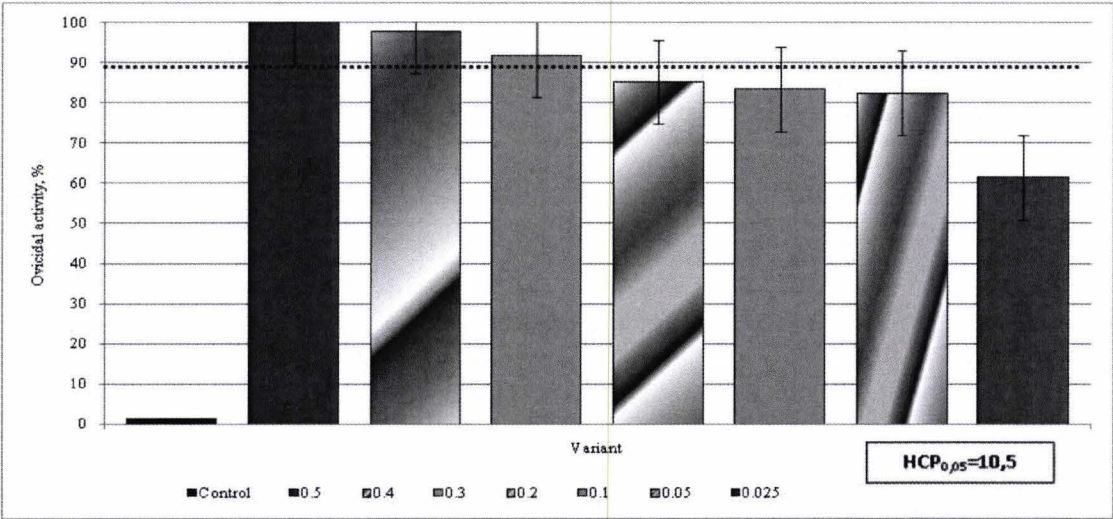


Figure 1. Ovicidal activity of the extract of *V. lobelianum* against *L. decemlineata* under laboratory conditions depending on the concentration.

Thus, it has been found that the extract of *V. lobelianum* possesses insecticidal, antifeedant and ovicidal activity against potato beetle (Coleoptera). In addition to potato beetle, we have also tested 0.1% extract of *V. lobelianum* against insects belonging to such orders as Lepidoptera and Homoptera, as well as against mites of Acariformes (Table 2).

As a result, it has been found that the extract shows high insecticidal activity (100%) against larvae of the age II-III of *M. brassicae* and *H. armigera*, as well as against colonies of aphids of *A. pisum* (Table 2).

Table 2. Insecticidal and acaricidal activity of the extract of *V. lobelianum* (0.1%) under laboratory condition sagainst various phytophagous species.

Class	Order	Pest species	Pest phase	Replication x times	Insecticidal activity, %	
					Reference	Extract of <i>V. lobelianum</i>
Insecta	Lepidoptera	<i>Mamestra brassicae</i> L. (Noctuidae)	II-III	4x5	0	100
		<i>Heliothis armigera</i> HBN.(Noctuidae)	Larvae of the age II-III	3x20	0	100
		<i>Galleria mellonella</i> L. (Pyralidae)	Larvae of the age II-III	4x5	0	47.0
	Homoptera	<i>Acyrtosiphon pisum</i> HARR. (Aphididae)	Colony	3x20	0	100
Arachnida	Acariformes	<i>Tetranychus viennensis</i> ZACHER. (Tetranychidae)	Imagoes	3x25	0	24.8
		<i>Tetranychus urticae</i> KOCH. (Tetranychidae)	Imagoes	3x25	0	23.4

The extract of *V. lobelianum* shows a medium level of insecticidal and antifeedant activity against larvae of the age II-III of *G. mellonella*. The death of insects reaches only 47.0% (Table 2), and the amount of eaten food is 25-30%.

We have noticed repellent activity of the extract, as in the first hours after being placed into Petri dishes with treated food insects travelled for a long time at the most remote distance from the food. Subsequently their behaviour also differed from that of reference individuals: most larvae in the experiment with the extract stayed at the edge of treated food, whereas larvae in the reference completely submerged into the food, embracing made holes with the silk.

It has been noticed that the extract of *V. lobelianum* possesses low acaricidal activity against both *T. viennensis* and *T. urticae*. The death of female mites reaches 23.4-24.8%. We have not revealed any repellent activity against both species of mites. It has been noticed that female mites lay eggs on leaves treated with the extract. The number of laid eggs in the experiment slightly differs from the reference.

Thus, we have not revealed any overall regularity proving that the extract of *V. lobelianum* possesses an activity at a certain level against insects belonging to a particular order. At the same time, it has been determined that the extract shows a quite low level of insecticidal activity against mites-phytophages and does not show any deterrent properties. This confirms the conclusions of other authors (HUMMELI, 2006; ISMAN, 2006; KHAMBAY & JEWESS, 2000) that plant extracts show selectivity action against different types of insects and mites and therefore may be low-toxic or non-toxic for entomophagous species. We have carried out studies to determine biological efficiency of the extract of *V. lobelianum* for reducing numerosity of *L. decemlineata* under field conditions. During vegetation period a single treatment was made, the terms of which have been determined depending on the numerosity of pests on tested plots. The average number of larvae on the date of June 4, 2012 was 55 individuals per 100 plants, exceeding economic limits of the harmfulness, and that was the reason for treating with the extract of *V. lobelianum* against potato beetle of the generation I. Plants were at the phase of budding-flowering.

Results show that the efficiency of tested extract of *V. lobelianum* is high (Figs.2a,b; 3a, b). Thus, it has been found that on the first day 100% of larvae died. On the 7th day after being treated, the efficiency of the extract against larvae of beetle was 99.6% and on the 21st – 92.0%, being at standard level (99.4 and 89.0% respectively). Three weeks after the treatment, potato leaf damage in the experiment did not exceed 5% (1 point). On reference plot the increase of pest number for this period resulted in complete destruction of leaf surface of plants (Table 3).



Figure 2. Death of larvae of *L. decemlineata* after treating with the extract of *V. lobelianum* (original).



Figure 3. Death of imagoes of *L. decemlineata* after treating with the extract of *V. lobelianum* (original).

Table 3. Biological efficiency of the extract of *V. lobelianum* against *L. decemlineata* under field conditions.

Variant	Biological efficiency on a corresponding day after being treated, %					Antifeedant activity on the 21 st day after being treated, points
	1	3	7	14	21	
Reference	0	0	0	0	0	5
Standard - 'Confidor Maxi 70 WG'	100	100	99.4	95.0	89.0	1
Experiment - Extract of <i>V. lobelianum</i>	100	100	99.6	97.0	92.0	1

HSD_{0.05}= 5.4

Statistical processing of the data obtained using variance analysis method allows us to reveal that the efficiency of tested extract of *V. lobelianum* against potato beetle larvae slightly differed from the standard and significantly differed from reference variant.

In field experiments we have also noticed that 80 to 95% of larvae hatch out of egg clutches treated with *V. lobelianum*. It has been found that in the experiment larvae hatched out of treated egg clutches do not spread across leaves and do not start eating but die (in the first 24 hours) when gnawing through and eating their chorions and unhatched eggs. It has been revealed that after the death larvae tend to blacken. At the same time, in the reference after hatching larvae eat their chorions and unhatched eggs and start eating first on same leaf and then on other leaves, spreading as a rule to younger top parts of plants.

As a result of the experiments made under field conditions, it has been found that the extract of *V. lobelianum* does not show any phytotoxicity. Plants in the experiment are well developed, with green leaves and growing young shoots. Potato plants do not retard in the growth compared to the standard.

One of the objectives of our research was to study the impact of the extract of *V. lobelianum* under field conditions on some species of predators and entomophagous species found on potato plants. As a result of field observations on a plot treated with the extract of *V. lobelianum*, we have found the following species of predators: egg clutches, larvae, pupae and imagoes of *C. septempunctata* egg clutches, larvae and imagoes *C. carnea*, egg clutches, larvae and imagoes of *Z. caerulea*.

According to our observations for the entire recorded period (21 days), on potato plants treated with the extract of *V. lobelianum* we have not found any dead larvae or imagoes of the golden-eye (*C. carnea*) or the ladybug (*C. septempunctata*). In addition, fresh egg clutches, hatching of healthy larvae, as well as further normal nutrition, development and pupation of larvae of both predator species has been observed (Figs. 4; 5; 6; 7). On the 7th day after being treated, in chemical standard the number of larvae of *C. septempunctata* was 0.1 individuals per plant, while in both the experiment and the reference their number was 5 times higher. On standard plot treated with 'Confidor Maxi 70 WG', a few death cases have been recorded for imagoes of *C. septempunctata*. On the 7th day after being treated, in the experiment and the reference the number of egg clutches of *C. carnea* (Fig. 4) reached 0.4 eggs per plant and larvae – 0.3 individuals per plant. The number in chemical standard – 0.1 eggs and 0.05 larvae per plant.



Figure 4. Eggs of *C. carnea* on potatoes (original).



Figure 5. Egg-laying of *L. decemlineata* attacked by larvae of *C. carnea* (original).



Figure 6. Larvae of *C. septempunctata* on aplot treated with the extract of *V. lobelianum* (original).



Figure 7. Pupa of *C. septempunctata* on a plot treated with the extract of *V. lobelianum* (original).

It has been noticed that *Z. caerulea*, a predatory bug, colonizes potato plants much later than terms of treating against the first generation of potato beetle. We have observed imagoes of this bug (Figs. 8; 9) in the third decade of June, with increased number of larvae of *L. decemlineata* on plants. At the same time, on the experiment plot and reference plot the numerosity of this predator was growing rapidly and by mid-July it reached 2 to 5 imagoes per plant. A few egg clutches of *Z. caerulea* have been observed. The number of imagoes was 4 times lower in chemical standard. It also has been noticed that imagoes and larvae started colonizing plants in chemical standard 7 days later than in the experiment and the reference.

Thus, we have found that at a concentration of 0.1% of active substance the extract of a plant of *V. lobelianum* species has intense insecticidal, antifeedant and ovicidal properties ensuring efficient protection of potatoes from summer generation of *L. decemlineata* for at least three weeks. For maximum effect, the treatment with plant extract is best done during mass hatching of larvae and not later than the apparition of larvae of the age II-III.

Our studies show that the treatment with plant extract of *V. lobelianum* has no negative impact on beneficial entomofauna that allows keeping natural balance of agricultural ecosystem.



Figure 8. Imago of *Z. caerulea*, eatinga larva of *L. decemlineata*. (original).



Figure 9. Coupling ofimagoes of *Z. caerulea* on a plot treated with the extract of *V. lobelianum*. (original).

CONCLUSIONS

As a result of laboratory testing, it has been proved that the extract of *V. lobelianum* shows high insecticidal (60.0-100%), antifeedant (1-2 points) and ovicidal (61.5 to 100%) properties that are dependent on the concentration of active substances. It has been revealed that the extract possesses both intestinal and contact action. However, contact action of the extract is weaker, so it is less toxic for imagoes of Coleoptera, as well as for individuals that are not exposed to the extract directly or do not eat treated food.

It has been determined that the extract shows a low level of insecticidal activity and does not show any deterrent properties against mites-phytophages (Tetranychidae). It has been experimentally confirmed that the extract possesses repellent activity against larvae of *G. mellonella*.

Made research has proved that a single treatment with 0.1% extract of *V. lobelianum* (water-alcohol solution, 7.5 l/ha, 40 g/l of active substance) is enough to reduce the numerosity of the first generation of potato beetle. On the 21st day after the treatment, the efficiency remained at standard level (92.0%), and plant damage did not exceed 5% (1 point).

It has been determined that the treatment of potatoes with the extract of *V. lobelianum* has no negative impact on entomophagous species (*C. septempunctata*, *C. carnea* and *Z. caerulea*), as their number remained at reference level and was significantly higher than chemical standard (1.5-4.0 times). Thus, it has been proved that the extract of *V. lobelianum* with a high biological efficiency can be used as a means of natural origin for reducing numerosity of potato beetle without any negative influence on beneficial entomofauna of the agrobiocoenosis of a potato field.

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***Trichogramma* - EFFECTIVE SOLUTION FOR THE BIOLOGICAL CONTROL OF THE *Laspeyresia pomonella* L. IN APPLE TREE ORCHARDS**

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Abstract. The entomophagous *Trichogramma embryophagum* HTG. is one of the most important biological agents which may be utilized against a complex of pests both in biological control, and in the system of integrated plant protection. The utilization of the eggs of *Sitotroga cerealella* OLIV. irradiated preliminary by gamma rays essentially increases *Trichogramma* biological indices and the efficiency in the field. Biological efficiency of *T. embryophagum* and the degree of damage in the first version (on irradiated eggs of grain moth) when compared with the second option (on the non-irradiated eggs) are significantly different.

Keywords: biological indices, prolificacy, effectiveness, *Trichogramma*, biological efficacy.

Rezumat. *Trichogramma* - soluție efektivă pentru protecția biologică în combaterea dăunătorului *Laspeyresia pomonella* L. în livezile de măr. Entomofagul *Trichogramma* este unul din cei mai importanți agenți biologici care poate fi utilizat în combaterea unui complex întreg de dăunători, atât în controlul biologic, cât și în sistemul integrat de protecție a plantelor. Utilizarea speciei *T. embryophagum* înmulțită în masă pe ouă de *S. cerealella* preliminar iradiate cu raze gama, permite creșterea esențială a indicilor biologici și a eficacității ei în câmp. Eficacitatea biologică a *T. embryophagum* este mult mai avantajoasă în prima variantă, unde ouăle de *S. cerealella* au fost preliminar iradiate. În acest caz și gradul de dăunare al dăunătorului se micșorează, în comparație cu varianta a doua, unde ouăle de molie, pe care a fost înmulțit entomofagul nu au fost iradiate.

Cuvinte cheie: indicii biologici, prolificitatea, eficacitatea, *Trichogramma*, eficacitatea biologică.

INTRODUCTION

The predominance of the chemical pressure to reduce the number of pests in fruit plantings causes significant disruption of biodiversity of arthropods fauna, changing the number and behaviour of insects, which subsequently causes disruption of the numerical connection in the food chains as well as of stable agrocoenosis, resulting in unintended environmental consequences. Relevant and promising for plant protection practice is the development and implementation of integrated systems for the protection of fruit crops from pests, which combine elements of different methods. However long-range integrating elements in such systems are alternative means and the entomophagous *Trichogramma* is the most important and effective biological agent, limiting the number of many species of Lepidopterans including orchard pests (GULII & PAMUJAC, 1994).

The bibliographic sources contains many positive results on the effectiveness of *Trichogramma* in the orchards, as a promising entomophagous for regulating the number of *Lepidopterans* (ALMATNI et. al., 2001; BOTTO, 2004; BREEDVELD & TANIGOSHI, 2007).

The most common and economically damaging pest of the apple tree is apple moth (*L. pomonella*), against which entomophagous *Trichogramma* is used at the egg stage. The most widely spread specie of *Trichogramma* in the orchards of the Republic of Moldova is *T. embryophagum* HTG. For breeding *Trichogramma* in the laboratory conditions the grain moth (*Sitotroga cerealella* OLIV.) is traditionally used as the host, which is at the same time more technological in production than other types. In order to improve test quality and effectiveness of *Trichogramma*, dilution was held on the host eggs, irradiated with gamma rays.

In The Institute of Protection of Plants and Ecological Agriculture there was an intensive research conducted in order to find solutions for technological and applied aspects of *Trichogramma* application, especially in programs of producing ecologically clean products in different cultures. The aspect of *Trichogramma* application is studied less in permanent crops.

The objective of our research was to improve the production technology and application of the entomophagous *T. embryophagum*, as well as to determine its place in the integrated system of protection of apple from apple moth.

MATERIAL AND METHODS

The studies were conducted between 2002 and 2009, both in the laboratory and field conditions – in the Institute of Protection of Plants and Ecological Agriculture orchards, agricultural farms Mereni and Puhoi, Ialoveni county, using laboratory populations of *T. embryophagum* (based on isolates collected in an apple orchard).

Issues of entomophagous in agrocoenosis were done by placing caps and bags at the bottom of the tree crowns. The rate of release of *Trichogramma* in the orchard was 500-600 thousand species individuals per hectare.

The collecting, identification, storage and accumulation of *Trichogramma* were conducted according to the set procedure (DIURICI, 2008).

Breeding of grain moth and *Trichogramma*, evaluation of biological effectiveness of entomophagous and the extent of fruit damage by pest and mathematical data processing were conducted according to the relevant procedures and guidelines for the mass breeding and use of *Trichogramma* (ABAȘCHIN et al., 1979; MENCER & ZIMERMAN, 1986).

RESULTS AND DISCUSSION

1. Determination of biological indicators of the *T. embryophagum*.

In laboratory conditions, the entomophagous *T. embryophagum* was reactivated after prolonged diapause and accumulated for 3-4 generations, while defining the biological indicators of species representatives bred on irradiated with gamma rays grain moth eggs in comparison with non-irradiated eggs option. The biological indicators *T. embryophagum* (1st variant, radiation) were characterized by high fertility from 27.5 to 32.0 egg/female, good survival from 88 to 93 % and the absolute predominance of females to 100% in the static quality criteria of 25.0 to 28.8. For *T. embryophagum*, bred on unexposed grain moth eggs (II version), these rates differed significantly – lower fecundity of females from 16.6 to 18.0 eggs/female, species survival from 80 to 90 % and lower static performance index of 13.4 to 16.4 (Table 1, Fig. 1).

Table 1. Biological indicators of *T. embryophagum*.

Species	Breeding performance, eggs/female, P1	Hatching, %, a1	Quota of females, %, a2	Statistic quality criterion, Y1
<i>T. embryophagum</i> , option I	27.5 – 32.0 1.2 ± 1.6	88.0 – 93.0 2.6 ± 3.2	100.0	25.0-28.8 0.4 ± 1.5
<i>T. embryophagum</i> , option II	16.6-18.0 0.4 ± 1.0	80.0 – 90.0 3.1 ± 3.6	100.0	13.4- 16.4 0.3 ± 0.5

2. Application *T. embryophagum* in the apple tree orchards.

During the period 2002-2009, experiments were carried out in apple orchards with different densities of apple moth populations in the Institute of Protection of Plants and Ecological Agriculture of the ASM, and agricultural enterprises Mereni and Puhoi, applying *T. embryophagum* for the development of two full and part-time third-generations of apple moth pest. The launch scheme of *T. embryophagum* in the field in small bags is represented in Fig. 2. The results of the efficacy of *T. embryophagum* against apple moth in the period from 2002 to 2009 are presented in Table 2. Research was conducted in three variants: the 1st version of *T. embryophagum* was bred on grain moth eggs irradiated with gamma rays, in the 2nd variant *T. embryophagum* was bred on the non-irradiated grain moth eggs; the 3rd option - control - without *Trichogramma* releases, where it was the detection of entomophage presence in nature.

To monitor the pest the pheromone traps were set in the garden. Direct recording of density apple moth eggs and the percentage of infected *Trichogramma* was carried on there. The density of eggs according to generations and years ranged from 8.0 - 90.0 eggs per 100 fruits and leaves. The total area of the orchids where entomophage issues were conducted was 33.15 hectares. After six issues of *T. embryophagum* the biological efficacy ranged from 68.2 to 90% in the 1st variant and 64.0 - 84.7% in the 2nd version. Control check reflected that the number of pest-parasitized eggs ranged from 2.0 to 21.5% and fruit-damage ranged from 45.0-93.0%.

Table 2. Biological efficiency of *T. embryophagum* on the apple tree crop.

Year, area	Variants	Biological effectiveness, %	Apple fruit damage, %
2002 Mereni	I	75.5±2.5	4.7±0.3
	II	64.0±1.9	6.8±0.2
	III	3.5 ±0.2	45.0±1.1
2003 Chișinău	I	90.0±2.8	11.0±1.6
	II	84.7±2.5	16.0±1.80
	III	4.0±0.3	81.7±2.9
2005 Chișinău	I	86.0±3.0	9.0±0.8
	II	80.0±2.8	15.0±0.9
	III	5.0±0.1	80.0±2.9
2006 Chișinău	I	80.0±3.0	11.2±0.8
	II	73.3±2.1	18.7±1.0
	III	5.0±2.0	70.0±2.1
2007 Chișinău	I	83.3±3.5	25.5±1.9
	II	78.1±2.5	27.8±1.0
	III	5.7±0.7	90.1±3.5
2007 Puhoi	I	83.3±2.7	3.5±0.3
	II	78.1±2.1	4.4±0.4
	III	2.0±0.1	34.0±1.2
2008 Chișinău	I	68.2±2.0	42.3±1.7
	III	8.8 ±1.0	92.5±3.6
2009 Chișinău	I	78.5±2.8	38.0±1.5
	III	21.5±0.9	93.0±3.6

Legend: Variant I - *T. embryophagum* bred on irradiated grain moth eggs; Variant II - *T. embryophagum* bred on non-irradiated grain moth eggs; Variant III - control, without the release of *Trichogramma*.

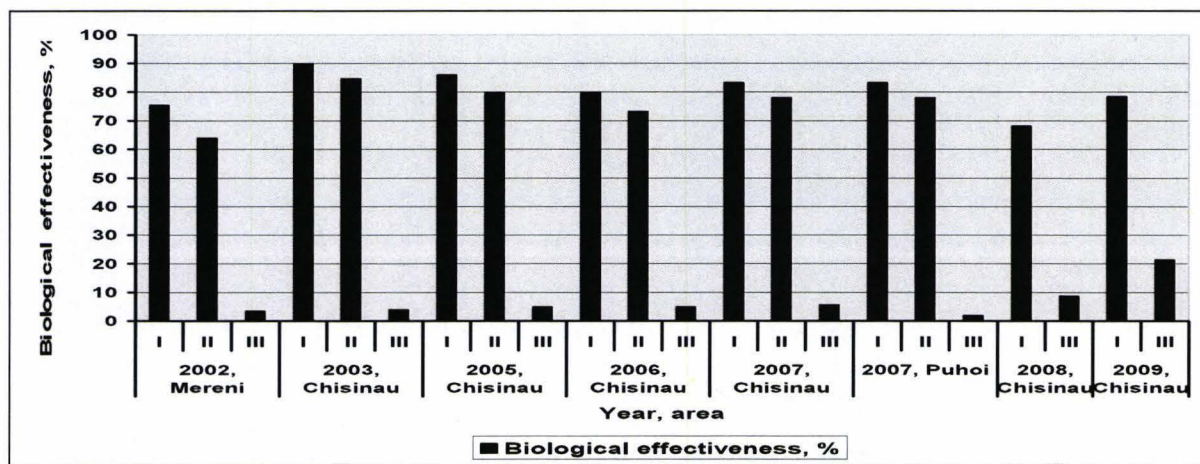


Figure 1. Biological effectiveness of *T. embryophagum* biological control over the *L. pomonella* in orchards.



Figure 2. Packed *T. embryophagum* launched (original).



Figure 3. *L. pomonella* eggs parasitized by *T. embryophagum* (original).

Therefore flooding issues of the entomophagous *T. embryophagum* to reduce the number of codling moth, the major pest of apple, can become one of the main methods of integrated garden protection, allowing the reduction of the number of insecticide treatments to a minimum.

The entomophagous *Trichogramma* is one of the most important biological agents which may be utilized against a complex of pests both in biological control and in the system of integrated plant protection. The utilization of the eggs of *S. cerealella*, irradiated preliminary by gamma rays essentially increases *Trichogramma* biological indices and the efficiency in the field. The biological efficacy of *Trichogramma* and the degree of damage in the first version (on irradiated eggs of grain moth) when compared with the second option (on the non-irradiated eggs) are significantly different.

The priorities of entomophagous in plant protection are: to reduce the financial expenses for protection, to save useful organisms in nature, to increase the biological effectiveness in the field, to increase the volume of agricultural production to provide food quality, to reduce the number of chemical treatments to a minimum of integrated system that prevents contamination of the environment, low price, safe, good-quality, easy usage, ecological cleanliness.

The entomophagous *Trichogramma* is one of the most important biological agents which may be used against a complex of pests both in biological control and in the Integrated Pests Management. The biological indicators of *T. evanescens* rearing on the eggs of different hosts irradiated with gamma rays increased by 2-2.5 times and its biological effectiveness in the field is more than 10-15%. Figure 3 summarizes *L. pomonella* eggs parasitized by entomophagous *T. embryophagum* in apple orchard.

CONCLUSIONS

Grain moth eggs (*Sitotroga cerealella*) irradiated with gamma radiation provide higher biological indicators of *Trichogramma* than its cultivation on non-irradiated eggs.

Consequential issues (5-6) - *T. embryophagum* in the garden provide a higher level of eggs parasitism thus significantly reducing the percentage of damaged fruit.

After six issues of *T. embryophagum* the biological efficacy ranged from 68.2 to 90% in the 1st variant and 64.0 - 84.7% in the 2nd variant. Control check reflected that the number of pest-parasitized eggs ranged from 2.0 to 21.5% and fruit-damage ranged from 45.0 to 93.0%.

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THE HYBRIDS CARP-CRUCIAN AS PROSPECTIVE OBJECT OF FRESHWATER AQUACULTURE

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Abstract. The comparative fish-breeding assessment under underyearlings and three-years-old fish of carp-crucian and of different carp breeds farmed in ponds and the morphofunctional characteristic of the gonads of carp-crucian hybrid females. For hybrid individuals are installed differences of left gonad oocytes from the right gonad on morphological features and on dimensional composition. The diameter of oocytes in the phase "E" of the left gonad authentically is smaller. $P > 0.95$, from sex cells of the right gonad.

Keywords: hybrids, carp-crucian, females, oocyte, gonad-somatic index.

Rezumat. Hibrizii crap-caras obiect de perspectivă al acvaculturii de apă dulce. Este prezentată evaluarea comparativă piscicolă a puietului de crap-caras de o vară și a peștilor de trei veri cu diferite rase de crap, crescute în eleștee și caracteristica morfofuncțională a gonadelor femelelor de crap-caras. La indivizii hibrizi au fost stabilite diferențe ale oocitelor gonadei din stânga comparativ cu gonada din dreapta conform indicilor morfologiei și compoziției dimensionale. Diametrul oocitelor în faza „E” a gonadei din stânga este veridic mai mic. $P > 0.95$, decât cel al gonadei din dreapta.

Cuvinte cheie: hibrizi, crap-caras, femele, oocite, indexul gonado-somatic.

INTRODUCTION

In fisheries a great interest is represented by distant hybridization, which can be used to obtain industrial hybrids, as well as for selection of hybridogeneous rocks. Also, is very important in theory and practice, is the study of fertile hybrids of carp with crucian.

Area of distribution of *Carassius gibelio* covers a large territory - from Japan to Western Europe. In the eastern part of the area circulates mainly the bisexual populations and as we move to the West the percentage of males in the populations *C. gibelio* gradually is decreasing as a result of this species is often represented as same-sex gynogenetic form (GOLOVINSKAIA et al., 1965; KIRPICHNIKOV, 1987).

A study of same-sex female's gynogenetic *C. gibelio* demonstrates that they are triploid (TCHERFAS & SHART, 1970). Bisexual forms are similar to the karyotype of carp. This species are called tetraploid ($2n \sim 100$).

Many interspecific hybrids can combine the valuable qualities of parent species. According to some authors, for the vast majority of distant hybrids are characteristic their sterility, which is caused by mismatch of chromosome sets (TCHERFAS & TSOY, 1984).

In such cases, in fish industry are used only the first hybrid generation, the so-called industrial hybrids.

The possibility of selection of hybrids appears at sufficient fecundity of females, which can be used to obtain of backcross hybrids by crossing them with males of the parental species. Increasing the share of heredity in the absorptive crossing one of the parental components can result to increase fecundity of backcross hybrids.

Interspecific hybrids of carp with *C. gibelio* are attractive as objects of fish farming through the possession of a high growth rate, resistance to deficiency of oxygen and some diseases, more complete use of natural forage of ponds – detritus (YARZHOMBEK, 1981).

The aim of our study was to comparative fish-breeding assessment under underyearlings and three-years-old fish of carp-crucian and of different carp breeds farmed in ponds and study the morphofunctional state of the gonads of female's hybrid carp-crucian.

MATERIALS AND METODS

The hybrids of carp-crucian (CC) of the first generation were obtained by artificial reproduction from cross of females of carp breed Teleneshtskiy Frame with genotype ssnn and males of *C. gibelio* (Cg). Growing under-yearlings of hybrids carp-crucian was carried out in separate nursery ponds, and two-and three-years-old fishes - in feeding ponds in policulture with carp and herbivorous fishes under continuous cultivation method.

As a control, were used pure-bred descendants of three breeds of carp: Teleneshtskiy Scaly (Ts) Teleneshtskiy Frame (Tf) and Kuboltskiy Scaly (Ks).

The test and control groups of fish were obtained at the same time. Simultaneously was conducted rearing of larvae and stocking of the similar nursery ponds at stocking density 30 thousand hectares.

During the time of cultivation of under-yearlings were fed 2 times a day of cereal forage mixture, the daily ratio was counted by dates of control catches.

Samples of sexual cells were fixed in Bouin liquid and filled in paraffin-wax with further histological processing by the standard technique. All the studied of females are subjected to the general biological analysis with calculation of gonad-somatic index (GSI). The stages of gonad maturity were determined according to the recommendation of SAKUN &

BUTSKAIA (1963), and development degree of oocytes – after classification of KAZANSKII (1949) with additions proposed by MAKEEVA & EMELIANOVA (1989). Cuts of gonads were colored after Mallory’s method (ROSKIN & LIVENSON, 1957). Diameter of oocytes was determined using the ocular-micrometer. All data were processed statistically (LAKIN, 1980). Microphotos were made with the help of the microscope with videocamera “Lomo, Mikmed-2”.

RESULTS AND DISCUSSIONS

Comparative growing of pure-bred under-yearlings of carp three breeds and of carp-crucian the first generation showed, that hybrids during the cultivation season had good growth rates, but somewhat inferior to the carp. All hybrid individuals had completely scaly covering. At the late growing season the temp growth of carp-crucian hybrids and Kuboltskiy Scaly carp was higher with respect to Teleneshtskiy Scaly and Teleneshtskiy Frame carps (Fig. 1).

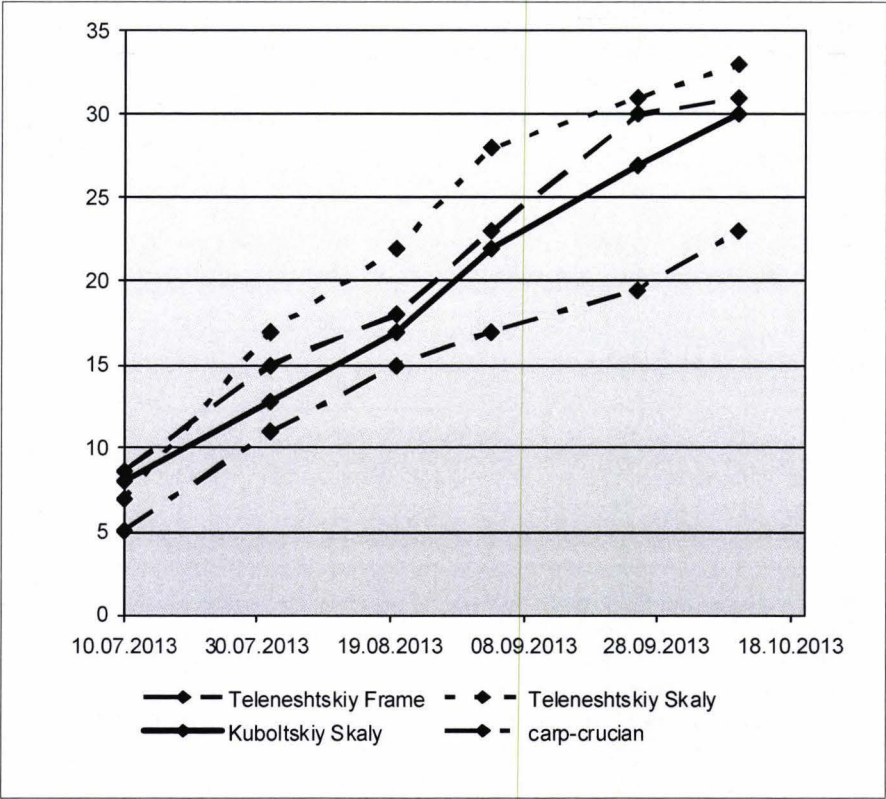


Figure 1. The growth rate under-yearlings of carp-crucian hybrids and of carp three breeds.

Despite the fact that hybrid underyearlings are somewhat inferior to the average weight of carp breeds, but at the expense to their substantial survival (82%), they had a high fish productivity - 570 kg / ha. On forage costs the carp-crucian hybrids are inferior by carp breeds (Table 1).

Table 1. Results of growing under-yearlings of carp and of carp-crucian hybrids.

Fish breeds, hybrid	Were caught			Fish productivity, kg/hectare	Food costs, kg/hectare
	Thousand units	Average body weight, g	Yield, %		
Ts	22.5	33.0	75.0	740.0	2.43
Tf	22.0	31.2	73.0	680.0	2.64
Ks	23.2	30.3	76.0	700.0	2.57
CC	24.6	23.0	82.0	570.0	3.15

Legend: Teleneshtskiy Scaly - (Ts), Teleneshtskiy Frame - (Tf), Kuboltskiy Scaly - (Ks), Carp-crucian - (CC).

Increased viability of carp-crucian hybrids compared to the carp manifested during winter: the yield was 93.4%, with less weight loss body – 5.8%. In order to estimate the consumption of natural food base of ponds, growing of carp-crucian hybrids was held with carps, *C. gibelio* and the *Silver carp* in fattening ponds with continuous process for two growing season without feeding. The total stocking density yearlings equaled 2400 units/hectare (Table 2).

Table 2. Results of growing in continuous process of the carp fishes.

Fish breeds, hibrid, species	Were stocked of yearlings		Average body weight of three-year-olds, g	Yield, %	Gain of weight, g	Fish productivity, kg/hectare
	Units/hectare	Average body weight, g				
Ts, Tf, Ks	500	27.0	$\frac{758}{510-940}$	61.0	731.0	230.0
CC	400	22.0	$\frac{567}{350-830}$	62.5	545.0	140.0
Cg	300	25.0	$\frac{318}{170-480}$	65.0	293.0	60.0
SC	1200	36.0	$\frac{1170}{1050-1390}$	58.3	1134	810.0

Legend: Teleneshitskiy Scalpy - (Ts), Teleneshitskiy Frame - (Tf), Kuboltskiy Scalpy - (Ks), Carp-crucian - (CC), *C. gibelio* - (Cg), *Silver carp* - (SC).

The results of co-cultivation in polyculture of three-years-old carp fishes showed that the level of the trophic utilization of ponds was high. Natural productivity of the ponds was 430 kg / hectare, excluding herbivorous fish. The best gain of body weight that is characterized by the three-years old carp - 731 g, intermediate held carp-crucian hybrids - 545 g, *C. gibelio* increase weight by 293 g.

The share of productivity of carp-crucian hybrids was 32.6% or 140 kg / ha.

Thus, you can use the carp-crucian hybrids when organize the pasture of fish farming for the effective use of natural forage, excluding the costs for concentrated feed, which will increase the productivity of water bodies and decrease costs of farmed commercial fish.

Very often a wide hybrids in the first generation of fish is detected phenomenon of same-sex male sterility.

According to some authors (TCHERFAS, 1971) when crossed females unisexual forms of *C. gibelio* with a males bisexual forms, or with males closely related species, obtain the posterities inherit only maternal characteristics and preserve the natural fecundity.

We investigated the females of carp-crucian hybrids and *C. gibelio* for the duration of works on reproduction

The females *C. gibelio* in the period were of the spawning process and in the pre-spawning state. And right and left gonads in both females hardly differed by their weight (Table 3).

Table 3. Biological characteristic of females *C. gibelio* and hybrid of carp-crucian.

Fish species, hybrid	Body length cm	Body weight, g	Gonad weight, g		Weight of all gonads, g
			Right	Left	
<i>Carassius gibelio</i> ♀	20.0	242	23.0	18.0	41.0
<i>Carassius gibelio</i> ♂	26.0	566	22.0	20.0	42.0
<i>Carp-crucian</i> ♀	32.0	820	11.0	143.0	154

In the gonads of females spawning finishes *C. gibelio* are present follicular emptied membranes, yolk oocytes not spawned in a state of resorption and sex cells of period trophy-plasmatic growth of the new generation, which is also touched upon process of degeneration. In the oocytes in the intensive phase of vitellogenesis, there is a process of destruction of cortical vacuoles, swelling of your own membrane with the release of the content of the oocyte under follicular epithelium (Fig. 2).

Gonad-somatic index (GSI) of females spawning finished *C. gibelio* is 8.71%. As at the given individual is a total resorption of oocytes of the next generation, her spawning in the current season is finished.

Females with a smaller body or weight are in the process of spawning, and their gonads correspond V stages maturity. Shedding of eggs occurs in the small portions, as at the time of catch of females, in the gonads contain a small amount of empty follicles, oocytes are in maturing phases (phase F), and finished vitellogenesis (phase E), the size of which is an average of $886 \pm 9,0 \mu\text{m}$, as well as younger cells in all phases of vacuolization. The Gonad-somatic index rather high also makes 23; 29%.

Its important to mention be noted that some of the oocytes in the phase of "E" have been subjected to degenerative changes (Fig. 3).



Figure 2. Resorption of the oocyte *C. gibelio*. Plot of follicular epithelium in the process phagocytosis of yolks granules (original).



Figure 3. Degenerative changes of the oocyte *C. gibelio* in a phase "E".
Destruction of cortical vacuoles (original).

By references of GOLOVINSKAIA et al., (1965) sexual maturation of *C. gibelio* in ponds of Moldova there comes at the age of 1+ and 2+ with a length of 12.6; 17.0 cm and average weight of 58; 160g, respectively. Investigated by us individuals are re-maturing fishes. According to the GOLOVINSKAIA et al., (1965) at the beginning of June, at such females happens shedding the third portion of eggs.

In the same time hybrid females of carp-crucian, which also participate in spawning are investigated. The process of spawning at females of carp-crucian at the time of the study is not finished. The presence in an ovary the emptied follicular membranes indicates the ovulation and spawning matured oocytes (Fig. 4).

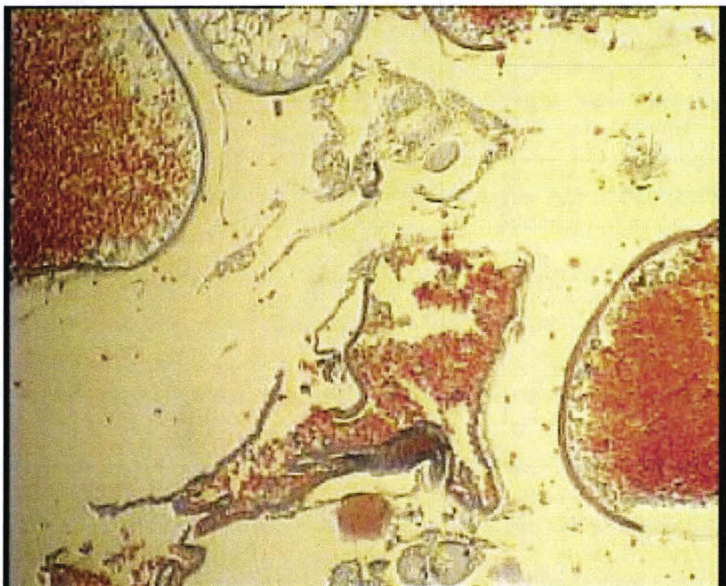


Figure 4. The fragment of ovarie of hybrid female in the process spawning. One can see the empty follicular membranes and not ovulated oocyte in the process resorption (original).

The morphometric analysis the gonads of hybrid females showed the different dimensions of gonads. The histological analysis revealed differences of oocytes left gonad from right by morphological characters and dimensional structure (Table 4).

Table 4. Reproductive ability of *C. gibelio* and hybrid of carp-crucian.

Fish species, hybrid	Body weight, g	Gonad-somatic index (GSI),%			Oocyte dimension,μm	
		Right gonad	Left gonad	all gonads	Right gonad	Left gonad
<i>Carassius gibelio</i> ♀	242	13.06	10.23	23.29	886±9.0	
Carp-crucian ♀	820	1.86	24.20	26.06	858±10.3	818±7.5

Analysis of oocytes right gonad revealed similarities with oocytes of *C. gibelio* on dimensional composition. Their diameters are in the phase of "E" authentically are not different (Table 4). At the same time is traced their similarities by morphological characters (Fig. 5).

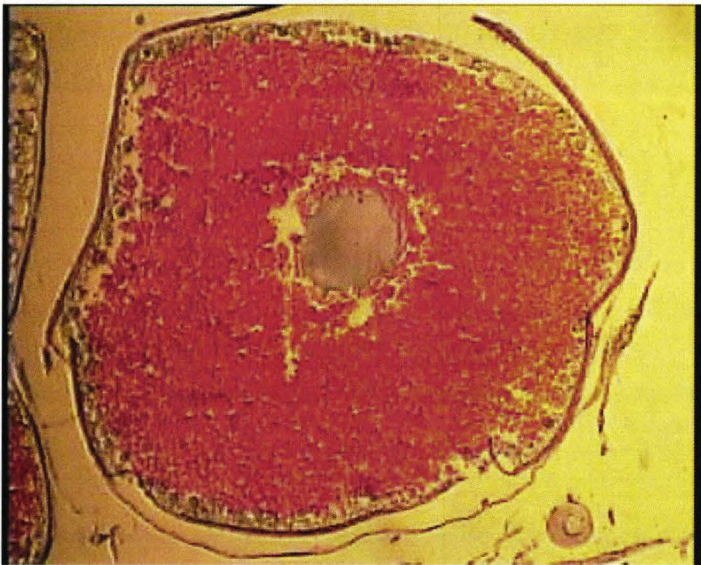


Figure 5. The oocyte in a phase "E" at hybrid female of carp-crucian in right gonad (original).

And when we compare the size of oocytes left gonad of hybrid female with oocytes of *C. gibelio* noted their reliable difference $P > 0.999$. The diameter and morphology of oocytes left gonad of hybrid female differed from oocytes the right: oocytes of left gonad next generation were in a phase of intensive vitellogenesis (D6) and has a smaller diameter. We believe that the structure of the sex cells in the left gonad similar to that of carp oocytes (Fig. 6).

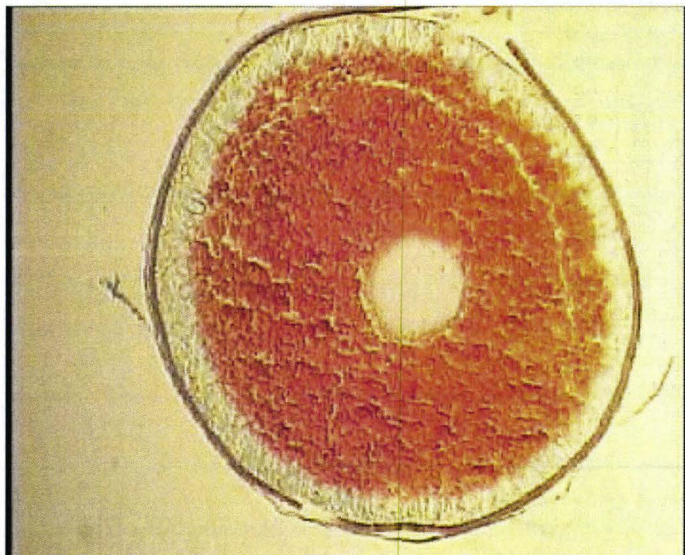


Figure 6. The oocyte of hybrid female carp-crucian in left gonad in a phase of intensive vitellogenesis "D₆" (original).

It should be noted that all oocytes in left gonad, aren't affected by how - either destructive changes, unlike sexual cells - in the right. In the latter, all the cells of tropho-plazmatic growth of the next generation affected the process of resorption.

CONCLUSIONS

After the research that was made, we can mention the main ideas. So we had revealed that:

- the under-yearlings carp-crucian hybrids have substantial survival and a high productivity;
- the carp-crucian hybrids can be effectively used for growing in continuous process of commercial fishing at the expense to natural forage, but only up to the age of three – beginning of their sexual maturity;
- there is a difference between morphofunctional state of the gonads of females *C. gibelio* and carp-crucian hybrids.

Also we had discovered the different-dimension of gonads and different-quality of oocytes in the right and left gonads of hybrid females.

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INFLUENCE OF SELECTION FOR INCREASING RESISTANCE TO INFECTIOUS DISEASES ON MORPHOLOGICAL AND REPRODUCTIVE CHARACTERISTICS OF MOLDAVIAN CARP BREEDS

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Abstract. It was revealed that the selection for increasing resistance to infectious diseases has led to some changes in morphotype of carp breeds and clarified necessary conditions when choosing of selection-valuable genotypes in selection. Is presented the comparative rating of reproductive characteristics of Moldavian breeds carp (*Cyprinus carpio*). Was defined the efficiency of selection: to the fifth generation were increased main indicators of reproduction in comparison to the standards of these breeds and norms.

Keywords: morphotype, breed, fecundity, generation, reproduction.

Rezumat. Influența selecției pentru mărirea rezistenței la bolile infecțioase asupra caracteristicilor morfologice și reproductive ale raselor de crap din Moldova. A fost determinat faptul, că selecția la mărirea rezistenței la bolile infecțioase a dus la unele modificări în morfotipul raselor de crap și au fost identificate condițiile necesare pentru alegerea genotipurilor selectiv-valorose în selecție. Este prezentată evaluarea comparativă a caracteristicilor de reproducere a raselor de crap din Moldova (*Cyprinus carpio*). A fost determinată eficacitatea de selecție: în a cincea generație au fost ameliorați indicii de reproducere în raport cu standardele acestor rase și normative.

Cuvinte cheie: morfotip, rasă, prolificitate, generație, reproducție.

INTRODUCTION

A target of selection the important point is to evaluate the prospects of selection in the source material and the choice of selection-valuable genotypes.

Important role is represented by the so-called "signal genes", as which can be used by the genes of the morphological and biochemical characteristics (KIRPICHNIKOV, 1987; MERLA, 1959; IZIUMOV & KASYANOV, 1981).

Moldova is realised a program of selective breeding of carps selection the role is to increase resistance to infectious diseases with the use of mass selections, also combined selections and family selection (KURKUBET, 1994). Consequently were created and tested two carp breeds: Teleneshtskiy Scaly and Teleneshtskiy Frame carp with the increased resistance to infectious diseases (KURKUBET & DOMANCHUK, 2005).

Sometimes, in the course of selection to increase the resistance of carp to rubella, are observed a negative correlation between resistance and growth of the rate of fish (KIRPICHNIKOV et al., 1987).

That's why the task of breeders is to strengthen of the subsequent generations also the results of selection and maintaining of the high productivity characteristics of breeds at the level of their standards.

The aim of this work is to determine the influence of selection for increase the resistance to infectious diseases on the morphological and reproductive characteristics of the Moldavian carps, based on previously identified and genetically determined relations between morphotype and signs of viability (KURKUBET, 1994; 2010).

MATERIALS AND METHODS

Analyses was performed on the base of data collected from 1981-2011 in the result of selection years of Carp Teleneshtskiy Scaly (Ts) and Carp Teleneshtskiy Frame (Tf) in hatcheries of Verezhny of Teleneshty Branch of the E.S. "Aquaculture-Moldova".

To determine the degree of the influence of selection on morphotype created breeds and on their reproductive characteristics was performed on breeding material 1 - 5 generations of selection (F_1 - F_5) of the aforementioned breeds.

The reproductive indicators of females of new generations of the approved Moldavian carp breeds: of Carp Teleneshtskiy Scaly (Ts5) and Carp Teleneshtskiy Frame fifth generation (Tf5) were evaluated in comparison with the previous generations (F_1 - F_4); data standards breeds (F_3) and existent norms.

RESULTS AND DISCUSSIONS

In the selection of Moldavian carp breeds for increase the resistance to infectious diseases by the most rigid selection (with a total tension for Carp Teleneshtskiy Frame - 9.2%, for Carp Teleneshtskiy Scaly - 20.2%) had a mass selection in the initial bloodstock (F_0).

In carrying out of family selection (F_1 , F_2) in the result of testing of carp at different stages of viability, of resistance to aeromonoz in bioassay and of susceptibility to diseases under fields conditions (cultivation in ponds on the

provocative background) were distributed to families in these contrasting characteristics of the group. We have identified differences between morphotypes among families with high and low viability (KURKUBET. 1994, 2010).

In the most differentiated groups of families (F2), the stepwise regression is identified several traits that are associated with the overall viability in the ponds and sustainability in the bioassay: body length (l), head length (C), head height (hC), the maximum body height (H). These signs are commonly used by breeders in the form of indices: the index body height l / H and the index of ratio of the head C / hC. In the least differentiated groups - (F3) are released by only one informative sign hC.

Based on this, on the investigated material were counted values of these indices for families with high viability: l / h - 2.5 and C / hC - 1.5 and with reduced: l / h - 2.0 and C / hC - 1.0. These indices can be used in the selection of the target standard and in the elaboration of selection methods.

According to our data there is a negative correlation between body height and overall viability, including the resistance to disease.

In the process of the formation of new broodstocks of Carp Teleneshtskiy Scaly (Ts) and Carp Teleneshtskiy Frame (Tf), conducted individual, mass and combined selections for 4 generations of selection resulted to increase the resistance to infectious diseases, and as a consequence, to change the main exterior indices. The average value of the index of body height (l/H) of Carps scaly increased from 2.09 to 2.30 - in the females and 2.16 to 2.28 - in males; of Carps Frame - from 2.11 to 2.34 and from 2.14 to 2.40, respectively (Table 1, Fig. 1).

Indicators of ratio of head length to its height also increased: in females scaly up to 1.38, in males - up to 1.33; in females frame - 1.44 and 1.44, respectively (Fig. 2).

Table 1. Change the morphometric parameters of breeds carp in result of selection for increasing resistance to infectious diseases.

Exterior indices	Females				Males			
	Ts(F ₁)	Tf(F ₁)	Ts(F ₃)	Tf(F ₃)	Ts(F ₁)	Tf(F ₁)	Ts(F ₃)	Tf(F ₃)
Body weight, P.g	5855	5350	2400-4800	2900-4500	5600	4580	2400-4100	3600-4300
Body length, l.cm	49.4	48.2	43.0-50.0	40.0-44.0	49.8	46.7	41.0-56.0	40.0-42.0
Indices:								
Head length/ body length, %C	25.5	25.7	25.0-27.9	26.0-28.0	25.4	25.2	24.0-29.3	26.2-28.0
Body height, l _h	2.09	2.11	2.09-2.47	2.05-2.44	2.16	2.14	2.21-2.31	2.20-2.56
Body thickness, l _{br}	21.4	21.2	19.1-20.9	18.1-21.4	21.1	20.7	18.5-21.9	17.5-19.5
Body girth, l _o	0.85	0.86	1.10-1.18	1.04-1.19	0.91	0.88	1.07-1.14	0.97-1.09
Relations:								
Length of caudal peduncle /height of his. pl/h	1.13	1.12	1.06-1.14	1.00-1.14	1.13	1.10	1.00-1.05	1.00-1.16
Head length/height of its, C /hC	1.21	1.24	1.20-1.38	1.30-1.44	1.23	1.22	1.20-1.33	1.27-1.44

More intensive selection of carps frame, related to their greater susceptibility to infectious diseases, has led to more significant changes in their morphology, as compared with scaly forms.

Selection of carps for increasing resistance to infectious diseases was accompanied by a double effect: on one hand, the indices had been identified that determine the prospects of selection for increased stability, by the other hand, in the result of selections of the best families on the viability and sustainability in the bioassay, we approach their exterior to the counted values.

When we chose a target standard the most important is the determination of the optimal level of resistance, which would give the production effect and was not accompanied by negatively correlated effects - declined in productive characteristics (decrease in growth rate, fecundity or meatiness). That's why, in parallel, performed the selection by body weight at a moderate tension - 64.6% and intensity - 1.95 and maintaining exterior.

Fecundity is one measure of productivity and depends on environmental conditions, and is under the control of hereditary factors (MASLOVA, 2005).

Comparative evaluation of reproductive characteristics of females of Carp Teleneshtskiy Scaly and of Carp Teleneshtskiy Frame of 1-5 generation is shown by the result of the selection process in 4-5 generations observed.

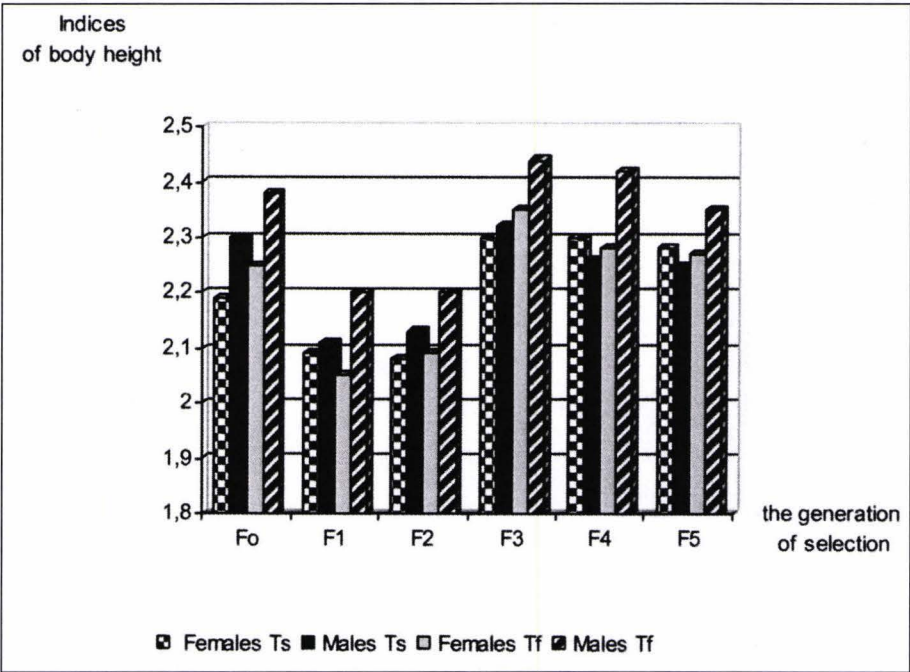


Figure 1. Change the indices of body height of Teleneshtskiy breeds carp in result of selection for increasing resistance to infectious diseases.

Increased in the percentage of fertilization to 93.3% in carps scaly and to 92.6% - in carps frame; percent of development of eggs - up to 82 and 80% and yield the three-day larvae - up to 490 and 480 thousand units, respectively. Yield of larvae from eggs increased in 1.9-1.7 times, respectively on breeds (Table 2).

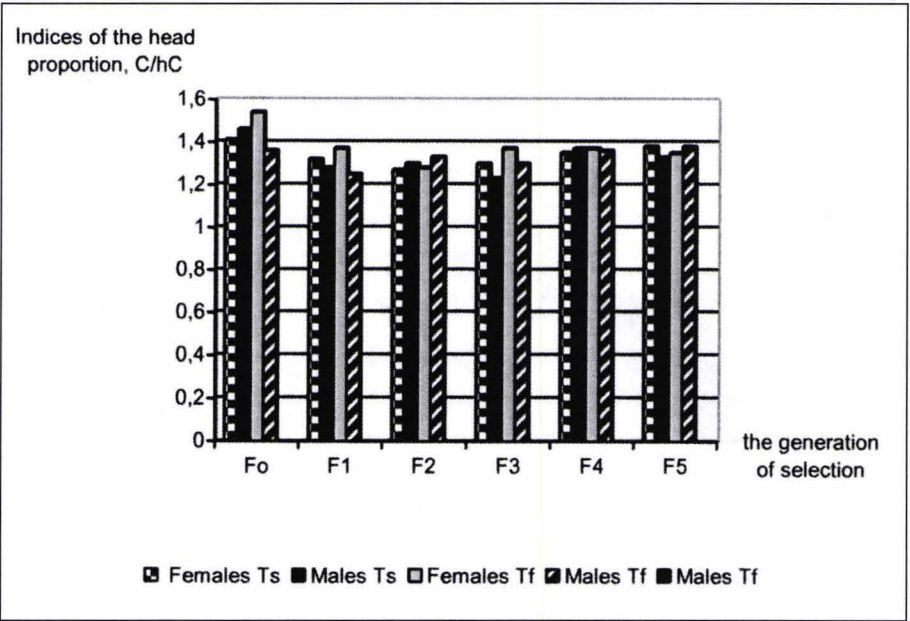


Figure 2. Change the indices of the head proportion of Teleneshtskiy breeds carp in result of selection for increasing resistance to infectious diseases.

Analysis of reproductive characteristics of Carps Teleneshtskiy of third generation of selection at the time approbation rocks (breed standard) is showed by working fecundity, yield of the three-day larvae and productivity of females on I-st year of both breeds are superior exceed standards of values, with some advantage Teleneshtskiy Scaly carp (Fig. 3).

Table 2. Change the reproductive parameters of breeds of carp in result of selection for increasing resistance to infectious diseases.

Generations, breeds	Average weight of females, g	Fertilization, %	Development of eggs, %	Yield the three-day larvae, thousand units	Yield of larvae from eggs, %
1 generation					
Carp Teleneshtskiy Scaly Carp	6500	91	76.3	330	30.2
Teleneshtskiy Frame	5800	82	71.3	320	33.2
2 generation					
Carp Teleneshtskiy Scaly Carp	5600	88	73.9	350	49.8
Teleneshtskiy Frame	5700	86	73	320	40.2
3 generation					
Carp Teleneshtskiy Scaly Carp	6000	84.2	73	429	51
Teleneshtskiy Frame	5700	85.8	73.4	399	50.4
4 generation					
Carp Teleneshtskiy Scaly Carp	5900	93.3	74	490	56.2
Teleneshtskiy Frame	5700	92.6	73.7	480	55.8
5 generation					
Carp Teleneshtskiy Scaly Carp	5280	93	82	410	57
Teleneshtskiy Frame	5360	90	80	430	56.6

Percent of yield larvae from eggs and the survival underyearlings from eggs of carps Teleneshtskiy (F3), relating to carps of "fattening-type", selection which was carried out in the direction to conservation of body height, meatiness, increasing the rate of growth, fecundity and resistance to infectious diseases, not to exceed normative (DOMANCHUK & KURKUBET, 2002; KURKUBET & DOMANCHUK, 2005).

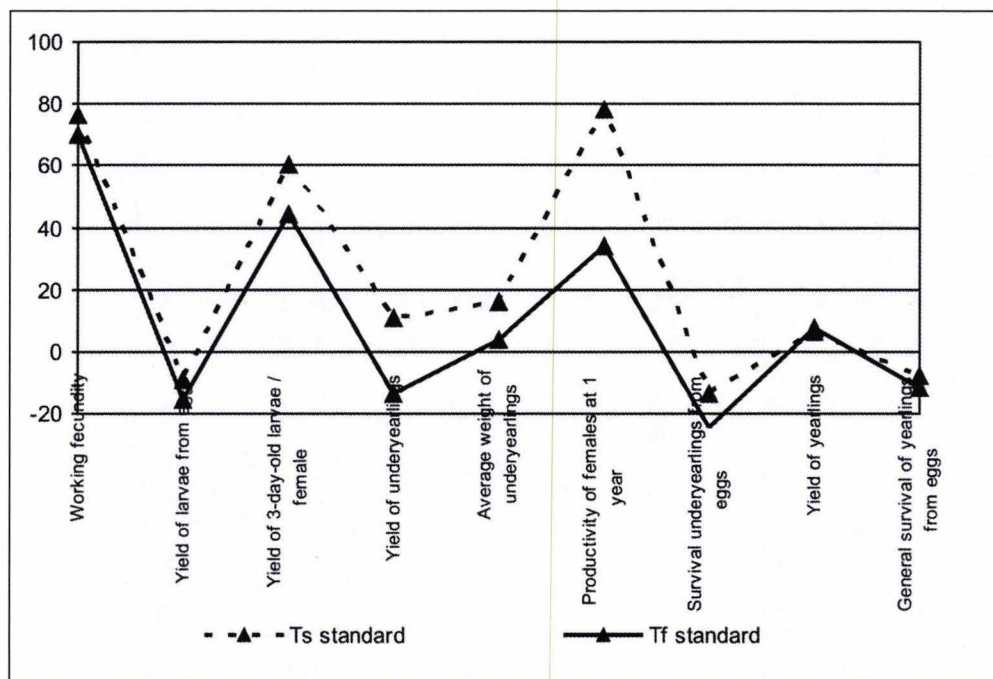


Figure 3. Comparative evaluation standards (F3) of the two breeds on indicators productivity with the norms.

Females and males of a new generation of two breeds of the 5th generation of selection were characterized by typical data breeds exterior indices: index body height of Carp Teleneshtskiy Scaly and of Carp Teleneshtskiy Frame was within 2.28-2.38 and showed good adaptability to the artificial methods of reproduction, to respond positively to hormonal stimulation. Number of spawned ranged from 86.7 to 90.0%. The working fecundity of females reaches 910-930 thousand of eggs and the relative fecundity meet the standards of rocks: 150-152 thousand units per kilogram of body weight. Realized fecundity, is expressed as a yield of three-day larvae was significant: 495-500 thousand per female. Survival of larvae from eggs was 53-55% (Table 3).

Table 3. Reproductive characteristics of females of the two breeds carp of subgeneration new fifth generation at the age six years.

Breed	Parameters	norms	Ts 5	Tf 5
Average female weight, g		5500	6000	6200
Number of spawned females, %		85,0	86.7	90,0
Eggs per female, g		400-600	1180	1200
Maturity rate, %		14,0	19.7	19,4
Working fecundity, thousand		300-500	910	930
Relative fecundity, thousand / kg		110-120	152	150
% of fertilization		80,0	91,0	88,0
Development of egg, %		70,0	74,0	77,0
Yield of 3-day-old larvae/female, thousand		150-250	500	495
Yield of larvae from eggs, %		50,0	55,0	53,0

In general, the usage in the reproduction of the females of new generations (F5) had higher reproductive performance and a number of indicators significantly exceeded standards.

Relative to the standard of breads in the next two generations (F4; F5) were increased in virtually all the analyzed productive factors: most benefits were manifested in productivity of the females at the first year: Carp Teleneshtskiy Scaly (F5) - 91.6%, Carp Teleneshtskiy Frame (F5) - 138.8%, both by increasing the body weight and survival underyearlings - 91.6% (Fig. 4).

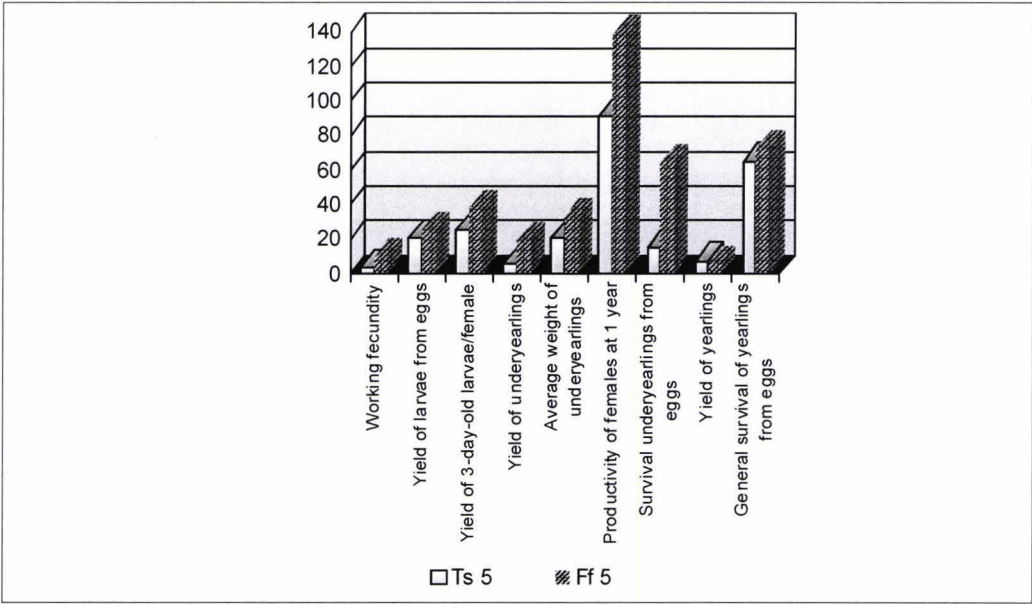


Figure 4. The advantage of the new generations of the two breeds on indicators productivity over their standards (%).

A clear advantage of the researched breeds of new generation in comparison to their standard is also observed on survival of yearling from eggs: Carp Teleneshtskiy Scaly (F5) - 65%; Carp Teleneshtskiy Frame (F5) - 72.6%; on survival underyearlings from eggs: 15.4%; 64.8% and on yield three days of larvae per female: 25.0%, 37.5%, respectively.

It should be noted that after approbation of breeds (2000) over the next two generations we carried only correcting selection on body weight, exterior and degree of sexual characteristics with the moderate intensity.

As a result of selection in several generations with greater intensity (to approbation) were increased three reproductive parameters that were included in the target program: realized fecundity (working fecundity + yield of three-day larvae) and productivity of females on underyearlings.

The next two generations of selection is indicated the excess of all breeds over their standards for all investigated indicators of productivity, with precedence on productivity females at the first year, general survival of yearling from eggs and survival underyearlings.

CONCLUSIONS

The effectiveness of selection of carps Teleneshtskiy for increased resistance to infectious diseases throughout 3-4 generations has led to an increased resistance and, as a consequence, to changes in the main exterior indicators (change of morphotype); much tension selection among carps frame contributed to more significant change in their morphological features compared with scaly carps.

The target selection of Moldovan carp breeds for increasing resistance to disease, directed at maintaining the productivity of characteristics led to a significant improvement in the last two generations of selection the main indicators of reproduction is compared to the standards of these rocks and norms.

When you select a target standard for selection for resistance to disease, they can not be conducted by one-way match only on the counted indices, should be guided by them, while maintaining a balance between the indicators of viability and maintaining high productivity characteristics.

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NEW DATA REGARDING THE AQUATIC AVIFAUNA ON THE TERRITORY OF CRAIOVA CITY, DOLJ COUNTY

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Abstract. The present paper brings to light new data about the aquatic birds from Craiova city after 2002 as a result of the bird monitoring action that took place here. The bird monitoring activity is part of a larger project concerning the biodiversity of the city fauna. The aquatic avifauna is well represented here because of the city geographical location on the left side of the Jiu river and because of the great variety of the habitats found here. The number of observed aquatic species rises up to 46, systematically classified into 9 orders and 16 families. In this paper there are also mentioned the species that live and breed in the reed-beds (reed warblers, reed buntings) or near waters (wagtails, largely spread species from the Passeriformes order). The number of species and of individuals has fluctuated according to the climatic factors and the availability of food, both seasonally and annually. In the city were found 16 species with varying degrees of threat (vulnerable, endangered, declining) both at European and national level.

Keywords: aquatic birds, anthropogenic habitat, protection of birds.

Rezumat. Noi date referitoare la avifauna acvatică de pe teritoriul municipiului Craiova (județul Dolj). Lucrarea aduce noi date despre păsările acvatice din municipiul Craiova, după anul 2002, în urma monitorizării speciilor de păsări din oraș. Monitorizarea se înscrie într-un proiect mai amplu referitor la biodiversitatea faunei orașului. Avifauna acvatică este bine reprezentată, fiind favorizată de localizarea geografică a orașului pe malul stâng al râului Jiu și de varietatea habitatelor. Numărul speciilor acvatice observate se ridică la 46, distribuite în 9 ordine și 16 familii. În lucrare sunt menționate și speciile ce trăiesc și cuibăresc în stufăriș (lăcari, presuri de stuf) sau pe lângă ape (codobaturi, specii eurtope ce aparțin ordinului Passeriformes). Numărul speciilor și al indivizilor a fluctuat în funcție de factorii climatici și de hrană, atât sezonier, cât și anual. Pe teritoriul orașului au fost evidențiate 16 specii cu diferite grade de amenințare (vulnerabile, periclitate, în declin) atât pe plan european cât și național.

Cuvinte cheie: păsări acvatice, habitat antropic, ocrotire a păsărilor.

INTRODUCTION

The cities are not complete without the presence of birds, which are important parts of the biocenosis, of the biological diversity and which contribute to the ecological balance in nature.

The refuge and adaptation of birds to town lead to a more profound study of synanthropic birds by researchers. Thus, during the last decades, there was a blast of information regarding the bird urbanization problem in Romania, the relations established between bird and human communities and also regarding the problems related to their protection (BÉRES, 1980; CROITORU, 2009; GACHE, 2004; GIURGINCĂ 1997; GLĂVAN & TOADER, 2001; ION, 1992; ION & GACHE, 1997; MUNTEANU, 1998; PAPADOPOUL & PETRESCU, 1991, etc.).

Craiova city, an important urban location in the Oltenia region and the county seat of Dolj county, is situated in the South of Romania, on the left bank of the Jiu river, with an altitude between 75 and 116 m. It is situated in a plain, having a very irregular shape on the North and South sides, while the Central part is compact. It has an approximate surface of 6676 ha.

In Craiova there are 14 parks, many of them being marshes in the past that have been transformed into more modern habitats. The study of aquatic birds was made at Craiovița Lake and Park, Romanescu Park, Botanical Garden, Tineretului Park and along the Jiu river and the moors on the outskirts of Mofleni neighbourhood (Fig. 1).

Craiovița Lake is situated in the N-W region of Craiova. It is an artificial lake created on the course of the Cornișoiu river in 1966 - 1977. Over the years, the lake has suffered modifications being divided by an isthmus into two unequal lakes. The lake, with small isles, surrounded by reed, rush, willows has gained in time the characteristics of an alluvial plain. The park near the lake, has alleys and a mosaic of trees and bushes, and thus represents an attraction for numerous birds. In 1985 the whole establishment, the lake and the park, occupied a surface of approx. 85 ha, and the water surface was of about 32 ha (CIOBOTEA et al., 1999). There was a time when the lake had an embankment area, boats, swimming areas, etc. being a perfect relaxing place for the weekends. After the year 1990, Craiovița Lake and Park have undergone some modifications, being disputed by the ex-owners and the city authorities. The modifications made by reducing the habitat space in favour of new constructions (the Flormang hotel/pension in the north, the Real hypermarket in the east) led to changes in the initial habitat. This is why nowadays we do not possess official data regarding the surface of the place. Still, estimations are made at about 55 ha. From an administrative point of view, the lake is assigned to a private person, who has not valued the habitat properly, leaving it deserted.

“Alexandru Buia” Botanical Garden is located in the SW part of Craiova. It extends over a surface of approx. 17 ha and it is divided in separate sectors, which comprise approx. 6000 taxa. It is crossed from east to west by a small river that comes from Iancu Jianu fountain. On the river course, there have been created 3 lakes with a surface of approximately 0.3 ha. The lakes contain aquatic plants with numerous water lilies. The lake found in the sector called “The floristic provinces of the world” is surrounded by a specific type of vegetation (reed, rush, willows) adequate for aquatic birds.

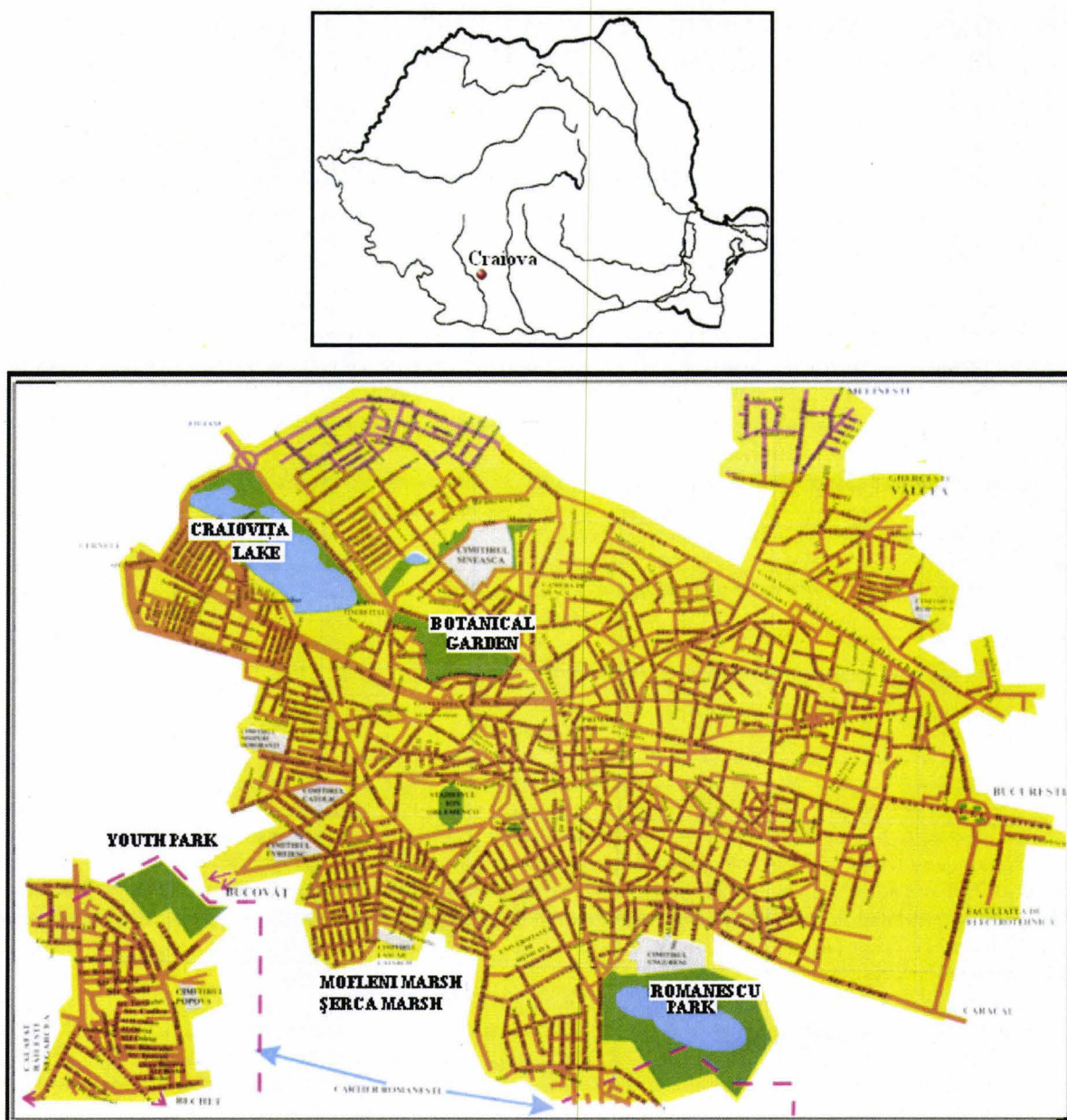


Figure 1. The map of Craiova city and of the studied habitats (from CIOBOTEA et al., 1999).

Tineretului Park, also known as Lunca Jiului Park till recently, is located in the western part of the city, on the left bank of the Jiu river. It first appeared as a result of a development program of a large sylvan area, having an initial surface of about 51 ha. It was modernized in the years 2008-2010. It contains a rich and diverse vegetation. Șerca creek, which crosses the park and the old swimming area that was abandoned due to lack of funds have attracted semi-aquatic and limicolous birds.

At the outskirts of Mofleni neighbourhood, area which contains the park, there is Mofleni Moor, permanently supplied by underground waters, with a surface of about 4 ha, and also Șerca Moor, a temporary moor which dries during the very droughty summers.

Nicolae Romanescu Park, located in the southern part of the city, is one of the most picturesque and one of the largest parks in our country (about 96 ha). Due to its unique view, the park is one of a kind in Romania and it is a special touristic attraction. It comprises forest and ornamental plantations with more than 250 tree and shrub species, numerous roads, alleys and footpaths. It also has greenhouses, race tracks and a zoo. The water surface is over 4 ha and it consists of the main lake in the central area of the park and of an artificial creek which crosses the park. On its course there is a line of lakes, all of them being supplied by underground waters.

The present paper is a continuation of a larger study regarding the city avifauna monitoring action, which began in the year 2000 (BĂLESCU 2000, 2002-2008; BĂLESCU & RIDICHE, 2001). Until now, a number of about 125 bird species has been observed in the habitats in Craiova. We will get back on this subject with more information.

The purpose of this paper is to do a brief analysis of the aquatic bird species of Craiova city from several points of view: the phenological status, the seasonal frequency and the recorded numbers. In order to fulfill this purpose the following objectives have been stated:

- the identification and the assessment of the aquatic bird species;
- the updating of the list of aquatic bird species that have been observed so far;
- the classification of the categories of birds found in Craiova;
- the diversity of the aquatic bird community in various seasons;
- the elaboration of a classification list containing the protected status of the aquatic bird species that have been identified.

MATERIAL AND METHODS

The used methods and materials were the classic ones, according to the existing guidelines. We also used data collected in the field.

The field studies have been performed monthly and twice a month in all phenological seasons. The observations have been realised from stationary locations, both with a binocular (Norconia 10x50) and without any optical equipment. For the identification of the birds I used bird guides (BRUUN et al., 1999; PETERSON et al., 1989). I also used FujiFilm FinePix S5700 and Canon SX40 HS photo cameras for taking photos and video equipment for recording the birds in various aspects of their lives and behaviour. The field observations were recorded on work sheets and analysed from a systematic, biological and ecological perspective. I also studied law statements with regard to the conservative aspect of bird species.

The information in this paper with regard to the aquatic birds from the city has been processed according to the observations recorded between 2003 and 2012. There were approached the reed species and also species which are connected to the water habitat, eurytopic species of wagtail birds which nesting on the banks of the rivers and lakes.

RESULTS AND DISCUSSIONS

In the city parks and surrounding areas, 46 aquatic species classified in 9 orders and 16 families were identified (Table 1).

Table 1. Classification of the observed number of bird species according to superior xonomical units (families, orders).

No.	Order	Family	Number of species
1.	Podicipediformes	Podicipedidae	3
2.	Pelecaniformes	Phalacrocoracidae	2
3.	Ciconiiforme	Ardeidae	7
		Ciconiidae	1
4.	Anseriformes	Anatidae	8
5.	Accipitriformes	Accipitridae	1
6.	Gruiformes	Rallidae	2
7.	Charadriiformes	Recurvirostridae	2
		Charadriidae	2
		Scolopacidae	2
		Laridae	3
		Sternidae	3
8.	Coraciiformes	Alcedinidae	1
9.	Passeriformes	Motacillidae	3
		Sylviidae	5
		Emberizidae	1

The aquatic avifauna known seasonal changes. Weather conditions, food availability, human activities in certain situations, all of these have influenced the presence or absence of the aquatic birds in certain periods of time, as well as the number variation inside of the same species (Table 2).

During the winter season (November - February) there have been monitored the species belonging to all the above-mentioned orders (with one exception - Accipitriformes) - not all of them, and not in all the years.

Tachybaptus ruficollis – in certain years, it stayed until the end of November. In other years, it stayed over the winter, but in a small number, in the Botanical Garden, Tineretului Park, Mofleni Moors, in the winter of 2004, 2005, 2006, 2008, 2010.

Podiceps nigricollis (Black Necked Grebe) – it remained over the winter in December 2007 – February 2008 at Romanescu Park and the Botanical Garden.

Among the cormorants – *Phalacrocorax pygmaeus* stayed here during mild winters – the first time it was observed being in February 2002. On February the 5th 2010 we observed 30 specimens in Romanescu Park trees.

Phalacrocorax carbo was observed during winter time in Romanescu Park in February 2008, 2010 and in March 2011. Its presence in this habitat is not constant.

Starting with the winter of 2007, *Tringa ochropus* appeared in Romanescu Park. It was systematically observed in this season. In the winter of 2010 it was also seen at Mofleni Moors.

In January 2008 there were made observations regarding the presence of *Mergus albellus* on the Jiu river. Some species have been constantly observed during winter time: *ralidae* (*Fulica atra* and *Gallinula chloropus*), *anatidae* (*Anas platyrhynchos* and *A. crecca*) and *laridae* (*Larus ridibundus* and *L. cachinnans*).

Regarding the number of specimens, the coots have dominated the moor hen. Frequently, the most mature specimens used to stay. A small moor hen population remained permanently in the Botanical Garden.

Starting with the winter of 2009, the sea-gulls have been recorded in big numbers on the Jiu river, in the proximity of the landfill, which served as an important source of food in this season. The number of specimens ranged between 20 – 100/150 specimens. From here, they made short distance flights to the parks of Craiova, being rarely observed and in a small number of specimens. Starting with the winter of the year 2010, *Anas platyrhynchos* has been constantly observed on the lake from Romanescu Park. This happened because it remained close to the already acclimated species: *Cygnus olor*, *Ciconia alba*.

Cygnus olor was identified in February 2006 at Craiovița Lake, on the Jiu river in 2010, 2011; in January 2012 there were identified 14 specimens, both adults and juveniles.

Ardea cinerea, *Egretta alba* have been observed during extremely cold winters, either as isolated specimens, or in small groups of up to 4 specimens.

Alcedo atthis at Romanescu Park and the Botanical Garden – maximum 1-2 specimens in the winters of 2005, 2007, 2008, 2009, 2011.

Emberiza schoeniclus is an uncommon species, hidden in the reed. I noticed it in the winter of 2008, 2009, 2011 at Craiovița Lake, in small groups of up to 6 specimens.

The presence of an important number of aquatic birds in the researched habitats proves the fact that over time, the birds have adapted to the synanthropic conditions. Unlike the open areas, inside cities, birds manage to face the cold winters and to find food as well. They have adapted to a type of food that is not what they usually eat – that is the garbage that people throw in the parks.

In the spring time (March, April, May) some of the aquatic bird species that come out of the place where they have spent their winter remain in the area for the nesting period (Ferruginous Duck, Little Bittern – Fig. 2a, Night Heron, Northern Lapwing, Whiskered Tern, different species of Warbler, White Wagtail and Yellow Wagtail etc.)

Other species are just passing through on their way between their nesting places and the place where they are about to stay for the winter (Purple Heron, Shoveler, Garganey, Little Ringed Plover, Black Tern etc.). For example: *Ardea purpurea* was observed in flight over the Jiu river on the 29th of April 2009, but also at Craiovița Lake in May 2011, September 2011 and 2012.

During this season, the winter visitors, which were common during the winter time, get ready to leave, in turns, depending on the weather conditions, either in February, or in March, in order to find new places for their nests (Mute Swan, Grey Wagtail, Kingfisher, Reed Bunting etc.)

The spring migration can extend till the second half of May. During this period of time, from the second half of March – till the first half of May there could be made observations of many of the above-mentioned species.

During summer (June - August) most of the species are active around their nesting spot and around their new-borns. Many species make short flights in order to find food after the nesting period is over. There can be observed how adults and juveniles fly from the nesting places to the eating ones. That is why, during this period of time, in this habitat there have been seen other species that nest in the surrounding areas and come looking for food: *Ardea cinerea*, *Egretta garzetta*, *E. alba*, *Chlidonias niger*, *Recurvirostra avosetta* etc.

At the end of the summer (when the fall migration begins) at Șerca and Mofleni Moors found at the outskirts of Mofleni neighbourhood I observed an increase in the number of gulls, herons (little egret, night heron, grey heron), ducks, etc. In some years we could even observe purple heron, cormorants, etc. Some of the specimens of cormorants are found on Craiovița Lake as well. Also, in this time frame, we have observed in the area different species of the genera *Tringa*, *Calidris*, *Charadrius*.

Autumn (September, October) is the season of actual migration. The autumn migration extends over a longer period of time. The summer guests leave the area making room for the winter guests.

The weather conditions are the ones which rule a quicker or a slower rhythm in which the aquatic bird species leave the habitats of Craiova. Thus, some species have left the earliest in September, most of them in October (Night Heron, Little Egret, Warblers etc.), others in November (Little Grebe, Grey Heron). During this period, aquatic birds could be observed both solitary and in groups of tens, even hundreds of specimens (*anatidae*, *laridae*).

As a result of the field studies made in 2003, I have not observed the following species anymore: *Cygnus cygnus*, *Rallus aquaticus* (BĂLESCU, 2002; 2003; 2004).

The aquatic habitats (lakes, moors, rivers) and semi-aquatic habitats (reed, with or without willows, wetlands, swamps, sandy shores, etc.) from the parks or at the outskirts of the city have the necessary conditions to allow the birds to cross them or even to find shelter or to build nests. A great fact in favour of this situation is the lack of specific

habitat predators. The frequency in a certain area, over a longer or a shorter period of time is also influenced by the weather conditions.

Between 2003 and 2012 the majority of the aquatic bird species have been seen in the habitats of the city. Cormorants, grebes, great egret, kingfisher have chosen as a place to stay over the winter, the parks in the city (the Botanical Garden and Romanescu Park). Although the main lakes usually freeze during harsh winters, there are still a few unfrozen areas. Thus, the group of lakes from Romanescu Park and the first lake in the Botanical Garden do not usually freeze, offering food for these birds. During summer, the ardeidae, the anatidae, the charadriidae have preferred the lakes and moors at the outskirts of the city (Craiovița Lake, Mofleni moors, Șerca, Șerca creek, Jiu river).

Over the years I have recorded changes in the aquatic bird dynamics. Thus, during the first years of observation, some species were present in almost all habitats. In the last 5 years, the frequency and the number of certain species decreased due to weather conditions, urban factors and the competition for food and nesting. Although at Craiovița lake all aquatic bird species can be found (with few exceptions), these do not stay there for the nesting period. Some of them have been observed in great numbers (anatidae), others in small numbers and rarely observed (great crested grebe, pygmy cormorant). For example, *Tachybaptus ruficollis*, nests at Craiovița lake in certain years. Starting with the year 2010 I have recorded its presence in a very small number and often just in flight. It was replaced by other species that remained here for the nesting period, such as *Chlidonias hybrida*, *Nycticorax nycticorax*, etc. There is a permanent change and variability of species.

Data regarding the nesting/non-nesting aquatic birds show that there are more non-nesting species.

Most of the aquatic and semi-aquatic species have nested at Craiovița Lake, Mofleni Moor and Șerca creek, in small numbers and not in all the years. Out of the summer guests I consider a number of 15 species to be nesting or possibly nesting.

Most aquatic species nesting in the aquatic type of vegetation (in the reed): *Tachybaptus ruficollis*, *Ixobrychus minutus*, *Anas platyrhynchos*, *Aythya nyroca*, *A. ferina*, *Gallinula chloropus*, *Fulica atra*, *Locustella luscinioides*, species of *Acrocephalus* sp. *Vanellus vanellus* nesting on a grassy field near Șerca and Mofleni moors. The wagtails nest on the ground (in reed piles, in banks holes, etc.). Regarding the Yellow Wagtail, there is a preponderance of *Motacilla flava feldegg*. There have been reported *M. flava thunbergi*, *M. f. flava* și *M. f. flavissima* as well.

Nycticorax nycticorax nesting at the Craiovița Lake. In the year 2012, 3 pairs of herons bred in the willows from the little isle on the great lake. The adults carried sticks and willow branches in their beak (Fig. 2c). At the beginning of August I observed juveniles hidden in the rush-bed near the willows.

It is important to notice that in 2010 a pair of storks started to build a nest at the outskirts of Mofleni neighbourhood, near the bridge on the Jiu (Fig. 2b). The nest was built on a telegraph pole. After a long period of time, of about 10 years of observations, the White Stork found the proper conditions to nest at the outskirts of the city. In the year 2011, the pair of storks had 2 juveniles. In 2012 it reached the nest on the 30th of March and it had 3 juveniles. This year, in 2013, it arrived on the 29th of March. We keep monitoring this pair of storks in order to better record the biological and ecological aspects of this species.

In certain years, at Craiovița Lake and Mofleni Moor we have observed breeding activities of the black winged stilt: spectacular flights over the reed, loud sounds at the sight of unwanted guests, etc. However, we did not find the nest. Still, I consider that it is very likely for this bird to have bred there. Because of the fact that in the past years this species has been continuously observed in the aquatic habitats of the city it is necessary to continue monitoring it in order to find the exact nesting spot, to study the materials used for building it and to count the eggs. This should also be done for other bird species that have been constantly observed in all the seasons.

Data regarding the breeding of the aquatic species will be the subject of a future paper.

The changes in the lives of the birds are caused by two major factors: the climatic factors and the anthropogenic factors.

The influence of the anthropogenic factors on the studied habitats had the following negative consequences: the limitation of the park areas-consequence of the urbanistic management which intended to change the natural ecosystems (which had a poor economic potential) into anthropogenic ones (with a higher economic-potential); the noise of the daily activities, of the cars that pass near the studied ecosystems, the loud music, the recreational activities, the fishing – all elements that can cause changes in the biorhythm of these birds; the cutting of the reed in inappropriate periods (in some years this was done exactly in the nesting period); the fact that the reed caught fire from a cigarette thrown there; the domestic animals left by the locals to eat on the fields near the moors that destroy the nests built on the ground, etc.

In spite of all these we have observed that the birds have chosen to cohabit in the company of people. In time, the birds have managed to transform the anthropogenic disadvantages in advantages, thus managing to survive and adapt to the new life conditions. The abandonment of the rehabilitation plans of Craiovița Lake and Park had a beneficial consequence on the plant and animal species found here leading to an increase in their number. The bio-ecological conditions offered by the aquatic and semi-aquatic habitats in Craiova led to the cohabitations and nesting of many aquatic species in the same habitat. Most of them are found on Craiovița Lake, Mofleni and Șerca Moors, during spring, summer and autumn.

At national level, the aquatic birds are protected by numerous laws and ordinances, these being on the same direction with the ones given by the European Union. We mention only a few of them: Law no. 13/1993, Law no. 13/1998, Law no. 407/2006 and 197/2007, The Bird Directive 2009/47/EC.

11 of the aquatic species recorded in Craiova can be found in the Red Book of Vertebrates in Romania (MUNTEANU, 2005), as endangered species: 7 bird species are vulnerable (*Phalacrocorax pygmaeus*, *Nycticorax nycticorax*, *Ardeola ralloides*, *Ciconia ciconia*, *Aythya nyroca*, *Mergus albellus*, *Recurvirostra avosetta*) and 4 species are endangered (*Egretta garzetta*, *E. alba*, *Ardea pupurea*, *Himantopus himantopus*).

The majority of the aquatic bird species recorded in Craiova (35 species) have a favourable status of conservation, being secure (Spec 4 and Non Spec category) (Table 3). Out of these, 6 species can become vulnerable or endangered at any time, having a temporary status. Taking into consideration the European status of conservation (HAGEMEIJER et al., 1997), we have recorded the presence of 13 aquatic species in the city, species that have the following conservation status: 8 species are vulnerable: *Phalacrocorax pygmaeus*, *Ixobrychus minutus*, *Ardeola ralloides*, *Ardea purpurea*, *Ciconia ciconia*, *Anas querquedula*, *Aythya nyroca*, *Mergus albellus*; 4 species are in decline: *Nycticorax nycticorax*, *Chlidonias hybrida*, *C. niger*, *Alcedo atthis* and one species has a broad area of distribution: *Recurvirostra avosetta*.

Table 3. The threat and the protection status of the aquatic avifauna of Craiova.

No.	Species	Category Spec	Threat status		Protection status			
			Europe	Romania	Birds Directive	Law 13/1993	Law 13/1998	Law 407/2006
1.	<i>Tachybaptus ruficollis</i>	Non Spec	S	-		Annex II	-	Annex 2
2.	<i>Podiceps cristatus</i>	Non Spec	S	-		Annex III	-	A2
3.	<i>Podiceps nigricollis</i>	Non Spec	S	-		AII	-	A2
4.	<i>Phalacrocorax carbo</i>	Non Spec	S	-		AIII		Annex I
5.	<i>Phalacrocorax pygmaeus</i>	Spec 2	V	V	Annex I	A II	AII	A2
6.	<i>Ixobrychus minutus</i>	Spec 3	(V)	-	A I	AII	AII	A2
7.	<i>Nycticorax nycticorax</i>	Spec 3	D	V	A I	AII	-	A2
8.	<i>Ardeola ralloides</i>	Spec 3	V	V	A I	AII	-	A2
9.	<i>Egretta garzetta</i>	Non Spec	S	V	A I	AII	-	A2
10.	<i>Egretta alba</i>	Non Spec	S	E	A I	AII	AII	A2
11.	<i>Ardea cinerea</i>	Non Spec	S	-		AIII	-	A2
12.	<i>Ardea purpurea</i>	Spec 3	V	E	A I	AII	AII	A2
13.	<i>Ciconia ciconia</i>	Spec 2	V	V	A I	AII	AII	A2
14.	<i>Cygnus olor</i>	Non Spec	S	-		AIII	AII	A2
15.	<i>Anas crecca</i>	Non Spec	S	-		AIII	AII	A1
16.	<i>Anas platyrhynchos</i>	Non Spec	S	-		AIII	AII	A1
17.	<i>Anas querquedula</i>	Spec 3	V	-		AIII	AII	A1
18.	<i>Anas chlypeata</i>	Non Spec	S	-		AIII	AII	A1
19.	<i>Aythya ferina</i>	Spec 4	S	-		AIII	AII	A1
20.	<i>Aythya nyroca</i>	Non Spec	V	V	A I	AIII	AII	A2
21.	<i>Mergus albellus</i>	Non Spec	V	V	A I	AII	AII	A2
22.	<i>Circus aeruginosus</i>	Non Spec	S	-	A I	AII	AII	A2
23.	<i>Gallinula chloropus</i>	Non Spec	S	-		AIII	-	A1
24.	<i>Fulica atra</i>	Non Spec	S	-		AIII	-	A1
25.	<i>Himantopus himantopus</i>	Non Spec	S	E	A I	AII	AII	A2
26.	<i>Recurvirostra avosetta</i>	Non Spec	L*	V	A I	AII	AII	A2
27.	<i>Charadrius dubius</i>	Non Spec	(S)	-	-	AII	AII	A2
28.	<i>Vanellus vanellus</i>	Non Spec	(S)	-	-	AIII	AII	A2
29.	<i>Calidris minuta</i>	Non Spec	(S)	-	-	AII	AII	A2
30.	<i>Tringa ochropus</i>	Non Spec	(S)	-	-	AII	AII	A2
31.	<i>Larus ridibundus</i>	Non Spec	S	-	-	AIII	-	A2
32.	<i>Larus fuscus</i>	Spec 4	S	-	-	-	-	A2
33.	<i>Larus cachinnans</i>	Non Spec	S	-	-	-	-	A2
34.	<i>Chlidonias hybrida</i>	Spec 3	D	-	A I	AII	-	A2
35.	<i>Chlidonias niger</i>	Spec 3	D	-	A I	AII	AII	A2
36.	<i>Chlidonias leucopterus</i>	Spec 3	S	-		AII	AII	A2
37.	<i>Alcedo atthis</i>	Spec 3	D	-	A I	AII	-	A2
38.	<i>Motacilla flava</i>	Non Spec	S	-	-	AII	-	A2
39.	<i>Motacilla cinerea</i>	Non Spec	S	-	-	AII	-	A2
40.	<i>Motacilla alba</i>	Non Spec	S	-	-	AII	-	A2
41.	<i>Locustella luscinioides</i>	Spec 4	(S)	-	-	AII	-	A2
42.	<i>Acrocephalus schoenobaenus</i>	Spec 4	(S)	-	-	AII	-	A2
43.	<i>Acrocephalus palustris</i>	Non Spec	S	-	-	AIII	-	A2
44.	<i>Acrocephalus scirpaceus</i>	Spec 4	S	-	-	AII	-	A2
45.	<i>Acrocephalus arundinaceus</i>	Non Spec	S	-	-	AII	-	A2
46.	<i>Emberiza schoeniclus</i>	Non Spec	S	-	-	AII	-	A2

Legend: SPEC category: SPEC 1 – species of global conservation concern; SPEC 2 – unfavourable conservation status concentrated in Europa. SPEC 3 – unfavourable conservation status not concentrated in Europa. SPEC 4 – favourable conservation status concentrated in Europa. Non-SPEC – favourable conservation not concentrated in Europa. Threat status: S – secure, V – vulnerable, D – declining, E – endangered, L – localized, () – provisional status; Protection status: Birds Directive: A I/Annex I = species for which measures of habitat conservation must be taken in order to ensure the survival and reproduction in the area of distribution; Law 13/ 1993: AII/Annex II – species that are strictly protected; AIII/Annex III – protected species; AII/Annex II – migratory species with unfavorable conservation status, which need of protection; Law 407/ 2006: A I/Annex 1 – wild species that can be hunted; AII/Annex 2 – wild species that cannot be hunted.

As for maintaining and increasing the aquatic species diversity there is a strong need of actual measures and local implementation. For example: maintaining and protecting already existing parks, rehabilitation of Craiovița Lake and Mofleni moor, arranging an artificial moor with proper vegetation in Tineretului Park, resolving the problem of street dogs that destroy the nests on the ground, implementing measures of public ecological education regarding the protection of the birds in the city, etc.

CONCLUSIONS

The placement of the city on the left bank of the Jiu river and its generous anthropogenic habitat creates the most favourable life conditions for the aquatic birds, mostly in case of those with a high adapting potential. It offers food resources, shelter and nesting places for numerous aquatic birds. The area is situated on the migration route of some birds (ducks, storks, herons, caradriiforms).

The number of the species and of the individuals has fluctuated according to weather and food conditions, both seasonally and annually. The largest bird groups and number variations have been observed during the prevernal, serotinal and autumnal seasons. The majority of the identified species are migratory (41 sp.), most of them not being strictly enclosed in a certain phenological category.

We have identified some scarce species in the aquatic habitats: *Podiceps nigricollis*, *Ardea purpurea*, *Recurvirostra avosetta*, *Mergus albellus*.

Out of the summer visitors species with a constant presence we mention: *Tachybaptus ruficollis*, *Ixobrychus minutus*, *Nycticorax nycticorax*, *Egretta garzetta*, *Ardea cinerea*, *Aythya ferina*, *A. nyroca*, *Circus aeruginosus*, *Vanellus vanellus*, *Chlidonias hybridus*, *Motacilla alba*, *M. flava*, *Acrocephalus schoenobaenus*, *A. scirpaceus*, *A. arundinaceus*.

With only a few exceptions, the majority of the aquatic and semi-aquatic species recorded in Craiova are protected by the Romanian laws. Looking at the threat categories on the national level, we have identified 3 endangered species (*Egretta alba*, *Ardea purpurea*, *Himantopus himantopus*) and 8 vulnerable species (*Egretta garzetta*, *Phalacrocorax pygmaeus*, *Nycticorax nycticorax*, *Ardeola ralloides*, *Ciconia ciconia*, *Aythya nyroca*, *Mergus albellus*, *Recurvirostra avosetta*), of which three are declared Monuments of Nature: *Egretta alba*, *E. garzetta*, *Himantopus himantopus*.

In order to maintain the avifaunistic component in the area and to protect the birds, a firm implication of the authorities is required to elaborate a complex and clear set of measures, all with the purpose of minimizing the negative impact of people on the birds in the parks. Only by informing the public opinion and by showing how importance is the anthropogenic ecosystems we can fulfill our purpose, that of conserving biological diversity and life in big cities.

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Table 2. The spatial distribution, the phenology and some observations regarding the aquatic avifauna of the city of Craiova.

No.	Species	Studied habitats				Period of observation phenology	Phenological category	Observations
		Craiovița Lake	Tineretului Park	Botanical Garden	Romanescu Park			
1.	<i>Tachybaptus ruficollis</i>	*	*	*		I, II, III, IV- XI, XII,	SV, RWR, N	in the past years - rare
2.	<i>Podiceps cristatus</i>	*	*		*	II, III, VII, VIII, IX, X	P, SV, PN	not constantly observed
3.	<i>Podiceps nigricollis</i>				*	I, II	WV	rare
4.	<i>Phalacrocorax carbo</i>		*		*	I, II, III, VIII	P, WR	rare
5.	<i>Phalacrocorax pygmaeus</i>	*	*	*	*	I, II, III, VII, IX, X, XI, XII	P, WR	not constantly observed
6.	<i>Ixobrychus minutus</i>	*	*	*		V-IX	SV, N	constant
7.	<i>Nycticorax nycticorax</i>	*	*	*	*	V-X	SV, N	constant
8.	<i>Ardeola ralloides</i>	*	*			V, VIII	P	rare
9.	<i>Egretta garzetta</i>	*	*			IV-X	SV, PN	constant
10.	<i>Egretta alba</i>	*	*	*	*	I, II, III, VIII, IX, X, XI, XII	P, WR	2,4 -14 ex
11.	<i>Ardea cinerea</i>	*	*	*	*	I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII	SV, WR	frequent, with a small number of specimens
12.	<i>Ardea purpurea</i>	*	*			IV,V, IX	P	very scarce, observed in 2009, 2011, 2012
13.	<i>Ciconia ciconia</i>	*	*			IV, VIII, III - IX	P, SV, N	nesting the third consecutive year
14.	<i>Cygnus olor</i>	*	*			I, II, III, IV, VI, XI,XII	P, WR	5-14 specimens
15.	<i>Anas crecca</i>	*	*	*	*	I, II, III, IV, V, X, XI, XII	WV, P	frequent
16.	<i>Anas platyrhynchos</i>	*	*	*	*	I-XII	MP, N	common
17.	<i>Anas querquedula</i>	*	*			III, IV, V	P, SV	not constantly observed
18.	<i>Anas clypeata</i>	*	*			III, IV, V, IX	P	not constantly observed
19.	<i>Aythya ferina</i>	*	*			III, IV,V, VI; VII, VIII-X	SV, P, N	not constantly observed
20.	<i>Aythya nyroca</i>	*	*			III-X	SV, N	frequent
21.	<i>Mergus albellus</i>		*			I	WV	rare
22.	<i>Circus aeruginosus</i>	*	*			III – IX	SV, PN	frequent
23.	<i>Gallinula chloropus</i>	*	*	*	*	I – XII	MP, N	common
24.	<i>Fulica atra</i>	*	*	*	*	I – XII,	MP, N	common
25.	<i>Himantopus himantopus</i>	*	*			IV – X	SV, PN	frequent
26.	<i>Recurvirostra avosetta</i>		*			V, VII, VIII	P, OV-P	rare
27.	<i>Charadrius dubius</i>	*	*			VIII, IX	P	not constantly observed
28.	<i>Vanellus vanellus</i>	*	*			V – X	SV, N	frequent
29.	<i>Calidris minuta</i>	*	*			VII, VIII, IX	P	infrequent appearances
30.	<i>Tringa ochropus</i>	*	*	*	*	I, II, III, IV, V, VIII, IX, X	P, WR	constant appearance in the last five years
31.	<i>Larus ridibundus</i>	*	*	*	*	I – XII	R	constantly observed
32.	<i>Larus fuscus</i>		*			X, XI, XII	P-WV	rare
33.	<i>Larus cachinnans</i>	*	*	*	*	I – XII	R	constantly observed
34.	<i>Chlidonias hybrida</i>	*	*	*		IV – X	SV, N	frequent
35.	<i>Chlidonias niger</i>	*	*			V, VII, VIII, IX	P	rare
36.	<i>Chlidonias leucopterus</i>		*			VIII	P	rare
37.	<i>Alcedo atthis</i>			*	*	XII – III	WV	only in cold winters
38.	<i>Motacilla flava</i>	*	*			III – XI	SV, N	common
39.	<i>Motacilla cinerea</i>			*	*	XI, XII, I, II, III	WV	frequent
40.	<i>Motacilla alba</i>	*	*	*	*	III – XI	SV, N	common
41.	<i>Locustella luscinioides</i>	*	*			V – X	SV,PC	regularly appears
42.	<i>Acrocephalus schoenobaenus</i>	*	*	*		IV-X	SV, N	frequent

43.	<i>Acrocephalus palustris</i>	*	*			V, VI, VII, VIII, IX	SV, PC	regularly appears
44.	<i>Acrocephalus scirpaceus</i>	*	*			IV-X	SV, N	frequent
45.	<i>Acrocephalus arundinaceus</i>	*	*	*	*	IV – X	SV, N	common
46.	<i>Emberiza schoeniclus</i>	*	*			XII, I, II	WV	rare

Legend: IXII – the months of the year; Phenological type: R – resident; MP – migratory partly; SV– summer visitor; WV– winter visitor; RWV – rarely winter visitors; N – nesting; PN – possible nesting.



a.



b.



c.

Figure 2. The aquatic species nesting in Craiova: a. *Ixobrychus minutus* – Botanical Garden, The 18th of July 2011; b. *Ciconia ciconia* – Mofleni District, The 29th of March 2012; c. *Nycticorax nycticorax* – Craiovița Lake, The 31st of May 2012. (Original photographs).

DIVERSITY AND ECOLOGICAL PECULIARITIES OF TERRESTRIAL VERTEBRATE FAUNA OF CHISINAU CITY, REPUBLIC OF MOLDOVA

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Abstract. The studies were accomplished during the spring-winter period of 2012 in various types of ecosystems from Chișinău city and its surroundings. The mammal fauna of the city is rather rich, being registered 39 mammal species: 6 insectivore species, 9 bat species, 16 rodent species, 1 lagomorph species, 5 carnivorous species and 2 artiodactyl species. 117 bird species were registered on the territory of the city during all phenological periods. The domination degree of bird species decreases from cold period toward summer, reaching its minimum in nesting period, when bird distribution is more uniform. In post-breeding period and during autumn migration the dominance degree increases again, fact connected to the accumulation of some species in flocks and their uneven distribution. Among reptiles 4 species were registered and among amphibians 9 species.

Keywords: urban ecosystems, terrestrial vertebrates, diversity, dominance, abundance.

Rezumat. Diversitatea și particularitățile ecologice ale faunei de vertebrate terestre din municipiul Chișinău, Republica Moldova. Cercetările au fost efectuate în perioada primăvară - iarnă a anului 2012 în diverse tipuri de ecosisteme ale orașului Chișinău. Fauna de mamifere este diversă, fiind înregistrate 39 de specii: 6 specii de insectivore, 9 specii de chiroptere, 16 specii de rozătoare, o specie de lagomorfe, 5 specii de carnivore și 2 specii de copitate. Pe parcursul tuturor perioadelor fenologice, pe teritoriul orașului au fost semnalate 117 specii de păsări. Gradul de dominanță al speciilor de păsări scade din perioada rece către cea estivală și atinge valori minime în perioada cuibăritului, când distribuția păsărilor este mai uniformă. În perioada postreproductivă și în timpul migrațiilor de toamnă dominanța păsărilor scade din nou, datorită acumulării indivizilor în stoluri și distribuției lor neuniforme. Printre reptile au fost identificate 4 specii, iar printre amfibieni 9 specii.

Cuvinte cheie: ecosisteme urbane, vertebrate terestre, diversitate, dominanță, abundență.

INTRODUCTION

At present the processes of anthropization and degradation of natural ecosystems occur intensely throughout the country. In the last decades a high growth of urban areas and, consequently, an increase in urban population density has been registered. From evolutionary aspect the cities represent new type of biota, with a complex of permanently changing ecological conditions. The city area and the adjacent territories subject to disturbances are constantly expanding. In such conditions modifications of faunistic community structure occur and the animal species gradually adapt to new conditions.

The terrestrial vertebrate fauna is an indispensable component of the urban environment, having a huge importance in the maintenance and functioning of the ecosystems strongly affected by anthropogenic activity. At the same time the vertebrate species can serve as ecological indicators of the ecosystem stability and of the urban coenoses status. There are only few studies concerning urban fauna in the past century (ANISIMOV, 1966; ANISIMOV & COJUHARI, 1978). In the last years the study of Chișinău vertebrate fauna was more intense (BOGDEA et al., 2008; NISTREANU & CARAMAN, 2009; NISTREANU et al., 2011; VASILASCU & MUNTEANU, 2008; VASILASCU, 2008; TIKHONOV et al., 2009, 2010; TIKHONOVA et al., 2012, etc.). Still, there are no complex studies on diversity and ecology of terrestrial vertebrate species from Chișinău city and its surroundings. Therefore, the aim of this study was to check the diversity of terrestrial vertebrate species of Chișinău city and to emphasize some ecological peculiarities of the studied groups.

MATERIALS AND METHODS

The studies were accomplished during the spring-winter period of 2012 in various types of ecosystems from Chișinău city and its surroundings. The parks are represented by city parks: La Izvor, Valea Trandafirilor, Dendrium, Ștefan cel Mare and Botanical Garden. The forest ecosystems are represented by forest plantations and remains of natural woods around the city (Durești, Dănceni, Băcioi, Suruceni, Sociteni, Vadul-lui-Voda). The open land ecosystems are represented by various types of cultivated lands (orchards, vineyards, cereals, alfalfa), fallow grounds, grasslands, pastures and rocky biotopes, including caves and stone quarries (Criuleni). The wet biotopes are represented by river banks, swamp sectors, lakes and ponds. Various types of buildings were also considered: houses, industrial deposits, tall buildings with adjacent territories, usually grown with tree and shrub vegetation.

The used methods were the direct observations during several days each month (March-October), collecting and determination of trophic remains, catching with traps (small mammals) and with nets (passerine birds). The large and medium-sized mammals, the birds, the reptiles and amphibians were counted during routes within a certain area; the route length varied from 1 to 10 km. The ecological analysis of vertebrate communities was based on the following parameters:

frequency, abundance, diversity (Shannon and Simpson indexes), Sorensen coefficient. In order to determine the influence degree of biotic and abiotic factors upon terrestrial vertebrate fauna, the factorial analysis was applied.

The statistical analysis was performed using the programs Statistics Workbook, Microsoft Excel, BiodiversityPro. In our studies the following equipment was used: binoculars, telescope, laser rangefinder, night vision monocular, GPS E-Trex-10, digital cameras Nikon and Panasonic, digital video camera, dictaphone, ultrasonic detector D-230, snap traps, live traps.

RESULTS AND DISCUSSIONS

The mammal fauna of Chişinău city and its surroundings is rather rich, being registered 39 species. There were recorded 6 insectivore species, 9 bat species, 16 rodent species, 1 lagomorph species, 5 carnivorous species and 2 artiodactyl species (Table 1).

Table 1. Mammal species registered in the urban ecosystems of Chişinău city and surroundings.

No.	Species	Ecosystems								Buildings
		Forest	Shelter belts	Parks	Wet biotopes	Orchards and vineyards	Cereals	Fallow ground	Rocky	
1	<i>Erinaceus concolor</i>	+	+	+	+	+	+	+	+	+
2	<i>Talpa europaea</i>	+	+	+	-	+	+	+	-	-
3	<i>Sorex araneus</i>	+	+	+	+	+	-	+	-	-
4	<i>Sorex minutus</i>	+	+	-	+	+	-	-	-	-
5	<i>Crocidura leucodon</i>	+	+	-	+	+	-	+	+	-
6	<i>Crocidura suaveolens</i>	+	+	+	+	+	+	+	+	+
7	<i>Myotis daubentonii</i>	+	-	+	+	+	-	-	+	-
8	<i>Myotis dasycneme</i>	+	-	-	+	-	-	-	+	-
9	<i>Myotis mustacinus</i>	+	-	-	+	-	-	-	+	-
10	<i>Myotis bechsteinii</i>	+	-	-	+	-	-	-	+	-
11	<i>Myotis blythii</i>	-	+	+	-	+	-	-	+	+
12	<i>Eptesicus serotinus</i>	+	-	+	-	+	-	+	+	-
13	<i>Plecotusaus triacus</i>	-	+	+	-	+	-	-	+	+
14	<i>Plecotusaus auritus</i>	+	+	+	-	+	-	-	+	+
15	<i>Pipistrellus pipistrellus</i>	+	+	+	-	+	-	-	+	+
16	<i>Sciurops vulgaris</i>	+	+	+	+	+	-	-	-	+
17	<i>Dryomys nitedula</i>	+	+	+	-	+	-	-	-	-
18	<i>Muscardinus avellanarius</i>	+	+	+	-	+	-	-	-	-
19	<i>Nannospalax leucodon</i>	-	+	-	-	+	+	+	-	-
20	<i>Ondatra zibethicus</i>	+	-	+	+	-	-	-	-	-
21	<i>Arvicola terrestris</i>	-	-	-	+	-	-	-	-	-
22	<i>Rattus norvegicus</i>	-	-	+	+	+	+	+	-	+
23	<i>Mus musculus</i>	-	+	+	+	+	+	+	+	+
24	<i>Mus spicilegus</i>	-	-	-	-	+	+	+	-	-
25	<i>Apodemus sylvaticus</i>	+	+	+	+	+	+	+	+	-
26	<i>Apodemus tralensis</i>	+	+	-	-	+	+	+	-	-
27	<i>Apodemus flavicollis</i>	+	+	+	-	+	+	+	-	-
28	<i>Apodemus agrarius</i>	+	+	-	+	+	+	+	-	-
29	<i>Microtus sp.</i>	-	+	-	+	+	+	+	+	-
30	<i>Clethrionomys glareolus</i>	+	+	+	-	+	-	-	-	-
31	<i>Pitymys subterraneus</i>	+	-	-	-	-	-	-	-	-
32	<i>Lepus europaeus</i>	+	+	-	-	+	+	+	-	-
33	<i>Vulpes vulpes</i>	+	+	-	-	+	-	+	+	-
34	<i>Meles meles</i>	+	-	-	-	-	-	-	-	-
35	<i>Mustela nivalis</i>	+	+	-	-	+	-	+	+	-
36	<i>Mustela putorius</i>	+	+	-	-	+	-	-	-	+
37	<i>Martes foina</i>	+	-	-	-	-	-	-	+	-
38	<i>Capreolus capreolus</i>	+	-	-	-	-	-	-	-	-
39	<i>Sus scrofa</i>	+	-	-	-	-	-	-	-	-

The forest ecosystems have rich and abundant fauna. There were registered all insectivorous, carnivorous and artiodactyl species, as well as the majority of bat and rodent species. The shelter belts also provide favourable conditions for the majority of insectivore, bat, rodent and carnivore species. In city parks the fauna is represented by the most spread insectivore and rodent species and by several bat species.

Among agricultural ecosystems the richest fauna was registered in orchards, where open-land species, forest species and some carnivorous mammals can find favourable conditions. The wet biotopes are suitable for hygrophilous insectivore, bat and rodent species. In rocky biotopes all bat species, some insectivore, carnivore and only few rodent species were recorded. The lowest number of species was registered in buildings and adjacent territories: hedgehog, squirrel, lesser shrew – the most anthropophilous species among shrews, some bat species hibernating inside the buildings, in attics, the polecat – find shelter in attics of private houses, where it feed on small rodents, birds, chicken, and a high number of house mouse and rats, strongly connected to anthropogenic environment.

The small mammal communities from city ecosystems were subjected to more deep study, being evaluated their ecological features. The distribution according the biotopes of small mammal species within city limits, it can be seen that the most suitable for small mammal fauna are the biotopes similar to natural ones: forests, shelter belts and landscape parks situated at city limits, as well as various types of agrocoenoses (Fig. 1). The biotopes situated closer to city centre have rather low diversity and the small mammal fauna is represented by several most eurytopic and widespread rodent species. The diversity of small mammals is rather high; the Shannon index varies between 0.99 and 1.77, being the highest in shelter belts and the lowest – in grasslands (Fig. 1). The dominant species are those from genus *Apodemus* in the majority of studied biotopes, in wet forest *Clethrionomys glareolus* (50%) is dominant and in cereal crops – *Mus spicilegus* (61%) with more than half of all caught rodents. The shrew species were found in wet biotopes, in forest ecosystems, at forest edge and in grasslands. In forest and paludous ecosystems the dominant species is *Sorex araneus*, while in open lands *Crocidura suaveolens* is dominant.

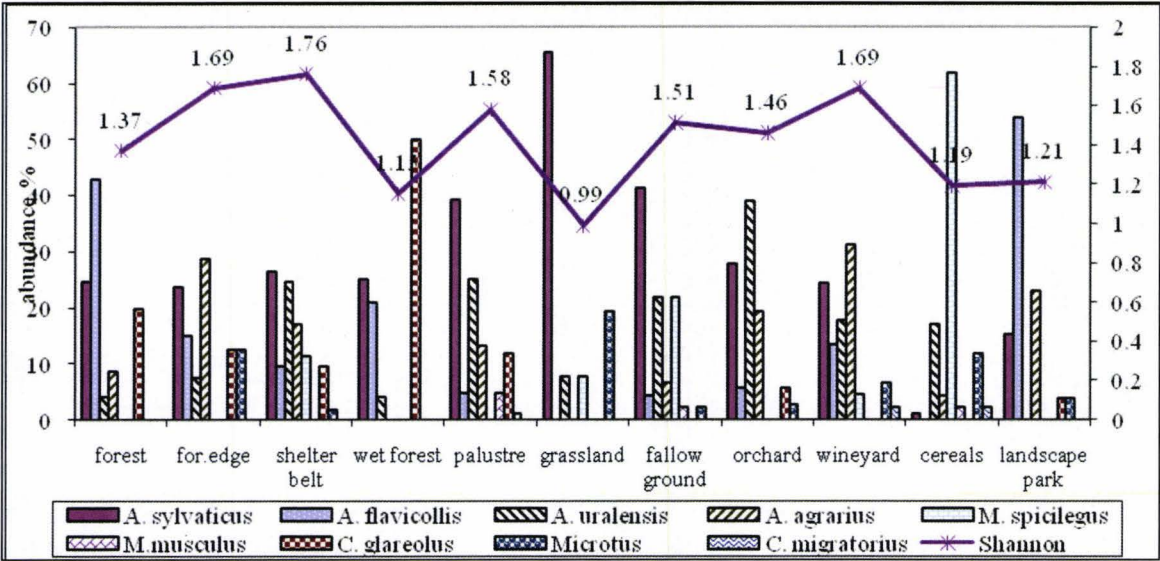


Figure 1. Abundance and diversity of rodent species in the ecosystems of Chișinău city.

Bird populations. The observation on bird populations in the studied ecosystems city allow to emphasize their distribution and density peculiarities in various types of biotopes within Chișinău city and adjacent territories. On the whole, 117 bird species were registered on the territory of the city during all phenological periods (Table 1). Some features of bird communities were emphasized, such as frequency, phenological category within Chișinău city compared to the whole country. Among phenological categories the most representative is the SV one (summer visitors), with 47% in the republic and 38.4% in Chișinău city. For some bird species the urban habitats serve as rest sites (12.8%) and in winter period these biotopes provide favourable conditions for 13.6% of bird species.

The most abundant and diverse avifauna lives in the green areas of the city. Thus, 73 species were registered in the park “La Izvor”, 62 species in the Botanical Garden, and the lowest species number (16 species) – in central public Garden “Ștefan cel Mare” (BOGDEA et al., 2008; VASIŁAȘCU, 2008). This situation is caused by its location in the city centre, low surface, high degree of disturbance, poor vegetation structure, absence of paludous biotopes. Therefore, according to its birdfauna diversity, the public Garden “Ștefan cel Mare” is more similar to residential neighbourhoods than to other investigated parks. The comparative study of bird communities in various biotopes shows that at city outskirts can be met many species that are not observed in the central part – *Corvus corax*, *C. monedula*, *Jynx torquilla*, *Luscinia luscinia*, *Lanius collurio*, *Saxicola torquata*, etc. (VASIŁAȘCU & MUNTEANU, 2008). Birds are more often attracted by forest plantations merging with urban parks such as forest with a relatively high surface and larger variety of biotopes.

Table 2. Bird species recorded in Chișinău city ecosystems.

No.	Species	Species frequency				Phenology	
		A	R	F	FF	Chișinău city	R. Moldova
1	<i>Podiceps cristatus</i>	+				P	SV
2	<i>Ciconia ciconia</i>		+			P	SV
3	<i>Ardea cinerea</i>		+			P	SV
4	<i>Ixobrychus minutus</i>			+		SV	SV
5	<i>Nycticorax nycticorax</i>		+			P	SV
6	<i>Anas platyrhynchos</i>				+	PM	PM
7	<i>Aythya ferina</i>	+				P	SV
8	<i>Aythya fuligula</i>	+				WV	WV
9	<i>Larus ridibundus</i>			+		SV	PM

10	<i>Larus cachinnans</i>			+		PM	PM
12	<i>Accipiter nisus</i>			+		S	S
13	<i>Accipiter gentilis</i>			+		P	S
14	<i>Aquila pomarina</i>	+				SV	SV
15	<i>Milvus migrans</i>	+				SV	SV
16	<i>Circus aeruginosus</i>		+			SV	SV, RI
17	<i>Buteo buteo</i>		+			PM	PM
18	<i>Falco columbarius</i>		+			WV	WV
19	<i>Falco vespertinus</i>		+			P	SV
20	<i>Falco subbuteo</i>		+			P	SV
21	<i>Falco tinnunculus</i>			+		PM	PM
22	<i>Phasianus colchicus</i>			+		S	S
23	<i>Coturnix coturnix</i>		+			P	SV
24	<i>Perdix perdix</i>		+			S	S
25	<i>Gallinula chloropus</i>			+	+	SV	SV
26	<i>Fulica atra</i>				+	PM	PM
27	<i>Columba livia domestica</i>				+	S	S
28	<i>Columba palumbus</i>				+	SV	SV
29	<i>Sreptopelia turtur</i>		+			P	SV
30	<i>Sreptopelia decaocto</i>			+		S	S
31	<i>Cuculus canorus</i>			+		SV	SV
32	<i>Asio otus</i>			+		S	S
33	<i>Athene noctua</i>		+			SV	S
34	<i>Srix aluco</i>		+			S	S
35	<i>Scops otus</i>			+		S	S
36	<i>Apus apus</i>				+	SV	SV
37	<i>Alcedo atthis</i>			+		SV	SV
38	<i>Upupa epops</i>			+		SV	SV
39	<i>Dendrocopos syriacus</i>				+	S	S
40	<i>Dendrocopos major</i>				+	S	S
41	<i>Dendrocopos medius</i>		+			WV	S
42	<i>Dendrocopos minor</i>			+		S	S
43	<i>Dryocopus martius</i>	+				WV	S
44	<i>Picus canus</i>				+	S	S
45	<i>Jynx torquilla</i>			+		SV	SV
46	<i>Galerida cristata</i>				+	S	S
47	<i>Alauda arvensis</i>			+		SV	SV
49	<i>Hirundo rustica</i>				+	SV	SV
50	<i>Delichon urbica</i>				+	SV	SV
51	<i>Anthus trivialis</i>			+		SV	SV
52	<i>Anthus pratensis</i>			+		SV	SV
53	<i>Anthus campestris</i>			+		SV	SV
54	<i>Motacilla alba</i>				+	SV	SV
55	<i>Motacilla flava</i>			+		SV	SV
56	<i>Bombycilla garrulus</i>		+			WV	WV
57	<i>Lanius collurio</i>			+		SV	SV
58	<i>Lanius excubitor</i>	+				RWI	WV
59	<i>Oriolus oriolus</i>				+	SV	SV
60	<i>Sturnus vulgaris</i>				+	SV	PM
61	<i>Garrulus glandarius</i>				+	S	S
62	<i>Pica pica</i>				+	S	S
63	<i>Corvus monedula</i>			+		S	S
64	<i>Corvus frugilegus</i>				+	S	S
65	<i>Corvus corone cornix</i>				+	S	S
68	<i>Corvus corax</i>			+		S	S
70	<i>Troglodytes troglodytes</i>			+		WV	SV
71	<i>Acrocephalus arundinaceus</i>			+		SV	SV
72	<i>Acrocephalus scirpaceus</i>			+		SV	SV
73	<i>Sylvia atricapilla</i>			+		SV	SV
74	<i>Sylvia curruca</i>			+		SV	SV
75	<i>Sylvia borin</i>			+		SV	SV
76	<i>Sylvia communis</i>			+		SV	SV
77	<i>Phylloscopus collybita</i>				+	SV	SV
78	<i>Phylloscopus sibilatrix</i>			+		P	SV
79	<i>Phylloscopus trochilus</i>		+			P	SV
80	<i>Regulus regulus</i>			+		WV	PM
81	<i>Hippolais icterina</i>		+			SV	SV
82	<i>Muscicapa striata</i>			+		SV	SV
83	<i>Ficedula albicollis</i>				+	SV	SV
84	<i>Ficedula hypoleuca</i>		+			P	SV
85	<i>Ficedula parva</i>			+		SV	SV
86	<i>Phoenicurus phoenicurus</i>			+		SV	SV

87	<i>Phoenicurus ochruros</i>				+	SV	SV
88	<i>Saxicola rubetra</i>			+		SV	SV
89	<i>Saxicola torquata</i>			+		SV	SV
90	<i>Oenanthe oenanthe</i>			+		SV	SV
91	<i>Luscinia luscinia</i>			+		SV	SV
92	<i>Erithacus rubecula</i>				+	SV	PM
93	<i>Turdus merula</i>				+	SV,PM	PM
94	<i>Turdus philomelos</i>				+	SV	SV
95	<i>Turdus pilaris</i>			+		WV	WV
96	<i>Turdus iliacus</i>		+			WV	WV
97	<i>Parus major</i>				+	S	S
98	<i>Parus caeruleus</i>				+	S	S
99	<i>Parus palustris</i>			+		S	S
100	<i>Aegitalus caudatus</i>			+		WV	SV
101	<i>Remiz pendulinus</i>			+		SV	SV
102	<i>Sitta europaea</i>			+		S	S
103	<i>Certhia familiaris</i>			+		S	S
104	<i>Passer domesticus</i>				+	S	S
105	<i>Passer montanus</i>				+	S	S
106	<i>Fringilla coelebs</i>				+	PM	PM
107	<i>Fringilla montifringilla</i>			+		WV	WV
108	<i>Coccothraustes coccothraustes</i>				+	S	S
109	<i>Phyrrhula phyrrhula</i>			+		WV	WV
110	<i>Loxia recurvirostra</i>	+				WV	WV
111	<i>Emberiza citrinella</i>			+		S	S
112	<i>Emberiza calandra</i>			+		SV	SV
113	<i>Emberiza schoeniclus</i>		+			WV	PM
114	<i>Carduelis chloris</i>				+	S	S
115	<i>Carduelis carduelis</i>				+	S	S
116	<i>Carduelis cannabina</i>			+		S	S
117	<i>Carduelis spinus</i>			+		WV	WV

Legend: A – accidental, R – rare, F – frequent, FF – very common; SV – summer visitor, WV – winter visitor, S – resident, PM – partial migrant, P – passage, RWV – rare winter visitor.

During a year the bird biotopic distribution is changing. The domination degree of bird species decreases from the cold period toward summer, reaching its minimum in the nesting period, when bird distribution is more uniform. In post-breeding period and during autumn migration the dominance degree increases again, a fact connected to the accumulation of some species in flocks and their uneven distribution. In built zones a similar aspect of bird distribution is observed as in green areas, but with a distance between limit values. Here, the species diversity in prevernal period is minimal $SI - 3.66$, then increases to $SI - 5.99$ in serotinal period and insignificantly decreases in autumn period. The dominance index (Soerenssen coefficient) in bird population from building zones is characterized by very high values, even higher than in green areas. Nevertheless, the index dynamics remain the same – the highest in spring ($Sc - 0.27$) and the lowest in post-breeding period ($Sc - 0.17$). This fact is due to differentiated bird biotopic distribution, conditioned by a different degree of their ecological capacity, by ethological peculiarities, especially of the synanthropic species.

The diversity analysis of bird species regarding the degree of habitat anthropozization was performed according to MacArthur method (Fig. 3). This method proves that the species number and density (individuals/ha) vary depending on biotope conditions and on the influence of anthropogenic factors.

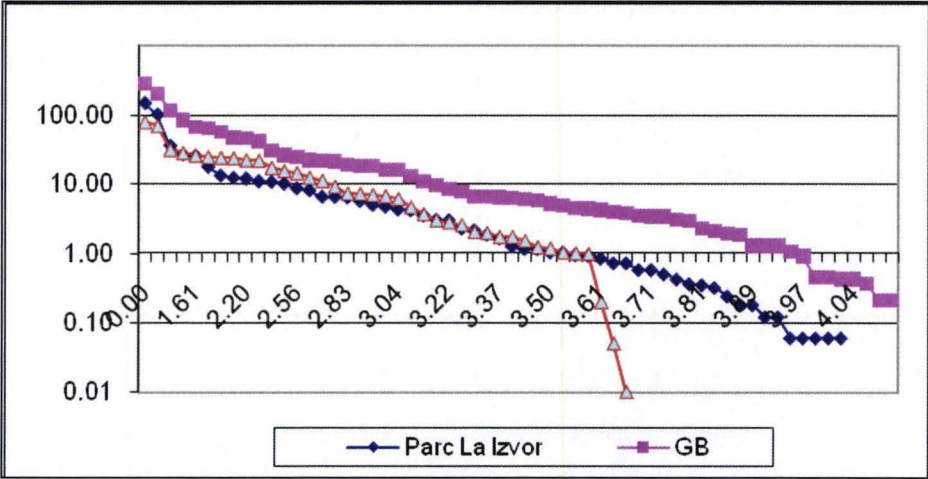


Figure 2. Comparative analysis of bird community diversity in natural, recreational and urban ecosystems (GB – Botanical Garden).

The species number and density is rather constant, but the natural biotopes can maintain a higher species density, due to homogeneous phytocenotic structure. The species number is higher in the forest park “La Izvor”, decreases in the Botanical Garden, in the forest belt “Ghidighici” and is the lowest in the shelter belt near Chetrosu locality.

Depending on preferences to different breeding sites the bird species registered within the studied ecosystems during nesting period were assigned to the following ecological groups:

- a) tree species breed in tree crown;
- b) shrub species breed on low height and build their nests in bushes or on undergrowth;
- c) soil species breed on soil surface, in galleries, or beside tree trunks;
- d) cavity species have a high plasticity, can breed on poles, in pipes, under house roof;
- e) building species connected to human localities and build their nests on buildings;
- f) hollow species nest in tree hollows. Some species are not able to build hollows themselves and use the existent ones;
- j) paludous species connected with water biotopes, breed in reedbeds.

Table 3. Bird distribution by ecological groups in breeding period (ind./km²).

Studied sector	Bird ecological groups according to nesting places						
	Tree	Shrub	Soil	Cavities	Buildings	Hollow	Reed
Park “La Izvor”	123.5	51.2	105	293	18.8	206	31.0
Park “Ștefan cel Mare”	140.00	30.00	40.00	580.00	80.00	100.00	0
5 floor buildings	85.76	19.6	0	437.50	49.09	76.47	0
Shelter belt	1252	841	180.0	-	-	312	-

The highest bird density was recorded in the species nesting in tree crowns (1.252 individuals/km²) from shelterbelts. The highest density of birds nesting in cavities and hollows was registered in the public garden “Ștefan cel Mare”, a situation that can be explained by the high number of hollow trees, lampposts, columns, poles and trays that provide suitable nesting places. The lowest value of this index (19.6 individuals/km²) was registered in a shrub group from the 5 floor building sectors, because of the general absence of shrub vegetation in these sectors.

The **amphibian and reptile fauna** in urban ecosystems of the city was studied during spring-autumn period. Thus, among reptiles 4 species were found, of which the pond turtle is a rare species, one snake and two lizard species (Table 4). The amphibian fauna is better represented, being registered 9 species, of which both newt species recorded on R. Moldova territory, 3 toad species, 3 frog species and the rare species common spadefoot (Table 4).

This rather high diversity of herpetofauna is due to the green sectors of the city, with relatively large surface, including the recreational parks Valea Morilor, Valea Trandafirilor, La Izvor, Valea Rîșcanilor, Dendrarium and the Botanical Garden, which provide favourable shelter and reproductive conditions for most of the reptiles and, especially, amphibian species. The amphibian tadpoles were found in the water basins from the above mentioned green areas of the city, on various stages of development.

Table 4. Spreading of herpetofauna species within the limits of Chișinău city.

Family	Species	Ecosystems				
		Forest	Green sectors	Paludous	Open biotopes	Buildings
Emydidae	<i>Emys orbicularis</i>	-	-	+	-	-
Lacertidae	<i>Lacerta agilis</i>	+	+	-	+	-
	<i>Lacerta viridis</i>	+	+	-	+	-
Colubridae	<i>Natrix natrix</i>	+	+	+	+	+
Salamandridae	<i>Triturus vulgaris</i>	+	-	+	-	-
	<i>Triturus cristatus</i>	+	-	+	-	-
Discoglossidae	<i>Bombina bombina</i>	-	-	+	-	-
Pelobatidae	<i>Pelobates fuscus</i>	-	+	+	-	+
Bufonidae	<i>Bufo bufo</i>	-	+	+	-	-
	<i>Bufo viridis</i>	-	+	+	+	+
Hylidae	<i>Hyla arborea</i>	+	+	+	-	+
Ranidae	<i>Rana esculenta</i>	-	-	+	+	-
	<i>Rana ridibunda</i>	-	-	+	+	-

The snakes are represented by one species – the grass snake *Natrix natrix*, which is common in all studied ecosystems. It is rather often met in Byc river meadow, which can be considered as the spreading ecological corridor for herpetofauna on the territory of the city. The lowest diversity was registered in the sectors with buildings, where only the green toad can survive, mostly due to its nocturnal way of life. The industrial zone as well as the small green squares does not provide any favourable conditions for herpetofauna existence.

The terrestrial vertebrate fauna in urban ecosystems of Chișinău city has a huge importance. It was grouped in the following categories: economically important species (115), among which regulatory species (98), game species (12) and invasive species (5).

CONCLUSIONS

The urban fauna of Chișinău city and its surroundings consists of 39 mammal species, 117 bird species, 4 reptile species and 9 amphibian species, among which 4 species are included in the Red Book of Moldova and 7 are rare species. The mammal fauna of the city is rather rich, being registered species from the orders Insectivora (6 species), Chiroptera (9 species), Rodentia (16 species), Lagomorpha (1 species), Carnivora (5 species) and Artiodactyla (2 species).

The most suitable biotopes for small mammal fauna are those areas which are similar to natural ones: forests, shelter belts and landscape parks situated at city limits, as well as various types of agrocoenosis. The diversity index of small mammal communities in various types of ecosystems is rather high and varies from 0.99 in grasslands and 1.77 in shelter belts.

Among bird species phenological categories the most representative is the Summer guest group with 47% in the republic and 38.4% in Chișinău city. The domination degree of bird species decreases from the cold period toward summer, reaching its minimum in the nesting period, when bird distribution is more uniform. In post-breeding period and during autumn migration the dominance degree increases again, a fact connected to the accumulation of some species in flocks and their uneven distribution. The dominance index in building zone is higher than in green sectors, being the highest in spring (0.27) and the lowest in post-breeding period (0.17), which show the differentiated biotopic distribution of birds within the city.

The rather high diversity of herpetofauna is due to the green sectors of the city, with relatively large surface, which provide favourable shelter and reproductive conditions for most of the reptile and, especially, amphibian species. The lowest diversity was registered in the sectors with buildings, while the industrial zone, as well as small green squares do not provide any favourable conditions for herpetofauna existence.

Note

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ARE HUMAN SETTLEMENTS ECOLOGICAL SYSTEMS?

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Abstract. Even though “human ecology” is an old and often used concept, many debates are present in the ecological literature whether human settlements are ecological systems or not. The paper analyses the evolution, structure and functions of human settlements in relationship to their scale. The results indicate that, despite of the altered structure and functions resulting from the presence and activities of the dominant human species, human settlements are ecological systems, ranging based on their size from “ecosystems” to “complexes of ecosystems” (landscapes).

Keywords: urban ecosystem, functional structure, biodiversity, human species, scale.

Rezumat. Sunt așezările umane sisteme ecologice? Deși termenul „ecologie urbană” datează de ceva vreme și este des folosit, în literatura de specialitate din domeniul ecologiei sunt prezente multe dezbateri privind posibilitatea de a considera așezările umane sisteme ecologice. Lucrarea analizează evoluția, structura și funcțiile așezărilor umane în funcție de scara acestora. Rezultatele arată că, în pofida modificării structurii și funcțiilor datorită prezenței și activității speciei umane dominante, așezările umane sunt sisteme ecologice, situate în funcție de mărime pe nivelul ecosistemelor sau complexelor de ecosisteme.

Cuvinte cheie: ecosistem urban, structură funcțională, biodiversitate, specia umană, scară.

INTRODUCTION

Analysing different definitions of “systems”, it can easily be seen that the concept describes a sum of elements working together as a whole (BOTNARIUC & VĂDINEANU, 1982); the elements are objects and their relationships (IANOȘ & HELLER, 2006). Other authors consider that systems are functional structures (IANOȘ, 2000; VĂDINEANU, 1998, 2004; PETRIȘOR, 2008). The later view expands the first one showing that the structure (interrelated elements) is adapted to its functions in a double sense: functions modify the structure, and a certain structure can carry only one or more specific functions (PETRIȘOR, 2011). This feature characterizes mechanical systems (e.g., a car must have a certain structure in order to function as a vehicle), living systems and other systems too. In ecology, Tansley’s definition (TANSLEY, 1935) identifies the two components of an ecosystem: the living component (called in the British literature “biocoenosis” and in the American one “community”) and the non-living component (biotope).

While “urban ecology” is said to trace its roots in the 50’s, but its first dated use was during the 1968 symposium “Challenge for survival in megalopolis” (PETRIȘOR, 2008). Since ecology embraced the systemic theory, urban ecology followed the same pattern and authors started using the term “urban ecosystem”. In 1997, a new journal, “Urban Ecosystems”, was started in the Netherlands. The first Editorial defines the urban ecosystem as a particular type of ecosystem (based on Tansley’s view), where man is the “*keystone species controlling ecosystem structure and function*” (WALBRIDGE, 1997).

The dominant presence of humans in their settlements, and the control exercised over the structure and functions of ecosystems made many authors reluctant in accepting the fact that human settlements are ecosystems (METZGER, 1994; MCINTYRE et al., 2000; REES, 2003; PICKETT & GROVE, 2009; COLLINS et al., 2012), while others seem to embrace the new concept (CRISTEA & BACIU, 2000; EUROPEAN ENVIRONMENT AGENCY, 2010). One of the underlying causes could be the lack of interest for carrying out research in inhabited areas (MARRIS, 2009; CORBYN, 2010; COLLINS et al., 2012). Other authors underline structural differences, particularly related to biodiversity (CRISTEA & BACIU, 2000; SAVARD et al., 2000; EUROPEAN ENVIRONMENT AGENCY, 2010; ȘUSTEK, 2011, 2012; RIDICHE & BĂLESCU, 2012) or altered biotope conditions due to human activities (GAVRILESCU, 2011; CORNEANU et al., 2012), functional differences (BOLUND & HUNHAMMAR, 1999; CRISTEA & BACIU, 2000; DECKER et al., 2000; LUNDHOLM, 2006), or scale (METZGER, 1994; SAVARD et al., 2000; CLERGEAU et al., 2006).

Especially in regard to the scale, based on Gaia theory (LOVELOCK, 1979), other authors assimilate the city, despite of its size, with an organism, and discuss about the “urban metabolism” (DECKER et al., 2000; CRĂCIUN, 2008; GOLUBIEWSKI, 2012), up to identifying “normal” and “pathological” forms of it (STAN, 2011). At the opposite pole, other authors consider that cities are situated at the level of “complexes of ecosystems” or “landscapes” (METZGER, 1994; CLERGEAU et al., 2006; PETRIȘOR & PETRIȘOR, 2008; PETRIȘOR, 2010; 2011).

The aim of this study is to discuss the structure and functions of human settlements from an ecological perspective, in order to see whether they can be assimilated to ecological systems.

EVOLUTION

During the historical evolution, human settlements were first similar to what we call “rural” nowadays. Gradually, some of them became “urban areas”. Therefore, the anthropization process consists of a first transformation of natural systems into rural systems, and later into urban systems. The presence of humans is more intense in cities.

resulting into additional elements that exacerbate the difference between natural and man-dominated systems along the gradient natural – rural – urban; for this reason, the conceptual model displayed in figure 1 shows a transversal line symbolizing the balance between natural and anthropic elements, leaning towards the latest in urban systems.

In this context, it is noteworthy mentioning that the definition of “urban” areas has a different meaning for ecologists, economists, sociologists, psychologists or planners (MCINTYRE et al., 2000); in general, the United States definitions are based on the density of human population, while the European ones focus on key indicators (such as educational or cultural infrastructure, but also from other social or economic areas) (PETRIȘOR, 2008).

During the process, man-dominated systems couple functionally to the natural ones. Resources are taken directly or from agro-ecosystems, a component of the socio-economic system, using technology. Energy is present in fertilizers, pesticides, soil and green space works. Humans change the structure of geographic spaces. Altered biogeochemistry and loss of biodiversity decrease the stability of natural systems and increased their dependence on the man-dominated ones (COLLINS et al., 2012). The process is assessed using the concept of eco-energy – initial energy, before conscious human interventions (IANOȘ, 2000); the concentration of population and economic activities consume primary eco-energies as the level of anthropization and complexity of geosystems increase. Eco-diversity and geodiversity increase at the expense of biodiversity (IANOȘ et al., 2011; PETRIȘOR & SÂRBU, 2010).

STRUCTURE

Even though the structure of natural, rural and urban systems does not differ in terms of the names of components, detailed variation is visible along gradients of anthropization (ȘUSTEK, 2011; 2012). In the introductory section, the living component of an ecosystem was called in the British literature “biocoenosis” and in the American one “community”. The latter term can produce confusions, especially when authors discuss about “mammal communities” or “bird communities”. The biocoenosis consists of all plant and animal species of an ecosystem (BOTNARIUC & VĂDINEANU, 1982); “community” can be used equivalently or in order to refer to a specific group. Especially in man-dominated systems, fragmentation, loss of biodiversity and functional disturbances induced by human activities determine the assembly of species to work as a group of “communities” instead of being a whole.

The overall biodiversity of species is lower than in natural systems, resulting into shorter food webs, and into the fact that man-dominated systems are “incomplete” and the reduced biodiversity prevents the specialization of its components (PETRIȘOR, 2008). However, some groups (particularly invertebrates) are favoured. Man replaces species in time introducing new ones or eliminating the existing ones directly or indirectly; as a result, the entire biocoenosis is changed over longer periods. Researchers have found a variety of niches and habitats even in urban areas (PETRIȘOR, 2008; 2010). They are inhabited by species seeking for human habitats – so-called hemerophilous species, including the ones that are found only in these areas (synanthropic), indifferent to the presence of humans (hemerodiaphore) (NOBLET, 1994, 2005; PETRIȘOR, 2008, 2010). From a different viewpoint, man-dominated systems include many ubiquitous and opportunistic species (the latter can be eventually favoured by shifting conditions due to human activities), and to a lesser extent by random species (CLERGEAU et al., 2006; PETRIȘOR, 2008, 2010). In addition to them, people bring allochthonous species, some even invasive (PETRIȘOR, 2008; COLLINS et al., 2012), and proliferate the domestic species (PETRIȘOR, 2008). Most often, the species characterizing natural systems from the same region are confined to the green spaces (PETRIȘOR, 2008; EUROPEAN ENVIRONMENT AGENCY, 2010). In a spatial perspective, the type of biodiversity (α , β etc.) can be correlated with the size of the human settlement and described in relationship with the diversity of habitats, land use or cover, biogeographical region, etc. (PETRIȘOR, 2012a). However, if biodiversity is diminished, the diversity of man-generated structures is increased with the level of anthropization.

The biotope is altered too. Over long periods of time, even the geography is changed by modifying water courses and through the pressure exercised by the increased mass of constructions. The soil is removed or covered by asphalt, or, if it exists, does not have too much to offer to vegetation (PETRIȘOR, 2008). Microclimate is affected especially by urban areas, which become heat islands, particularly during the warm season (CHEVAL et al., 2009; EUROPEAN ENVIRONMENT AGENCY, 2010; COLLINS et al., 2012) due to the alteration of biogeochemical cycles, such as the water circuit discussed in the next section. However, these changes are the least evident. What becomes obvious in man-dominated system is the presence of infrastructure (built capital, physical capital) and pollutants (METZGER, 1994; REES, 2003; PETRIȘOR, 2008, 2010; PICKETT & GROVE, 2009; GAVRILESCU, 2011; CORNEANU et al., 2012). All these are influenced by what could be called the “human mind”, and assessed by psychological, social, anthropological, cultural structures, resulting into the socio-economic, political, legislative and administrative conditions that set their fingerprint over the layout and operation of human settlements (BOȘTENARU, 2005; PETRIȘOR, 2008, 2010; PICKETT & GROVE, 2009).

The separation between man-dominated systems and the adjacent ones is naturally done by ecotone areas. Researchers have identified urban fringes as having the same function (STAN, 2009; COLLINS et al., 2012). However, based on its social analysis, other authors consider that urban fringes have a separating role instead of a joining one, and certainly do not account for the productivity of the two ecosystems (PETRIȘOR, 2012b).

Figure 1 displays the components of human settlements in this view, as well as their connections. Based on the definition of territorial systems (IANOȘ, 2000) and ecological standpoint (TANSLEY, 1935; BOTNARIUC & VĂDINEANU, 1982; VĂDINEANU, 2004; PETRIȘOR, 2008, 2010, 2011), the figure has four areas, corresponding to the division between “natural” vs. “man-dominated” and “living” vs. “non-living”.

FUNCTIONS

Again, in theory man-dominated systems carry out the same functions as the natural ones: biogeochemical cycles and self-regulation. Nevertheless, they are radically changed. Biogeochemical cycles are disturbed, often resumed to a straight, unidirectional linear flow (CRISTEA & BACIU, 2000; PETRIȘOR, 2008). Some of the examples relate to the water circuit: in cities, water reaches the sewerage system, and is taken outside; consequently, urban planners find a strong need to create artificial water bodies or courses, or at least fountains (CRISTEA & BACIU, 2000; DECKER et al., 2000). Also, the input of natural energy is diminished due to the scarcity of primary producers. Consequently, man-dominated systems need external matter and energy sources, found in the natural ones (CRISTEA & BACIU, 2000; DECKER et al., 2000). From this perspective, they become “energetic parasites” of these systems (VĂDINEANU, 2004; PETRIȘOR, 2008). The dissipative character is more evident in man-dominated system than in the natural ones, due to the structuring interventions of humans (REES, 2003; PETRIȘOR & SÂRB, 2010).

Moreover, the food levels can be seen as part of an entire hierarchy of levels, starting with the traditional trophic ones, derived from physical consumption of food, and continuing with technotrophy (consumption of energy and resources by technology) and nootrophy (their consumption in the activities supporting human development, such as research, education, management, administration etc.) (IANOȘ, 2000; REES, 2003; PETRIȘOR, 2011).

Self-regulation is inhibited by the loss of biodiversity (CRISTEA & BACIU, 2000; EUROPEAN ENVIRONMENT AGENCY, 2010) and, in general, by the control exercised by humans. If the evolution of general systems is described by concepts like “succession” (BOTNARIUC & VĂDINEANU, 1982; DECKER et al., 2000; MCINTYRE et al., 2000), “panarchy” or “adaptive cycles” (HOLLING, 2004; VĂDINEANU, 2004), the model proposed for the human settlements is a spiral, suggesting the fact that humans change the characteristics of their system through each intervention (IANOȘ et al., 2011; PETRIȘOR, 2011). Nevertheless, from the succession standpoint, man-dominated systems are young, immature (PETRIȘOR & IANOȘ, 2011).

Functions are also affected by the action of natural laws. One of the limiting ones is the “minimal law”, meaning that many parameters required for the normal life of species are reduced to a minimum and act as constraining factors. Mitscherlich’s law, describing the decrease in intensity of the favourable action of some factors as their dosage increases, explains the need to use extra-energy in order to maintain natural areas like urban green spaces (PETRIȘOR, 2008).

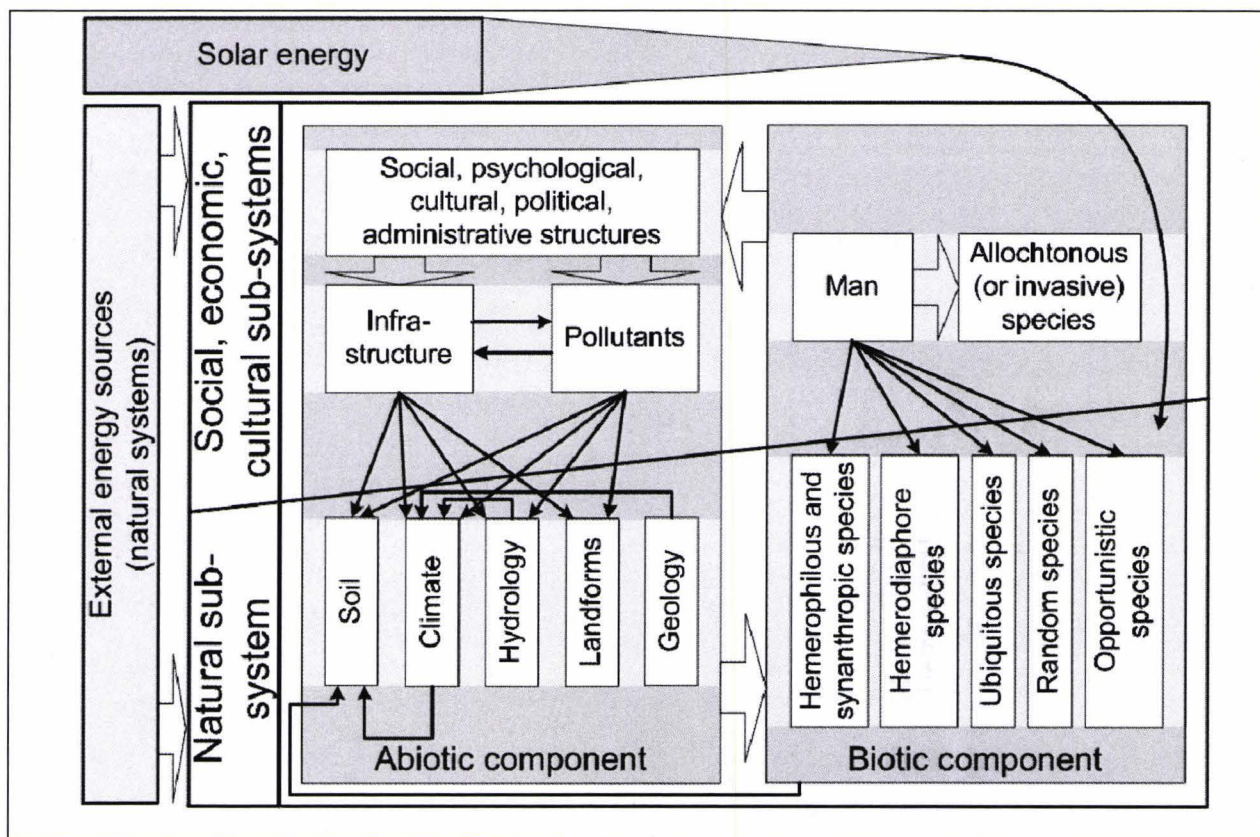


Figure 1. Conceptual model of the structure of man-dominated systems in relationship to their functions.

Last but not least, specific functions oriented by the role of human settlements (military, commercial, capital etc.) influence their structure and functions seen from an ecological perspective, resulting into diminishing or favouring some of them, but also in increased pollution (PETRIȘOR, 2008; PEPTENATU et al., 2010, 2012), depending on the attitude towards the environment (IANOȘ et al., 2009).

Figure 1 displays the functional characteristics of human settlements, with a particular focus on energy. The focus is determined by the relationship between energy, land use, climate change and composition of biocoenoses, and its consequences over the planning process (DALE, 1997; BOLUND & HUNHAMMAR, 1999; DALE et al., 2009, 2011; MEIȚĂ et al., 2011; PETRIȘOR et al., 2011; PETRIȘOR, 2012a). Land cover refers to a biophysical interpretation of what lies on the ground surface (JENSEN, 2000), while land use indicates its use by human communities or a more detailed classification of natural systems (PETRIȘOR et al., 2010).

SCALE

Apart from the diversity of habitats, human settlements are, from the standpoint of land cover and use, a mosaic of vegetated areas (pastures within rural areas, green spaces within the urban ones), semi-natural areas (parks, green spaces, encroached portions of natural systems), human-built infrastructure and occasionally other elements. For this purpose, some authors suggest that, on a scale starting with ecosystems, continuing with the complex of ecosystems or landscapes and ending with the planetary system – ecosphere, large human settlements, especially cities, through their complexity exceed the first level approaching the second (METZGER, 1994; BOLUND & HUNHAMMAR, 1999; CLERGEAU et al., 2006; PETRIȘOR, 2010, 2011).

At the next spatial level, man-dominated system and adjacent infrastructure (roads) form the socio-economic system, expanding over the natural ones. The characteristics of its dynamics are: (1) spatial expansion by substituting, simplifying and fragmenting natural systems; (2) increase of inner complexity and fluxes of resources taken from natural systems and pollutants dispersed into them; (3) linearization of biogeochemical cycles; (4) accumulation of waste; and (5) regionalization and globalization through increased connectivity (VĂDINEANU, 1998).

CONCLUSIONS

The paper aimed to answer the question whether man dominated systems, particularly human settlements, can still be considered ecological systems. Many differences have been found between the extremes of an evolution scale starting with natural system, including the rural systems and ending with the urban systems, in order to reflect the degree of anthropization and consumption of natural energy and resources, but also between the three levels. The differences are found in their structure (loss of biodiversity and presence of man-induced infrastructure and pollutants in man-dominated systems) and functions (linearization of biogeochemical cycles, increased dependence of human activities, energy and resources of other systems, and low self-regulation capacity of man-dominated systems). Despite of these differences, the essential components of ecological systems are still present. Based on this, human settlements can be considered ecological systems with a different homomorphous model, situated, depending on their scale, at the level of “ecosystems” or “complexes of ecosystems” (landscapes).

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CONSIDERATIONS ON THE CLIMATIC CONDITIONS IN OLTENIA DURING THE WARM WINTER OF 2012-2013

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Abstract. The paper analyses the climatic evolution during the warm winter of 2012-2013, caused by the radical weather change occurred on December 24, 2012. The excessively droughty autumn of 2012, and the cool December, was followed by a warm January and February. There was only one cold interval, December 10–23, 2012, which caused agricultural material damages, because autumn crops were not prepared for wintering conditions. The analysis is a continuation of some extended studies on the growing climatic oscillations and risks as a consequence of the climatic variability increase in the south-west of Romania. The paper is useful for a broad category of specialists interested in the climatic, climatic risks and agroclimatic field.

Keywords: monthly temperature means, Hellman criterion, severe winter phenomena, vegetative processes.

Rezumat. Considerații privind condițiile climatice în Oltenia din iarna caldă 2012-2013. În lucrare este analizată evoluția climatică din iarna caldă 2012-2013, determinată de schimbarea radicală a vremii survenită în data de 24 decembrie 2012. După toamna excesiv de secetoasă 2012, după luna decembrie în ansamblul său răcoasă au urmat lunile ianuarie și februarie calde. S-a înregistrat un singur interval cu vreme rece 10-23 decembrie 2012 care a determinat unele pagube materiale în agricultură, ca urmare a surprinderii culturilor de toamnă neadaptate la condițiile de iarnă. Analiza este o continuare a unor studii extinse privind oscilațiile și riscurile climatice tot mai numeroase ca urmare a creșterii variabilității climatice în sud-vestul României. Lucrarea este utilă unei categorii largi de specialiști interesați de domeniul climei, riscului climatic și agroclimatic.

Cuvinte cheie: medii lunare de temperatură, criteriul Hellmann, fenomene de iarnă severă, procese vegetative.

INTRODUCTION

The extremely warmish summer and the droughty and extremely warmish autumn of 2012 in the first half was followed by the early and cold winter of 2012-2013 in December. Then, the radical change of the thermal regime, after the precipitations on December 5 – 6, 2012, starting with December 7, 2012 when the daily temperature means became negative, marked a sudden weather change, during which not only in Romania, but also in the entire European continent, the winter thermal regime arrived, and the snow layer extended in most of Europe. This type of climatic evolution is an extremely dangerous climatic risk, because most of the population was caught unprepared from different points of view by the fast setting of the excessively cold weather. In consequence, there have been significant material damages, especially in agriculture, caused by the early low temperatures and the frost at the soil' surface. The interval December 7-31 clearly stands out from most of the winter as a thermal and excessive anomaly, which occurred fast. Then, even from the beginning of January, the air temperature means became positive on long intervals of time.

The paper is a continuation of some extended studies on the growing climatic oscillations and risks as a consequence of the climatic variability increase in the south-west of Romania, as well as of their effects on the environment, society and bioclimate in general (BOGDAN et al., 2008, 2010; BOGDAN & MARINICĂ, 2009; MARINICĂ & CHIMIȘLIU, 2008; MARINICĂ et al., 2010, 2011, 2012; MARINICĂ & MARINICĂ, 2012).

DATA AND METHODS

For this paper, we analysed the data from Oltenia MRC¹ Archive, the results of the daily processing with special software from the weather forecast, the current maps from the operative activity, and those provided by the analysis and forecast international centres and NAM Bucharest (National Administration of Meteorology). We used the facilities provided by the Office for drawing the tables and charts.

The paper analyses the climatic conditions during the winter of 2012-2013, on the basis of the thermal and pluviometric regime of December 2012, January and February 2013 and the thermal and pluviometric regime on the whole of the winter of 2012-2013.

RESULTS

1a. The thermal regime of December 2012

In December 2012, *air temperature means* were comprised between -3.0°C in Voineasa and 0.1°C in Drobeta Turnu Severin, and their deviations from the multiannual means were comprised between -3.3°C in Bechet and -0.6°C in Băcș. According to Hellmann criterion, the thermal time type in December 2012 at the meteorological stations in

¹ MRC= Regional Meteorological Center Oltenia

Oltenia was comprised between cold (CL)² in the extreme south in Bechet, in the south Carpathian area in Apa Neagră and in the intra-Carpathian depression Voineasa and normal (N) in Râmnicu Vâlcea, with in the Olt Couloir and in the high mountainous area in Parâng. The monthly temperature mean for the entire region was -1.4°C, and its deviation from the multiannual mean was -1.3°C, which classifies December as a cool month for the entire region (Table 1).

The monthly minimum air temperatures were comprised between -19.6°C in Târgu Logrești and Apa Neagră registered on December 14 and -9.5°C registered in Drăgășani on the same date, and in the high mountainous area the minimum thermal value was -23.5°C in Obârșia Lotrului. All the monthly minimum thermal values were registered in the middle of the second decade in the interval December 14-15.

The monthly minimum thermal values at the soil surface were mostly registered in the middle of the second decade of the month and were comprised between -12.1°C in Drobeta Turnu Severin on December 15 and -25.0°C in Apa Neagră on December 14. In the interval December 4-31, the phenomenon of frozen soil occurred. These values show that, in most of Oltenia, the critical biological limits of plants' resistance to the air temperature regime (-20 -15°C) were exceeded, the lowest values being registered in the Getic Plateau and the Subcarpathian area.

The maximum thermal values were comprised between 8.3°C in Voineasa registered on December 1 and 14.1°C in Caracal on the same date, and in the mountainous area the maximum thermal value was 14.2°C in Parâng registered on December 25 (Table 1).

Table 1. The air thermal regime in Oltenia and the minimum thermal values at the soil surface in December 2012.

Meteorological Station	Hm	NXII	M	AT=M-N	CH	MinT		MaxT		MinsoilT	
						(°C)	Date	(°C)	Date	(°C)	Date
Drobeta Turnu Severin	77	1.4	0.1	-1.3	CO	-10.1	14	11.8	1	-12.1	15
Calafat	66	1.0	-0.8	-1.8	CO	-12.7	15	12.1	1	-19.0	14
Bechet	65	0.4	-2.9	-3.3	CL	-16.9	14	13.5	1	-20.5	14
Băilești	56	0.4	-1.3	-1.7	CO	-14.5	15	11.8	1	-19.5	14
Caracal	112	-0.1	-1.2	-1.1	CO	-13.4	15	14.1	1	-16.0	15
Craiova	190	0.1	-1.1	-1.2	CO	-13.5	15	13.4	1	-15.0	15
Slatina	165	0.3	-1.0	-1.3	CO	-14.5	14	13.8	1	-20.1	14
Băcleș	309	-0.4	-1.0	-0.6	N	-9.9	14	11.0	1.26	-	-
Târgu Logrești	262	0.1	-1.5	-1.6	CO	-19.6	14	13.7	1	-21.1	15
Drăgășani	280	0.6	-0.4	-1.0	CO	-9.5	15	13.4	1	-14.8	14
Apa Neagră	250	0.1	-2.1	-2.2	CL	-19.6	14	12.5	1	-25.0	14
Târgu Jiu	210	0.1	-1.0	-1.1	CO	-15.4	14	13.3	1	-21.2	13
Polovragi	546	0.1	-1.0	-1.1	CO	-15.2	13	12.8	26	-22.3	14
RâmnicuVâlcea	243	0.5	-0.2	-0.7	N	-12.2	14	13.8	1	-22.6	13
Voineasa	573	-1.9	-3.0	-1.1	CO	-17.4	14	8.3	1	-	-
Parâng	1585	-3.7	-4.0	-0.3	N	-13.6	13	14.2	25	-	-
Media Oltenia	-	-0.1	-1.4	-1.3	CO	-14.3		12.7		-19.2	-
Obârșia Lotrului	1348	-4.9	-5.7	-0.8	N	-23.5	14	4.3	1	-	-

(Source: processed data)

In figure 1 there are represented the air temperature variation, the daily means, daily minimum and maximum temperatures mean, calculated for the entire region in December 2012. There were registered 23 days in which the daily means were negative (in the intervals December 6-16, December 19-26, December 28-31) and only 8 days with positive means. There were registered 22 days with air and soil temperatures of 0°C (frost and thaw phenomena).

The number of frost units in December 2012 was comprised between 33.9 in Drobeta Turnu Severin in the west of the region and 111.5 in Bechet in the south of the Romanian Plain, designating a soft month on average from an agrometeorological point of view.

The number of frost units³ was comprised between 0 on extended areas in Oltenia and 7.6 in Târgu Logrești, designating an insignificant phenomenon from an agrometeorological point of view. The sums of the active daily average temperatures were comprised between 19.0°C in Voineasa and 42.7°C in Râmnicu Vâlcea, and in the mountainous area 17.4°C in Parâng.

² The thermal time type according to Hellmann criterion are: excessively warm (EW), very warm (VW), warm (W), warmish (WS), normal (N), cool (CO), cold (CL), very cold (VC) and excessively cold (EC).

³ The degree of winter bitterness in agrometeorology (winter type) classifies according to the sum of frost units (Σ differences between the daily minimum temperature values <-15°C and the agroclimatic critical threshold of -15.0°C, in the interval December - February). Therefore, a frost unit is the difference of 1°C between the critical threshold of -15.0°C and an air minimum thermal value ≤ -15°C (for example for T min = -16.0°C then the difference -15.0°C - (-16.0°C) = 1, namely a frost unit. (SANDU et al., 2010).

1.b. The pluviometric regime of December 2012

The monthly quantities of precipitations registered in December 2012 were comprised between 31.4 l/m² in Băilești in the Oltenian Plain and 118.0 l/m² in Apa Neagră in the Subcarpathian area; the percentage deviations from the normal values were comprised between -32.9% in Băilești and 50.3% in Polovragi in the Subcarpathian depressions area, and in the submountainous area -27.5% in Parâng (Table 2). According to Hellmann criterion, the pluviometric time type at the meteorological stations in Oltenia was comprised between very droughty (VD) in Băilești and excessively rainy (ER) in Polovragi.

Table 2. Quantities of precipitations registered during the winter of 2012-2013 (Σ), in comparison with the normal values.

Meteorological Station	Hm	December 2012				January 2013				February 2013				Winter 2012 – 2013			
		ΣII	N	Δ%	CH	ΣI	N	Δ%	CH	ΣII	N	Δ%	CH	ΣW	N	Δ%	CH
Drobeta Turnu Severin	77	69.2	61.2	13.1	LR	45.7	51.4	-11.1	LD	141.2	47.9	194.8	ER	256.1	160.5	59.6	ER
Calafat	66	54.4	45.5	19.6	LR	27.7	40.4	-31.4	VD	78.5	38.0	106.6	ER	160.6	123.9	29.6	VR
Bechet	65	32.9	36.3	-9.4	N	18.5	33.5	-44.8	VD	38.8	34.8	11.5	LR	90.2	104.6	-13.8	LD
Băilești	56	31.4	46.8	-32.9	VD	34.4	38.5	-10.6	LD	57.3	36.1	58.7	ER	123.1	121.4	1.4	N
Caracal	112	34.5	39.5	-12.7	LD	20.2	34.7	-41.8	VD	51.4	34.5	49.0	VR	106.1	108.7	-2.4	N
Craiova	190	44.9	41.8	7.4	N	33.9	37.5	-9.6	N	61.0	30.4	100.7	ER	139.8	109.7	27.4	VR
Slatina	165	44.4	42.8	3.7	N	33.5	36.0	-6.9	N	57.6	38.4	50.0	VR	135.5	117.2	15.6	LR
Târgu Logrești	262	66.8	44.8	49.1	VR	36.5	35.9	1.7	N	77.2	41.0	88.3	ER	180.5	121.7	48.3	ER
Drăgășani	280	59.0	44.6	32.3	VR	41.2	34.1	20.8	R	57.0	35.4	61.0	ER	157.2	114.1	37.8	VR
Apa Neagră	250	118.0	82.3	43.4	VR	65.9	70.9	-7.1	N	200.1	66.4	201.4	ER	384.0	219.6	74.9	ER
Târgu Jiu	210	64.8	64.0	1.3	N	53.3	53.9	-1.1	N	115.6	52.0	122.3	ER	233.7	169.9	37.6	VR
Polovragi	546	84.3	56.1	50.3	ER	54.1	48.9	10.6	LR	60.7	48.4	25.4	R	199.1	153.4	29.8	R
Râmnicu Vâlcea	243	68.6	46.2	48.5	VR	48.4	35.5	36.3	VR	48.1	38.4	25.3	R	165.1	120.1	37.5	VR
Parâng	1585	39.5	54.6	-27.7	D	58.2	57.7	0.9	N	40.2	47.7	-15.7	LD	137.9	160.0	-13.8	LD
Media Oltenia	-	58.1	50.5	15.0	LR	40.8	43.5	-6.1	N	77.5	42.1	84.0	ER	176.4	136.1	29.6	VR

(Source: processed data)

The overall mean for the entire region of Oltenia was 58.1 l/m², and its percentage deviation from the normal value was 15.0%, which shows that for the entire region of Oltenia December 2012 was a little rainy month on average, thus confirming the interruption of the excessive drought from the autumn of 2012, in the first month of winter. However, due to the low thermal air regime and the poor water consumption of the vegetal canopy, the water soil reserve in the layer of 0-100 cm on December 28, 2012 was satisfactory (S)⁴ close to optimum and optimum, within the entire the territory of Oltenia.

In these conditions, the vegetative sleep continues in crops, fruit and wine growing species. Therewith, as a consequence of the drifting snow on fields with a discontinuous, superficial (below 10 cm) or even absent snow layer, of the low minimum air temperatures which were below the critical biological thresholds of plants` resistance (Tmin < -10-15-20°C), damages of the foliar apparatus occurred through scalds and scorches of the leaves margin.

The maximum snow layer thickness, for the entire winter of 2012-2013, was registered on December 12, 2012 after the abundant snowfalls in the interval December 11-12, 2012 (Fig. 2), when it was comprised between 11 cm in Halânga and 76 cm in Balta in Mehedinți County. Subsequently, the snow layer thickness has decreased, and on December 31, 2012 there was a snow layer in the Subcarpathian and mountainous area.

The snowfalls, winter thermal regime and wintry climatic phenomena started in the night of December 4/5, 2012, thus registering an *early winter* for Oltenia and a fast passing from positive to negative temperature values. This fast variation of the air and soil thermal regime caught unprepared for winter many crops, because the agricultural works of setting up the crops as well as the germination and spring processes occurred late as a consequence of the autumn excessive drought. Consequently, the most significant damages of crops and vineyards occurred in December, since January and February were warm.

2.a. The thermal regime of January 2013

The monthly thermal means were comprised between -2.6°C in Voineasa and +1.3°C in Drobeta Turnu Severin and Calafat, and the deviations from the monthly multiannual means were comprised between 1.2°C in Apa Neagră and 3.1°C in Polovragi.

According to Hellmann criterion, the thermal time types in January 2013 at the meteorological stations were comprised between warmish (WS) in the extreme south in Bechet, in the Subcarpathian depression area (Apa Neagră) and in the mountainous area and warm (W) in most part of Oltenia (Table 3).

The overall monthly mean for the entire region was -0.5°C, and its deviation from the normal mean was 2.3°C, which according to Hellmann criterion led to the conclusion that January 2013 was warm (W).

⁴ SP = pedological drought. SM = moderate drought. AS = almost satisfactory. ApO = almost optimum. AO = optimum (O) or very close of optimum (AO).

The monthly maximum air temperatures were mostly registered in the third decade of the month (January 22-31) and in the second decade (most of them on January 22) and were comprised between 7.2°C in Drăgășani and 13.5°C in Craiova, and the maximum temperatures mean was 11.3°C.

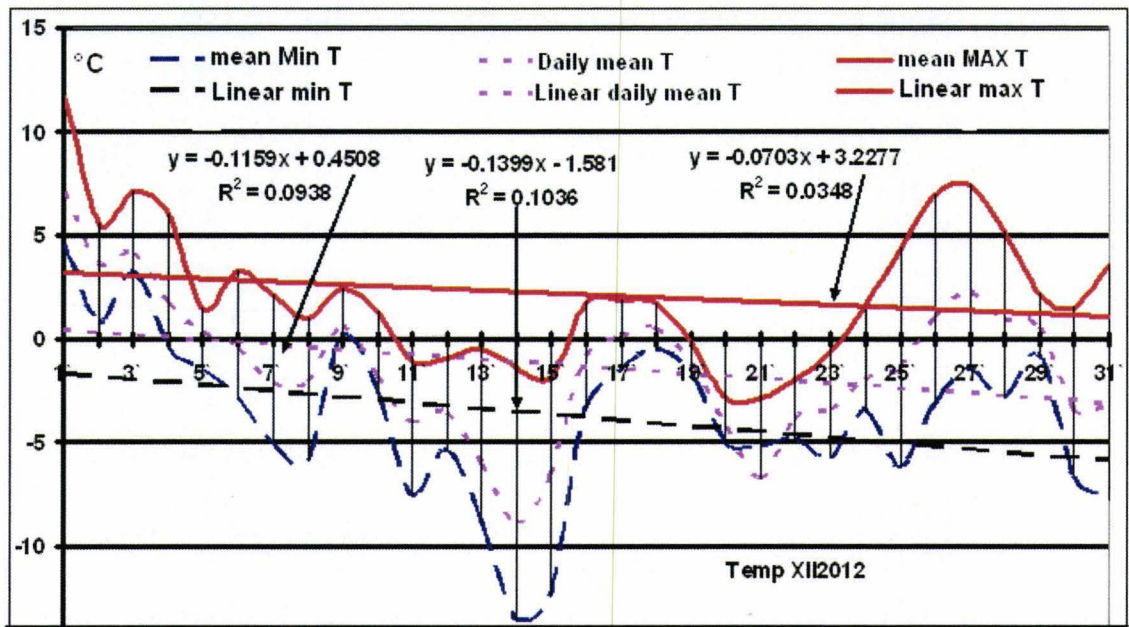


Figure 1. The air temperature variation, the daily means (daily mean T), daily minimum (mean Min T) and maximum temperatures mean (mean MAX T), calculated for the entire region in December 2012.

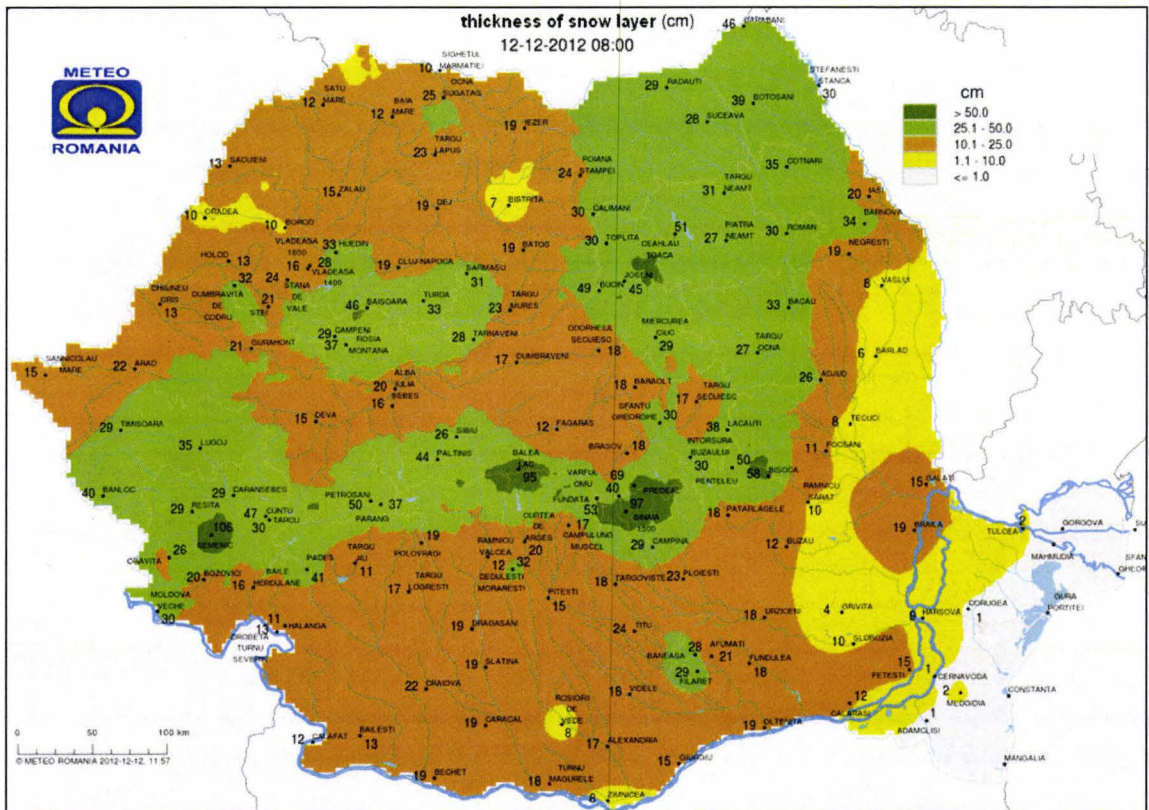


Figure 2. The maximum snow layer thickness in the winter of 2012-2013 registered on December 12, 2012 (according to NAM Bucharest).

The monthly minimum air temperatures were mostly registered in the first decade of the month, and in the south of Oltenia Plain in the last five days and were comprised between -15.8°C in Voineasa in the intra-Carpathian depression (registered on January 9) and -7.5°C in Calafat (on January 28), and in the mountainous area -16.0°C in Parâng (on January 8).

The monthly minimum thermal values mean was -11.0°C. The low thermal minimum temperatures were caused by the cold wave from the end of the first decade of January, and the cooling process started in the night of January 7/8, 2013 (Table 3 and Fig. 3).

The minimum temperature values at the soil surface were comprised between -17.6°C in Apa Neagră (registered on January 10) and -9.0°C in Craiova, which were registered in the same interval as the minimum thermal air values.

The monthly minimum temperatures mean at the soil surface, for the entire region was -12.2°C. There were registered 27 days when the air and soil temperature reached the critical threshold of 0°C and 27 days with frost and thaw, and the great number of days with average and maximum positive temperature values led to the slow continuation of the vegetative processes in certain periods.

Table 3. The thermal air regime and the thermal minimum temperatures at the soil surface in January 2013, in Oltenia.

Meteorological Station	Hm	NI	M	AT=M-N	CH	MinT		MaxT		MinsoilT	
						(°C)	Date	(°C)	Date	(°C)	Date
Drobeta Turnu Severin	77	-1.1	1.3	2.4	W	-7.6	9	11.9	31	-10.0	9
Calafat	66	-1.8	0.6	2.4	W	-7.5	28	11.7	31	-9.2	28
Bechet	65	-2.2	-0.3	1.9	WS	-12.6	28	12.4	22	-11.0	28
Băilești	56	-2.3	-0.2	2.1	W	-9.6	28	11.2	31	-14.5	28
Caracal	112	-2.9	-0.4	2.5	W	-9.2	10	13.2	22	-9.1	9
Craiova	190	-2.6	-0.3	2.3	W	-9.6	9	13.5	22	-9.0	9
Slatina	165	-2.4	-0.2	2.2	W	-10.4	10	12.7	22	-12.3	10
Băcleș	309	-3	-0.1	2.9	W	-9.5	9	11.1	22	-	-
Târgu Logrești	262	-2.7	-0.6	2.1	W	-12.8	8	11.6	22	-14.4	9
Drăgășani	280	-2.2	0.3	2.5	W	-9.7	10	12.3	22	-13.4	10
Apa Neagră	250	-2.6	-1.4	1.2	WS	-15.0	8	11.8	22	-17.6	10
Târgu Jiu	210	-2.6	-0.1	2.5	W	-10.1	9	12.3	31	-11.6	8
Polovragi	546	-3.2	-0.1	3.1	W	-10.8	10	10.5	3	-15.8	9
Râmnicu Vâlcea	243	-2.2	0.5	2.7	W	-9.8	9	12.7	22	-10.3	10
Voineasa	573	-4.7	-2.6	2.1	W	-15.8	9	7.2	4	-	-
Parâng	1585	-5.9	-4.5	1.4	WS	-16.0	8	4.6	21	-	-
Media Oltenia	-	-2.8	-0.5	2.3	W	-11.0		11.3	-	-12.2	-
Obârșia Lotrului	1348		-5			-23.1	8	4.5	22	-	-

(Source: processed data)

The negative temperatures and the frost at the soil surface, which occurred again, caused the vulnerability of plants to frost in the cultivated areas.

The frost was insignificant from an agrometeorological point of view. In January, the frost units⁵ were comprised between 66.4 in Drobeta Turnu Severin and 181.3 in Apa Neagră, and the mean for the entire region was 114.7, designating a “mild” month from an agrometeorological point of view.

The sums of the daily active temperature means were comprised between 12.6°C in Voineasa and 63.5°C in Drobeta Turnu Severin, while in the mountainous they are 3.6°C in Parâng.

2.b. The pluviometric regime of January 2013

The monthly quantities of precipitations were comprised between 18.5 l/m² in Bechet and 65.9 l/m² in Apa Neagră in the Subcarpathian area, and the percentage deviations from the multiannual means were comprised between -44.8% in Bechet and 20.8% in Drăgășani (Table 2). According to Hellmann criterion, the pluviometric time type at the meteorological stations in Oltenia was comprised between very droughty (VD) on extended areas in the Oltenia Plain (Calafat, Bechet and Caracal) and very rainy (VR) in the Olt Couloir in Râmnicu Vâlcea.

The quantities of precipitations mean for the entire region was 40.8 l/m², and its percentage deviation was -6.1%, which classifies January as a normal month (N) from a pluviometric point of view for the entire region.

There were registered 21 days with precipitations, 15 (71.4%) days with liquid precipitations and 8 days with snowfall⁶ representing 38.1%.

⁵ “Frost” units, a specific notion of agrometeorology, is calculated with the formula ΣTdaily mean <0°C, and their values are established for the entire cold season (1 November-31 March);
⁶ The number of days with sleet is added not only to the number of days with liquid precipitations, but also to the number of days with solid precipitations, according to the meteorological instructions.

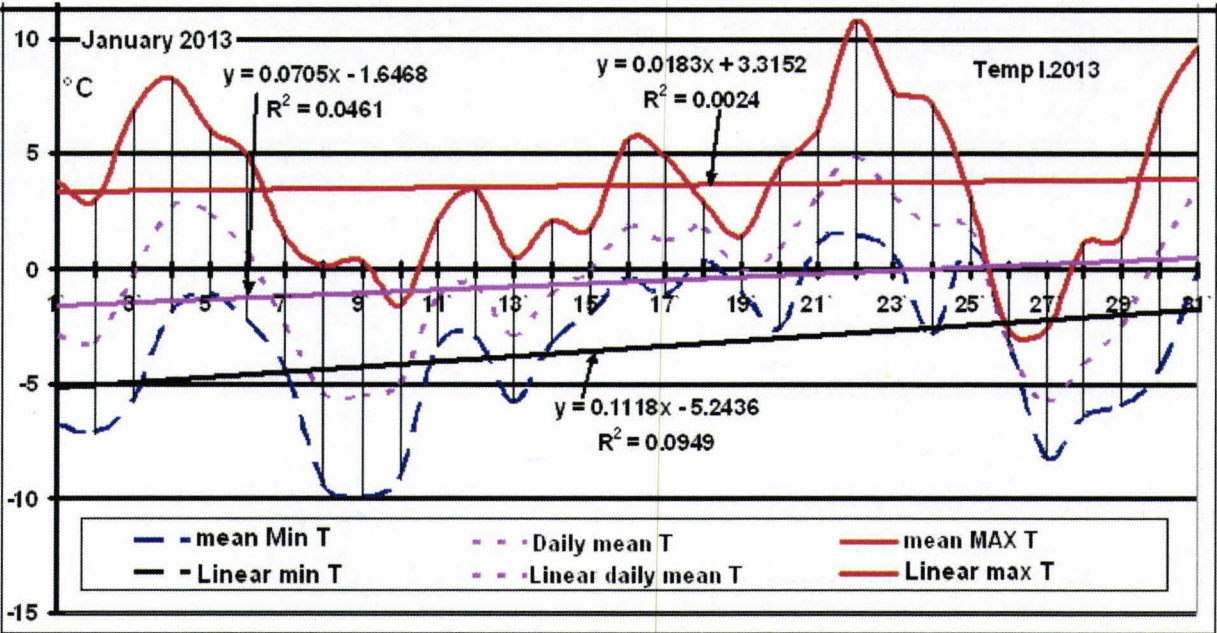


Figure 3. The air temperature variation, the daily means (Daily mean T), daily minimum (mean min T) and maximum temperatures mean (mean MAX T), calculated for the entire region in January 2013.

As climatic risk phenomena we record that on January 17, for the interval January 18, between 6 a.m. and 6 p.m., a yellow code warning for dangerous meteorological phenomena was remitted, related to significant quantities of precipitations in Mehedinți County, in the mountainous area of Gorj and the northern half of Vâlcea County, and for the interval January 24, 6 p.m. January 26, 8 p.m., for abundant rain, sleet and snowfall. Thus, in the aforementioned interval, in the south and south-east of the country there were significant precipitations, locally exceeding 25 l/square meters, predominantly in the form of rain in the southern half of Muntenia and Dobrogea, mixed in the first hours and then snowfall in the south of Banat, Oltenia and northern Muntenia (Fig. 4).

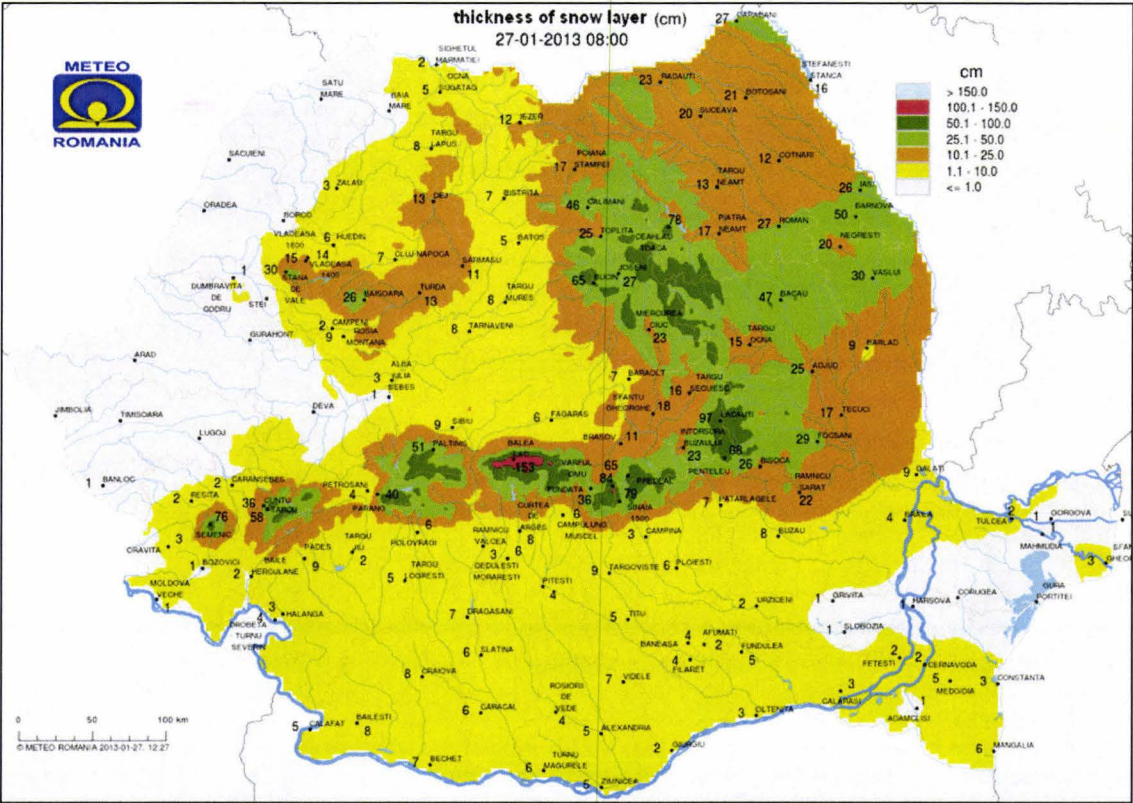


Figure 4. The maximum snow layer thickness in January 2013 registered on January 27, 2013 (according to NAM Bucharest).

On extended areas there was glazed frost. In all these regions there were unceasing wind gusts, with speeds exceeding 50 - 60 km/h. In the counties from the southern half of Moldavia, in the Curvature Carpathians area and Bucegi Mountains it snowed, the wind gust exceeding 70 km/h and there was a snowstorm, too.

The snow layer was absent in the interval January 1-25, in most of the region (excepting the mountainous and submountainous area) and its reduced thickness was formed beginning with January 28 and was comprised between 2 cm in Târgu Jiu 9 cm in Apa Neagră, and in the mountainous area 41 cm in Parâng (Fig. 4).

On January 31, the water reserve in the soil layer of 0-100 cm, was satisfactory, close to optimum and optimum, in all crop regions.

3.a. The thermal regime of February 2013

The monthly average air temperature values were comprised between 0.1°C in the intra-Carpathian Depression Voineasa and -3.5°C in Drobeta Turnu Severin in the western region, and their deviations from the multiannual means were comprised between 2.6°C in Voineasa and Râmnicu Vâlcea in the Olt Couloir. According to Hellmann criterion the thermal time types in Oltenia were comprised between warm (W) in most of the region excepting the mountainous area Obârșia Lotrului (Table 4), where the multiannual mean is a little significant, being calculated for a short series of data.

The monthly air temperature mean calculated for the entire region was 2.1°C, and its deviation from the multiannual monthly mean⁷ was 2.9°C, thus confirming the classification of warm month (W) for the entire region.

The minimum air temperature values were comprised between -12.6°C in Voineasa registered on February 11 and -2.5°C registered in Calafat on February 5, and the monthly minimum temperature mean was -6.0°C, higher than that of January.

The monthly maximum temperature values were mostly registered on 1 (excepting the mountainous and submountainous area), an atypical situation for February and were comprised between 8.3°C in Voineasa registered on February 25 and 14.1°C registered on February 1.

The maximum temperature values mean for the entire region was 11.5°C.

The daily maximum temperature values were positive in the entire region and in all days, excepting the mountainous area, which shows and confirms a warm weather in February.

Table 4. The air thermal regime in Oltenia and the minimum thermal values at the soil surface in February 2013.

Meteorological Station	Hm	NI	M	ΔT=M-N	CH	MinT		MaxT		MinsoilT	
						(°C)	Date	(°C)	Date	(°C)	Date
Drobeta Turnu Severin	77	0.9	3.5	2.6	W	-4.8	5	13.4	1	-6.4	12
Calafat	66	0.4	3.4	3.0	W	-2.5	5	14.1	1	-4.2	5
Bechet	65	-0.1	3.4	3.5	W	-4.1	5	13.7	1	-5.0	5;6
Băilești	56	-0.1	3.0	3.1	W	-4.6	11	12.6	1	-5.4	11
Caracal	112	-0.7	2.9	3.6	W	-3.7	5;12	11.9	1	-5.8	5
Craiova	190	-0.4	2.6	3.0	W	-4.2	11	12.9	1	-4.4	5
Slatina	165	-0.2	2.7	2.9	W	-4.4	12	12.1	1	-5.2	5;12
Băcleș	309	-0.9	1.9	2.8	W	-6.8	11	11.4	1	-	-
Târgu Logrești	262	-0.7	2.0	2.7	W	-6.8	5	10.8	1	-5.4	5
Drăgășani	280	-0.2	2.5	2.7	W	-3.5	5	11.8	1	-6.2	12
Apa Neagră	250	-0.6	2.2	2.8	W	-6.5	5	9.6	1	-7.0	1
Tîrgu Jiu	210	-0.4	2.7	3.1	W	-4.9	5	13.1	1	-5.6	5;12
Polovragi	546	-1.4	1.8	3.2	W	-9.4	11	9.1	1	-17.6	11
Rm. Vâlcea	243	0.0	2.9	2.9	W	-5.0	5	12.2	1	-5.9	2;5
Voineasa	573	-2.5	0.1	2.6	W	-12.6	11	8.3	25	-	-
Parâng	1585	-5.6	-3.5	2.1	W	-11.6	11	7.6	25	-	-
Media Oltenia	-	-0.8	2.1	2.9	W	-6.0	-	11.5	-	-6.5	-
Obârșia Lotrului	1348	-5.5	-3.6	1.9	WS	-20.2	12	5.9	5	-	-

(Source: processed data)

The chart of the maximum temperature values presented a linear decreasing tendency after the warming in the first part of the month, and the daily means and the daily minimum temperature values had a linear increasing tendency, which confirms the warm weather during the entire month (Fig. 5). The weather warming, due to the spring arrival, as a consequence of some macroprocesses at the level of the entire northern hemisphere and of the continent was more obvious starting with February 10.

⁷ The multiannual means used as a comparison terms were calculated for the interval 1901-1990, which gives to the conclusions obtained a very good meaning.

There were registered 13 days when the air temperature reached the *critical threshold of 0°C*⁸ (days with frost and thaw) and 15 days in which the daily minimum temperature values were positive in most of the region.

The *minimum temperatures at the soil surface* were extremely low and were comprised between -17.6°C in Polovragi on February 11 and -4.2°C in Calafat, registered on February 5, and at some meteorological stations on February 6, 11 and 12, which shows the cooling not only in the air and soil, but also at the surface of rivers and lakes, causing an in-depth soil frost and a thick ice layer at the surface of rivers and lakes in the interval February 5-12. The soil has been thawed during long periods of time, and in the periods when it occurred it was superficial in general.

The *number of frost units* in February was comprised between 0 in most of the Romanian Plain and in the Olt Couloir in Râmnicu Vâlcea and 18.4 in Voineasa, which confirms the fact that from an agrometeorological point of view, February 2013 was a warm month, in which the vegetative processes occurred again in some intervals of time.

The *agrometeorological⁹ frost* was not registered in February.

The *sums of the active daily average temperatures* were comprised between 19.5°C in Voineasa and 99.0°C in Drobeta Turnu Severin, and in the mountainous area 4.9°C in Parâng, which confirms the predominance of warm weather on extended periods of time.

3.b. The pluviometric regime of February 2013

The *monthly quantities of precipitations* in February were comprised between 38.8 l/m² in Bechet and 200 l/m² in Apa Neagră, the Subcarpathian depression, and their deviations from the multiannual means were comprised between -11.5% in Bechet (the only negative deviation being in the mountainous area -15.7% in Parâng) and 201.4% in Apa Neagră, and according to Hellmann criterion, the pluviometric time types at the meteorological stations in Oltenia were comprised between little rainy (LR) in Bechet and exceedingly rainy (ER) in most part of the region (Drobeta Turnu Severin, Calafat, Băilești, Craiova, Târgu Logrești, Drăgășani, Apa Neagră, Tg Jiu), and in the mountainous area a little droughty (LD) in Parâng (Table 2).

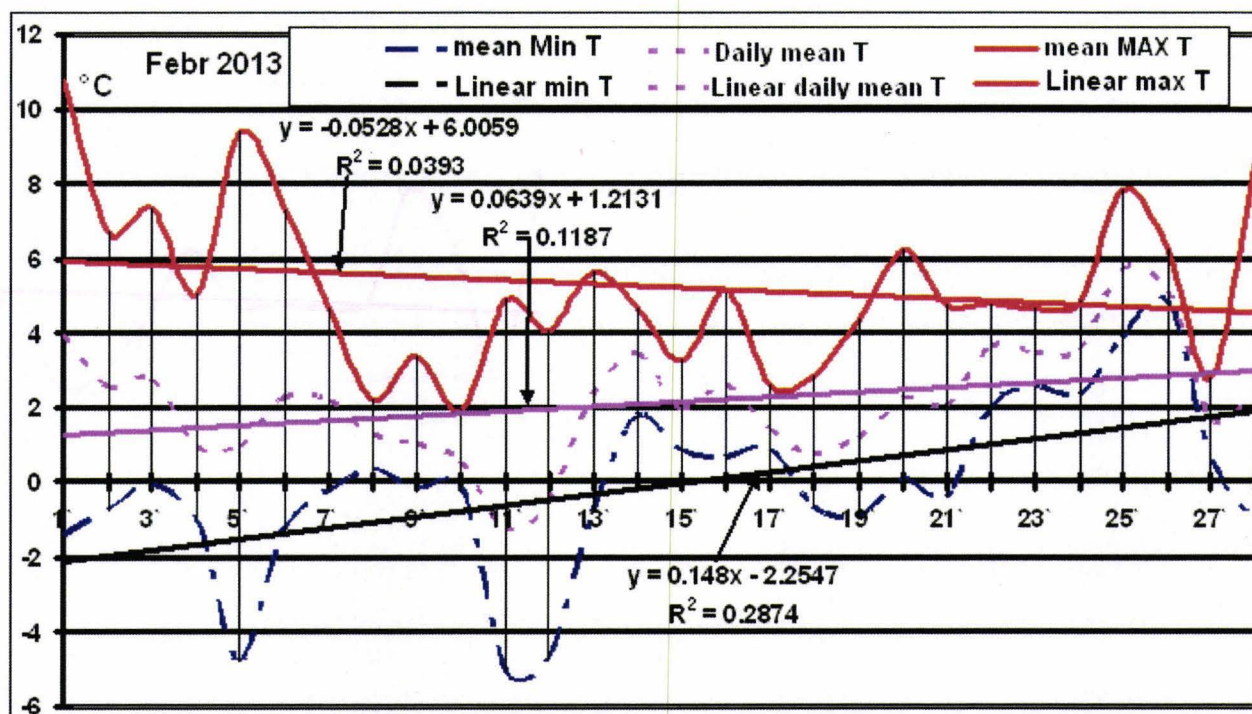


Figure 5. The air temperature variation, the daily means (Daily mean T), daily minimum (mean min T) and maximum temperatures mean (mean MAX T), calculated for the entire region in February 2013.

⁸ The temperatures value exceeding 0°C, in agrometeorology are active temperatures, and their persistence during some consecutive days establishes the start over of the vegetative processes. The decrease of the air and soil surface temperature below 0°C induces the vegetative sleep.

⁹ For weather forecast and in general in mass-media, for people and animals, the notion of *frost* means temperature values of $\leq -10^{\circ}\text{C}$, but in agrometeorology and for plants the term of frost means air temperatures of $< -15.0^{\circ}\text{C}$. The threshold of -15.0°C is the critical threshold of crops resistance to frost, below this threshold there are registered frostbites of crops and irremediable damages. These different thresholds of resistance to low temperatures show that plant are better adapted to low temperatures than animals, a normal aspect if we take into account that during the geological eras plants were the first to appear and afterwards the animals, an aspect which is also justified by the different cellular structures of the two kingdoms.

The monthly quantities of precipitations mean for the entire region was 56.6 l/m², and its percentage deviation from the multiannual mean was 33.6%, which classifies February as a very rainy (VR) month for the entire region.

The monthly quantities of precipitations mean for the entire region was 77.5 l/m², and its percentage deviation from the multiannual mean was 84.0%, which classifies February as an excessively rainy (VR) month for the entire region, meaning the interruption of the droughty period which has extended from the beginning of the summer of 2012 to the end of January 2013.

The snow layer was absent, excepting the mountainous area some days when it was insignificant and temporary on restricted areas. *The water soil reserve* in the end of February (in the end of winter) in the depth of 0-100 cm, in the autumn wheat crop was satisfactory (S), close to optimum (CIO) and optimum (O), in the entire Oltenia.

4.a. The overall thermal regime of the winter of 2012-2013

The seasonal temperature means for the winter of 2012-2013 were comprised between -1.8°C in the intra-Carpathian depression Voineasa and 1.6°C in Dobeta Turnu Severin, and their deviations from the multiannual means were comprised between 0.6°C in Apa Neagră and 1.7°C in Băcleș, Polovragi and Râmnicu Vâlcea.

According to Hellmann criterion (CH) applied to seasonal means the thermal time types for the winter of 2012-2013 were comprised between warmish (WS) in Bechet, Apa Neagră and Obîrșia Lotrului (spatial-temporal extension of 17.7%) and warm (W) in most of the region (at 15 of 18 meteorological stations) having a spatial-temporal extension of 83.3% (Table 5).

The seasonal thermal mean was 0.1°C, and its deviation from the normal was 1.3°C, which according to Hellmann criterion classifies it as a warm winter (W) on the whole. This general aspect is due to the increase of the overall mean because of the high values in January and February.

The interval comprised between December 5 and December 27 is that of *severe winter* during which the air temperature decreased at extremely low temperatures and the climatic risk phenomena: snowfalls, snowstorms, snow layer with a significant thickness occurred, which shows that the severe winter lasted only 22 days.

However, this climatic risk phenomena manifested also in this winter especially through the great number of days with frost and thaw (62 days with the isotherm of 0°C, namely 68.9% of the total number of days of the season), which made vulnerable some crops and particular species of fruit trees such as the apricot tree, which is extremely reactive to weather warming.

The number of frost units for the entire winter was comprised between 100.3 in Drobeta Turnu Severin and 296.4 in Voineasa close to the lower limit of the mountainous area, and in the mountainous area 437.0 in Parâng, which designates a mild winter from an agrometeorological point of view.

The sum of the active average temperatures for the entire winter was comprised between 51.5°C in Voineasa and 198.9°C in Drobeta Turnu Severin, prefiguring an early spring, and in the mountainous area 50.5 in Parâng. For the interval December 1, 2012 March 31, 2013 the sum of the active average temperatures was comprised between 128.8°C in Voineasa and 366.4°C in Drobeta Turnu Severin, which designates the values of a mild winter.

Table 5. Overall average thermal values of the winter of 2012-2013.

Meteorological Station	Hm	meanT N Winter	Mean Winter 2012-2013	Δ=meanT-N	CH
Drobeta Turnu Severin	77	0.4	1.6	1.2	W
Calafat	66	-0.1	1.1	1.2	W
Bechet	65	-0.6	0.1	0.7	WS
Băilești	56	-0.7	0.5	1.2	W
Caracal	112	-1.2	0.4	1.6	W
Craiova	190	-1.0	0.4	1.4	W
Slatina	165	-0.8	0.5	1.3	W
Băcleș	309	-1.4	0.3	1.7	W
Târgu Logrești	262	-1.1	0.0	1.1	W
Drăgășani	280	-0.6	0.8	1.4	W
Apa Neagră	250	-1.0	-0.4	0.6	WS
Târgu Jiu	210	-1.0	0.5	1.5	W
Polovragi	546	-1.5	0.2	1.7	W
Râmnicu Vâlcea	243	-0.6	1.1	1.7	W
Voineasa	573	-3.0	-1.8	1.2	W
Parâng	1585	-5.1	-4.0	1.1	W
Media Oltenia		-1.2	0.1	1.3	W
Obârșia Lotrului	1348	-5.5	-4.8	0.7	WS

(Source: processed data)

4.b. The overall pluviometric regime of the winter of 2012-2013

The seasonal quantities of precipitations were comprised between 90.2 l/m² in Bechet in the extreme south and 384.0 l/m² in Apa Neagră, and their percentage deviations from the normal values were comprised between -13.8% in Bechet and 74.9% in Apa Neagră. According to Hellmann criterion applied to the seasonal quantities of precipitations, the pluviometric time types at the meteorological stations in Oltenia were comprised between little droughty (LD) in Bechet and in the mountainous area and exceedingly rainy (ER) in Drobeta Turnu Severin, Târgu Logrești and Apa Neagră.

The spatial-temporal extension of the rainy (R) + very rainy (VR) and exceedingly rainy (ER) pluviometric time types was of 60.0%, which shows that on the whole, pluviometrically, the winter of 2012-2013 was very rainy (VR). This aspect is also sustained by the overall precipitations mean for the entire region of 176.4 l/m² whose percentage deviation from the normal values is of 29.6%, and according to Hellmann criterion on the whole the winter was very rainy (VR) (Table 2).

DISCUSSIONS

It is widely known that the interval January 15 February 15 is winter's peak period in Romania, when, usually there occur the most intense cooling and the most abundant snowfalls associated with snowstorms which produce a thick snow layer, burying in snow the communication routes and often causing the death of people and material damages. In the winter of 2012-2013, the thermal regime was contradictory compared to the normal, registering (with few exceptions) the monthly maximum thermal values for January and February in the end and respectively the beginning of the month. The high frequency of the Mediterranean Cyclones caused an excessive pluviometric regime and interrupted the drought, which had persisted until the end of autumn.

The causes of this warm winter were determined by the appearance and development of some Mediterranean cyclones which evolved in the intervals: December 1-4, December 5-6, December 8-10, December 11-13, December 17-20, December 26-27, December 26-27, December 28-29, January 5-6, January 10-12, January 13-27, January 28-29, February 2-4, February 7-11, 11-17 February and February 19-26 and led to warm and wet air advections from the south of the continent, and in the intervals in which they did not act, the Azores High was there bringing oceanic warm air masses above Oltenia and sunny days, and the East European Anticyclone acted during short intervals of time. The North-Atlantic Oscillation maintained in a positive phase during some intervals of time.

Economic and bioclimatic effects on the environment of the winter of 2012-2013

The cold and early winter episode appeared even from the first decade caught unprepared some autumn crops for winter because of the cooling intensity. The significant destructive effects were observed in general on all biotopes¹⁰. Significant surfaces of rape crops were damaged and destroyed because the plants were caught unprepared for winter, due to the severe autumn drought, which delayed their spring.

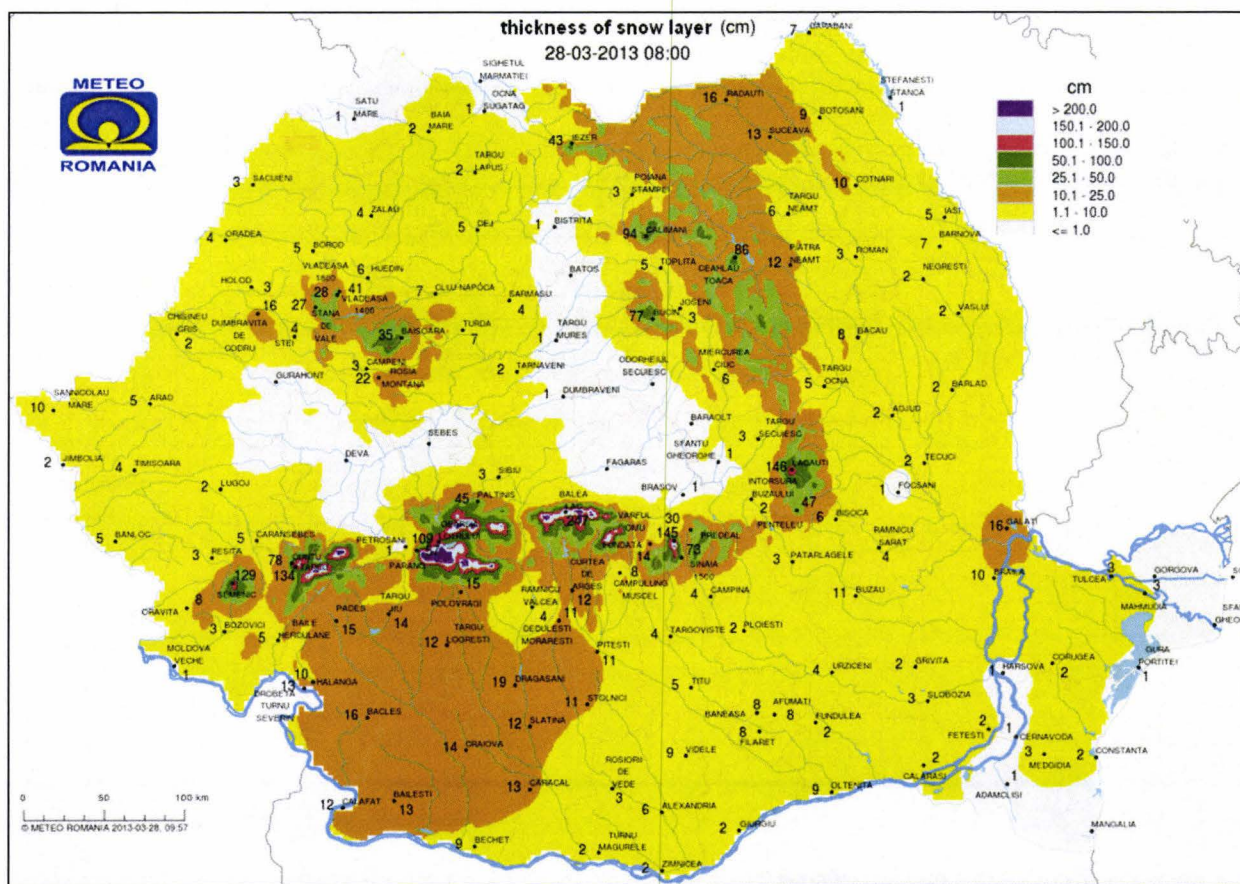


Figure 6. The maximum snow layer thickness in March 2013 registered on March 28, 2013 (according to NAM Bucharest).

¹⁰ BIOTOPE, biotopes, (Biol.) The natural environment in which a group of plants or animals lives in homogenous conditions. [Pr.: bi-o-] – From the engl. biotope (DEX).

The warm weather on long periods of time in January and February led to the slow start over of the vegetation phases in particular intervals of time, and often the frost-thaw episodes made some cultures vulnerable (for example rape and apricot trees crops).

The climatic risk of warm winters is related to the effects of active temperatures on the vegetal carpet and biotopes. These phenomena occur on extended surfaces of the continent not only in the south-west of Romania, but also on the entire biosphere. Therefore, even from the first day of March the early arrival of migratory birds in Romania, the late cooling and the cold wave from the last five days of March caused the death of a significant number of migratory birds, which were surprised by weather cooling and did not have the possibility to feed themselves, their resistance to cold being low.

Weather cooling in the last 5 days of March was extremely intense (for that calendar date), and the minimum temperature values registered in the morning of March 27 were comprised between -5.2°C in Polovragi and -0.8°C in Drobeta Turnu Severin and in Parâng (-9.9°C), and constitute climatic records for that date being associated with very low values of the cooling index (CI), due to the wind cooling effect, which contributed essentially to destructive effects. The snow layer in Oltenia registered in the morning of March 28 the maximum thickness in the country being comprised between 12 and 21 cm in Târmigani in Mehedinți County (Fig. 6), which shows the risk intensity if we take into account the advanced vegetation phases in some crops. On March 24, 2013 at some stone fruits (for example apricot tree) the floral sprouts were developed, and the blossoming was imminent. Weather cooling in the interval March 25-27, 2013, snowfalls and the snow layer caused damages in orchards, so that in the morning of March 28 it was observed the fall of the floral sprouts of apricot trees. In the night of March 25/26, the cooling index (CI) reached the value of -17°C (in Slatina), being a bioclimatic record for this calendar date, and the effects caused shows that even the vegetal carpet is sensitive to low values of the CI index, thus confirming its usefulness in the bioclimate study. As a consequence of these effects the early spring vegetables appeared later and had a poor quality.

In the Danube Meadow, there were flood as a consequence of the increase of the river level caused by the fast snow layer melting from the continent.

CONCLUSIONS

After an early and cold winter beginning in the interval December 10-23, 2012, in the south-west of Romania weather radically changed, following a weather warming which maintained with slight fluctuations in January and February.

Our study shows that the climatic and agroclimatic indexes are thus bioclimatic indexes, with a good significance for the biosphere.

Earth has more than a climate as the other planets of our solar system¹¹, it has a climate that has created life and has preserved its perpetuity during the geological eras, and therefore in our opinion Earth has a bioclimate, something different from the other planets. The entire biosphere is fragile and extremely sensitive to climate variations, and this is why these studies are necessary in order to highlight the effects of these variations which became very frequent as a consequence of the climatic variability increase, and their thorough study can help find some solutions in the situations in which these variations become dangerous.

This severe winter interval was caused by the appearance and development of some Mediterranean cyclones, which evolved in some intervals of time mentioned in the paper, which in interaction with the anticyclone field that had covered most of the continent led to abundant snowfalls associated with wind gusts (snowstorm).

The snowfalls were followed by weather intense cooling because of the advection of the northern, northeastern and eastern extremely cold air advection, and the local phenomena of thermal inversion and intense nocturnal cooling in conditions of sunny sky and thick snow layer worsened the cooling.

The winter of 2012-2013 marked an important climatic oscillation not only in the south-west of Romania, but also in the entire European continent, where on extended areas, mild winter phenomena occurred and only in the northern part predominated the cold winter and the prolonged frost.

The intense weather cooling as well as snowstorms and snowfalls manifested on extended areas on the continent, not only in Oltenia and in Romania.

The abundant snowfalls as well as the rains of December, January and February restored the water soil reserve at the optimum level ensuring the good development of crops and vegetal carpet in the first part of spring. We conclude that the phenomenon of global climatic warming is not uniform, although the increasing trend continues, leading to the climatic variability increase and surpassing of the thermal and pluviometric extremes in both senses.

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¹¹ Earth bioclimate and climate is mainly due to the position of our planet in the solar system ("geometry" Earth-Sun as well as the significant quantity of water forming the hydrosphere which has the role of heat storage, and the thermodynamic processes and the general atmosphere circulation essentially contribute to its redistribution in the atmosphere and planetary Ocean, leading to a relative stability of the bioclimate.

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HALOPHILIC ARCHAEA IN THE NEOGENE SALT MASSIF FROM SLĂNIC PRAHOVA, ROMANIA

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Abstract. The archaeal component of salt embedded microbiota from a Neogene salt massif located in Unirea salt mine, Slănic, Prahova (Romania), was isolated and characterized. Salt crystals were collected from the subterranean salt block. After washing by immersion in 50 ml of sterile 20% NaCl for 5 minutes, the crystals were transferred in a fresh NaCl solution and incubated at 37°C until completely dissolved. The number of c.f.u. present at the surface of salt crystal was at least double than that present inside the crystal, indicating most probably a contamination with salt tolerant microorganisms, rather than naturally occurring species. Biochemical and molecular analyses revealed a low diversity of haloarchaea, the predominant strains belonging to *Halorubrum* or *Haloarcula* genera. Eight out of 18 investigated strains were cultivated in the presence of 2-5.2 M NaCl, with an optimum varying from 2.5-3 M to 4.0-4.5 M. The remaining ten strains were able to grow in the presence of 2.5 M NaCl until saturation concentrations, with an optimum around 4 M NaCl. Three of the strains did not catalyse the formation of H₂S from thiosulphate. All strains were catalase positive, and able to grow in the presence of chloramphenicol and ampicillin (50 µg/ml) but not in the presence of sodium deoxycholate and novobiocin.

Keywords: rock salt, halophilic archaea, salt mine, hypersaline environments.

Rezumat. Aspecte privind prezența unor microorganisme halofile arheane în zăcămintul de sare format în zona Slănic Prahova, România în perioada Neogenă. Cristalele de sare prelevate din Mina Unirea din zona Slănic Prahova au fost imersate pentru cinci minute în 50 ml soluție sterilă de clorură de sodiu 20% în vederea spălării acestora după care au fost transferate în soluție proaspătă de NaCl și ținute la 37°C până la dizolvarea completă. Numărul de u.f.c. prezente la suprafața cristalului de sare a fost mai mare comparativ cu numărul celor provenite din interiorul cristalului arătând cel mai probabil un grad ridicat de contaminare cu microorganisme capabile să tolereze concentrații mari de sare. Analizele moleculare și de biochimie au arătat o diversitate scăzută a haloarheelor, majoritatea aparținând genurilor *Halorubrum* și *Haloarcula*. Un număr de 8 tulpini dintre cele 18 investigate au crescut pe medii de cultură conținând concentrații de clorură de sodiu între 2 și 5.2 M cu un optim de dezvoltare în prezența unor concentrații cuprinse fie în intervalul 2.5-3 M fie 4-4.5 M. Cele zece tulpini rămase au fost capabile să crească în prezența a 2.5 M NaCl până la concentrațiile de saturație, cu un optim în jurul valorii de 4 M NaCl. Trei dintre tulpinile investigate nu au produs H₂S din tiosulfat. Toate tulpinile testate au avut activitatea cataliză pozitivă și au fost capabile să se dezvolte în prezența cloramfenicolului și a ampicilinei (50 µg/ml) însă nu și în prezența deoxicolatului de sodiu și a novobiocinei.

Cuvinte cheie: evaporite, halofile arhea, mina de sare, medii hipersaline.

INTRODUCTION

Halophilic microorganisms belonging to the domain Archaea have been identified in a variety of saline and hypersaline environments. Due to their unique structural and functional characteristics, which support their growth, they flourish in such extremely severe conditions. Haloarchaea were isolated or detected by PCR techniques (even if in some cases they are non-cultivable) in a wide range of environments with salinity varying from negligible (ELSHAHED et al., 2004; LEUKO et al., 2008) to saturation (32 g/L NaCl), such as Dead Sea (OREN, 1983; 2002), solar salterns (BENLLOGH et al., 2001; LITCHFIELD & GILLVERT, 2002; OCHSENREITER et al., 2002) or unusual salted habitat like nostrils salt glands of *Calonectris diomedea*, a sea bird (BRITO-ECHEVERRIA et al., 2009).

Such kind of microorganisms were isolated from a fluid inclusion in salt crystals (NORTON et al., 1993) and in the last decade a novel member, *Halobacterium noricense* was characterized as a new species of halophilic archaea as a novel member of the genus *Halobacterium* (GRUBER et al., 2004). Moreover, a recent study revealed that *Halobacterium* species are adapted to survive in halite for long periods of time (GRAMAIN et al., 2011), and a number of viable haloarchaea were isolated from the rock salts (GRANT et al., 1998; MCGENITY et al., 2000), suggesting that several and various populations of halophilic microorganisms are relict populations derived from the ancient sea.

Salt (halite) deposits resulting from the precipitation turnover through the years of older salty environments were identified worldwide. Taking into account that one of the oldest data which could be attributed to the presence of microbial species associated with rock salts (FISH et al., 2002; KAMEKURA, 2003; 2007), they can be also considered as salted relics of brines that presumably hosted some populations of halophilic archaea.

Some salt deposits that were formed in the Neogene period are distributed in Romania, mainly in the proximity of the Carpathian areas, such as Slănic, Prahova (HAR et al., 2006; MĂRUNȚEANU, 1999). The exploitation of the halite from this deposit was active since 1685 until nowadays using several technologies. However, little is known about their microbiota, except recent investigations that revealed for the first time the presence of both halophilic archaea and bacteria (COJOC et al., 2009; ENACHE et al., 2000; 2008ab; 2012). The underground salt deposit from Slănic is located 45.5 m to 499 m deep (DRĂGĂNESCU, 1990), and most probably dated from the Neogene period (23.03 - 2.588 MY ago). The deposit showed a grey and swarthy colour, and is variegated as a consequence of turnovers that took place during precipitation processes, due to climatic and sedimentary variations (HAR et al., 2006). Currently this deposit

represents one of the most important salt exploitation in Romania. In this study, we investigated whether haloarchaea inhabit this salt mine, and if they represent relict populations of ancient haloarchaea.

MATERIALS AND METHODS

Sampling of rock salt and isolation of halophilic microorganisms

The rock salt crystal were collected from subterranean salt mine Unirea, located in Slănic, Prahova, Romania, at about 208 meters depth. The crystal with no apparent strong clay or soil content was broken in three pieces and washed with sterile 20% NaCl solution. The resulted brine was used to remove the microorganisms located on the crystal surface. After washing, the crystal was sterilized in flame for few seconds, and dissolved in fresh sterile 20% NaCl. The resulted brine was used to isolate haloarchaeal strains located inside the crystal. Aliquots of 1 ml of resulted brines (surface and inside) were mixed with 25–30 ml of molten agar medium JCM 168 which contained (g/L): Bacto casamino acids (5), Bacto yeast extract (5), sodium glutamate (1), trisodium citrate (3), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (29.5), KCl (2), NaCl (175.5), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.036), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.36 mg). The medium pH was 7.0–7.2 before autoclaving. After that the samples were incubated at 37°C for several weeks until the appearance of red and pink colonies.

Biochemical assays

A biochemical characterization of the isolated haloarchaeal strains was performed following the minimal standard procedure for description of new taxa, as previously described in the case of halobacteria (ENACHE et al., 2000; 2007; 2008ab; 2009). The NaCl concentration range and the optimum NaCl concentration for growth were determined by cultivation at 37°C in JCM 168 medium for up to seven days. The catalase and oxidase activities and the presence of metabolic pathways for producing H_2S from thiosulphate, of indole from tryptophan and starch hydrolysis were investigated according to previous protocols (ENACHE et al., 2007; 2008ab). The antibiotic and bile salts resistance was also tested, using 50 µg/ml of chloramphenicol, penicillin, ampicillin erythromycin and novobiocin and sodium deoxycholate in order to differentiate archaeal and bacterial strains, as previously indicated (ENACHE et al., 2007). In addition, the Gram staining and the cell shape were determined as previously described (ENACHE et al., 2007; 2009).

16S rRNA gene sequence analysis

Total DNA was extracted and purified using the method of Tamaoka adapted for halophilic archaea (ENACHE et al., 2008ab). The 16S rRNA genes were amplified by PCR, using the archaeal specific forward and reverse primers 5'-TCCGTTGATCCTGCCG (position 8–24) and 5'-GGAGGTGATCCAGCCG (position 1540–1525), respectively. The resulted DNA fragments were sequenced using BigDye Terminator Cycle Sequencing Kit (Pharmacia Biotech) and ABI Prism DNA genetic analyzer (Applied Biosystems). The sequences obtained were analysed using BLAST and aligned with other reported haloarchaeal 16S rRNA gene sequences using CLUSTAL W 1.7 software. A phylogenetic tree was reconstructed by the neighbour-joining method.

RESULTS AND DISCUSSIONS

Isolation of halophilic microorganisms and number of strains

The analysis of the salt crystal extracted from Slănic salt mine revealed the presence of inhabiting halophilic microorganisms able to grow in the presence of sodium chloride concentrations varying from 2M to saturation (5.2M). The number of colonies from the surface of the crystal ranged from 340 c.f.u./weight of crystal part (Table 1), in the case of the soil-contaminated crystal region, to none for another part of the crystal. In another part of the crystal the number of colonies was huge. The brine obtained from dissolving the washed crystal contained a variable c.f.u. number ranging from 6 to 120/weight of crystal part (Table 1). This number appears to be relatively high as compared with those reported in literature (DOMBROWSKI, 1963; NORTON et al., 1993). Figures 1 and 2 showed colonies obtained during the experimental procedure from the brine obtained by washing the salt crystal with sterile 20% NaCl solution.

Considering the number of colonies and based on the appearance of their pigmentation, predominantly dark red, 18 colonies were randomly picked up from the plate and transferred to agar slant for further investigation (Table 1). The strains unable to grow in the presence of taurocholic acid but able to grow in the presence of chloramphenicol were assigned as belonging to archaea.

Table 1. Samples characterization.

Crystal	Apparent contamination	Weight of crystal before washing step	Total observed colonies (colony forming units)		Strains		Observations
			Surface	Inside	Surface	Inside	
1	Apparent contaminated with trace of soil	5.86 g	340	120	7, 8, 9, 10	12, 13, 14, 15	Colony 14 is difficult to cultivate on agar slant
2	No contamination	4.05 g	absent	20	-	4, 5, 6	
3	No contamination	1.73 g	high number	6	3, 11	1, 2, 16, 17, 18, 19	Colonies observed on brine from surface appear to be small and similar.

All the selected strains appear to belong to Archaea, suggesting that, at least inside of the salt crystal, the microorganisms associated to the salt crystal are not members of Eubacteria. In the case of crystal 3, colonies observed on brine from surface appear to be similar and were located in the small area of the plate. The strain 16 was lost after the second passage. The strains 1, 2 and 14 are difficult to cultivate on agar slant.

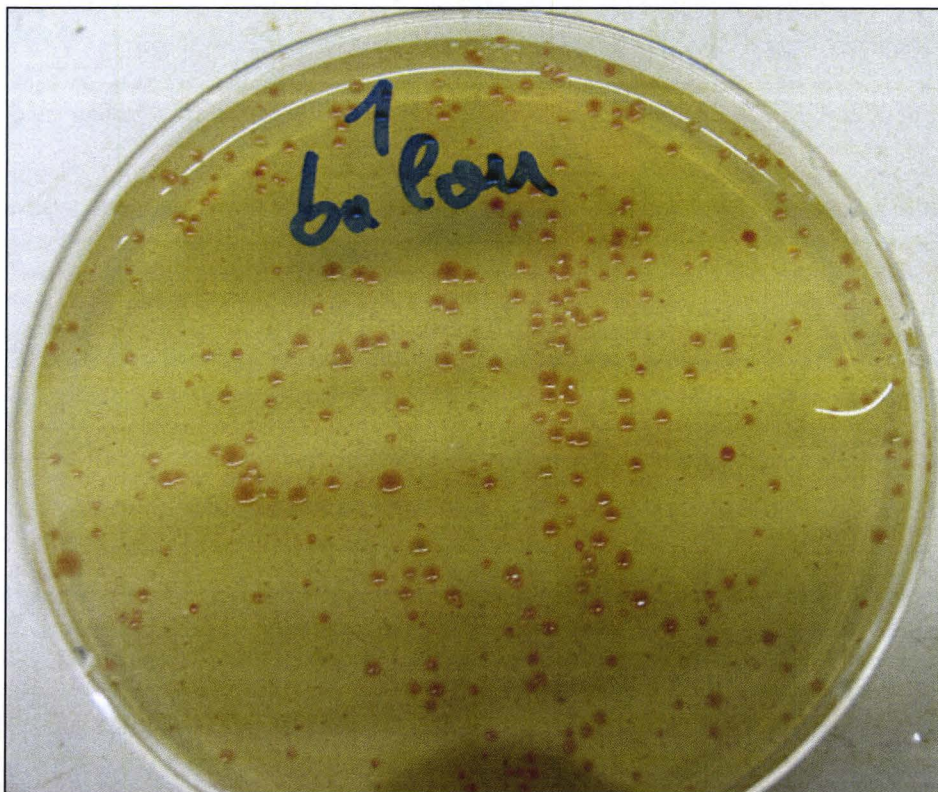


Figure 1. Colonies of red-pigmented haloarchaea obtained from the brine resulted by washing the salt crystal part 1 with sterile 20% NaCl solution (original).



Figure 2. Colonies of red-pigmented haloarchaea obtained from the brine resulted by washing the salt crystal part 3 with sterile 20% NaCl solution (original).

Biochemical characterization

The biochemical characteristics of the investigated strains are shown in table 2. All strains showed a negative Gram staining, and presented catalase activity. All strains were rod-shaped, with the exception of strain 6, isolated from inside of the crystal region that was not contaminated with soil traces, which showed an irregular shape.

The growth range of NaCl concentrations is indicated for all strains (Table 2). Eight out of the investigated strains were able to grow in a range of 2M to 5.2M, seven strains from 2.5M to 5.2M NaCl, strain 17 from 1.5M to 5.2M, and strain 19 from 3.0 to 5.2M. The optimum NaCl concentrations were also determined (Table 2). All these strains presented cell lysis when the cell suspension was diluted in distilled water, indicating halophilic specific behaviour.

Moreover, these strains were not catalysing the tryptophan deamination reaction. Strains 5, 6 and 14 were not able to catalyse the thiosulphate reduction to H₂S. Nine strains (3, 4, 5, 6, 7, 8, 9, 11 and 12) were able to hydrolyse the starch. In addition, three strains (13, 18 and 19) were not capable to catalyse oxidative reactions (Table 2).

The sensitivity to antibiotics (50µg/ml) of strains 3-17 was tested for chloramphenicol, penicillin, ampicillin erythromycin and novobiocin (Table 3). All the strains investigated presented resistance to chloramphenicol and ampicillin, and were not able to grow in the presence of sodium deoxycholate and novobiocin. Among the surface and inside strains, only one strain (13) showed no resistance to penicillin, and five strains (5, 12, 13, 15, 17) were not capable to grow in the presence of erythromycin.

Table 2. Biochemical features of investigated strains.

Test Tested strain	A	B	C	D	E	F	G	H	I
1	2.0-5.2	2.5-3.0	N	N	N	N	N	N	N
2	2.0-5.2	3.0-4.0	N	N	N	N	N	N	N
3	2.0-5.2	2.5-3.5	-	B	+	+	+	-	+
4	2.5-5.2	3.5-4.0	-	B	+	+	+	-	+
5	2.5-5.2	4.0-4.5	-	CB	+	+	-	-	+
6	2.5-5.2	4.0-4.5	-	I	+	+	-	-	+
7	2.0-5.2	2.5-3.0	-	B	+	+	+	-	+
8	2.5-5.2	3.5-4.0	-	CB	+	+	+	-	+
9	2.5-5.2	2.5-3.0	-	B	+	+	+	-	+
10	2.5-5.2	3.5-4.0	-	CB	+	+	+	-	-
11	2.0-5.2	3.5-4.0	N	N	+	+	+	-	+
12	2.0-5.2	3.5-4.5	-	B	+	+	+	-	+
13	2.0-5.2	2.5-3.5	-	B	+	-	+	-	-
14	N	N	-	B	+	+	-	-	-
15	2.0-5.2	4.0-4.5	-	B	+	+	+	-	-
17	1.5-5.2	4.0-4.5	-	B	+	+	+	-	-
18	2.5-5.2	4.0-4.5	-	CB	+	-	+	-	-
19	3.0-5.2	4.0-4.5	-	CB	+	-	+	-	-

Legend: N = no data available; B = rod form; CB = irregular rod; I = irregular form; + = activity is present; - = activity is absent; red number represent strains isolated from the surface of the salt crystal; A = range of NaCl for growth (M); B = Optimum NaCl (M); C = Gram staining; D = Shape; E = Catalase; F = Oxidase; G = H₂S from thiosulphate; H = Indole from tryptophan; I = Starch hydrolysis.

Table 3. Antibiotic and bile salt resistance. Strain growth was carried out in the presence of various antibiotics (50 µg/ml) and bile salt, as described in methods. Strains isolated from the surface of the salt crystal (red), and inside the salt crystal (black); (+) strains able to grow; (-) strains not able to grow.

Antibiotic Strain	Chl	NaDeox	Pen	Amp	Eryth	Nov
3	+	-	+	+	+	-
4	+	-	+	+	+	-
5	+	-	+	+	-	-
6	+	-	+	+	+	-
7	+	-	+	+	+	-
8	+	-	+	+	+	-
9	+	-	+	+	+	-
10	+	-	+	+	+	-
11	+	-	-	+	-	-
12	+	-	+	+	-	-
13	-	-	+	+	-	-
15	+	-	+	+	-	-
17	+	-	+	+	-	-

Legend: Chl = chloramphenicol, NaDeox = Sodium deoxycholate; Pen = penicillin; Amp = ampicillin; Eryth = erythromycin; Nov = novobiocin.

Phylogenetic tree reconstruction

The phylogenetic tree reconstructed from the 16S rRNA gene sequences (Fig. 3) revealed that most of the investigated strains grouped closely with *Halorubrum saccharovorum*. Other strains (18 and 19) were grouping with *Halobacterium noricense*, an organism isolated from Permian salt deposit in Austria (GRUBER et al., 2004), and strain 14 with *Haloarcula japonica*. This distribution is in accordance, most probably, with a high intragenomic heterogeneity occurring within this haloarchaeal genus. The strains 12 and 13, isolated from the inside of crystal apparently contaminated with trace of soil, clustered together with *Halorubrum* genus, suggesting that they constitute a new species. The other 11 strains (3, 4, 5, 6, 7, 8, 9, 10, 11, 15 and 17) (Fig. 3) were grouped in a tight cluster with *Halorubrum saccharovorum*, suggesting an intragenomic heterogeneity also within genus *Halorubrum*.

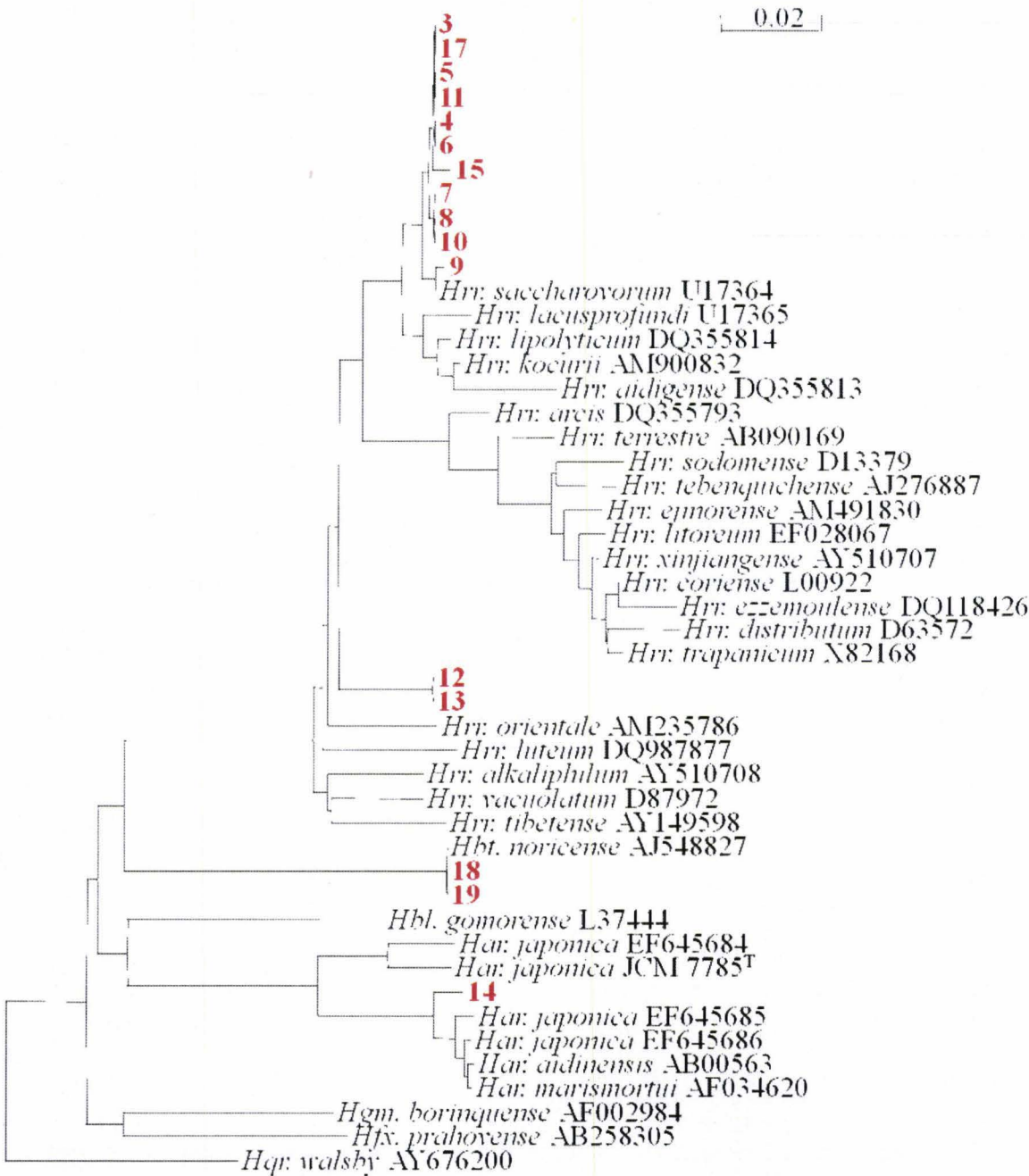


Figure 3. Phylogenetic tree based on partial sequences of 16S rDNA revealed the position of the investigated strains (in red) among species of genera *Halorubrum*, *Halobacterium* and *Haloarcula*. The tree was reconstructed by neighbour-joining method. Bootstrap values $\geq 70\%$ (1000 replicates) are shown. Bar 0.02 substitutions per nucleotide position.

Predominant presence of Halorubrum strains

As mentioned above, the majority of the investigated strains (13 out of 18) appear to be either new species of *Halorubrum* (strains 12 and 13) or strains closely related to *H. saccharovorum* (11 strains). The strains 12 and 13 were isolated from inside of a salt crystal. From the total of 11 strains grouping with *Hrr. saccharovorum*, five were also isolated from inside of the salt crystal.

Taking into account the origin of salt deposit from Slănic, Prahova and previous data (ENACHE et al., 2008a, b) concerning halophilic microorganisms from the salted lakes surface from the same area, the predominance of *Halorubrum* species both inside and on the surface of the salt crystal suggested that the underground salt deposit host *Halorubrum* species as the dominant biota component of ancient origin, in contrast with surface salted lakes where the predominant biota are represented by members of the genus *Haloferax*.

CONCLUDING REMARKS

This work reported the first isolation and cultivation of haloarchaeal species from an underground salt massif, formed in the Neogene period, located in the area of Slănic, Prahova.

The phylogenetic tree of the 16S rRNA gene sequences from the investigated 18 strains revealed that these isolates belong to the *Halorubrum*, *Haloarcula* and *Halobacterium* genera. The strains grouping within *Halobacterium* genus were very similar with *Halobacterium noricense*, a cultivable haloarchaeal strain identified from ancient salt, salt mine environments, halite crust, and halite crystal from a saltern (GRAMAIN et al., 2011). The age of the salt hosting *Hbt. noricense* varies from 1.8 until to 250 MY (GRAMAIN et al., 2011), supporting the hypothesis of an ancient age for this haloarchaeal strains.

In accordance, our data revealing the absence of a tryptophan metabolizing pathway suggest the older age of the strain, while the capacity to transform thiosulphate argued for an age of at least 2.8 – 3.2 MY (BLANK, 2009).

Since the salt deposit from Slănic was formed during the Neogene period, there is a possibility that microorganisms isolated from this salt block to be relics of the life forms that existed in that area, since the occurrence of the first elements which subsequently created the deposit of salt.

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LONG - TERM ANALYSIS OF CYANOBACTERIAL BLOOMS IN LAKE ROȘU (DANUBE DELTA)

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Abstract. Cyanobacteria became increasingly dominant as concentrations of total phosphorus and total nitrogen increased during the eutrophication of the Danube Delta ecosystems. As large, inedible algae, they induce a bottleneck in the carbon and energy flow of the plankton food web. Taking into account these reasons, our work aimed at highlighting the dynamics of cyanobacterial blooms in Lake Roșu at a wider temporal scale (1975-2002) and update the scientific data in the year 2011. The hypertrophy, a characteristic stage of the deltaic ecosystems in the critical period after 1980, involves increased concentration of nutrients and the nitrogen factor-limiting role of phytoplankton development. In the new environmental conditions, the chance of intense proliferation of cyanobacteria group increased, becoming dominant in the ecosystem. After 1980, yearly averages of biomass exceeded 10-30 times the "water blooms" threshold. Monthly averages in July-September period exceeded 100-125 times the mentioned limit. The spectacular values of cyanobacterial abundance and biomass between 1980 and 1990 triggered also the dominance of potential toxic species. The Mc Naughton and Wolf dominance index of phytoplankton biomass between 1982 and 1985 exceeded the threshold of 0.5 in all seasons. In general, the dominant species belong to toxin-forming Cyanobacteria: *Microcystis aeruginosa* KÜTZING 1846, *M. flos-aquae* (WITTR.) KIRCHNER 1898, *M. pulvereae* (WOOD) MIGULA 1849, *Anabaena hassalii* (KÜTZ.) WITTRÖCK 1909, *A. scheremetievi* ELENKIN 1909, *Oscillatoria tenuis* AGARDH 1813. The edibility degree of these species is very low, the herbivorous zooplankton being forced to feed on detrito-bacterial aggregates to be able to survive. While in 2001 the diatoms and cyanobacteria biomass decreased, especially in the warm seasons, and the ecosystem tended to reach a functional regime, more stable, due to a lower nutrient pressure, in 2011, the high values of phytoplankton biomass (78.72 wet weigh mg l⁻¹) have shown new eutrophication signals, including intense cyanobacterial blooms episodes.

Keywords: cyanobacterial blooms, long-term studies, Lake Roșu, phytoplankton biomass, Danube Delta.

Rezumat. Analiza pe termen lung a înfloririlor cianobacteriene din lacul Roșu (Delta Dunării). Grupul Cianobacteria devine dominant odată cu creșterea concentrațiilor fosforului total și a azotului total pe parcursul procesului de eutrofizare din Delta Dunării. Cianobacteriile de dimensiuni mari, necomestibile, induc o barieră în fluxul de carbon și energie în rețeaua planctonică. Luând în considerare aceste argumente, lucrarea noastră și-a propus să evidențieze dinamica înfloririlor cianobacteriene în lacul Roșu la o scară mare de timp (1975-2002) și să actualizeze informațiile științifice în anul 2011. Hipertrofia, stadiu caracteristic ecosistemelor deltaice în perioada critică de după 1980, a implicat concentrații crescute ale nutrienților, precum și rolul azotului ca factor limitant în dezvoltarea fitoplanctonului. În noile condiții de mediu, a crescut șansa de proliferare a cianobacteriilor, ele devenind grupul dominant în ecosistem. După 1980, media anuală a biomasei a depășit de 10-30 ori pragul de înflorire al apelor. Media lunară din perioada iulie-septembrie a depășit de 100-125 ori limita menționată. Valorile spectaculoase ale abundenței și biomasei cianobacteriene din perioada 1980-1990 au declanșat dominanța unor specii potențial toxice. Indicele de dominanță al biomasei Mc Naughton și Wolf în perioada 1982-1985 a depășit pragul de 0.5 în toate sezoanele. În general, speciile dominante au aparținut grupului Cianobacteria, cu potențial de producere a toxinelor: *M. aeruginosa* KÜTZING 1846, *M. flos-aquae* (WITTR.) KIRCHNER 1898, *M. pulvereae* (WOOD) MIGULA 1849, *A. hassalii* (KÜTZ.) WITTRÖCK 1909, *A. scheremetievi* ELENKIN 1909, *Oscillatoria tenuis* AGARDH 1813. Gradul de edibilitate al acestor specii este foarte scăzut, zooplanctonul ierbivor fiind obligat să acceseze agregatele detrito-bacteriene ca sursă de hrană pentru supraviețuire. În anul 2001, biomasa diatomeelor și cianobacteriilor a scăzut, în special în sezonul cald, ecosistemul a tins către un regim de funcționare mult mai stabil datorită scăderii presiunii nutrienților. În anul 2011, valorile ridicate ale biomasei fitoplanctonului (78.72 s. um. mg l⁻¹), au arătat noi semnale de eutrofizare, incluzând intense episoade de înfloriri cianobacteriene.

Cuvinte cheie: înfloriri cianobacteriene, studii de lungă durată, lacul Roșu, biomasa fitoplanctonică, Delta Dunării.

INTRODUCTION

Nutrient and hydrological conditions strongly influence harmful planktonic and benthic cyanobacterial bloom dynamics in aquatic ecosystems ranging from streams and lakes to coastal ecosystems. Numerous freshwater genera within the diverse phyla comprising the phytoplankton are capable of forming blooms; however, the cyanobacteria are the most notorious bloom formers (PAERI et al., 2001; TÖRÖK, 2008).

The temporal dynamics of Cyanobacteria blooms is variable, with a wide range of possible biological impacts including potentially toxic effects and impacts on food web functionality. Toxin production by certain cyanobacteria (e.g., *Anabaena circinalis* RABENHORST ex BORNET & FLAHAULT 1886, *Aphanizomenon flos-aquae* RALFS ex BORNET & FLAHAULT 1886, *Cylindrospermopsis raciborskii* (WOLOSZYNSKA) SEENAYYA & SUBBA RAJU 1972, *Microcystis aeruginosa*) may lead to a wide array of biological impacts. These include: allelopathic effects on other phytoplankton (SUIKKANEN et al., 2004); suppression of zooplankton grazing, (GILBERT, 1990; FERRAO-FILHO et al., 2000; GHADOUANI et al., 2003); hepatotoxic effects on fish (ANDERSEN et al., 1993); and accumulation of toxins in tissues of invertebrates (LIRAS et al., 1998, LEHTINIEMI et al., 2002) and fish (MAGALHAES, 2001).

Lake Roșu is representative for the lacustrine-type of ecosystems, being the largest lake (1375 ha) of the fluvio-maritime delta.

The objectives of this paper were to highlight the dynamics of cyanobacterial blooms in Lake Roşu at a wider temporal scale (1976-2002) and to update the existing information by presenting the evolution of Cyanobacteria in the year 2011.

MATERIALS AND METHODS

The long-term results belong to the database of the Institute of Biology Bucharest. Updated data (2011) is the original work of the authors.

The samples were taken monthly or seasonally during 1976-2011, covering the same 5 sampling points during the entire period (Fig. 1).

Phytoplankton sampling was performed using a Patalas-Schindler (5 litres) device on water column.

The phytoplankton conservation was made in 500 ml plastic containers, with 4% formaldehyde solution. In the laboratory, phytoplankton samples were concentrated by sedimentation and filtration, using an Ø 65 mm network (VOLLENWEIDER, 1969; BRITTON & GRESSION, 1987). The identification of phytoplankton species and abundance assessment were made using a Zeiss inverted microscope according to UTERMÖHL (1958). Phytoplankton biomass was established by volumetric and gravimetric measurements (OLRIX et al., 1998).

Statistical analyses were performed using SPSS 15.0 Windows Evaluation Version and BioDiversity Pro.

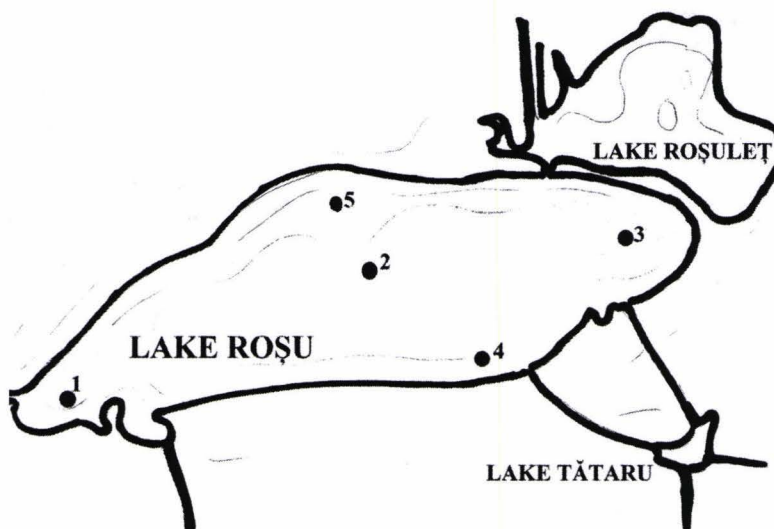


Figure 1. The map of Lake Roşu (the Danube Delta) with sampling points.

RESULTS AND DISCUSSION

The evaluation of bloom frequency highlights the high percents of cyanobacterial blooms during the hypertrophy period (1982-1986) (Table 1). Accelerated eutrophication of the water began to be evident within the Danube Delta from 1980-1982 because of the increased nutrient load in the Danube River (POSTOLACHE, 2006).

The hypertrophy, a characteristic stage of the deltaic ecosystems in the critical period after 1980, involves increased concentration of nutrients and the nitrogen factor-limiting role of phytoplankton development. In the year 2011 constant cyanobacterial blooms was recorded.

After 1980, yearly averages of biomass exceeded 10-30 times the "water blooms" threshold (5 wet weight mg^{-1} , OLTEAN, 1985). Monthly averages in July-September period exceeded 100-125 times the mentioned limit (Fig. 2).

Among the causes for this dramatic increase in phytoplankton biomass, we mention high abundance values and dominance of filamentous, cenobial and colonial species, belonging mainly to Cyanobacteria, followed by Bacillariophyceae. The Mc Naughton and Wolf dominance index (DI) of phytoplankton biomass between 1982 and 1985 exceeded the threshold of 0.5 in all seasons. In general, the dominant species belong to toxin-forming Cyanobacteria (Table 2).

It has been reported high dominance of the species belonging to the genus *Microcystis*, other species of filamentous cyanobacteria (*Aphanizomenon flos-aquae*) and filamentous species of diatoms (*Aulacoseira granulata*, *A. granulata* var. *angustissima*) (Table 2).

Table 1. The frequency of cyanobacterial blooms during long-term studies.

Year	3	4	5	6	7	8	9	10	11	12	%	Frequency level
1976											0	
1977											0	
1978											0	
1982							*				50	constant
1983				*	*	*	*				66	constant
1984				*	*	*	*		*		55	constant
1985					*	*	*	*		*	71	constant
1986					*		*	*	*		57	constant
1987											0	
2000				*							33	accessories
2001					*			*			66	constant
2002					*						33	accessories
2011				*		*		*			100	constant
1976-2011											40	accessories

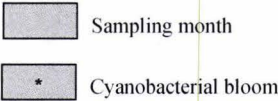


Table 2. The Mc Naughton and Wolf dominance index of phytoplankton biomass.

Year	Month	DI	Dominant species
1982	6	0.3642	<i>Microcystis aeruginosa</i> KÜTZING <i>Chroococcus limneticus</i> LEMM.
	9	0.6986	<i>Microcystis aeruginosa</i> KÜTZING <i>Microcystis flos-aquae</i> (WITTROCK) KIRCHNER
1983	4	0.5751	<i>Cyclotella chaetoceras</i> LEMM. <i>Aulacoseira granulata</i> (EHR.) RALFS
	5	0.3566	<i>Aulacoseira granulata</i> var. <i>angustissima</i> MÜLL. <i>Cyclotella chaetoceras</i> LEMM.
	6	0.3349	<i>Aphanizomenon flos-aquae</i> (L.) RALFS <i>Microcystis aeruginosa</i> KÜTZING
	7	0.3462	<i>Aphanizomenon flos-aquae</i> (L.) RALFS <i>Aulacoseira granulata</i> var. <i>angustissima</i> MÜLL.
	8	0.3789	<i>Aulacoseira granulata</i> (Ehr.) RALFS <i>Aphanizomenon flos-aquae</i> (L.) RALFS
	9	0.5508	<i>Microcystis flos-aquae</i> (WITTROCK) KIRCHNER <i>Microcystis pulvereae</i> (WOOD) MIGULA
1984	3	0.5584	<i>Cyclotella chaetoceras</i> LEMM. <i>Oscillatoria tenuis</i> AGARDH
	4	0.7784	<i>Cyclotella chaetoceras</i> LEMM. <i>Diatoma elongatum</i> AGARDH
	5	0.5210	<i>Cyclotella chaetoceras</i> LEMM. <i>Aulacoseira granulata</i> var. <i>angustissima</i> MÜLL.
	6	0.5977	<i>Microcystis flos-aquae</i> (WITTROCK) KIRCHNER <i>Aulacoseira granulata</i> var. <i>angustissima</i> MÜLL.
	7	0.4789	<i>Microcystis pulvereae</i> (WOOD) MIGULA <i>Chroococcus minutus</i> (KÜTZ.) NÄGELI
	8	0.6141	<i>Microcystis aeruginosa</i> KÜTZING <i>Aphanizomenon flos-aquae</i> (L.) RALFS
	9	0.3984	<i>Aulacoseira granulata</i> (EHR.) RALFS <i>Aphanizomenon flos-aquae</i> (L.) RALFS
	11	0.6636	<i>Aphanizomenon flos-aquae</i> (L.) RALFS <i>Cyclotella chaetoceras</i> LEMM.
1985	4	0.6159	<i>Cyclotella chaetoceras</i> LEMM. <i>Diatoma elongatum</i> AGARDH
	6	0.5365	<i>Melosira varians</i> C. A. AG. <i>Aulacoseira granulata</i> var. <i>angustissima</i> MÜLL.

	7	0.3158	<i>Anabaena hassalii</i> (KÜTZ.) WITTROCK <i>Anabaena scheremetievi</i> ELENKIN
	8	0.4956	<i>Aulacoseira granulata</i> var. <i>angustissima</i> MÜLL. <i>Aphanizomenon flos-aquae</i> (L.) RALFS
	9	0.5938	<i>Microcystis pulverea</i> (WOOD) MIGULA <i>Oscillatoria tenuis</i> AGARDH
2011	6	0.8885	<i>Aulacoseira granulata</i> (EHR.) RALFS <i>Aulacoseira granulata</i> var. <i>angustissima</i> MÜLL.
	8	0.1566	<i>Aulacoseira granulata</i> (EHR.) RALFS <i>Microcystis flos-aquae</i> (WITTROCK) KIRCHNER
	10	0.7756	<i>Aulacoseira granulata</i> (EHR.) RALFS <i>Aulacoseira granulata</i> var. <i>angustissima</i> MÜLL.

When blooms (or dense surface scums) are formed, the risk of toxin contamination of surface waters increases especially for some species of algae with the ability to produce toxins and other noxious chemicals (PAERL et al., 2011). During eutrophication, in the evolution of phytoplankton, there was an important phenomenon influencing the structural and functional dynamics of zooplankton and bacterioplankton, namely dimensional variation of phytoplankton community, the nanoplankton being replaced by large and filamentous species (NICOLESCU & OLTEAN, 1984). The phytoplankton populations have not the same nutritional value to the food web of the ecosystem. Cyanobacteria biomass has ensured a low energy value used by consumers, compared with diatoms and green algae. All these changes in the structure and function of phytoplankton fundamentally transform the ecosystem state that affects the offer of ecological services (PARPALĂ et al., 2008).

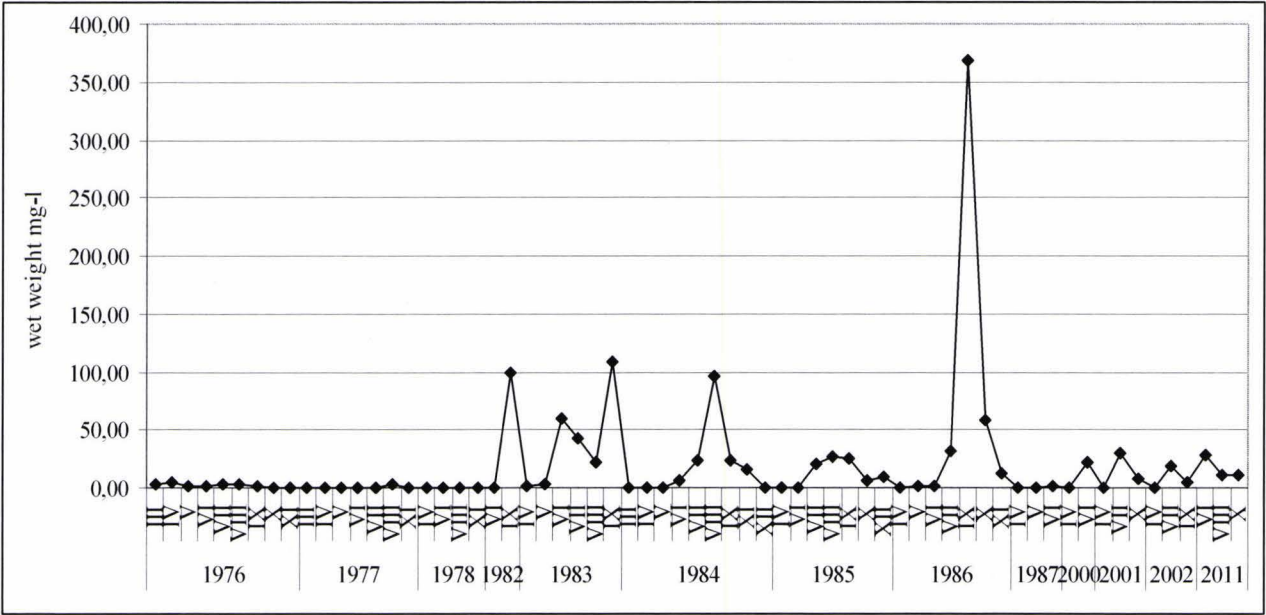


Figure 2. The long-term dynamics of cyanobacteria biomass in Lake Roșu.

When phytoplankton biomass increases during eutrophication, there are coincident changes in the taxonomic structure. Most notably the relative biomass of cyanobacteria increased with eutrophication while in 1977 (mesotrophy stage) the diatoms dominated (Fig. 3). In 2001 the diatoms and cyanobacteria biomass decreased, especially in the warm seasons, and the ecosystem tended to reach a functional regime, more stable, due to a lower nutrient pressure (Fig. 3). There was an increasing trend in the cyanobacterial species richness in the taxonomic composition of phytoplankton in the analysed period. It reached 35% in 2011 (Fig. 4). The Ward's distance indicated a similarity cluster among 2000, 2001, 2002 and 2011, not far from 1983-1984 cluster (hypertrophy period) (Fig. 5). This analysis shows that eutrophication is a long-term process and the cyanobacterial blooms continue today. The edibility degree of cyanobacterial species is very low, the herbivorous zooplankton being forced to feed on detrito-bacterial aggregates to be able to survive. As large, inedible algae, they induce a bottleneck in the carbon and energy flow of the plankton food web (GILBERT, 1990). According to Pearson correlation, in 2011, the zooplankton biomass is explained by an inverse relationship of the cyanobacteria biomass ($r = -0.559$, $p = 0.029$) (Fig. 6).

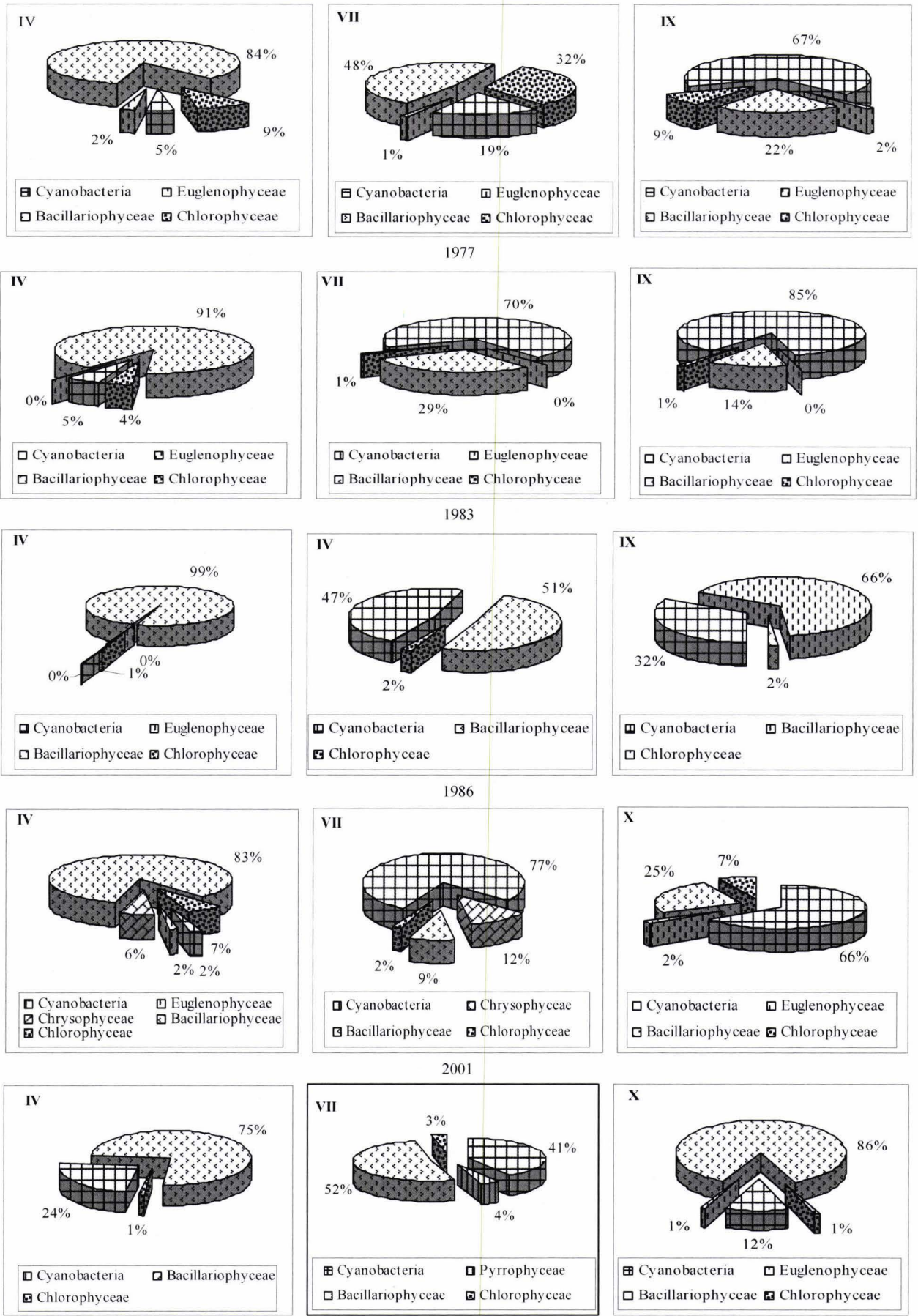


Figure 3. The seasonal variation of the intensity of the cyanobacterial blooms in the selected years.

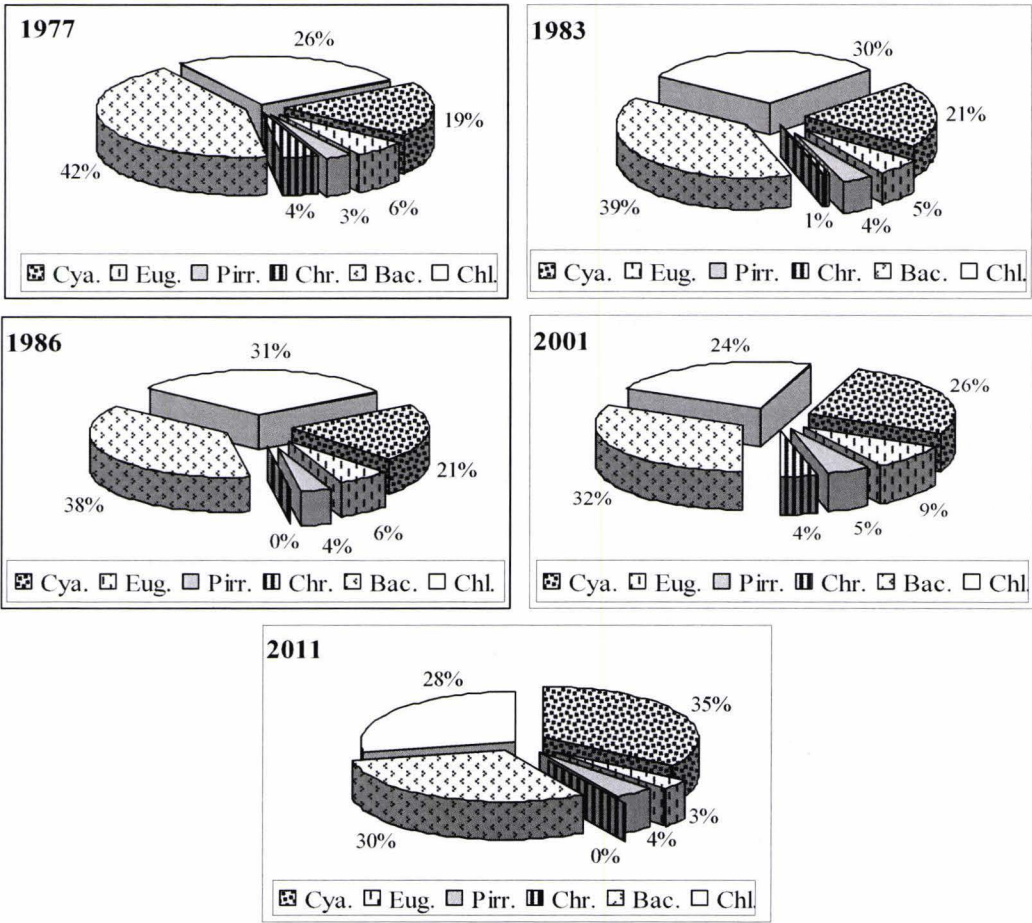


Figure 4. The annual variation of Cyanobacteria species proportion from the total phytoplankton in the selected years.

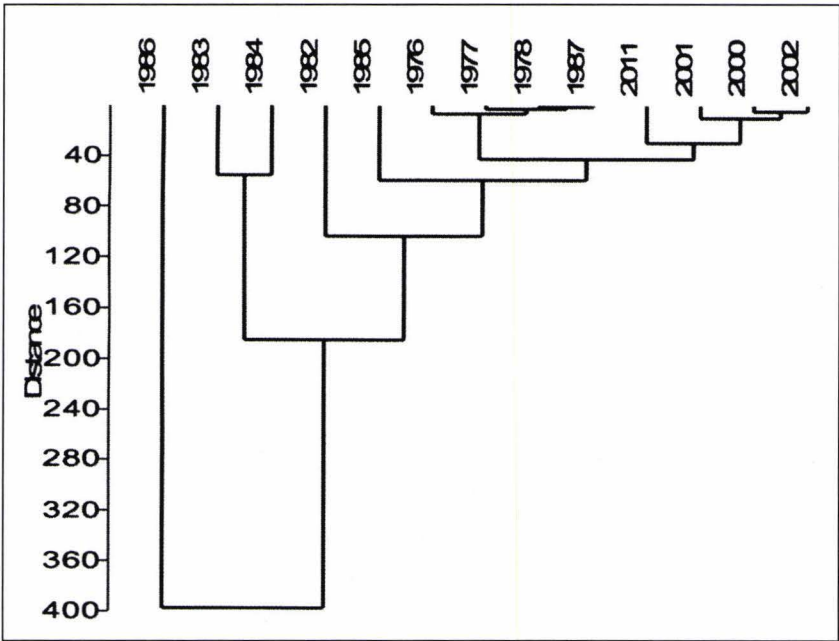


Figure 5. The similarity (Ward's method) of the studied years based on cyanobacterial blooms.

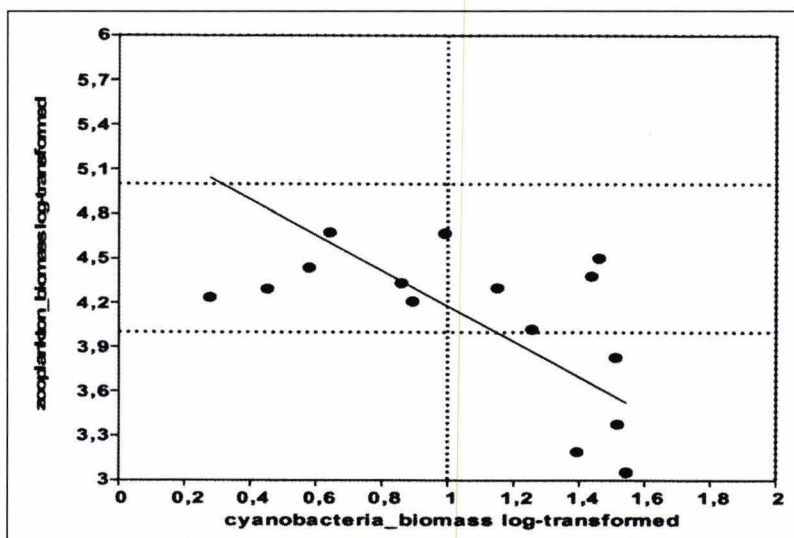


Figure 6. The Pearson correlation between zooplankton and cyanobacteria biomass in 2011 conditions.

CONCLUSIONS

In 2011, the high values of phytoplankton biomass (78.72 wet weight mg^{-1}) show new eutrophication signals, including intense cyanobacterial blooms episodes.

The frequency of cyanobacterial blooms reaches 40% in entire period.

Further investigations will establish if the cyanobacterial blooms are constant and triggered by other factors, as climate change and global warming.

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THE ACIDOPHILIC BACTERIA ABILITY TO PRODUCE METALLOENZYMES RESPONSIBLE FOR THE STARCH DEGRADATION IN THE PRESENCE OF HEAVY METAL IONS

CISMAȘIU Carmen Mădălina

Abstract. An action of removing heavy metal ions from aqueous solutions consists in their biosorption using the acidophilic heterotrophic bacteria of the *Acidiphilium* genus present in aquatics and ground environments. The assimilative capacity of heavy metals by the aerobic bacterial biomass has been investigated and compared to different types of biomass, grown in laboratory conditions by exploiting synthetic wastewater. Some of these types of biomass have a high potential for biosorption, appreciated as competitive in the biotechnological detoxification of industrial effluents. Efficiency of the extracellular enzymatic activity of the acidophilic heterotrophic bacteria is correlated in direct proportion to the amount of organic substances from the environment, the concentration of oxygen and heavy metallic ions. In this context, this paper presents the influence of Cu^{2+} , Zn^{2+} and Ni^{2+} on the enzymatic activity of the extracellular starch degradation in the presence of acidophilic heterotrophic bacterial populations of the *Acidiphilium* genus in order to increase the efficiency of organic substances deterioration ability for contaminated environments with heavy metal ions. The obtained results showed a stimulation of starch degradation by the action of extracellular amylolytic enzymes synthesized by acidophilic bacterial populations in the presence of heavy metal ions. In addition, the performed studies allowed the selection of bacterial cultures with increased degradation capacity of organic substances under extreme environmental conditions for removal of heavy metallic ions from industrial waste waters.

Keywords: metalophiles, biocatalytic application, starch hydrolyzing, enzymes.

Rezumat. Capacitatea bacteriilor acidofile de a produce metaloenzyme responsabile cu degradarea amidonului în prezența ionilor de metale grele. O metodă de îndepărtare a ionilor de metale grele din soluții apoase o reprezintă biosorbția acestora folosind bacterii heterotrofe acidofile din genul *Acidiphilium* prezente în mediile acvatice și terestre. Capacitatea de asimilare a metalelor grele de către biomasa aerobă de bacterii a fost investigată și comparată la diferite tipuri de biomasă, crescute în condiții de laborator utilizând ape reziduale sintetice. Unele din aceste tipuri de biomasă au un potențial ridicat de biosorbție, apreciate ca biotehnologii competitive în detoxifierea efluenților industriali. Eficiența activității enzimactice extracelulară a bacteriilor heterotrofe acidofile este corelată direct proporțional cu cantitatea de substanță organică din mediu, concentrația de oxigen și ioni metalici. În acest context, studiul de față prezintă influența Cu^{2+} , Zn^{2+} și Ni^{2+} asupra activității enzimactice de degradare a amidonului în prezența populațiilor de bacterii heterotrofe acidofile din genul *Acidiphilium* în vederea creșterii eficienței capacității de degradare a substanțelor organice din medii poluate. Rezultatele obținute au evidențiat o stimulare a hidrolizei amidonului sub acțiunea enzimelor amilolitice extracelulare sintetizate de populațiile bacteriene în prezența ionilor de metale grele. De asemenea, studiile efectuate au permis selectarea unor culturi bacteriene cu capacități crescute de degradare a substanțelor organice în condiții extreme de mediu pentru îndepărtarea ionilor metalici din ape reziduale industriale.

Cuvinte cheie: metalofile, aplicație biocatalitică, hidrolizarea amidonului, enzime.

INTRODUCTION

The uncontrolled discharge of industrial waste waters into the river systems containing metallic ions induce profound changes of water quality which are translated in disrupting the ecosystems due to perturbation processes in the trophic chain, with inhibiting mineralization and accumulating of heavy metals in elevated concentrations in certain aquatic organisms. The presence of metallic ions in the solution and the need for their removal has opened two important ways to approach research, namely: their extraction in order to recover heavy metal ions and industrial waste water treatment to reduce the concentrations of heavy metal ions to values mentioned in international standards (VOLESKY, 1994; GUPTA & MOHAPATRA, 2003; SUD et al., 2008; SINGH et al., 2011).

In polluted habitats, the most sensitive species removed environmental pollutants, reduce the competitive interactions and also promote the proliferation of stress-tolerant species; therefore, biodiversity is greatly reduced in the proximity of the discharges of industrial waste water and the acidophilic bacteria are reduced to several dominant species. The mechanism of the action to chemical agents is realized by their effect on one of the significant structures of the cell, namely cell membranes, proteins, the cytoplasmic enzyme or the nuclear apparatus, whose normal operating mode is absolutely necessary to acidophilic heterotrophic bacteria for adaptation at elevated concentrations of metal ions from the surrounding environment (LEUNG et al., 2001; KIRK et al., 2002; GOMES & STEINER, 2004; ENACHE & KAMEKURA, 2010; LU et al., 2010; ALMEIDA et al., 2011).

Acidophilic heterotrophic bacterial populations participate in the mineralization of organic substances from the soil, in these ways vegetal and animal residues shall be brought in a form that is useable in the nutrition of plants or other microorganisms. They are involved in the soil solubilisation of mineral stocks, facilitating their use by acidophilic heterotrophic bacteria with implication in reduced environmental pollution by industrial mining activities. It is considered that the chemical and physiological reactions taking part in the retention of heavy metal ions was depended on the microbial cell of physiological requirements, the chemical status of the heavy metal ions in the cells of impact with the products secreted by the cells. As a whole, all of these are substantially influenced by surrounding environment (VIEIRA & VOLESKY, 2000; AGRAWAL et al., 2005; AIYER 2005; GAYRIVRILESCU & CHISTIL, 2005).

The populations of acidophilic heterotrophic bacteria isolated from acid mine waters shall establish a series of relationships leading to the formation of community structures that ensure an optimum use of energy flow in extreme environments and a given acid homeostasis. Their diversity shall be amended under the influence of the stress factors from the acidic environment (GOYAL et al., 2003; LENTZEN & SCHWARZ, 2006; JOHNSON & HALLBERG, 2008).

The heterogeneity of physical-chemical factors of the surrounding environment stimulates the ecosystem stability. The diverse nature of acidophilic heterotrophic bacterial populations present in extremes habitats. The environmental disturbance as frequency and intensity, correlated to the degree of heterogeneity has determined the speed and range of progressive flexibility in a continuous ecosystem. The heterogeneity of physico-chemical requirements, such as concentration and type of organic and inorganic substances, conditions of pH, Eh, degree of illumination, humidity, osmotic pressure, the concentration of dissolved gases (mainly O₂ and CO₂), of toxic or inhibitory substances create physico-chemical conditions favourable to certain species and unacceptable for others. As a consequence, the environmental compartments show a discontinuous spatial distribution of acidophilic heterotrophic bacterial populations (TILMAN et al., 1997; KONSULA & LIAKOPOULOU-KYRIAKIDES, 2004; KIMURA et al., 2011; SHARMA et al., 2012).

The acidophilic heterotrophic bacteria have metabolism products of the type that can be synthetically chemically used as a carbon source of organic compounds but behave differently regarding the nitrogen of the source nature, as some of them still maintain the capacity to use inorganic chemicals, while others require amino acids and use just the organic part of nitrogen compounds and of the chemical complex. They are also called organotrophic bacteria because they use the organic substances as a carbon source for the biosynthesis and energy production. They show a large adaptability to the environmental conditions regarding the temperature and pressure and the mineralization. (BERTOLDO & ANTRANIKIAN, 2002; HAKI & RAKSHIT, 2003; REDDY et al., 2003; GUIDASZ et al., 2010; KRISHNANI et al., 2012).

The biodegradation of the carbon compounds by acidophilic heterotrophic bacterial populations is very different in aerobic and anaerobic niches. Thus, in aerobic environments, by natural synthesis, organic chemicals are completely degraded, whereas in anaerobic environments they are incorporated as organic connections of the carbon substances becoming recalcitrant to biodegradation. They may be converse to the other recalcitrant forms or deposited in the layer and the exposure to physical and chemical processes determine the digenesis of fossil fuels under the impact of the complex community of acidophilic heterotrophic populations (HOOPER & VITOUSEK, 1997; GIANFREDI & RAO, 2004; BERGGREN et al., 2010; PANDEY & FULEKAR, 2012).

The data in specialized literature have pointed out that the submissions of calcareous rocks reduce the amount of carbon stock available for the biological systems from the surrounding environment. This may return to circulation following the erosion processes or the indirect action of acidophilic heterotrophic bacterial populations. Furthermore, coals contain sulphur compounds in quantities varying between 0.5 to 11% in the form of organic or inorganic complexes (sulphides of lead, iron, zinc, copper or as CaSO₄ in the water and only very rarely as sulfur content) (YOUNGER, 2004; SAJEDI et al., 2005; AZKI, 2008; 2009; SINGH et al., 2011; AXINI, 2012).

It is well known that the physiological group of heterotrophic bacteria present in the acid mining drainage has an ecological importance, as well as a practical importance, being a source of new bacteria with biotechnological potential. The study of the interactions between acidophilic heterotrophic bacteria and heavy metal ions can be achieved through active processes, involving the metabolic sequences of living microorganisms or passive processes, independent of cellular metabolism. The residual biomasses of heterotrophic bacteria belonging to the *Acidiphilium* genus proved an affinity for a wide variety of heavy metal ions such as Cr⁶⁺, Cr³⁺, Cu²⁺, Zn²⁺ and Ni²⁺ (ATKINSON et al., 1998; BOUKHIFI & BENCHEIKH, 2000; AZKI, 2003; WANG & CHEN, 2009; FAROOQ et al., 2010).

In view of the above, the present study is aimed at evaluating the extracellular starch degradation enzyme activity of the *Acidiphilium* populations, isolated from representative mining sites in Romania, in the presence of the heavy metallic ion solution and at selecting some bacterial cultures with high starch degradation process in the presence of these ions in the environment.

MATERIAL AND METHODS

To cultivate the acidophilic heterotrophic bacteria from the *Acidiphilium* genus it was used a selective medium, organic medium with pH=3.0, in which the source of energy is the glucose (CISMAȘIU et al., 2010). From samples of complex ores, mining waters and sediments (Baia and Rosia Poieni County) bacterial strains and populations belonging to this genus were obtained after 21-day incubation at 28°C. The isolated colonies obtained on agar medium are cultivated in GYE liquid medium in continuous agitation conditions at a temperature of 28°C for 7 days.

In order to increase the efficiency of the starch degradation process using *Acidiphilium* populations in the presence of the metallic ions solution, experiments were accompanied by chemical controls (heavy metallic ions and selective culture medium). Chemical controls were made in six Erlenmeyer flask with GYE medium at the starch concentration of 2.0g/l as an optimum substrate, which contains solutions of CuSO₄ and CuCl₂ respectively, ZnSO₄ and ZnCl₂ respectively, NiSO₄ and NiCl₂ respectively. The tests about the influence of CuSO₄-CuCl₂ solutions, ZnSO₄-ZnCl₂ solutions and NiSO₄-NiCl₂ solutions on the starch degradation enzymatic activity of bacterial populations were performed in GYE medium with 2.0 g/l starch at a temperature of 28°C in stirring incubation conditions. Growth experiments were carried out at different species type, heavy metal ion type and contact times between cell and these

ions. Regarding the influence of metallic ions solution on extracellular starch degradation process of the acidophilic heterotrophic bacteria it was followed the bacterial density of *Acidiphilium* populations (measuring spectrophotometer turbidity at a wavelength of 660nm) and extracellular starch enzymatic activity of bacterial populations (the spectrophotometer determination of starch at 580nm) by Wohlgemuth method (CISMAȘIU, 2012).

RESULTS

Comparative studies made on the *Acidiphilium* populations cultivated in GYE medium with 2.0g/l starch in the presence of 0.1% CuSO₄ and CuCl₂ solution, of 0.1% ZnSO₄ and ZnCl₂ solution and of 0.1% NiSO₄ and NiCl₂ solution are illustrated in figures 1-6. Regarding the influence of 0.1% CuSO₄ solution, respectively CuCl₂, on the growth of two bacterial populations, it is shown that the P₄ population, isolated from Roșia Poieni area, is more sensitive to 0.1% CuSO₄ compared to 0.1% CuCl₂ in the same experimenting conditions after 7 days of incubation. It was also established that, during the period, they present a reduced growth, although they are cultivated in the same experimental conditions. Thus, in an organic medium with 0.1% CuSO₄ solution, the P₄ population had an optical density of only 0.213, compared to the P₇ population in the presence of 0.1% CuCl₂, for which the values reached 0.235 after 7 incubation days (Figs. 1-2).

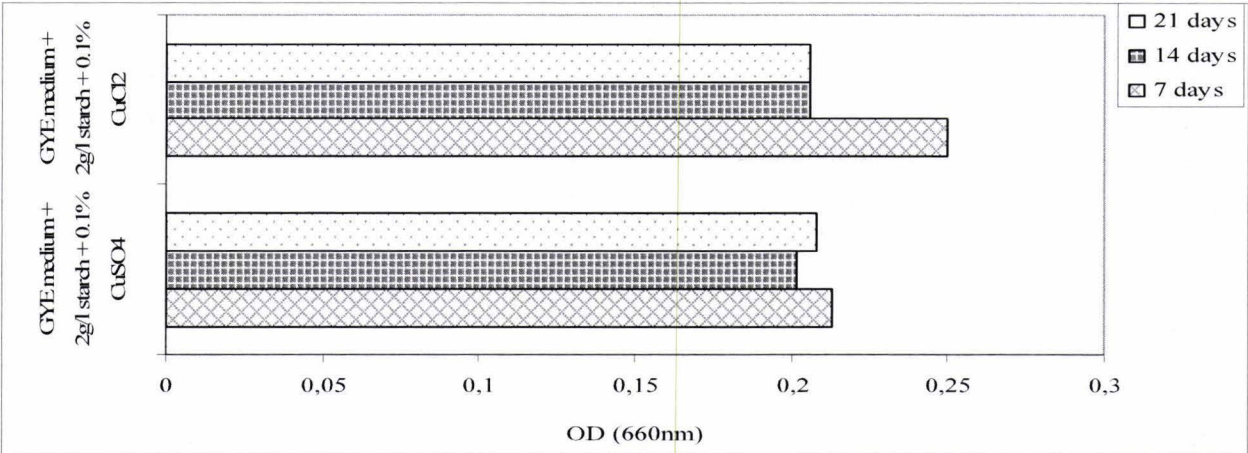


Figure 1. The bacterial density of the P₄ population from the *Acidiphilium* genus in the presence of 0.1% CuCl₂, respectively 0.1% CuSO₄, in the GYE medium with 2g/l starch, at intervals of 7 days incubation.

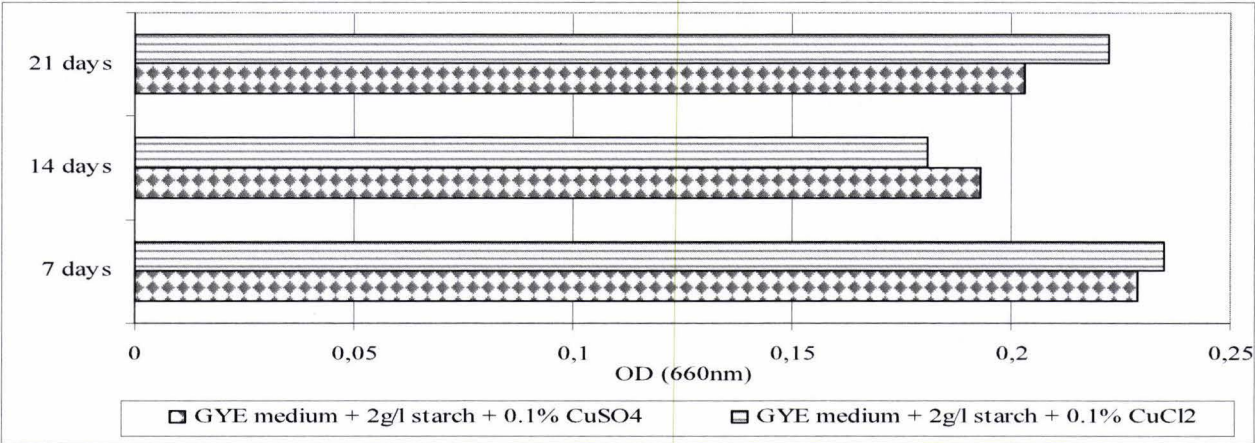


Figure 2. The bacterial density of the P₇ population from the *Acidiphilium* genus in the presence of 0.1% CuSO₄, respectively 0.1% CuCl₂, in the GYE medium with 2g/l starch.

The comparative results regarding the bacterial density of the two *Acidiphilium* populations in the presence of 0.1% ZnSO₄, ZnCl₂ respectively, proved to be efficient in the extracellular starch degradation processes in the GYE medium with 2g/l starch. It was also demonstrated that the final pH values of treated organic medium with 2g/l starch were lower than the initial values of 3.0, a fact correlated with the higher bacterial density of the acidophilic heterotrophic population isolated from mining effluents in different experimental conditions after 21 days of incubation (Figs. 3-4).

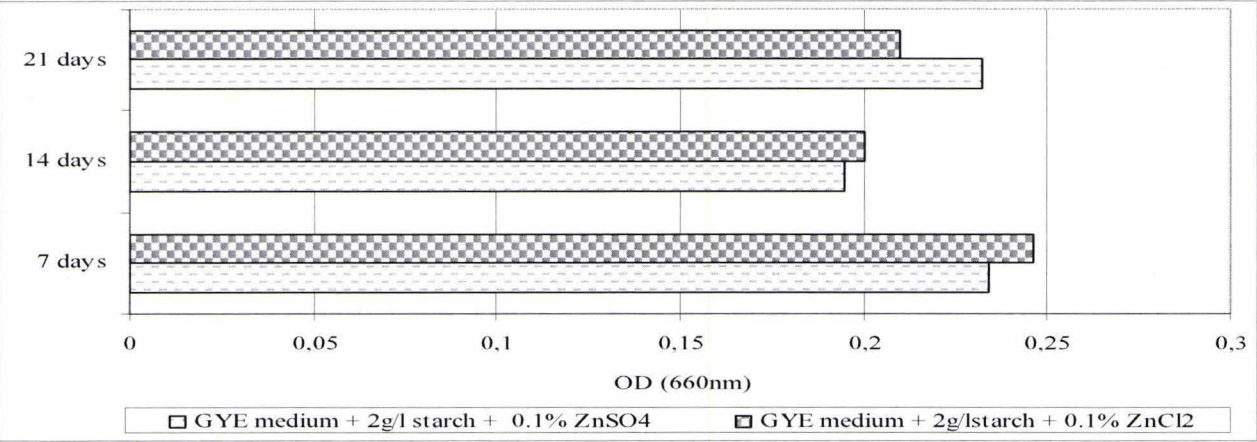


Figure 3. The bacterial density of the P₄ population from the *Acidiphilium* genus in the presence of 0.1% ZnSO₄, respectively 0.1% ZnCl₂, in the GYE medium with 2g/l starch.

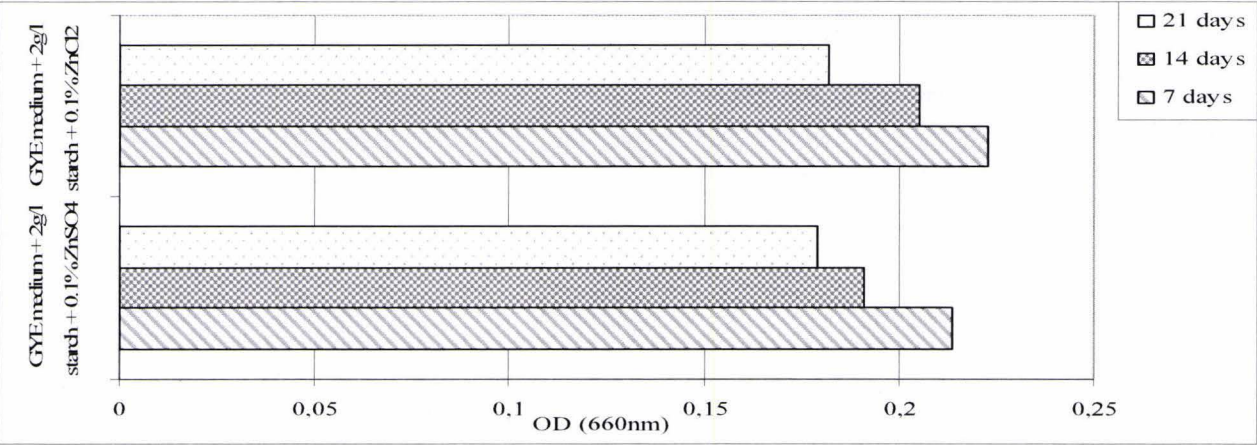


Figure 4. The bacterial density of the P₇ population from the *Acidiphilium* genus in the presence of 0.1% ZnCl₂, respectively 0.1% ZnSO₄, in the GYE medium with 2g/l starch.

Treating the GYE medium with 2g/l starch with biomass of the two *Acidiphilium* populations in the presence of 0.1% NiSO₄ and NiCl₂ solutions proved that the highest optical density of the bacterial culture was got at pH=3.0 by up to 7 and 21 days of incubation periods at the same heavy metal ion concentration (Figs. 5-6).

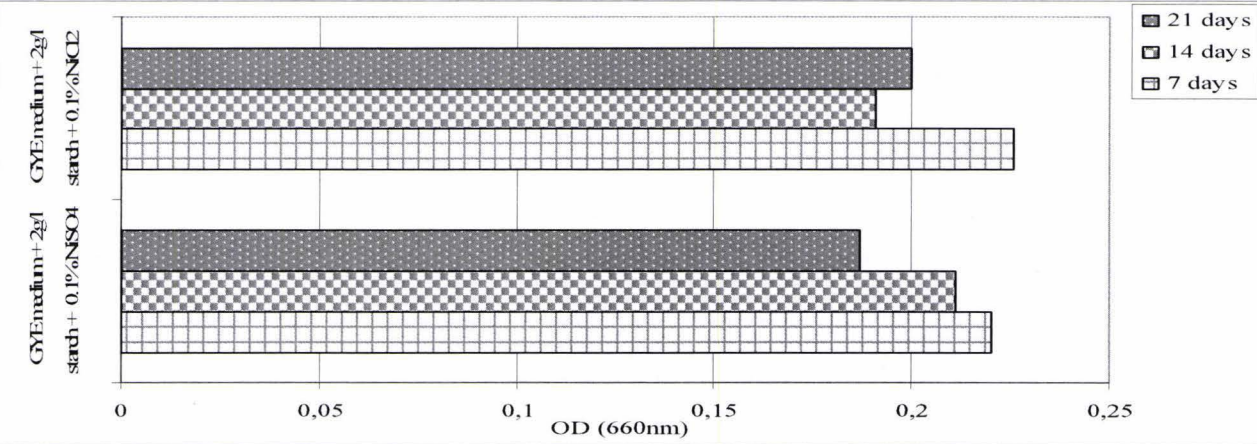


Figure 5. The bacterial density of the P₄ population from the *Acidiphilium* genus in the presence of 0.1% NiCl₂, respectively 0.1% NiSO₄, in the GYE medium with 2g/l starch.

The most important physical and chemical parameters that influence the growth of the *Acidiphilium* populations, isolated from water mining effluents of Baia and Roșia Poieni areas, in the presence of 0.1% NiSO₄-NiCl₂ solution are the contact time between biomass and the GYE medium with 2g/l starch as energy source, the type of

heavy metal ions and the metal ion concentration from this selective medium in optimal experimental conditions. This fact is correlated with the high fixation capacity of acidophilic heterotrophic bacterial populations in order to establish their distribution in polluted environments with higher content of heavy metallic ions, namely Cu^{2+} , Ni^{2+} and Zn^{2+} acting as enzyme cofactors in the oxidation-reduction reactions (Figs. 1-6).

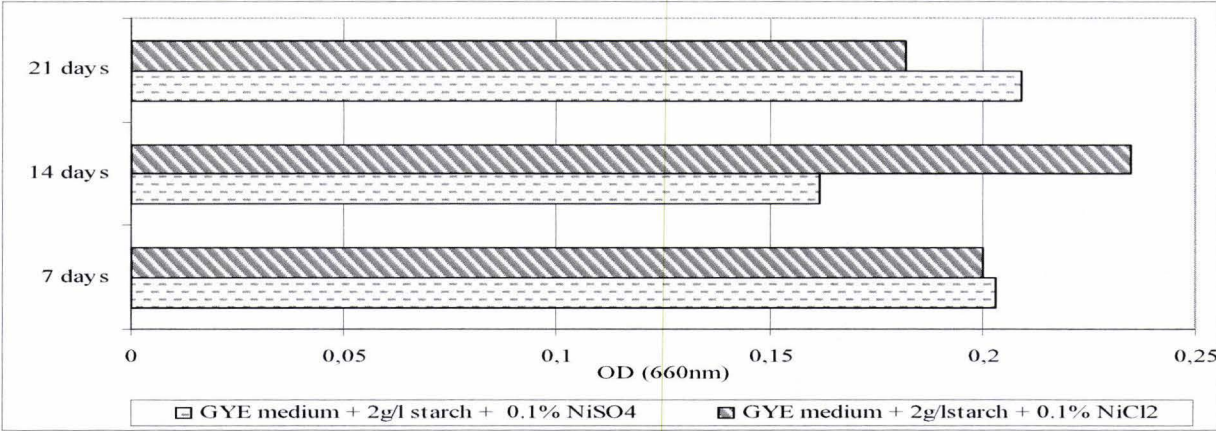


Figure 6. The bacterial density of the P₇ population from the *Acidiphilium* genus in the presence of 0.1% NiSO₄, respectively 0.1% NiCl₂, in the GYE medium with 2g/l starch.

The compared data within studying the extracellular starch degradation enzymatic activity of the two bacterial populations in the same optimum growth conditions with the 0.1% CuSO₄ and CuCl₂ solutions, the 0.1% ZnSO₄ and ZnCl₂ solutions and the 0.1% NiSO₄ and NiCl₂ solutions are illustrated in figures 7-9. The results regarding the influence of the 0.1% CuSO₄, NiSO₄ and ZnSO₄ concentration, in different experimental variants, concerning the extracellular hydrolytic activity of the acidophilic heterotrophic bacteria from *Acidiphilium* sp., isolated from the two mining sites mentioned above, permitted the selection of some bacterial strains and populations with a higher resistance to the presence of these ions in the medium regarding the development of the biosorption processes (Figs. 7-8).

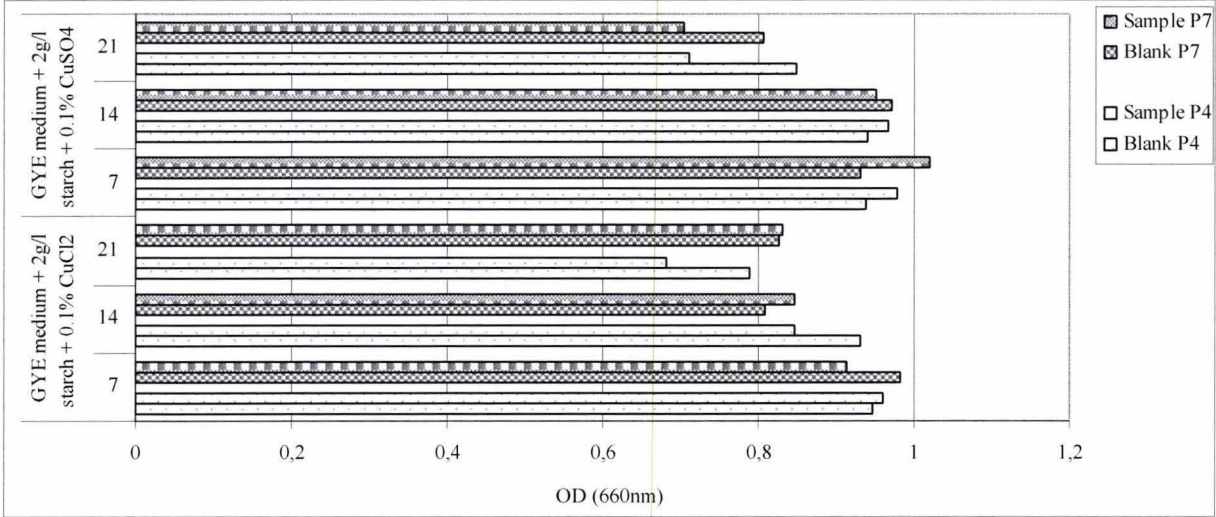


Figure 7. The extracellular starch degradation enzyme activity of the *Acidiphilium* populations in the presence of 0.1% CuSO₄, respectively 0.1% CuCl₂, at 2g/l starch in GYE medium.

In all performed experiments the extracellular activity of the starch degradation by the *Acidiphilium* populations isolated from water and sediment samples from Baia and Roșia Poieni areas was stimulated by the presence of Cu^{2+} in the form of CuSO₄, Zn^{2+} in the form of ZnSO₄, Ni^{2+} in the form of NiSO₄, acting as cofactors, in the concentration of 0.1% on the selective medium with 2g/l starch (Figs. 7-9). The comparative analysis of the results obtained in this study, illustrated in figure 9 demonstrated that the P₄ population (isolated from Roșia Poieni) are less sensitive to the tested concentration, compared to the P₇ population (isolated from Baia) on the whole incubation period. The comparative studies regarding the influence of the metallic ions solution on the growth and the starch degradation activity are developed with a maximum intensity up to 14 days of incubation periods at the same substrate concentration. High percentage degradation of the substrate obtained by using populations of heterotrophic bacteria compared with purified strains, which confirms the adaptation of the two *Acidiphilium* populations to the used concentrations of heavy metal ions, are illustrated in figures 7-9.

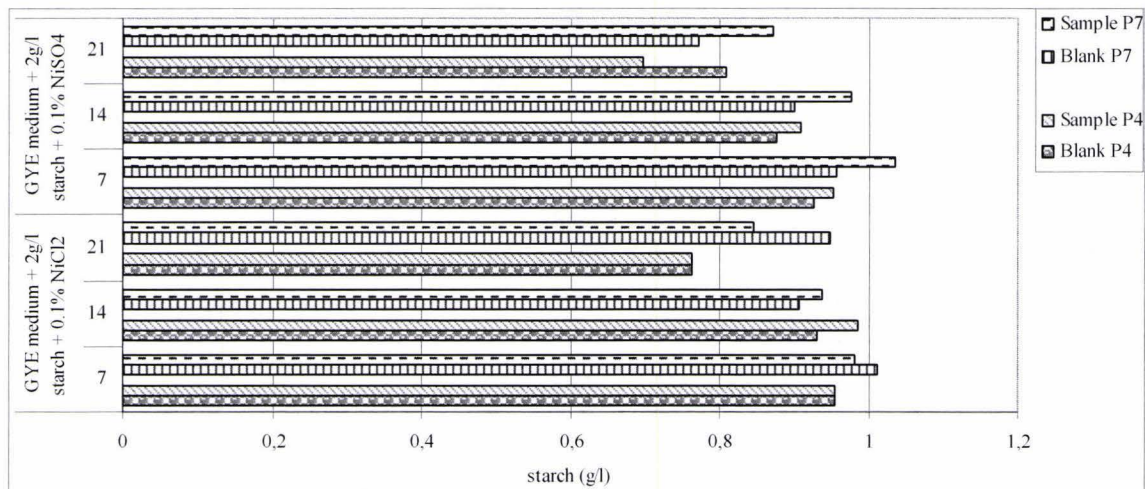


Figure 8. The extracellular starch degradation enzyme activity of the *Acidiphilium* populations in the presence of 0.1% Ni SO₄, respectively 0.1% Ni Cl₂, at 2g/l starch in GYE medium.

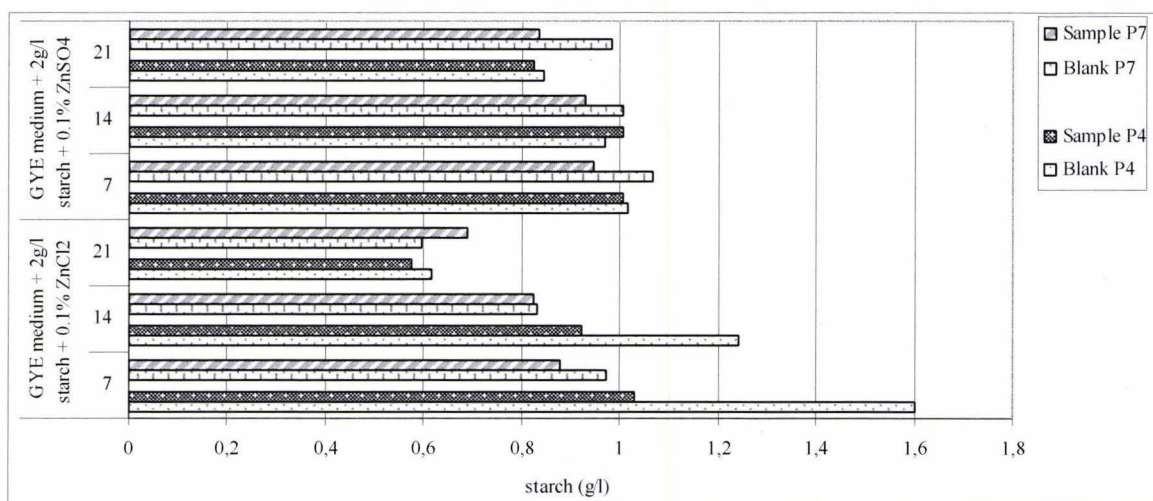


Figure 9. The extracellular starch degradation enzyme activity of the *Acidiphilium* populations in the presence of 0.1% ZnSO₄, respectively 0.1% ZnCl₂, at 2g/l starch in GYE medium.

The comparative analyses regarding the influence of the heavy metallic ions (Cu²⁺, Zn²⁺ and Ni²⁺) on the extracellular starch degradation activity using acidophilic heterotrophic bacterial biomass evidenced the fact that the cultures grown in the presence of 0.1% CuCl₂, ZnCl₂ and NiCl₂ solutions have a slower enzymatic activity at a substrate concentration established at 2g/l starch. The comparative studies made on the influence of heavy metallic ion solutions on the growth and the extracellular enzymatic activity of the *Acidiphilium* populations cultivated in GYE medium with starch, as carbon and energy source, proved that the influence occurs at a great intensity up to 7 days of incubation periods at the same heavy metal ion concentration in continuous agitation conditions. The comparative results of the research regarding extracellular starch degradation enzyme activities of the bacterial populations in the presence of 0.1% CuSO₄, ZnSO₄ and NiSO₄ solutions evidenced the bacterial density in the GYE medium with 2g/l starch was raised, a fact correlated with higher percentages of the hydrolytic activity, thus confirming the data from the specialty literature on the higher abilities of the mixed populations to adapt to acidic conditions. In accordance with the specialty literature, an advantage in the extracellular hydrolysis process of starch is the fact that the value of the pH and the optimum temperature coincide with the optimum values to increase the bacterial density of acidophilic heterotrophic bacterial populations concerned to the decontamination of polluted environments with high concentrations of metal ions (GOYAL et al., 2003; GIANFREDI & RAO, 2004; AIYER, 2005; WANG & CHEN, 2009; CISMAȘIU et al., 2010).

CONCLUSIONS

The extracellular starch degrading activity of acidophilic heterotrophic bacterial populations in the presence of 0.1% CuSO₄, ZnSO₄ and NiSO₄ solutions revealed a correlation among the bacterial density and the chemical valence of heavy metallic ions that act as cofactors. Through their extracellular hydrolytic activities, they lead to changes in the values of the oxidation-reduction potential by modifying the medium composition correlated to the decrease of the

initial pH value of the organic medium. High percentage degradation of the substrate was observed by using acidophilic heterotrophic bacterial populations, which modify the medium composition correlated to the increase of the bacterial density after 14 days at 28°C, which confirms the adaptation of these bacteria to the used concentration of heavy metal ions. The study on metabolism of the acidophilic heterotrophic bacteria is of great importance for the selection of some bacterial strains and populations, which can offer a higher efficiency to the biosorption processes when they are used, having a significant increase of bacterial density and a maximum extracellular starch degradation enzymatic activity even in the presence of some heavy metallic ions concentrations. Regarding the dynamics of the physiological conditions in the organic medium specific to the *Acidiphilium* genus, it was proved that the ability to secrete extracellular substances involved in the starch degradation activities of bacterial populations depends on the chemical valence of the heavy metallic ions, the physical-chemical factors in the selective medium, all of them being important in the removal processes of carbon compounds from industrial waste waters and explanation of biodiversity in extreme environments.

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CHOROLOGY OF THE *PHYSICIA* (SCHREB.) MICHAUX (1803) GENUS IN ROMANIA

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Abstract. Within this paper it is presented the lichen species chorology of the *Physcia* (SCHREB.) MICHAUX (1803) genus. For each species there have been indicated, especially the counties, localities, UTM codes, and substratum. The majority of the recorded data has been published by Romanian lichenologists and a few of these has been published by foreign lichenologists. A part of the records represents author's published and unpublished contributions. The most numerous data on lichen species chorology from *Physcia* genus on Romania territory, have been recorded for *P. adscendens* (FR.) OLIV., *P. stellaris* (L.) NYL. em. HARM. and *P. tenella* (SCOP.) DC. in LAM. & DC. A lower number of data found in literature has been recorded in case of *Physcia endochrysoides* NYL., *P. tribacia* (ACH.) NYL. and *P. tribacoides* NYL. Regarding the author's own contributions, most of the recorded data refer to *P. adscendens*, *P. aipolia*, *P. stellaris* and *P. tenella* within the forests from Ilfov County.

Keywords: chorology, UTM code, *Physcia* genus, Romania.

Rezumat. Corologia genului *Physcia* (SCHREB.) MICHAUX (1803) în România. În cadrul acestei lucrări este prezentată corologia speciilor de licheni din genul *Physcia* (SCHREB.) MICHAUX (1803). Pentru fiecare specie au fost indicate, în special județele, localitățile, codurile UTM și substratul. Majoritatea datelor înregistrate au fost publicate de lichenologi români și câteva dintre acestea au fost publicate de către lichenologi din străinătate. O parte din înregistrări sunt contribuții personale publicate în reviste de specialitate și nepublicate. Cele mai multe date referitoare la răspândirea speciilor de licheni din genul *Physcia* pe teritoriul României, au fost înregistrate pentru *P. adscendens* (FR.) OLIV., *P. stellaris* (L.) NYL. em. HARM. și *P. tenella* (SCOP.) DC. în LAM. & DC. Un număr mai redus de citări în literatura de specialitate a fost înregistrat pentru *Physcia endochrysoides* NYL., *P. tribacia* (ACH.) NYL. și *P. tribacoides* NYL. În ceea ce privește contribuțiile personale, cele mai multe date au fost înregistrate pentru *P. adscendens*, *P. aipolia*, *P. stellaris* și *P. tenella* în păduri din județul Ilfov.

Cuvinte cheie: corologie, codul UTM, genul *Physcia*, România.

INTRODUCTION

In Romania, the species tabulated within the *Physcia* (SCHREB.) MICHAUX (1803) genus are widely distributed in mountainous, hilly and plain areas (MORUZI & TOMA, 1971; CIURCHEA, 2004). A serious contribution on *Physcia* genus chorology in Romania was carried out by MORUZI et al. (1967) and CIURCHEA (2004).

An important role for the distribution of lichen species is attributed to the substratum characteristics. Accordingly, in Romania, the species of the *Physcia* genus have been identified, especially on corticolous substrata (MORUZI & MANTU, 1965; CRIȘAN & ARDELEAN, 2010; VICOL, 2010a; VICOL, 2011b; VICOL, 2012) but they are also reported widespread on man-made substrata (MORUZI & PETRIA, 1961; MANOLIU et al., 1998), saxicolous substrata (CIURCHEA & CRIȘAN, 1991-1992), and lignicolous substrata (VICOL, 2010a). Regarding the distribution in macrohabitats, *Physcia* genus is well represented within forests (BURLACU, 1967; BARTÓK, 1988; VICOL, 2011a).

This study is based on the knowledge of the spatial distribution of the *Physcia* genus in Romania. The main objective of this work consists in mapping of the species of *Physcia* genus on the Romania territory.

MATERIALS AND METHODS

The researches on the chorology of the *Physcia* genus are based on the data gathered from the field, the herbarium specimens, the literature and the author's unpublished data. According to the field observations the species of the *Physcia* genus were identified on corticolous and lignicolous substrata. The original studies on *Physcia* genus chorology in the field were performed in the forest ecosystems and in the agro-ecosystems (in a *Vitis vinifera* L. crop situated at the edge of Igești village, Vaslui County) between 2009 and 2012.

The collected species from the field were determined using the bibliography as it follows: CIURCHEA (2004), MORUZI & TOMA (1971). The lichen species were investigated using a stereomicroscope (Zeiss Stereo CL 1500 ECO) and an optical microscope (Zeiss Scope A 1). The identification of each species was based on morphology, colour reaction of the upper cortex and medulla, and anatomical investigation. The collected lichen species were determined using as chemical reagent KOH (potassium hydroxide). The nomenclature is according to CIURCHEA (2004). The nomenclature for cornophytes is according to CIOCĂRIAN (2009). A part of the identified species are in the Mycology Herbarium, Lichen Collection, abbreviated as BUCM L (Bucharest Mycology Lichen) in the numbers: [BUCM L1306-1307], [BUCM L 1311], [BUCM L 1316], [BUCM L 1318], [BUCM L 1321], [BUCM L 1325], [BUCM L 1328], [BUCM L 1339-1341], [BUCM L 1343], [BUCM L 1345-1347], [BUCM L 1349], [BUCM L 1356], [BUCM L 1366] and [BUCM L 1397].

The mapping of the localities was performed using UTM code according to the work of LEHRER & LEHRER (1990). The maps of the species chorology were constructed using Corolog software (ȘTEFĂNUȚ et al., 2009).

RESULTS AND DISCUSSIONS

In Romania, the *Physcia* genus is represented by the following species: *P. adscendens* (FR.) OLIV., *P. aipolia* (EHRH. ex HUMB.) FÖRNR., *P. caesia* (HOFFM.) HAMPE, *P. dubia* (HOFFM.) LETT., *P. endochrysoides* NYL., *P. semipinnata* (J. F. GMELIN) MOBERG, *P. stellaris* (L.) NYL. em. HARM., *P. tenella* (SCOP.) DC. in LAM. & DC., *P. tribacia* (ACH.) NYL., *P. tribacoides* NYL. (CIURCHEA, 2004).

Original data performed in the investigated macrohabitats on the species of the *Physcia* genus have revealed that these are common and widespread within anthropogenic and ruderalised areas, agro-ecosystems and forests surrounded by agricultural land. During the field work, there have been identified the following species: *P. adscendens*, *P. aipolia*, *P. dubia*, *P. semipinnata*, *P. stellaris*, *P. tenella*, and *P. tribacoides*.

The studies performed in the North-West of Hungary have indicated that in the centre of Komárom locality with a continuous build-up area and relatively high pollution (CO, Pb) caused by increased traffic, there predominate especially nitrofrequency corticolous lichen species, such as: *P. adscendens*, *P. tenella*, accompanied by *Xanthoria parietina* (L.) TH. FR. and *Phaeophyscia orbicularis* (NÉCK.) MOBERG. (FARKAS et al., 2001).

MARMOR & RANDLANE (2007) have carried out a study in Tallinn (Estonia) on the influence of the car traffic in relationship to bark pH and epiphytic lichen species. In this study, authors indicate that *P. stellaris* accompanied by *Lecanora carpinea* (L.) VAIN. and *Xanthoria parietina* have preferred small-leaved lime with a higher bark pH. Traffic did not affect the bark pH of small-lived lime because it is normally subneutral and therefore, alkaline dust does not change its value significantly. Significant correlation between bark pH of *Pinus sylvestris* L. and distance from roads were recorded. Thus, the car traffic has changed the bark pH of *P. sylvestris* from acidic to subneutral, which is favourable to nitrophilous species (*P. tenella*, *Lecanora hagenii* (ACH.) ACH., *Caloplaca holocarpa* (HOFFM. ex ACH.) WADE, and *Candelariella xanthostigma* (PERS.) LETT.

In other study regarding the effect of forest management on epiphytic lichen species in remnants forests distributed in Central Spain, species from *Physcia* genus were related to high management intensity, low shrub cover and areas with no steeper slopes (ARAGÓN et al., 2010).

An interesting study performed in apple orchards from Poland, Slovakia and Italy have pointed out that *P. adscendens*, *P. tenella*, *X. parietina*, *P. orbicularis* were found in most of the investigated orchards. The bark pH *Malus* sp. is naturally higher which favours the occurrence of nitrophilous lichen species (ZARABSKA et al., 2009).

In New Zealand, the species of the *Physcia* genus occur most commonly on coastal and inland (rarely high-alpine) rocks, on a variety of human made substrata (especially concrete), on living bark of trees and shrubs, mainly of planted and ornamental trees (especially fruit trees) in urban and agricultural areas, and more occasionally on native trees and shrubs in forest and shrub communities (GALLOWAY & MOBERG, 2005).

The chorology of lichen species tabulated within *Physcia* genus on Romania territory is presented as it follows:

Physcia adscendens (FR.) OLIV. (Fig. 1).

Alba County

Apuseni Mountains, within forests from Avram Iancu locality surroundings (FS 33), on beech and ash rhytidome (CIURCHEA & CODOREANU, 1967; CIURCHEA, 2004); Roşia Montană (FS 62/63) on *Populus tremula* L., *Salix alba* L., and *Prunus domestica* L. rhytidome (CRIŞAN & ARDELEAN, 2010); Bihorului Mountains, Răchita Peak (FS 43), altitude 1300 m, on fir, spruce and maple rhytidome (BARTÓK, 1982; CIURCHEA, 2004).

Botoşani County

Within Dersca, Lozna, Hilişeu, Pădureni and Şendriceni forests (MP 41), on *Quercus* sp., *Acer campestre* L., *Fraxinus excelsior* L., *Cornus* sp., *Tilia cordata* MILL., altitude 300 m (BURLACU, 1967; CIURCHEA, 2004); Vârful Câmpului Forest (MN 49), Horlăceni and Gorovei forests (MP 50) (BURLACU, 1969c; CIURCHEA, 2004).

Bucharest

Botanical Garden (MK 21/22/31/32), on *Abies nordmanniana* (STEVEN) SPACH, *Pinus strobus* L., *P. nigra* J. F. ARNOLD, *Gleditsia triacanthos* L., and *A. campestre* L. rhytidome (MORUZI & PETRIA, 1961; CIURCHEA, 2004); Băneasa Forest (MK 21/22/31/32), on lignicolous substrata, leg. et det. Vicol Ioana, 02.06.2010 [BUCM L 1316] (unpublished data), on lignicolous substrata, leg. et det. Vicol Ioana, 18.03.2011 (unpublished data), on corticolous substrata (VICOL, 2010b).

Caraş-Severin County: the Danube Gorge, within forests between Cozla and Pescari (EQ 54), on *A. campestre*, *Carpinus orientalis* MILL., *Fagus sylvatica* L., *Fraxinus ornus* L., *Quercus cerris* L., and *Tilia tomentosa* MOENCH (*T. argentea* DC.) rhytidome (BURLACU et al., 1969; CIURCHEA, 2004).

Călăraşi County

Călăreţilor Forest (MK 62), on *Quercus robur* L. rhytidome, leg. Vicol Ioan, 04.06.2010, det. Vicol Ioana, 04.06.2010 [BUCM L 1397] (VICOL, 2012); Goştilele Forest (MK 62), on *Robinia pseudacacia* L., leg. Vicol Ioan, 03.06.2010, det. Vicol Ioana, 03.06.2010 (VICOL, 2012).

Cluj County

Botanical Garden from Cluj (FS 97), on *Hippophaë rhamnoides* L. rhytidome (CODOREANU et al., 1960; CIURCHEA, 2004); Transilvaniei Plain, Băile Sărate, Turda (GS 15), on *Fraxinus excelsior* L. rhytidome (TODOR, 1947; CIURCHEA, 2004).

Giurgiu County

Crețești Forest (MK 30), on lignicolous substrata, leg. et det. Vicol Ioana, 24.08.2010 (unpublished data); Crevedia Forest (MK 02), on lignicolous substrata, leg. et det. Vicol Ioana, 17.03.2011 (unpublished data); Bolintin-Deal Forest (MK 02), on *Quercus pedunculiflora* K. KOCH (VICOL, 2011c).

Constanța County

Constanța, Trofeul Traiani, Adamclisi (PJ 28/29/38/39), altitude 150 m, 44°06'07.00"N, 27°57'20.80"E, 16.05.2007, on *Juglans* sp., *Tilia* sp. (YAVUZ & ÇOBANOĞLU, 2008).

Harghita County

Căpâlnița Village (LM 83), on fruit trees (BARTIL, 1905; CIURCHEA, 2004).

Iași County

Dealul Mare-Hârlău Forest (MN 78), on *Fagus sylvatica* L. rhytidome; Iași, Ciric Park Forest, on *Robinia pseudoacacia* L. (BURLACU, 1969a; CIURCHEA, 2004).

Ifov County

Mogoșoaia Forest (MK 13), on *A. campestre* L. rhytidome (MORUZI & MANTU, 1965; CIURCHEA, 2004); Snagov Forest (MK 34), on corticolous substrata (MANTU, 1965; CIURCHEA, 2004); Brănești Forest (MK 42), on *Tilia* sp., *Ulmus minor* MILL., *Prunus cerasifera* EHRL., *Acer tataricum* L., *Fraxinus ornus* L., *Crataegus monogyna* JACQ. rhytidome (MORUZI & KLOIS, 1970; CIURCHEA, 2004); Andronache Forest (MK 32), on *F. excelsior* L. rhytidome, leg. Vicol Ioan, 18.06.2009, det. Vicol Ioana 03.07.2009 [BUCM L 1341], on *Prunus cerasifera* Ehrh. rhytidome, leg. Vicol Ioan, 24.03.2009, det. Vicol Ioana 30.06.2009 [BUCM L 1345], on *Quercus robur* L. rhytidome, leg. Vicol Ioan, 18.06.2009, det. Vicol Ioana, 06.07.2009 [BUCM L 1346], on *Q. cerris* L., *C. monogyna* JACQ., *Sophora japonica* L., *Q. robur* L., *A. campestre* L. (VICOL, 2010a); Cernica Forest (MK 41), on *Q. cerris* L. rhytidome, leg. Vicol Ioan, 26.03.2009, det. Vicol Ioana, 30.06.2009 [BUCM L 1325] (unpublished data); Pustnicul Forest (MK 42), on *Q. cerris* L. rhytidome, leg. Vicol Ioan, 30.04.2009, det. Vicol Ioana 25.06.2009 [BUCM L 1318], VICOL (2010a); Vlădiceasca Forest, on lignicolous substrata, leg. Vicol Ioana, 26.04.2010, det. Vicol Ioana 06.06.2010 (unpublished data); Biglaru Forest (MK 34), on lignicolous substrata, leg. Vicol Ioana, 27.04.2010, det. Vicol Ioana, 17.05.2010 [BUCM L 1347] (VICOL, 2011b); Snagov Forest (MK 34/35), on lignicolous substrata, leg. et det. Vicol Ioana, 08.06.2010 [BUCM L 1343], leg. Vicol Ioana, 26.04.2010, det. Vicol Ioana 07.05.2010 [BUCM L 1349] (unpublished data); Pustnicul Forest (MK 42), on *Fraxinus* sp. and *Quercus* sp. rhytidome (VICOL, 2012).

Mehedinți County

The Danube Gorge at Cazanele Mici (FQ 04), Cernei Valley (FQ 17), Ieșelniței Valley (FQ 05) on oak, hornbeam, and ash rhytidome; Forest Reserve Ogradena (FQ 04) on stones (MORUZI & TOMA, 1972; CIURCHEA, 2004).

Neamț County

Cheile Bicazului, Cheile Mici and Cheile Mari (MM 18), on beech and willow, respectively rhytidome (BURLACU, 1969b; CIURCHEA, 2004); Ceahlău Mountain (MN 20), on *Acer* sp. at Piciorul Maicilor, *Alnus incana* (L.) MOENCH. at Piciorul Muntelui, *Populus* sp. on Horștei Peak, *Ribes aureum* PURSH at Bâta Durăului, on the boards of the fences near the tourist complex Durău (MANOLIU et al., 1998).

Sibiu County

Agnita (LL 19), on oak rhytidome, altitude 560 m (MORUZI & TOMA, 1967; CIURCHEA, 2004); Cindrel (Cibinului) Mountains (GR 15), on *Quercus* sp., *Q. cerris* L. altitude 450-500 m, *Robinia pseudoacacia* L., altitude 500-550 m, *Crataegus* sp., altitude 600 m, *Tilia* sp., altitude 450- 650 m, *Fraxinus* sp., altitude 900 m, *Acer* sp., *Fagus* sp., altitude 1200 m (MORUZI & TOMA, 1970; CIURCHEA, 2004); Tâlmăciu Forest (KL85/86), on lignicolous substrata, leg. et det. Vicol Ioana, 28.03.2011 (unpublished data).

Tulcea County

Tulcea, Horia (PL 40), altitude 175 m, 45°00'45.2"N, 28°26'53.1"E, 13.04.2008, on *Juglans* sp.; Valea Teilor, near Nicolîțel, altitude 270 m, 45°10'3.1"N, 28°29'20.5"E, 13.04.2008, on *Juglans* sp., *Platanus* sp. (YAVUZ & ÇOBANOĞLU, 2008).

Vaslui County

Mălușteni Nature Reserve (NM 71), on *Populus nigra* L. (VICOL, 2011a); Igești Village (NM 71), at the edge of this locality, on *Vitis vinifera* L. rhytidome, leg. Vicol Ioana, 04.10.2011, det. Vicol Ioana, 20.10.2011 (unpublished data); Forestry Nature Reserve Bălteni Forest (NM 46), on lignicolous substrata, leg. Vicol Ioana, 17.08.2012 det. Vicol Ioana, 14.09.2012 (unpublished data).

Vâlcea County

Cozia National Park (KL 92), on beech (ÇOBANOĞLU et al., 2009).

Vrancea County

Cârligata Forest (ML 79), on *Fagus sylvatica* L. rhytidome (BURLACU, 1969a; CIURCHEA, 2004).

Physcia aipolia (EHRL. ex HUMB.) FÜRN. (Fig. 2).

Alba County

Sebeș (FR 99), on the alder branches (BORZA, 1959; CIURCHEA, 2004); Bihorului Mountains, Răchita Peak, altitude 1300 m, on *Acer* sp. rhytidome (BARTÓK, 1982; CIURCHEA, 2004).

Botoșani County

Within Pădureni Forest on *Quercus* sp., Dersca and Șendriceni forests (MP 41), on *A. campestre* L. (BURLACU, 1967; CIURCHEA, 2004); in Horlăcenii Forest, on *A. campestre* and *Populus* sp., Gorovei Forest, on *Quercus* sp., and Văculești Forest, on *Populus* sp. (MP 50), Vârful Câmpului Forest (MN 49), on *Populus* sp. rhytidome (BURLACU, 1969c; CIURCHEA, 2004).

București

Băneasa Forest (MK 21/22/31/32), on lignicolous substrata (VICOL, 2011b).

Caraș-Severin County

The Danube Gorge, within forests between Cozla and Pescari (EQ 54), on *Fraxinus ornus* L., *F. sylvatica* L., and *Morus* sp. (BURLACU et al., 1969; CIURCHEA, 2004).

Călărași County

Goștilele Forest (MK 62), on *Quercus robur* L. rhytidome (VICOL, 2012) and lignicolous substrata (unpublished data).

Cluj County

Bocului Mountain, Boc hamlet (FS 86), on ash rhytidome (TOTHĂZAN & CRIȘAN, 2008)

Ilfov County

Mogoșoaia Forest (MK 13), on *Quercus cerris* L., *Q. robur* L., *A. campestre* L., *F. excelsior* L., and *Tilia tomentosa* MOENCH. (MORUZI & MANTU, 1965; CIURCHEA, 2004); Snagov Forest (MK 34/35), on *Quercus* sp., *Tilia* sp., *Fagus* sp., and *Carpinus* sp. rhytidome (MANTU, 1965; CIURCHEA, 2004); Brănești Forest (MK 42), on *Q. robur* L., *Q. pedunculiflora* K. KOCH., *F. ornus* L., *A. campestre* L., and *Ulmus procera* SALISB. (MORUZI & KLOHS, 1970; CIURCHEA, 2004); Vlădiceasca Forest (MK 24), on lignicolous substrata, leg. Vicol Ioana, 26.04.2010, det. Vicol Ioana, 11.05.2010 [BUCM L 1339] (unpublished data); Snagov Forest (MK 34/35), on *Pinus nigra* L. trunk, leg. Vicol Ioana, 08.06.2010, det. Vicol Ioana [BUCM L 1340] (VICOL, 2011c); Biglaru Forest (MK 34), on lignicolous substrata, leg. Vicol Ioana, 27.04.2010, det. Vicol Ioana, 14.05.2010 (VICOL, 2011b); Cernica Forest (MK 41), on lignicolous substrata, leg. Vicol Ioana, 25.04.2009, det. Vicol Ioana, 28.04.2010 [BUCM L 1321] (VICOL, 2010a).

Mehedinți County

Along of Ieșelnița-Ogradena and Orșova-Ogradena (FQ 05) roads, on mulberry and walnut, respectively rhytidome; the former Ada-Kaleh island (FQ 24), on the poplar rhytidome (MORUZI & TOMA, 1972; CIURCHEA, 2004).

Neamț County

Ceahlău Mountain, at Bâta Durăului (MN 20), on *F. sylvatica* L., *Ribes aureum* PURS., and *Rosa* sp. (MANOLIU et al., 1998).

Sibiu County

Cindrel (Cibin) Mountains, Crinț Pasture (GR 16), on beech rhytidome, altitude 1150 m; along the Săliștei road (GR 27), on lime and pear rhytidome, altitude 1200 m; Agnita (LL 19), on oak rhytidome, altitude 560 m (MORUZI & TOMA, 1967; CIURCHEA, 2004).

Vaslui County

Igești Village (NM 71), at the edge of this locality, on *Vitis vinifera* L. rhytidome, leg. Vicol Ioana, 04.10.2011, det. Vicol Ioana, 20.10.2011 (unpublished data).

Vâlcea County

Cozia National Park (KL 92), on ash rhytidome (ÇOBANOĞLU et al., 2009).

Physcia caesia (HOFFM.) HAMPE (Fig. 3).

Alba County

Apuseni Mountains, Râmetului Gorges (FS 93), on saxicolous substrata (CODOREANU et al., 1968; CIURCHEA, 2004).

București

Botanical Garden (MK 21/22/31/32), on limestone substrata (MORUZI & PETRIA, 1961; CIURCHEA, 2004).

Caraș-Severin County

The Danube Gorge, at Cozla, between Cozla and Pescari: Mare Valley, Stâncă Mare, Stâncă Popa, Stâncă Toza (EQ 54/84), on saxicolous substrata (CIURCHEA et al., 1968; CIURCHEA, 2004); Pescari (EQ 54), on saxicolous substrata (CODOREANU & CIURCHEA, 1970; CIURCHEA, 2004).

Cluj County

Pietrele Albe Mountain (FS 39), situated in south-west part of Vlădeasa Peak, altitude 1514 m, on saxicolous substrata (CODOREANU & CIURCHEA, 1966; CIURCHEA, 2004); Vlădeasa Mountains (FS 39), on western side of this mountain, on saxicolous substrata (BARTÓK & CODOREANU, 1979; CIURCHEA, 2004); Someșului Cald Valley, between Fântânele dam (Beliș locality) and Târnița dam (FS 56), on saxicolous substrata (CIURCHEA & CRIȘAN, 1991-1992; CIURCHEA, 2004).

Harghita County

Căpâlnița locality (LM 83), on a tile (BARTH, 1905; CIURCHEA, 2004).

Ilfov County

Mogoșoaia Forest (MK 13), on *Quercus pedunculiflora* K. KOCH and *Acer campestre* L. rhytidome (MORUZI & MANTU, 1965; CIURCHEA, 2004); Snagov Forest (MK 34), corticolous (MANTU, 1965; CIURCHEA, 2004).

Mehedinți County

The former Ada-Kaleh island (FQ 24), on siliceous rocks (MORUZI & TOMA, 1973; CIURCHEA, 2004).

Mureș County

Gănești Village (KM 93), on old boards (CRETZOIU, 1939; CIURCHEA, 2004); Călimani Mountains, at Neagră Valley, within Defileul Deda-Toplița Landscape Reserve (LN 50), on *Populus* sp. (CRIȘAN & BANC, 2007).

Neamț County

Ceahlău Mountain, Ocolașul Mic (MN 20), on saxicolous and corticolous substrata (MANOLIU et al., 1998).

Sibiu County

Cindrel (Cîbin) Mountains, Căldarea Iezerului Mare, altitude 2050 m and Cindrel Peak (GR 26), altitude 2230 m, on saxicolous substrata (MORUZI & TOMA, 1967; CIURCHEA, 2004).

Vâlcea County

Cozia Mountain, on crystalline schists (CODOREANU & CIURCHEA, 1965; CIURCHEA, 2004); Olt Valley, at Călinești (KL 82), on saxicolous substrata (CIURCHEA, 1969; CIURCHEA, 2004); Lotrului Mountains, Călinești Valley (KL 82), on saxicolous substrata (CIURCHEA, 1970; CIURCHEA, 2004).

Physcia dubia (HOFFM.) LEFT. (Fig. 4).

Alba County

Apuseni Mountains, Râmetului Gorges (FS 93), on saxicolous substrata (CODOREANU et al., 1968; CIURCHEA, 2004).

București

Botanical Garden (MK 21/22/31/32), on stones (MORUZI & PETRIA, 1961; CIURCHEA, 2004).

Caraș-Severin County

Between Liubcova and Coronini localities (EQ 74), on saxicolous substrata, the Danube Gorge at Cozla, Stâncă Popa, Stâncă Mare, and Stâncă Toza (EQ 84), on saxicolous substrata (CIURCHEA et al., 1968; CIURCHEA, 2004).

Călărași County

Călăreților Forest (MK 62), on *Quercus robur* L. rhytidome, leg. Vicol Ioana, 24.08.2011, det. Vicol Ioana, 29.08.2011 (unpublished data).

Cluj County

Someșului Cald Valley, between Fântânele dam (Beliș locality) and Tarnița dam (FS 56), on saxicolous substrata (CIURCHEA & CRIȘAN, 1991-1992; CIURCHEA, 2004).

Ilfov County

Râioasa Forest (MK 12) on *Fraxinus* sp. rhytidome (VICOL, 2011c).

Giurgiu County

Căscioarelor Forest (LK 92), corticolous, leg. Vicol Ioana, 17.03.2011, det. Vicol Ioana, 16.05.2011 (unpublished data); Bolintin-Deal Forest (MK 02), on *Quercus* sp. (VICOL, 2011c).

Neamț County

Ceahlău Mountain, at Bâta Fântânilor (MN 20), on saxicolous substrata (MANOLIU et al., 1998).

Vâlcea County

Lotrului Mountains, Călinești Valley (KL 82), on saxicolous substrata (CIURCHEA, 1970; CIURCHEA, 2004); Cozia National Park (KL 92), on calcareous rock, 45°17'49.51"N, 24°18'25.63"E, 10.07.2007, altitude 661 m; 45°22'15.83"N, 24°18'26.11"E, 27.07.2007, altitude 382 m (ȚOBANOȚIU et al., 2010).

Physcia endochrysoides NYL. (Fig. 5).

Hunedoara County

Retezat Mountains, Râul Mare Valley (FQ 69), on saxicolous substrata (CRETZOIU, 1940; CIURCHEA, 2004).

Gorj County

Vâlcanului Mountains, near Gureni (FR 32), on saxicolous substrata (CRETZOIU, 1940; CIURCHEA, 2004).

Physcia semipinnata (J. F. GMELIN) MOBERG. (Fig. 6).

Botoșani County

Within Pădureni Forest, on *Quercus* sp. and *Fraxinus excelsior* L., Șendriceni and Dersca forests, on *A. campestre* L. (MP 41); within Gorovei Forest, on *A. campestre* L. and *Quercus* sp., Horlăceni Forest, on *A. campestre* L. and *Populus* sp., Vârful Câmpului Forest, on *Populus* sp., Văculești Forest (MP 50) BURLACU (1969c); CIURCHEA, 2004).

București

Botanical Garden, on corticolous substrata (MORUZI & PETRIA, 1961; CIURCHEA, 2004).

Caraș-Severin County

The Danube Gorge, within the forests between Cozla and Pescari (EQ 54), on *Tilia cordata* MILL., *Cynops orientalis* DAVID, *Fagus sylvatica* L., *F. ornus* L., *Quercus cerris* L., and *T. tomentosa* MOENCH (BURLACU et al., 1969; CIURCHEA, 2004).

Ilfov County

Mogoșoaia Forest (MK 13), on *T. tomentosa* MOENCH, *A. campestre* L., and *F. excelsior* L. rhytidome (MORUZI & MANTU, 1965; CIURCHEA, 2004); Snagov Forest (MK 34), on *Quercus* sp., *Alnus* sp., and *Carpinus* sp.

(MANTU, 1965; CIURCHEA, 2004): Snagov Forest (MK 34), on lignicolous substrata, leg. Vicol Ioana, 26.04.2010, det. Vicol Ioana, 14.05.2010 (unpublished data).

Mehedinți County

The Danube Gorge, at Cazanele Mici (FQ 04), on hornbeam and walnut rhytidome; on the side of the road Orșova (FQ 05/15)-Ogradena (EQ 93/FQ 04), on mulberry rhytidome (MORUZI & TOMA, 1972; CIURCHEA, 2004).

Neamț County

Ceahlău Mountains, Bâta Durăului (MN 20), on *Acer* sp., *Malus sylvestris* (L.) MILL., *Populus* sp., *Ribes aureum* PURS. (MANOLIU et al., 1998).

Sibiu County

Cindrel (Cibinului) Mountains (KL 76), on *Crataegus* sp., altitude 600 m (MORUZI & TOMA, 1970; CIURCHEA, 2004); Plaiul Tiliștei (GR 27), on beech rhytidome, altitude 1270 m (MORUZI & TOMA, 1967; CIURCHEA, 2004).

Vaslui County

Igești Village (NM 71), at the edge of this locality, on *Vitis vinifera* L. rhytidome, leg. Vicol Ioana, 04.10.2011, det. Vicol Ioana, 20.10.2011 (unpublished data).

Vâlcea County

Cozia National Park (KL 92), on ash and beech rhytidome (ÇOBANOĞLU et al., 2009).

Physcia stellaris (L.) NYL. em. HARM. (Fig. 7).

Alba County

Apuseni Mountains, within the forests from the surroundings of Avram Iancu locality (FS 33), on corticolous substrata (CIURCHEA & CODOREANU, 1967; CIURCHEA, 2004); Roșia Montană (FS 62/63), on *Salix alba* L. and *Prunus domestica* L. (CRIȘAN & ARDELEAN, 2010).

Bistrița-Năsăud County

Rodnei Mountains, Rodna, Vinului Valley (LN 36), on oak rhytidome (ZSCHACKE, 1911; CIURCHEA, 2004); Șieului Valley, Arcalia Village, Arcalia Scientific Stationary Park, on corticolous substrata (CIURCHEA & SZABÓ, 1966; CIURCHEA, 2004).

Boțoșani County

Within Dersca, Lozna, Hilișeu, and Pădureni forests (MP 41), on *Quercus* sp., *F. sylvatica* L., and *A. campestre* L. rhytidome (BURLACU, 1967; CIURCHEA, 2004); within Gorovei Forest, on *A. campestre* L. and *F. excelsior* L., Vârful Câmpului Forest, on *Populus* sp., Horlăceni and Văculești forest, on *Quercus* sp., *Betula* sp., and *Acer* sp. (MP 50) BURLACU (1969c); CIURCHEA, 2004).

Brașov County

Brașov (LL 85/95), on walls (ZSCHACKE, 1911; CIURCHEA, 2004).

București

Botanical Garden (MK 21/22/31/32), on *Pinus strobus* L., *Populus alba* L., *Abies numidica* COSS, and *Rhus hirta* (L.) SUDW. (MORUZI & PETRIA, 1961; CIURCHEA, 2004).

Cluj County

Hoia Hill (FS 97/98/GS 08), altitude 400 m, on corticolous substrata (BORZA, 1938; CIURCHEA, 2004); on the western side of Vlădeasa Mountain (FS 39), on *Juniperus communis* L. (BARTÓK & CODOREANU, 1979; CIURCHEA, 2004); Vlădeasa Mountain, Răcad Valley (FS 38), altitude 1200-1300 m, on conifers rhytidome (BARTÓK, 1988; CIURCHEA, 2004); Botanical Garden from Cluj (FS 97), on corticolous substrata (CODOREANU et al., 1960; CIURCHEA, 2004) Bocului Mountain, at Roșala (FS 86) (TOTIĂZAN & CRIȘAN, 2008).

Constanța County

Constanța, Trofeul Traiani, Adamclisi (PJ 28/29/38/39), altitude 150 m, 44°06'07.00"N, 27°57'20.80"E, 16.05.2007, on *Elaeagnus* sp., *Tilia* sp. (YAVUZ & ÇOBANOĞLU, 2008).

Dâmbovița County

Leota Mountain, Romanescu Peak, Poiana Marginea Domnească Valley, altitude 850 m, on *A. alba* trunk (BURLACU & DIACONESCU, 1969; CIURCHEA, 2004).

Harghita County

Harghitei Mountains (LM 62/MM 00), on fruit trees (BARTH, 1905; CIURCHEA, 2004).

Giurgiu County

Crevedia Forest (MK 02), on lignicolous substrata, leg. Vicol Ioana, 17.03.2011, det. Vicol Ioana, 08.04.2011; Crețești Forest (MK 30), on lignicolous substrata, leg. Vicol Ioana, 24.08.2010, det. Vicol Ioana, 09.2010 (unpublished data).

Iași County

Dealul Mare-Hârlău Forest (MN 95), on *Quercus* sp. rhytidome, Cîric Park Forest from Iași (NN 41), on *R. pseudoacacia* rhytidome, Sadoveni Forest (NP 01/11), on *Quercus* sp. rhytidome (BURLACU, 1969a; CIURCHEA, 2004).

Ifov County

Mogoșoaia Forest (MK 13), on lignicolous substrata, on *A. campestre* L. and *Q. cerris* L. rhytidome (MORUZI & MANTU, 1965; CIURCHEA, 2004); Snagov Forest (MK 34/35), on corticolous and saxicolous substrata (MANTU, 1965; CIURCHEA, 2004); Brănești Forest (MK 42), on *Q. robur* rhytidome (MORUZI & KLOHS, 1970; CIURCHEA, 2004); Pustnicul Forest (MK 42), on lignicolous substrata (VICOL, 2010a); Snagov Forest (MK 34/35), on lignicolous

substrata, leg. Vicol Ioana, 08.06.2010, det. Vicol Ioana, 07.2010; Vlădiceasca Forest (MK 24), on lignicolous substrata, leg. Vicol Ioana, 06.04.2010, det. Vicol Ioana, 11.05.2010 [BUCM L 1366] (unpublished data); Biglaru Forest (MK 34), on lignicolous substrata (VICOL., 2011b); Pustnicul Forest (MK 42), on lignicolous substrata, leg. Vicol Ioana, 26.08.2011, det. Vicol Ioana, 02.09.2011 (unpublished data).

Mehedinți County

Porțile de Fier I, at Cazanele Mici (FQ 04) and Ieșelniței Valley (FQ 05), on walnut, hornbeam, and hawthorn (MORUZI & TOMA, 1972; CIURCHEA, 2004).

Mureș County

Gănești (KM 93), on old boards (CRETZOIU, 1939; CIURCHEA, 2004); Călimani Mountains, Neagră Valley, Defileul Deda-Toplița Reserve (LN 50), on oak rhytidome (CRIȘAN & BANC, 2007).

Neamț County

Bicazului Gorges, Mici Gorges (MM 18), on beech rhytidome (BURLACU, 1969a, b, c; CIURCHEA, 2004); Ceahlău Mountain, Bâta Durăului and Horștei Peak (MN 20), on *Acer* sp., *Malus sylvestris* MILL., *Sorbus aucuparia* L., and *Populus* sp., respectively (MANOLIU et al., 1998).

Sibiu County

Sibiu (KL 77/87) (CRETZOIU, 1940; CIURCHEA, 2004); Sibiu (KL 77/87), on *R. pseudacacia* L. rhytidome (MORUZI & TOMA, 1970; CIURCHEA, 2004); Cindrel (Cibin) Mountains, Pășunea Crinț (GR 16), altitude 1190 m, on beech rhytidome, Gușteriței Hill (KL 87), altitude 460 m, on plum rhytidome, Agnita (LL 19), altitude 750 m, on oak rhytidome (MORUZI & TOMA, 1967; CIURCHEA, 2004); Râșinari (KL 76), on *R. pseudacacia* L. rhytidome (MORUZI & TOMA, 1970; CIURCHEA, 2004); Tâlmăciu Forest (KL 85), on lignicolous substrata, leg. Vicol Ioana, 28.03.2011, det. Vicol Ioana, 05.04.2011, on *R. pseudacacia* rhytidome, leg. Vicol Ioana, 28.03.2011, det. Vicol Ioana, 05.04.2011 (unpublished data); Nature Reserve Lacul Tătarilor, on *Prunus spinosa* L., leg. Vicol Ioana, 29.03.2011, det. Vicol Ioana, 05.04.2011 (unpublished data).

Tulcea County

Tulcea, Horia (PL 40), altitude 175 m, 45°00'45.2"N, 28°26'53.1"E, 13.04.2008, on *Juglans* sp. (YAVUZ & ÇOBANOĞLU, 2008).

Vaslui County

Mălușteni Nature Reserve (NM 71), on *Populus* sp. rhytidome (VICOL., 2011a).

Vâlcea County

Cozia National Park (KL 92), on beech rhytidome (ÇOBANOĞLU et al., 2009).

Physcia tenella (SCOP.) DC. in LAM. & DC. (Fig. 8).

Alba County

Apuseni Mountains, Râmețului Gorges, Uzmezău (FS 93), on saxicolous substrata (CODOREANU et al., 1968; CIURCHEA, 2004); Bihorulului Mountain, Răchita Peak (FS 43), altitude 1300 m, on *Abies alba* L. and *Picea abies* (L.) H. KARST. (BARTÓK, 1982; CIURCHEA, 2004).

Botoșani County

Within Dersca and Pădureni forests, on *Quercus* sp., *A. campestre*, Lozna Forest, on *Quercus* sp., Hilișeu Forest (MP 41), on *A. campestre* and *Carpinus betulus* L. (BURLACU, 1967; CIURCHEA, 2004); within Vârful Câmpului Forest, on *Quercus* sp., Văculești Forest, on *Populus* sp., and Horlăceni Forest (MP 50), on *Acer* sp. and *Quercus* sp. (BURLACU, 1969c; CIURCHEA, 2004).

București

Botanical Garden (MK 21/22/31/32), on corticolous substrata (MORUZI & PETRIA, 1961; CIURCHEA, 2004); Băneasa Forest (MK 21/22/31/32), on *A. campestre* rhytidome, leg. Vicol Ioana, 26.03.2009, det. Vicol Ioana, 15.06.2009 [BUCM L 1307] (VICOL., 2010b).

Caraș-Severin County

Pescari (EQ 54), on saxicolous substrata (CODOREANU & CIURCHEA, 1970; CIURCHEA, 2004).

Cluj County

Someșului Cald Valley, between Fântânele dam (Beliș locality) and Tarnița dam (FS 56), on saxicolous substrata (CIURCHEA & CRIȘAN, 1991-1992; CIURCHEA, 2004).

Ifov County

Mogoșoaia Forest (MK 13), on *Ulmus minor* MILL., *Prunus spinosa* L., and *T. tomentosa* L. rhytidome (MORUZI & MANTU, 1965; CIURCHEA, 2004); Snagov Forest (MK 34), on corticolous substrata (MANTU, 1965; CIURCHEA, 2004); Brănești Forest (MK 42), on *Q. robur* L., *Q. pedunculiflora* K. KOCH, *Prunus cerasifera* L., *F. ornus* L., and *F. excelsior* L. (MORUZI & KLOHS, 1970; CIURCHEA, 2004); Andronache Forest (MK 32), on *F. excelsior* and *P. cerasifera* leg. Vicol Ioan, 18.06.2009, det. Vicol Ioana, 07.07.2009 [BUCM L 1306] (VICOL., 2010a); Snagov Forest (MK 34), on lignicolous substrata, leg. Vicol Ioana, 22.04.2010, det. Vicol Ioana, 05.2010 [BUCM L 1311] (unpublished data); Cernica Forest (MK 41), on *Q. cerris* rhytidome (VICOL., 2010a); Pustnicul Forest (MK 42), on lignicolous substrata, leg. Vicol Ioana, 19.06.2009, det. Vicol Ioana, 07.07.2009 [BUCM L 1328] (unpublished data).

Mehedinți County

Within the forests between Cozla and Pescari (EQ 54), on *A. campestre* L., *C. orientalis* DAVID, *F. sylvatica* L., *F. ornus* L., and *Q. cerris* L. (BURLACU et al., 1969; CIURCHEA, 2004); Cazanele Mici and Ogradena Forest Reserve (FQ 04), Ieșelniței Valley (FQ 05), Cernei Valley (FQ 37), on hornbeam, ash, poplar and hawthorn rhytidome (MORUZI & TOMA, 1972; CIURCHEA, 2004).

Prahova County

Sinaia (LL 81/82), on corticolous substrata (ZSCHACKE, 1911; CIURCHEA, 2004).

Sibiu County

Sibiului Depression, at Tâlmăciu (KL 85/86) ZSCHACKE (1911), CIURCHEA (2004); Cindrel (Cibin) Mountains, along the Săliștei Road (GR 27), on linden rhytidome, at Agnita (LL 19), on saxicolous substrata (MORUZI & TOMA, 1967; CIURCHEA, 2004); at Rășinari (KL 76), on *Quercus* sp., *Crataegus* sp., *Tilia* sp., *Fraxinus* sp., *Acer* sp., and *R. pseudacacia* L., altitude 500-1200 m (MORUZI & TOMA, 1970; CIURCHEA, 2004).

Vâlcea County

Oltului Valley, between Proeni and Călinești (KL 82), on saxicolous substrata (CIURCHEA, 1969; CIURCHEA, 2004).

Physcia tribacia (ACH.) NYL. (Fig. 9).

Sibiu County

Micăsasa, Radac Forest (KM 70), on beech (CRETZOIU, 1939; CIURCHEA, 2004); Cindrel (Cibin) Mountains, Rășinari (KL 76), on *Quercus* sp., *Crataegus* sp., *Tilia* sp., *Fraxinus* sp., *Acer* sp., *R. pseudacacia* rhytidome (MORUZI & TOMA, 1970; CIURCHEA, 2004).

Physcia tribacoides NYL. (Fig. 10).

Botoșani County

Lozna Forest (MP 41), on *Quercus* sp. rhytidome (BURLACU, 1967; CIURCHEA, 2004).

Ilfov County

Cernica Forest (MK 41), on lignicolous substrata, leg. Vicol Ioan, 25.03.2009, det. Vicol Ioana, 26.06.2009 [BUCML 1356] (VICOL, 2010a).

Tulcea County

Tulcea, Horia (PL 40), altitude 175 m, 45°00'45.2"N, 28°26'53.1"E, 13.04.2008, on *Juglans* sp. (YAVUZ & ÇOBANOĞLU, 2008).

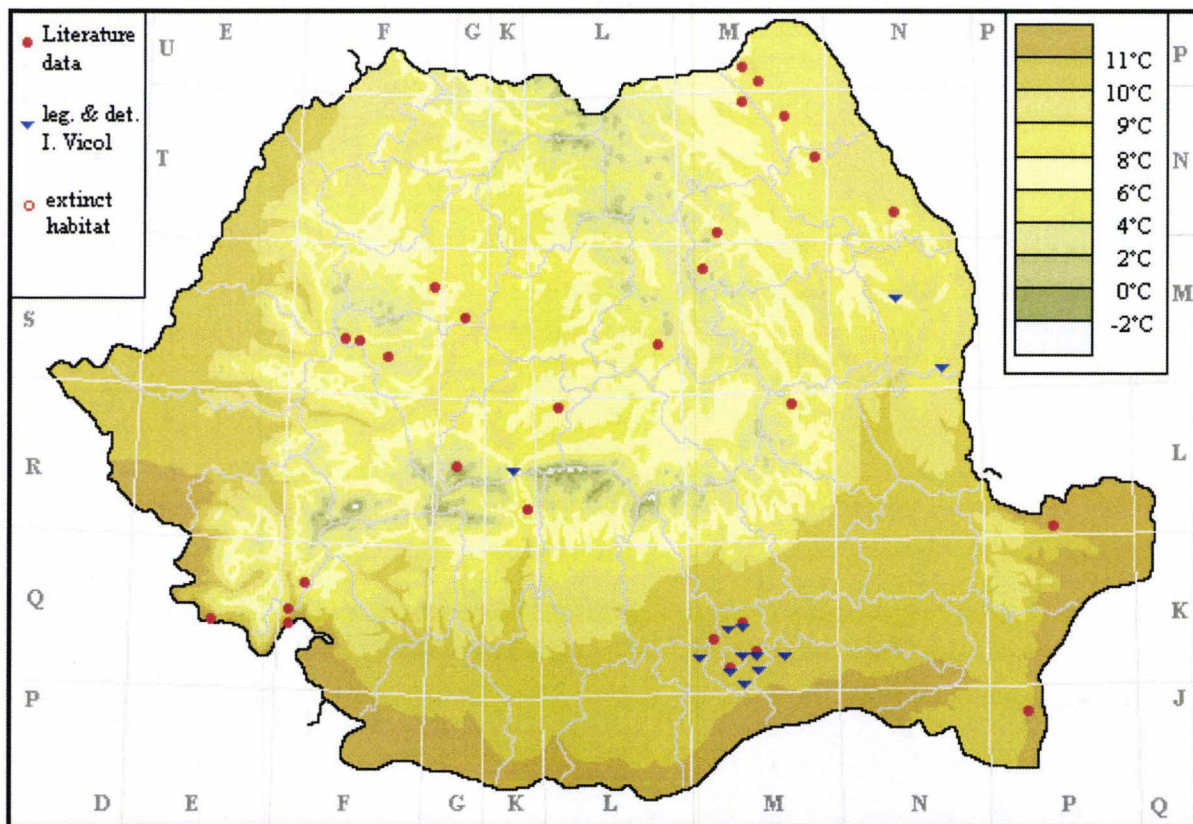


Figure 1. Chorology of the *Physcia adscendens* (Fr.) OLIV. in Romania.

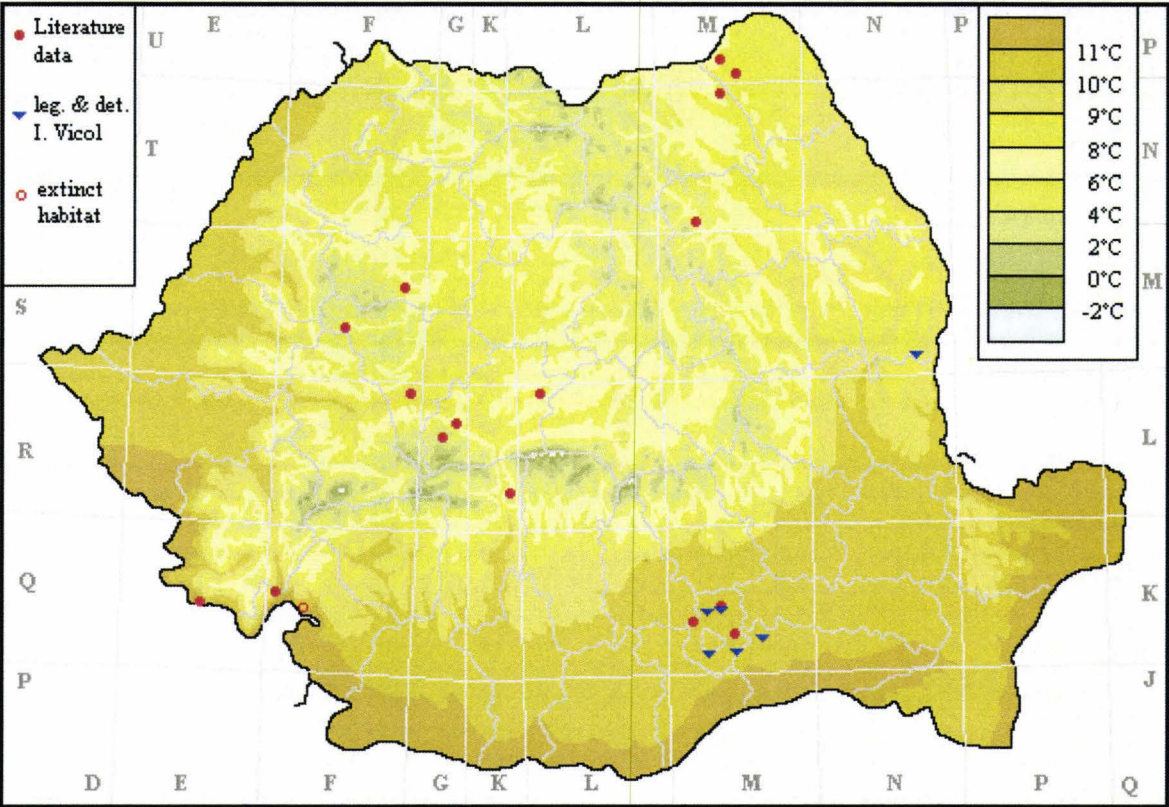


Figure 2. Chorology of the *Physcia aiipolia* (EHRH. ex HUMB.) FÜRNRR. in Romania.

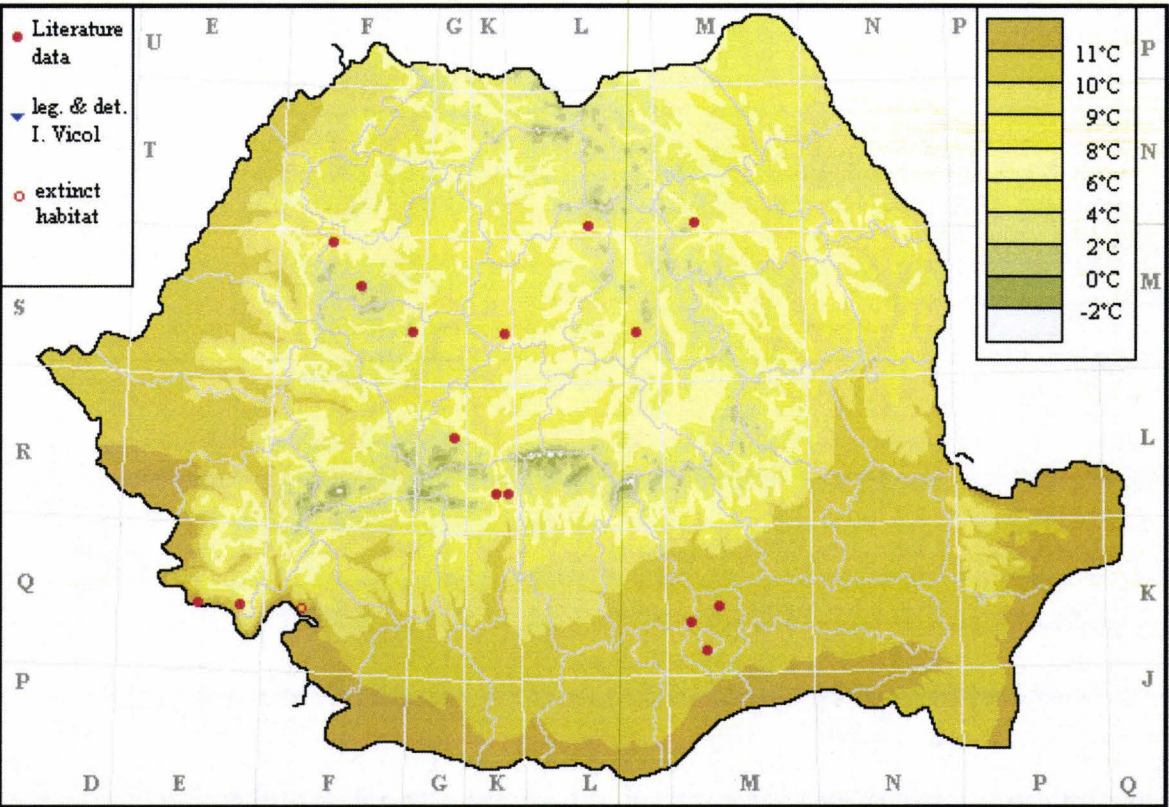


Figure 3. Chorology of the *Physcia caesia* (HOFFM.) HAMPE in Romania.

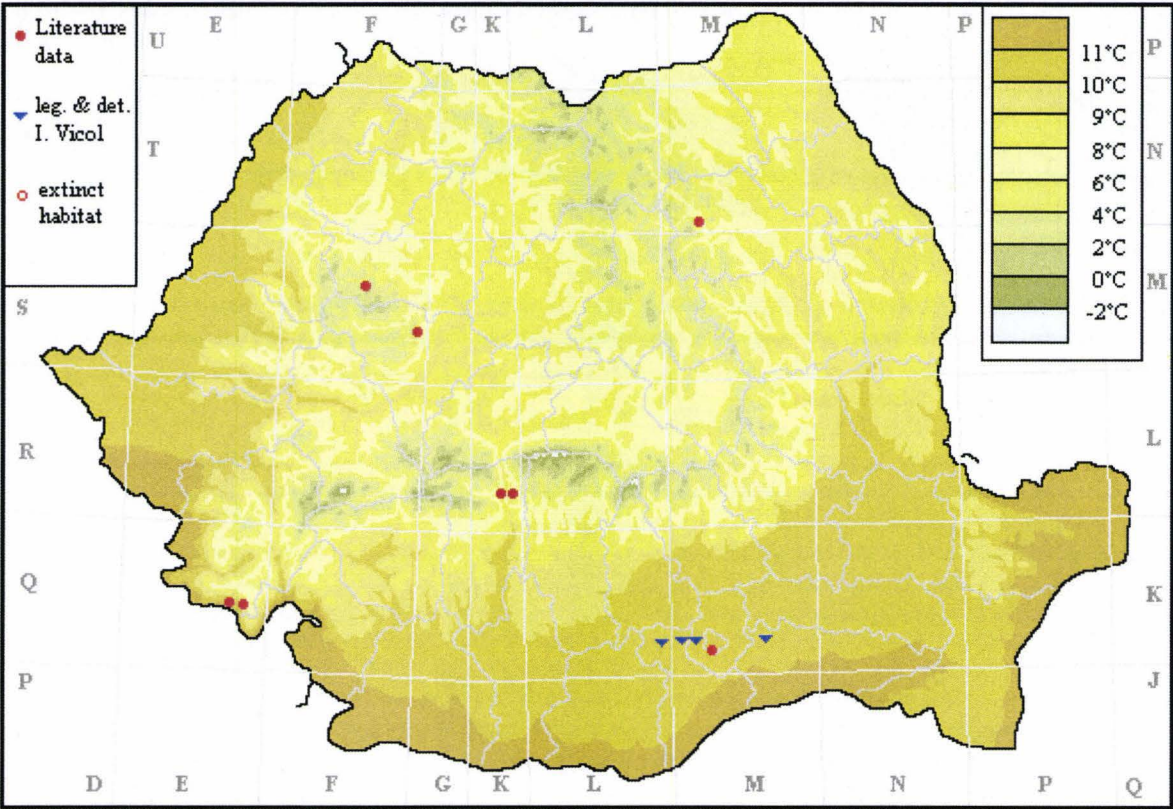


Figure 4. Chorology of the *Physcia dubia* (HOFFM.) LETT. in Romania.

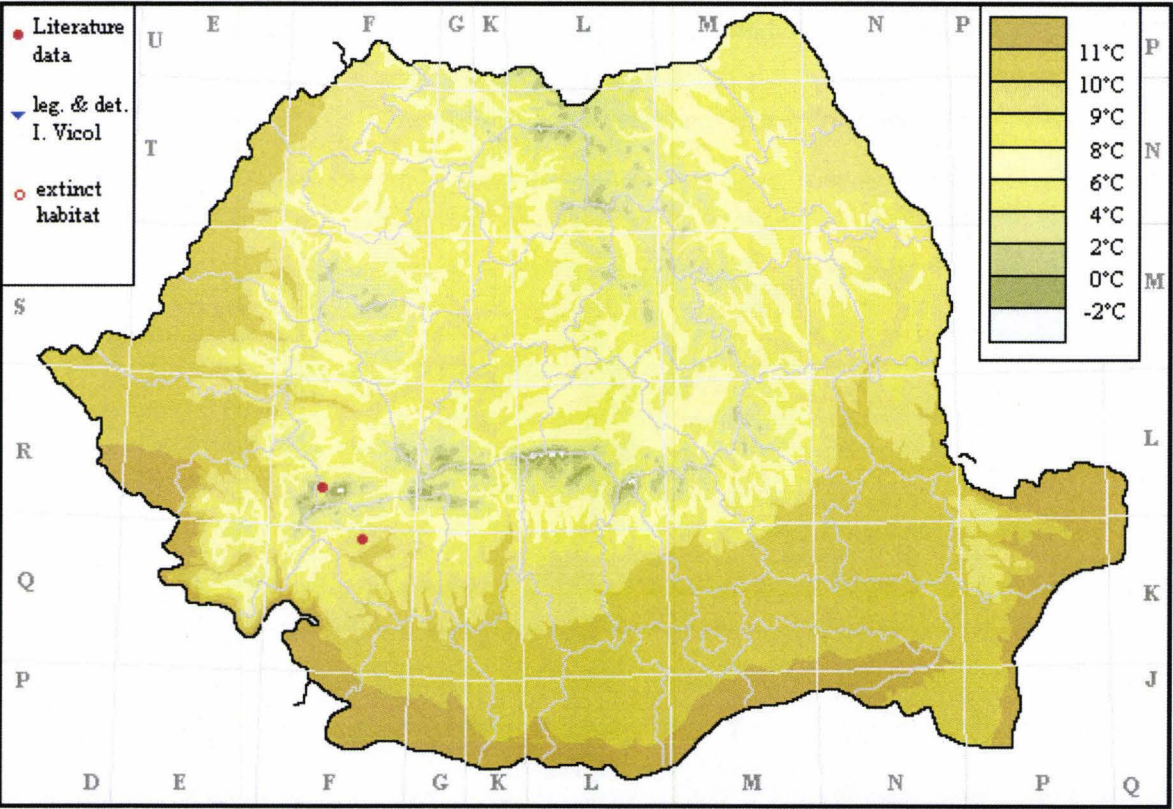


Figure 5. Chorology of the *Physcia endochrysoides* NYL. in Romania.

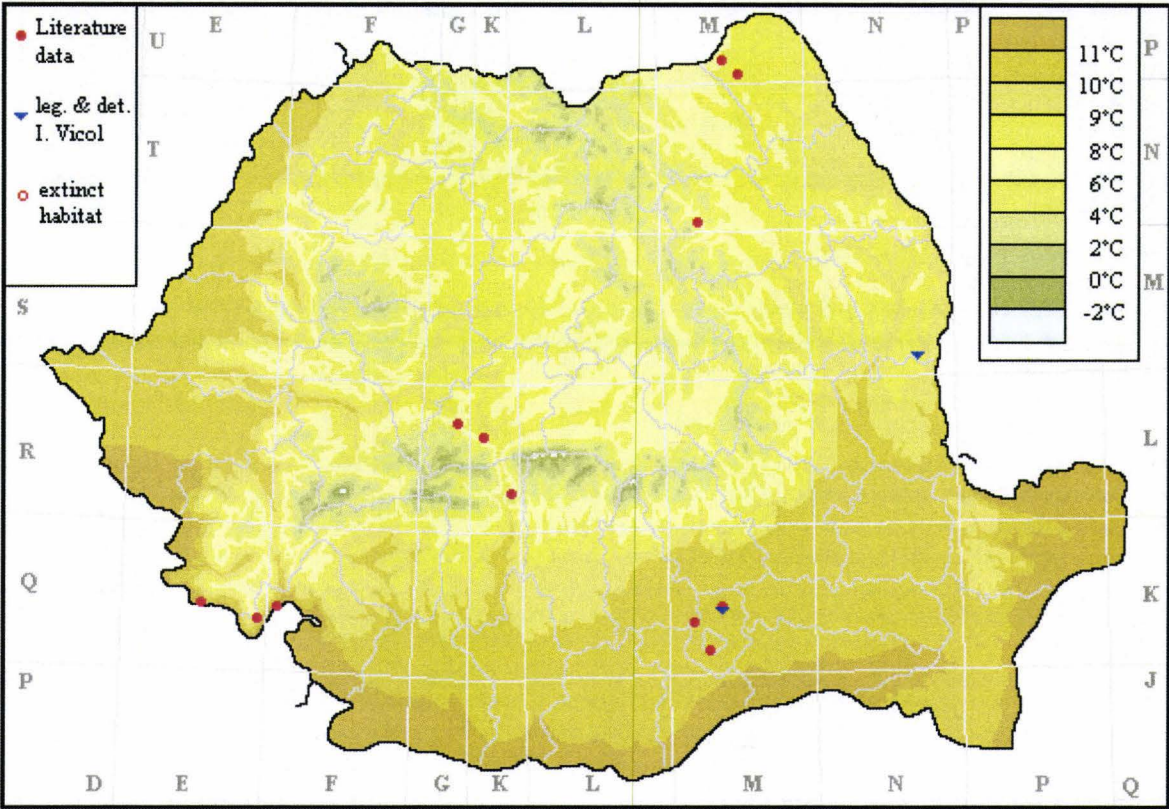


Figure 6. Chorology of the *Physcia semipinnata* (J. F. GMELIN) MOBERG. in Romania.

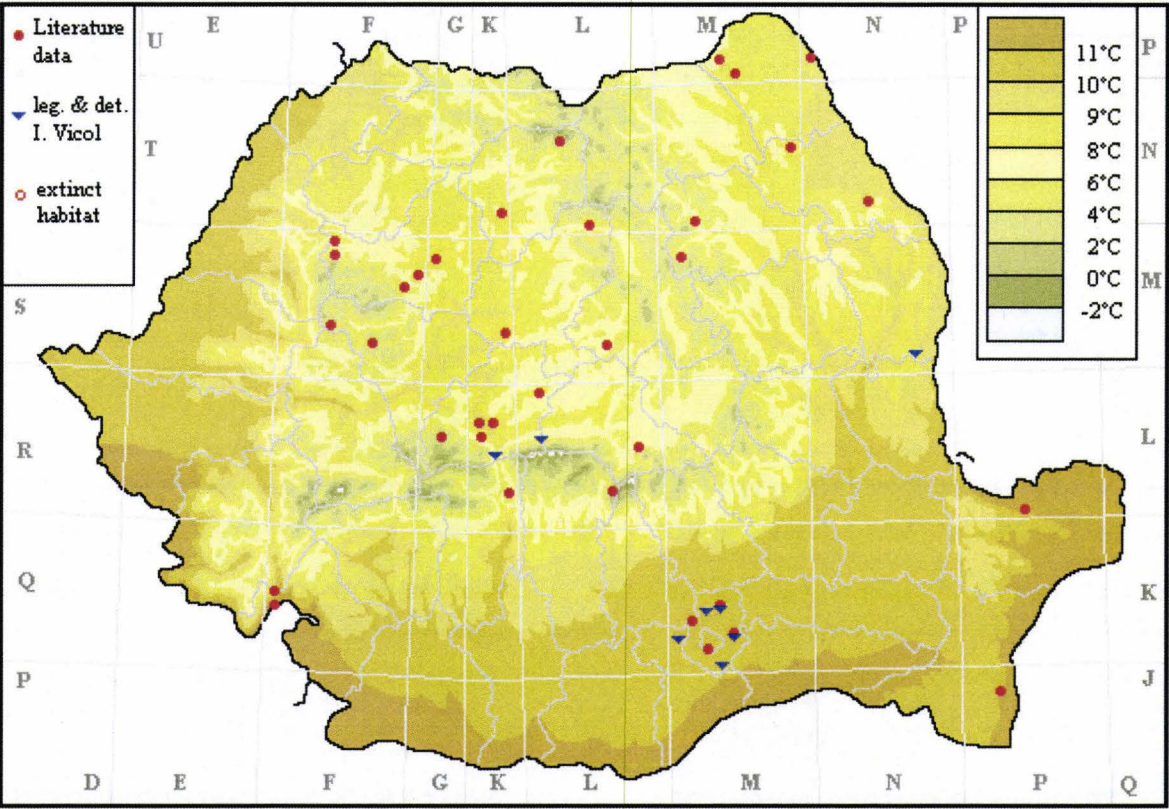


Figure 7. Chorology of the *Physcia stellaris* (L.) NYL. em. HARM. in Romania.

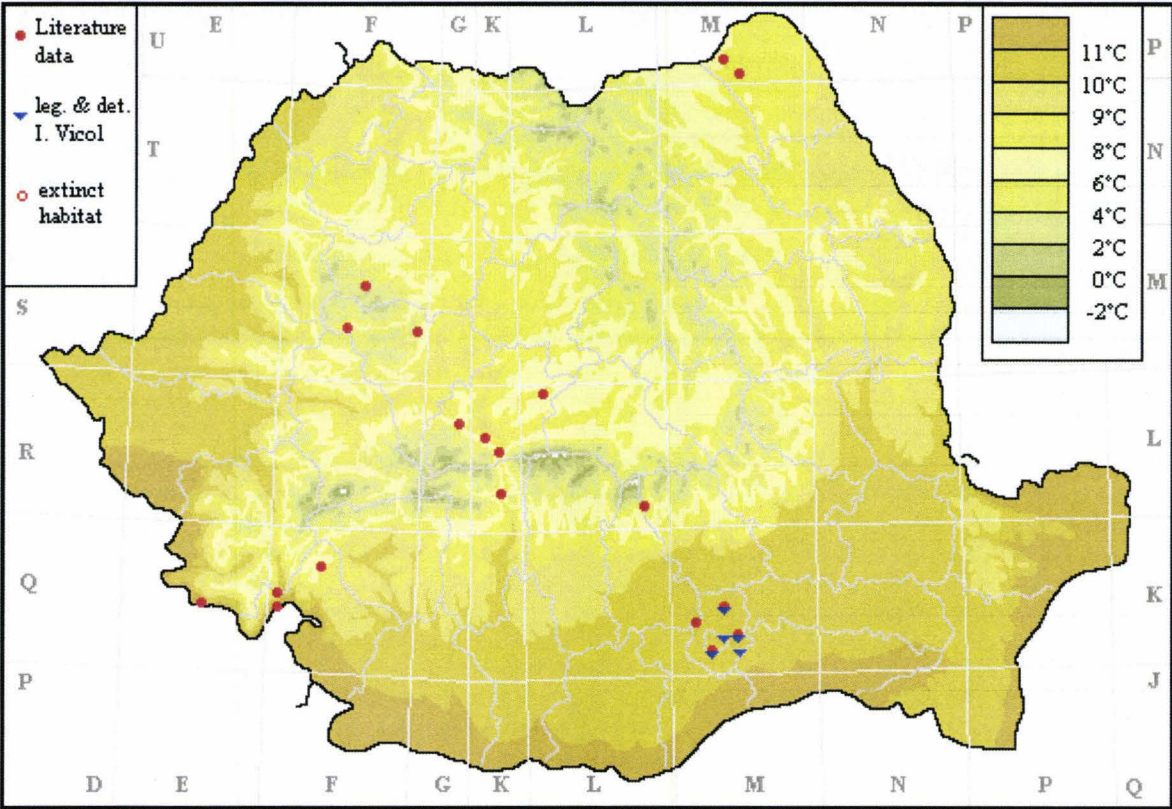


Figure 8. Chorology of the *Physcia tenella* (SCOP.) DC. in LAM. & DC. in Romania.

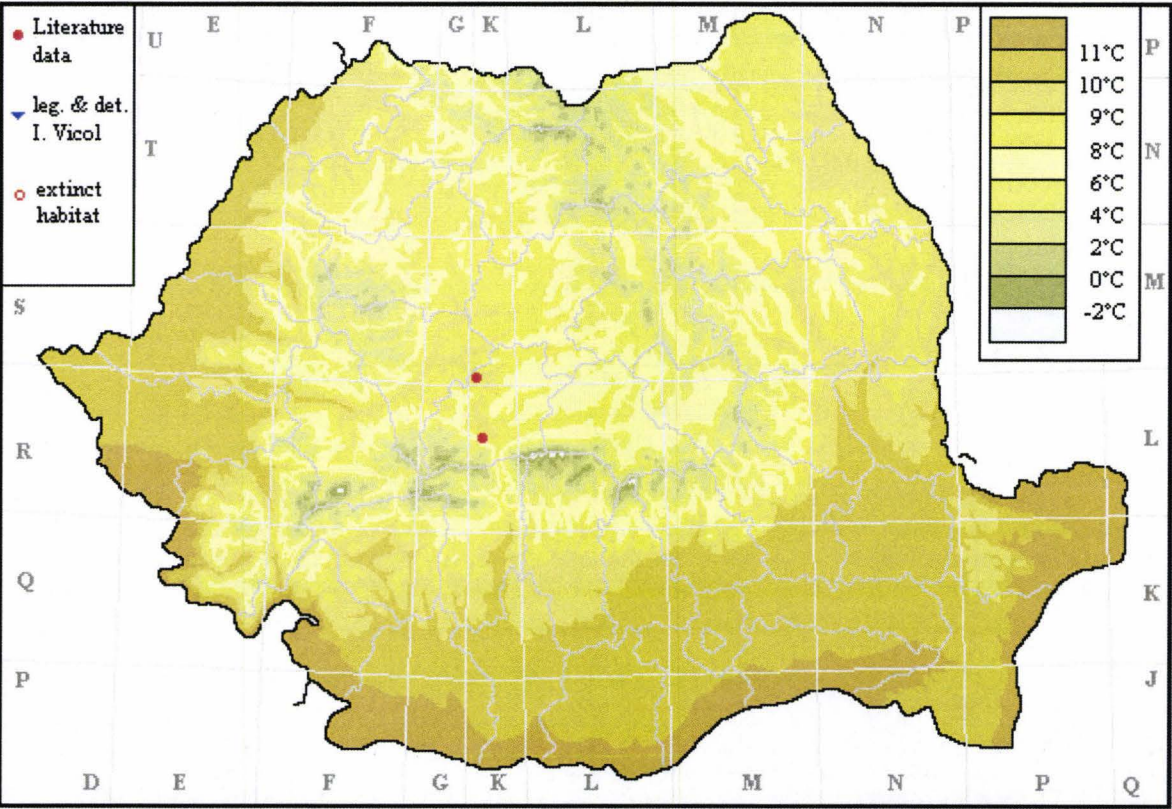


Figure 9. Chorology of the *Physcia tribacia* (ACH.) NYL. in Romania.

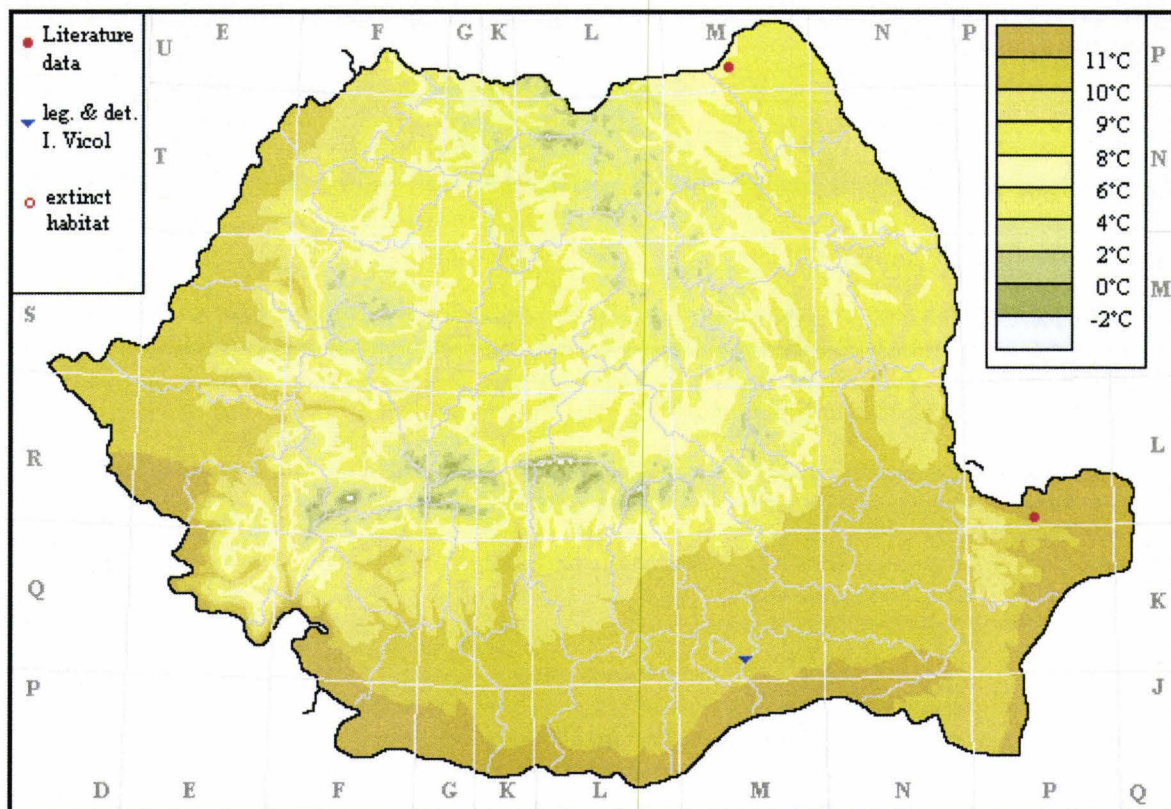


Figure 10. Chorology of the *Physcia tribacoides* NYL. in Romania.

CONCLUSIONS

The most widespread lichen species from *Physcia* genus in Romania are *P. adscendens*, *P. stellaris*, *P. tenella*. Compared with the above mentioned species, *P. aipolia*, *P. dubia*, *P. caesia*, *P. semipinnata* were distributed in a lower number of localities. A few data on the spatial distribution were recorded in case of *P. endochrysoides*, *P. tribacia* and *P. tribacoides*. Within own fieldwork, more widespread species of *Physcia* genus were attributed to *P. adscendens*, *P. aipolia*, *P. stellaris* and *P. tenella* found especially on outer bark of trees in the forest ecosystems from Ilfov County. A few original data were recorded from Bucharest and Călărași, Giurgiu, Sibiu, and Vaslui counties.

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SOME DATA CONCERNING THE APPLICATION OF WEBB METHOD TO SEDIMENT SAMPLES FROM THE MURAT RIVER (AĞRI REGION – TURKEY)

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Abstract. The Murat River (in Turkish Murat Nehri, Murat Suyu or Murat Irmağı) is the eastern branch of the Euphrates. The documentation activity about the Murat River started in September 2011. This direction of research is new and original, as there have not been published any scientific papers in Turkey so far, especially dedicated to this ecosystem. Webb method is applied for the separation of ciliates from the Murat River sediments and there were obtained good results. The slides were analysed regularly for five days to establish the qualitative and quantitative composition of ciliate fauna. There were identified individuals belonging to 13 ciliate species on the slides during the observation days.

Keywords: Ciliates, the Murat River.

Rezumat. Câteva date privind aplicarea metodei Webb la probe de sediment provenite din Râul Murat (Regiunea Ağrı - Turcia). Râul Murat (în limba turcă Murat Nehri, Murat Suyu ori Murat Irmağı) reprezintă ramura estică a Eufratului. Activitatea de documentare privind râul Murat a început în septembrie 2011. Această direcție de cercetare este nouă, în Turcia nu sunt multe articole științifice dedicate acestui ecosistem. Metoda Webb a fost aplicată cu rezultate bune pentru separarea ciliatelor de sedimentele râului Murat. Lamelele au fost analizate cu regularitate timp de patru zile în scopul stabilirii compoziției calitative și cantitative a faunei de ciliate. În timpul observațiilor au fost identificați indivizi aparținând unui număr de 13 specii.

Cuvinte cheie: ciliate, Râul Murat.

INTRODUCTION

The observation and diagnosis of benthic ciliates is sometimes difficult taking into account their thigmotaxis capacity. In order to separate ciliates from origin sediments, there were discovered different methods, which present much or less advantages concerning the application time and distinct action according to the species fragility degree.

The separation method proposed by Uhlig (DRAGESCO & DRAGESCO-KERNÉIS, 1986b; UHLIG, 1964) is very advantageous if referring to the short time necessary to separate ciliates from sediments (only two hours), but aggressive for cells (especially fragile ciliates).

Although the duration of application is longer (maximum five days), Webb method consists in ciliates migration on the slides disposed at the surface of sediments looking for food and oxygen. We tested this method and the results are encouraging in case of the samples of sediment taken from the median part of the seashore and paramarine lakes (DUMITRACHE-KERKMANN, 2005). Our scientific paper presents results of Webb method application of sediments from the Murat River (Ağrı Region, Turkey). The Murat River (in Turkish Murat Nehri, Murat Suyu or Murat Irmağı) is the eastern branch of the Euphrates and it springs near the small town Doğubeyazıt. Ağrı city is the capital of the region with the same name and is located in eastern Anatolia; in year 2008, the population of this city was officially estimated to 91.817 inhabitants (WIKIPEDIA, 2012).

MATERIAL AND METHODS

The water and sediment samples collected from the second station of the Murat River (established in the area where the city sewerage system discharges into the Murat river; water temperature was 23.5 °C) (KERKMANN et al., 2012; KERKMANN, 2012) were left to rest into laboratory for a few hours; after the removal of the water from the sediment, the first centimetres of the samples were distributed in three Petri Dishes noted WI (Photo 1).

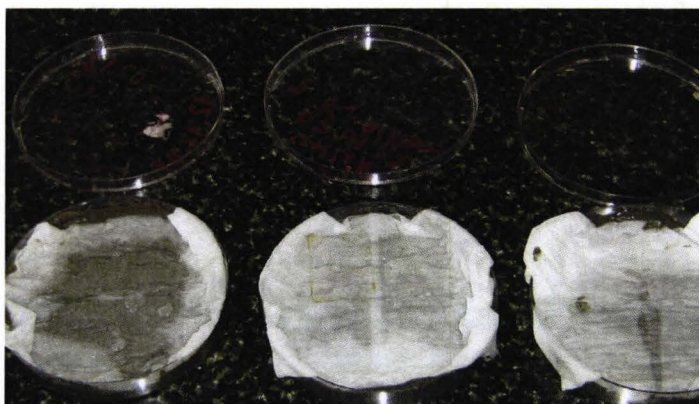


Photo 1 – Webb Method (original).

On the sediments wetted with distilled water (to prevent ciliates inflow from the water of the original ecosystem), it was applied a laboratory tissues and four slides. The lower sediment layer of the samples was put in another Petri dishes noted WII (see Table 1, legend) and subjected to the same procedure.

Table 1. Evidence of ciliates species on the slides of first sample.

Species	HP	W.I.I.1	W.I.I.2	W.I.I.3	W.I.I.4	W.I.II.1	W.I.II.2	W.I.II.3	W.I.II.4	W.I.III.1	W.I.III.2	W.I.III.3	W.I.III.4
<i>Holophrya</i> sp.	R	-	4	-	3	3,4	-	-	-	-	-	-	-
<i>Urotricha globosa</i> CLAPAREDE et LACHMANN 1857	Ba,Al	3	3	-	-	-	-	-	3	3	2,3	3	3
<i>Urotricha</i> sp.	Ba,Al	4	4	4	4	4	4	4	4	4	4	4	4
<i>Prorodon</i> sp.	R	4	-	-	-	-	-	-	3,4	-	-	-	-
<i>Didinium</i> sp.	R	-	-	-	-	-	-	-	3	3	-	3	-
<i>Plagiocamp arouxi</i> KAHL 1932	Ba,Al	-	-	-	-	-	1,2	-	-	-	-	-	-
<i>Plagiolyla nasuta</i> STEIN 1860	Ba,Sb, Al,Fl	3,4	4	-	3,4	-	3,4	-	3	-	-	-	-
<i>Colpidium colpoda</i> (LOSANA 1829) STEIN 1860	Ba,Fl, Al	4	4	4	4	-	-	-	-	-	-	-	-
<i>Paramecium cf. aurelia</i> EHRENBERG 1838	Ba	-	-	-	-	4	-	4	-	-	-	-	-
<i>Uronema nigricans</i> (MÜLLER 1786) FLORENTIN 1901	Ba,Fl	1,3,2,4	3,4	3,4	3,4	2,3,4	1,2,3,4	2,3,4	2,3,4	2,3,4	2,3,4	1,3,4	2,3,4
<i>Vorticella campanula</i> EHRENBERG 1833	Ba,Al	3,4	3,4	3,4	3,4	2,3,4	2,3,4	1,2,3,4	2,3,4	2,3,4	1,3,4	1,2,4	2,3,4
<i>Oxytricha</i> sp. 1	Ba,Fl	-	-	-	4	-	3	-	-	-	-	-	1,3
<i>Stylonychia</i> sp.	Ki,Fl	-	3,4	-	4	4	4	4	4	4	4	-	-
Forms under 10 µm	?	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3	1,2,3,4	1,2,4	2,3,4	1,2,3,4	1,2,3 4	1,2,3 4	1,2,3 4	1,2,3 4
Flagellata	-	1	-	1	1	-	-	1	-	-	-	-	-
Nematoda	-	1	-	-	1	-	2	1	-	1	1	-	1

Legend of Table 1 and 2: 1,2,3,4 – days of samples examination; **W I.I.1** = sample I, Petri dish I, slide 1; **W I.I.2** = sample I, Petri dish I slide 2; **W I.I.3** = sample I, Petri dish I, slide 3; **W I.I.4** = sample I, Petri dish I, slide 4; **W I.II.1** = sample I, Petri dish II, slide 1; **W I.II.2** = sample I, Petri dish II, slide 2; **W I.II.3** = sample I, Petri dish II, slide 3; **W I.II.4** = sample I, Petri dish II, slide 4; **W I.III.1** = sample I, Petri dish III, slide 1; **W I.III.2** = sample I, Petri dish III, slide 2; **W I.III.3** = sample I, Petri dish III, slide 3; **W I.III.4** = sample I, Petri dish III, slide 4; **W II.I.1** = sample II, Petri dish I, slide 1; **W II.I.2** = sample II, Petri dish I, slide 2; **W II.I.3** = sample II, Petri dish I, slide 3; **W II.I.4** = sample II, Petri dish I, slide 4; **W II.II.1** = sample II, Petri dish II, slide 1; **W II.II.2** = sample II, Petri dish II, slide 2; **W II.II.3** = sample II, Petri dish II, slide 3; **W II.II.4** = sample II, Petri dish II, slide 4; **W II.III.1** = sample II, Petri dish III, slide; **W II.III.2** = sample II, Petri dish III, slide 2; **W II.III.3** = sample II, Petri dish III, slide 3; **W II.III.4** = sample II, Petri dish III, slide 4; **Al** = algae (except of diatoms but inclusive autotrophic flagellates); **Ba** = bacteria; **Fl** = heterotrophic flagellates; **Ki** = diatoms; **R** = predator; **Sb** = sulphur bacteria (FOISSNER & BERGER, 1986).

The daily control of the slides aimed at establishing the qualitative and quantitative composition of ciliates (forms larger than 10 µm) migrated on the slides. The experiment finished after only three days considering that a complete and diversity ciliate fauna installed on the slides. The maximum recommended duration is five days. Some ciliates were studied “in vivo”, while in case of others, there were applied colorations such as methyl green or haematoxylin.

Some quantitative data were obtained by daily counting of the forms larger than 10 µm. During the experiment, the Petri dishes were maintained in laboratory conditions, temperature ranging between 18 and 21°C. There were also taken pictures. The systematic arrangement of the determined forms respects the systematics proposed by Puytorac et al. (DRAGESCO & DRAGESCO-KERNÉIS, 1986a).

RESULTS AND DISCUSSIONS

After the systematic examination of the slides disposed on the sediment surface, 13 species of Ciliata were found; 6 of them only to genus. During the experiment there were observed small forms (up to 10 µm), unidentifiable with the available equipment, as well as the presence of representative groups of protozoans or metazoans installed on the organic pellicle, which covers the exposed slides (Table 1).

The ciliate fauna from the sediments of the Murat River (Ağrı Region) interested us since September 2011, the first scientific results already making the subject of a scientific paper. Two ciliate forms completed the initial list proposed by us, *Prorodon* sp. and *Didinium* sp. (KERKMANN et al., 2012).

The ciliates migration on the slides disposed at the surface of the sediments is a dynamic phenomenon, migration being triggered by the search for food; most of the identified forms are bacterivorous, microalgivorous, while others feed on zooflagellates (FOISSNER & BERGER, 1986). When the parameters of one or many abiotic factors do not

correspond to ciliates requirements, they return into the first millimetres of the sediments or transform into cysts until favourable conditions return (personal observations).

In terms of ciliates fauna installed on the slides with sediments of the first sample, after analysing table 1, one may notice that in the first examination day, the number of identified species was small, except small forms (up to 10 μm), which were identified constantly during the experiment and were probably bacterivorous.

From the total of identified species in the four days of the experiment only two species seem to have adapted to the new conditions imposed by the presence of the artificial substrate. Thus, the individuals of *Vorticella campanula* (EHRENBERG 1833 and *Uronemanigricans* (MÜLLER 1786; FLORENTIN 1901) were identified on 3 of the 12 slides from the first examination day, representing the constant presence throughout the observations.

Other individuals of other species appeared on the slides in the second (*Urotricha globosa* CLAPAREDE et LACHMANN 1857), third (*Prorodon* sp., *Holophrya* sp., *Stylonychia* sp.) and even in the last day of the experiment *Urotricha* sp., *Colpidium colpoda* (LOSANA 1829) STEIN 1860, *Paramecium* cf. *aurelia* EHRENBERG 1838 respectively *Stylonychia* sp. Among the identified species, there are some which even if they appeared in the last day of observations, they were found on all the slides of sample 1 or on more than half of them (*Stylonychia* sp.). A possible explanation could be their ecological plasticity and the species evolution through the differentiation of cilia.

In terms of the evolution of the species number during the four observation days (Fig. 1), the situation is relatively balanced, their number increasing with the time passed from the initial moment.

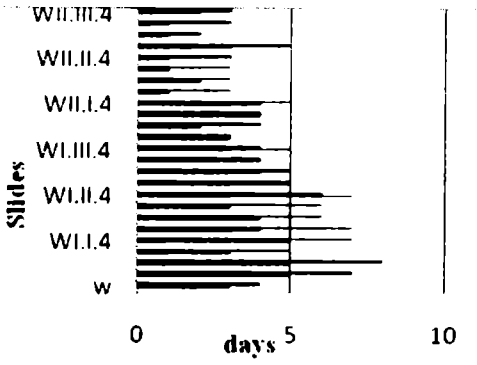


Figure 1. WEBB – Daily number of species on each slide.

Regarding the frequency of ciliates species developed on the slides of the second sample of sediments (WII), the situation is the same: the ciliates belonging to *Urotrycha* and *Stylonychia* genus appeared on the majority of the exposed slides in the last experiment day (Table 2).

Table 2. Evidence of the ciliates species on the slides of second sample.

Species	W.II.I.1	W.II.I.2	W.II.I.3	W.II.I.4	W.II.II.1	W.II.II.2	W.II.II.3	W.II.II.4	W.II.III.1	W.II.III.2	W.II.III.3	W.II.III.4
<i>Holophrya</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-
<i>Urotricha globosa</i> CLAPAREDE et LACHMANN 1857	2	2	2.3	-	-	-	-	-	-	-	-	-
<i>Urotricha</i> sp.	4	4	4	4	4	4	4	4	4	-	-	-
<i>Prorodon</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-
<i>Didinium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-
<i>Plagiocamp arouxi</i> KAHL 1932	-	-	-	-	-	-	-	-	-	-	-	-
<i>Plagiopyla nasuta</i> STEIN 1860	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpidium colpoda</i> (LOSANA 1829) STEIN 1860	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paramecium</i> cf. <i>aurelia</i> EHRENBERG 1838	-	-	-	-	-	-	-	-	-	-	-	-
<i>Uronema nigricans</i> (MÜLLER 1786) FLORENTIN 1901	1.2.3.4	2.3.4	1.2.3.4	2.3.4	-	-	-	-	4	-	3.4	4
<i>Vorticella campanula</i> EHRENBERG 1833	3	2	1.2.3	2.4	4	2.3.4	2.4	4	2.3.4	4	3.4	3.4
<i>Oxytricha</i> sp. 1	-	-	-	-	-	-	-	-	3	-	-	4
<i>Stylonychia</i> sp.	-	4	4	4	-	-	-	-	4	-	-	-
Forms under 10 μm	1.2.3.4	1.2.3.4	1.2.3.4	1.2.3.4	1.2.3.4	1.2.3.4	1.2.3.4	1.2.3.4	1.2.3.4	1.2.3.4	1.2.3.4	1.2.3
Flagellata	-	-	-	-	-	-	-	-	-	-	-	-
Nematoda	-	4	4	-	4	4	-	4	-	-	-	-

Regarding the qualitative composition of the ciliates fauna (Figs. 2-4), during the first day of observation, there were identified only small forms (up to 10 µm); the same situation was observed in the second day, when on the slides surfaces there were identified the first larger ciliates forms (number variation between 1 and 7 individuals). The most abundant forms were observed in the third day of the experiment, the maximum value reaching 315 individuals (W.I.II.4), while in the fourth day, the number increased to 2.510.

Regarding the qualitative variation of the ciliate individuals belonging to different species, in the second day of the experiment, there were identified only two individuals of *Vorticella campanula* (EHRENBERG 1833) on slide W.I.III.1.; in the third day, the number reached 42, while in the fourth, it increased to 341; for *Uronema nigricans* (MÜLLER 1786; FLORENTIN 1901), we registered a similar evolution - 3 individuals (first day), 18 (second day), respectively 401 (fourth day). A possible explanation of their quantitative evolution could be the abundance of food - small algae and bacteria (Table 1).

CONCLUSIONS

- 1. During the four days of the experiment there were identified 13 ciliate species on the slides, most of them feeding on bacteria and small algae.
- 2. If in the first two days of the experiment the small forms (up to 10 µm) dominated, the number of larger forms increased in the next two days (Figs. 2-4), because most of them consume small algae and bacteria (Table 1).
- 3. Despite the longer time of observations and difficulty of work consisting in daily control of the slides, compared to other methods (UHLIG, 1964), the present method is recommended especially for fragile forms because it is not so brutal.

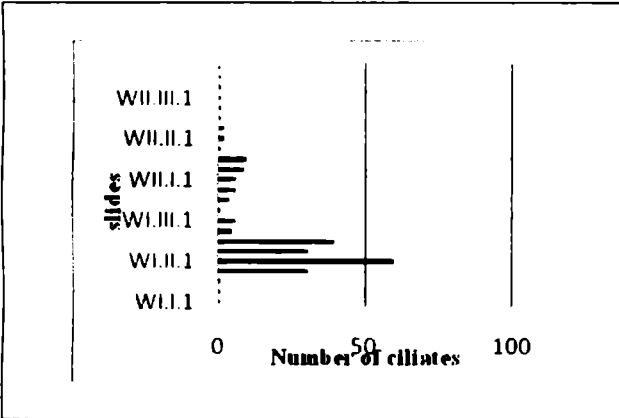


Figure 2. WEBB – Quantitative variation of the ciliates during the second day of the experiment.

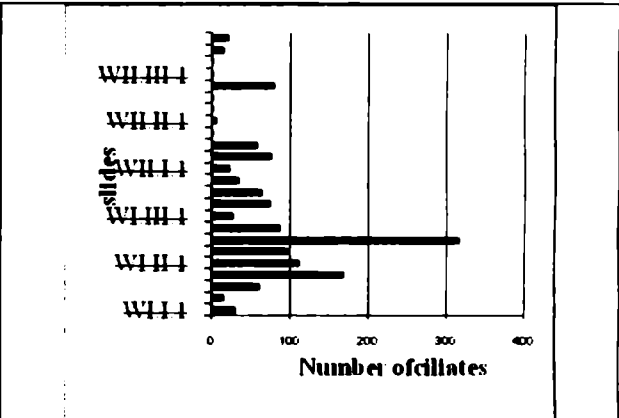


Figure 3. WEBB – Quantitative variation of the ciliates during the third day of experiment.

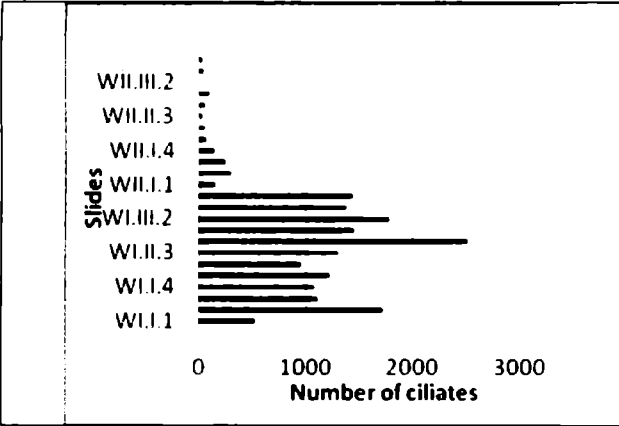


Figure 4 -WEBB – Quantitative variation of the ciliates during the fourth day of experiment

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MULTIANNUAL VARIABILITY OF ROTIFER PRODUCTION IN SFÂNTU GHEORGHE BRANCH

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Abstract. Rotifers are minute metazoans, widespread in aquatic ecosystems involving both classical and microbial food web (microbial loop). The assessment of biomass and production are useful in order to have an overview of the whole community, in terms of its trophic and ecological significance. A detailed knowledge of a community production can highlight the key species (or groups) involved in "top-down" control of matter and energy flow in trophic networks. The studies conducted in the period 2008-2010 in Sfântu Gheorghe branch, revealed differences among the three seasons in terms of rotifer secondary production. Spring and summer showed high values of secondary production compared to the autumn season when it was installed a decline period. Bray-Curtis similarity analysis confirmed that the distribution of rotifers production was mainly determined by the seasons and to a lesser extent by hydro-geomorphological differences among areas. Based on ANOVA analysis it was evaluated the significance degree of the differences among the studied areas and seasons. The rotifers production of the three years of study ranged from 0.07 to 24.85 μg wet weight $\text{L}^{-1}/24\text{h}$, typical to lotic ecosystems, characterized by a poor production. The study has also demonstrated that, the secondary production was influenced by variations of specific richness ($R^2 = 0.104$, $p = 0.000218$). The B/P turnover ratio showed the time of generations ranged from 1.374-7.831 days, while the P/B ratio varied from 0.124 to 0.949. The opposite relation between these two ratios emphasizes that the high renewing time of biomass is characterized by a low rate of productivity.

Keywords: rotifers, secondary production, Sfântu Gheorghe branch.

Rezumat. Variabilitatea multianuală a producției rotiferelor în Brațul Sfântu Gheorghe. Rotiferele sunt organisme cu durată scurtă de viață, foarte larg răspândite în ecosistemele acvatice cu implicare atât în rețeaua trofică clasică cât și în rețeaua trofică microbială (bucla microbială). Evaluarea biomasei și estimarea producției sunt utile pentru a avea o imagine de ansamblu a întregii comunități în ceea ce privește importanța ei trofică și semnificația ecologică a acesteia. O cunoaștere detaliată a producției unei comunități poate scoate în evidență și așa numitele specii (grupe) cheie care exercită un control „top-down” în circuitul materiei și energiei în rețelele trofice. Studiile desfășurate în perioada 2008-2010 în Brațul Sfântu Gheorghe, au evidențiat diferențe între cele trei sezoane în ceea ce privește producția secundară a rotiferelor. Primăvara și vara au prezentat valori ridicate ale producției secundare comparativ cu sezonul de toamnă când s-a instalat o perioadă de declin. Analiza de similaritate Bray-Curtis, a confirmat că distribuția producției rotiferelor a fost în special determinată de sezoane și într-o măsură mai mică de diferențele hidrogeomorfologice dintre zone. Pe baza analizelor ANOVA s-a putut evalua gradele de semnificație a diferențelor apărute dintre zonele studiate și sezoniere. Producția rotiferelor celor trei ani de studiu a variat între 0.07-24.85 μg subst. umedă $\text{L}^{-1}/24\text{h}$, tipic ecosistemelor lotice, care sunt caracterizate de o producție slabă. Studiile au mai demonstrat că producția secundară a fost influențată de variațiile bogăției specifice ($R^2 = 0.104$, $p = 0.000218$). Raportul de turnover B/P_{24h}, a arătat că în cei 3 ani de studiu timpul de reînnoire a unei generații a variat de la 1.374-7.831 zile în timp ce raportul P_{24h}/B 0.124-0.949 se comportă exact invers timpului de turn over, ceea ce se poate interpreta prin faptul că timpul mare de reînnoire a biomasei este caracterizat de o viteză redusă a producției.

Cuvinte cheie: rotifere, producție secundară, brațul Sfântu Gheorghe.

INTRODUCTION

The zooplankton community plays an important role in aquatic ecosystems being involved in the biogeochemical cycles. Thus, as main grazers of primary production, detrito-bacterial aggregates consumers or predators and also as food for fish, the zooplankton participates in the carbon and nutrient cycles (MOLDOVEANU & IONICĂ, 2011, LEGENDRE & LE FEVRE, 1995). To estimate the amount of matter and energy that cycles in ecosystems, it is necessary to know the biological production of these communities (MACFADYEN, 1948). Knowledge of populations' productivity allows the evaluation of the energetic role and their contribution in providing nutrients to upper trophic levels. Also, the turnover can contribute to the knowledge of their ecological importance in energy flow and nutrient cycling (LOHRENZ et al., 1992). The zooplankton communities are characterized by a certain complexity, due to seasonal variations, abiotic parameters, predators or competitive pressures. As a result, the distribution of planktonic rotifers cannot be described as having a random character (RUTTNER-KOLISKO, 1974).

Sfântu Gheorghe branch is the oldest branch of the Danube River and delineates the southern side of the Danube Delta. Being difficult to navigate on this branch, between 1985 and 1990, hydrotechnical engineering was carried out. The branch suffered major changes by cutting-off six important meanders and building of straight canals. Given the new configuration, the cut-off meanders became inoperative in terms of water flow, the current very weak, so, in time, they transformed into puddles and thus will be subject to clogging (GĂȘTESCU & ȘTIUCĂ, 2006). As a result of these interventions, there have resulted several types of ecosystems that develop biocoenoses whose structure and functionality will vary: natural sectors, cut-off meanders and new build canals.

The aim of this work was to analyse the functional parameters of rotifer communities under variable pressure in time and space exercised by some natural and anthropogenic factors.

MATERIAL AND METHODS

For this study, 7 sampling stations with coastal and medial points were established: 1, 4 and 7 in natural sectors, 2, 5 in meanders and 3, 6 in canals (Fig. 1).

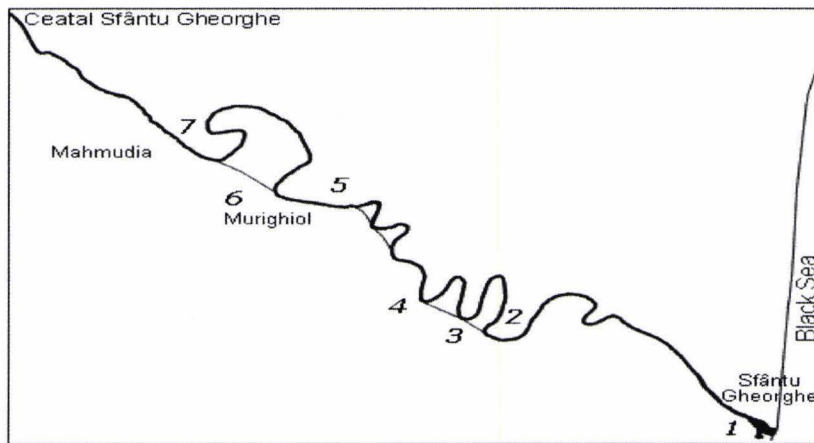


Figure 1. Sfântu Gheorghe branch with the sampling points.

The rotifers samples were taken seasonally (spring, summer and autumn), from 2008 to 2010, on whole water column with a Patalas Schindler plankton trap. The rotifers samples were collected by filtering 50 liters of water through standard plankton net (65 μm \varnothing mesh) and the samples were fixed with 4% formaldehyde.

For the species identification a Zeiss inverted microscope was used according to the following references: RUDESCU (1960), VOIGHT (1956). The density was calculated as individuals L^{-1} and for the biomass calculations it was used the wet weight of the organism (μg wet weight L^{-1}) according to DUMONT et al. (1975). The production per day was assessed based on the method described by Edmondson and Winberg (WINBERG, 1971; EDMONDSON & WINBERG, 1971) and expressed in μg wet weight $\text{L}^{-1}/24\text{h}$. For statistical analysis a free version of PAST software (HAMMER et al., 2001) and a trial version of XLSTAT were used.

RESULTS AND DISCUSSIONS

Secondary production of rotifers presented different percentages in total zooplankton production in the three studied areas. In natural sectors, the rotifers accounted for over 60% of the total production, but only in spring and autumn. In meanders, the cladocerans were those that prevailed throughout the research period while the rotifers showed low values (9-11%). In channels, the rotifers were found only in the spring season in high percentages (over 60%). The comparative analysis of the spatial and seasonal distribution of secondary rotifers production showed clear differences among seasons, the highest values being reported in spring and summer. Instead, the spatial distribution shows close values of the rotifers production (Fig. 2).

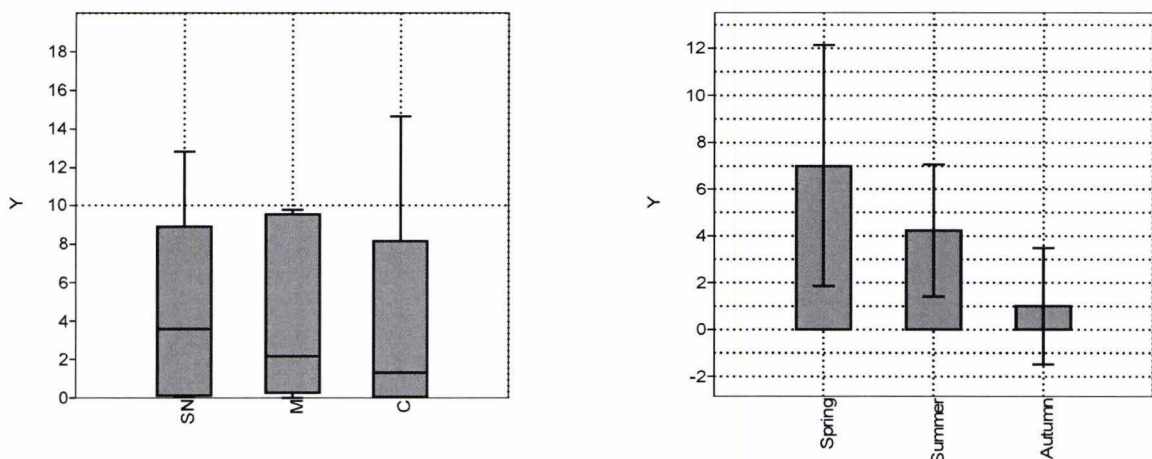


Figure 2. Box plot of the rotifers production: the middle line-median, the bottom of the boxes-25th percentile and the top -75th percentile. The T bars -the minimum or maximum values.

A detailed dendrogram based on Bray-Curtis similarity analysis (single link) of areas and seasons of the three years of study was made. The similarity clusters associated the areas and periods with high levels of similarity and thus provide the opportunity to establish a complex overview of community in terms of the approached parameter. The highest similarity degrees were found between natural areas (spring 2008) and channels (spring 2008) (79.03%), meanders (spring 2009) and channels (spring 2009) (77.65%), natural sectors (spring 2008) and natural sectors (autumn 2008) (77.32%) (Fig. 3). The Bray-Curtis dendrogram showed that the distribution of rotifers production was mainly due to the seasons and to a lesser extent to the hydro-geomorphological differences between areas.

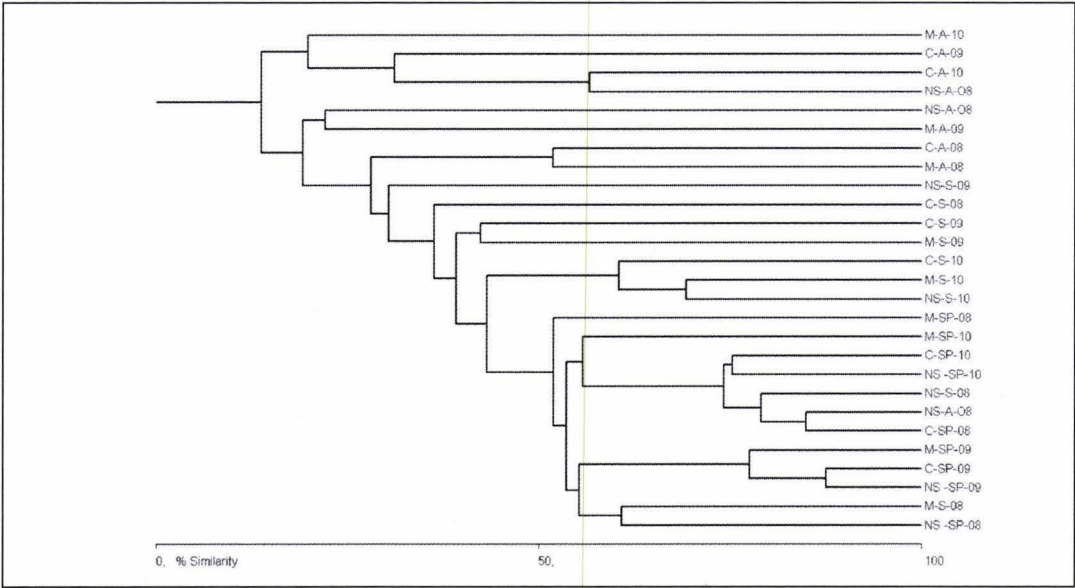


Figure 3. Bray Curtis dendrogram of the rotifers production in the studied period 2008-2010.
NS-natural sectors, M- meanders, C-canal, SP-Spring, S-Summer, A-autumn 08-2008, 09-2009, 10-2010.

Based on ANOVA (single factor) analysis, the Bray Curtis dendrogram results were statistically confirmed. There was found a significant difference ($p = 0.05$, $F = 2.981$) among the three sampling seasons. Applying ANOVA to evaluate differences among areas showed no significant differences.

The rotifers production ranged from 0.07 to 24.85 μg wet weigh $\text{L}^{-1}/24\text{h}$. In the histogram of the rotifers production the most frequent were the small values, under 6.91 μg wet weigh $\text{L}^{-1}/24\text{h}$ (Fig. 4). In lotic ecosystems, due to unfavourable conditions, the rotifers are found in low densities resulting a production much lower than in lentic ecosystems (ODUM, 1971; LAIR, 2005).

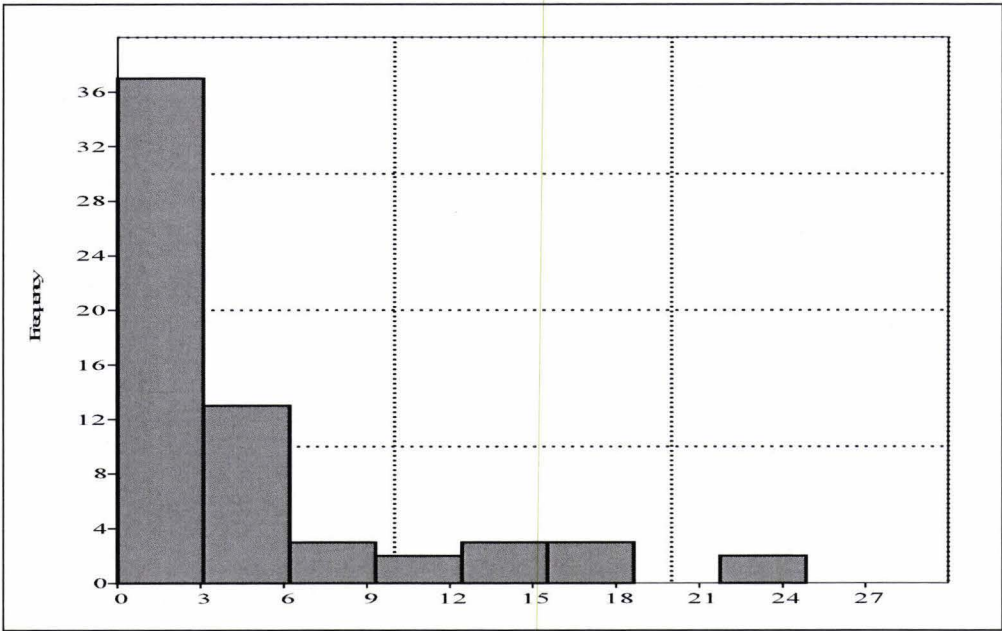


Figure 4. Histogram of the rotifers seasonal production.

The turnover of B/P (biomass/production) based on annual average values shows that, in the three years of study, the generation time ranged from 1.374 to 7.831 days (Table 1). 2008 is characterized by close values of B/P in the three sampling periods, which are no longer found in the other two years. The highest values of turn-over time were found in spring 2009 (7.831 days) and autumn (5.983). On the other hand, summer is the most active period in the rotifers productivity, defined by the shortest generation time throughout entire research period (1.374-2.491 days).

Table 1. Dynamics of annual mean of turnover time (B/P) and turnover rate (P/B) of rotifers community in Sfântu Gheorghe branch.

	Spring			Summer			Autumn		
	2008	2009	2010	2008	2009	2010	2008	2009	2010
B/P	3.93	7.831	2.597	1.374	1.195	2.491	3.423	5.983	5.815
P/B	0.261	0.129	0.401	0.742	0.949	0.124	0.631	0.192	0.249

The annual averages of turnover rate (P/B) ranged between 0.124 and 0.949 and behaved opposite then the turnover time, which can be interpreted that the renewing time of biomass is described by a low rate of production.

The productivity in an ecosystem is strongly related to biomass and taxonomical composition of the communities. Rotifers represent a major component in zooplankton production by a quick generation time (B/P) and also high turnover rate (P/B) (PARK & MARSHALL, 2000). The analysis of Person correlation emphasised a significant relationship between biomass and rotifer productivity ($R = 0.29$, $R^2 = 0.164$, $p = 0.001$). The changes of the abundance and biomass are influenced by productivity and mortality dynamics (KIORBOE & NIELSEN, 1994). Also, a high significant connection ($R = 0.40$, $R^2 = 0.164$, $p = 0.0001$) between production and turnover rate (P/B) was found. In Sfântu Gheorghe branch, during the survey, the daily P/B weight growth rate and production presented the same trend (Fig. 5).

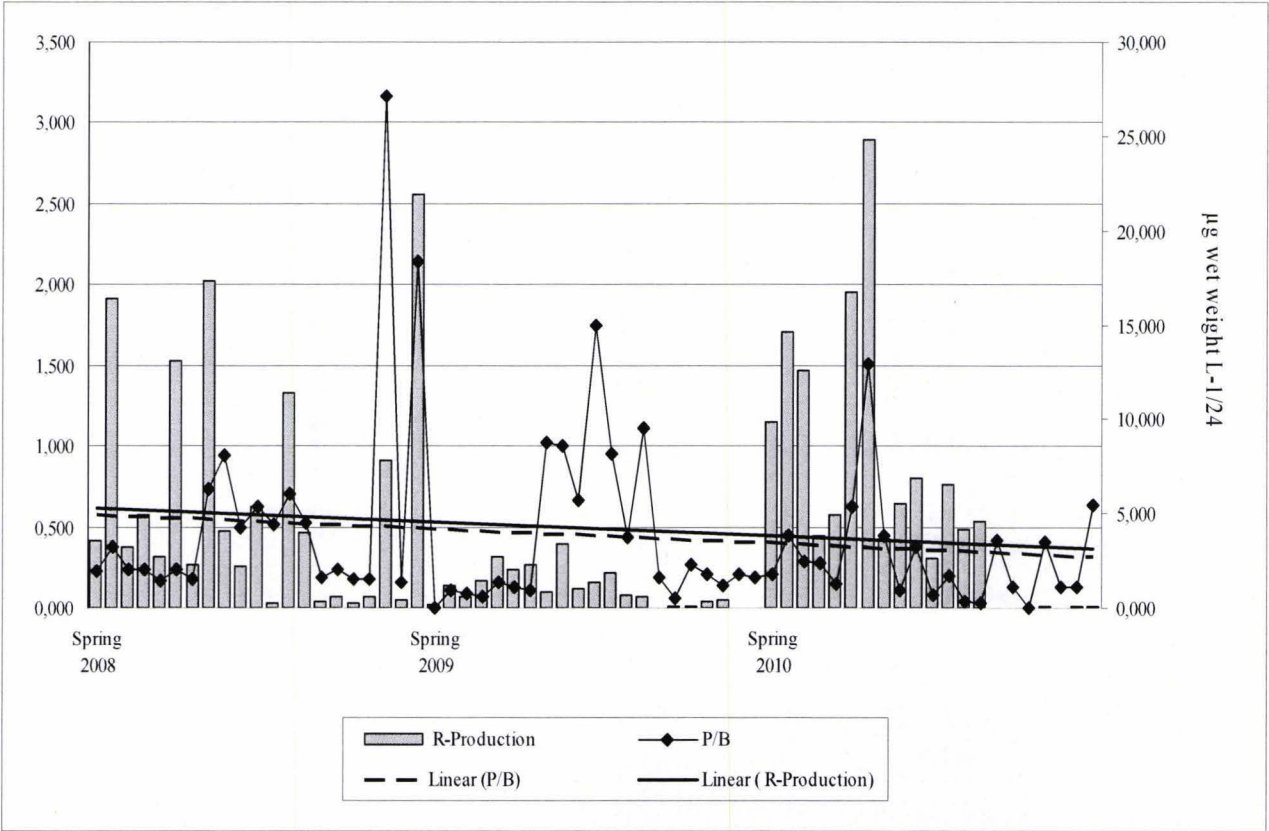


Figure 5. Dynamics of rotifers production and P/B ratio during the survey.

Previous research mentions that the deltaic zooplankton is characterized by high values of daily turnover rate ($P_{24\text{ h}}/B$) (0.113 to 0.220), a situation favoured by the predominance of small organisms, with short generation times. The analysis of systematic groups and developmental stages reveals that the rotifers present short periods of biomass recycling (2.85 days - 3.86 days). At the other extreme, the juvenile forms of copepods have the longest recycling biomass time (20.01 to 39.92 days). The zooplankton found in the Danube Delta streams and channels had a turnover rate (P/B) around 0.13 and the turnover time of 8.39 days. The changing of the values of daily turnover rate and turnover time is achieved by appropriate changes in the taxonomic spectrum of zooplankton, to form an adequate trophic structure for the new features of planktonic primary producers (ZINEVICI & PARPALĂ, 2007).

Numerous studies have demonstrated a relationship between biodiversity and community functions leading to the conclusion that the species presents an important ecological role both by their number (species richness) and dominance (abundance). The functional diversity is a measure of diversity based on functional traits of species in a community (BARNET et al., 2007). Functional characteristics of a species are those traits that define it in terms of its ecological roles, how the environment interacts with other species (for example, the body size, their growth rates, metabolism, sensitivity to environmental conditions, their ability to move) (TELESH, 2001; OBERTEGGER & MANCA, 2011).

From this point of view, it could be appreciated that in Sfântu Gheorghe branch, both species richness and dominant species had an important contribution to rotifers production. In order to confirm this assumption, a linear regression was performed. A high significance level of species richness in determining the secondary production ($R^2 = 0.104$, $p = 0.000218$, significance ****) was observed.

The taxonomic analysis has revealed a taxonomic profile of the branch. The rotifer communities in Sfântu Gheorghe branch tend to be composed by closely related species, most of them, loricate. During the study period, 83 species belonging to 9 families were recorded (Table 2). Among the common species encountered, we mention *Brachionus calyciflorus* (PALLAS 1766), *Asplanchna priodonta* (GOSSE 1850), *Synchaeta oblonga* (EHRENBERG 1832).

Table 2. List of planktonic rotifer families and number of species recorded in Sfântu Gheorghe branch.

Families	Natural sectors	Meanders	Canals
Fam. Gastropodidae	1	1	1
Fam. Asplanchnidae	1	2	1
Fam. Brachionidae	29	32	24
Fam. Notommatidae	3	2	1
Fam. Testudinellidae	5	6	4
Fam. Habrotrochidae	1	2	4
Fam. Lecanidae	2	2	0
Fam. Synchaetidae	7	9	7
Fam. Trichocercidae	6	7	3

Brachionidae had the largest contribution in production, as they dominated both the number of species and high densities. An important contribution to rotifers production was also registered by Asplanchnide; even if there were only two species, they compensated by higher density and biomass of individuals. The dominant species in terms of biomass are also known as cosmopolitan species (Fig. 6).

In order to identify the distribution tendency of rotifer production data, a Principal Component Analysis (PCA) was performed. The first result is a correlation matrix of rotifers production of the three areas. Notice the similarity degree of meanders and natural sectors (0.8), at a level of significance of 0.05 (two-tailed test) (Table 4).

The goal of the Principal Component Analysis (PCA) was to look at the data on a two-dimensional map and to identify trends. We can see that *Anuaeropsis fissa* and *Polyarthra remata* share common characteristics and are linked with the second axis and to canal production. These species have not a contribution to the meanders and natural sectors. In contrast, all other species joins the first axis and represent an assemblage in terms of meanders and natural sectors production. The first and second axis explain 94.48% of the rotifer production variability in studied zones (Fig. 7).

Table 4. Correlation matrix of the rotifers production in the three studied areas.

	Natural sectors	Meanders	Canals
Natural sectors	1	0.833	-0.009
Meanders	0.833	1	0.034
Canals	-0.009	0.034	1

Legend: in bold, significant values (except diagonal) at the level of significance $\alpha=0,050$ (two-tailed test).

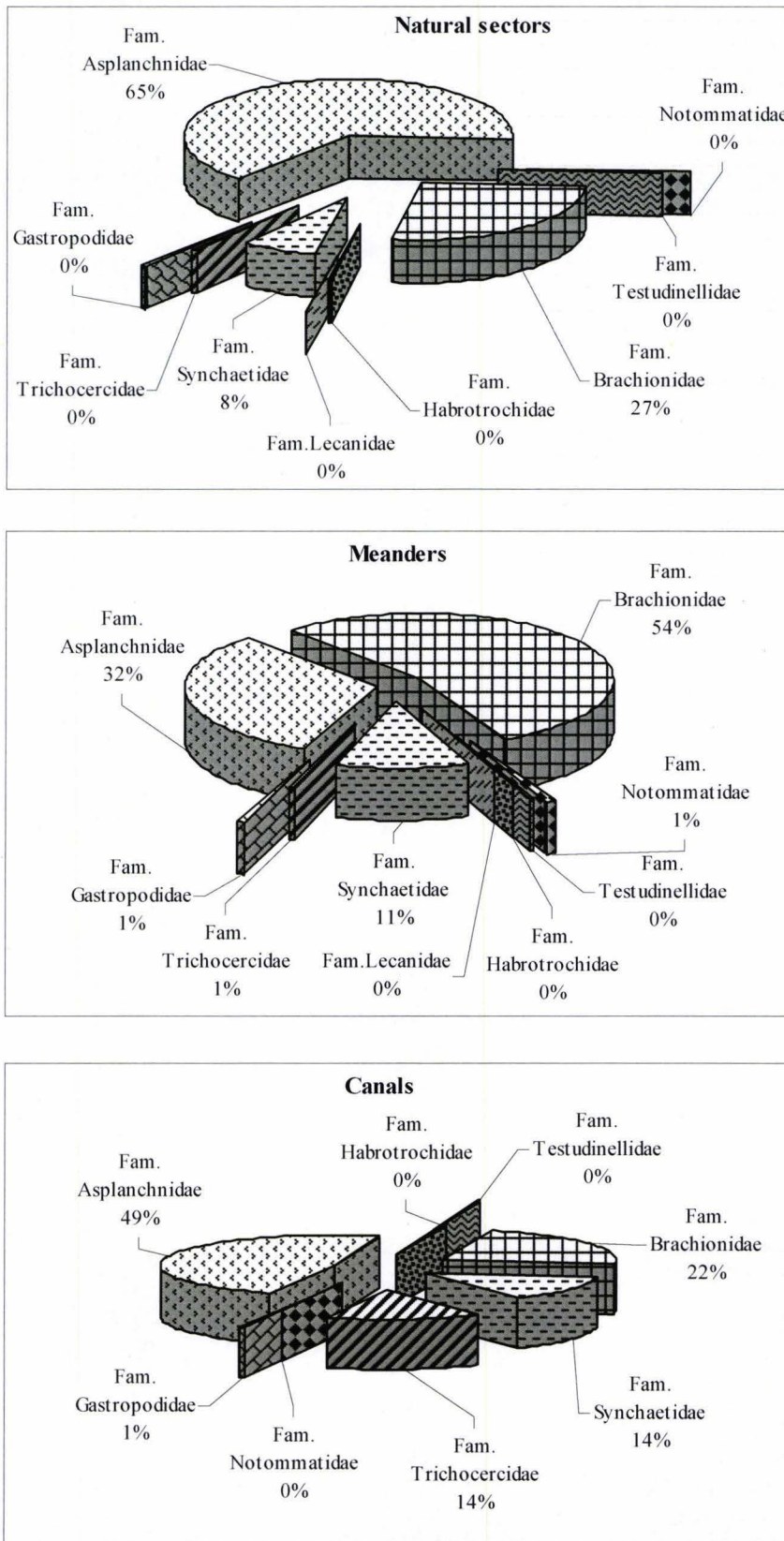


Figure 6. The contribution of rotifer families to secondary production in studied areas in Sfântu Gheorghe branch.

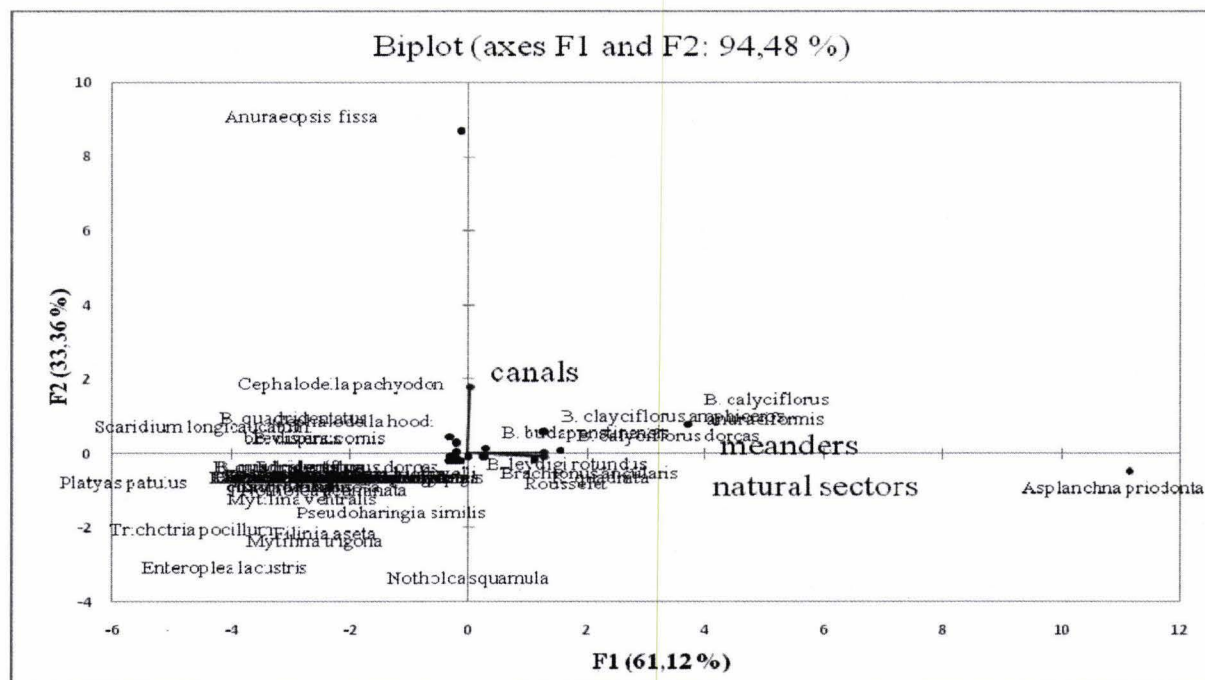


Figure 7. PCA biplot for species distribution in the three studied areas, based on their annual production averages.

CONCLUSIONS

During the study, 9 rotifer families were determined, whose production ranged from 0.07 to 24.85 μg wet weigh $\text{L}^{-1}/24\text{h}$. Most species have low values of production, typical to lotic ecosystems (under 6.91 μg $\text{L}^{-1}/24\text{h}$).

A comparison among the three seasons of study highlights that the highest values of production were found in spring and summer while the spatial review shows close values.

The highest similarities based on Bray Curtis analysis were described between natural areas (spring 2008) and channels (spring 2008) (79.03%), meanders (spring 2009) and canals (spring 2009) (77.65%), natural sectors (spring 2008) and natural sectors (autumn 2008) (77.32%).

The ANOVA analysis of variance showed a statistical difference among the three sampling seasons while in the study areas there were not exhibit significant differences.

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THE DISTRIBUTION OF THE GASTROPOD POPULATIONS ALONG THE CHARACTERISTIC SECTORS OF THE DANUBE

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Abstract. The Danube represents one of the European areas with the richest fauna of gastropods (81 species). There appear 3 zoogeographical sectors (the upper sector, the middle sector, the lower sector), characterised by their own structure of gastropod populations. Duo to their ecological valences, in the upper sector, there are characteristic 6 species, in the middle sector 5 species, in the lower sector 14 species. Most of the species display an ubiquist character (35 species) as they are present within all the three aforementioned sectors. The global evaluation of the Gastropod populations from the Danube established that they represent 32 per cent of the European malacofauna.

Keywords: gastropods, distribution, the Danube, the global evaluation.

Rezumat. Distribuția populațiilor de gastropode în sectoare caracteristice Dunării. Dunărea reprezintă unul din fluviile europene cu cea mai bogată diversitate a populațiilor de gastropode (81 specii). Se disting trei sectoare zoogeografice (superior, mijlociu, inferior) caracterizate prin structura populațiilor de gastropode. Prin valențele ecologice în sectorul superior sunt caracteristice 6 specii, în sectorul intermediar 5 specii, în sectorul inferior 14 specii. Cele mai multe specii au un caracter ubicvist (35 specii) fiind prezente în cele trei sectoare. Evaluarea globală a populațiilor de gastropode din Dunăre reprezintă 32% din malacofauna europeană.

Cuvinte cheie: gastropode, distribuție, Dunăre, evaluare globală.

INTRODUCTION

The Danube represents one of the European areas with the richest fauna of gastropods (RUSSEV, 1998; CIOBOIU, 2008). Its geographical position and ecosystem structure represent main factors that ensure the conditions necessary for the development of gastropod populations (CIOBOIU CODOBAN, 2003; BREZEANU et al., 2011).

There appear 3 zoogeographical sectors (the upper sector, the middle sector, the lower sector), characterized by specific populations of gastropods (Fig. 1). The upper or alpine sector that refers to the spring area and the torrent-like course (Km 2857 – 1880); The middle or Pannonian sector characterized by a rapid flow of the river (Km 1879 – 1103); The lower or Carpathian-Balkan sector, where the river has a large bed, slow flowing speed, and variable depths (Km 1102 – 0) (ARDELEAN et al., 1967; CIOBOIU, 2006, 2010; CIOBOIU & BREZEANU, 2000).



Figure 1. The Danube (Km 2826 – 0) (after ICPDR, Vienna).

MATERIAL AND METHODS

According to the data rendered in the literature in the field and according to my own research, there has been made a synthesis that allowed a global evaluation of the species from the Danube (BREZEANU et al., 2011; CIOBOIU, 2006, 2010; CSANYI, 1996; CUTTELOD et al., 2011; FRANK, 1987; GROSSU, 1987, 1993; JURGEN et al., 1988; LIASHENKO et al., 2010; LIASHENKO & ZORINA-SAKHAROVA, 2012; IVANYI et al., 2012; OERTEL & NOSEK, 2006; RUSSEV, 1998; TITTIZER et al., 1997; ZIERITZ & WARINGER, 2006; Fauna Europaea, 2013).

RESULTS AND DISCUSSIONS

The populations of gastropods from the Danube totalize 81 species (Table 1).

Table 1. Taxonomic composition of the Gastropods from the Danube.

No.	Species	The Danube		
		Upper sector	Middle sector	Lower sector
1.	<i>Theodoxus (Th.) danubialis</i> (C. PFEIFFER 1828)	+	+	+
2.	<i>Theodoxus (Th.) d. stragulatus</i> (C. PFEIFFER 1828)		+	+
3.	<i>Theodoxus (Th.) euxinus</i> (CLESSIN 1887)			+
4.	<i>Theodoxus (Th.) fluviatilis</i> (LINNAEUS 1758)	+	+	+
5.	<i>Theodoxus (Th.) pallasi</i> LINDHOLM 1924			+
6.	<i>Theodoxus (Th.) prevostianus</i> (C. PFEIFFER 1828)			+
7.	<i>Theodoxus (Th.) transversalis</i> (C. PFEIFFER 1828)		+	+
8.	<i>Viviparus acerosus</i> (BOURGUIGNAT 1862)	+	+	+
9.	<i>Viviparus ater</i> (DE CRISTOFORI & JAN 1832)	+		
10.	<i>Viviparus comectus</i> (MILLET 1813)		+	+
11.	<i>Viviparus mamillatus</i> (KUSTER 1852)		+	+
12.	<i>Viviparus viviparus</i> (LINNAEUS 1758)			+
13.	<i>Viviparus viviparus penthicus</i> (SERVAIN 1884)	+		
14.	<i>Valvata (Cincinna) piscinalis</i> (O. F. MULLER 1774)		+	+
15.	<i>Valvata (Cincinna) studeri</i> BOETERS & FALKNER 1998	+		
16.	<i>Valvata (Cincinna) piscinalis antiqua</i> MORRIS 1838			+
17.	<i>Valvata (Tropidina) macrostoma</i> MORCH 1864	+		
18.	<i>Valvata (Valvata) cristata</i> O. F. MULLER 1774	+	+	+
19.	<i>Borysthenia naticina</i> (MENKE 1845)		+	+
20.	<i>Pseudamnicola (P.) dobrogica</i> GROSSU 1986			+
21.	<i>Pseudamnicola (P.) penchinati</i> (BOURGUIGNAT 1870)			+
22.	<i>Bythinella austriaca</i> (FRAUENFELD 1857)	+		
23.	<i>Bythinella cylindrica</i> (FRAUENFELD 1857)	+		
24.	<i>Bythinella hungarica</i> HAZAY 1880		+	
25.	<i>Potamopyrgus antipodarum</i> (J. E. GRAY 1843)			+
26.	<i>Lithoglyphus apertus</i> (KUSTER 1852)			+
27.	<i>Lithoglyphus fuscus</i> (C. PFEIFFER 1828)		+	
28.	<i>Lithoglyphus naticoides</i> (C. PFEIFFER 1828)	+	+	+
29.	<i>Lithoglyphus pygmaeus</i> FRAUENFELD 1863			+
30.	<i>Bithynia (Bithynia) mostarensis</i> MOELLENDORFF 1873		+	
31.	<i>Bithynia (Bithynia) tentaculata</i> (LINNAEUS 1758)	+	+	+
32.	<i>Bithynia (Codiella) troschelii</i> (PAASCH 1842)	+	+	+
33.	<i>Bithynia (Codiella) leachii</i> (SHEPPARD 1823)	+		+
34.	<i>Turricaspia (Clessimiola) variabilis</i> (EICHWALD 1838)			+
35.	<i>Turricaspia (Oxypyrgula) ismailensis</i> (GOL. & STAROB. 1966)			+
36.	<i>Turricaspia (Turricaspia) dimidiata</i> (EICHWALD 1841)			+
37.	<i>Esperiana esperi</i> (A. FERUSSAC 1823)		+	+
38.	<i>Esperiana (Microcolpia) daudebardii</i> (PREVOST 1821)		+	+
39.	<i>Esperiana (M.) daudebardii acicularis</i> (A. FERUSSAC 1823)		+	+
40.	<i>Amphimelania holandri</i> (C. PFEIFFER 1828)		+	+
41.	<i>Physa fontinalis</i> (LINNAEUS 1758)	+	+	+
42.	<i>Physella (Costatella) acuta</i> (DRAPARNAUD 1805)			+
43.	<i>Physella (Costatella) heterostropha</i> (SAY 1817)		+	+
44.	<i>Aplexa hypnorum</i> (LINNAEUS 1758)		+	+
45.	<i>Lymnaea stagnalis</i> (LINNAEUS 1758)		+	+
46.	<i>Stagnicola corvus</i> (GMELIN 1791)	+	+	+
47.	<i>Stagnicola fuscus</i> (C. PFEIFFER 1821)	+		
48.	<i>Stagnicola palustris</i> (O. F. MULLER 1774)		+	+
49.	<i>Stagnicola turricula</i> HELD 1836	+	+	+
50.	<i>Radix ampla</i> (W. HARTMANN 1821)	+	+	+
51.	<i>Radix auricularia</i> (LINNAEUS 1758)		+	+
52.	<i>Radix balthica</i> (LINNAEUS 1758)		+	+
53.	<i>Radix labiata</i> (ROSSMASSLER 1835)		+	+

54.	<i>Radix lagotis</i> (SCHRANK 1803)	+		
55.	<i>Galba truncatula</i> (O. F. MULLER 1774)	+	+	+
56.	<i>Ancylus fluviatilis</i> O. F. MULLER 1774	+	+	+
57.	<i>Ferrissia</i> (<i>Pettancylus</i>) <i>clessiniana</i> (JICKELI 1882)	+	+	+
58.	<i>Acroloxus lacustris</i> (LINNAEUS 1758)		+	+
59.	<i>Planorbis</i> (<i>Planorbis</i>) <i>carinatus</i> O. F. MULLER 1774	+	+	+
60.	<i>Planorbis</i> (<i>Planorbis</i>) <i>planorbis</i> (LINNAEUS 1758)	+	+	+
61.	<i>Anisus</i> (<i>Anisus</i>) <i>calculiformis</i> (SANDBERGER 1874)		+	+
62.	<i>Anisus</i> (<i>Anisus</i>) <i>leucostoma</i> (MILLET 1813)		+	
63.	<i>Anisus</i> (<i>Anisus</i>) <i>spiroboris</i> (LINNAEUS 1758)			+
64.	<i>Anisus</i> (<i>Disculifer</i>) <i>vortex</i> (LINNAEUS 1758)		+	+
65.	<i>Anisus</i> (<i>Disculifer</i>) <i>vorticulus</i> TROSCHEL 1852		+	+
66.	<i>Bathymphalus contortus</i> (LINNAEUS 1758)	+	+	+
68.	<i>Gyraulus</i> (<i>Armiger</i>) <i>crista</i> LINNAEUS 1758		+	+
69.	<i>Gyraulus</i> (<i>Gyraulus</i>) <i>acronicus</i> (A. FERUSSAC 1807)	+	+	+
70.	<i>Gyraulus</i> (<i>Gyraulus</i>) <i>albus</i> (O. F. MULLER 1774)	+	+	+
71.	<i>Gyraulus</i> (<i>Gyraulus</i>) <i>chinensis</i> (DUNKER 1848)	+		
72.	<i>Gyraulus</i> (<i>Lamorbis</i>) <i>riparius</i> (WESTERLUND 1865)	+	+	
73.	<i>Gyraulus</i> (<i>Lamorbis</i>) <i>rossmaessleri</i> (AUERSWALD 1852)	+		
74.	<i>Gyraulus</i> (<i>Torquis</i>) <i>laevis</i> (ALDER 1838)			+
75.	<i>Hippeutis complanatus</i> (LINNAEUS 1758)		+	+
76.	<i>Segmentina nitida</i> (O. F. MULLER 1774)		+	+
77.	<i>Planorbarius corneus</i> (LINNAEUS 1758)	+	+	+
78.	<i>Oxyloma</i> (<i>Oxyloma</i>) <i>dunkeri</i> (L. PFEIFFER 1865)		+	+
79.	<i>Oxyloma</i> (<i>Oxyloma</i>) <i>elegans</i> (RISSO 1826)		+	+
80.	<i>Oxyloma</i> (<i>Oxyloma</i>) <i>pinteri</i> GROSSU 1987			+
81.	<i>Oxyloma</i> (<i>Oxyloma</i>) <i>sarsii</i> (ESMARK 1886)	+		

According to the analysis of the taxonomic composition of the populations of gastropods it can be noticed that there have been identified 33 species within the upper sector, so far (Table 1). The species *Viviparus ater* (DE CRISTOFORI & JAN 1832), *Valvata* (*Cincinna*) *studerii* BOETERS & FALKNER 1998, *V. (Tropidina) macrostoma* MORCH 1864, *Bythinella austriaca* (FRAUENFELD 1857), *B. cylindrica* (FRAUENFELD 1857), *Stagnicola fuscus* (PFEIFFER 1821), *Radix lagotis* (SCHRANK 1803) are characteristic to torrents and mountain streams. These represent 3.6 percent of the populations of gastropods present into the Danube (BREZEANU et al., 2011; FRANK, 1987; TITTIZER et al., 1997; ZIERITZ & WARINGER, 2006). Besides these, within the mentioned sector, there also appear the species *Theodoxus* (*Th.*) *danubialis* (PFEIFFER 1828), *Th. (Th.) fluviatilis* (LINNAEUS 1758), *Viviparus acerosus* (BOURGUIGNAT 1862), *Valvata* (*V.*) *cristata* MULLER 1774, *Lithoglyphus naticoides* (PFEIFFER 1828), *Bithynia* (*B.*) *tentaculata* (LINNAEUS 1758), *Physa fontinalis* (LINNAEUS 1758), *Stagnicola corvus* (GMELIN 1791), *S. turricula* HELD 1836, *Radix balthica* (LINNAEUS 1758), *Galba truncatula* (MULLER 1774), *Planorbarius corneus* (LINNAEUS 1758) that display a more or less ubiquist character (CIOBOIU, 2008; GROSSU, 1993).

Within the middle sector, the structure of which is characterized by relatively uniform biotopes, with a benthall facies, mostly sandy-clayish, the populations of gastropods are represented by the species *Theodoxus* (*Th.*) *transversalis* (PFEIFFER 1828), *Valvata* (*C.*) *piscinalis* (MULLER 1774), *Borysthenia naticina* (MENKE 1845), *Bythinella hungarica* HAZAY 1880, *Lithoglyphus fuscus* (PFEIFFER 1828), *Bithynia* (*B.*) *mostarensis* MOELLENDORFF 1873, *Esperiana esperi* (FERUSSAC 1823), *Amphimelania holandri* (PFEIFFER 1828), *Lymnaea stagnalis* (LINNAEUS 1758), *Stagnicola palustris* (MULLER 1774), *Ferrissia* (*P.*) *clessiniana* (JICKELI 1882), *Anisus* (*A.*) *leucostoma* (MILLET 1813), *Gyraulus* (*Lamorbis*) *riparius* (WESTERLUND 1865), *Hippeutis complanatus* (LINNAEUS 1758), *Segmentina nitida* (MULLER 1774), *Oxyloma* (*O.*) *elegans* (RISSO 1826) (BOSCHKE, 1990; CSANYI 1996; OERTEL, 2000; OERTEL & NOSEK, 2006; IVANYI et al., 2012). As it can be noticed, some of them also appear within the upper sector (Table 1). In other words, 10.8 percent of the total number is characteristic to this area (Table 1, Fig. 2).

Within the lower sector, due to the relation between the river and its liable to floods area and due to the influence of its many tributaries, the diversity of the gastropods species is higher (BREZEANU & GRUIȚĂ, 2002; CIOBOIU, 2006, 2010; LIASHENKO & ZORINA-SAKHAROVA, 2012; RUSSEV, 1998). Within the river, there develop both species characteristic to the eutrophic lacustrine ecosystems: *Theodoxus* (*Th.*) *danubialis stragulatus* (PFEIFFER 1828), *Th. (Th.) pallasii* LINDHOLM 1924, *Viviparus acerosus* (BOURGUIGNAT 1862), *V. viviparus* (LINNAEUS 1758), *Valvata* (*C.*) *piscinalis* (MULLER 1774), *Lymnaea stagnalis* (LINNAEUS 1758), *Stagnicola corvus* (O. F. MULLER 1774), *Radix auricularia* (LINNAEUS 1758), *Planorbis planorbis* (LINNAEUS 1758), *Gyraulus* (*G.*) *acronicus* (FERUSSAC 1807) and species that prefer the conditions of the rheophilic ecosystems: *Lithoglyphus naticoides* (PFEIFFER 1828), *L. pygmaeus* FRAUENFELD 1863, *Esperiana* (*M.*) *daubebardii acicularis* (FERUSSAC 1823), *Radix ampla* (HARTMANN 1821), *Segmentina nitida* (MULLER 1774).

On the other hand, a series of species prefer the sandy facies *Theodoxus* (*Th.*) *prevostianus* (PFEIFFER 1828), *Turricaspia* (*Oxytyrgula*) *ismailensis* (GOL. & STAROB. 1966), *Radix balthica* (LINNAEUS 1758), while others the clayish facies *Lithoglyphus apertus* (KUSTER 1852), *Galba truncatula* (MULLER 1774). Within the areas characterized by rocky banks, there appear the species *Potamopyrgus antipodarum* (GRAY 1843), *Bythinella austriaca* (FRAUENFELD 1857), *Amphimelania holandri* (PFEIFFER 1828). Thus, it results that the variable environmental factors of the Danube

(the water flowing velocity, the nature of the bed, the trophic state) determine this distribution of the populations of gastropods (BREZEANU & ENĂCEANU, 1969; BUȘNIȚĂ & BREZEANU, 1970; BREZEANU et al., 2011; CIOBOIU & BREZEANU, 2000; CIOBOIU, 2008; CUTTELOD et al., 2011; GROSSU, 1987, 1993; JURGEN et al., 1988; MARINESCU, 1992; NEGREA & MARINESCU, 1992; NEGREA, 1994; OERTEL, 2000).



Figure 2. The Danube along the middle and the lower sectors (original).

Of course, there are not strict limits between these sectors; this is why it can be noticed an interference of the gastropod species between different sectors of the Danube, the most numerous, respectively 85.6 percent being characteristic to the lower sector (Table 1, Fig. 3).

- the upper sector 3.6 percent
- the middle sector 10.8 percent
- the lower sector 85.6 percent

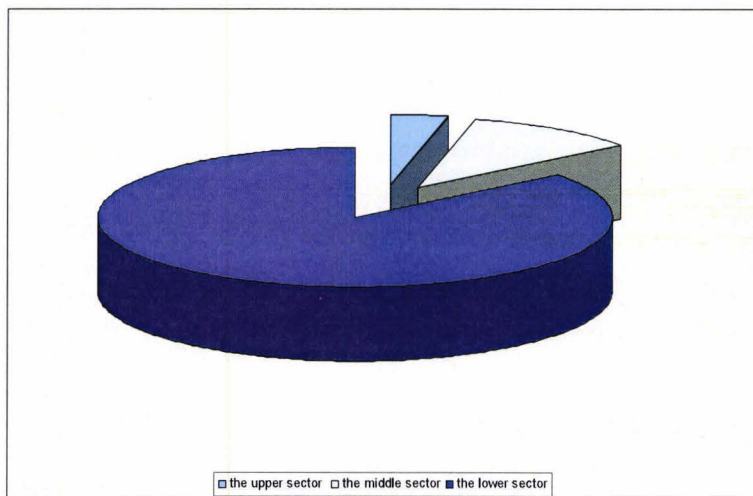


Figure 3. The distribution of the gastropod species in the Danube.

CONCLUSIONS

The global evaluation of the populations of gastropods from the Danube represents an important part of the European malacofauna (Fauna Europaea, 2013). Due to their ecological valences, the species *Viviparus ater* (DE CRISTOFORI & JAN 1832), *Valvata (Cincinna) studeri* BOETERS & FALKNER 1998, *V. (Tropidina) macrostoma* MORCH 1864, *Bythinella austriaca* (FRAUENFELD 1857), *B. cylindrica* (FRAUENFELD 1857), *Stagnicola fuscus* (PFEIFFER 1821), *Radix lagotis* (SCHRANK 1803) are cryophilic species and they are characteristic to the upper sector; the species *Bithynia (Bithynia) mostarensis* MOELLENDORFF 1873, *Amphimelania holandri* (PFEIFFER 1828), *Anisus (Anisus) leucostoma* (MILLET 1813) rheophilic along the middle sector; the species *Theodoxus (Th.) d. stragulatus* (PFEIFFER 1828), *Th. (Th.) euxinus* (CLESSIN 1887), *Valvata (Cincinna) piscinalis antiqua* MORRIS 1838, *Pseudamnicola (P.) dobrogica* GROSSU 1986, *Potamopyrgus antipodarum* (GRAY 1843), *Lithoglyphus apertus* (KUSTER 1852), *Gyraulus (Torquus) laevis* (ALDER 1838) are fluvial-lacustrine characteristic for the lower sector (Table 1, Fig. 3). It is worth

mentioning that the highest diversity of species (Table 1, Fig. 1) characterizes the lower sector of the Danube, especially the Romanian part of the river, where the area liable to floods, the delta and the tributaries represent factors that enrich the diversity of populations.

The synthesis we made may represent a parameter in evaluating the distribution of the gastropods populations within the Danube basin.

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SOME DATA UPON THE TERRESTRIAL ISOPOD ASSEMBLAGE FROM A NORTH-WESTERN ROMANIAN WETLAND

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Abstract. In 2009, we identified nine terrestrial isopod species in a wetland near Valea lui Mihai locality (Bihor County, Romania): *Hyloniscus riparius*, *H. transsilvanicus*, *Haplophthalmus danicus*, *Cylisticus convexus*, *Porcellium collicola*, *Trachelipus arcuatus*, *T. rathkii*, *T. nodulosus*, *Armadillidium vulgare*. Considering certain ecologic and zoogeographic characteristics of the nine species, we divided them in three groups: 1. species the presence of which is due to the actual features of the habitat (*H. riparius*, *T. rathkii*, *A. vulgare*, *P. collicola*, *C. convexus*, *T. nodulosus*); 2. species that probably represent survivors of a colder climate and the forest from the past (*H. transsilvanicus* and *T. arcuatus*); 3. species rarely found in traps (*H. danicus*). The surface activity is low in July, probably due to the high temperatures, which reduce the humidity of the habitat.

Keywords: wetland, Oniscidea, habitat, surface activity.

Rezumat. Date asupra comunității de izopode terestre dintr-o zonă umedă din nord-vestul României. Într-o mlaștină de lângă localitatea Valea lui Mihai (Bihor, România) am identificat în anul 2009, nouă specii de izopode terestre: *Hyloniscus riparius*, *H. transsilvanicus*, *Haplophthalmus danicus*, *Cylisticus convexus*, *Porcellium collicola*, *Trachelipus arcuatus*, *T. rathkii*, *T. nodulosus*, *Armadillidium vulgare*. Luând în considerare anumite caracteristici ecologice și zoogeografice ale acestora, am împărțit cele nouă specii în trei grupe: 1. specii ale căror prezență se datorează caracteristicilor actuale ale habitatului (*H. riparius*, *T. rathkii*, *A. vulgare*, *P. collicola*, *C. convexus*, *T. nodulosus*); 2. specii care probabil reprezintă urmele unui climat mai rece și a pădurilor din trecut (*H. transsilvanicus* și *T. arcuatus*); 3. specii rare în capcane (*H. danicus*). Activitatea de suprafață este redusă în iulie, probabil datorită temperaturilor ridicate, care diminuează umiditatea habitatului.

Cuvinte cheie: zonă umedă, Oniscidea, habitat, activitate de suprafață.

INTRODUCTION

Wetlands from north-western Romania have a distinct importance both for the flora and fauna (e.g. KARACSONYI, 1987; FERENTI et al., 2012; COVACIU-MARCOV et al., 2009a, b; ARDELEAN & KARACSONYI, 2005). From this perspective, Carei Plain is an area with numerous wetlands, extremely important from a zoogeographic and conservative point of view (KARACSONYI, 1987; FERENTI et al., 2012; COVACIU-MARCOV et al., 2009a). Remains of the natural wetland fauna have been recently mentioned near some canals from north-western Romania following their regularisation (e.g. SAS et al., 2008; COVACIU-MARCOV et al., 2007, 2008a, b). Such results were obtained by collecting terrestrial isopods through pitfall traps (FERENTI & DIMANCEA, 2012). However, qualitative studies with direct method were previously made in the wet areas of Carei Plain (FERENTI et al., 2012), quantitative data being recorded in only one wetland (TOMESCU et al., 2008). Thus, our study started from the hypothesis that the terrestrial isopod fauna from a wetland from Valea lui Mihai is similar to the one from the wet areas from Carei Plain. Considering that the remains of this fauna are found in the artificial canals from north-western Romania (FERENTI & DIMANCEA, 2012), we wanted to establish the composition of the terrestrial isopod fauna from a relatively natural wetland, through quantitative collections, in order to compare what and how many of it survives in the anthropogenic canals from the region. Regarding the herpetofauna, the wetland from Valea lui Mihai shelters mountainous elements at low altitudes (COVACIU-MARCOV et al., 2009b). Assuming that the climatic conditions from the past have had the same influence upon the vertebrate and invertebrate fauna, it is possible that those conditions will also be felt by the terrestrial isopods.

MATERIAL AND METHODS

The study was realised in 2009, in a wetland near Valea lui Mihai locality, from north-western Romania. The habitat is a typical wetland from north-western Romania, having permanent humidity, being formed on the sides of a low flowing stream. The natural vegetation generally consists of grassy vegetation, pewter, reed and willow in certain places. The wetland is mainly affected by the nearby anthropogenic areas (households and fields), representing a natural island between them. The samples were prevailed with pitfall traps, once a month, from April to September. We set four traps / month in a representative area, at 5 m distance from each other. However, this number of traps was not the one that we collected, because many were destroyed by animals or humans due to the close proximity of the town. Therefore, we collected only 16 traps, respectively about 2-3 traps/month. The aim of the study was not to observe the differences between the microhabitats within the wetland, but to make an inventory of the species from it and to establish certain quantitative parameters. The isopods were preserved in alcohol, and afterwards determined in the laboratory using the scientific literature (e.g. RADU, 1983, 1985). The following parameters were analysed: numeric abundance, relative abundance, species frequency of appearance, species richness and diversity (Shannon-Wiever index, SHANNON & WIEVER, 1949).

RESULTS

In the wetland from Valea lui Mihai we identified 9 terrestrial isopod species: *Hyloniscus riparius* (C. KOCH 1838), *H. transsilvanicus* (VERHOEFF 1901), *Haplophthalmus danicus* BUDDE-LUND 1880, *Cylisticus convexus* (DE GEER 1778), *Porcellium collicola* (VERHOEFF 1907), *Trachelipus arcuatus* (BUDDE-LUND 1885), *T. rathkii* (BRANDT 1833), *T. nodulosus* (C. KOCH 1838) and *Armadillidium vulgare* (LATREILLE 1804). From a whole of 674 individuals, 273 were males and 401 females. The males only belonged to seven species, while the females were identified from each of the nine species. *H. riparius*, *T. arcuatus* and *A. vulgare* dominated the isopod assemblage. The same species also had high frequency (Table 1).

The largest number of individuals was registered in April. From the first months of the study a gradual decrease regarding the species richness can be observed, reaching the minimum value in August, and recovering in September. Concerning the diversity, the maximum value was reached in July, while the minimum in August (Table 1).

The structure of the terrestrial isopod assemblages was different from one month to another. Although, *H. riparius* had the highest abundance during the whole study, it only held a majority in the first two months, period in which *T. arcuatus* and *A. vulgare* were also greatly active. *T. rathkii* dominated the next month, while *T. nodulosus* held a majority in July. The total number of individuals was very low in August, *H. riparius*, *T. rathkii* and *A. vulgare* being the dominant ones. The *T. arcuatus* population presented a more intense surface activity in the last month.

Table 1. Relative abundance of the individuals of each species, numeric abundance, species richness and Shannon-Wiever (H) diversity in each study month; numeric (A) and relative (A%) abundance and frequency of appearance (F) of the individuals of each species, and assemblage diversity (H) throughout the whole study period; (IV – April, V – May, VI – June, VII – July, VIII – August, IX – September).

	IV	V	VI	VII	VIII	IX	Total		
	A / month						A	A%	F
<i>H. riparius</i>	46.45	54.42	37.08	12.58	10.52	8.47	233	34.57	87.50
<i>H. transsilvanicus</i>	-	0.68	-	-	-	-	1	0.14	6.25
<i>H. danicus</i>	1.29	1.36	1.98	1.39	-	1.69	10	1.48	37.50
<i>C. convexus</i>	0.64	0.68	-	-	5.26	3.38	5	0.74	31.25
<i>P. collicola</i>	1.93	2.04	1.32	-	-	1.69	9	1.33	31.25
<i>T. arcuatus</i>	15.48	19.72	10.59	32.86	-	49.15	145	21.51	75.00
<i>T. rathkii</i>	-	2.04	43.70	-	57.89	-	80	11.86	18.75
<i>T. nodulosus</i>	-	-	-	34.26	-	5.08	52	7.71	12.50
<i>A. vulgare</i>	34.19	19.04	5.29	18.88	26.31	30.50	139	20.62	100
No. of individuals	155	147	151	143	19	59	674		
No. of species	6	8	6	5	4	7	9		
<i>H</i>	1.17	1.25	1.25	1.36	1.06	1.32	1.64		

DISCUSSIONS

Considering certain ecologic and zoogeographic characteristics of the nine identified terrestrial isopod species, these can be divided in three groups: 1. species the presence of which is determined by the actual features of the habitat; 2. species the presence of which can be due to the past of the area; 3. rare species in traps. The presence of the dominating species throughout the study was determined by two characteristics of the habitat: humidity and anthropogenic impact. Humidity determined the dominance of *H. riparius* and *T. rathkii*, these species being characteristic or frequent in the wet areas (e.g. RADU, 1983, 1985; WIJNHOFEN, 2000, see in: JASS & KLAUSMEIER, 2003). The high abundance of the synanthropic species *A. vulgare* (RADU, 1985) is a result of the presence of affected areas surrounding the wetland, some individuals probably migrating in the natural areas, situated at the limit of the anthropogenic areas.

H. transsilvanicus and *T. arcuatus* are present in the wetland due to its past. The first one is characteristic to the wet areas from higher altitudes (RADU, 1983; TOMESCU et al., 2011), while the second one is typical of the forested areas (RADU, 1985; TOMESCU et al., 2005). The wetlands from north-western Romania represent extremely important habitats for the preservation of the mountain species found in the plain (e.g. COVACIU-MARCOV et al., 2009a; FERENTI et al., 2012). The areas with cold and wet climate from the north-western part of Hungary also proved to be capable of sheltering *H. transsilvanicus* population in the Tisa flood plain (VILISICS & HORNUNG, 2010). *H. transsilvanicus* is also frequent in the wetlands from Carei Plain (FERENTI et al., 2012). Therefore, although the wetland from Valea lui Mihai is small and surrounded by anthropogenic areas, it still preserves a terrestrial isopod fauna similar to the one of other wetlands from the region, fact also observed for the herpetofauna (COVACIU-MARCOV et al., 2009b). The presence of these terrestrial isopod species probably denotes their ecologic plasticity during times.

Although *T. arcuatus* is considered a forest species (RADU, 1985), it has been exceptionally observed in opened areas, in favourable microhabitats (TOMESCU et al., 2000). However, although the previous situation was observed in a mountain area (TOMESCU et al., 2000), the phenomenon seems to be more common in north-western Romania (FERENTI & DIMANCEA, 2012). Thus, identifying *T. arcuatus* in the wetland from Valea lui Mihai can suggest that the presence of the species in the canal from Caraseu (FERENTI & DIMANCEA, 2012) does not necessarily represent

a consequence of the forest disappearing from the area, but of the disappearing of the wetland. However, it is possible that forests were found in the past in Valea lui Mihai exactly in, or near the wetland, forests that have disappeared following the anthropogenic pressure. An argument in this direction is the high number of species, in other cases the isopods being more numerous in forests than in opened areas (SPUNGIS, 2008). On the other hand, it seems that in the past forests were well represented in the area (ARDELEAN & KARACSONYI, 2005), being present in the neighbouring areas in Hungary, even in the last glacial maximum (WILLIS et al., 1995).

H. danicus is a rare presence in pitfall traps, because of its low mobility (TOMESCU et al., 2008). Previously, the species was identified in wet areas, both natural and artificial (RADU, 1983), thus the habitat from Valea lui Mihai being a favourable one. Although represented by low numbers, the permanent presence of the species, with the exception of the warmest month, suggests the fact that it is a characteristic element of this assemblage. *C. convexus* appears in low numbers, and its presence can be due to its synanthropic character (RADU, 1985).

One of the most important parameters that influences the surface activity of the terrestrial isopods is the temperature of the environment (see in: WARBURG et al., 1984; MA et al., 1991; KUZNETSOVA & GONGALSKY, 2012). This is also highlighted in the manner in which the number of individuals and species varies throughout the warm season. The more intense surface activity from spring – beginning of summer and beginning of autumn - is probably due to the moderate temperatures from these periods. It has been observed that in some cases the dynamics of the terrestrial isopod populations is affected especially by habitat changes and not by weather (DAVIS, 1984). Thus, the lowest species and individuals diversity from August can be a consequence of the decrease of the isopods surface activity because of the modifications produced in the habitat by excessive heat.

Despite the fact that the number of terrestrial isopod species collected using pitfall traps is small (e.g. RADU & TOMESCU, 1976; TOMESCU et al., 2000), the number of species collected from Valea lui Mihai is high, considering the low number of traps. This probably indicates the existence of a high biodiversity in the wetland. Although the wetland from Valea lui Mihai is surrounded by anthropogenically affected areas, it represents one of the few relatively natural islands from the region, fact that probably determined the concentration of the existing species on a limited surface.

The structure of the terrestrial isopod assemblages from the wetland from Valea lui Mihai is similar to the one from the wet natural areas from Carei Plain (FERENTI et al., 2012). This fact was expectable, because these wetlands were connected with each other in the past, even if they are presently separated following anthropogenic activities. However, in comparison to the isopod fauna from near the recently studied anthropogenic canal (FERENTI & DIMANCEA, 2012), in the wetland from Valea lui Mihai, the species assemblages is much more diversified, the number of species being greater. This underlines the fact that natural wetlands are more favourable for the terrestrial isopod fauna. Although the artificial canals can shelter a part of the original wetland fauna, they are limited to a small part of the terrestrial isopods. This fact proves the distinct importance of natural wetlands for the terrestrial isopod fauna from north-western Romania, and meanwhile represents a new argument for the preservation of these habitats.

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STRUCTURAL CHARACTERISTICS OF SOIL MITE POPULATIONS (ACARI- MESOSTIGMATA) FROM THE OAK-HORNBEAM FORESTS FROM SOUTHERN ROMANIA

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Abstract. The paper present the structural modifications of soil mite populations from four forest ecosystems, characterized by the two vegetal associations (*Quercus petraeae-Carpinetum* SOO et POCS 1957 and *Q. robori-Carpinetum* BORZA 1937), taking into account the distribution of these invertebrates on soil layers (litter-fermentation, humus and soil). In total, 35 species of Mesostigmata were identified, three species being common: *Pachyseius humeralis* (BERLESE 1910), *Prozercon traegardhi* (HALBERT 1923) and *Feigaia nemorensis* (C. L. KOCH 1836). The analysis of the dominance and constancy indices showed that the eudominant-dominant and euconstant-constant species represented 15.79 %-30 % from the total number of mites. Based on the Jaccard index, the similarities and differences between mite populations from four investigated forest and from all three soil layers were identified.

Keywords: mite, dominance, constancy, similarity, soil.

Rezumat. Caracteristicile structurale ale populațiilor de acarieni de sol (Acari-Mesostigmata) din stejăreto-cărpinele din sudul României. Lucrarea prezintă modificările structurale ale populațiilor de acarieni de sol din patru ecosisteme forestiere, caracterizate de două tipuri de asociații vegetale (*Quercus petraeae-Carpinetum* SOO et POCS 1957 și *Q. robori-Carpinetum* BORZA 1937), ținând cont de distribuția acestor nevertebrate pe straturi de sol (litieră-fermentație, humus și sol). În total, 35 mesostigmatide au fost identificate, trei specii fiind comune: *Pachyseius humeralis* (BERLESE 1910), *Prozercon traegardhi* (HALBERT 1923) și *Feigaia nemorensis* (C. L. KOCH 1836). Analiza indicilor de dominanță și constanță a arătat că speciile eudominate-dominate și cele euconstante-constante reprezintă 15.79 % - 30 % din numărul total de acarieni. Bazându-ne pe indicele Jaccard, au fost identificate similaritățile și diferențele dintre populațiile de acarieni din cele patru păduri studiate, dar și din straturile de sol.

Cuvinte cheie: acarian, dominanță, constanță, similaritate, sol.

INTRODUCTION

The edaphic Mesostigmata mites are considered bioindicators for soil quality, especially in forest ecosystems, where they find proper environmental conditions for their development. This characteristic was offered after numerous studies, which demonstrated that any anthropogenic impact determines modifications of the qualitative and quantitative parameters. Being, in their majority predator species, Mesostigmata communities are influenced primarily by the availability of the trophic source and then by the abiotic factors (WALTER & PROCTOR, 2009; SALMANE & BRUMELIS, 2010; BEDANO & RUF, 2010; PEVERIERI et al., 2011). Analysing their ecological requirements it was observed that the mite population structure and dynamics are correlated with biotic and abiotic factors from investigated ecosystems. If we take into consideration one of the biocoenosis components (edaphic invertebrates fauna), the ecological characterization of soil from forest ecosystems can be done with the aid of mites (COJA & BRUCKNER, 2006; GULVIK, 2007; MANOLE et al., 2011).

Studies concerning the soil fauna from deciduous forest ecosystems were also conducted in Romania (Moldova, Romanian Plain, Doftana and Prahova valleys), highlighting the high biodiversity and the characteristic structure of invertebrate populations for each type of investigated areas (CĂLUGĂR & HUȚU, 1999; CIORNET et al., 2003; FALCĂ et al., 2003; HONCIUC & STĂNESCU, 2004; STĂNESCU & HONCIUC, 2004; SANDA et al., 2006a, b; OROMULU-VASILIU et al., 2007-2008; CĂLUGĂR, 2009). However, studies on the structural characteristics of edaphic mite communities from these types of forests (oak and hornbeam) are very few.

If we refer to Europe, ecological studies on the Mesostigmata populations from oak and hornbeam forest have been made in France, Austria, Spain, Slovakia, Poland and Italy (SADAKA & PONGE, 2003; SKORUPSKI, 2003; FENDA & CICEKOVA, 2005; MORANZA, 2006; GWIAZDOWICZ, 2007; NIEPARALA & BLOSZYK, 2009; MADEJ et al., 2011; PEVERIERI et al., 2011).

The aim of the present study is to investigate for the first time the structural characteristics of the Mesostigmata soil populations from four oak-hornbeam forests located in southern Romania, highlighting the similarities and differences between them. The originality of this work is the study of population structures taking into account the distribution of these invertebrates on soil layers (litter-fermentation, humus and soil).

MATERIAL AND METHODS

Study area

The study was made in 2005 in four deciduous forests, characterized by two vegetal associations: *Quercus petraeae-Carpinetum* SOO et POCS 1957 (QpC), described in ecosystems from Cobia (QpC1), Sărata Monteoru (QpC2)

and *Q. robori-Carpinetum* BORZA 1937 (QrC), identified in areas from Călugăreni (QrC1) and Căscioarele (QrC2). All are natural forests from the south part of Romania (Fig. 1).

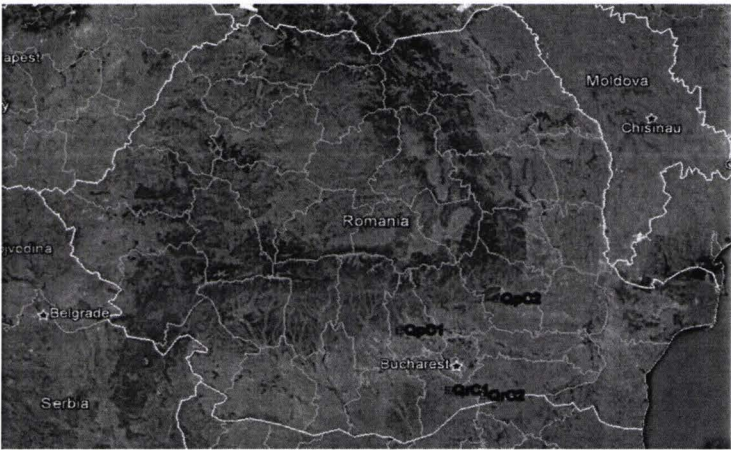


Figure 1. Geographical position of the investigated forest ecosystems {Cobia (QpC1), Sărata Monteoru (QpC2), Călugăreni (QrC1) and Căscioarele (QrC2)} (original).

The geomorphologic characteristics and description of vegetation from the investigated forests are presented below (Table 1) (SANDA et al., 2006a, b).

Table 1. The geomorphological characteristics and description of the vegetation from the investigated forests.

Characteristics	Cobia (QpC1)	Sărata Monteoru (QpC2)	Călugăreni (QrC1)	Căscioarele (QrC2)
Geographical coordinates	N: 44°51'00.15" E: 25°21'17.80"	N: 45°24'35.11" E: 25°25'20.07"	N: 45°44'46.84" E: 25°27'22.45"	N: 44°07'41.35" E: 26°27'32.46"
Altitude	447 m	516 m	152 m	127m
Vegetal association	<i>Quercus petraeae-Carpinetum</i> SOO et POCs 1957	<i>Quercus petraeae-Carpinetum</i> SOO et POCs 1957	<i>Quercus robori-Carpinetum</i> BORZA 1937	<i>Quercus robori-Carpinetum</i> BORZA 1937
Forest consistency	0.8-0.9	0.8-0.9	0.7-0.8	0.7-0.8
Coverage	75-80%	80-85%	80%	75%
Type of forest	Young forest (35 years) regenerated from offshoots	Mature forest, over 100 years	Mature forest, over 100 years	Mature forest, over 100 years
Dominant tree species	<i>Quercus petraea</i> , <i>Q. frainetto</i>	<i>Quercus petraea</i> , <i>Carpinus betulus</i> , <i>Q. frainetto</i>	<i>Quercus robur</i> , <i>Tilia tomentosa</i> , <i>C. betulus</i>	<i>Quercus robur</i> , <i>Q. cerris</i>
Dominant shrub species		<i>Cornus sanguinea</i> , <i>Carpinus betulus</i>		<i>Ligustrum vulgare</i> , <i>Cornus mas</i>
Dominant herbaceous species	<i>Carex pilosa</i> , <i>Lathyrus vernus</i> , <i>C. sylvatica</i>	<i>Melittis melissophyllum</i> , <i>Tamus communis</i> , <i>Lychnis coronaria</i>	<i>Allium ursinum</i> , <i>Lamium galeobdolon</i> , <i>Alliaria petiolata</i>	<i>Ranunculus ficaria</i> , <i>Lamium galeobdolon</i> , <i>Alliaria petiolata</i>
Exposure	S	S	N- NE	N
Slope	15°	30°	10°-15°	10°-15°
Soil	Brown forest soil, medium podzolic	Brown forest soil, medium podzolic	Alluvial soils	Alluvial soils

Soil samples

From each ecosystem 15 samples of soil were collected with MacFadyen corer (5 cm diameter), up to depth of 10 cm. Each soil samples was divided in three subunits, taking into account the soil layers: litter-fermentation (L), humus (S1) and soil (S2). The soil samples were taken three times in one year: in May, July and September. The sampling periods were the same for all ecosystems. The extraction was performed with a modified Berlese-Tullgren extractor, in ethylic alcohol and the mites samples were clarified in lactic acid. The identification of the mites from the Mesostigmata order was made up to the species level, using the latest taxonomic literature. In total, 60 soil samples were analysed, divided in 180 subunits, with 35 species and 136 individuals.

Statistical analysis

After taxonomical identification, the numerical abundance (number of individuals) was the base for the quantification of some structural index as: numerical density (x/sq.m.); dominance (D%); constancy (C%) and Jaccard similarity index (q). The numerical density (x/sq.m.) was calculated using the following formula:

$$x/sq.m = (\sum \text{no. of individuals/no. of samples}) \times 1m^2/\text{surface of the soil core}$$

Where: 1 m²= 10000 cm²; surface of the soil core = 10 cm² (BOTNARIUC & VĂDINEANU, 1982).

The analysis of the results was conducted with the aid of the BioDiversityPro 2.0 program (MCALEEE et al., 1997). Using this program the index Jaccard (q) for mite populations from the four studied forests was calculated:

$$q = c / a+b-c .$$

In which: a = number of species from ecosystem A; b = number of species from ecosystem B; c = number of common species from ecosystems A and B.

The dominance was calculated using the formula:

$$D=100*n/N$$

Where: n-number of individuals of one species from one sample; N-total number of individuals of all species from one sample.

The dominance classes for the identified gamasid mites were: eudominant species with dominance over 10% (D5); dominant species with dominance between 5.1 and 10% (D4); subdominant species with dominance between 2.1 and 5% (D3); recedent species with dominance between 1.1 and 2% (D2) and subrecedent species with dominance under 1.1% (D1) (ENGELMANN, 1978).

The constancy was obtained using the formula:

$$C=100*pA/P$$

Where: pA-number of samples with species A, P-total number of samples.

The mite species were classified in four constancy classes: euconstant species having constancy of 75.1-100% (C4), constant species having constancy of 50.1-75% (C3), accessory species having constancy of 25.1-50% (C2) and accidental species having constancy of 1-25% (C1) (SELVIN & VACCA, 2004).

RESULTS AND DISCUSSIONS

The taxonomical structure of mite communities revealed the presence of 34 species in all investigated forests, with a numerical density of 27.200 ind./sq.m. These species were included in 19 genera and 10 families (Ascidae, Laelapidae, Macrochelidae, Parasitidae, Pachylaelapidae, Rhodacaridae, Trachytidae, Uropodidae, Veigaidae and Zerconidae). The most increased species diversity was recorded in QrC2, followed by the QpC1 and QrC1. The lowest number of species was obtained in QpC2 (Table 2).

Taking account of the soil layers, in litter-fermentation the number of species and the numerical density were the highest, the medium values being recorded in humus and the lowest values in soil layer. The differences between the investigated parameters from the four forests were not so significant, with one exception, the mite communities from QpC2. Due to the more increased slope and to the southern exposure from this area, the litter-fermentation layer, which was thinner and dryer, in comparison with the other forests, does not offer such proper conditions for the development of Mesostigmata, being recorded almost a half from the numerical density obtained in QpC1, QrC1 and QrC2 (Table 2).

Table 2. Some population parameters of Mesostigmata communities from the investigated forests and soil layers.

Population parameters	Cobia (QpC1)	Sărata Monteoru (QpC2)	Călugăreni (QrC1)	Căscioarele (QrC2)	Litter-fermentation (L)	Humus (S1)	Soil (S2)
No. of species	17	13	17	19	26	19	12
No. of ind./sq.m.	7400	3600	6800	9400	15200	8000	4000
No. of eudominant/dominant species	4/3	3/9	2/5	1/3	1/7	2/2	2/1
No. of euconstant/constant species	0/4	0/0	0/4	2/0	3/5	2/1	1/0

These data were similar with those obtained by other specialists from Romania and Europe, where in deciduous forest, characterized by *Quercus* sp. and *Carpinus* sp. there were identified between 15 species and 54 species, the taxonomical spectrum being similar with that from the Romanian forests (CĂLUGĂR & HUȚU, 1999; SADAKA & PONGE, 2003; SKORUPSKI, 2003; FENDA & CICEKOVA, 2005; MORANZA, 2006; MADEJ et al., 2011; PEVERIERI et al., 2011).

Pachyseius humeralis BERLESE 1910 was a common species for all investigated forests and *Prozercon traegardhi* (HALBERT 1923) for all soil layers. *Veigaia nemorensis* (C. L. KOCH 1836) was identified in all studied forests and in all soil layers, as well. These species have a wide ecological plasticity, being predators, in generally. These characteristics allowed them to occur in various microhabitats, being distributed from lowlands (100 m a.s.l) up to mountain areas (1400 m a.s.l) (MASAN & FENDA , 2004; SALMANE INETA & BRUMELIS, 2010; MANU, 2012)

If we take into consideration the dominance, in QpC1 23.53 % from the total number of species are eudominant (*Hypoaspis aculeifer* (G. CANESTRINI 1884), *Prozercon kochi* SELLNICK 1943, *Prozercon traegardhi* and *Veigaia nemorensis*); 23.53% are dominant and 52.94% are subdominant. *Pergamasus barbarus* (BERLESE 1905) and the eudominant species were classified as euconstant as well, the rest of 76.47% being accidental. In QpC2. only

30.77% are eudominant species (*Leptogamasus tectegynellus* (ATHIAS-HENRIOT 1967), *Pachylaelaps furcifer* OUDEMANS 1902, *Pseudolaelaps doderoi* (BERLESE 1910) and *Prozercon fimbriatus* (C. L. KOCH 1839)), 53.85 % are dominant and 15.38% are subdominant. The species *Leptogamasus tectegynellus* and *P. doderoi* accessory, the rest of them being accidental (84.62%) (Table 3).

In QrC1, *Pseudolaelaps doderoi* (BERLESE 1910) and *Trachytes aegrota* (C. L. KOCH 1841) are eudominant, 29.11% from the total number of species being dominant and the rest (58.82% subdominant). If we analyse the constancy, only 17.65% are constant species, 11.76% accessory and 70.69% accidental. In QrC2, *Hypoaspis aculeifer*, *Pachyseius humeralis* and *Veigaia nemorensis*, represented 15.79% as eudominant species and euconstant as well, 5.26% being dominant and 78.95% subdominant. 26.32% were accessory and 63.16% accidental species (Table 3).

The analysis of the dominance and constancy indices revealed the absence of the recedent-subrecedent species and a high number of accessory and accidental ones. This fact demonstrates the high mobility of these edaphic mites, being capable to looking after food from different microhabitats from forest ecosystems.

Table 3. The dominance (D%) and constancy (C%) of the investigated Mesostigmata populations.

Species	Cobia (QpC1)			Sărata Monteoru (QpC2)			Călugăreni (QrC1)			Căscioarele (QrC2)		
	Soil layer	D%	C%	Soil layer	D%	C%	Soil layer	D%	C%	Soil layer	D%	C%
Family Parasitidae												
<i>Leptogamasus parvulus</i> (BERLESE 1903)	L, S1	5.41	40									
<i>Lysigamasus neoruncatellus</i> SCHWEIZER 1961		2.70	20							L, S1	4.26	20
<i>Lysigamasus</i> sp.	S2	2.70	20									
<i>Leptogamasus tectegynellus</i> (ATHIAS-HENRIOT 1967)				L	11.11	40				L	4.26	40
<i>Leptogamasus parvulus</i> (BERLESE 1903)	L, S1	5.41	40									
<i>Pergamasus barbarus</i> (BERLESE 1905)	L	8.11	60				L	2.94	20			
<i>Pergamasus laetus</i> JUVARA-BALȘ 1970	L	2.70	20				L	8.82	40	L	2.13	20
<i>Pergamasus quisquiliarum</i> (CANESTRINI & CANESTRINI 1882)				S2	5.56	20						
<i>Vulgarogamasus kraepelini</i> BERLESE 1905				L	5.56	20	S1	5.88	20	L	2.13	20
Family Veigaidae												
<i>Veigaia cervus</i> (KRAMER 1876)				L	5.56	20						
<i>Veigaia exigua</i> (BERLESE 1916)	L	2.70	20	L	5.56	20	S1	2.94	20			
<i>Veigaia kochi</i> (TRĂGĂRDH 1901)	L	2.70	20									
<i>Veigaia nemorensis</i> (C. L. KOCH 1836)	L, S1	10.81	60	S2	5.56	20	L, S1	8.82	60	L, S1, S2	21.28	80
<i>Veigaia transisalae</i> (OUDEMANS 1902)										S1	4.26	40
Family Rhodacaridae												
<i>Dendrolaelaps</i> sp.										L	4.26	20
<i>Rhodacarus denticulatus</i> BERLESE 1921							S2	2.94	20			
Family Ascidae												
<i>Asca aphidoides</i> (LINNEAUS 1758)										L	2.13	20
<i>Proctolaelaps pomorum</i> (OUDEMANS 1929)							S1	5.88	20			
Family Macrochelidae												
<i>Macrocheles montanus</i> (C. L. KOCH 1839)							S1	2.94	20	S1	2.13	20
<i>Neopodocimum mrciaki</i> SELLNICK 1968				S2	5.56	20						
<i>Geholaspis longispinosus</i> (KRAMER 1876)	S1	2.70	20				S1	2.94	20			
Family Laelapidae												
<i>Hypoaspis aculeifer</i> (G. CANESTRINI 1884)	L, S1	13.51	60							L, S2	10.64	40
<i>Hypoaspis oblonga</i> (HALBERT 1915)							L	2.94	20			
<i>Pseudolaelaps doderoi</i> (BERLESE 1910)				L, S1	11.11	40	L	11.76	60	L	4.26	40
Family Pachylaelapidae												
<i>Olopachys suecicus</i> SELLNICK 1950				L	5.56	20	L	2.94	20			
<i>Pachylaelaps dubius</i> HIRSCHMANN & KRAUSS 1965	S2	2.70	20									

<i>Pachylaelaps furcifer</i> OUDEMANS 1902				L, S2	11.11	20	L	2.94	20	S1	2.13	20
<i>Pachylaelaps imitans</i> BERLESE 1920							L	2.94	20			
<i>Pachyseius humeralis</i> BERLESE 1910	S1	2.70	20	L	5.56	20	L	8.82	60	L, S1, S2	14.89	80
Family Zerconidae												
<i>Prozercon fimbriatus</i> (C. L. KOCH 1839)	L, S1	8.11	20	L	16.67	20				L, S2	4.26	40
<i>Prozercon kochi</i> SELLNICK 1943	L	10.81	20							S2	2.13	20
<i>Prozercon traegardhi</i> (HALBERT 1923)	L, S1, S2	10.81	60	L	5.56	20						
<i>Zercon fageticola</i> HALASKOVA 1969										L	4.26	20
<i>Zercon peltatus</i> C. L. KOCH 1836	L	2.70	20							S1	2.13	20
Family Trachytidae												
<i>Trachytes aegrota</i> (C. L. KOCH 1841)	L	8.11	40				L, S1	20.59	60	L	6.38	40
Family Uropodidae												
<i>Uropoda</i> sp.							S1	2.94	20	L	2.13	20

Analysing the Jaccard index, we observed the highest similarity for Mesostigmata communities from QrC1, QrC2 and QpC1 ($q_{QrC1-QrC2} = 33.33\%$; $q_{QrC2-QpC1} = 33.33\%$ and $q_{QrC1-QpC1} = 25.92\%$). The number of common species between the forest of QpC2 ecosystem and the other three forests was low ($q_{QpC1-QpC2} = 25\%$; $q_{QpC1-QrC2} = 28\%$). These results reflected the similarities between environment conditions from QrC1 and QrC2 forests, which are characterized by the same vegetation and abiotic factors (exposure, soil, slope) and by small distance from one to another (about 40 km). On the other hand, some habitat differences from QpC2 forest (high slope by 30°, south exposure) influence the mite community structures (Fig. 2A).

If we take into consideration the mite distribution on soil layers, for populations from forests characterized by *Quercus petraeae-Carpinetum* (QpC) and *Q. robori-Carpinetum* (QrC), there were recorded the highest similarities between litter-fermentation layers ($q_{LQpC-LQrC} = 44.44\%$) and humus-soil ($q_{S1QpC-S2QrC} = 40\%$). The lowest values of the Jaccard index were obtained between mite communities from soil layers ($q_{S2QrC-S2QpC} = 8.33\%$) and litter-fermentation-soil ($q_{LQrC-S2QpC} = 8\%$) (Fig. 2B).

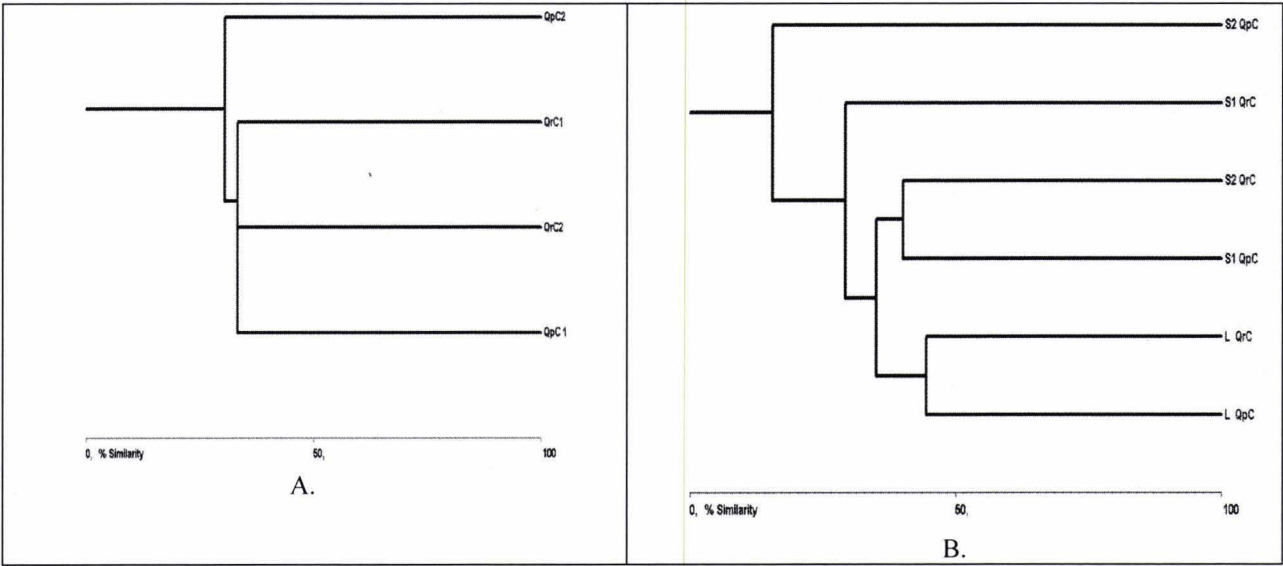


Figure 2 A, B. Dendrogram of Jaccard similarity index between Mesostigmata populations from: A- Cobia (QpC1), Sărata Monteoru (QpC2), Călugăreni (QrC1) and Căscioarele (QrC2) (A) and between Mesostigmata populations from soil layers: B – litter - fermentation (L), humus (S1) and soil (S2).

Many ecological studies revealed that the litter-fermentation layer is the most favourable habitat for Mesostigmata species. Due to the increased humidity and organic matter content, which is the trophic source for other soil invertebrates, the edaphic mites find proper conditions for their development (KOEHLER, 1999; MANU, 2012). This fact was highlighted by the high similarities obtained between mite populations from investigated forest, from the first soil layer.

We assumed that the alluvial soil (which is reach in organic matter) and northern exposure (which determines a high soil humidity) of *Quercus robori-Carpinetum* forest created similar habitats for mites on S2 level as those from humus layer S1 (mull or mull-like moder) from *Q. petraeae-Carpinetum*, which have a high level of organic matter especially close to the

surface (CHIRIȚĂ, 1974). Some differences in soil mite distribution were recorded between soil layers L, S1 and S2 from each investigated ecosystems. Even the type of vegetation influences the abundance and species diversity of the mite fauna (KOEHLER, 1999; CĂLUGĂR & HUȚU, 1999; FALCĂ et al., 2003; MANOLE et al., 2011; MADEJ et al., 2011).

CONCLUSIONS

Ecological investigations on the structure of Mesostigmata from four oak-hornbeam forest ecosystems from the south part of Romania revealed the presence of 34 species, with a numerical density by 27.200 ind./sq.m. The species number and numerical density had recorded closed values between forests, with one exception: Sărata Monteoru area (QpC2). The increased slope (which determines instability of the soil layers) and southern exposure of the forest (which causes dryness) from this forest, influence the abundance of Mesostigmata mites.

Due to their wide ecological plasticity, some common species were identified: *Pachyseius humeralis* found in all investigated forests and *Prozercon traegardhi* in all soil layers. *Veigaia nemorensis* was identified in all studied forests and in all soil layers, as well. The analysis of the dominance and constancy indices showed on one hand that the eudominant-dominant and euconstant-constant species represented 15.79 % - 30% from the total number of mites. On the other hand, these parameters revealed the absence of the recedent-subrecedent species and the presence of a high number of accessory and accidental ones, which proves the high mobility of these edaphic mites.

Significant similarities between mite communities from Călugăreni (QrC1) and QrC2 (Căscioarele) forests and between mites from soil layers are due to the same vegetation and abiotic factors (especially soil type and exposure). Unfavourable environment conditions (high slope, south exposure) are reflected by the decreased values of Jaccard index obtained between communities from Sărata Monteoru (QpC2), in comparison with the other two forests mentioned above.

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ECOTOURISM. PRESENT AND FUTURE

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Abstract. Mass tourism practiced during the last half of the century has generated a spectacular economic growth, tourism industry presently representing the first *industry* worldwide. However, this activity has also triggered negative impact upon the most visited tourist attractions. In response to these negative actions, there emerged other forms of tourism with less impact on tourist sights. Among these forms of tourism, starting with 1983 there appeared ecotourism. This represents a form of travel that respects and investigates attractions and state of local people within their natural areas through eco-volunteering.

Keywords: tourism, ecotourism, eco-volunteering, permafrost.

Rezumat. Ecoturismul. Prezent și viitor. Turismul de masă practicat în ultima jumătate de secol a generat o creștere economică spectaculoasă, activitatea turistică reprezentând în prezent prima *industrie* a planetei. Dar, această activitate a determinat și impacte negative asupra obiectivelor turistice vizitate. Ca reacție la acțiunile negative sesizate au apărut în timp și alte forme de turism cu mai puțin impact asupra obiectivelor turistice. Dintre aceste forme de turism s-a evidențiat, începând din anul 1983, ecoturismul. Acesta reprezintă o formă de călătorie care respectă și cercetează în spațiile naturale, obiectivele vizitate și starea populației locale, prin ecovoluntariat.

Cuvinte cheie: turism, ecoturism, ecovoluntariat, permafrost.

Tourism, as a form of travel of a person aims at relaxing, knowing and admiring natural and man-made attractions: mountain summits, caves, waterfalls, natural or anthropogenic landscapes, rare plants and animals, ancient and medieval remains, churches and monasteries, monuments of architecture and art, museums, memorial houses, farmhouses, economic, technical objectives, etc. (FREIDEL, 1980; NICULESCU, 1997; STĂNESCU, 1993; VOICA, 2002).

Globally, tourists' number exceeded 1.6 billion persons in 1997, and, it is expected to reach over 5 billion in 2017. Presently, tourism represents the first *industry* of the planet as more than 200 million men and women work in this field. According to the World Tourism Organization (WTO), tourism activity represents the first source of dividends in more than one third of the countries; it is among the first five categories of exports in 83% of the countries, generating between 3 and 5% of the world GDP.

In case of less developed countries, international tourism may confer considerable economic benefits. However, mass tourism practiced between 1960 and 1980 triggered negative impacts upon the environment (waste, pollution, landscape deterioration, etc.) (HALIOUA & CABOUT, 2001; PINCE, 2007). Parallel to the growth of tourism it has developed clandestine trade with various rare animals or their products (skins, shells of molluscs, ivory, rhino horn, feathers, stuffed animals, etc.). These challenges have jeopardized the survival of many rare species (SEAGER, 1993; NEACȘU & NEACȘU, 1994).

As a reaction to the excesses of mass tourism there have lately emerged new and less harmful forms of tourism, which take into account the conservation and protection of visited tourist attractions. Thus, in 1995, it appeared sustainable tourism, which, according to its Charter, has to be ecologically bearable in long time, economically viable, ethically and socially equitable for the local populations (PINCE, 2007).

Responsible tourism aims at respecting the development of ecological and social practices. In 1999, the World Tourism Organization elaborated a global code of ethics for tourism (PINCE, 2007).

Solitary tourism is based on the principle of solidarity both in the organizational system and in the size and structure of the market or exchange and investment instruments (PINCE, 2007).

Rural tourism represents the tourism in rural areas, consisting of three main independent elements:

- attraction to natural beauty, charm and specific events of country life;
- accommodation and meals, even if they are not at hotel standards, have to be of high quality and offered with hospitality;
- transport, access roads to rural areas is vital to ensure a continuous flow of tourists.

For a good collaboration between countries with tradition in rural tourism, in 1990, it was created the European Federation for Rural Tourism, in Strasbourg, which brought together 24 rural tourism associations from 18 European countries.

In Romania, the first rural tourism activities occurred in the early 1967 – 1968 for tourist groups visiting the Romanian coast of the Black Sea and the Danube Delta. In 1982, according to an Order of the Ministry of Tourism, the Research Centre for the Promotion of International Tourism, identified and selected the representative villages on the territory of the Romania. This study established that 118 rural settlements can be introduced in domestic and international tourism. Starting with 1973, by the Order 774 of the Ministry of Tourism, 13 rural settlements were declared experimental tourist attractions: Lerești, Rucăr (Argeș County), Fundata (Ialomița County), Șirnea, Sibiel, Rășinari (Sibiu County), Tismana (Gorj County), Vaideeni (Vâlcea County), Halmagiu (Hunedoara County), Bogdan Vodă (Maramureș County), Vatra Moldoviței (Suceava County), Murighiol and Sfântul Gheorghe (Tulcea County) (CIOBOIU, 2005; VOICA, 2000).

Ecotourism was defined in 1983 by the Mexican architect and urbanist HC Lascurian as a form of tourism that consists in visiting relatively intact or less disturbed areas, for research purposes, admiring the scenery, flora and fauna, and cultural past and present aspects - inherent in a given place.

In 1991, the International Society of Tourism formulated a new definition stating that: *Ecotourism is the responsible travel form in natural areas that contributes to environmental protection and welfare of the local population* (PINCE, 2007). This form of tourism involves individual trips or those trips organized in small groups. Small-scale activity promotes the observation of visited attractions, as well as the study and understanding of the environment and its inhabitants; the educational dimension is of great importance.

Ecotourism favourably influences on the development of local communities and the environment; it generates local resources, part of which is devoted to the management and protection of natural habitats and species. The traveller has the opportunity to know protected nature, preferably in the hotspots of the world biodiversity – mountains, deserts, plains, forests, etc. Ecotourists, traveling in small groups, strive to leave no other footprint behind them but their steps (PINCE, 2007).

Currently, ecotourists represent about 5% of all tourists in the world and the number of protected areas that can be visited is estimated at 30.300, totalizing a surface of 13.2 million sq km or 10% of the world land area.

In Romania, there are 155 parks and scientific reserves that can be visited by ecotourists (NEACȘU & APOSTOLACHE, 1982).

WTO defined a series of indicators that allow the estimation of the costs and environmental benefits of tourism:

- The indicator of maximum number of persons that may visit a tourist attraction;
- The indicator of the disturbance of the tourist attraction that allows us to assess the level of environmental impact, according to its natural and ecological peculiarities;
- The indicator of ecological perturbation meant to estimate the natural ecological disturbances of the visited tourist objective according to tourist attractiveness, evolution and frequency.

The application of these indicators provides a correct ecological surveillance of the visited landmarks (PINCE, 2007).

Eco-volunteering and protection of the world patrimony. Ecotourism can influence the growth and mass protection of tourist attractions, due to the fact that the participants in this type of tourism are attached to what they visit and many of them even participate in the restoration and their protection. This action resulted in eco-volunteering, namely many tourists participate voluntarily and without payment, along with specialists, in the restoration and maintenance of the visited objectives. The Italian NGO TETHYS whose objective is the protection of the marine environment, involved thousands of volunteers in its campaigns of study and protection of dolphins and whales. Eco-volunteers in this action have gained a better understanding of the environmental issues and an awareness of the studied animals.

Coral protection. The Great Barrier Reef from Australia is the largest marine protected area. This area of global importance allowed eco-volunteers to participate in coral research, working with researchers from EARTHWACH ecological organization. The mission of this organization was to dive for sampling and identification of those corals, which have a fluorescent protein with applications in medicine and in the survival of threatened coral communities.

Protection of Przewalski horse. Przewalski horse was found in its natural environment in Mongolia in 1970. The first specimens bred in captivity by eco-volunteers and specialists from the Netherlands and Germany were transported in Mongolia in 1992. In France, Przewalski Horse Association, created in 1990, was concerned with the breeding and release of this animal into nature. Ten years later, in the years 2004 – 2005, 22 specimens were taken in Mongolia. And in this action, the role of eco-volunteers was obvious.

Setting up Jatun Sacha Reserve. To promote research and education in environment and ecology, the state Ecuador in South America, founded Jatun Sacha Foundation. This foundation created in NAPO province Jatun Sacha Reserve (Great Forest), with an area of 2,500 hectares. In the reserve, the foundation allowed eco-volunteers to participate in research programs on nature.

In 2005, the foundation received 800 Ecuadorian and five foreign eco-volunteers who freely participated in the research projects initiated by the reserve administration. Eco-volunteers performed numerous works in the forest: tree planting, experimenting with some new species, collecting seeds, etc. Thus, in the greenhouse and garden of the foundation, there harmoniously combined the work of scientists and eco-volunteers.

Research of the Arctic coast. Along the Arctic coast of Canada, there is a strip of bogs, currently under intense scrutiny by specialists due to global warming. These bogs contain up to 20% terrestrial carbon contained in permafrost (frozen ground all year). Due to global warming, the soil will thaw and release large amounts of CO₂ and methane gas into the atmosphere. New environmental changes may lead to the emergence and development of plants that can contribute to carbon fixation. University of Alberta in Canada supports by eco-volunteering the collection of scientific data needed to predict environmental reaction to global warming.

Eco-volunteering actions are taken in other tourist spots in the world and aims at: protection of turtles in Madagascar and Senegal, protection of orangutans in Borneo island, regeneration of forests in Vietnam, opening sites of history and architecture for the protection of the medieval heritage in Belgium and France; discovery and preservation of aboriginal culture and history of Australia, development and preservation of cultural centres and national parks in India, etc. (PINCE, 2007).

CONCLUSIONS

Presently, there are many forms of tourism: sustainable, responsible, solitary, rural, ecological, etc.

Among these forms, the first position is held by eco-tourism, which, among other objectives, aims at the volunteer collaboration between tourists and experts in different fields of activity, such as protection, restoration and prosperity of visited tourist attractions.

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AQUAPORINOLOGY (THE STUDY OF WATER CHANNEL PROTEINS - AQUAPORINS AND RELATIVES) AS A NEW DOMAIN OF NATURAL SCIENCES

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Abstract. A new domain of natural sciences, for which I propose the scientific term “aquaporinology”, began with the discovery in 1985 by BENGA group in Cluj-Napoca, Romania, of the first water channel protein from the human red blood cell membrane. This protein was re-discovered in 1992 by AGRE group in Baltimore, USA, and was called aquaporin 1 (AQP1) in 1993. In subsequent years, hundreds of water channel proteins have been discovered in unicellular organisms (archaea, bacteria, yeasts, and protozoa) and multicellular organisms (plants, animals, and humans). In addition to aquaporins, other classes of water channel proteins (that I called “relatives of aquaporins”) have been discovered: aquaglyceroporins, S-aquaporins, etc. The study of water channel proteins became a new domain of natural sciences and a very hot field of research with a lot of theoretical and practical issues.

Keywords: Aquaporinology, aquaporins, water channel proteins, natural sciences, new scientific domain.

Rezumat. Aquaporinologia (studiul proteinelor canal pentru apă - aquaporine și rudele lor) ca un domeniu nou al științelor naturii. Un domeniu nou al științelor naturii, pentru care propun termenul științific de „aquaporinologie”, a început cu descoperirea în 1985 de către grupul BENGA la Cluj-Napoca, România, a primei proteine canal pentru apă din membrana celulei roșii umane. Această proteină a fost re-descoperită în 1992 de către grupul AGRE la Baltimore, USA, fiind numită în 1993 aquaporina 1 (AQP1). În anii următori s-au descoperit sute de proteine canal pentru apă în organisme unicelulare (arheea, bacterii, drojdii, protozoare) și organisme multicelulare (plante, animale, oameni). Pe lângă aquaporine s-au descoperit alte clase de proteine canal pentru apă (pe care le-am numit “rude ale aquaporinelor”: aquagliceroporinele, S-aquaporinele). Studiul proteinelor canal pentru apă a devenit un domeniu nou al științelor naturii și un foarte fierbinte domeniu de cercetare cu o mulțime de aspecte teoretice și practice.

Cuvinte cheie: aquaporinologie, aquaporine, proteine canal pentru apă, științele naturii, nou domeniu științific.

The discovery of the water channel proteins (WCPs) is considered by many scientists as being of utmost importance. As formulated by WOLBURG et al., (2011): “The detection of water-specific membrane channels in red blood cells belongs to the fundamental discoveries in biology of the twentieth century (BENGA et al., 1986a, b; DENKER et al., 1988; PRESTON et al., 1992).” The first WCP, called today aquaporin 1 (AQP1), was discovered in the red blood cell (RBC) membrane by BENGA group in 1985 in Cluj-Napoca, Romania, reported in publications in 1986 (BENGA et al., 1986a,b) and reviewed in subsequent years (BENGA et al., 1988; 1989a, b). I have previously reviewed the discovery of the first WCP and presented in detail the landmarks leading to this discovery (BENGA 2003; 2004; 2006a-c, 2009; 2011; 2012a, b).

This protein was purified by chance in 1988 by AGRE group in Baltimore, USA, and called CHIP28 (*C*Hannel forming *I*ntegral membrane Protein of 28 kDa) (DENKER et al., 1988). The AGRE group found the water transport property of this protein only in 1992 (PRESTON et al., 1992). In the same year, other WCPs were discovered and cloned: WCH-CD (*W*ater *C*Hannel of the kidney *C*ollecting *D*ucts) from the rat kidney (FUSHIMI et al., 1993) and γ -TIP (γ -*T*onoplast *I*ntrinsic Protein) from the vacuolar membrane (tonoplast) of *Arabidopsis thaliana* (MAUREL et al., 1993). Thus, it became obvious that the family of WCPs exists and the name “aquaporins” was proposed for this class of membrane proteins, from the Latin words: aqua=water and porus=passage (AGRE et al., 1993). The WCP first discovered by my group in 1985 and re-discovered by AGRE group in 1992 was called aquaporin 1 (AQP1).

I claim now that this discovery was in fact a crucial event in science, opening a new domain of natural sciences, dedicated to all aspects of WCPs, domain for which I suggest here the term of “aquaporinology”. As I have been working in the field for more than 25 years, I could see that this domain of science became a very hot area of research embracing many branches of natural sciences, as hundreds of WCPs have been discovered in organisms from all kingdoms of life, including unicellular organisms (archaea, bacteria, yeasts, and protozoa) and multicellular ones (plants, animals, and humans). These proteins are now studied from the molecular and cellular level (structure-function relationships, expression in various cells, regulation) to the level of whole organisms and of populations. New very important aspects are uncovered every day, the diversity of hundreds of WCPs is revealed, with increasing practical implications, including the physiological and medical implications. Thousands of publications appeared on these topics, including many reviews (see references in BENGA 2009). I am still actively working in the field as it can be seen from the articles of my group (reviewed by BENGA 2013).

The scientific importance of the discovery of WCPs was also recognized by the Nobel Foundation, as half of the 2003 Nobel Prize in Chemistry “for the discovery of water channels” was awarded to Peter AGRE (USA), a physician from Baltimore. Water channels are synonymous with water channel proteins. As a graduate in both medicine and chemistry field, I was pleased that The Nobel Committee for Chemistry selected the water channels as the area of interest in 2003. However, I was very disappointed that my landmark discovery of the first WCP in the human RBC

membrane was overlooked by The Nobel Committee and The Royal Swedish Academy of Sciences. My claim is now supported by thousands of scientists from the whole world.

A **water channel protein (WCP)** can be defined as a transmembrane protein that has a specific three-dimensional structure with a pore that provides a pathway for water permeation across biological membranes (BENGA 2009). The three-dimensional structure of AQP1 is represented in figure 1.

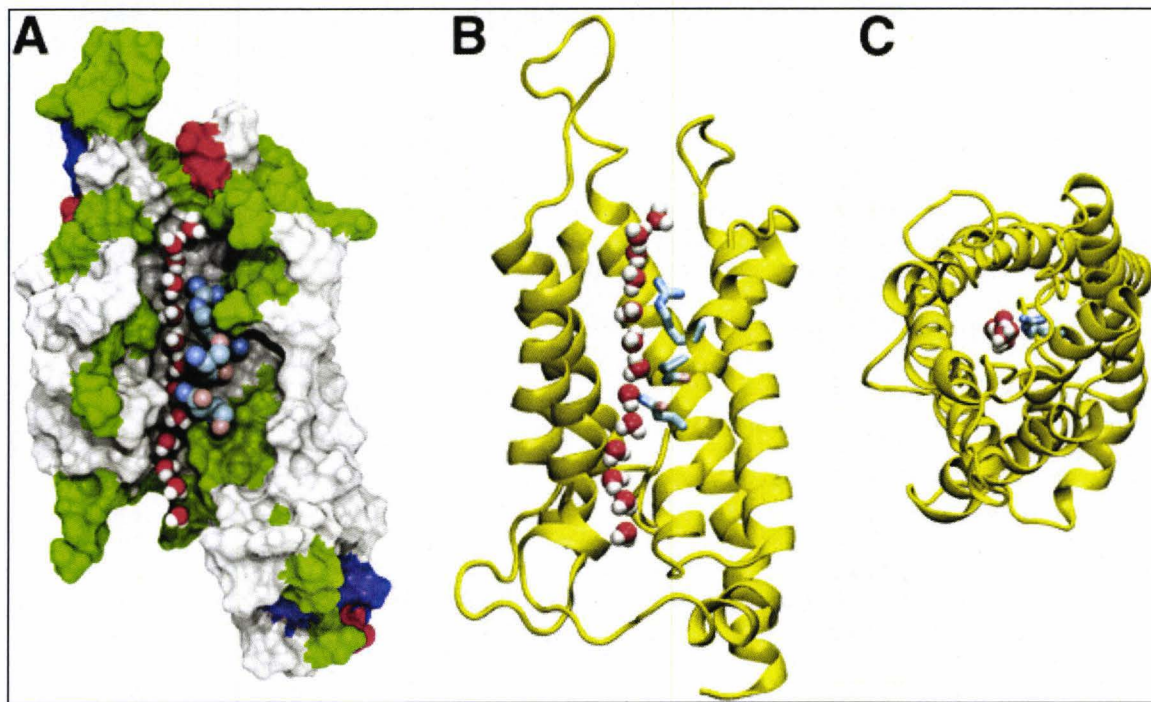


Figure 1. The structure of an AQP monomer (a,b: side views; c: topview).
(From WANG & TARKHORJID 2007).

The monomer is shown in surface representation in a and in yellow cartoon representation in b,c. In a, charged residues are drawn in red (negative) and blue (positive), polar residues in green, and nonpolar hydrophobic residues in white. Water molecules form a single file inside the channel, with a unique bipolar configuration that disfavours proton transfer through the file (24). The interior of the channel is mostly hydrophobic. Key hydrogen-binding residues that line the pore, namely an Arg at the *selectivity filter* and 2 asparagines from the conserved *asparagineproline-alanine (NPA) motifs*, are explicitly shown. Reproduced from Wang and Tajkhorshid, 2007, with permission of the copyright holder.

WCPs, as a family of membrane proteins, belong to the Membrane Intrinsic Proteins (MIPs) superfamily with more than 1000 members (reviewed by ZARDOYA 2005). In addition to WCPs, MIPs also include proteins with no identified channel activity. WCPs are characterized by a homotetrameric structure, with each monomer having a pore formed by two highly conserved regions in the amino acid sequence, called NPA boxes (or motifs) with three amino acid residues (asparagine-proline-alanine, NPA) and several surrounding amino acids. The NPA boxes have been called the “signature” sequence of WCPs (reviewed by BENGA 2012c).

WCPs family of proteins include three subfamilies. 1) *Aquaporins* (abbreviated as AQPs) are mainly water selective or specific water channels; they were also named by various authors as “orthodox”, “ordinary”, “conventional”, “classical”, “pure”, “normal”, or “sensu strictu” aquaporins; 2) *Aquaglyceroporins* are permeable to water, but also to other small uncharged molecules, in particular glycerol; 3) The third subfamily of WCPs have little conserved amino acid sequences around the NPA boxes, unclassifiable to the first two subfamilies. They received various names: “superaquaporins”, “aquaporins with unusual (or deviated) NPA boxes”, “subcellular aquaporins”, or “sip-like aquaporins”. I recommended (BENGA, 2012a) to use always for this subfamily the name *S-aquaporins*. I called aquaglyceroporins and S-aquaporins the “relatives of aquaporins”.

Some confusion regarding the nomenclature of WCP exists in the scientific literature. Some authors say: “Aquaporins are divided into aquaporins, aquaglyceroporins and superaquaporins”. It is like saying: “Men are divided into men, women and children” (with a note that “children” are “supermen”!). As discussed previously (BENGA 2012d) since it appears more appropriately to say: “People are divided into men, women and children”, I believe it is also more appropriate to say: “**Water Channel Proteins (WCPs) are divided into aquaporins, aquaglyceroporins and S-aquaporins**”. In this way the requirement of adding another specification for aquaporins (“sensu strictu”, “orthodox”, “ordinary”, “conventional”, “classical”, “pure” or “normal”) is avoided, as well as that of naming the WCPs with deviated NPA boxes as “superaquaporins” or “unorthodox” (why not “non-Catholic”?).

In order to debate all aspects of WCPs I organized in October 2011 *The First World Congress on Water Channel Proteins (Aquaporins and Relatives) Celebrating the 25th Anniversary of the Discovery of the First Water Channel Protein (Later Called Aquaporin 1)*. *The Second World Congress on Water Channel Proteins (Aquaporins and Relatives) Celebrating the 30th Anniversary of the Discovery of The First Water Channel Protein is already scheduled to take place in Cluj-Napoca (May 20-24, 2015).*

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RECOMMENDATIONS

regarding the elaboration of the papers for the scientific journal

“Oltenia. Studii și comunicări. Științele Naturii”

The journal is edited by the Oltenia Museum Craiova and it publishes original papers in the fields of vegetal and animal biology, ecology-environment protection, mineralogy-palaeontology, as well as scientific reports, reviews, anniversary or commemoration papers.

It appears annually, it is ISI indexed (<http://science.thomsonreuters.com/cgi-bin/jrnlst/jlresults.cgi?PC=MASTER&Word=oltenia>) **and accredited by CNC SIS as a B+ Journal.**

I. Structure (format) for original papers, scientific reports and reviews:

A	Original papers	will be structured according to the information rendered in the Table 1.
B	Scientific reports	will be structured according to the author’s (authors’) preferences, but it has to include abstract and key words, both in English and Romanian.
C	Reviews	there will be mentioned: author (authors) of the book (name and first name – CAPITAL LETTERS),comma, the title of the book, lowercase letters (Italic), publishing house, publishing location, year, number of pages. Use a free space and then render the text of the review with as fewer paragraphs as possible and the same characters as in the case of original papers.

Table 1.

STRUCTURE OF THE PAPER	CHARACTERISTICS	OBSERVATIONS
TITLE	capital letters, 12 pt., bold, centred	
two spare rows (12 pt.) between the title and the name of the author/s		
Author/Authors	name, capital letters, first name, noncapital, 11 pt., bold, normal, aligned right	between two or many authors, use comma
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Abstract (English)	from the beginning of the line, without tab, 9 pt., bold, normal	the abstract will be written with 9 pt., normal, maximum 300 words
One spare row, 9 pt.		
Keywords (English)	from the beginning of the line, without tab, maximum 5 words, 9 pt., normal	
One spare row, 9 pt.		
Rezumat (Romanian)	from the beginning of the line, without tab, 9 pt., bold, normal	Complete translation of the title in Romanian (no capital letters, except for the first letter of the title; 10 pt., bold). The content of the abstract – 9 pt., normal, maximum 300 words
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INTRODUCTION	10 pt. (capital letters, bold)	content – 10 pt., normal
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MATERIAL AND METHODS	10 pt. (capital letters, bold)	content – 10 pt., normal
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RESULTS	10 pt. (capital letters, bold)	content – 10 pt., normal
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DISCUSSIONS	10 pt. (capital letters, bold)	content – 10 pt., normal
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CONCLUSIONS	10 pt. (capital letters, bold)	content – 10 pt., normal
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ACKNOWLEDGEMENTS	10 pt. (capital letters, bold)	content – 10 pt., normal
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REFERENCES	10 pt. (capital letters, bold)	content – see bibliographical references
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Other details related to the papers:

Publishing language	English
Page format	A4 (21 x 29.7 cm), margins: top – 2.5 cm; bottom – 2.0 cm; left – 2.0 cm; right – 2.0 cm; gutter – 0 cm; header, footer – 1.27 cm. The papers will be elaborated in Microsoft Word, justified; font: Times New Roman, 10 pt., normal; single space.

- Latin names (genus, subgenus, species, subspecies) will be written with italic characters;
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The first mention of a taxon in the text will be followed by the taxon author's name and the publishing year of the description, according to the zoological nomenclature code (e.g.: <i>Cossus cossus</i> (LINNAEUS, 1758), afterwards, it will be used abbreviated.
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The materials sent for publication (printed and in electronic format) has to be between 2 and 8 pages (the number of pages must be even).

II. References

- **References** in the text (quotation) includes only the author's/authors' names (SMALL CAPS) and publishing year.
For example:
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 - when there are two authors, it is used "&": RĂDULESCU & SAMSON (1990) or (RĂDULESCU & SAMSON, 1990);
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Reference to a part of a collective paper; volume (with editors): IFTIME Al. 2005. Amfibieni și Reptile. In: Botnariuc & Tatole (Eds.) <i>Cartea Roșie a Vertebratelor din România</i> . Edit. Academiei Române. București: 1-325.
Papers presented at scientific manifestations and published in a volume without editors: CIOCHIA V. & STANCĂ-MOISE CRISTINA. 2001. Contributions to the knowledge of the Macrolepidoptera from natural complex “Dumbrava Sibiului”. <i>Sesiunea Științifică dedicată împlinirii a 75 de ani de la înființarea Stațiunii Biologice Marine „Prof. Dr. I. Borcea”</i> . Agigea-Constanța. 19-20 octombrie 2001: 125-131.
Official publications (laws, decrees, official reports): ***. România. Legea nr. 13 / 1993 pentru aderarea României la Convenția privind conservarea vieții sălbatice și a habitatelor naturale din Europa, adoptată la Berna la 19 septembrie 1979. <i>Monitorul Oficial al României</i> . An V, nr. 62/25 martie 1993. București: 1-20.
PhD thesis: COSTACHE I. 2005. <i>Flora and vegetation Motru River Lower Basin</i> . Ph. D. Thesis, University of Bucharest. Romania. 290 pp., 8 Pl.
Web pages: Muzeul Olteniei Craiova. Secția Științele Naturii. <i>Oltenia. Studii și comunicări. Științele Naturii</i> . (online). 2011. Publisher: Museum of Oltenia Craiova, Romania. www.olteniastudii.3x.ro (accesed: May 8, 2012).
Entire electronic document or service (data base): ***. Fauna Europaea: Chironomidae. In: <i>Fauna Europaea: Chironomidae, Diptera, Nemathocera</i> . (Ed. H. de Jong)

Fauna Europaea version 1.5, <http://www.faunaeur.org>. (accessed: June 23, 2012).

E-book:
AHMADJIAN V. 1967. *The Lichen Symbiosis*. Blaisdell Publishing Company. Massachusetts. Available from: http://books.google.ro/books?id=at7uXMn8iMC&printsec=frontcover&hl=ro&source=gbg_summary_r&cad=0#v=onepage&q&f=false. 152 pp. (accessed: January 15, 2013).

Electronic publication (papers):
DANILEVSKY M. L. 2007. A check-list of Longicorn Beetles (Coleoptera, Cerambycoidea) of Europe. Available online at: http://www.coleoptera-literatura.ic.cz./literatura/checklist_cerambycidae_2007.doc. (accessed: May 20, 2009).

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ALEXANDROVICH O. R. 1995. Reconstruction of the ways of the ground beetles (Coleoptera, Carabidae) fauna forming at the West of the Russian plain. In: I. K. Lopatin, Pisanenko A. D., Shklyarov L. P. (Eds.). *Fauna and taxonomy: Proceed. Zool. Museum Byel. University Minsk: Nauka Tekhnika*. **1**: 52-68. [In Russian].

III. Illustration

- Images (white/black or colour), tables, graphs and maps are inserted into the manuscript, but **the original versions have to be sent also separately**: high contrast photographs, electronic images in TIFF format at a minimum resolution of 300 dpi.
- The references to the illustrations (tables, images, photographs) will be made in the text as it follows: (Fig. 1), (Figs. 1a, b), (Figs. 3; 5); (Table 1); (Photo 1).
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The title of a table (in English) will be placed above the table (aligned right), 9 pt., normal.

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The editorial board has the right to reduce the number of figures and photos (if there are too many as compared to the text of the paper or if they do not correspond to the requirements) and not to accept papers sent after deadline, **March 31, 2014**.

With all the respect for the authors, papers that do not correspond to the recommendations will be sent back.

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