

IN MEMORIAM



ȘTEFAN NEGREA PhD
(November 09, 1930 – February 20, 2019)

**A life dedicated to the study on the creatures of the dark
and damp underground caves**
A life dedicated to speleology – Light from the dark

The scientific community of Romanian biologists, as well as many lovers of nature or hiking, have been deeply saddened by the news of the famous biospeleologist Dr. Ștefan Negrea's disappearance, witness to the renaissance of the Emil Racoviță Institute of Speleology in Bucharest, later becoming one of its most renowned scholars, known everywhere at home and abroad for his papers of speleology, ecology and zoology, especially for those on cladoceran crustaceans and chilopods.

Dr. Ștefan Negrea left us discreetly, lonely after the loss of his dear wife and collaborator on the road of science – Dr. Alexandrina Negrea – Didina, as he usually called her.

Dr. Ștefan Negrea left behind a radiant light of his speleological and hydrobiological research in Romania and abroad.

Dr. Ștefan Negrea highlighted the life and work of some great personalities of Romanian biology.

Dr. Ștefan Negrea spoke to scientists, and to the media, about the caves in our country and elsewhere, showing their treasures, the creatures of the world without sun, with their amazing adaptations.

Dr. Ștefan Negrea worked at the Institute of Speleology of the Romanian Academy, an institution reborn after 1956, under the direction of Emeritus Professor Constantin Motaș (1891–1980). We can say that Ștefan Negrea was linked by speleology, for life, mostly due to Professor Constantin Motaș, who later, prefacing a book, characterized him as follows: “*Ștefan Negrea is a naturalist by vocation, and in his veins is flowing a restless blood of an explorer...*”.

In these lines, we want to honor our colleague Ștefan Negrea’s memory, but we think it is absolutely necessary to interpose in our note a few words about Prof. C. Motaș, who was his model in life, mentor and colleague, true friend, without whom, with all his talent and hereditary heritage, they could not had been activated for the completion of the young biospeleologist.

Certainly, the passion for knowing life in the dark and humid caves of the earth was transmitted to Ștefan Negrea and other speleologists as a relay started from Emil Racoviță through Constantin Motaș and from here through the generation living in a troubled world, with many shortcomings, but strengthened and forced to survival, Stephen's generation, to the young generation of the new Millennium. But let the facts speak.

Constantin Motaș was an eminent young man recommended by Emil Racoviță for a scholarship in France, which he concluded with a doctorate for which he obtains the mark “très honorable avec félicitations du jury”.

Returned to the country, the young biologist with a brilliant training, as we were told, occupied a number of important academic dignities and, after 11 April 1949, Acad. C. Motaș became responsible for the series of volumes “Fauna Republicii Populare Române” [“Fauna of the Romanian People's Republic”], he is arrested (after a political trial and convicted at 25 years); after seven years in communist prisons, under the pressure of colleagues from abroad, he was released and asked to choose one of the institutions in which he worked. He chose the Institute of Speleology, which was being reorganized at national level, almost 10 years after Emil Racoviță’s death, its founder in Cluj. He was director of the institute from 1956 till 1969, but continued to work at the institute until 1980, sharing the same room with Ștefan Negrea for a long time. Emeritus Professor Constantin Motaș was followed by Prof. Dr. Traian Orghidan, Prof. Costin Rădulescu and Dr. Ioan Povară. Together with them, Ștefan Negrea contributed fundamentally to the “invention” and the development of stigobiology – the study of the creatures of all kinds of underground waters. Without the contribution of stigobiology, speleology would remain a field of geology – carstology, without the knowledge of biodiversity and the evolution of life in underground environments.

Ștefan Negrea was born on the 9th of November 1930 in a family with intellectual roots, that of Priest Vasile and Maria Negrea, in Gura Văii village, Neamț county. He was proud of his family, whom he described in warm,

melancholic tones, when he was at conferences in his native lands, in Piatra Neamț or in Bacău, for example. Important ancestors, as they were mentioned in Ștefan Negrea's CV, were: Ion Negrea (great-grandfather's brother, born in Gura Văii), a professor of science, who founded, along with the writer "literature professor" Calistrat Hogaș, Petru Rareș Theoretical High School from Piatra Neamț (now National College) in 1869; Vasile Conta (his grandfather's primary cousin, Ioan Trifan, born in Dachia) – philosopher, propagator of darwinism and author of „Teoria undulațiunii universale” [“Universal Wave Theory”]; Constantin Virgil Gheorghiu (his mother's primary cousin, born in Petricani in 1916, m. 1992) – priest from 1968 at the Orthodox Church in Paris, prolific writer (he wrote over 50 books, among which “Ard malurile Nistrului” [“Banks of Dniester River are burning”] as reporter of war and the novel “Ora 25” [“Hour 25”], in 1962, which brought him a world fame, and after which the French–Italian–Yugoslav film “The 25th Hour” was made.

ȘTEFAN NEGREA'S SCHOOLS

He spent his childhood and primary school (1936–1940) in his native village, then he attended the gymnasium at Petru Rareș High School in Piatra Neamț (1941–1949).

As a high school student was enchanted by the lessons of zoology which guided him to observe and to discover nature; sometimes he told us how he was waiting for the butterflies to fly, the hairy caterpillars rattling the leaves, bush-crickets singing in the grass, the “black as pitch” crickets taken out with the straw from holes, the ants carrying their colossal burdens.

In high school he was also noted for his literary skills and participated in the student competitions, obtaining for his papers on Soviet literature, compulsory at that time, the First Prize in 1947 and 1948, and in 1949 he became a member of the Literary Cenacle “The New Word” and founding member of the Romanian Writers' Union Branch in Piatra-Neamț. His skills in writing and literature would reach high levels all Ștefan's life. In 1949 Ștefan Negrea took the baccalaureate exams receiving the highest mark, “very well”.

A year later, he married (on August 16, 1950) with his former high school colleague – Alexandrina Budăi, the One, Didina as he used to tell her, remaining together all their life. Young Ștefan Negrea felt strongly attracted both to literature and to biology-zoology. Finally, discussing with Didina, he decided and had chosen the second option, the study of nature. Thus, together with his wife, he decides to study biology together and in 1951–1954 the young couple Ștefan and Alexandrina attended the courses of the Faculty of Biology of the University of Bucharest.

As a student he is remarked for his scientific interest and becomes president of a student scientific circle of hydrobiology, being awarded in Bucharest and country

competitions. In 1953 he wrote the paper “Hydrobiological and Fishery Research in Snagov Lake”, being supported by Prof. Traian Orghidan (1917–1985) – a remarkable naturalist and scientific personality who, after 1956, was to become one of the dynamic organizers of the Institute of Speleology, and which helped Ștefan Negrea to develop interest in the study of hydrobiology. The paper was awarded with an honorary diploma.

Also in 1953, as a student, Ștefan Negrea and Didina had the great chance to visit the Cave of Vadul Crișului, a cave with resurgent reliefs, with abundant water, very suited to speleological and bio-speleological research, the very first cave visited by them. Being fascinated by its beauties, Negrea spouses were glimpsed by an idea, which became a dream of their future activity; was the desire to explore the cave creatures as zoologists and hydrobiologists at the Speleology Institute of Emil Racoviță and as researchers, together with their masters, Professor Margareta Dumitrescu and associate professor Traian Orghidan, biologists “definitively converted to speleology”.

Next year, in 1954, Ștefan Negrea wrote the paper “Fauna de cladoceri a Lacului Snagov” [“Cladoceran Fauna of Snagov Lake”], which was awarded at the second Student Scientific Conference. For the Bachelor's degree exam, he continued the study of the tiny aquatic crustaceans and prepares the thesis “Contribuții la studiul faunei de cladoceri din lacurile glaciare ale R. P. Române” [“Contributions to the Study of Cladocerans of the Glacial Waters of the Romanian People's Republic”] and passes the exam with the grade “Very well”.

Under the guidance of Prof. Nicolae Botnariuc – the promoter of systemic conception in ecology, Ștefan Negrea prepares the PhD at Bucharest University, studying the small freshwater crustaceans from his group studied during studentship, Cladocera Order, included in Branchiopoda Class, a group he studied minutely his whole life. In 1971 he obtained a PhD in Biology with a doctoral thesis titled “Ecologia cladocerilor din lacurile Jijila, zona inundabilă a Dunării” [Cladoceran Ecology of Jijila lakes, flooded area of the Danube”].

In 1975 he attended a training and scientific study period in France (Paris, Moulis) in the Underground Laboratory of the National Center for Scientific Research, considered the most important biospeleological center in the world at that time.

SCIENTIFIC ACTIVITY

The period of the 1950s, when Ștefan Negrea made his university studies and began to work, was a difficult time, with many shortcomings and destructions, as after the war, but also as the setting up of a new political order in the country, the Soviet order brought with the tanks. Then, as a result of a political pressure to organize the Romanian Academy according to the Soviet model, the Committee of Hydrology, Hydrobiology and Ichthyology of Romanian Academy is founded. This

committee gathers around it some distinguished biologists with the initiative and desire to develop biology in Romania, such as Teodor Bușniță (1900–1997), Mihai Băcescu (1908–1999), Ludovic Iosif Urban Rudescu (Rodewald) (1908–1992), Nicolae Botnariuc (1915–2011), A.C. Banu (geographer), Radu Codreanu (1904–1987) and others, who organized a series of researches on the Danube and the Black Sea, employing university graduates.

After graduating his studies, Ștefan Negrea's career takes the lead of scientific research. In brief, after a brief period in Constanța and Brăila, Dr. Ștefan Negrea was a scientific researcher at the “Emil Racoviță” Institute of Speleology of the Romanian Academy, Bucharest, since 1956. In 1966, he was promoted to the position of scientific researcher gr. III, in 1990 he became scientific researcher gr. II, and in 1995 – scientific researcher gr. I. In 2005 he retired, and since 2008 he was associate researcher at “Emil Racoviță” Institute of Speleology.

However, we must mention that at the end of 1954, he was appointed as a research assistant at the Committee of Hydrology, Hydrobiology and Ichthyology of the Romanian Academy, with a job at the Constanța Marine Research Station, the former Bio-Oceanographic Institute, founded by Grigore Antipa. Here he stayed for a short time, but he gave us the “Observations sur la répartition du zooplancton sur le profil Est – Constanța”, published together with Alexandrina Negrea and Lucreția Elian in 1959.

In the period 1955–1956, Ștefan Negrea was transferred to the Brăila Hydrobiological Station of the University of Bucharest, thanks to Prof. Nicolae Botnariuc, who coordinated an important project on the “Study of the Crapina-Jijila Pool in the Danube Flooding Area”. He was among the young researchers; the results of this collaboration are included in the paper “Observații asupra biologiei speciei *Fagotia esperi* (Fér.) din complexul de bălți Crapina-Jijila”. [“Observations on the biology of the species *Fagotia esperi* (Fér.) in the Crapina-Jijila Pond Complex”], published in 1963 together with Nicolae Botnariuc and Alexandrina Negrea.

At the beginning of his career, Ștefan Negrea collaborated with Prof. Constantin Motaș and Lazăr Botășăneanu, making important researches on the biology of the springs and phreatic waters in the central part of the Romanian Plain; the results of the extensive studies were published in a volume of over 330 pages in the Romanian Academy Publishing House (1956).

Among the reference works of Dr. Ștefan Negrea, we mention:

- Cercetări asupra biologiei izvoarelor și apelor freatice din partea centrală a Câmpiei Române (C. Motaș, L. Botoșăneanu, Șt. Negrea, 1962, 366 pp.); [Researches on the biology of springs and phreatic waters in the central part of the Romanian Plain];
- Bibliographia Biospeologica Romanica (1937–1963) (V. Decu, Șt. Negrea, 1965);

- Recherches sur les grottes du Banat et d’Oltenie (L. Botoșăneanu, A. Negrea, Șt. Negrea, A. Decou, V. Decou, M. Bleahu, 1967, 397 pp.) [Studies on the caves of Banat and Oltenia];
- The three papers dedicated to parietal, guano and flooring biocenoses of the caves of the Banat Mountains (Șt. Negrea, A. Negrea, 1971–1977);
- Ecologia populațiilor de cladoceri și gasteropode din zona inundabilă a Dunării (Șt. Negrea, A. Negrea, 1975, 232 pp.) [Ecology of the cladoceran and gastropod populations of the Danube flooding area];
- The volume “Speleology” in the monograph series “The Iron Gates” (editors T. Orghidan, Șt. Negrea, 1979);
- Expediționari români la tropice (Șt. Negrea, 1980, 208 pp.) [Romanian Explorers in Tropics];
- “Cladocera” in the academic series “Romanian Fauna” (Șt. Negrea, 1983, 399 pp.);
- Pe urmele lui Grigore Antipa (Șt. Negrea, 1990) [On Grigore Antipa’s Footsteps];
- Biodiversitatea în mediile subterane din România (Șt. Negrea, A. Negrea, A. Ardelean, 2004, 248 pp.) [Biodiversity in the underground environments in Romania].

Dr. Ștefan Negrea was a member of a number of scientific organizations and associations:

- The International Myriapodology Center in Paris;
- The Senate of “Alexandru Ioan Cuza” University of Iași;
- Limnology Society of Romania, affiliated to the International Society of Limnology (SIL);
- Division of the History of Science of the Romanian Committee for the History and Philosophy of Science and Technology (CRIFST) of the Romanian Academy;
- The Leading Board of the Association of Journalists and Writers of Tourism in Romania.

Also, Ștefan Negrea was part of the Editorial Staff of scientific journals of Romania and abroad:

- Academic series “*Fauna României*” (deputy editor-in-chief since 1983);
- *Annales de Limnologie – International Journal of Limnology* in Toulouse;
- *Noema; Studii și Cercetări* – Divizia de Istoria Științei a CRIFST;
- *Travaux de l’Institut de Speologie “Emile Racovitza”*;
- *Oltenia – Studii și Comunicări, Științele Naturii* (the journal of “Oltenia” Museum in Craiova).

IMPORTANT ACHIEVEMENTS

Over the years, the results of the research undertaken by Dr. Ștefan Negrea materialized in the elaboration of an impressive number of works, with particular reference to the cladocerans (Cruciacea: Branchiopoda), the chilopods (Myriapoda) and the underground environments.

He has also carried out numerous expeditions for the scientific exploration of caves in Romania.

- The researches from 1956–1959 were carried out during scientific expeditions aimed at exploring the caves of the Southern Carpathians, Banat and Dobrogea.
- Between 1960–1986, Dr. Ștefan Negrea explored, together with his wife and Dr. Lazăr Botoșăneanu, hundreds of caves and fauna of the Banat Mountains, the Cerna Valley and the Poiana Ruscă, which became very well known in Romania, from biospeleological point of view.
- As a result of these studies, information on more than 240 caves and ravines from Romania were published, and about 30 km of cave galleries were mapped, mostly in the southwestern part of the country.

Since 1961, Dr. Ștefan Negrea has carried out a series of field trips together with members of the “Exploratorii” Speleological Club of Reșita, and since 1987 he did field applications at the National School of Biospeology. Negrea spouses, in collaboration with amateur biospeleologists from this center, studied dozens of caves from the hydrographic basins of Caraș and Bârzava (1980–1984).

Between 1969 and 1973, Dr. Ștefan Negrea participated in 40-day Romanian–Cuban Biospeleological Campaigns in the Cuban Tropical Karst Area, the results contributing to the formation of an overview of the cave fauna within the area. Obtained data were published in four volumes at the Romanian Academy Publishing House (1973, 1977, 1981 and 1983).

In 1990, at Prof. Univ. Dr. Francis Dov Por’s request (University of Jerusalem), in 1990, Ștefan Negrea was included in a team for a 40-day expedition in Israel for the collection of zoological material from the cave and terrestrial environment. The results of the researches were used in a series of papers published under the aegis of the Romanian Academy (1995).

During his long scientific career, Dr. Ștefan Negrea collaborated with numerous colleagues, both from the country (Motaș C., Orghidan T., Botnariuc N., Botoșăneanu L., Decu V., Sencu V.L., Karban G., Elian Lucretia, etc.), especially from “Emil Racoviță” Institute of Speleology and from abroad: Pospisil P. from Austria, Dumont H.J. from Belgium, Por F.D., Dimentman Ch. from Israel and other famous specialists from France, Spain, Germany, Hungary, Bulgaria, Macedonia, Poland, Czech Republic, Italy, Russia, Cuba, Mexico, USA, etc.

In his 55 years of scientific activity (1954–2009), Dr. Ștefan Negrea has published, single or as collaborator, 406 articles and books with subjects that dealt

with the various fields of biological sciences: classical taxonomy (Cladocera, Chilopoda) aquatic, terrestrial and underground ecology, zoogeography, phylogeny, nature conservation, history of biological sciences and some of its representatives.

- Among these, a valuable scientific contribution is the original, complex study of the crustaceans of the Branchiopoda Class, with reference to ecology, ethology, phylogeny and their classification. Dumont and Negrea made a cladistic analysis of all branchiopod groups, using a total of 42 morphological characters and establish that this class is composed of five superordinates and 11 orders (nine recent, two fossils); the results of these researches materialized in the publication of the monograph "Introduction to the Class Branchiopoda" (H.J. Dumont, Șt. Negrea, 2002, 398 pp.), published in the series "Guides to the identification of the invertebrates of the continental waters of the world". The paper was awarded the "Emil Racoviță" Prize of the Romanian Academy and now it is considered one of the most valuable carcinology works of the world;
- Dumont and Negrea (1996), reviewing of the cladocerans of the cave waters of the world, present, also, a synthesis of the current knowledge on cladocerans by providing a historical study on concept development, category identification among groundwater species and discussing the existing adaptations and lines of evolution; in addition, they consider that out of the total of 450 non-marine Cladocera, estimated in the world, only ~20% can occur in underground aquatic habitats, their number (stigobionites or stigobites) being relatively low (Dumont H. J., Negrea Șt., 1996 – "A conspectus of the Cladocera of the underground waters of the world", *Hydrobiologia* 325 (1): 1–30 DOI: 10.1007 / BF00023664);
- In Israel, Negrea and his collaboratories studied the morphology, zoogeography and ecology of scutigermorph centipeds and presented an identification key for species.

Dr. Ștefan Negrea spoke about the caves in our country and abroad, showing us their treasures, the creatures of the world without the sun, with their amazing adaptations. The description of over 40 new species for science, of myriapods (chilopods and diplopodes) and cladoceran crustaceans represents a significant contribution to the knowledge of the cave environment biodiversity.

This way, the specialist Ștefan Negrea evolved, the one who studied taxonomically, ontogenetically, phylogenetically, and biogeographically mostly the Branchiopod crustaceans from the freshwater, brackish, marine, hypersaline and underground regions of the palearctic, neotropical and ethiopian regions, for more than 50 years from embryonic development point of view, but also other invertebrate groups, in terms of ethology, ecology, and biogeography (including palaeobiogeography). Dr. Ștefan Negrea's books guided young people towards science adventure and decided the orientation to the biological knowledge and training of many people.

He left behind, the result of an agitated life, the search for science, numerous and valuable biological contributions, which, at this early hour after his departure into eternity, we mention by chance and partly as follows:

- Studiul zoologic al peșterilor din România: [*Heleobia (Semisalsa) dobrogica* (Grossu & Negrea, 1989). (Gastropoda: Rissoidea: Cochliopidae), Biodiversitatea în mediile subterane din România (Șt. Negrea, A. Negrea, A. Ardelean, 2004, 248 pp.). [Zoological study of the caves in Romania: [*Heleobia (Semisalsa) dobrogica* (Grossu & Negrea, 1989). (Gastropoda: Rissoidea: Cochliopidae), Biodiversity in the underground environments in Romania];
- Studiarea biocenozelor parietală, de guano și de planșeu a peșterilor din Munții Banatului (Șt. Negrea, A. Negrea, 1971–1977) [Study of the parietal, guano and flooring biocenoses of the caves in the Banat Mountains];
- Ecologia populațiilor de cladoceri și gasteropode din zona inundabilă a Dunării (Șt. Negrea, A. Negrea, 1975, 232 pp.) [Ecology of the cladoceran and gastropod population in the floodplain area of the Danube];
- Cladocera, in the serie “Fauna României” [Romanian Fauna] (Șt. Negrea, 1983, 399 pp.);
- Cunoașterea biologiei izvoarelor și apelor freatice din partea centrală a Câmpiei Române (C. Motaș, L. Botoșăneanu, Șt. Negrea, 1962, 366 pp.) [Knowledge of the biology of the springs and phreatic waters in the central part of the Romanian Plain];
- Cercetări asupra peșterilor din Banat și Oltenia (L. Botoșăneanu, A. Negrea, Șt. Negrea, A. Decou, V. Decou, M. Bleahu, 1967, 397 pp.) [Research on the caves in Banat and Oltenia];
- Contribuții speologice la realizarea Seriei monografice Porțile de Fier (editori T. Orghidan, Șt. Negrea, 1979) [Speleological contributions to the Iron Gates monographic series];
- Filogenia, evoluția și clasificarea Clasei Branchiopoda (Crustacea) (Ștefan Negrea, Nicolae Botnariuc, Henri J. Dumont) [Phylogeny, Evolution and Classification of Class Branchiopoda (Crustacea)];
- Conspectul Cladocercelor din fauna apelor subterane ale lumii (Ștefan Negrea, Henri J. Dumont) [Cladocerans Groundwater Fauna of the World];
- Evaluarea critică a speciilor de Chilopode din România și Israel – istoricul studiilor, sinonimele, actualizarea nomenclaturii a două înregistrări vechi: *Eupolyothrus* [Critical evaluation of Chilopod species in Romania and Israel - history of the studies, synonyms, updating the nomenclature of two old records: *Eupolyothrus*];
- Istoria biologiei românești și biografiile marilor săi slujitori: Pe urmele lui Grigore Antipa (Șt. Negrea, 1990); Nicolae Botnariuc la 95 de ani [The history of Romanian biology and of its great servants’ biographies: In Grigore Antipa’s footsteps of Grigore Antipa; Nicolae Botnariuc at his 95th anniversary];

- *Expediționari români la tropice* (Șt. Negrea, 1980, 208 pp.) [Romanian explorers in the Tropics].

Being contemporary with Dr. Ștefan Negrea, we can appreciate him as one of the most important specialists from Romania in the second half of the 20th century in the knowledge of organisms of the underground waters. The recognition of Dr. Ștefan Negrea's scientific contributions can be exemplified by the many new species and genera to science, which were dedicated to him by a number of Romanian and foreign specialists.

But as many aspects of life and work as Dr. Ștefan Negrea could add, nothing is more relevant than the impressive list of 343 scientific papers, plus 28 books (author and/or editor), 42 chapters of books or independent scientific volumes, approachably edited for the distribution of science all levels of education.

Looking at the list of the distinguished colleague's papers, we will have a better image of the passionate, pedantic naturalist, biospeleologist-zoologist Ștefan Negrea, the slightly proud-spirited one, but with literary talent and profound analyst of the researched subjects, that he presented clearly, by explicit texts, and where it was necessary, by beautiful scientific drawings. We will understand its ability to explain the evolution of organisms from the complex environments, revealing the apomorphic characters, as compared to the plesiomorphic ones, in order to establish cladograms, verified and confirmed later and by methods of molecular biology techniques.

During his career, Dr. Ștefan Negrea wrote valuable pages of the history of Romanian biology, about "people and facts", witnessing events that marked the development of biological sciences in our country.

In more than 50 years of scientific activity, Dr. Ștefan Negrea worked with passion and tenacity at the service of Biology in Romania, to whom he was devoted to his colleagues, collaborators, by all those who had the privilege to be in around.

Today, when he is in a better world, he left us a light behind, gathered along a life time the darkness of caves. Also, he left us a model of marriage rarely occurred, when spouses share the same concern, and together they go in field trips, share the lab together, consult together on scientific issues, write and publish together, participate in conferences and symposiums. This explains why, after Didina's eternal departure, Stephen did not find his peace of mind until February 20, 2019, when he also left for eternity.

Farwell beloved friend and colleague in your last underground trip!

Rest in peace, anxious soul in search of light in the darkness of the caves!

Prof. Dr. Marian-Traian Gomoiu,
Member of the Romanian Academy

Dr. Dumitru Murariu,
Corresponding Member of the
Romanian Academy

Dr. Sanda Maican,
Institute of Biology Bucharest of the Romanian Academy

DR. ȘTEFAN NEGREA – SELECTED PAPERS

1. **Negrea Șt.**, Negrea A., Elian L., **1959** – Observations sur la répartition du zooplancton sur le profil est – Constanța. Lucr. Ses. Șt. (15–17 sept. 1956) a Staț. Zool. Marine “Prof. I. Borcea”, Agigea. Univ. Al. I. Cuza, Iași, pp.: 9–24.
2. Botnariuc N., Negrea A., **Negrea Șt.**, **1963** – Observații asupra biologiei speciei *Fagotia esperi* (Fér.) din complexul de bălți Crapina – Jijila. Com. Zool. (SSNG), 2: 9–19.
3. **Negrea Șt.**, Negrea A., Sencu V., Botoșăneanu L., **1965** – Grottes du Banat (Roumanie) explorées en 1963. Int. J. Speleol., I (4): 397–439.
4. Botoșăneanu L., Negrea A., **Negrea Șt.**, **1965** – Colorări cu fluoresceină în carstul din zona Reșița – Moldova Nouă (Banat). Lucr. Inst. Speol. “E. Racoviță”, IV: 118–125.
5. Negrea A., Botoșăneanu L., **Negrea Șt.**, **1967** – Documents pour servir à la connaissance de la faune de mammifères des grottes du Banat (Roumanie). Int. J. Speleol., II, 4: 342–353.
6. Botoșăneanu L., Negrea A., **Negrea Șt.**, **1967** – Grottes du Banat, explorées de 1960 à 1962. In: Recherches sur les grottes du Banat et d’Olténie (Roumanie 1959–1962), Edit. CNRS, Paris, 397 pp.
7. **Negrea Șt.**, Negrea A., **1968** – Contribuție la studiul asociației parietale a peșterilor din Banat (Roumanie). Lucr. Inst. Speol. “E. Racoviță”, VII: 79–148.
8. **Negrea Șt.**, Negrea A., **1969** – Peșterile din Defileul Dunării. Lucr. Inst. Speol. “E. Racoviță”, VIII: 25–50.
9. Sencu V., **Negrea Șt.**, Negrea A., Botoșăneanu L., **1971** – Grottes explorées dans le bassin du Caraș (Banat, Roumanie). Trav. Inst. Speol. “E. Racovitza”, X: 43–70.
10. Negrea A., **Negrea Șt.**, **1971** – Sur la synusie du guano des grottes du Banat (Roumanie). Trav. Inst. Speol. “E. Racovitza”, X: 81–122.
11. **Negrea Șt.**, Negrea A., **1972** – Recherches sur l’association parietale des grottes du Banat (Roumanie). Acta Zool. Cracov., XVII (3): 45–96.
12. Negrea A., **Negrea Șt.**, **1972** – Zonele biospeologice și fauna peșterilor. Atlas complex “Porțile de Fier”, Edit. Acad. R.S Română, pp.: 152–153.
13. **Negrea Șt.**, Negrea A., **1975** – Ecologia populațiilor de cladoceri și gasteropode din zona inundabilă a Dunării. Edit. Acad. R.S.R., 232 pp.
14. Decu V., Negrea A., **Negrea Șt.**, **1974** – Une oasis biospéologique tropicale développée dans un région tempérée: “Peștera lui Adam” de la Băile Herculane (Carpates méridionales, Roumanie). Trav. Inst. Speol. “E. Racovitza”, XIII: 83–105.
15. **Negrea Șt.**, Negrea A., **1976** – Ecologie des populations de Cladocères et Gastéropodes de la zone inondable du Danube. Bull. d’ Ecologie, 7 (2): 246–251, Edit. Masson, Paris.
16. **Negrea Șt.**, Negrea A., **1977** – Sur les associations de plancher des grottes du Banat (Roumanie). Trav. Inst. Speol. “E. Racovitza”, XVI: 99–139.
17. Negrea A., **Negrea Șt.**, **1978** – Sur l’écologie et l’origine des populations cavernicoles du Defilé du Danube. Trav. Hist. Mus. “Grigore Antipa”, XIX: 340–351.
18. Negrea A., **Negrea Șt.**, **1978** – Considerații ecologice și biogeografice asupra faunei cavernicole terestre din Banat. Banatica, Ser. St. Nat. Reșița, 7: 225–246.
19. **Negrea Șt.**, **1979** – Prin peșterile lumii. Edit. Sport-Turism, București, 176 pp.
20. Negrea A., **Negrea Șt.**, **1979** – Peșterile din Defileul Dunării și fauna terestră. In: “Speologia”, Ser. Monografică “Porțile de Fier”, Edit. Acad. R.S.R : 30–75.
21. Negrea A., **Negrea Șt.**, **1979** – Sur la dynamique des populations de Cladocères et des Gastéropodes de zone inondable du Danube. XIX Jubil. Donauforschung, Sofia, pp.: 248–252.
22. Negrea A., **Negrea Șt.**, **1980** – Considérations sur la biocénose de plancher de la zone profonde des grottes du Banat (Roumanie). Trav. Mus. Hist. Nat. “Grigore Antipa”, XXI: 151–156.
23. Negrea A., **Negrea Șt.**, **1983** – Considérations sur les écosystèmes des grottes Comarnic et Popovăț (Banat, Roumanie). Trav. Inst. Speol. “E. Racoviță”, XXII: 47–55.
24. **Negrea Șt.**, Negrea A., **1988** – Îndrumător pentru colectarea, conservarea și recunoașterea faunei subterane terestre. Xerografie, București, 16 pp.

25. Negrea A., **Negrea Șt., 1991** – La liste des travaux publiés (1956–1990). Bull. de liaison de la Société de Biospéologie, 18: 25–29.
26. **Negrea Șt.,** Negrea A., Karban G., **1992** – Grottes explorées dans les Gorges du Caraș (Banat, Roumanie) et leur faune. Trav. Inst. Speol. “E. Racovița”, XXXI: 105–132.
27. **Negrea Șt.,** Negrea A., Karban G., **1993** – Grottes explorées dans les vallées Luca, Comarnic et Toplița (Banat, Roumanie) et leur faune. Trav. Inst. Speol. “E. Racovița”, XXXII: 131–154.
28. Negrea A., **Negrea Șt.,** Karban G., **1994** – Grottes explorées dans le bassin du Doman (Banat, Roumanie) et leur faune. Trav. Inst. Speol. “E. Racovița”, XXXIII: 179–194.
29. **Negrea Șt.,** Negrea A., **1995** – Necesitatea unor măsuri de protecție și a extinderii perimetrului rezervației Valea Mare (Moldova Nouă). A 2-a Conf. Naț. de Prot. Mediului Brașov, 29–31 mai 1992. Tipogr. Univ. Transilvania Brașov, pp.: 283–288.
30. **Negrea Șt.,** Negrea A., **1995** – Îndrumător pentru colectarea, conservarea și recunoașterea faunei subterane terestre. Edit. V. Horoi, București, 27 pp.
31. **Negrea Șt.,** Negrea A., **1996** – Din Defileul Dunării în Cheile Nerei (Munții Almăjului și Locvei). Edit. Sport–Turism, 111 pp.
32. **Negrea Șt. 1997** – Contributions à l’histoire de la Biospéologie. I. La période d’avant Emile Racovița. Noesis. Trav. Comit. Hist. Phil. Sci., 22: 65–69.
33. **Negrea Șt., 1998** – Contributions à l’histoire de la Biospéologie. II. La période d’Emile Racovița (1907–1947). Noesis. Trav. Comit. Hist. Phil. Sci., 23: 79–84.
34. **Negrea Șt., 1999** – Contributions à l’histoire de la Biospéologie. III. La période d’après Emile Racovița. Noesis. Trav. Comit. Hist. Phil. Sci., 24: 181–187.
35. **Negrea Șt.,** Negrea A., **2000** – Problema conservării Rezervației naturale “Complexul de izvoare de la Corbii Ciungi” – oază acvatică cu faună relictă în Câmpia Română. Ocr. Nat. Med. Înconj., 42–43: 33–45, București.
36. Negrea A., **Negrea Șt., 2000** – Problema protejării mediilor subterane din România. Oltenia. Stud. Com. Șt. Nat., XVI: 191–192, Craiova.
37. **Negrea Șt.,** Negrea A., **2002** – Ad Aquas Herculi sacras. Băile Herculane și Valea Cernei. In the collection: “Itinerare în Munții Banatului” nr. 2, 256 pp., Edit. Timpul, Reșița.
38. **Negrea Șt.,** Negrea A., **2002** – Parcul Național Domogled – Valea Cernei la 10 ani de la constituire. Anuarul Muzeului județean Argeș–Pitești. Stud. Com. Șt. Nat., 9–10: 423–432
39. **Negrea Șt.,** Negrea A., Gava R., **2002** – Contribuția profesorului George D. Vasiliu (1908–1989) la dezvoltarea biologiei românești. Argesis, Stud. Com. Ser. Șt. Nat., 9–10: 46–67, Pitești.
40. **Negrea Șt.,** Negrea A., **2003** – Conservarea mediilor și faunei subterane din România. I. Cauzele deteriorării habitatelor. Ecos, 15: 8–14, Pitești.
41. Negrea A., **Negrea Șt., 2003** – Evoluția relațiilor dintre om și peșteră de-a lungul timpului. Oltenia. Stud. Com. Șt. Nat., XIX: 235–236, Craiova.
42. **Negrea Șt.,** Negrea A., **2003** – Biodiversity of the aquatic subterranean environments from Romania. Studii și cercetări, Univ. Bacău, 8: 174–176.
43. **Negrea Șt.,** Negrea A., Ardelean A., **2004** – Biodiversitatea în mediile subterane din România (Inițiere în biologia, ecologia, conservarea și protecția mediilor subterane terestre și acvatice. Mic dicționar de termeni). “Vasile Goldiș” University Press, Arad, 248 pp.
44. **Negrea Șt.,** Negrea A., **2004** – Conservarea mediilor și faunei subterane din România. II. Cauzele deteriorării populațiilor. Ecos 16: 37–42, Pitești.
45. Negrea A., **Negrea Șt., 2004** – Colonization, evolution and speciation in the interstitial environments. Oltenia. Stud. Com. Șt. Nat., 20: 305–308.
46. **Negrea Șt.,** Negrea A., **2004** – Colonization, evolution and speciation in the speleic environments. Oltenia. Stud. Com. Șt. Nat., 20: 309–311.
47. **Negrea Șt.,** Negrea A., **2004** – Din Cheile Carașului pe Semenice. Itinerare în Munții Aninei, Dognecei și Semeniceului. Edit. Timpul, Reșița, 161 pp.
48. **Negrea Șt.,** Negrea A., **2005** – Conservarea mediilor și faunei subterane din România. III. Conservarea habitatelor și speciilor subterane. Ecos, 17: 11–22, Pitești.

49. **Negrea Șt.**, Negrea A., **2005** – Grigore Antipa and the problems of the Danube – ways contemporary. Univ. Bacău, Studii și Cercetări, Biologie, 10: 3–5.
50. **Negrea Șt.**, Negrea A., **2005** – René Jeannel (1879–1965): 40 de ani de la moartea principalului colaborator al lui Emil Racoviță (René Jeannel (1879–1965): 40 years from the death of the main collaborator of E. Racovitza). Oltenia. Stud. Com. Șt. Nat., 21: 233–234.
51. **Negrea Șt.**, Negrea A., **2006** – Colaborarea franco-română în domeniul biospeologiei. Oltenia. Stud. Com. Șt. Nat., 22: 231–236.
52. **Negrea Șt.**, Negrea A., **2007** – 110 years from the birth of the distinguished biologist Prof. Acad. Emil Pop (1897–2007). Oltenia. Stud. Com. Șt. Nat., 23: 251–254.
53. **Negrea Șt.**, Negrea A., **2008** – Academicianul Nicolae Botnariuc, nonagenar. Noema, Acad. Română, Comit. Rom. Ist. Filosof. Șt. Tehn., 7: 224–236.
54. **Negrea Șt.**, Negrea A., **2008** – Contribuția academicianului Mihai Băcescu la dezvoltarea etnozooologiei românești. Stud. Com. Divizia de Ist. Științei, Acad. Română, Comit. Rom. Ist. Filosof. Șt. Tehn., 1: 99–105.
55. **Negrea Șt.**, Negrea A., **2008** – Contribution to the history of science. Memoires about Prof. Acad. Constantin Motaș (1891–1980). Oltenia. Stud. Com. Șt. Nat., 24: 271–274.
56. **Negrea Șt.**, Negrea A., **2009** – Amintiri despre Prof. Acad. Constantin Motaș (1891–1980) – personalitate proeminentă a Biologiei românești, creatorul Freatobiologiei. Noema, Acad. Română, Comit. Rom. Ist. Filosof. Șt. Tehn., 8: 494–504.
57. **Negrea Șt.**, Negrea A., **2009** – Bicentenarul nașterii lui Charles Darwin (12 feb. 1809 – 19 apr. 1882): Omul și Opera. Stud. Com. Divizia de Ist. Științei, Acad. Română, Comit. Rom. Ist. Filosof. Șt. Tehn., 2: 233–259.
58. **Negrea Șt.**, Negrea A., **2009** – Two hundred years since the birth of Charles Darwin (12th of February 1809 – 19th of April 1882). The man and the work. Oltenia. Stud. Com. Șt. Nat., 25: 396–406.
59. **Negrea Șt.**, Negrea A., **2010** – Academicianul Nicolae Botnariuc la 95 de ani. Stud. Com., Divizia de Ist. Științei, Acad. Română, Comit. Rom. Ist. Filosof. Șt. Tehn., 3: 61–75.
60. **Negrea Șt.**, Negrea A., **2010** – Dr. Dumitru Murariu, membru corespondent al Academiei Române la 70 de ani. Stud. Com. Divizia de Ist. Științei, Acad. Română, Comit. Rom. Ist. Filosof. Șt. Tehn., 3: 91–106.
61. **Negrea Șt.**, Negrea A., **2011** – Despre un dicționar puțin cunoscut: primul dicționar enciclopedic de mediu din România. Noema, Acad. Română, Comit. Rom. Ist. Filosof. Șt. Tehn., 10: 649–651.
62. **Negrea Șt.**, Negrea A., **2011** – O culegere de documente inedite despre ctitorul Universității din Bacău: prof. dr. Gheorghe Hasan (6 iulie 1912 – 13 septembrie 2005). Stud. Com., Divizia de Ist. Științei, Acad. Română, Comit. Rom. Ist. Filosof. Șt. Tehn., 4: 461–463.

REFERENCES

- MURARIU D., 2012, *Alexandrina Negrea (23 August 1930 – 2 May 2011) – her life and scientific contributions*. Travaux de l'Institut de Spéologie "Émile Racovitza", Bucarest, **LI**: 91–104.
- NEGREA ȘT., 2006–2007, *Istoria dezvoltării biospeologiei în România după moartea lui Emil Racoviță*. Travaux de L'Institut de Speologie "Emile Racovitza", Bucarest, **XLV–XLVI**: 131–167.
- NEGREA ȘT., 2009, *Dr. Ștefan Negrea. Curriculum Vitae et Opera Omnia*. Studii și Comunicări, **II**: 81–86.

DIVERSITY AND SPATIAL DISTRIBUTION OF ZOOPLANKTON COMMUNITIES OF DANUBE DELTA LAKES

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The high diversity of aquatic communities offers stability and resilience to the ecosystems. The zooplankton has a particularly important role in aquatic ecosystems, by up-taking the organic matter synthesized by phytoplankton and transferring it to higher consumer orders, by grazing and facilitating bacterial decomposition processes, by contributing to the enrichment with nutrients of the water and benthic layers, etc. Our study was focused on assessing zooplankton diversity, spatial distribution and particularities in 21 selected lakes located in three main lake complexes of the Danube Delta. The structural parameters with key role for zooplankton diversity were assessed by linear regressions. The most representative taxonomic groups were Rotifera and Copepoda, the highest number of species and abundance being recorded in Matița-Merhei lake complex (164 species, 343 ind. L⁻¹). In the same complex, the Shannon index was positively correlated with species richness and evenness. In the Roșu-Puiu complex, a negative correlation between evenness and abundance was found as a result of the high number of accidental species; also, a positive correlation between evenness and Shannon index was recorded. The highest diversity was recorded in Isac-Gorgova lake complex, characterized by a positive relationship between abundance and the number of species and between Shannon index and evenness. The correlations with phytoplankton groups indicated the importance of Chlorophyceae and Cyanobacteria for the development of rotifers and copepods. The influence of environmental variables on zooplankton diversity was further tested to explain the dissimilarities between the lake complexes.

Keywords: Copepoda, diversity indices, population dynamics, Rotifera, shallow lakes.

INTRODUCTION

The zooplankton communities play a pivotal role in aquatic food webs as they represent a significant food for planktivorous fish and invertebrate predators. They graze on algae, bacteria, protozoa, and other small invertebrates.

Diversity is a very important trait of any community in a given ecosystem. The variation of diversity may strongly influence the functionality, stability and productivity of the ecosystem (Zhang *et al.*, 2012). Many authors use species richness as a measure of diversity (Stirling & Wilsey, 2001; Bock *et al.*, 2007; Gotteli & Chao, 2013). But community diversity includes two main components: number of species and evenness. Hence some authors recommend to treat these two components separately in order to investigate the determinants of diversity.

Examined along an increasing gradient of trophicity, the zooplankton community is represented by species like large cladocerans, diaptomids and cyclopids and small percentages of rotifers, in mesotrophic lakes, while in more eutrophic lakes, the structure is based on small species: small cladocerans, more copepod instars, and dominating rotifers (Gliwicz, 2005).

During early spring, the zooplankton community is dominated by small species, with high reproductive rates, like protozoans and rotifers, while large species, such as cladocerans and copepods, occur later; this dynamics is highly dependent on phytoplankton development and the predators pressure. Moreover, the particular conditions created by wind-induced mixing (Yoshida *et al.*, 2001; Gliwicz, 2005), raise additional difficulties to anticipate the distribution of zooplankton in shallow lakes.

With the beginning of summer, the zooplankton community is restructured as a consequence of temperature increase, dynamics of phytoplankton community and fish predation pressure; small zooplankton genera, such as *Thermocyclops*, *Mesocyclops*, *Bosmina*, *Chydorus*, *Diaphanosoma*, *Ceriodaphnia*, *Moina*, and rotifers prevail. As autumn progresses, large genera, like *Daphnia* and *Eudiaptomus*, start developing again. This is an effect of both the changes occurred in the phytoplankton community, when edible species have a distinct peak of development, and of the decreased predation pressure. During late autumn and winter, many zooplankton species enter a diapause phase (Sommer *et al.*, 1986).

Generally, the zooplankton diversity in Danube Delta lakes is very high, being influenced however by the natural and antropogenic drivers in the area. For instance, between 1975–1995, 562 species were recorded (Zinevici & Parpală, 2006), but their number fluctuated widely during this interval: e.g. due to a high impact of eutrophication, a drastic reduction of zooplankton diversity was recorded between 1975–1987 (53.08%) (Zinevici & Teodorescu, 1996; Zinevici & Parpală, 2000). Hence, investigations of freshwater zooplankton community structure could represent a valuable indicator for assessing aquatic ecosystem health (Rocha *et al.*, 1997; Pedrozo & Rocha, 2005; Şundri, 2015).

The objectives of this study were to assess the diversity of zooplankton and its spatial distribution in selected lakes of Danube Delta, as well as the state of these communities in 2013. Such information plays a major role for the conservation efforts of Danube Delta lakes.

MATERIAL AND METHODS

Study area

The Danube Delta Biosphere Reserve, located at 45°0'N latitude, 29°0'E longitude, in the Eastern part of Romania encompasses a complex of aquatic and terrestrial ecosystems unique in Europe.

The study was conducted during 2013, in 21 lakes belonging to three lake complexes of the Danube Delta: Roşu-Puiu, Matiţa-Merhei, Gorgova-Uzlina (Fig. 1).

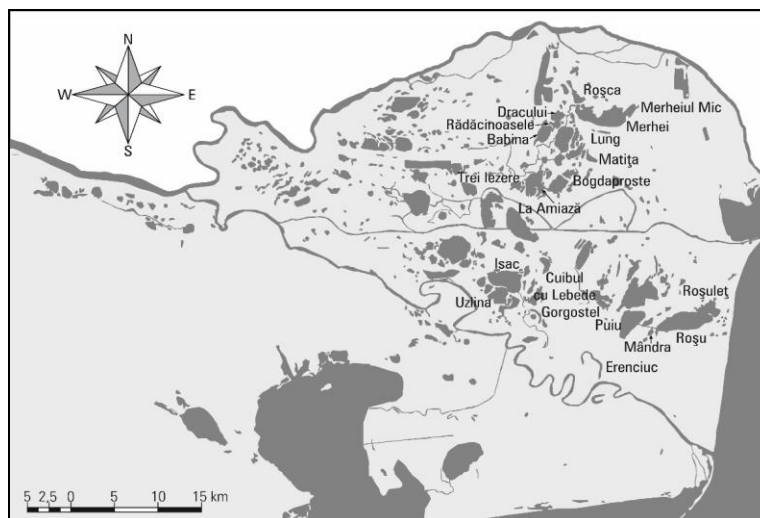


Fig. 1. The map of sampled lakes (orig.).

Table 1

List of the sampled lakes in the Danube Delta

Roșu-Puiu	Matia-Merhei	Gorgova-Isac
Roșuleț	Trei Iezere	Cuibul cu Lebede
Roșu	La Amiază	Isac
Mândra	Bogdaproste	Uzlina
Erenciuc	Matia	Gorgostel
Puiu	Merheiul Mic	
Tătaru	Lacul Lung	
	Merhei	
	Roșca	
	Dracului	
	Rădăcinoasele	
	Babina	

Field sampling and laboratory analysis

Three sampling campaigns were carried out seasonally: in May, July and September. The sampling point was located in the centre of the lake, samples being collected on water column. The redox potential, pH, conductivity, were measured in the field with a multiparameter WTW 340i. The turbidity was measured with a Hanna Instruments turbidimeter and the water velocity was estimated using a flowmeter. The phytoplankton samples (500 ml) were taken on the water column,

without filtration and conserved with formaldehyde 4% in order to estimate the abundance (cells L⁻¹). The total phytoplankton biomass and the biomass of different algal groups (expressed as chlorophyll *a* content, µg L⁻¹) were assessed *in situ* with a submersible fluorometer (Fluoroprobe III, bbe Moldaenke).

The zooplankton was sampled by filtering 50 L of water taken from the water column with a Patalas-Schindler plankton trap (5 L) and plankton nets (65 µm mesh size), concentrated in 10 ml and conserved with formaldehyde solution 4%.

Samples for chemical analyses were filtered through Whatman GF/F glass fibre filters and frozen for further analyses in the lab. Nutrients were determined spectrophotometrically following a modified Berthelot method for N-NH₄ (Krom, 1980), Griess-Ilosvay modified method for N-NO₂ (Keeney & Nelson, 1982), Tartari & Mosello (1997) for N-NO₃, P-PO₄ and TP.

The phytoplankton samples were counted in the lab using Utermöhl method (1958), a Zeiss inverted microscope, and specific keys.

Zooplankton species were identified using a Zeiss inverted microscope and the following keys: for Ciliata (Grospietsch, 1972; Foissner *et al.*, 1991–1995), Testacea (Bartoš, 1954), Lamellibranchia (Marsden, 1992), Rotifera (Voight, 1956; Rudescu, 1960), Cladocera (Brooks, 1959; Negrea, 1983), Copepoda (Damian-Georgescu, 1963, 1966–1970). At the same time with species identification, the individuals were counted to assess abundance (ind. L⁻¹) (Edmonson, 1971).

Data Analysis

For statistical processing, PAST (Hammer *et al.*, 2001) and XLSTAT software were used. The zooplankton diversity was evaluated by species richness, Shannon index and Evenness. A log transformation of zooplankton abundances, phytoplankton and physical-chemical data was applied for multivariate statistical analysis.

RESULTS AND DISCUSSION

The diversity assessment is based on species richness, abundance and also by the distribution of individuals in populations (Magurran, 1988; Legendre & Legendre, 1998; Vădineanu, 2004).

In our study, the highest value of species richness (164 species) was found in Matița-Merhei Lake complex and the lowest (117) in Gorgova-Isac complex (Table 2). The highest zooplankton abundance (343 ind. L⁻¹) was recorded also in Matița-Merhei complex, while the lowest abundance (216 ind. L⁻¹) was noticed in Gorgova-Isac.

Most of the zooplankton species belong to rotifers, ciliates and cladocerans (Table 2). In freshwater ecosystems, rotifers are known to be the dominant group, both, as species number and abundance (Berzins & Pejler, 1987; Barrabin, 2000; Saler, 2004).

Table 2

Species richness recorded in the three lake complexes of the Danube Delta in 2013

Taxa	Roşu-Puiu	Gorgova-Isac	Matîţa-Merhei
Ciliata	23	15	23
Testacea (Testate amoebae)	5	3	4
Lamellibranchia	1	1	1
Gastrotricha	1	1	1
Rotifera	78	80	103
Cladocera	22	12	20
Copepoda	3	4	11
Ostracoda	0	1	1
Total zooplankton	133	117	164

Also, in our study rotifers were the most abundant group, while cladocerans were not so well represented in abundance. The abundance of copepods (Crustacea) was very high, even if the number of species was low, due to the high number of juvenile stages (Table 3). The copepod communities are frequently dominated by juvenile instars (Pourriot *et al.*, 1997).

Table 3

Annual abundance (ind. L⁻¹) of the zooplankton communities
(1 – Ciliata; 2 – Testacea; 3 – Lamellibranchia; 4 – Gastrotricha; 5 – Rotifera;
6 – Cladocera; 7 – Copepoda; 8 – Ostracoda)

	1	2	3	4	5	6	7	8
Roşu-Puiu complex								
Roşuleţ	4.35	10.69	7.14	0.00	33.16	28.05	52.43	0.00
Roşu	16.95	35.76	6.91	0.00	94.55	8.56	39.69	0.00
Mândra	7.19	67.77	6.00	0.41	126.95	13.16	104.40	0.00
Erenciuc	4.67	57.00	0.00	0.21	45.79	1.49	73.17	0.00
Puiu	10.22	70.64	0.09	0.37	173.42	4.85	66.86	0.00
Tătaru	3.46	13.24	0.00	1.12	10.35	122.32	237.16	0.00
Gorgova-Isac complex								
Cuibul cu Lebede	1.51	7.46	0.00	0.26	26.17	0.36	14.14	0.00
Isac	2.40	2.42	0.10	0.00	29.24	1.66	236.03	0.00
Uzlina	3.16	5.87	1.27	0.00	129.12	0.99	102.15	0.28
Gorgostel	10.00	20.41	0.00	0.84	157.42	41.43	70.32	0.00

Table 3 (continued)

Matița-Merhei complex								
Trei Iezere	1.27	11.93	0.00	0.76	23.92	4.07	16.37	0.00
La Amiază	4.09	13.44	0.00	0.00	146.99	2.25	54.07	0.00
Bogdaproste	2.79	16.89	0.00	0.82	120.93	1.18	86.26	0.00
Matița	3.47	3.59	0.60	0.00	186.24	25.68	104.04	0.00
Merheiul Mic	19.01	12.61	0.07	0.28	257.62	12.27	61.00	0.13
Lacul Lung	1.19	17.03	0.00	0.00	21.92	10.36	78.66	0.40
Merhei	6.52	8.30	0.00	0.56	356.17	47.36	71.12	0.00
Dracului	11.50	8.33	0.00	0.82	656.67	14.80	126.83	0.00
Rădăcinoasele	9.33	3.79	0.00	0.00	342.75	11.15	92.68	0.00
Babina	1.73	2.05	0.00	0.11	113.45	3.77	219.97	0.00

Although the highest values of species richness and abundance were recorded in the lakes of Matița-Merhei complex, the Shannon-Wiener and evenness indices were lower than in other complexes. The highest diversity was recorded in Gorgova-Isac complex (Fig. 2).

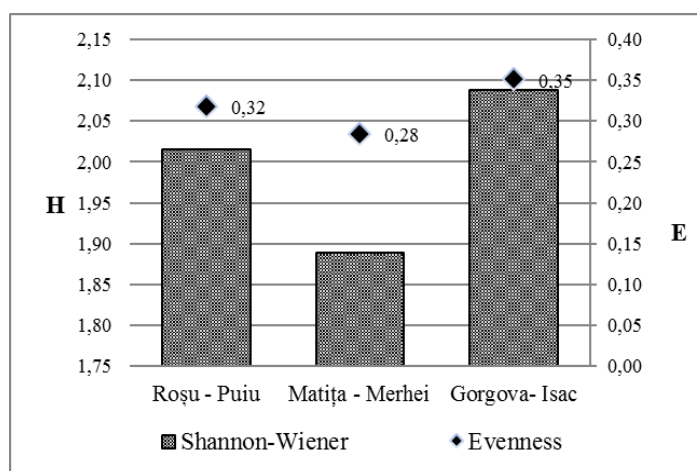


Fig. 2. Shannon-Wiener diversity index and evenness of the three investigated lake complexes.

In order to establish which structural parameter (species richness or abundance) had a decisive role in defining diversity, linear regressions were performed.

In Matița-Merhei complex, the Shannon-Wiener index was significantly influenced by the number of species in ecosystems (Table 4) while between abundance and evenness a negative relationship was found. Also, between Shannon-Wiener

and evenness indices a positive correlation was noticed. In addition, the evenness presented the lowest value in comparison with the other complexes.

Table 4

The relationship among diversity indices and abundance in Matița-Merhei complex

	R			p-values:
	Species richness	Abundance	Shannon	Evenness
Species richness		0.004	0.0001	0.39
Abundance	0.5		0.29	0.05
Shannon	0.73	0.2		0.01
Evenness	-0.16	-0.36	0.48	

All this explains that, even if the number of species and abundance was higher in Matița-Merhei comparing with Roșu-Puiu and Gorgova-Isac complexes, the individuals were not evenly distributed in the lakes. Some species were dominant or were concentrated in few lakes of the complex, which reduced the evenness and diversity. This was confirmed using Simpson's Index of dominance (D) that showed higher values, ranging between 0.59 and 0.72 in almost half of the lakes of the complex (Babina, Bogdaproste, Dracului, Matița, Lung, Merhei lakes).

In Gorgova-Isac, the complex with the highest diversity, a positive relationship was recorded between species richness and abundance (Table 5). As new species occurred, the abundance increased significantly, accompanied by evenness, in benefit of the diversity.

Table 5

The relationship among diversity indices and abundance in Gorgova-Isac complex

	R			p-values:
	Species richness	Abundance	Shannon	Evenness
Species richness		0.03	0.01	0.69
Abundance	0.62		0.99	0.08
Shannon	0.71	0.003		0.01
Evenness	0.13	-0.53	0.73	

The Shannon-Wiener diversity index from Roșu-Puiu complex was significantly influenced only by the evenness (Table 6). The abundance presented a positive correlation with species richness, while a negative relation was found with evenness. The output of these correlations supports the fact that abundance was the main parameter influencing the diversity, but this was reflected only for few species and in few lakes. As an example, 13% of the total number of species were present only once during the survey.

Table 6

The relationship among diversity indices and abundance in Roşu-Puiu complex

	R			p-values:
	Species richness	Abundance	Shannon	Evenness
Species richness		0.0008	0.11	0.07
Abundance	0.73		0.91	0.01
Shannon	0.40	-0.03		0.03
Evenness	-0.44	-0.60	0.52	

The diversity assessed in the 3 complexes reflects the environmental conditions existing in the ecosystems and also the functionality of the organisms forming the zooplankton communities. In our study, zooplankton was represented by eight taxonomic groups who contributed to diversity in various degrees, both through species richness and abundance. To assess the importance of different groups to the overall diversity of zooplankton community, Simper Analysis (similarity percentage analysis) was used. The results show that, in all the three studied complexes, Rotifera and Copepoda had the largest contribution (77.04 %) (Table 7).

Table 7

Results of SIMPER test indicating the contribution of major groups to zooplankton diversity

Taxa	Av. dissim	Contrib. %	Averages		
			Roşu-Puiu	Gorgova-Isac	Matia-Merhei
Rotifera	23.57	48.79	80.7	85.5	223
Copepoda	13.64	28.25	95.6	106	91.1
Testacea	4.94	10.22	42.5	9.04	9.8
Cladocera	4.46	9.23	29.7	11.1	13.3
Ciliata	1.07	2.21	7.81	4.27	6.09
Lamellibranchia	0.53	1.1	3.36	0.34	0.07
Gastrotricha	0.08	0.16	0.35	0.28	0.34
Ostracoda	0.02	0.03	0	0.07	0.05

The development of these groups was primarily based on the existing physical-chemical conditions and on the availability of the food resources. For zooplankton, there are two main food sources: phytoplankton communities and secondary detrito-bacterial particles/aggregates (Kim *et al.*, 2000; Freese & Martin-Creuzburg, 2013; Zinevici *et al.*, 2015).

During our investigation, a common trait of the three lake complexes was the fact that Rotifera consumed total phytoplankton, while copepods seem to prefer

cryptophyte algae, confirming the results of other studies (Antajan & Gasparini, 2004) (Tables 8–10). There is a big resemblance between the food sources used by zooplankton in Roşu-Puiu and Matia-Merhei complex. Although the rotifers and copepods groups prefer green algae and diatoms, in agreement with results for similar ecosystems (Work & Havens, 2003), in the Danube Delta they seem also well adapted to cyanobacterial consumption (Tables 8–9) even if these organisms could be inedible or even become toxic in certain conditions, especially in Roşu-Puiu complex, affected by a long-term eutrophication (Postolache, 2006). Also, the diatomids are able to use their long antenna and break the cyanobacterial filaments to consume them (Moriarty *et al.*, 1973). The third lake complex, Gorgova-Uzlina, shows a different pattern of biodiversity, due to different environmental factors (Table 10).

Table 8

Significant relationships of zooplankton, phytoplankton and environmental variables in Matia-Merhei ($p < 0.05$)

Variables	Ciliata	Testacea	Lamellibranchia	Gastrotrichia	Rotifera	Cladocera	Copepoda	Ostracoda
Bacillariophyceae ($\mu\text{g chl } a \text{ L}^{-1}$)					0.384	0.498	0.493	
Chlorophyceae ($\mu\text{g chl } a \text{ L}^{-1}$)					0.477		0.427	-0.363
Chryptophyceae ($\mu\text{g chl } a \text{ L}^{-1}$)		0.440			0.364		0.703	
Total chl. <i>a</i> ($\mu\text{g chl } a \text{ L}^{-1}$)					0.583	0.678	0.493	
Cyanobacteria (cells L^{-1})		0.471			0.600	0.483	0.734	
Pyrrophyceae (cells L^{-1})		0.492		0.375				0.480
Chrysophyceae (cells L^{-1})						0.354		
Bacillariophyceae (cells L^{-1})			-0.522				0.457	
Chlorophyceae (cells L^{-1})	0.398	0.437			0.686	0.526	0.780	
T (C)					0.361		0.548	
T (C) sediment		0.693		0.415	0.608	0.732	0.694	
pH		0.390			0.505		0.670	
pH sediment		0.689		0.413	0.633	0.748	0.705	
Cond. ($\mu\text{s/cm}$)							0.395	
Turbidity (NFU)		0.596			0.485	0.620	0.561	
Light intensity (lx)					0.345			
Water flow (counts/min)		0.669		0.436	0.648	0.643	0.614	
Water velocity (m/s)		0.462		0.394	0.526	0.664	0.544	
NH_4^+ ($\mu\text{g N L}^{-1}$)		0.535			0.549	0.559	0.779	
NO_3^- ($\mu\text{g N L}^{-1}$)							0.492	
PO_4^{3-} ($\mu\text{g P L}^{-1}$)		0.517			0.481	0.406	0.537	
TP ($\mu\text{g P L}^{-1}$)		0.384			0.425		0.585	

Table 9
Significant relationships of zooplankton, phytoplankton and environmental variables in Roşu-Puiu ($p < 0.05$)

Variables	Ciliata	Testacea	Lamellibranchia	Gastrotrichia	Rotifera	Cladocera	Copepoda	Ostracoda
Cyanobacteria ($\mu\text{g chl } a \text{ L}^{-1}$)	0.553	0.471			0.633		0.514	
Chlorophyceae ($\mu\text{g chl } a \text{ L}^{-1}$)		0.567					0.509	
Chryptophyceae ($\mu\text{g chl } a \text{ L}^{-1}$)		0.511					0.511	
Total chl. <i>a</i> ($\mu\text{g chl } a \text{ L}^{-1}$)	0.609	0.812			0.504		0.644	
Cyanobacteria (cells L^{-1})	0.576	0.550			0.722		0.744	
Euglenophyceae (cells L^{-1})			0.528					
Pyrrophyceae (cells L^{-1})	0.538			0.548			0.566	
Bacillariophyceae (cells L^{-1})		0.504			0.671		0.737	
Chlorophyceae (cells L^{-1})	0.513				0.750		0.796	
D (m)					0.490			
T (m)		-0.501						
T (C)					0.491		0.473	
T (C) sediment		0.626		0.471		0.494		
pH					0.640		0.617	
pH sediment	0.480	0.655					0.501	
Cond ($\mu\text{s/cm}$)					0.544		0.576	
Light intensity (lx)					0.541		0.535	
Water flow (counts/min)		0.652			0.469			
Water Velocity (m/s)		0.614						
NH_4^+ ($\mu\text{g N L}^{-1}$)		0.493						
PO_4^{3-} ($\mu\text{g P L}^{-1}$)				0.579			0.486	
TP ($\mu\text{g P L}^{-1}$)					0.585		0.649	

The environmental parameters are key drivers for the dynamics of biological communities, influencing both phyto- and zooplankton populations (Basu & Pick, 1997; Heneash *et al.*, 2015). Although there is no general agreement regarding all the factors regulating phytoplankton and zooplankton communities in different

aquatic ecosystems (Reynolds, 1988), evidence shows that light, water velocity, temperature, nutrients, and xenobiotics modulate the development of plankton communities and interspecific competitions.

Table 10
Significant relationships of zooplankton, phytoplankton and environmental variables in Gorgova-Isac ($p < 0.05$)

Variables	Ciliata	Testacea	Lamellibranchia	Gastrotrichia	Rotifera	Cladocera	Copepoda	Ostracoda
Chryptophyceae ($\mu\text{g chl } a \text{ L}^{-1}$)							0.591	
Total chl. <i>a</i> ($\mu\text{g chl } a \text{ L}^{-1}$)		0.643			0.694	0.688		
Cyanobacteria (cells L^{-1})				0.561				
Euglenophyceae (cells L^{-1})	0.716				0.678			
Pyrrophyceae (cells L^{-1})								
Chrysophyceae (cells L^{-1})								
Bacillariophyceae (cells L^{-1})	0.605							
pH							0.596	
NO_3^- ($\mu\text{g N L}^{-1}$)							-0.772	

The investigations carried out in the three lake complexes emphasized that pH was a common factor influencing the development of copepods (Tables 8–10), but the other physical and chemical parameters modulating zooplankton communities had a different influence in each lake complex: while in Gorgova-Isac only nitrates influenced copepods development, in the other two complexes TP and temperature influenced both rotifers and copepods (Tables 8–10). In Matia-Merhei complex, rotifers, cladocerans, copepods and also testaceans are influenced by turbidity, water velocity and NH_4^+ content (Table 8).

The hydrogeomorphological features of the ecosystems have also key role in defining the development of plankton communities, habitat heterogeneity influencing the distribution of zooplankton individuals. The distribution of zooplankton communities in the three investigated complexes was analyzed based on Detrended Correspondence Analysis (DCA). The results show that although rotifers did not seem affected by the hydrogeomorphological characteristics, being widely spread in all the lake complexes, crustaceans (Cladocera and Copepoda) were found especially in Roşu-Puiu and Gorgova-Isac complexes (Fig. 3).

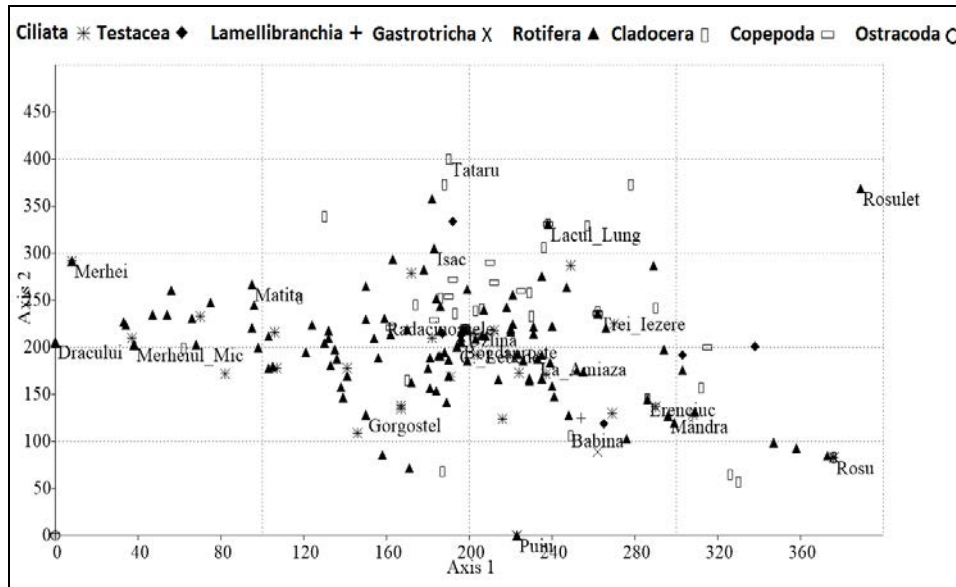


Fig. 3. Detrended correspondence analysis (DCA) of the zooplankton species distribution in the Danube Delta lakes.

CONCLUSIONS

Diversity is an important tool to assess the complexity of a community and its stability. In our study, the zooplankton diversity was assessed based on species richness and evenness. In lake complexes exhibiting a negative correlation between evenness and abundance, a higher presence of accidental species was found, determining a decrease of diversity indexes.

Taxonomic groups with significant role in defining traits of diversity were rotifers and copepods. Their role depended on both, community structure and spatial distribution. Water velocity, pH, temperature, turbidity, and nutrients modulated the development of zooplankton communities during the investigated period.

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REFERENCES

- ANTAJAN E., GASPARINI S., 2004, *Assessment of Cryptophyceae ingestion by copepods using alloxanthin pigment: a caution*. Marine Ecology Progress Series, **274**: 191–198.
- BARRABIN J. M., 2000, *The rotifers of Spanish reservoirs: Ecological, systematical and zoogeographical remarks*. Limnetica, **19**: 91–114.
- BARTOŠ E., 1954, *Koreôono ce Radu Testacea*, Vydavatel'stvo Slovenskej Akadémie Vied Bratislava, 190 pp. (in Slovak)
- BASU B. K., PICK F.R., 1997, *Phytoplankton and zooplankton development in a lowland, temperate river*. Journal of Plankton Research, **19** (2): 237–253.
- BERZINS B., PEJLER B., 1987, *Rotifer occurrence in relation to pH*. Hydrobiologia, **147**: 107–116.
- BOCK C. E., JONES Z. F., BOCK J. H., 2007, *Relationships between species richness, evenness, and abundance in a southwestern savanna*. Ecology, **88** (5): 1322–7.
- BROOKS J. L., 1959, *Cladocera*. Freshwater Biology. Ward H. B. and G. C. Whipple G. C., 587–656.
- DAMIAN-GEORGESCU A., 1963, *Fauna Republicii Socialiste România. Crustacea, vol. IV, Fascicula 6, Copepoda. Cyclopidae*. Edit. Acad. R. S. R., 204 pp..
- DAMIAN-GEORGESCU A., 1966, *Fauna Republicii Socialiste România. Crustacea vol. IV, Fascicula 8, Copepoda. Calanoida*. Edit. Acad. R. S. R., 130 pp..
- DAMIAN-GEORGESCU A., 1970, *Fauna Republicii Socialiste România. Crustacea vol. IV, Fascicula 11, Copepoda. Harpacticoida*, Edit. Acad. R. S. R., 248 pp..
- EDMONDSON W. T., 1971, *Counting zooplankton samples*. In: Edmonson W. T. & Winberg G. G. (Eds.), *A manual on the methods for the assessment of secondary productivity in freshwaters (IPB Handbook 17)*. Blackwell Scientific Publications, Oxford and Edinburg, 358 pp..
- FOISSNER W., BLATTERER H., BERGER H., KOHMANN F., 1991, *Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band I: Cyrtophorida, Oligotrichida, Hypotrichia, Colpodea*. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft, **1** (91), 478 pp..
- FOISSNER W., BERGER H., KOHMANN F., 1992, *Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems, Band II: Peritrichia, Heterotrichida, Odontostomatida*. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft, **5** (92), 502 pp..
- FOISSNER W., BERGER H., KOHMANN F., 1994, *Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems, Band III: Hymenostomata, Prostomatida, Nassulida*. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft, **1** (94), 548 pp..
- FOISSNER W., BERGER H., KOHMANN F., 1995, *Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band IV: Gymnostomatea, Loxodes, Suctorina*. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft, **1** (95), 540 pp..
- FREESE H. M., MARTIN-CREUZBURG D., 2013, *Food quality of mixed bacteria–algae diets for Daphnia magna*. Hydrobiologia, **715** (1): 63–76.
- GLIWICZ Z. M., 2005, *Food web interactions: why are they reluctant to be manipulated?* Verhandlungen des Internationalen Verein Limnologie, **29**: 73–88.
- GOTELLI N. J., CHAO A., 2013, *Measuring and Estimating Species Richness, Species Diversity, and Biotic Similarity from Sampling Data*. In: Encyclopedia of Biodiversity, Levin S.A. (Ed.) second edition, Vol. 5, Waltham, MA: Academic Press: 195–211.
- GROSPIETSCH TH., 1972, *Wechsel-tierchen (Rhizopoden)*. Kosmos-Verlag Franckh'sche Verlagshandlung, 80 pp..
- HAMMER O., HARPER D. A. T., RYAN P. D., 2001, *PAST: Paleontological statistics software package for education and data analysis*. Palaeontologia Electronica.
- HENEASH A. M. M., TADROSE H. R. Z., HUSSEIN M. M. A., HAMDONA S. K., ABDEL AZIZ N., GHARIB S. M., 2015, *Potential effects of abiotic factors on the abundance and distribution of the plankton in the Western Harbour, south-eastern Mediterranean Sea, Egypt*. Oceanologia, **57** (1): 61–70.

- KEENEY D. R., NELSON D. W., 1982, *Nitrogen-inorganic forms*. In: Page A. L.(Ed.), *Methods of Soil Analysis*. Part 2, Agronomy Monograph 9, 2nd ed., Madison, WI: ASA and SSS, pp.: 643–698.
- KIM H. W., S. J. HWANG G. J. JOO, 2000, *Zooplankton grazing on bacteria and phytoplankton in a regulated large river (Nakdong River, Korea)*. Journal of Plankton Research, **22** (8): 1559–1577.
- KROM M. D., 1980, *Spectrophotometric determination of ammonia: a study of a modified Berthelot reaction using salicylate and dichloroisocyanurate*. Analyst, **105**: 305–316.
- LEGENDRE P., LEGENDRE L., 1998, *Numerical ecology*. Elsevier, Oxford, 853 pp..
- MAGURRAN A. E., 1988, *Ecological diversity and its measurement*. Press Princeton, Princeton University, 256 pp..
- MARSDEN J. E., 1992, *Standard protocols for monitoring and sampling zebra mussels*. Illinois Natural History Survey Biological Notes, **138**, 37 pp..
- MORIARTY D. J. W., 1973, *The physiology of digestion of bluegreen algae in the cichlid fish, Tilapia nilotica*. Journal of Zoology, **171** (1): 25–39.
- NEGREA ȘT., 1983, *Cladocera*. In: Fauna Republicii Socialiste România. Edit. Academiei R. S. R., 4, 399 pp..
- PEDROZO C. D. A. S., ROCHA O., 2005, *Zooplankton and water quality of lakes of the Northern Coast of Rio Grande do Sul State, Brazil*. Acta Limnologica Brasiliensis, **17** (4): 445–464.
- POSTOLACHE C., 2006, *The chemistry of the Danube Delta*. In: Tudorancea C., Tudorancea M. M., (Eds.), *Danube Delta, Genesis and Biodiversity*. Backhuys Publishers, Leiden: 65–93.
- POURRIOT R., ROUGIER C., MIQUELIS A., 1997, *Origin and development of river zooplankton: Example of the Marne*. Hydrobiologia, **345**: 143–148.
- REYNOLDS C. S., 1988, *Potamoplankton: paradigms, paradoxes and prognoses*. In: Round F. F. (Ed.), *Algae and the aquatic environment*. Biopress: 285–311.
- ROCHA O., MATSUMURA-TUNDISI T., SAMPAIO E. V., 1997, *Phytoplankton and zooplankton community structure and production as related to trophic state in some Brazilian lakes and reservoirs*. Verhandlungen des Internationalen Verein Limnologie, **26**: 599–604.
- RUDESCU L., 1960, *Rotatoria*. In: Fauna României. Trochelinthes, 2 (2), Edit. Acad. R. P. R., 1192 pp..
- SALER S., 2004, *Observations on the seasonal variation of Rotifera fauna of Keban Dam Lake (Cemisgezek Region)*. Science and Engineering Journal of Firat University, **16** (4): 695–701.
- SOMMER U., GLIWICZ Z. M., LAMPERT W., DUNCAN A., 1986, *The PEG-model of seasonal succession of planktonic event in fresh waters*. Archiv für Hydrobiologie, **106** (4): 433–471.
- STIRLING G., WILSEY, B., 2001, *Empirical Relationships between Species Richness, Evenness, and Proportional Diversity*. The American Naturalist, **158**: 286–299.
- ȘUNDRI M. I., 2015, *Danube zooplankton diversity in Cernavoda area*. Annals of Maritime University, Constanța, **23**: 81–86.
- TARTARI G., MOSELLO R., 1997, *Metodologie analitiche e controlli di qualita nel laboratorio chimico dell'Istituto Italiano di Idrobiologia*. Documenta dell'Istituto Italiano di Idrobiologia, no 60, Consiglio Nazionale delle Ricerche, Verbania, Italia, 160 pp..
- UTERMÖHL H., 1958, *Zur Vervollkommnung der quantitative Phytoplankton-Methodik*. Mitteilungen Internationale Vereinigung für Theoretische und Angewandte, Limnologie, **9**: 1–38.
- VĂDINEANU A., 2004, *The management of development. A systemic approach*. Edit. Ars Docendi, University of Bucharest, 394 pp. (in Romanian)
- VOIGHT M., 1956, *Rotatoria – Die Rädertiere Mitteleuropa*. Edit. Gebrüder Borntraeger – Berlin – Nikolasse, 508 pp..
- WORK K. A., HAVENS K. E., 2003, *Zooplankton grazing on bacteria and cyanobacteria in a eutrophic lake*. Journal of Plankton Research, **25** (10): 1301–1306.
- YOSHIDA T., KAGAMI M., GURUNG T. B., URABE J., 2001, *Seasonal succession of zooplankton in the north basin of Lake Biwa*. Aquatic Ecology, **35** (1): 19–29.

- ZHANG H., JOHN R., PENG Z., YUAN J., CHU C., GUOZHEN D., SHURONG Z., 2012, *The Relationship between Species Richness and Evenness in Plant Communities along a Successional Gradient: A Study from Sub-Alpine Meadows of the Eastern Qinghai-Tibetan Plateau, China*. PLOS ONE, **7** (11): 1–9.
- ZINEVICI V., PARPALĂ L., 2000, *The zooplankton in the Danube lacustrine ecosystems. I. Multiannual means of the structure, productivity and biomass recylation*. Revue Roumaine de Biologie, Série de Biologie Animale, **45** (1): 13–25.
- ZINEVICI V., PARPALĂ L., 2006, *The zooplankton structure and productivity in Danube Delta lacustrine ecosystems*. In: Tudorancea C., Tudorancea M. M. (Eds.), *Danube Delta, Genesis and Biodiversity*, Backhuys Publishers, Leiden: 177–210.
- ZINEVICI V., TEODORESCU L., 1996, *The structure dynamics of the zooplankton from Danube Delta big lakes in the 1975-1995 period*. Scientific Annals of ICPDD, **5**: 63–75.
- ZINEVICI V., FLORESCU L., PARPALĂ L., MOLDOVEANU M., 2015, *The zooplankton of Lake Snagov: diversity, structure and production*. Romanian Journal of Biology – Zoology, **59** (2): 129–142.

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PRESENCE OF *BRANCHIOSTOMA LANCEOLATUM* (PALLAS, 1774) LARVAE IN THE BLACK SEA ROMANIAN WATERS

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The paper reports the first occurrence of *Branchiostoma lanceolatum* (Pallas, 1774) larvae in the Romanian waters in samples collected in July and August 2018 in four stations located in coastal and offshore waters of the Romanian shelf.

Keywords: *Branchiostoma lanceolatum*, Black Sea, Romania.

INTRODUCTION

After almost fifty years since the first observation of an adult of *Branchiostoma lanceolatum* (Pallas, 1774) in the benthic samples collected in shallow waters of the Romanian littoral (Gomoiu, *pers. comm.*), here we report the first occurrence of pelagic larvae of the species in the national waters.

Branchiostoma lanceolatum is known as a relatively rare species in the Black Sea. Grigore Antipa, during the expedition performed in 1893 all around the Black Sea, documented the presence of amphioxus from Sinope, on the northern Anatolian coast, and from Sevastopol, where it had been found by Ostroumov on coarse sands in association with *Ophelia taurica*, *Polygordius ponticus*, *Glycera* sp., and two species of *Synapta* (Antipa, 1941).

Only few recent records in literature were found reporting, for example, abundances of 1,109 indiv. m⁻² (Luth, 2004) in 2004. In the period 1999–2014, *B. lanceolatum* was found in 38% of the samples collected on the Crimean soft bottoms (Shalovenkov, 2017). Shiganova *et al.* (2010) signals the appearance of specimens of the lancelet larvae (1.5–5 mm in length) in the layer of 0–160 m above 500 m (sampling point: 44°513' N, 37°933' E) at a temperature of 26°C, inferring its potential intrusion in the Black Sea from the Mediterranean Sea. At the Bulgarian coast, it forms in shallow waters (10–30 m) on shelly, medium and coarse sand an association with *Protodorvillea kefersteini* and *Gouldia minima* (Todorova *et al.*, 2015; Todorova, 2017). Lately (2011–2017), *B. lanceolatum* larvae have been constantly observed in the summer-autumn season mainly at depths of

15–40 m in coastal and inner shelf area and rarely, on the outer shelf and open sea. Its average abundance varies insignificantly around 2 ind.m^{-3} ($SD \pm 5$), an exception being recorded in front of Ropotamo river where a peak density of 32 ind.m^{-3} was found in 2016 (Stefanova K. & Stefanova E., *pers. data*). To our knowledge, no data is available for the Black Sea, from the Turkish and Georgian coast.

This species has been included in the Black Sea Red Data Book and in the Vulnerable category according to the IUCN criteria, in 1996, due to its continuous decreasing population since the 60's as result of anthropogenic pressure (Konsulova, in Dumont, 1999). Most data in the literature suggest that its demise is generally related to the change in texture and eutrophication of the coastal sediments.

MATERIAL AND METHOD

The larvae were found in the zooplankton samples collected by a Juday net with 150μ mesh size, deployed at certain depths in the water column in July and August 2018. Out of the eight collected larvae, five specimens were found in the samples collected in front of Mamaia Bay at depths between 23–25 m, and the other three in the samples taken along Sf. Gheorghe and Constanţa transects in offshore waters (Fig. 1).

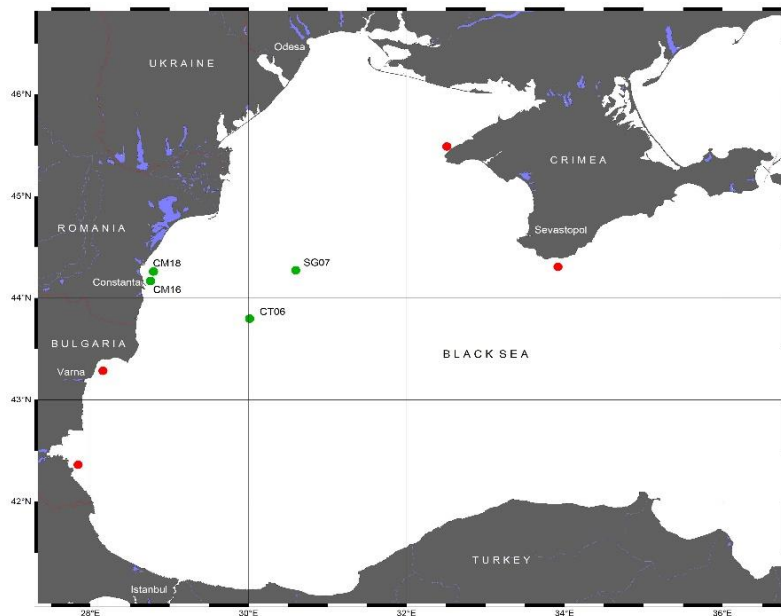


Fig. 1. The sampling stations (green points) where *B. lanceolatum* larvae occurred in July and August 2018 at the Romanian coast and its occurrence (red points) (according to Konsulova, 1999).

RESULTS

Description of larvae

Along the body three regions are seen: the head with the pharyngeal region, the midgut, the hindgut with the anus opening and the spear like tail. The notochord and the nerve chord with pigment spots span along entire body length. In the anterior head region the main observable morphological features were: the preoral organ, the club shaped gland and the anterior and posterior endostyle ridges (Fig. 2b). Within the pharyngeal part, 10–11 primary gill slits were accounted, while a canal-like atrial chamber formed from the level of the third posterior gill slit to the anterior midgut region (atriopore) was noticed (Fig. 2c). In the hindgut, the cells forming the wall corresponding to the future ileocolonic ring (Urata *et al.*, 2007) of brownish colour and the anal opening could be seen (Fig. 2d). The larvae were 5–5.5 mm long, flattened in transverse plan. After all appearances, the larvae were in their late stage of development (premetamorphic larvae). *B. lanceolatum* larvae metamorphose with at least 14 gill slits, 45 to 50 days after fertilization at 23°C (Fuentes *et al.*, 2007).

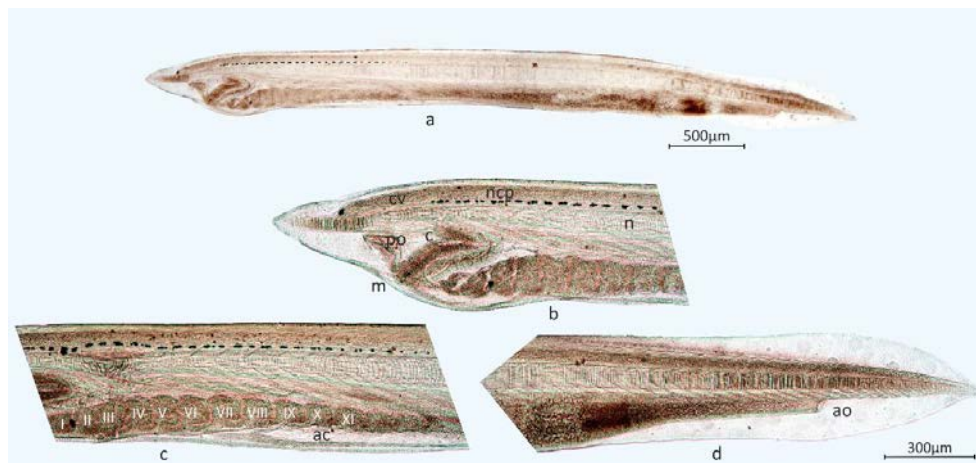


Fig. 2. a. Larva before metamorphosis into juvenile; b. Left sided head region of larva, cv. – cerebral vesicle, ncp.– nerve cord with pigment spots; c. – club-shaped gland; m. – mouth margin; n. – notochord; po. – preoral organ; c. Branchial region of larva with 11 primary gill slits (I–XI); ac – the canal-like atrial chamber formed from the level of the third gill slit to the anterior midgut region; d. Hindgut with brownish wall cells and the ao – anal opening on the left body side, and the spear shaped tail region (Photo: Teacă A.).

Distribution of specimens found at the Romanian littoral

The 4 specimens collected in July in the station CM 16 were found in the upper surface layer of 0–5 m depth as well as within the thermocline layer at 5–15 m, respectively. Likewise, in the station CM18 the fifth specimens was collected also within the thermocline layer at 10–18 m depth. The distribution of all three specimens

found in August was confined to the under thermocline layers. The larvae is believed to execute diurnal vertical migration due to their light-guided behaviour (Pergner & Kozmik, 2017). Their diet include algae such as diatoms, dinoflagellates, e.g., *Ceratium* (Kehayias, 2015), the latter being present in great abundances during sampling period. However, the larvae ingest also calanoid copepods or other organic material and small particles of a size similar to that of the larval mouth (Webb, 1969).

The salinity and temperature at the sampling site varied between 17.19 (surface layer) and 18.91 PSU (under thermocline layer) and 23.97°C (surface) and 8.41°C (bottom layer) (Table 1). In the Black Sea, the species has a reproductive peak during the summer season (Konsulova, 1992).

Table 1

Physico-chemical parameters of seawater at depths where larvae have been collected in July and August 2018

Station	CM16	CM16	CM18	SG07	CT06
Date	23.07.2018	23.07.2018	24.07.2018	20.08.2018	21.08.2018
Longitude [deg. E]	28.75747	28.75747	28.79524	30.59493	30.01361
Latitude [deg. N]	44.16838	44.16838	44.26112	44.27365	43.79941
Bot Depth [m]	27.4	27.4	27	107	89.7
Depth [m]	0–5	5–15	10–18	25–60	35–85
Chla [ug/L]	0.41	9.89	9.67		
T °C	23.97	12.73	13.65	10.11	8.41
PSU	17.19	18.49	18.57	18.46	18.91
O ₂ mg.L ⁻¹	7.24	7.06	7.95		6.99
Substrate type	coarse (shell debris)	coarse (shell debris)	sand	shelly mud	shelly mud
No. Ind./sample	2	2	1	2	1

DISCUSSION

At this moment there is no evidence for the species presence in the adult stage on the Romanian shelf. In our opinion, there are three hypothetic explanations that could be drawn concerning the larvae appearance.

Firstly, it is likely that adult populations to be actually present in the Romanian waters, but with distribution and abundances limited to few habitats, such as the sandy bottoms from the coast and/or circalittoral. For example, the habitats investigated in July correspond to the circalittoral sand and circalittoral coarse (shell debris) sediments (Teacă *et al.*, 2018) that are likely suitable for adults dwelling and spawning.

Secondly, the Romanian sector is on the north-south cyclonic current trajectory, thus the larvae could have been conveyed in our research area simultaneously with the water masses from the Crimean coast.

Third, the favourable hydrometeorological conditions in the collecting period and especially in the previous days, characterised by predominantly W-NW winds that favoured upwelling and increased surface and subsurface cyclonic and anticyclonic eddies in the region of the Romanian shelf.

CONCLUSIONS

After more than fifty years since first observation of an adult of *Branchiostoma lanceolatum* at the Romanian littoral (Gomoiu, *pers. comm.*), here we report the first occurrence of pelagic larvae of the species, eight specimens being found in the coastal and offshore Romanian waters within the surface and under thermocline layers.

Three hypothesis regarding their occurrence were assumed: one, the species actually lives within the Romanian habitats but its presence in adult stage has not been observed due to its limited distribution and abundance, the second, the larvae were drifted from the Crimean coast to the Romanian waters with the north-south cyclonic current, and the third, as result of favourable hydromorphological conditions prior and during the sampling period.

Acknowledging that the species has already been declared in the Vulnerable category at the Black Sea level, according to the IUCN criteria and has been enlisted in the 1999 Black Sea Red Data Book, we also propose re-evaluation of its status at the Romanian littoral if its presence will be further confirmed.

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REFERENCES

- ANTIPA GR., 1941, *Marea Neagră, Vol. I. Oceanografia, Bionomia și Biologia generală a Mării Negre*. Imprimeria Națională, București, 313 pp..
- FUENTES M., BENITO E., BERTRAND S., PARIS M., MIGNARDOT A., GODOY L., JIMENEZ-DELGADO S., OLIVERI D., CANDIANI S., HIRSINGER E., D'ANIELLO S., PASCUAL-ANAYA J., MAESO I., PESTARINO M., VERNIER P., NICOLAS J. F., SCHUBERT M., LAUDET V., GENEVIÈRE A. M., ALBALAT R., GARCIA FERNANDEZ J., HOLLAND N. D., ESCRIVA H., 2007, *Insights into spawning behavior and development of the European amphioxus (Branchiostoma lanceolatum)*. Journal of Experimental Zoology, Part B, Molecular and Developmental Evolution, **308** (4): 484–93.

- KEHAYIAS G., 2015, *Spatial and temporal variation of Branchiostoma lanceolatum larvae (Cephalochordata) in a hypoxic bay*. *Biologia*, **70** (9): 1234–1244.
- KONSULOVA T., 1992, *Seasonal structure and ecological status of Varna Bay (Black Sea) sandy and muddy macrozoobenthic coenoses*. *Rapp. Comm. Int Mer Medit.*, **33**: 42.
- KONSULOVA T., 1999, *Amphioxus lanceolatum* Pallas, 1774. In: DUMONT H., 1999. *Black Sea Red Data Book*, UNOPS, New York, 413 pp..
- LUTH U., 2004, *The benthos of the oxic/anoxic interface in the western Black Sea: comparative macro-and meiofauna investigations on transects from the Ukrainian, Romanian and Turkish shelf: 2–70*. In: ÖZTÜRK B., MOKIEVSKY V. O., TOPALOĞLU B. (Eds.), *International Workshop on Black Sea Benthos*. Published by Turkish Marine Research Foundation Turkey, 245 pp..
- PERGNER J., KOZMIK Z., 2017, *Amphioxus photoreceptors - insights into the evolution of vertebrate opsins, vision and circadian rhythmicity*. *International Journal of Developmental Biology*, **61**: 665–681.
- SHALOVENKOV N., 2017, *Non-native zoobenthic species of the Crimean Black Sea Coast. Mediterranean Marine Science*. DOI: <http://dx.doi.org/10.12681/mms.1925>
- SHIGANOVA T. A., MUSAIEVA E. I., LUKASHEVA T. A., STUPNIKOVA A. N., ZAS'KO D. N., ANOKHINA L. L., SIVKOVICH A. E., GAGARIN V. I., BULGAKOVA YU. V., 2012, *Increase in Findings of Mediterranean Nonnative Species in the Black Sea*. *Russian Journal of Biological Invasions*, **3** (4): 255–280.
- TEACĂ A., MUREŞAN M., BEGUN T., POPA A., 2018, *Marine benthic habitats within a physical disturbed site from the Romanian coast of the Black Sea*. Pp.: 231-236. In: ÖZCAN G., TARKAN A. S., ÖZCAN T. (Eds.), *Proceeding Book, International Marine & Freshwater Sciences Symposium, 18–21 October 2018, Kemer-Antalya, Turkey*, 402 pp..
- TODOROVA V., 2017, *Macrozoobenthos*. In: *Monitoring report "Analysis of marine environmental status – 2016" Agreement -33-5/28.01.2016 between MEW and IO-BAS for implementation of obligations under the monitoring of the Black Sea, on the grounds of Article 17, Paragraph 2, item 3 of the Water Act*, pp.: 207–232 (in Bulgarian)
- TODOROVA V., DIMITROV L., DONCHEVA V., TRIFONOVA E., PRODANOV B., 2015, *Benthic Habitat Mapping in the Bulgarian Black Sea*. *Proceedings of the Twelfth International Conference on the Mediterranean Coastal Environment 15, 06-10 October 2015, Varna, Bulgaria, Mediterranean Coastal Foundation, Dalyan, Mugla, Turkey*, **1**: 251–262.
- URATA M., YAMAGUCHI N., HENMI Y., YASUI K., 2007, *Larval Development of the Oriental Lancelet, Branchiostoma belcheri, in Laboratory Mass Culture*. *Zoological Science*, **24**: 787–797.
- WEBB J. E., 1969, *On the feeding and behaviour of the larva of Branchiostoma lanceolatum*. *Marine Biology*, **3**: 58.

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EFFECT OF TEMPERATURE ON THE VIRULENCE OF ENTOMOPATHOGENIC NEMATODES

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MZIA KOKHIA*, MADONA KUCHAVA*

Nowadays, the use of entomopathogenic nematodes as a biological control agent is a key component in IPM system. Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* (Nematoda: Rhabditida) are extraordinarily lethal to many important insect pests, yet are safe for plants and animals. They are the only insect-parasitic nematodes possessing an optimal balance of biological control attributes. The effect of temperature on the virulence of three species of entomopathogenic nematodes, *Steinernema thesami*, *Heterorhabditis bacteriophora* and *Steinernema feltiae* was investigated. Last instar of *Tenebrio molitor* Linnaeus, 1758 larvae were chosen for experiment. In the laboratory, all three nematode species successfully reproduced inside *T. molitor* larvae. *H. bacteriophora* produced the highest number of infective juveniles per larva at 30°C than *S. feltiae* and *S. thesami*. *S. feltiae* caused the highest mortality of larvae at 20°C, whereas *S. thesami* infected *T. molitor* larvae at the widest temperature range and killed insects between 10–33°C. Based on the present study, we indicate that entomopathogenic nematodes have well-defined thermal breadths for their development and reproduction.

Keywords: entomopathogenic nematodes, *Heterorhabditis*, *Steinernema*, *Tenebrio molitor*, biocontrol.

INTRODUCTION

Entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) are soil-inhabiting insect parasites that possess potential as biological control agents (Gaugler & Kaya, 1990; Kaya & Gaugler, 1993). These nematodes have a symbiotic association with bacteria of the genus *Xenorhabdus* (Akhurst & Boemare, 1990). The bacteria convert the insects into a suitable environment for development and reproduction of the nematodes' parasitic stages (Poinar, 1990). The only function of the infective juveniles is to locate and parasitize new host (Grewal *et al.*, 1994). Variation among species of entomopathogenic nematodes for temperature tolerance has been reported (Grewal *et al.*, 1993). The aim of this work was to determine thermal factors for infection and reproduction of three species of entomopathogenic nematode: *Steinernema thesami*, *Heterorhabditis bacteriophora* and *Steinernema feltiae* (Gorgadze *et al.*, 2016) in laboratory conditions. The species *Neoplectana*

(= *Steinernema*) *thesami* was isolated in Mtskheta-Mtianeti Region of Georgia, from infected pupa of a winter moth, *Operophtera brumata* Linnaeus, 1758 (Lepidoptera: Geometridae). The isolate of *S. thesami* was maintained in the laboratory of enthomopathogenic nematodes of the Institute of Zoology of Ilia State University, Tbilisi, Georgia.

MATERIAL AND METHODS

Nematodes were reared at 25°C in last instar larvae of the wax moth, *Galleria mellonella*, according to procedures described by (Woodring & Kaya, 1998). The infective juveniles (IJs) that emerged from cadavers were recovered using modified White traps (White, 1927), and stored at 7°C for 7–14 days before use (Kaya & Stock, 1997).

Infectivity of nematodes to last instar of *Tenebrio molitor* at 8–35°C was tested in a sand – based assay (Grewal *et al.*, 1994). Fifty infective juveniles of a nematode species in 200 µl of distilled water were inoculated into a 5 cm diameter Petri dish containing 3 g dry sand. One last instar larva of *T. molitor* was placed on dish. The dishes were wrapped with parafilm to reduce desiccation. Insect mortality was recorded during 20 days. Dead larvae were removed from sand, washed in distilled water, dissected and the number of nematodes established recorded. Three Petri dishes were prepared for each species and for each temperature.

Nematode reproductive potential was evaluated in *T. molitor* larvae. Five last instars larvae were exposed to 500 IJs of each species on a filter paper in 10 cm diameter Petri dish. Dead larvae were transferred to White traps for the recovery of a new generation of IJs. After the start of emergence (from 5 to 14 days), IJs were collected and counted. Total number of IJs produced per host was then determined.

Both treatment was replicated four times, included untreated control dishes, which received only distilled water. Mortality percentage was recorded and corrected with Abbott formula (Abbott, 1925).

RESULTS AND DISCUSSION

Thermal effect for infection and reproduction differed among nematode species.

Infection. *S. thesami* infected *T. molitor* larvae at the widest temperature range and killed insects at 10–33°C, *H. bacteriophora* infected host at between 10–32°C. The two species of nematodes *S. thesami* and *H. bacteriophora* wide killed insects between 20–32°C and 10–30°C, whereas *S. feltiae* infected host at the narrow temperature range (10–25°C) and caused the highest mortality to larvae at 20°C (Fig. 1).

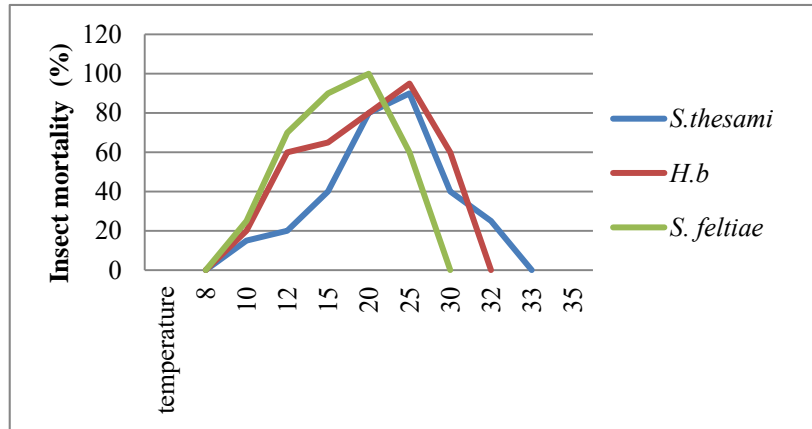


Fig. 1. Mortality of *Tenebrio molitor* larvae by entomopathogenic nematodes at different temperature.

Reproduction. Thermal breadth for reproduction was for *S. thesami* (20–32°C), for *H. bacteriophora* from 15–30°C, whereas *S. feltiae* reproduced at cooler temperatures (12–25°C) (Fig. 2).

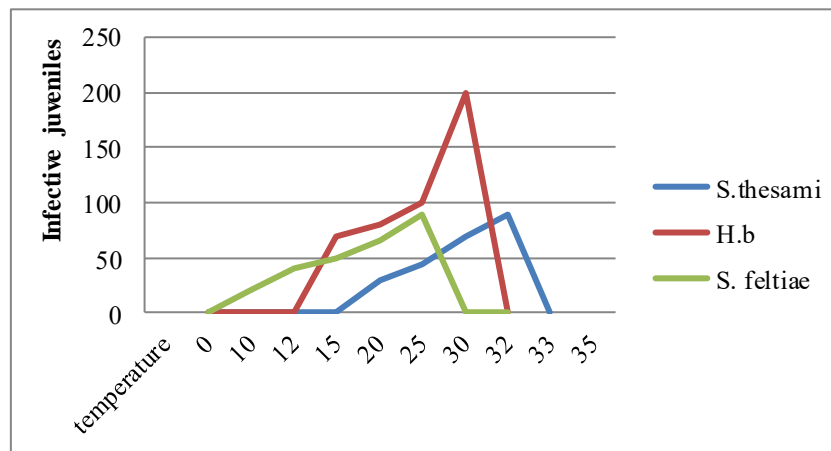


Fig. 2. Number of infective juveniles produced per *Tenebrio molitor* larvae at different temperature.

After 5 days' exposure, each larva was transferred to a separate White trap containing filter paper with distilled water and the total number of emerging IJs, were counted every two days until there was no further recovery. *H. bacteriophora* produced the highest number of infective juveniles – 200, 000 per cadaver at 30°C, compared with *S. thesami* and *S. feltiae*. The number of infective juveniles produced by *S. thesami* was 90,000, whereas for *S. feltiae* – 85,000 per insects (Fig. 3).

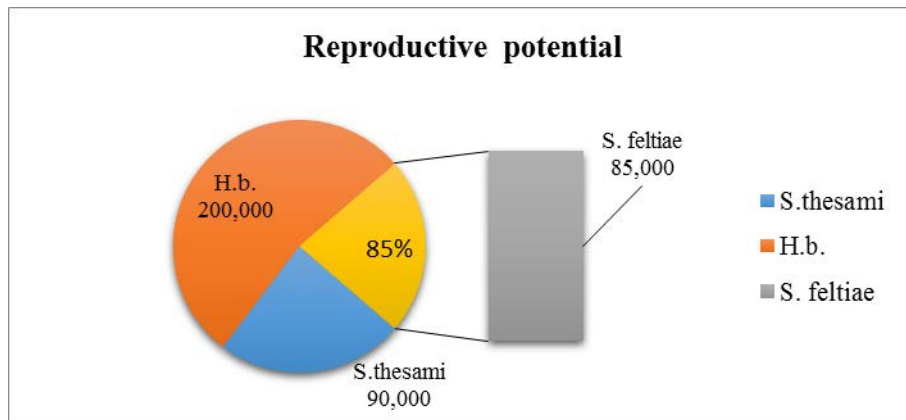


Fig. 3. Reproductive potential of entomopathogenic nematode species.

H. bacteriophora produced the highest number of infective juveniles per larva at 30°C than *S. feltiae* and *S. thesami*. *S. feltiae* caused the highest mortality of larvae at 25°C, whereas *S. thesami* infected *T. molitor* larvae at the widest temperature range and killed insects between 12–32°C. *S. feltiae* was the only species that killed the larvae at 10°C, *S. thesami* and *H. bacteriophora* were effective at 15–30°C. The temperature significantly affected the host searching ability of all tested species.

CONCLUSIONS

Thermal effect for infection differed among nematode species. Both species *S. thesami* and *H. bacteriophora* were more adapted to warm temperature reproduction, whereas *S. feltiae*, to cooler temperatures.

In the laboratory, all three nematode species successfully reproduced inside *T. molitor* larvae, *H. bacteriophora* produced the highest number of infective juveniles per larva, followed *S. thesami* and *S. feltiae*. *H. bacteriophora* treatment generally caused the highest mortality levels to *T. molitor* at high temperature (30°C), whereas *S. feltiae* caused the highest mortality to *T. molitor* larvae at low temperature (25°C).

Temperature influences the nematodes' survival, infection, and reproduction, is one of the most important factors limiting the practical uses of the nematodes as biocontrol agents (Jagdale & Gordon, 1998). It has been established that the nematodes are able to adapt physiologically to environmental temperatures. In future research, for field tests will be used the most suitable nematode species for biological control of different pest insects.

Recommendations. All nematodes were significantly different from each other in effectiveness against last instars of *T. molitor* larvae. Temperature is the most influential environmental factor, which has great biological significance. Mortality of larvae and production of IJs in *T. molitor* increased with increasing exposure time and temperature in both experiment.

As an environmental factor, temperature is variable both in space and time (Prosser, 1973). Temperature influences nematode mobility, reproduction and development (Mason & Hominik, 1995).

Farmer through the activities should be acquire knowledge and choose the time and appropriate conditions for the application of entomopathogens in the field, i.e. season of the year, a-biotic factors such as temperature, humidity of soils etc. and biotic factors including living organisms. Environmental conditions – cool weather, dry conditions, UV radiation during application, climatic variation, pests, disease and price risks as well as natural disasters such as droughts and floods, free information about the weather and temperature.

REFERENCES

- ABBOTT W. S., 1925, *A method of computing the effectiveness of an insecticide*. Journal of Economic Entomology, **18**: 265–276.
- JAGDALE G. B., GIRDON R., 1998, *Effect of propagation temperatures on temperature tolerances of entomopathogenic nematodes*. Fundamental and Applied Nematology, **21**: 177–183.
- AKHURST R. J., BOEMARE N. E., 1990, *Biology and taxonomy of Xenorhabdus*. Pp.: 75–90. In: GAUGLER R., KAYA H. K. (Eds.), *Entomopathogenic nematodes in biological control*. CRC Press, Boca Raton, FL.
- GAUGLER R., KAYA H. K., 1990, *Entomopathogenic Nematodes in Biological Control*. CRC. Boca Raton, FL.
- GORGADZE O. A., IVANOVA E. S., LORTKIPANIDZE M. A., SPIRIDONOV S. E., 2016, *Redescription of Steinernema thesami Gorgadze, 1988 (Rhabditida: Steinernematidae) from Georgia*. Russian Journal of Nematology, **24** (1): 17–31.
- GREWAL P. S., GAUGLER R., KAYA H. K., WUSATY M., 1993, *Infectivity of the entomopathogenic nematode (Steinernema scapterisci (Nematoda Steinernematidae))*. Journal of Invertebrate Pathology, **6**: 22–28.
- GREWAL P. S., LEWIS E. E., GAUGLER R., CAMPBELL J. F., 1994, *Host finding behavior as a predictor of foraging strategies in entomopathogenic nematodes*. Parasitology, **108**: 207–215.
- KAYA H. K., GAUGLER R., 1993, *Entomopathogenic nematodes*. Annual Review of Entomology, **38**: 181–206.
- KAYA H. K., STOCK S. P., 1997, *Techniques in insect nematology*. Pp.: 281–324. In: LACEY L. (Ed.), *Manual of Techniques in Insect Pathology*, Academic Press, San Diego,.
- MASON J. M., HOMINIK W. M., 1995, *The effect of temperature on infection, development and reproduction of Heterorhabditids*. Journal of Helminthology, **69**: 337–45.
- GREWAL P. S., SELVAN SEN, GAUGLER R., 1994, *Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction*. Journal of Thermal Biology, **19** (4): 245–253.
- POINAR G. O. Jr., 1990, *Taxonomy and biology of Steinernematidae and Heterorhabditidae*. Pp.: 23–64. In: GAUGLER R., KAYA H. K. (Eds.), *Entomopathogenic nematodes in biological control*. CRC Press, Boca Raton, FL.

- PROSSERC L., 1973, *Comparative Animal Physiology*. 3rd Edn. Philadelphia: W. B. Saunders Co.
WHITE GF., 1927, *A method for obtaining infective nematode larvae from cultures*. *Science*, **66**: 302–303.
WOODRING J. L., KAYA H. K., 1998, *Steinernematid and Heterorhabditid nematodes: A handbook of techniques*. South. Co-operative Serv. Bull.

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DATA ON THE COLEOPTERA (STAPHYLINIDAE,
CERAMBYCIDAE AND CHRYSOMELIDAE)
IN THE FĂGĂRAȘ MOUNTAINS AREA
(SOUTHERN CARPATHIANS, ROMANIA)

SANDA MAICAN*, RODICA SERAFIM**, MELANIA STAN**

The paper presents data on the diversity of Coleoptera (Staphylinidae, Cerambycidae and Chrysomelidae families) in the Făgăraș Mountains and its surroundings, based on the study of material preserved in the collections of the “Grigore Antipa” National Museum of Natural History (Bucharest) and the Institute of Biology Bucharest of the Romanian Academy. A total of 148 species belonging to 89 genera and 20 subfamilies are reported. Among the valuable species recorded in this area, we mention: *Cornumutilla lineata* (Letzner, 1844) – a highly rare representative of the fauna of Palearctic coniferous forests and *Ocypus kuntzeni* (G. Müller, 1926) – a mountainous species, possibly endemic in the Romanian Carpathians. Also, *Rosalia alpina alpina* (Linnaeus, 1758) and *Morimus asper funereus* Mulsant, 1863 are species of community interest, included in the Annex II of the Habitats Directive.

Keywords: faunistic diversity, Staphylinidae, Cerambycidae, Chrysomelidae, Făgăraș Mountains, Romania.

INTRODUCTION

The first data on the distribution of the coleopterans in the Făgăraș Mountains area were published beginning with the end of the 19th century by several authors, such as: Kuthy (1896), Bielz (1887), Petri (1912; 1925–1926), followed later by Panin (1944), Eliescu *et al.* (1949), Panin & Săvulescu (1961), Roșca (1973, 1974), Gruev *et al.* (1993), Szél *et al.* (1995), Rözner (2007), Rözner & Rözner (2007), Tăușan & Bucșa (2010), Dascălu *et al.* (2012), Cuzepan & Tăușan (2016), etc. Data on the Natura 2000 Cerambycidae species reported in this area, respectively *Morimus asper funereus* and *Rosalia alpina alpina*, were published by Tatole *et al.* (2009).

Recently, Linnell *et al.* (2016) had published a report that aims to summarise the existing knowledge concerning the biodiversity of the Făgăraș Mountains, giving a list with 125 beetle species based on data published and unpublished sources.

MATERIAL AND METHODS

This paper is based on published and unpublished data on the distribution of staphylinids, cerambycids and chrysomelids in the Făgăraş Mountains and adjacent areas.

The studied material is preserved in the collections of the “Grigore Antipa” National Museum of Natural History (Bucharest) and the Institute of Biology Bucharest of the Romanian Academy. The specimens from the “Grigore Antipa” museum were collected during several trips between 1949 and 2006, that were made mainly in the Avrig River Valley, in the Vâlsan River Valley and Vidraru Lake surroundings.

Most rove beetle species were collected in August 2004 on the occasion of a project regarding the diversity of Făgăraş Mountains fauna, other specimens/species were donated by the Hungarian Natural History Museum (collected in 2003, 2006 and 2008) and only a few specimens/species were collected before the 2000s.

A part of Cerambycidae material belongs to Dr. Nicolae Săvulescu Collection from the “Grigore Antipa” National Museum, which includes material collected between 1952 and 1974 especially in the Capra Peak, the Oticu Peak, the Buda Peak, Pleaşa Mts, “Sâmbăta” Chalet. The other part of material was collected in 1985, 1994 and 2004 on the occasion of a Museum’s Research Projects of the Făgăraş Mountains Biodiversity especially Poiana Neamţului Chalet (Valley of Avrig River), Bâlea Waterfall, Vama Cucului Chalet (Transfăgărăşan mountain road), Vidraru Lake area (the valleys of Buda, Cumpăna, Oticu Rivers, and so on), Brăduleţ (Vâlsan Valley). This material was included in the Museum's Cerambycidae Palaearctic Collection.

The Chrysomelidae specimens that were collected between 2000 and 2002 are recorded mainly from the “Vâlsan Valley” Site of Community Importance ROSCI0268.

The collecting sites in the Făgăraş Mountains and its surroundings:

Argeş County: Arefu, Argeş Valley, Brăduleţ (Brădetu and Galeşu, Vâlsan Valley), Buda Peak, Buda Valley, Capra Chalet, Capra Peak, Clăbucet Peak, Clearings of Vâlsan Valley (Brădetu), Cumpăna Valley, Cumpăna Chalet, Cumpăna Peak, Curtea de Argeş, Făgăraş Mountains, Iezer Păpuşa Mountains (Lereşti), Lunca Gârtii (Stoeneşti), Moliviş, Muşăteşti, Oticu Valley, Oticu Peak, Pleaşa Mountains, Stan Valley, “Valea cu Peşti” Chalet, Vidraru Lake area, Valley of Oticu River.

Sibiu County: Avrig, Bâlea Lake, Bâlea Waterfall, Bârcaciu Chalet, Cârţişoara, Laiţa River, Negoiu Peak, Negru Peak (Piscul Negru), Poiana Neamţului Chalet (Valley of Avrig River), Porumbacu de Sus, Sebeşu de Sus, Suru Chalet, Turnu-Roşu, Vama Cucului Chalet (Transfăgărăşan mountain road).

Braşov County: Sâmbăta de Sus, Sâmbăta Chalet.

Abbreviations for collector's name:

A.B. – Anca Bănărescu; A.Ba. – Adalbert Balog; A.P. – Angela Petrescu; A.P.G. – Aurelian Popescu - Gorj; A.S. – Aurora Stănescu; At.S. – Atanasie Săftoiu; C.P. – Corneliu Pârvu; C.C. – Cristina Constantinescu; C.H. – Cristina Hoinic; D.M. – Dumitru Murariu; G.C. – Gabriel Chișamera; Gy.M. – György Makraczy; I.C. – Ioana Cacoveanu; I.D. – Ion Drăghia; I.M. – Ioana Matache; M.S. – Melania Stan; Ma.S. – Mihaela Stănescu; N.S. – Nicolae Săvulescu; R.S. – Rodica Serafim; S.M. – Sanda Maican; S.S. – Steluța State; S.M.P. – Șerban Mihai Procheș; X.S.P. – Xenia Scobiola Palade.

Other abbreviations:

a.s.l. – above sea level; ID – identification; leg. – legit; Mts – Mountains; spec./specs – specimen/specimens.

RESULTS

The paper reports 148 species belonging to 89 genera and 20 subfamilies from Făgăraș Mountains and surroundings, based on the study of the material preserved in the collections of the “Grigore Antipa” National Museum of Natural History (Bucharest) and the Institute of Biology Bucharest of the Romanian Academy.

The nomenclature and the systematical order follow the *Catalogue of Palaearctic Coleoptera* (Löbl & Smetana, 2004, 2010).

Part of the data presented here have been published (Serafim, 2005, 2006; 2007, 2009, 2010; Stan, 1998, 2003, 2010, 2012; Makranczy, 2014; Maican, 2003, 2006; Maican & Serafim, 2016, 2017, 2018 a, b).

Among the valuable species recorded in this area, we mention the species *Cornumutilla lineata* Letzner, 1844, a very rare representative of the fauna of Palaearctic coniferous forests (*Picea*, *Larix*, *Pinus*, *Abies*). *C. lineata* is a boreo-mountainous species, distributed in Central and Eastern Europe and Siberia. In the Romanian fauna, it was less mentioned: Făgăraș Mountains, Bârcaciu Chalet (Panin & Săvulescu, 1961); Făgăraș Mts, Negoiu Peak (Serafim, 2006), Făgăraș Mts (Lazarev, 2009); Rarău Mt., Valea Putnei (Eastern Carpathians) (Serafim & Maican, 2011).

Ocypus kuntzeni (G. Müller, 1926) is a mountainous species, distributed in the southern and eastern Carpathians and the Transylvanian Plateau and it is possibly an endemic species of the Romanian Carpathians.

Rosalia alpina alpina (Linnaeus, 1758) and *Morimus asper funereus* Mulsant, 1863 are species of community importance, included in the Annex II of the Habitat Directive.

Family Staphylinidae Latreille, 1802

Subfamily Omaliinae MacLeay, 1825

Anthophagus alpestris Heer, 1839

Material: 1 spec., surroundings of Capra Chalet, N: 45°34'58", E: 24°37'40", 1535 m a.s.l., 05.08.2004, M.S.

Geodromicus plagiatus (Fabricius, 1798)

Material: 20 specs, bank of stream Laița, 2 km S Cârțișoara, near bridge, 1 km W stream Cârțișoara, N: 45°42'17", E: 24°34'11", 520 m a.s.l., 16.05.2003, Gy.M., ID Adriano Zanetti.

Subfamily Tachyporinae Macleay, 1825

Lordithon lunulatus (Linnaeus, 1760)

Material: 1 spec., the bank of Vidraru Lake close to forest road (N: 45°27'14", E: 24°36'13", 860 m a.s.l.), in mushrooms grown on dried elm, 04.08.2004, M.S.; 7 specs, the bank of Vidraru Lake (close to "Valea cu Pești" Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

Lordithon trinotatus (Erichson, 1839)

Material: 2 specs, the bank of Vidraru Lake (near the "Valea cu Pești" Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

Tachinus laticollis Gravenhorst, 1802

Material: 5 specs, Buda Valley, in cow dung on forest road, 04.08.2004, M.S.; 2 specs, 1 km upstream "Valea cu Pești" Chalet, horse dung, 6.08.2004, M.S.; 1 spec., the bank of Vidraru Lake close to forest road (N: 45°27'14", E: 24°36'13", 860 m a.s.l.), in mushrooms grown on dried elm, 04.08.2004, M.S.; 6 specs, Stan Valley, in cow dung on the forest road, N: 45°22' 45", E: 24°36'56", 960 m a.s.l., 07.08.2004, M.S.

Tachyporus chrysomelinus (Linnaeus, 1758)

Material: 1 ♂, Galeșu, Vâlsan Valley, 7–21.07.2001, C.P. (Stan, 2003).

Tachyporus dispar (Paykull, 1789)

Material: 6 ♂, 1 ♀, Galeșu, Vâlsan Valley, 7–21.07.2001, C.P. (Stan, 2003).

Tachyporus hypnorum (Fabricius, 1775)

Material: 8 specs, Galeșu, Vâlsan Valley, 7–21.07.2001, C.P.

Subfamily Aleocharinae Fleming, 1821

Aleochara bilineata Gyllenhal, 1810

Material: 1 spec., surroundings of Capra Chalet, N: 45°34'58", E: 24°37'40", 1535 m a.s.l., 05.08.2004, R.S.

Atheta aeneicollis (Sharp, 1869)

Material: 1 spec., the bank of Vidraru Lake close to forest road (N: 45°27'14", E: 24°36'13", 860 m a.s.l.), in mushrooms grown on dried elm, 04.08.2004, M.S.

Atheta castanoptera (Mannerheim, 1830)

Material: 5 specs, the bank of Vidraru Lake close to forest road (N: 45°27'14", E: 24°36'13", 860 m a.s.l.), in mushrooms grown on dried elm, 04.08.2004, M.S.; 28 specs, bank of Vidraru Lake (near the "Valea cu Pești" Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

Atheta crassicornis (Fabricius, 1792)

Material: 22 specs, the bank of Vidraru Lake (near the "Valea cu Pești" Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

Atheta leonhardi Bernhauer, 1911

Material: 1 spec., surroundings of Bâlea Lake, 2075 m a.s.l., 26.07.2006, A.Ba., ID Ádám Laszlo.

Atheta liturata (Stephens, 1832)

Material: 18 specs, the bank of Vidraru Lake close to forest road (N: 45°27'14", E: 24°36'13", 860 m a.s.l.), in mushrooms grown on dried elm, 04.08.2004, M.S.; 3 specs, bank of Vidraru Lake (near the "Valea cu Pești" Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

Atheta nigrifula (Gravenhorst, 1802)

Material: 3 specs, the bank of Vidraru Lake (near the "Valea cu Pești" Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

Atheta pallidicornis (Thomson, 1856)

Material: 1 spec., bank of Vidraru Lake (near the "Valea cu Pești" Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., in mushrooms, 06.08.2004, M.S.

Gyrophana minima Erichson, 1837

Material: 28 specs, the indicator area Valea Cumpăna Mare-Clăbucet 9,2 km, in mushrooms (mixed forest), 08.08.2004, M.S.

Gyrophana strictula Erichson, 1839

Material: 2 specs, the indicator area Valea Cumpăna Mare-Clăbucet 9,2 km, in mushrooms (mixed forest), 08.08.2004, M.S.

Liogluta alpestris (Heer, 1839)

Material: 5 specs, surroundings of Bâlea Lake, N: 45°36'17", E: 24°36'10", 2046 m a.s.l., 26.07.2006, A.Ba., ID Ádám Laszlo.

Myllaena intermedia Erichson, 1837

Material: 6 specs, bank of stream Laița, 2 km S Cârțișoara, near bridge, 1 km W stream Cârțișoara, N: 45°42'17", E: 24°34'11", 520 m a.s.l., 16.05.2003, Gy.M., ID Ádám Laszlo.

Oxyroda deubeli Bernhauer, 1899

Material: 1 spec., surroundings of Bâlea Lake, 25.02.2008, 2075 m, A.Ba., ID Ádám Laszlo.

Subfamily Oxytelinae Fleming, 1821

Anotylus mutator (Lohse, 1963)

Material: 1 spec., Buda Valley, N: 45°28', E: 24°38', 930 m a.s.l., cow dung (forest road), 4.08.2004, M.S.

Ochtheophilus praepositus Mulsant & Rey, 1878

Material: 1 spec., bank of stream Laița, 2 km S Cârțișoara, near bridge, 1 km W stream Cârțișoara, N: 45°42'17", E: 24°34'11", 520 m a.s.l., 16.05.2003, Gy.M., ID György Makraczy (Makranczy, 2014).

Oxytelus laqueatus (Marsham, 1802)

Material: 4 specs, Buda Valley, N: 45°28', E: 24°38', 930 m a.s.l., cow dung (forest road), 4.08.2004, M.S.; 3 specs, the bank of Vidraru Lake (near the "Valea cu Pești" Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., horse dung, 06.08.2004, M.S.; 3 specs, Stan Valley, N: 45°22' 45", E: 24°36'56", 960 m a.s.l., cow dung, 7.08.2004, M.S.

Platystethus arenarius (Geoffroy, 1785)

Material: 3 specs, Buda Valley, N: 45°28', E: 24°38', 930 m a.s.l., cow dung (forest road), 4.08.2004, M.S.; 1 spec., Stan Valley, N: 45°22' 45", E: 24°36'56", 960 m a.s.l., cow dung, 7.08.2004, M.S.

Thinobius angusticeps Fauvel, 1889

Material: 1 spec., Porumbacu de Sus, Șerbota stream, 18.05.2013, leg. & ID György Makraczy.

Subfamily Steninae MacLeay, 1825.

Stenus comma LeConte, 1863

Material: 1 spec., Curtea de Argeș, the bank of Argeș river, 3.09.1979, leg. A.P.G.; 3 specs, Buda Valley, the bank of Buda stream, N: 45°28', E: 24°38', 930 m a.s.l., M.S.

Subfamily Paederinae Fleming 1821

Lathrobium laevipenne Heer, 1839

Material: 1 ♂, Arefu, the bank of Oticu valley, in detritus, N: 45°27'20", E: 24°38'12", 925 m a.s.l., 04.08.2004, M.S.

Paederus littoralis Gravenhorst, 1802

Material: 3 specs, Vâlsan Valley, the confluence Galeș-Vâlsan, grassland, 19.08.1995, M.S. (Stan, 1998).

Subfamily Staphylininae Latreille, 1802

Bisnius fimetarius (Gravenhorst, 1802)

Material: 3 specs, Buda Valley, N: 45°28', E: 24°38', 930 m a.s.l., cow dung (forest road), 4.08.2004, M.S.; 1 spec., the bank of Vidraru Lake (near the "Valea cu Pești" Chalet), N: 45°23'37", E: 24°37'50", 830 m a.s.l., horse dung, 06.08.2004, M.S.

Philonthus cruentatus (Gmelin, 1790)

Material: 2 ♂, 2 ♀, Stan Valley, N: 45°22' 45", E: 24°36'56", 960 m a.s.l., cow dung, 7.08.2004, M.S. (Stan, 2012).

Philonthus decorus (Gravenhorst, 1802)

Material: 1 spec., Galeșu, 7-21.07.2001, C.P. (Stan, 2012).

Philonthus marginatus (Müller, 1764)

Material: 1 ♀, Stan Valley, N: 45°22' 45", E: 24°36'56", 960 m a.s.l., cow dung, 7.08.2004, M.S. (Stan, 2012).

Philonthus spinipes Sharp, 1874

Material: 1 ♂, 1 ♀, Stan Valley, N: 45°22' 45", E: 24°36'56", 960 m a.s.l., cow dung, 7.08.2004, M.S. (Stan, 2012).

Philonthus succicola Thomson, 1860

Material: 1 ♂, Vâlsan Valley, 06.1989, A.B. (Stan, 2012).

Philonthus varians (Paykull, 1789)

Material: 1 ♀, Stan Valley, N: 45°22' 45", E: 24°36'56", 960 m a.s.l., cow dung, 7.08.2004, M.S. (Stan, 2012).

Quedius cincticollis Kraatz, 1857

Material: 1 spec., Cumpăna Valley (surroundings Cumpăna Chalet), N: 45°26'09.24'', E: 24°36'18.22'', 853 m a.s.l. in moos, 03.08.2004, M.S.; 1 spec., Bâlea Lake, 2075 m a.s.l., 26.07.2006, A.B., ID Ádám Laszlo.

Ocypus aeneocephalus (De Geer, 1774)

Material: 1 ♂, 1 ♀, Galeșu (Vâlsan Valley), meadow in plum orchad, between the streams, 600 m a.s.l., 7-21.07.2001, C.P. (Stan, 2010).

Ocypus fulvipennis Erichson, 1840

Material: 1 ♂, Galeșu (Vâlsan Valley), meadow in plum orchad, between the streams, 600 m a.s.l., 7-21.07.2001, C.P. (Stan, 2010).

Ocypus kuntzeni (G. Müller, 1926)

Material: 4 ♂, Galeșu (Vâlsan Valley), meadow in plum orchad, between the streams, 600 m a.s.l., 7-21.07.2001, C.P. (Stan, 2010).

Ocypus picipennis picipennis (Fabricius, 1793)

Material: 1 ♂, Galeșu (Vâlsan Valley), meadow in plum orchard, between the streams, 600 m a.s.l., 7-21.07.2001, C.P. (Stan, 2010).

Platydracus stercorarius (Olivier, 1795)

Material: 10 ♂, 4 ♀, Galeșu (Vâlsan Valley), meadow in plum orchard, between the streams, 600 m a.s.l., 7-21.07.2001, C.P.

Quedius alpestris (Heer, 1839)

Material: 7 specs., surroundings of Bâlea Lake, 2075 m alt, 26.07.2006, A.Ba., ID Ádám Laszlo.

Quedius punctatellus (Heer, 1839)

Material: surroundings of Bâlea Lake: 2 specs, N: 45°36'17", E: 24°36'10", 2046 m alt and 6 specs, 2075 m a.s.l., 26.07.2006, A.Ba., ID Ádám Laszlo.

Family Cerambycidae Latreille, 1802

Subfamily Prioninae Latreille, 1802

Prionus coriarius (Linnaeus, 1758)

Material: 1 spec., Cumpăna Peak, 2.08.1952, N.S.; 2 specs, Capra Peak, 17.08.1959, N.S.; 1 spec., Poiana Neamțului Chalet surroundings, 17.07.1985, I.M. 1 spec., Cumpăna Chalet surroundings, 4.08.2004 (Serafim, 2005).

Subfamily Lepturinae Latreille, 1802

Alosterna tabacicolor Degeer, 1775

Material: 5 specs, Buda Peak, 12-21.08.1959; 7 specs, Oticu Peak, 15.08.1959 N.S.; 9 specs, Capra Peak, 17.08.1959, N.S. (Serafim, 2006); 1 spec., Valley of Buda River, 4.08.2004, R.S.; 1 spec., "Valea cu Pești" Chalet surroundings, 6.08.2004, M.S.; 1 spec., Moliviș, 7.08.2004, M.S.

Anastrangalia dubia (Scopoli, 1763)

Material: Capra Peak, 2.08.1957 (4 specs), 17-20.08.1959 (25 specs); 27 specs, Buda Peak, 12-21.08.1959, N.S.; Oticu Peak, 15-16.08.1959 N.S. (15 specs), 29.VII.1974 N.S. (1 spec.); 7 specs, Vama Cucului Chalet surroundings, 29.VII.-1.08.1994, C.P., A.S.; 1 spec., Bâlea Waterfall, 25.07.1994, C.H. (Serafim, 2006); 1 spec., Valley of Buda River, 12.08.1959, N.S.; 1 spec., Valley of Oticu River, 4.08.2004.

Anastrangalia sanguinolenta (Linnaeus, 1761)

Material: Capra Peak, 2.08.1957 N.S. (9 specs), 17-20.08.1959, N.S. (10 specs), 28.VII.1974 (63 specs); Buda Peak, 12-18.08.1959, N.S. (15 specs), 30.VII.1974 N.S. (21 specs); Oticu Peak, 15-16.08.1959, N.S. (9 specs), 29.07.1974 N.S.

(19 specs); 59 specs, Negru Peak (Piscu Negru), 27.07.1974 N.S.; 7 specs, Vama Cucului Chalet (Cârțișoara), 29.07.–1.08.1994, C.P., A.S. (Serafim, 2006); 2 specs, Bâlea Waterfall, 25.07.1994, A.S., C.H.

Cornumutilla lineata Letzner, 1844

Material: 5 specs, Negoiu Peak, 11.08.1957, N.S. (Serafim, 2006).

Judolia sexmaculata (Linnaeus, 1758)

Material: 1 spec., Capra Peak, 2.08.1957, N.S.; 2 specs, Suru Chalet, 28.07.1974, N.S. (Serafim, 2006).

Leptura annularis Fabricius, 1801 (syn. *arcuata* Panzer, 1793)

Material: Turnu-Roșu, without other data (Serafim, 2006).

Leptura quadrifasciata quadrifasciata Linnaeus, 1758

Material: 4 specs, Oticu Peak, 15–16.08.1959, N.S.; 3 specs, Capra Peak, 17–20.08.1959, N.S.; 16 specs, Buda Peak, 12–21.08.1959, N.S.; 3 specs, Suru Chalet, 5.07.1977; 1 spec., Avrig, 16.07.1985, R.S.; 5 specs, Vama Cucului Chalet surroundings, 29.07.–1.08.1994, C.P., A.S., C.H.; 1 spec., valley of Oticu River, 4.08.2004 R.S.; 3 specs, valley of Cumpăna River, 8.08.2004 R.S.; 1 spec., “Valea cu Pești” Chalet surroundings, 6.08.2004, R.S. (Serafim, 2006); 1 spec., Galeșu (Brăduleț), 8.07.2001, C.P.

Lepturobosca virens (Linnaeus, 1758)

Material: Buda Peak, 12–21.08.1959 (19 specs), 30.07.1974 (47 specs) N.S.; Oticu Peak, 15–16.08.1959, (5 specs), 29.07.1974 (3 specs), N.S.; Capra Peak, 17–20.08.1959 (5 specs), 28.07.1974 (48 specs), N.S.; 46 specs, Negru Peak (Piscu Negru), 27.07.1974, N.S.; 1 spec., Bâlea Waterfall, 26.07.1994, A.S.; 1 spec. Vama Cucului Chalet surroundings, 29.07.1994 (Serafim, 2006); 1 spec., valley of Cumpăna River, 8.08.2004, R.S.

Pachytodes cerambyciformis (Schrank, 1781)

Material: 20 specs, Buda Peak, 12.08.1959, N.S.; 4 specs, Oticu Peak, 15.08.1959, N.S.; Capra Peak, 17–20.08.1959 (24 ex.), 27.07.1974 (17 ex.), N.S.; 55 specs, Poiana Neamțului Chalet, 18–21.07.1985, I.D., R.S., I.M., I.C.; 8 specs, Vama Cucului Chalet surroundings, 29.07.–1.08.1994, C.P., A.S.; 1 spec., Valley of Buda River, 4.08.2004, R.S.; 3 specs, valley of Oticu River, 4.08.2004, R.S.; 4 specs, valley of Cumpăna River, 8.08.2004, R.S. (Serafim, 2006).

Pachytodes erraticus erraticus (Dalman, 1817)

Material: 2 specs, Suru Chalet, 27.07.1974, N.S.; Capra Peak, 28.07.1974, N.S. (Serafim, 2006); 2 specs, Făgăraș Mts, 27.07.1974.

Pseudovadonia livida livida (Fabricius, 1776)

Material: 1 spec., Pleașa Mts, 12.08.1959, N.S.; 2 specs, Buda Peak, 12–21.08.1959, N.S.; 3 specs, Oticu Peak, 15–16.08.1959, N.S.; 3 specs, Capra Peak, 17.08.1959,

N.S.; 10 specs, Poiana Neamțului Chalet surroundings, 19.07.1985, R.S., I.M. (Serafim, 2006); 1 spec., “Valea cu Pești” Chalet surroundings, 850 m, 6.08.2004, R.S.; 1 spec., Stan Valley, 7.08.2004, R.S.; 3 specs, Moliviș, 7.08.2004, M.S.

Rutpela maculata maculata (Poda, 1761)

Material: 2 specs, Pleașa Mts, 12.07.1959, N.S.; 30 specs, Poiana Neamțului Chalet surroundings, 18–21.07.1985, I.D., R.S., I.M.; 17 specs, Vama Cucului Chalet surroundings, 29.07.–2.08.1994, C.P., A.S., C.H.; 1 spec., Cumpăna Chalet surroundings, 3.08.2004, R.S.; 1 spec., Valley of Oticu River, 4.08.2004, R.S.; 16 specs, “Valea cu Pești” Chalet surroundings, 6.08.2004, R.S., M.S.; 2 specs, Moliviș, 7.08.2004, R.S.; 8 specs, Stan Valley, 7.08.2004, R.S., S.M. (Serafim, 2006).

Stenurella bifasciata bifasciata (Müller, 1776)

Material: 1 spec., Pleașa Mts, 12.08.1959, N.S. (Serafim, 2006); 1 spec., Galeșu, (Vâlsan Valley), 7.08.1997, C.P.

Stenurella melanura (Linnaeus, 1758)

Material: 2 specs, Pleașa Mts, 12.08.1959, N.S.; 16 specs, Poiana Neamțului Chalet surroundings, 19–20.07.1985, I.D.; 65 specs, Vama Cucului Chalet surroundings, 29.07.–1.08.1994, C.P., A.S. (Serafim, 2006); 1 spec., valley of Buda River, 4.08.2004, A.P.; 1 spec., “Valea cu Pești” Chalet surroundings, 850 m, 6.08.2004, R.S.; 7 specs, Moliviș 7.08.2004, R.S., M.S.; 1 spec. valley of Cumpăna River, 8.08.2004, R.S.

Stenurella nigra (Linnaeus, 1758)

Material: 1 spec., Sebeșu de Sus (Racovița), 12.V.1986, I.M.

Stenurella septempunctata septempunctata (Fabricius, 1792)

Material: Turnu-Roșu, without other data (Serafim, 2006).

Stictoleptura rubra rubra (Linnaeus, 1758)

Material: Capra Peak, 28.07.1957, N.S. (2 specs), 17–20.08.1959 N.S. (11 specs); 3 specs, Suru Chalet, 7.08.1957, N.S.; Buda Peak, 18–21.08.1959, N.S. (13 specs), 30.07.1974, N.S. (47 specs); Oticu Peak, 15–16.08.1959, N.S. (6 specs), 29.07.1974, N.S. (3 specs); Negru Peak (Piscu Negru), 27.07.1974, N.S.; 13 specs, Poiana Neamțului Chalet surroundings, 18–21.VII.1985, I.D., R.S.; 26 specs, Vama Cucului Chalet surroundings, 29.07.–1.08.1994, C.P., A.S., C.H.; 1 spec., Stan Valley, 7.08.2004, R.S.; 6 specs, Moliviș, 7.08.2004, R.S., M.S. (Serafim, 2006); 1 spec., valley of Oticu River, 08.2004, A.P.

Stictoleptura maculicornis maculicornis (Degeer, 1775)

Material: Capra Peak, 2.08.1957, N.S. (1 spec.), 17–20.08.1959, N.S. (19 specs); Oticu Peak, 15.08.1959, N.S. (1 spec.), 29.07.1974, N.S. (3 specs); 11 specs, Buda Peak, 12–18.08.1959, N.S.; 5 specs, valley of Oticu River, 4.08.2004, R.S. (Serafim, 2006); 1 spec., valley of Buda River, 4.08.2004, M.S.

Stictoleptura scutellata scutellata (Fabricius, 1781)

Material: 10 specs, Buda Peak, 12–21.08.1959, N.S.; 1 spec., Oticu Peak, 16.08.1959, N.S.; 3 specs, Capra Peak, 20.08.1959, N.S.; 9 specs, Poiana Neamţului Chalet surroundings, 18–21.07.1985, I.D., R.S.; 1 spec., Vama Cucului Chalet surroundings, 1.08.1994, C.P.; 1 spec., Moliviş, 7.08.2004, M.S. (Serafim, 2006).

Strangalia attenuata (Linnaeus, 1758)

Material: 1 spec., Suru Chalet, 12.08.1959, N.S.; 1 spec., Avrig, 16.07.1985, R.S.; 1 spec., Vama Cucului Chalet surroundings, 1.08.1994, C.P.; 1 spec., Moliviş, 7.07.2004, M.S. (Serafim, 2006).

Oxymirus cursor (Linnaeus, 1758)

Material: 1 spec., Turnu-Roşu, 13.05.1986, I.D. (Serafim, 2005).

Evodinus clathratus (Fabricius, 1792)

Material: 5 specs, Făgăraş Mts, Capra Peak, 27–29.07.1974, N.S.

Gaurotes virginea virginea (Linnaeus, 1758)

Material: Capra Peak, 2.08.1957, N.S. (3 specs), 17–20.08.1959, N.S. (2 specs), 27–29.VI.1974, N.S. (69 specs); Oticu Peak, 15.08.1959, N.S. (21 specs), 29.07.1974, N.S. (34 specs); Buda Peak, 18.08.1959 (10 specs), 30.07.1974, N.S. (29 specs), 12–21.08.1959, N.S. (26 specs); 34 specs, Negru Peak (Piscu Negru), 27.07.1974, N.S.; 36 specs, Poiana Neamţului Chalet surroundings, 18–21.07.1985, R.S.; 3 specs, Bâlea Waterfall, 24–25.07.1994, C.P.; 2 specs, valley of the Oticu River, 4.08.2004, R.S., M.S. (Serafim, 2005).

Pachyta lamed lamed (Linnaeus, 1758)

Material: 1 spec., Făgăraş Mts, the road to Clăbucet Peak, 2.08.1954, N.S. (Serafim, 2005).

Pachyta quadrimaculata (Linnaeus, 1758)

Material: Capra Peak, 27–28.07.1957, N.S. (4 specs), 17–20.08.1959, N.S. (10 specs), 28.07.1974, N.S. (2 specs); Oticu Peak, 21 specs., Buda Peak, 12–21.08.1959, N.S.; 1 spec., Poiana Neamţului Chalet surroundings, 6.08.1985; 2 specs, valley of Cumpăna River, 8.08.2004, R.S. (Serafim, 2005).

Pidonia lurida (Fabricius, 1792)

Material: Capra Peak, 17–20.08.1959, N.S. (4 specs), 27–28.07.1974, N.S. (25 specs); 10 specs, Oticu Peak 15–16.08.1959, N.S.; 8 specs, Buda Peak, 12–21.08.1959, N.S.; 5 specs, Negru Peak (Piscu Negru) 27.07.1974, N.S.; 1 spec., Poiana Neamţului Chalet surroundings, 21.07.1985, R.S.; 1 spec., Bâlea Waterfall, 25.07.1994, C.P. (Serafim, 2005).

Rhagium (Megarhagium) mordax (Degeer, 1775)

Material: Capra Peak, 2.08.1957, N.S. (1 spec.), 27.07.1974, N.S. (2 specs); 1 spec., Negru Peak (Piscu Negru), 27.08.1957, N.S. (Serafim, 2005).

Subfamily Spondylidinae

Tetropium castaneum (Linnaeus, 1758)

Material: Capra Peak, 28.VI.1957, N.S. (5 specs), 17.08.1959, N.S. (1 spec.); 2 specs, Buda Peak, 12.08.1959, N.S.; 1 spec., Oticu Peak, 15.08.1959, N.S.; 1 spec., Poiana Neamțului Chalet surroundings, 15.05.1986 (Serafim, 2007).

Tetropium fuscum (Fabricius, 1787)

Material: 1 spec., Capra Peak, 28.07.1957, N.S. (Serafim, 2007).

Saphanus piceus piceus (Laicharting, 1784)

Material: 1 spec., Bârcaciu Chalet surroundings, 10.08.1958, N.S. (Serafim, 2007).

Spondylis buprestoides (Linnaeus, 1758)

Material: 2 specs, Cumpăna Chalet surroundings, 17.08.1957, N.S. (Serafim, 2007).

Subfamily Cerambycinae Latreille, 1802

Aromia moschata moschata (Linnaeus, 1758)

Material: 1 spec., Galeșu (Vâlsan Valley), 16.08.1990, C.P. (Serafim, 2009).

Callidium coriaceum Paykull, 1800

Material: 3 specs, Capra Peak, 26–28.07.1957, N.S. (Serafim, 2009).

Callidium violaceum (Linnaeus, 1758)

Material: 1 spec., Sâmbăta Chalet surroundings, 3.VI.1949, A.P.G.; 1 spec., Făgăraș Mts, 18.05.1962, X.S.P. (Serafim, 2009).

Ropalopus clavipes (Fabricius, 1775)

Material: 1 spec., Capra Peak, 28.07.1957, N.S. (Serafim, 2010); 1 spec., Buda Peak, 18.08.1959, N.S.

Chlorophorus sartor (Müller, 1766)

Material: 2 specs, Galeșu (Vâlsan Valley), 20.08.1998, C.P.

Chlorophorus varius (Müller, 1766)

Material: 1 spec., Pleașa Mts, 12.08.1959, N.S.; 4 specs, Galeșu (Vâlsan Valley), 25.07.1995, C.P. (Serafim, 2009); 3 specs, Galeșu, 20.08.1998, C.P.

Clytus lama Mulsant, 1847

Material: 1 spec., Cumpăna Peak, 2.08.1958, N.S. (Serafim, 2009).

Clytus rhamni Germar, 1817

Material: 1 spec., Galeșu (Vâlsan Valley), 25.07.1995, C.P. (Serafim, 2009).

Plagionotus floralis (Pallas, 1773)

Material: 1 spec., Galeșu (Vâlsan Valley), 8.07.2001, C.P. (Serafim, 2009).

Molorchus minor minor (Linnaeus, 1767)

Material: 1 spec., Poiana Neamțului Chalet surroundings, 15.05.1986, I.D.

Rosalia alpina alpina (Linnaeus, 1758)

Material: 6 specs, Pleașa Mts., 12.08.1959, N.S. (Serafim, 2009); 3 specs, Făgăraș Mts, 18.07.1979, N.S.; 1 spec., Brădetu (Vâlsan Valley) (beech forest), N: 45.32555°, E: 24.74644°, 721 m a.s.l., 29.07.2015, observed by M.S., C.C., G.C. (Fig. 1).



Fig. 1 – *Rosalia alpina alpina* (Photo: Melania Stan).

Stenopterus flavicornis Küster, 1846

Recorded: 1 spec., Galeșu (Vâlsan Valley), 8.07.2001, C.P. (Serafim, 2009).

Stenopterus rufus rufus Linnaeus, 1767

Material: 1 spec., Pleașa Mts., 12.08.1959, N.S.; 1 spec., Poiana Neamțului Chalet surroundings, 21.07.1985, R.S.; 1 spec., Galeșu (Vâlsan Valley), 25.07.1995, C.P. (Serafim, 2009); 1 spec., Galeșu, 7.08.1997, C.P.

Subfamily Lamiinae Latreille, 1825

Leiopus nebulosus (Linnaeus, 1758)

Material: 1 spec., Capra Peak, 28.07.1957, N.S. (Serafim, 2010).

Aegomorphus clavipes (Schrank, 1781)

Material: 1 spec., Capra Peak, 28.07.1957, N.S. (Serafim, 2010); 1 spec., Buda Peak, 18.08.1959, N.S.

Agapanthia (Epopetes) dahli (Richter, 1821)

Material: 1 spec., Turnu-Roșu, 13.05.1986, I.D. (Serafim, 2010).

Agapanthia (Epopetes) villosoviridescens (Degeer, 1755)

Material: 1 spec., Făgăraș Mts, 8.VI.1976, X.S.P. (Serafim, 2010).

Monochamus sartor (Fabricius, 1787)

Material: 1 spec., Bârcaciu Chalet, 06.1924; 4 specs, Negoiu Chalet, 28.07.1957, N.S.; 6 specs, Suru Chalet, 7.08.1957, N.S.; 9 specs, Capra Peak, 28.07.1957–15.09.1959, N.S.; 1 spec., Iezer Păpușa Mts, Lerești, 19.07.2003, Ma.S.; 3 specs, Cumpăna Chalet surroundings, 3 - 7.08.2004, R.S., D.M. (Serafim, 2010).

Monochamus sutor sutor (Linnaeus, 1758)

Material: 1 spec., Bârcaciu Chalet, 06.1924; 21 specs, Capra Peak, 20.08.–17.08.1959, N.S.; 9 specs, Oticu Peak, 16.08.1959, N.S.; 2 specs, Iezer Păpușa Mts, Lerești, 14.08.1997–22.07.2003, Ma.S. (Serafim, 2010); 1 spec., surroundings of Vidraru Lake, 7.08.2004.

Morimus asper funereus Mulsant, 1863

Material: 1 spec., Brădetu (Vâlsan Valley) (beech forest), N: 45°19'16.968", E: 24° 45'2.916", 682 m a.s.l., 29.07.2015, observed by M.S., C.C., G.C. (Fig. 2).



Fig. 2 – *Morimus asper funereus* (Photo: Melania Stan).

Phytoecia (Phytoecia) nigricornis (Fabricius, 1781)

Material: 1 spec., Bâlea Waterfall, 24.07.1994, C.P. (Serafim, 2010).

Family Chrysomelidae Latreille, 1802

Subfamily Donaciinae Kirby, 1837

Plateumaris consimilis (Schrank, 1781)

Material: 1 spec. ♂, Brădetu, upper end of Vâlsan dam lake, 25.07.2000, S.M. (Maican, 2003); 28 specs, Poiana Neamțului Chalet surroundings, 14–21.05.1986, I.M., R.S., I.D. (Maican & Serafim, 2016).

Subfamily Criocerinae Latreille, 1807

Lilioceris lili (Scopoli, 1763)

Material: 4 specs, Mușătești, 25.07.2000, S.M. (Maican, 2003); 1 spec., Stan Valley, 7.08.2004, R.S. (Maican & Serafim, 2016).

Lilioceris merdigera (Linnaeus, 1758)

Material: 1 spec. ♀, Brădetu, upper end of Vâlsan dam lake, 25.07.2000, S.M. (Maican, 2003).

Oulema erichsonii (Suffrian, 1841)

Material: 1 spec. ♂, Galeșu (Vâlsan Valley), 1.08.1995, C.P. (Maican, 2003).

Oulema gallaeciana (Heyden, 1870)

Material: 1 spec. ♀, Galeșu (Vâlsan Valley), 1.07.1995, C.P. (Maican, 2003).

Oulema melanopus (Linnaeus, 1758)

Material: 2 specs, Cârțișoara, Vama Cucului Chalet, 1–2.08.1994, C.H., C.P. (Maican & Serafim, 2016).

Subfamily Cassidinae Gyllenhal, 1813

Cassida rubiginosa rubiginosa O.F. Müller, 1776

Material: 1 spec., Clearings of Brădetu (Vâlsan Valley), 27.07.2000, S.M. (Maican, 2003).

Cassida vibex Linnaeus, 1767

Material: 1 spec., Brădetu (Vâlsan Valley), 25.07.2000, S.M. (Maican, 2003).

Cassida viridis Linnaeus, 1758

Material: 11 specs, Brădetu (Vâlsan Valley), 24.07.2000, S.M.; 2 specs, Mușătești, 6.08.2001, S.S. (Maican, 2003); Poiana Neamțului Chalet surroundings, 19–20.07.1985, I.M., R.S. (6 specs), 14–15.05.1986, I.M., I.D. (14 specs) (Maican & Serafim, 2017); 1 spec., Oticu Valley, 4.08.2004, R.S. (Maican & Serafim, 2017).

Subfamily Chrysomelinae Latreille, 1802

Chrysomela vigintipunctata vigintipunctata (Scopoli, 1763)

Material: 3 specs, Stoenеști, 30.04.1995, S.M.P. (Maican, 2003).

Plagioder a versicolor a (Laicharting, 1781)

Material: 1 spec. ♀, Brădetu, upper end of Vâlsan dam lake, 25.07.2000, S.M. (Maican, 2003); valley of Buda River, Făgăraș Mts (Maican & Serafim, 2018 a).

Plagiosterna aenea aenea (Linnaeus, 1758)

Material: 2 specs, Stoenеști, 30.04.1995, S.M.P.; 37 specs, “Valea cu Pești” (on *Alnus incana*), 28.07.2000, S.M. (Maican, 2003); 58 specs, valley of Buda River, 4.08.2004, R.S., M.S., A.P.; 75 specs, Vidraru Lake, 5 km away from Cumpăna Chalet, 4.08.2004, R.S.; 17 specs, valley of Oticu River, 4.08.2004, R.S.; 146 specs, Vidraru Lake, “Valea cu Pești” Chalet surroundings, 6.08.2004, M.S., R.S.; 43 specs, Vidraru Lake, Stan Valley, 7.08.2004, R.S.; 13 specs, Vidraru Lake, Moliviș, 7.08.2004, M.S.; 16 specs, Vidraru Lake, Cumpăna Valley, 8.08.2004, R.S.; 17 specs, Vidraru Lake, Cumpăna–Clăbucet Mt. route, 8.08.2004, M.S. (Maican & Serafim, 2018 a).

Gastrophysa polygoni polygoni (Linnaeus, 1758)

Material: 1 spec., Brădetu (Vâlsan Valley), 16.05.2002, S.S. (Maican, 2003).

Gastrophysa viridula viridula (DeGeer, 1775)

Material: 4 specs, Brădetu (Vâlsan Valley), 25–26.07.2000, S.M.; 6 specs, Vâlsan dam, 6.08.2001, S.S.; 1 spec., “Valea cu Pești” Chalet, 28.07.2000, S.M.; 5 specs, Lunca Gârtii (Stoenеști), 7.05.2002, At.S. (Maican, 2003); Oticu River Valley, 4.08.2004, R.S.; 7 specs, valley of Buda River, 4.08.2004, R.S., A.P.; 1 spec., Vidraru Lake, “Valea cu Pești” Chalet, 6.08.2004, R.S. (Maican & Serafim, 2018 a).

Phaedon armoraciae (Linnaeus, 1758)

Material: 2 specs, Clearings of Vâlsan Valley, 27.07.2000, S.M. (Maican, 2003).

Phaedon cochleariae cochleariae (Fabricius, 1792)

Material: 2 specs, Clearings of Vâlsan Valley, 27.07.2000, S.M.; 1 spec., Brădetu (Vâlsan Valley), 6.08.2001, S.S. (Maican, 2003).

Chrysolina haemoptera haemoptera (Linnaeus, 1758)

Material: 3 specs, Stoenеști, 25.08.1996, S.M.P. (Maican, 2003).

Chrysolina marcasitica marcasitica (Germar, 1824)

Material: 1 spec. ♂, Stoenеști, 30.04.1995, S.M.P. (Maican, 2003).

Chrysolina polita polita (Linnaeus, 1758)

Material: 2 specs, Stoenеști, 7.07.1995, S.M.P.; 12 specs, Brădetu (Vâlsan Valley), 25.07.2000, 5.08.2002, S.M., S.S.; 6 specs, Mușătești, 6.08.2001, S.S. (Maican, 2003).

Chrysolina fastuosa fastuosa (Scopoli, 1763)

Material: 2 specs, Brădetu, upper end of Vâlsan dam lake, 25.07.2000, S.M.; 4 specs, Brăduleț, 26.07.2000, S.M.; 1 spec., Lunca Gârtii, Stoenești, 7.05.2002, At.S. (Maican, 2003).

Chrysolina olivieri olivieri (Bedel, 1892)

Material: 5 specs, Brădetu (Vâlsan Valley), 24.07.2000, S. M. (Maican, 2003).

Chrysolina varians (Schaller, 1783)

Material: 1 spec., Brădetu, upper end of Vâlsan dam lake, 25.07.2000, S.M. (Maican, 2003).

Chrysolina herbacea herbacea (Duftschmid, 1825)

Material: 292 specs, Poiana Neamțului Chalet surroundings, 15–22.VII.1985, 700 m a.s.l., I.C., I.D.; 34 specs, Brădetu (Vâlsan Valley) (on *Mentha longifolia*), 25.07.2000, 27.09.2000, 5.08.2002, S.M., S.S. (Maican, 2003).

Oreina cacaliae senecionis Schummel, 1844

Material: 1 spec. ♂, Brădetu, Vâlsan dam, 2.04.2002, S.S. (Maican, 2003).

Oreina intricata intricata (Germar, 1824)

Material: 10 specs ♂, Brădetu, Vâlsan dam, 2.04.2002, S.S. (Maican, 2003).

Oreina virgulata virgulata (Germar, 1824)

Material: 1 spec. ♂, Brădetu, Vâlsan dam, 25.07.2000, S.M. (Maican, 2003).

Leptinotarsa decemlineata (Say, 1824)

Material: 3 specs, Brădetu (Vâlsan Valley), 26.07.2000, S.M.; 1 spec., Mușătești, 25.07.2000, S.M. (Maican, 2003).

Gonioctena pallida (Linnaeus, 1758)

Material: Brădetu, Vâlsan dam (Maican, 2003).

Subfamily Galerucinae Latreille, 1802

Galeruca pomonae pomonae (Scopoli, 1763)

Material: 1 spec., Brădetu, Clearings of Vâlsan, 27.07.2000, S.M. (Maican, 2003).

Galeruca tanacetii tanacetii (Linnaeus, 1758)

Material: 1 spec., “Valea cu Pești” Chalet surroundings, 28.07.2000, S.M. (Maican, 2003).

Galerucella lineola lineola (Fabricius, 1781)

Material: 1 spec. ♂, Mușătești, 6.08.2001, S.S.; 1 spec. ♀, Brădetu (Vâlsan Valley), 26.07.2000, S.M.; 10 specs, Brăduleț, 26.07.2000, S.M. (Maican, 2003).

Lochmaea caprea (Linnaeus, 1758)

Material: 1 spec., “Valea cu Pești” Chalet surroundings, 28.07.2000, S.M. (Maican, 2003).

Pyrrhalta viburni (Paykull, 1799)

Material: 2 specs ♂, Brădetu (Vâlsan Valley), 26.07.2000, S.M. (Maican, 2003).

Agelastica alni alni (Linnaeus, 1758)

Material: 3 specs, Brădetu (Vâlsan Valley) (on *Alnus incana*), 25.09.2000, S.M.; 2 specs, Brădetu, Vâlsan dam, 25.07.2000, S.M. (Maican, 2003).

Subfamily Alticinae Newman, 1834

Crepidodera aurata (Marsham, 1802)

Material: 2 specs, Galeșu (Vâlsan Valley), 1.08.1995, C.P.; 4 specs, Brădetu (Vâlsan Valley), 25–26.07.2000, 27.09.2000, S.M. (Maican, 2003).

Neocrepidodera femorata (Gyllenhal, 1813)

Material: 2 specs, Brădetu, upper end of Vâlsan dam lake, 6.08.2001, S.S. (Maican, 2003).

Neocrepidodera transversa (Marsham, 1802)

Material: 3 specs, Brădetu (Vâlsan Valley), 6.08.2001, S.S.; 2 specs, Mușătești, 6.08.2001, S.S. (Maican, 2003).

Phyllotreta ochripes (Curtis, 1837)

Material: 1 spec. ♀, Mușătești, 6.08.2001, S.S. (Maican, 2003).

Phyllotreta tetrastigma (Comolli, 1837)

Material: 5 specs, Brădetu, Vâlsan dam lake, 25.07.2000, S.M. (Maican, 2003).

Sphaeroderma testaceum (Fabricius, 1775)

Material: 1 spec. ♂, Brădetu (Vâlsan Valley), 25.07.2000, S.M. (Maican, 2003).

Subfamily Cryptocephalinae Gyllenhal, 1813

Labidostomis longimana (Linnaeus, 1761)

Material: 1 spec. ♀, Galeșu (Vâlsan Valley), 1.08.1995, C.P. (Maican, 2003); 1 spec. ♂, Vama Cucului, Cârțișoara 30.07.1994, C.H. (Maican, 2006).

Smaragdina flavicollis (Charpentier, 1825)

Material: 1 spec., Mușătești, 6.07.2001, S.S. (Maican, 2003).

Smaragdina xanthaspis (Germar, 1824)

Material: 1 spec. ♂, Galeșu (Vâlsan Valley), 25.07.1995, C.P.; 1 spec., Stoenesti, 7.07.1990, S.M.P. (Maican, 2003).

Cryptocephalus ocellatus ocellatus Drapiez, 1819

Material: 2 specs ♀, Brădetu (Vâlsan Valley), 24.07.2000, S.M.; 4 specs, Brăduleț (Vâlsan Valley), 26.07.2000, S.M.; 1 spec. ♂, Mușătești, 6.08.2001, S.S. (Maican, 2003).

Cryptocephalus octacosmus Bedel, 1891

Material: 1 spec., Galeșu (Vâlsan Valley), 25.07.1995, C.P. (Maican, 2003).

Cryptocephalus bipunctatus bipunctatus (Linnaeus, 1758)

Material: 1 spec., Stoenesti, 7.07.1995, S.M.P. (Maican, 2003).

Cryptocephalus frenatus Laicharting, 1781

Material: 1 spec., Brădetu, Vâlsan dam, 6.08.2001, S.S. (Maican, 2003).

Cryptocephalus hypochoeridis (Linnaeus, 1758)

Material: 1 spec. ♂, Galeșu (Vâlsan Valley), 25.07.1995, C.P. (Maican, 2003).

Cryptocephalus moraei (Linnaeus, 1758)

Material: 1 spec. ♂, Brădetu (Vâlsan Valley), 25.07.2000, S.M.; 1 spec. ♂, Brădetu, Vâlsan dam, 6.08.2001, S.S. (Maican, 2003).

Pachybrachis sinuatus (Mulsant & Rey, 1859)

Material: 2 specs, Brădetu (Vâlsan Valley) (on *Salix capreae*), 25.07.2000, S.M. (Maican, 2003).

Subfamily Eumolpinae Thompson, 1859

Pachnophorus villosus (Duftschmid, 1825)

Material: 1 spec., Mușătești, 6.08.2001, S.S. (Maican, 2003).

CONCLUSIONS

This study completes the data regarding the occurrence of Staphylinidae, Cerambycidae and Chrysomelidae species in the Făgăraș Mountains area, based on the study of the material preserved in the collection of the “Grigore Antipa” National Museum of Natural History and the Institute of Biology Bucharest of the Romanian Academy, and also on literature sources (published by authors between 1998 and 2018).

A total of 148 species (including 44 Staphylinidae species, 53 species of Cerambycidae and 51 Chrysomelidae species) belonging to 20 subfamilies and 89 genera are listed.

According to IUCN Red List of Threatened Species, *Morimus asper funereus* and *Rosalia alpina alpina* are listed as Vulnerable species.

Coleoptera fauna of the Făgăraş Mountains is still less known, and the potential of the area is very large. Therefore, based on the data known so far, we consider that it is necessary to continue the research in order to have an inventory as complete as possible.

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REFERENCES

- BIELZ A., 1887, *Die Erforschung der Kaferfauna. Verhandlungen und Mitteilungen des Siebenbürgischen Vereins für Naturwissenschaften zu Hermannstadt*, **37**: 27–114.
- CUZEPAN G., TĂUŞAN I., 2016, *Saproxylic beetles (Insecta: Coleoptera) of community interest in the natural history museum collections of Sibiu (Romania)*. Brukenthal, Acta Musei, **XI** (3): 433–442.
- DASCĂLU M. M., CUZEPAN G., TĂUŞAN I., 2012, *The Catalogue of Dorcadionini species (Coleoptera: Cerambycidae) from the Natural History Museum of Sibiu (Romania)*. Analele Ştiinţifice ale Universităţii “Alexandru Ioan Cuza” din Iaşi, Seria Biologie Animală, **LVIII**: 77–84.
- ELIESCU GR., ENE M., RĂDULESCU T., NEGRU ŞT., 1949, *Atacuri cauzate de insecte. Starea fitosanitară forestieră în anii 1948 și 1949*. Institutul de Cercetări și Experimentări Forestiere, **2** (78): 41–45. (in Romanian)
- GRUEV B., MERKL O., VIG K., 1993, *Geographical distribution of Alticinae (Coleoptera, Chrysomelidae) in Romania*. Annales Historico-Naturales Musei Nationalis Hungarici, Budapest, **85**: 75–132.
- KUTHY D., 1896, *Ordo Coleoptera. In: A Magyar Birodalom Állatvilága (Fauna Regni Hungariae)*. A Királyi Magyar Természettudományi Társulat, Budapest, 213 pp. (in Hungarian and Latin)
- LAZAREV M., 2009, *Cornumutila quadrivittata (G:7A:G, 1830) and C. lineata (L:IOC:G, 1844), stat. rest. (Coleoptera, Cerambycidae) from Western Europe and Russia*. Special Bulletin of the Japanese Society of Coleopterology, Tokyo, **7**: 117–126.
- LINNELL J. D. C., KALTENBORN B., BREDIN Y., GJERSHAUG J. O., 2016, *Biodiversity assessment of the Făgăraş Mountains, Romania*. NINA Report 1236, 86 pp.
- LÖBL I., SMETANA A. (eds.), 2004, *Catalogue of Palaearctic Coleoptera. Hydrophiloidea- Histeroidea- Staphylinoidea*. 2, Apollo Books, Stenstrup, 942 pp.
- LÖBL I., SMETANA A. (eds.), 2010, *Catalogue of Palaearctic Coleoptera. Chrysomeloidea*. 6, Apollo Books, Stenstrup, 924 pp.
- MAICAN S., 2003, *Leaf-beetles (Coleoptera: Chrysomelidae) from the Argeş River Basin*. Proceedings of the Institute of Biology, Bucharest, **5**: 81–92.
- MAICAN S., 2006, *Clytrins (Coleoptera: Chrysomelidae: Clytrinae) from the collections of the “Grigore Antipa” National Museum of Natural History (Bucharest)*. Travaux du Muséum National d’Histoire Naturelle “Grigore Antipa”, Bucharest, **49**: 239–257.
- MAICAN S., SERAFIM R., 2016, *The catalogue of Donaciinae and Criocerinae species (Coleoptera: Chrysomelidae) from the new leaf beetles collection from “Grigore Antipa” National Museum of Natural History (Bucharest) (Part I)*. Travaux du Muséum National d’Histoire Naturelle “Grigore Antipa”, Bucharest, **59** (2): 179–194.
- MAICAN S., SERAFIM R., 2017, *Catalogue of Cassidinae (Coleoptera: Chrysomelidae) from the New Leaf Beetles Collection from “Grigore Antipa” National Museum of Natural History (Bucharest) (Part II)*. Travaux du Muséum National d’Histoire Naturelle “Grigore Antipa”, Bucharest, **60** (2): 477–494.

- MAICAN S., SERAFIM R., 2018 a, *Catalogue of Chrysomelini Species (Coleoptera: Chrysomelidae: Chrysomelinae) from the New Leaf Beetles Collection of "Grigore Antipa" National Museum of Natural History (Bucharest) (Part III)*. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa", Bucharest, **61** (1): 23–42.
- MAICAN S., SERAFIM R., 2018 b, *Diversity of Chrysomelidae and Cerambycidae (Coleoptera: Chrysomeloidea) in the Făgăraş Mountains area*. In: Făgăraş Mountains. An inventory towards a new National Park, Book of Abstracts: 50.
- MAKRANCZY Gy., 2014, *Revision of the genus Ochtheophilus Mulsant & Rey, 1856 (Coleoptera: Staphylinidae, Oxytelinae)*. Revue Suisse de Zoologie, **121** (4): 457–694.
- PANIN S., 1944, *Les Chrysomela de la Roumanie*. Bulletin Section Scientifique de l'Académie Roumaine, **26** (9): 601–625.
- PANIN S., SĂVULESCU N., 1961, Cerambycidae. In: Fauna României, **10** (5). Edit. Academiei Române, 523 pp. (in Romanian)
- PETRI K., 1912, *Siebenbürgens Käferfauna auf Grund ihrer Erforschung bis zum Jahre 1911*. Verhandlungen und Mitteilungen des Siebenbürgischen Vereins für Naturwissenschaften zu Hermannstadt: 1–376.
- PETRI K., 1925–1926, *Ergänzungen und Berichtigungen zur Käferfauna Siebenbürgens 1912*. Verhandlungen und Mitteilungen des Siebenbürgischen Vereins für Naturwissenschaften zu Hermannstadt, **76**: 165–206.
- ROȘCA A., 1973, *Contributions à la connaissance du genre Cryptocephalus Foudr. (Coleoptera, Chrysomelidae) en Roumanie*. Travaux du Muséum d'Histoire Naturelle "Grigore Antipa", **13**: 143–154.
- ROȘCA A., 1974, *Contribution à la connaissance du genre Chrysomela L. (Coleoptera: Chrysomelidae) en Roumanie*. Travaux du Muséum d'Histoire Naturelle "Grigore Antipa", **14**: 251–259.
- ROZNER I., 2007, *Adatok Erdély és a Bánság cincérfajához (Coleoptera: Cerambycidae)*. Acta Siculica: 133–141.
- ROZNER I., ROZNER Gy., 2007, *Adatok Erdély és a Bánság levélbogár-fajához (Coleoptera: Chrysomelidae)*. Acta Siculica: 115–131.
- SERAFIM R., 2005, *The Catalogue of the Palaearctic species of Prioninae and Lepturinae (Coleoptera: Cerambycidae) from the patrimony of "Grigore Antipa" National Museum of Natural History (Bucharest) (Part I)*. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa", **48**: 103–117.
- SERAFIM R., 2006, *The Catalogue of the Palaearctic species of Lepturinae (Coleoptera: Cerambycidae) from the patrimony of "Grigore Antipa" National Museum of Natural History (Bucharest) (Part II)*. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa", **49**: 203–238.
- SERAFIM R., 2007, *The catalogue of the Palaearctic species of Spondylidinae (Coleoptera: Cerambycidae) from the patrimony of "Grigore Antipa" National Museum of Natural History (Bucharest) (Part III)*. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa", **50**: 221–230.
- SERAFIM R., 2009, *The catalogue of the Palaearctic species of Necydalinae and Cerambycinae (Coleoptera: Cerambycidae) from the patrimony of "Grigore Antipa" National Museum of Natural History (București) (Part IV)*. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa", **52**: 263–292.
- SERAFIM R., 2010, *The catalogue of the palaearctic species of Lamiinae (Coleoptera: Cerambycidae) from the patrimony of "Grigore Antipa" National Museum of Natural History (Bucharest) (Part V)*. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa", **53** (1): 235–272.
- SERAFIM R., MAICAN S., 2011, *Catalogue of Cerambycidae, Megalopodidae and Chrysomelidae (Coleoptera: Chrysomeloidea) recently entered in the patrimony of "Grigore Antipa" National Museum of Natural History (Bucharest) "Igor Ceianu" Collection*. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa", **54** (2): 425–460.
- STAN M., 1998, *Noi contribuții asupra faunei de stafilinide (Coleoptera) din România*. Studii și Cercetări de Biologie, seria Biologie Animală, **50** (1): 3–10. (in Romanian)

- STAN M., 2003, *Tachyporus dispar* (Paykull, 1789) and *Haploglossa nidicola* (Fairmaire, 1852) (Coleoptera: Staphylinidae) two new mentions in the staphylinid fauna of Romania. Travaux du Museum d'Histoire Naturelle "Grigore Antipa", **45**: 187–192.
- STAN M., 2010, *On the species of Ocypus Leach of the Carpathian Basin with special reference to the species of Romania* (Coleoptera: Staphylinidae: Staphylininae: Staphylinini). Acta Entomologica Serbica, **15** (2): 171–193.
- STAN M., 2012, *On the species of Philonthus Stephens* (Coleoptera: Staphylinidae: Staphylininae: Staphylinini: Philonthina) in the collections of Romanian natural history museums. Travaux du Museum National d'Histoire Naturelle "Grigore Antipa", **55** (2): 233–276.
- SZÉL G., ROZNER I., KOCS I., 1995, *Contribuții la cunoașterea Coleopteleror din Transilvania (România) pe baza colectărilor din ultimii ani*. Acta: 73–92.
- TATOLE V., IFTIME AL., STAN M., IORGU E. I., IORGU I., OȚEL V., 2009, *Speciile de animale Natura 2000 din România*. 174 pp. (in Romanian).
- TĂUȘAN I., BUCȘA C., 2010, *Palaeartic Longhorn Beetles (Coleoptera: Cerambycidae) from "Dr. Karl Petri" Collection of the Natural History Museum of Sibiu (Romania). Part I: Lepturinae Subfamily*. Travaux du Muséum National d'Histoire Naturelle „Grigore Antipa”, **LIII**: 223–233.

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OTOLITH - BODY LENGTH RELATIONSHIP OF KAIS KINGFISH (*CYPRINION KAIS* HECKEL, 1843) IN TIGRIS RIVER, TURKEY

MUHAMMED YAŞAR DÖRTBUDAK*, GÜLNAZ ÖZCAN**

The relationships between standard length (SL) and otolith length (OL), and width (OW) were examined for kais kingfish (*Cyprinion kais* Heckel, 1843) from Güçlükönak location of Tigris River. Sampling was carried out every month using nets from January to December 2015. Otolith samples for analysis were obtained from 135 specimens (101 females and 34 males). Standard length and otolith length measured for all fish and sex was identified. Samples consisted of fish varying in standard length from 6.0 to 14.8 cm, weight from 2.8 to 75.55 g and otolith length from 1.51 to 3.07 mm. The relationship between fish length (SL) and otolith length (OL) was as $y=0.1596x + 0.4445$ ($R^2=0.7606$), between fish length (SL) and otolith width (OW) was as $y=0.1201x + 0.5561$ ($R^2=0.8155$), between fish length (SL) and otolith weight (OWe) was as $y=0.0003x - 0.0017$ ($R^2=0.7632$), between otolith length (OL) and otolith width (OW) was as $y=0.6655x + 0.4192$ ($R^2=0.8385$). Analysis for the relation between right and left otolith did not reveal any significant differences (t-test, $p>0.05$).

Keywords: *Cyprinion kais*, otolith, kais kingfish, Tigris River, Turkey.

INTRODUCTION

Cyprinion kais Heckel, 1843 is an endemic freshwater fish seen in the Tigris-Euphrates river basin (Nasri *et al.*, 2010). *Cyprinion* species spread in a region extending from the river Indus to Orontes, Tigris and Euphrates rivers. The description of the two types of *Cyprinion* was made by Heckel in Tigris and Euphrates river systems. One of these species, *Cyprinion macrostomum* Heckel, 1843, has been mentioned in subsequent studies. The other *Cyprinion kais* has been reported in studies in Iraq and Syria. *Cyprinion kais* species have not been mentioned in studies for determination of freshwater fish in our country. However, specimens collected from the Tigris River and the Batman Creek were identified by Bănărescu & Herzig-Straschil (1995) as *Cyprinion kais*. And shows the distribution in freshwater of Turkey, Syrian Arab Republic, Iran and Iraq. In the case of wide distribution, it is under the danger of extinction in Turkey. *Cyprinion kais* is a valuable species for edible and angling. Despite its wide distribution and endangered species, it has not been studied extensively. Compared with *C. macrostomum*, there

is much doubt about its validity and sometimes it is considered synonymous with *C. macrostomum* (Nasri *et al.*, 2010).

Bănărescu & Herzing-Sraschil (1995) and Geldiay & Balık (2007), reported in their studies the number of branched rays in dorsal fin of *C. kais* is around 13–15 and this number is 9–11 in *C. macrostomum*. *C. kais* is flattened from side and its head length is less than the body height. The ratio between the standard length and body depth is ranged from 2.9 to 3.4. The mouth of fish is small and on the ventral and there is a lobe on it. There are a couple of barbels around the mouth. The last branched ray of the dorsal fin is boned and there are denticles on the ray (Bilici *et al.*, 2016).

Although kais kingfish is endemic Tigris and Euphrates basin, biology and ecology have not been well studied (Alkan-Uçkun & Gökçe, 2015). For this reason, in this study, morphometric and meridional properties of *Cyprinion kais* species were tried to be given comparatively in the samples obtained from Dicle River at Şırnak province, Güçlükonak.

MATERIAL AND METHODS

A total of 135 specimens of kais kingfish was caught monthly using nets from Tigris River, Şırnak from January to December 2015. Each fish samples was measured to the nearest 1 mm for standard length (SL). Sagittal otoliths were removed, cleaned and preserved dry in labeled boxes. Otolith length and otolith width were measured to the nearest 1 µm using Olympus SZ61TR+Olympus LC20. The right and left otoliths were dealt with separately. Broken and damaged otoliths were excluded from the calculations.

Differences between the lengths of the right and left otoliths for each species were tested using paired t-test. The relationships between otolith length and fish length were calculated using least squares regression equations to predict the standard length and weight of the original fish from otolith length. The otolith dimensions-standard length relationships were examined by using the following equation: ($y=a+bx$), where y is otolith length, x is fish standard length, a is intercept value, b is coefficient value (Başusta *et al.*, 2013).

RESULTS AND DISCUSSION

The sagittal otoliths of 135 kais kingfish specimens were examined. The standard length of all individuals ranged from 6.0 to 14.8 cm SL (mean 11.37 ± 0.15 cm) and weight from 2.8 to 75.55 g (mean 29.77 ± 1.19 g). The otolith length ranged between

1.51 and 3.07 mm (mean 2.27 ± 0.029 mm), otolith width from 1.28 to 2.48 mm (mean 1.93 ± 0.021 mm) and otolith weight from 0.0004 to 0.0034 g (mean 0.0018 ± 0.00005 g). The age of kais kingfish ranged from one to six years.

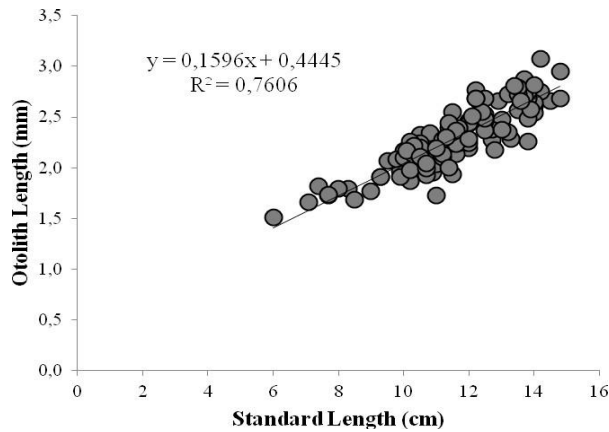


Fig. 1. Standard length-otolith length relationship of *Cyprinion kais*.

The relationship between fish length (SL) and otolith length (OL) was found as $y = 0.1596x + 0.4445$ ($R^2 = 0.7606$) (Fig. 1), between fish length (SL) and otolith width (OW) was $y = 0.1201x + 0.5561$ ($R^2 = 0.8155$) (Fig. 2), between fish length (SL) and otolith weight (OWe) was $y = 0.0003x - 0.0017$ ($R^2 = 0.7632$) (Fig. 3), between otolith length (OL) and otolith width (OW) was calculated as $y = 0.6655x + 0.4192$ ($R^2 = 0.8385$) (Fig. 4), between fish total weight (W) and otolith weight (OWe) was determined as $y = 0.00003x - 0.0008$ ($R^2 = 0.6367$) (Fig. 5). Analysis for the relation between right and left otolith did not reveal any significant differences (t-test, $p > 0.05$).

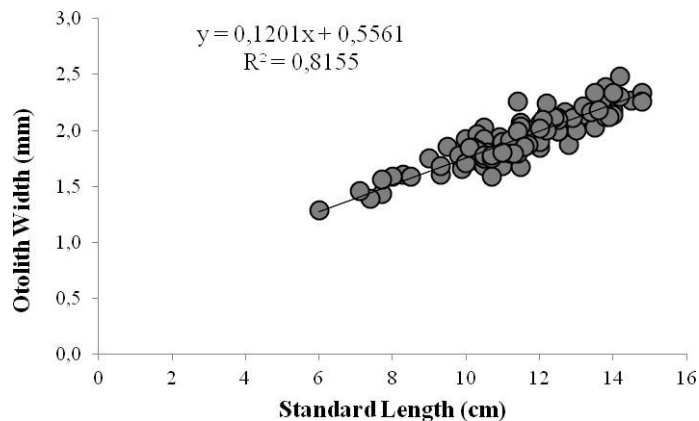


Fig. 2. Standard length-otolith width relationship of *Cyprinion kais*.

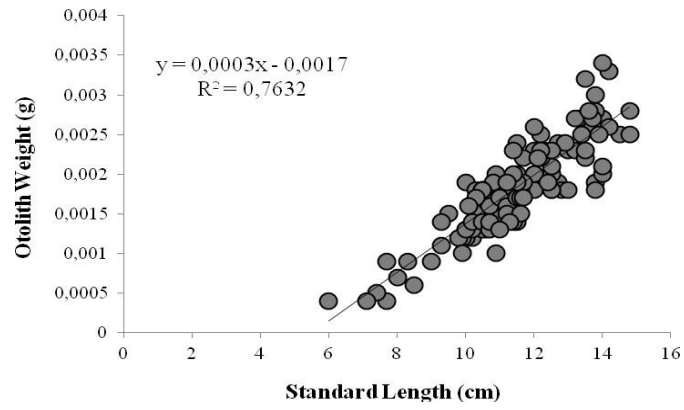


Fig. 3. Standard length-otolith weight relationship of *Cyprinion kais*.

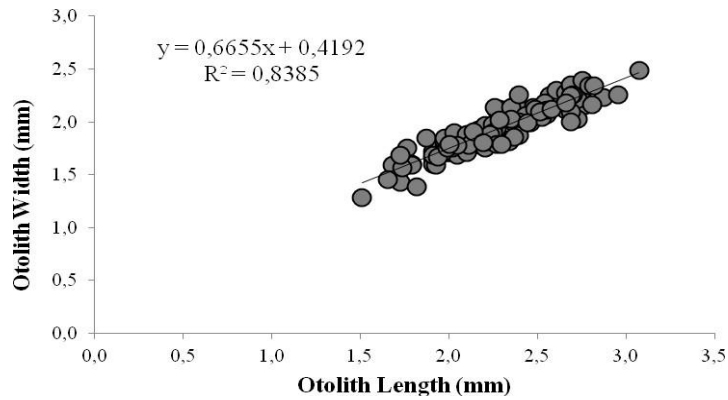


Fig 4. Otolith length-otolith width relationship of *Cyprinion kais*.

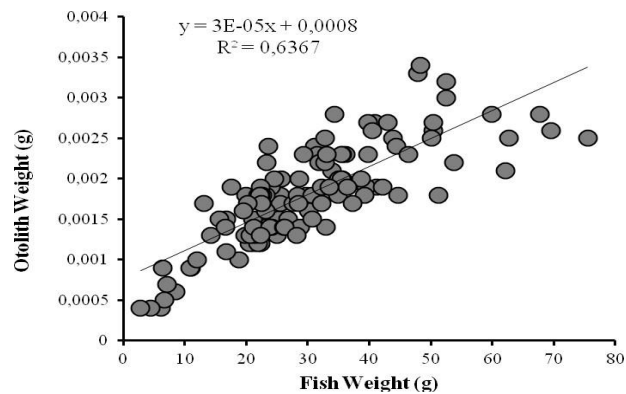


Fig. 5. Total weight-otolith weight relationship of *Cyprinion kais*.

Despite endemic kais kingfish in Tigris and Euphrates basin, otolith biometry have not been studied. Therefore, this is the first information on otolith biometry of *C. kais* from Şırnak, Turkey. Otolith length and otolith width were found to highly reliable measurement for determining the fish length.

The maximum estimated age was 6 years for kais kingfish in Şırnak. Alkan-Uçkun & Gökçe (2015) reported a maximum age of four years for *C. kais* and four years for *C. macrostomum* from Karakaya Dam Lake. Five years of age was reported for *C. macrostomum* from Gamasiab River in the Tigris River drainage in Iran (Faghani-Langroudi & Mousavi-Sabet, 2018).

There are a strong linear relationship between fish length and otolith length, and between fish length and otolith width ($R^2 > 0.76$). Başusta *et al.* (2013) reported that strong correlation between fish length-otolith length have been found in *Salmo trutta macrostigma* in Munzur River. Likewise, a strong positive correlation between fish length and otolith length have been found in some other fish species (Şen *et al.*, 2001; Ross *et al.*, 2005; İlkyaz *et al.*, 2011; Jawad *et al.*, 2011a, b; İşmen *et al.*, 2013; Yilmaz *et al.*, 2014; Dörtbudak & Özcan, 2015; Bostancı *et al.*, 2017; Mat-Piah *et al.*, 2017).

Also, a relationship between otolith weight and fish length has been used by some authors (Jawad *et al.*, 2011b; İlkyaz *et al.*, 2011; Yilmaz *et al.*, 2014; Bostancı *et al.*, 2017, Mat-Piah *et al.*, 2017). These all studies found that there was a positive linear relationship between fish length and otolith weight. In this study, a strong correlations was found between fish length and otolith weight.

CONCLUSIONS

Consequently, studies on the otolith biometry of kais kingfish are generally unavailable. Hence, relationship between fish length and otolith size of kais kingfish in Tigris River given in this study provides some tools for the study of food habits of piscivores and size of fish in archaeological samples.

REFERENCES

- ALKAN - UÇKUN A., GÖKÇE D., 2015, *Growth and reproduction of Cyprinion macrostomum* (Heckel, 1843) and *Cyprinion kais* (Heckel, 1843) populations in Karakaya Dam Lake (Euphrates River), Turkey. Turkish Journal of Zoology, **39**: 685–692.
- BĂNĂRESCU P. M., HERZIG-STRASCHIL B., 1995, *A revision of the species of the Cyprinion macrostomus-group (Pisces: Cyprinidae)*. Annalen des Naturhistorischen Museums in Wien, Serie B, **97**: 411–420.
- BAŞUSTA A., ÖZER Eİ., GİRGİN H., 2013, *Otolith Dimensions-Fish Length Relations Between Of Red-Spotted Trout (Salmo trutta macrostigma (Dummeril, 1858)) in Munzur River*. Journal of Fisheries Sciences.com, **7** (1): 22–29.

- BİLİCİ S., KAYA A., ÇİÇEK T., DÖRTBUDAK MY., 2016, *Investigation of size and shape differences depend to sex, age and season on scales of smallmouth lotak (Cyprinion kais)*. Journal of Survey in Fisheries Sciences, **3** (1): 37–45.
- BOSTANCI D., YEDİER S., KONTAŞ S., KURUCU G., POLAT N., 2017, *Regional variation of relationship between total length and otolith size in the three Atherina boyeri Risso, 1810 populations, Turkey*. Ege Journal of Fisheries and Aquatic Sciences, **34** (1): 11–16.
- DÖRTBUDAK M. Y., ÖZCAN G., 2015, *Relationship Between Otolith Length and Fish Length of Shiraz Barb (Capoeta umbla Heckel, 1843) in İkizce Stream (Şırnak)*. Yunus Araştırma Bülteni, **1**: 67–72. (in Turkish)
- FAGHANI - LANGROUDI H., MOUSAVI - SABET H., 2018, *Reproductive biology of lotak, Cyprinion macrostomum Heckel, 1843 (Pisces: Cyprinidae), from the Tigris River drainage*. Iranian Journal of Fisheries Sciences, **17** (2): 288–299.
- GELDİAY R., BALIK S., 2007, *Freshwater Fishes in Turkey*. Ege University Publishing, İzmir, **46**: 1–644. (in Turkish)
- İLKİYAZ A. T., METİN G., KINACIGİL H. T., 2011, *The use of otolith length and weight measurements in age estimations of three Gobiidae species (Deltentosteus quadrimaculatus, Gobioides niger and Lesueurigobius friesii)*. Turkish Journal of Zoology, **35** (6): 819–827.
- İŞMEN A., ARSLAN M., GÜL G., YİĞİN C. Ç., 2013, *Otolith morphometry and population parameters of red porgy, Pagrus pagrus (Linnaeus, 1758) in Saros Bay (North Aegean Sea)*. Ege Journal of Fisheries and Aquatic Sciences, **30** (1): 31–35.
- JAWAD L., AMBUALI A., AL-MAMRY J. M., AL-BUSAİDİ H. K., 2011a, *Relationship between fish length and otolith length and width in the lutjanid fish, Lutjanus bengalensis (Lutjanidae) collected from Muscat City coast on the Sea of Oman*. Journal of the Black Sea/Mediterranean Environment, **17** (2): 116–126.
- JAWAD L., AL - MAMRY J., AL - BUSAİDİ H., 2011b, *Relationships between fish length and otolith length, width and weight of the Indian Mackerel Rastrelliger kanagurta (Cuvier, 1817) collected from Sea of Oman*. Ribarstvo, **69** (2): 51–61.
- MAT - PIAH R., KAMARUDDİN S. A., ABDUL-KADİR N. H., AMBAK M. A., 2017, *Relationship between otolith measurements with the size of Areolate grouper, Epinephelus areolatus in Terengganu Waters, Malaysia*. Journal of Fisheries and Aquatic Science, **12** (2): 90–94.
- NASRİ M., KEİVANY Y., DORAFSHAN S., 2010, *First karyological analysis of smallmouth lotak, Cyprinion kais Heckel, 1843, an endemic Cyprinid fish from the Tigris-Euphrates Basin*. Italian Journal of Zoology, **77** (3): 272–276.
- ROSS R. M., JOHNSON J. H., ADAMS C. M., 2005, *Use of fish-otolith-length regressions to infer size of double-crested cormorant prey fish from recovered otoliths in Lake Ontario*. Northeastern Naturalist, **12** (2): 133–140.
- ŞEN D., AYDİN R., ÇALTA M., 2001, *Relationships between fish length and otolith length in the population of Capoeta capeta umbla (Heckel, 1843) inhabiting Hazar Lake, Elazığ, Turkey*. Archives of Polish Fisheries, **9** (2): 267–272.
- YILMAZ S., YAZIÇIOĞLU O., SAYGIN S., POLAT N., 2014, *Relationships of otolith dimensions with body length of european perch, Perca fluviatilis L., 1758 from Lake Ladik, Turkey*. Pakistan Journal of Zoology, **46** (5): 1231–1238.

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REPORT ON THE ACCUMULATION OF HEAVY METALS IN THE FEATHERS OF SOME WETLAND BIRDS IN THE DANUBE DELTA (ROMANIA)

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LILIANA TEODOROF, MIHAELA ȚIGĂNUȘ, LUCIAN-EUGEN BOLBOACĂ,
KATARINA TOSIC, JANOS BOTOND KISS, MARIAN TUDOR

An assessment was carried out in the Danube Delta Biosphere Reserve (Romania, 2015) regarding heavy metal residues in the feathers of native wild bird species that depend on wetlands for all or part of their life cycle. The concentrations of trace elements (As, Cr, Mn, Ni, Pb and Zn) were determined in the feathers of Ferruginous Duck, White Stork, Common Little Bittern, Little Egret, Great White Pelican, Pygmy Cormorant, Common Tern and Western Marsh-harrier. Arsenic concentrations varied between 1.610 mg/kg (Ferruginous Duck) and 3.437 mg/kg (Great White Pelican). The highest chromium concentrations were found in Pygmy Cormorant (11.063 mg/kg) and Great White Pelican (12.368 mg/kg); the highest zinc concentration was found in Ferruginous Duck (163.237 mg/kg). The highest concentrations of manganese and nickel were found in the White Stork (66.541 mg/kg – Mn; 6.276 mg/kg – Ni), Western Marsh-harrier (59.037 mg/kg; 4.075 mg/kg) and Little Egret (64.347 mg/kg; 5.900 mg/kg). The concentration of lead showed values between 3.474 mg/kg (Western Marsh-harrier) and 0.426 mg/kg (Ferruginous Duck), while the other studied species had concentrations up to 0.9 mg/kg. Laboratory analyses revealed important interspecific differences and a high capacity of waterbirds for storing heavy metals in plumage.

Keywords: heavy metals, feathers, birds, Danube Delta, Romania.

INTRODUCTION

At the European level, a series of natural and anthropic limiting factors affect wild bird species, most of which are protected by a number of European and international directives and conventions. Heavy metals are among these limiting factors. There have been several studies regarding heavy metal bird poisoning in Europe (Lebedeva *et al.*, 1997; Hernandez *et al.*, 1999; Berglund *et al.*, 2011; Kitowski *et al.*, 2014).

In Romania, there have been several studies regarding heavy metal concentrations in abiotic elements, plants and fish (Dinescu *et al.*, 2004; Tudor *et al.*, 2006; Vignati *et al.*, 2013; Ilie *et al.*, 2014; Ionescu *et al.*, 2015; Burada *et al.*, 2014; 2015; 2016; 2017; Ștefănuț *et al.*, 2018). However, studies focusing on bird species

are lacking (Fasolă-Mătășaru *et al.*, 2017). In order to evaluate the degree of heavy metal accumulation in bird species of Community interest from the Danube Delta Biosphere Reserve, a non-invasive protocol was applied. According to literature, this type of monitoring can show annual variations in heavy metal concentrations due to the fact that these substances are integrated into new plumage during the moulting period (Frantz *et al.*, 2012; Gushit *et al.*, 2016).

The present study was carried out during a colonial waterbird breeding census and the monitoring of bird species of Community interest from the Danube Delta Biosphere Reserve on the Romanian territory.

MATERIAL AND METHODS

All the samples were collected (May to August, 2015) in the Danube Delta Biosphere Reserve [DDBR, Gâștescu & Știucă (2008)] and its immediate vicinity, in the eastern part of Romania. Sampling locations included several reas: Murighiol, Uzlina Lake, Nufăru, Maliuc fishpond, Sălchioara fishpond, Nebunu Lake, Fortuna Lake and Draghilea Channel (Fig. 1).

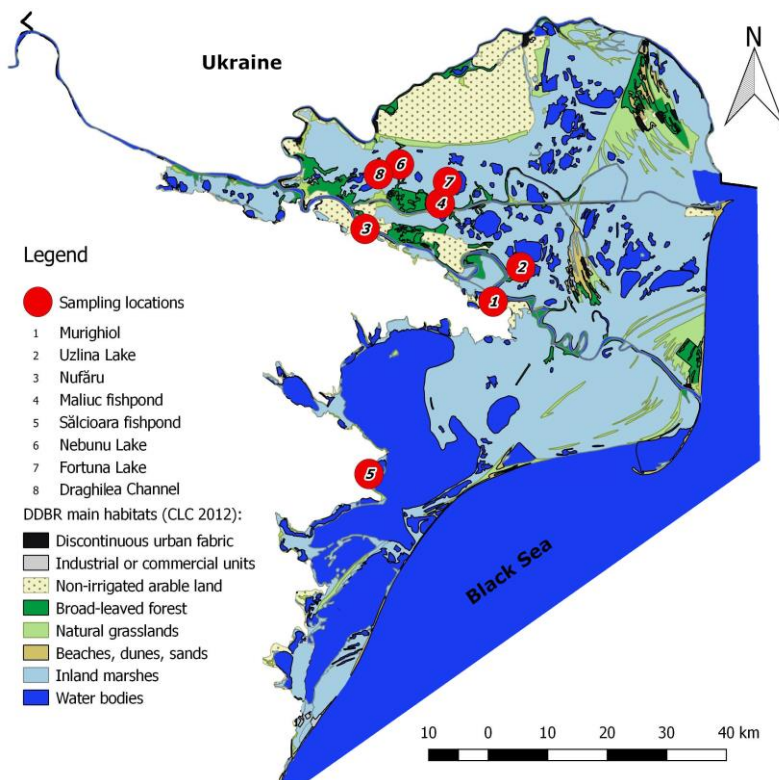


Fig. 1. Locations of sample collection in the Danube Delta Biosphere Reserve.

We sampled moulted flight feathers in feeding and roosting areas, as well as feathers collected from dead individuals (approximately 5 g of feathers per individual). All the samples were primary flight feathers (remiges), as it has been shown that different feathers accumulate trace elements in different concentrations (Guo *et al.*, 2001). The concentration of trace elements (As, Cr, Mn, Ni, Pb and Zn) was determined for Ferruginous Duck – *Aythya nyroca* (1 individual), White Stork – *Ciconia ciconia* (5 individuals), Common Little Bittern – *Ixobrychus minutus* (1 individual), Little Egret – *Egretta garzetta* (2 individuals), Great White Pelican – *Pelecanus onocrotalus* (1 individual), Pygmy Cormorant – *Phalacrocorax pygmeus* (1 individual), Common Tern – *Sterna hirundo* (3 individuals) and Western Marsh-harrier – *Circus aeruginosus* (1 individual).

Sample analysis was conducted by acid mineralization, necessary for trace elements determination, with the help of an Anton Paar Multiwave 3000 microwave oven. The trace element content was analysed using the ICP-MS Elan DRC-e, which is applicable for the determination of small concentrations of a large number of elements. Approximately 1–2 g of each sample was weighed and 5 ml HNO₃ and 2 ml of H₂O₂ were added. After a short pre-reaction time (15 minutes), the recipients were closed with special caps and placed in the protection layer with secured cap, after which it was introduced into the rotor. The power of the device was gradually raised to 600 W over a period of 5.5 minutes and maintained at this level for 4.5 minutes. In the last stage, the power was raised to 1000 W and maintained for 10 minutes. The total mineralization time was 20 minutes, while the total cooling time was 20–25 minutes.

Following the completing of the mineralization program and the cooling time, the quartz recipients were opened, and the contents transferred into 50-ml volumetric flasks that were then filled to the mark with acidified water.

The content of heavy metals was analysed with the help of *Elan DRC-e ICP-MS* applicable in the determination of small concentrations of a large number of elements. Trace elements are nebulized and transformed into aerosols which are then carried by argon into the plasma torch. The resulting ions are trained in plasma and introduced via an interface into a mass spectrometer according to SR EN ISO 17294-2, 2005 (****2005).

RESULTS AND DISCUSSION

We analysed a total of 15 individual samples from eight locations, belonging to eight species (Table 1). The highest concentrations were recorded for Zn, with values between 12,307 to 158,237 mg/kg.

Table 1
Trace element concentrations for sampled feathers

Prelevation place	Species	Values (mg/Kg)					
		As	Cr	Mn	Ni	Pb	Zn
Murighiol	<i>Ciconia ciconia</i>	2.856	3.948	108.827	7.868	2.007	130.485
Nufăru	<i>Ciconia ciconia</i>	2.534	1.2	153.43	6.508	0.74	143.761
Maliuc fishpond	<i>Ciconia ciconia</i>	1.625	0.683	37.714	5.208	0.672	146.654
Murighiol	<i>Ciconia ciconia</i>	3.12	2.924	16.31	6.018	3.019	136.401
Murighiol	<i>Ciconia ciconia</i>	2.659	3.607	16.424	5.78	1.31	145.925
Draghilea Channel	<i>Phalacrocorax pygmeus</i>	1.589	4.063	22.258	1.694	0.608	12.572
Murighiol	<i>Sterna hirundo</i>	3.226	2.803	14.109	1.445	3.337	20.402
Murighiol	<i>Sterna hirundo</i>	2.776	2.257	41.063	2.557	1.803	18.849
Murighiol	<i>Sterna hirundo</i>	1.796	1.926	15.895	1.855	1.697	12.307
Uzlina Lake	<i>Circus aeruginosus</i>	2.924	4.619	59.037	4.075	3.474	157.986
Nebunu Lake	<i>Egretta garzetta</i>	3.584	7.542	79.688	4.585	0.966	119.627
Nebunu Lake	<i>Egretta garzetta</i>	1.709	0.848	49.005	7.215	1.408	119.307
Maliuc fishpond	<i>Ixobrychus minutus</i>	2.239	6.815	23.418	1.905	1.263	93.527
Fortuna Lake	<i>Pelecanus onocrotalus</i>	3.437	12.368	25.215	2.789	1.558	136.357
Salcioara fishpond	<i>Aythya nyroca</i>	1.61	4.58	18.431	1.391	0.426	158.237
Average		2.5122	4.012	45.38	4.059	1.61	103.49
Max		3.584	12.368	153.43	7.868	3.474	158.23
Min		1.589	0.683	14.109	1.391	0.426	12.307

All of the six studied elements (As, Cr, Mn, Ni, Pb and Zn) have bioaccumulative and toxic effects on the organism (Hill, 2010). It should be noted that the accumulation of heavy metals (internally, from food and water) occurred during the period of growth of feathers, for about 1–3 months during the previous year, very likely in the Danube Delta (all birds were adults).

The studied birds either moult after the breeding period, as in the case of *Aythya nyroca* (Cramp & Simmons, 1977), or during the breeding period until the start of migration, for the other species. However, in the case of large species, the moulting of flight feathers can occur over longer time periods (Ramirez & Panuccio, 2019), and the accumulation of these elements could have occurred outside the DDBR.

Also, the question of the origins of these trace elements should be treated with care, as it is very likely that the samples suffered from external contamination, either by atmospheric deposition or by contact with water, as has been shown in the case of Ni, Pb and Zn (Dauwe *et al.*, 2003). In this situation the origin of the contaminants can also be from outside of the DDBR, as all the sampled species belong to species that have migratory populations.

Moreover, some species (the Great White Pelican at least) can frequently feed at great distances from the colony during the nesting period and, again, the accumulation (from food, water and atmospheric deposition) of these elements have occurred outside the DDBR. A comparison of our results with previous studies regarding background concentrations of DDBR ecosystems (water and sediments, Burada *et al.*, 2014; Ibram *et al.*, 2019) showed our values to be lower.

There are also differences ($p < 0.05$) between trace element concentrations found in bird feathers and those found in aquatic macroinvertebrates (Ibram *et al.*, 2019) and fish (Burada *et al.*, 2017). However, these comparisons must be regarded with caution, since the sampling periods were different and samples were collected from different places in the DDBR. Thus, we cannot make definitive conclusions of this kind regarding bioaccumulation phenomena for the studied elements.

The concentration values for arsenic varied between 1.610 mg/kg for the Ferruginous Duck and 3.437 mg/kg for the Great White Pelican. In general, the arsenic concentrations show little variation ($S^2 = 0.496$) for the studied species (Fig. 2). The differences between values may be caused by the trophic factor or phenological characteristics of each species. However, when concentrations have low variation, we can consider that these are caused by the natural level of arsenic in the environment.

Comparing the levels of arsenic found in birds with the ones found in fish in the DDBR (Burada *et al.*, 2017), we found higher concentrations in our samples, which can be an indicator of a bioaccumulation process via the trophic pyramid.

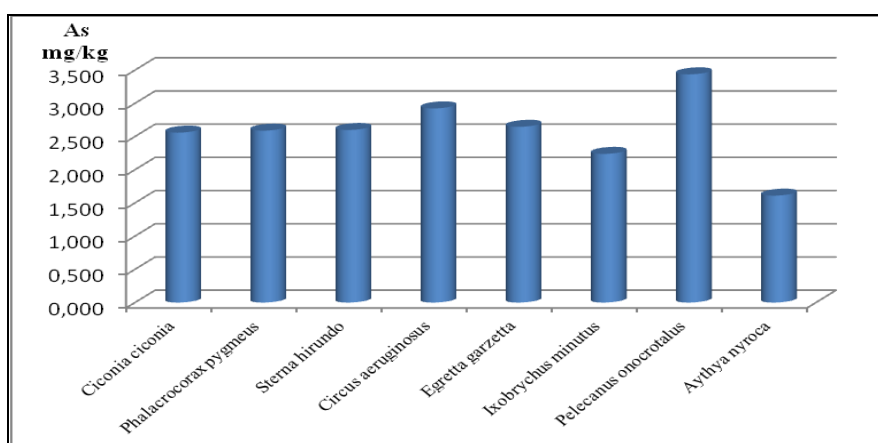


Fig. 2. Arsenic concentrations in feathers of bird species of Community interest in the Danube Delta Biosphere Reserve.

The graphical representation of chromium concentrations (Fig. 3) shows a significant difference of accumulation capacities of this heavy metal between the Pygmy Cormorant – 11.063 mg/kg and the Great White Pelican – 12.368 mg/kg, and other species. These differences are most likely linked to the size of fish that are consumed by different species of birds (including benthic fish that are in close contact with the substrate). The differences between these concentrations can also be linked to the feeding areas, as well as with the different migration periods, different stopover sites and especially with wintering areas.

The minimum values, up to 2.472 mg/kg, were identified for White Storks, a species that frequently consumes its prey from dry habitats, especially amphibians, snakes and lizards, and much less fish (unlike fish, many amphibians and reptiles spend less time or none at all in water).

For some species, the variation of chromium shows no clear pattern. In the case of the Common Tern, a fish eating bird, chromium was found in smaller concentrations than in the Ferruginous Duck which is a species that predominantly feeds on plants of aquatic origin and rarely eats invertebrates (Kiss *et al.*, 1980; Kiss *et al.*, 1984; Kiss *et al.*, 1986; Ciochia, 1992).

The values of chromium in birds were smaller than those found in sediments (Ibram *et al.*, 2019), though larger than the average concentrations found in macroinvertebrates and in most of the studied fish (Burada *et al.*, 2017; Ibram *et al.*, 2019).

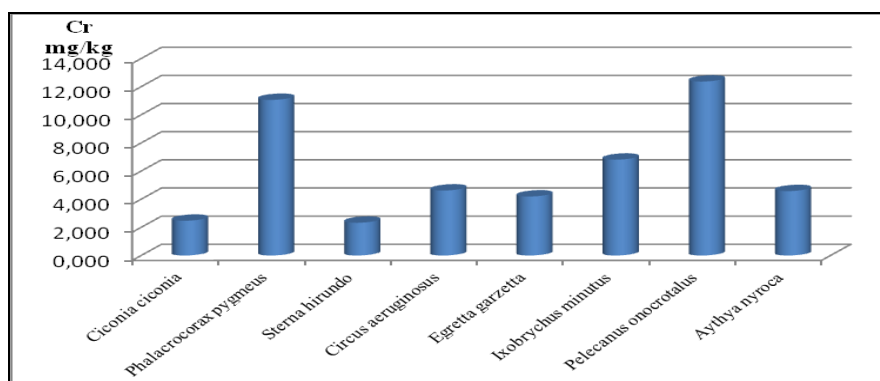


Fig. 3. Chromium concentrations in feathers of bird species of Community interest in the Danube Delta Biosphere Reserve.

Out of all the studied trace elements, manganese was highest in concentration values after zinc. The highest concentrations were recorded for the White Stork (66.541 mg/kg), Western Marsh-harrier (59.037 mg/kg) and Little Egret (64.347 mg/kg) (Fig. 4). For the rest of the species it varied between 18.431 mg/kg and 25.215 mg/kg.

By analysing these differences, we observed that the bird species that feed mainly in the riparian or marsh areas have the highest concentration levels. In contrast, in species that feed in open waters (except the Common Little Bittern), the manganese concentrations were lower.

Manganese is known to be a common element in aquatic ecosystems, occurring in large quantities (Burada, 2015). At concentrations over 0.2 mg/kg, in the presence of oxygen, it precipitates and is deposited in sediments (Allen, 1989), from where it is assimilated in large quantities by aquatic organisms.

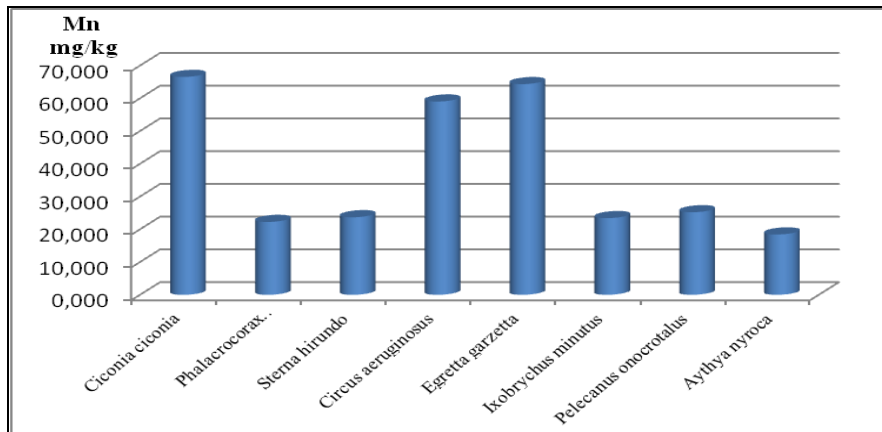


Fig. 4. Manganese concentrations in feathers of bird species of Community interest in the Danube Delta Biosphere Reserve.

In the case of nickel (Fig. 5), much like in the case of manganese, we noted a high accumulation capacity for the species that feed mainly in riparian and marsh areas (White Stork – 6.276 mg/kg, Little Egret – 5.900 mg/kg and Western Marsh-harrier – 4.075 mg/kg). From our results, it seems that both nickel and manganese have a similar concentration distribution among species and it is most likely that they have the same contamination source. The smallest concentration levels of nickel were identified for the Ferruginous Duck (1.391 mg/kg).

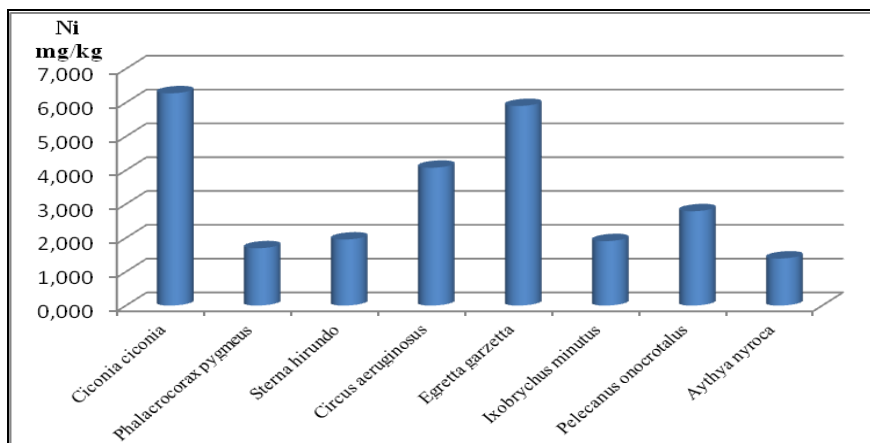


Fig. 5. Nickel concentrations in feathers of bird species of Community interest in the Danube Delta Biosphere Reserve.

The nickel values from sampled feathers are much lower than those found in sediments (Ibram *et al.*, 2019), though relatively similar (with the exception of the White Stork, Little Egret and Western Marsh-harrier that showed higher concentrations) to medium concentrations found in aquatic macroinvertebrates and in most fish species (Burada *et al.*, 2017; Ibram *et al.*, 2019).

Regarding lead, it is important to note that it is an accumulative poison and has no essential biological function, as all of its compounds are toxic. Lead forms strong bonds with enzymes and proteins (e.g. haemoglobin), thus disrupting many metabolic processes (Bolcu & Király, 2012). Lead concentration levels (Fig. 6) varied between 36.474 mg/kg for the Western Marsh-harrier and 0.426 mg/kg for the Ferruginous Duck. Overall, the lead concentrations varied only slightly ($S^2=0.953$).

Considering that the sample size was small and a definitive pattern cannot be observed, preliminary analysis shows that lead levels increase directly in proportion to bioaccumulation along the trophic pyramid. The highest lead concentration was found in the Western Marsh-harrier, a raptor species that feeds mainly on birds and mammals (Ciochia, 1992), species with higher accumulation potentials compared to herbivorous, insectivorous and piscivorous birds.

Thus we can explain a lower lead concentration in the Ferruginous Duck, which is a herbivorous species (Ciochia, 1992). The values of lead concentrations from bird feathers (Fig. 6) were much lower than those from sediments (Ibram *et al.*, 2019), but much higher than those found in fish tissues (Ibram *et al.*, 2017).

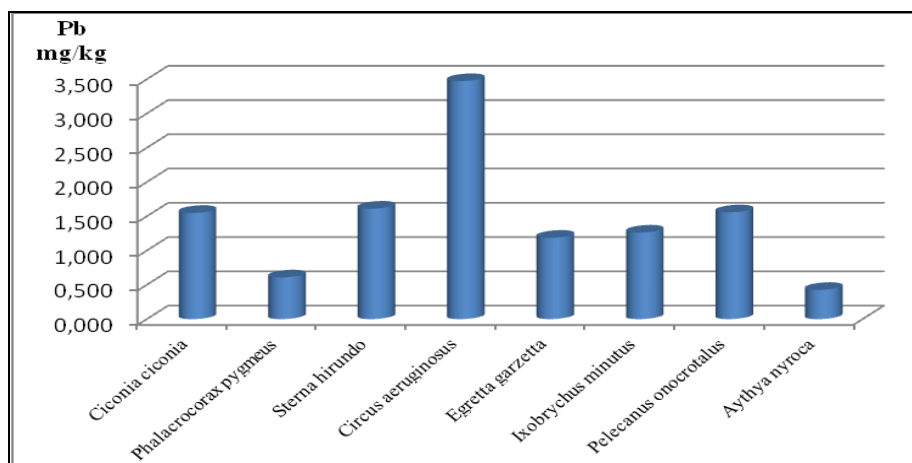


Fig. 6. Lead concentrations in feathers of bird species of Community interest in the Danube Delta Biosphere Reserve.

The zinc concentrations found in bird feathers (Fig. 7) are higher than those from sediments and much higher than those found in aquatic macroinvertebrates (Ibram *et al.*, 2019).

The highest values, up to 163.237 mg/kg were observed for the Ferruginous Duck, while lowest, at 93.527 mg/kg, were identified for the Common Little Bittern. These high values recorded for Ferruginous Ducks may be caused by the metabolism of some forms of zinc in aquatic vegetation (the basic food of this species), a hypothesis mentioned by Iordache (2009).

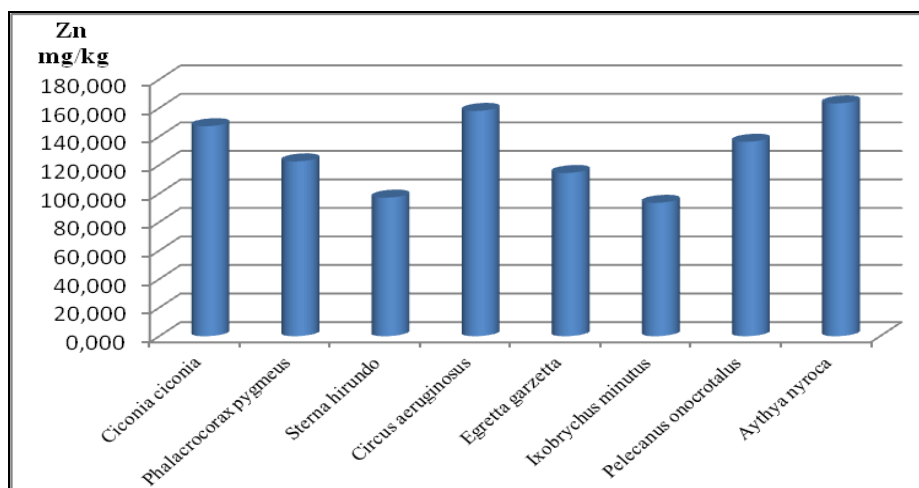


Fig. 7. Zinc concentrations in feathers of bird species of Community interest in the Danube Delta Biosphere Reserve.

CONCLUSIONS

Our investigations regarding heavy metals in samples collected from eight bird species of Community interest revealed inter-specific differences, most likely caused by differences in diet and other ecological features.

Laboratory analyses revealed that the studied species have a high capacity for accumulation of heavy metals and arsenic in their plumage. However, the values were generally lower than those found by other studies to be present in the sediments and water of the DDBR.

Arsenic concentrations varied between 1.610 mg/kg for *Aythya nyroca* and 3.437 mg/kg for *Pelecanus onocrotalus*, whereas variations were minor in the rest of the studied species.

There was a significant difference in the chromium accumulation capacity in medium-sized piscivorous species compared to large species (*Phalacrocorax pygmeus* – 11.063 mg/kg and *Pelecanus onocrotalus* – 12.368 mg/kg).

Out of all the studied elements (with the exception of zinc), manganese had the highest values. The highest concentrations were identified for species that feed in marshy and riparian areas (*Ciconia ciconia* – 66.541 mg/kg, *Circus aeruginosus* – 59.037 mg/kg and *Egretta garzetta* – 64.347 mg/kg).

In the case of nickel, there was also a similarity in storage capacity among the species that predominantly feed in riparian and marshy areas (*Ciconia ciconia* – 6.276 mg/kg, *Circus aeruginosus* – 4.075 mg/kg and *Egretta garzetta* – 5.900 mg/kg).

The lead concentration range varied between 3.474 mg/kg in the case of the Western Marsh-harrier and 0.426 mg/kg for the Ferruginous Duck. The levels of lead increased in proportion to bioaccumulation along the trophic pyramid.

In comparison to background values, zinc concentrations were higher, up to 165.237 mg/kg, as identified in *Aythya nyroca*, while the lowest, 93.527 mg/kg, was found in *Ixobrychus minutus*.

We consider it necessary for the future to repeat these analyses on a larger number of birds. With a larger sample we would be able to analyse individual concentrations of trace elements from different areas of the DDBR. In order to have a better understanding of the feather accumulation phenomenon in DDBR, sampling from bird juvenile specimens is recommended.

REFERENCES

- ALLEN S., 1989, *Chemical Analysis of Ecological Materials*. Blackwell Scientific Publications, Second ed., Oxford.
- BERGLUND A. M. M., KOIVULA M. J., EEVA T., 2011, *Species and age-related variation in metal exposure and accumulation of two passerine wild bird*. *Environmental Pollution*, **159**: 2368–2374.
- BOLCU C., KIRÁLY Z., 2012, *Efectele nocive ale plumbului asupra organismelor vii*. Revista de Informare, Universitatea de Vest, Facultatea de Chimie-Biologie.
- BURADA A., ȚOPA M. C., GEORGESCU L. P., TEODOROF L., NĂSTASE C., SECELEANU-ODOR D., NEGREA B. M., ITICESCU C., 2014, *Heavy metals accumulation in plankton and water of four aquatic complexes from Danube Delta area*. *AAEL Bioflux*, **7** (4): 301–310.
- BURADA A., ȚOPA C.M., GEORGESCU L.P., TEODOROF L., NĂSTASE C., SECELEANU-ODOR D., ITICESCU C., 2015, *Heavy Metals Environment Accumulation in Somova – Parches Aquatic Complex from the Danube Delta Area*. *Chemistry Magazine, Bucharest*, **66** (1): 48–54.
- BURADA A., TEODOROF L., IONAȘCU A., ȚOPA M. C., GEORGESCU L. P., TUDOR I.-M., IBRAM O., TUDOR M., 2016, *Temporal trends and evolution of heavy metals concentrations in Somova-Parches Aquatic complex – last area of the Danube floodplain*. *Journal of Environmental Protection and Ecology*, **17** (3): 864–973.
- BURADA A., TEODOROF L., DESPINA C., SECELEANU-ODOR D., TUDOR I.-M., IBRAM O., NAVODARU I., MURARIU G., ȚOPA C. M., TUDOR M., 2017, *Trace elements in fish tissue with commercial value of the Danube Delta Biosphere Reserve*. *Environmental Engineering and Management Journal*, **16** (3): 731–738.
- CIOCHIA V., 1992, *Păsările clocitoare din România*. Editura Științifică, București, 386 pp.
- CRAMP S., SIMMONS K. E. L., 1977, *Handbook of the birds of Europe, Middle East and North Africa*. Vol. 1, Oxford University Press.
- DAUWE T., BERVOETS L., PINXTEN R., BLUST R., EENS M., 2003, *Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination*. *Environmental Pollution*, **124**: 429–436.
- DINESCU L. C., STEINNES E., DULIU O. G., CIORTEA C., SJOBACK T. E., DUMITRU D. E., GUGIU M. M., HARALAMBIE M., 2004, *Distribution of some major and trace elements in Danube Delta lacustrine sediments and soil*. *Journal of Radioanalytical and Nuclear Chemistry*, **262**: 345–354.

- FASOLĂ-MĂȚĂȘARU L., STRUNGARU Ș. A., SIMIONOV I. A., NICOARĂ M. N., COJOCARU D., 2017, *The presence of different elements in Herons eggshells used as bioindicators of wetland health in Moldova region, Romania: preliminary study*. The 9th International Zoological Congress of "Grigore Antipa" Museum, Bucharest, Book of Abstracts, pp.: 173.
- FRANTZ A., POTTIER M.-A., KARIMI B., CORBEL H., AUBRY E., HAUSSY C., GASPARINI J., CASTREC-ROUELLE M., 2012, *Contrasting levels of heavy metals in the feathers of urban pigeons from close habitats suggest limited movements at a restricted scale*. Environmental Pollution, **168**: 23–28.
- GĂȘTESCU P., ȘTIUCĂ R. (eds.), 2008, *Delta Dunării Rezervație a Biosferei/Danube Delta Biosphere Reserve*. Institutul de Geografie, INCDDD Tulcea, Editura CD Press, 400 pp.
- GUO D. L., ZHOU M. S., XI Y.Y., 2001, *Preliminary studies on the level and distribution of mercury in feathers of birds*. Acta Zoologica Sinica, **47**: 139–149.
- GUSHIT J. S., TURSHAK L. G., CHASKDA A. A., ABBA B. R., NWAEZE U. P., 2016, *Avian feathers as bioindicator of heavy metal pollution in urban degraded woodland*. Ewemen Journal of Analytical & Environmental Chemistry, **2** (2): 84–88.
- HERNÁNDEZ L. M., GÓMARA G., FERNÁNDEZ M. A., JIMÉNEZ B., GONZÁLEZ M. J., BAOS R., HIRALDO F., FERRER M., BENITO V., SUÑER M. A., DEVESA V., MUÑOZ O., MONTORO R., 1999, *Accumulation of heavy metals and As in wetland birds in the area around Donana National Park affected by the Aznalcollar toxic spill*. The Science of the Total Environment, **242**: 293–308.
- HILL M. K., 2010, *Understanding Environmental Pollution*. Third ed., Cambridge University Press.
- IBRAM O., SPIRIDON C., TUDOR I.-M., SULIMAN I., TEODOROF L., DESPINA C., SECELEANU-ODOR D., BURADA A., 2019, *Heavy Metals in Aquatic Macroinvertebrates and Danube River Sediments*. Scientific Annals of the Danube Delta Institute, **24**: 41–50.
- ILIE M., MARINESCU F., GHITA G., DEAK GY., TANASE G. S., RAISCHI M., 2014, *Assessment of heavy metal in water and sediments of the Danube River*. Journal of Environmental Protection and Ecology, **15** (3): 825–933.
- IONESCU P., RADU V. M., DEAK G., 2015, *Spatial and seasonal variations of heavy metals in water and sediment samples collected from the Lower Danube River*. Scientific Annals of the Danube Delta Institute, **21**: 43–50.
- IORDACHE V., 2009, *Ecotoxicologia metalelor grele în Lunca Dunării*. Editura Ars Docendi, București, 339 pp.
- KISS J. B., RÉKÁSI J., STERBETZ I., 1980, *Cercetări privind hrana raței mari (Anas platyrhynchos L.) și raței ochi albi (Aythya nyroca Gmel.) în bunurile piscicole din Delta Dunării*. Trav. Staț. "Stejarul", **8**: 229–239.
- KISS J.B., RÉKÁSI J., STERBETZ I., 1984, *A Duna-Deltában (România) élő tőkés (Anas platyrhynchos) - és cigányrécék (Aythya nyroca) táplálékának vizsgálata*. Puszta, **2** (11): 39–52.
- KISS J. B., RÉKÁSI J., RICHNOVSZKY A., 1986, *Mollusken als Vogelnahrung*. Proceedings of the 8th International Malacological Congress, Budapest, 1983, pp.: 109–111.
- KITOWSKI I., SUJAK A., WIACEK D., STROBEL W., RYAMARZ M., 2014, *Trace element residues in eggshells of Grey Heron (Ardea cinerea) from colonies of East Poland*. North-Western Journal of Zoology, **10** (2): 436–354.
- LEBEDEVA N. V., 1997, *Accumulation of Heavy Metals by Birds in the Southwest of Russia*. Russian Journal of Ecology, **28** (1): 41–46 (Translated from Ekologiya, **1**: 45–50).
- RAMIREZ J., PANUCCIO M., 2019, *Flight feather moult in Western Marsh Herriers during autumn migration*. Avian Research, **10** (7).
- ȘTEFĂNUȚ S., MANOLE A., ION C. M., CONSTANTIN M., BANCUIU C., ONETE M., MANU M., VICOL I., MOLDOVEANU M. M., MAICAN S., COBZARU I., NICOARĂ G.R., FLORESCU I. L., MOGÎLDEA D. E., PURICE M. D., NICOLAE D. C., CATANĂ D. R., TEODOSIU G., DUMITRACHE A.C., MARIA M.G., VÂTCĂ C., OANȚĂ M., ÖLLERER K., 2018, *Developing a novel warning-informative system as a tool for environmental decision-making based on biomonitoring*. Ecological Indicators, **89**: 480–487.

- TUDOR M. I., TUDOR M., DAVID C., TEODOROF L., TUDOR D., IBRAM O., 2006, *Heavy metals concentrations in aquatic environment and living organisms in the Danube Delta*. Pp.: 435–442. In: Simeonov L. and Chirilă E. (eds.), *Romania Chemicals as International and Accidental Global Environmental Threats*, Series C: Environmental Security, Springer, Dordrecht, The Netherlands.
- VIGNATI D.A., SECRIERU D., BOGATOVA Y.I., DOMINIK J., CÉRÉGHINO R., BERLINSKY N.A., OAIE G., SZOBOTKA S., STANICĂ A., 2013, *Trace element contamination in the arms of the Danube Delta (Romania/Ukraine): current state of knowledge and future needs*. *Journal of Environmental Management*, **125**: 169–178.
- *** 2005, SR EN ISO 17294-2. The water quality. The application of the Inductively-Coupled Plasma Mass Spectrometry Method. Two part – The 62 elements determination. US EPA 3015 / 1994 – Microwave assisted acid digestion of aqueous samples and extracts.

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EFFECTS OF DIFFERENT LEVELS OF SAVORY (*SATUREJA HORTENSIS* L.) POWDER ON PERFORMANCE, SOME BLOOD BIOCHEMICAL AND INTESTINAL CHARACTERISTICS OF COBB 500 BROILER CHICKS

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In this study we evaluated the effects of Savory powder on the performance, blood biochemical and intestinal characteristics of female Cobb 500 broiler chicks. Total of 240 one day broiler chicks were randomly divided into 4 treatments. Treatments were further divided into 6 replicates. Broilers were fed a basal diet as control group, basal diet with 0.2% Savory powder (S1), basal diet with 0.4% Savory powder (S2) and basal diet with 0.6% Savory powder (S3). The experiment was carried out for 6 weeks. Feed intake (FI) and body weight gain (BWG) was calculated for estimation of feed conversion ratio (FCR). At the end of the experimental period four birds from each replicate were randomly slaughtered to determine carcass characteristics and other parameters. The blood serum samples were subjected to biochemical analysis. For determination of intestinal characteristics and small intestine tissues samples were collected. All data were analyzed as a completely randomized design using General Linear Models (GLM). The significance of the differences among least square means of main effects was tested by Duncan's new multiple-range test. Data from this study showed BWG and FCR in broilers improved underfed different levels of Savory powder ($P \leq 0.05$). There were significant differences between carcasses traits under effect of feed Savory powder levels ($p \leq 0.05$). The results of this study showed that serum triglyceride, cholesterol LDL content decreased in groups fed by savory powder and HDL increased significantly ($p \leq 0.05$) instead. In addition, villus height decreased in S1 and S2 groups. An increasing crypt depth was seen on S2. According to these data villus widths, epithelium layer and goblet cells increased by using Savory, especially by using S2 ($p \leq 0.05$). In conclusion we could demonstrate that the savory powder may be used as ingredient in broilers ration without harming effects on carcass characteristics, blood biochemical parameters, and intestinal morphology of Cobb 500 broiler chicks.

Keywords: blood biochemical, performance, intestinal characteristics, Cobb 500 broiler.

INTRODUCTION

Natural feed additives had beneficial effect for stimulation and activity of digestive system by improving the diet palatability and enhancing appetite of poultry, thus increasing the amount of feed consumed (Kamel, 2001). Aromatic plants powder, and their associated essential oils or extracts are used as potentially

growth promoters (Dibner & Richards, 2005). In this case, the scientists are working to improve feed efficiency and growth rate of livestock using herbal plants (Bunyaphatsara, 2007). Savory plant (*Satureja hortensis* L.) is an annual, herbaceous aromatic and medicinal plant belonging to the Lamiaceae family (Zargari, 1997). It is known as summer savory, native to southern Europe, Asia and naturalized in parts of North America (Ghalamkari *et al.*, 2011). It is widely distributed in different parts of Iran as one of the most important of classified twelve *Satureja* species. Its essential oil contains considerable amounts of two phenolic ketones that is carvacrol (Ultee *et al.*, 1998) and thymol (Ghannadi, 2001). The savory is traditionally used in foods for herbal tea and flavor component and in folk and traditional medicine and to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhea and infectious diseases (Leung & Foster, 1996). Also, Zamani Moghaddam *et al.* (2007) reported that the beneficial effects of savory in poultry disease treatment. The pharmacological action of herbal extracts and their active materials in humans is also well known, but in animal nutrition the number of experimental studies is relatively low (Faghani *et al.*, 2014), hence the objectives of this study was to explore the potential use of different levels of savory powder as a feed additive and growth promoter, on performance, some blood biochemical and intestinal characteristics in Cobb 500 broiler chicks.

MATERIAL AND METHODS

This study was conducted last during 42 days at the poultry farm of the Veterinary College, Islamic Azad University, Shahrekord branch, ShahreKord, Iran during April until June 2017.

Experimental plan

A total of 240 commercial (Cobb 500) one days old broiler chicks were divided into four treatment groups and they were further sub-divided into six replicates within 10 birds per each. The savory powder purchased from herbalists, cleaned was mixed and adding different levels to control (basal) diet as four experimental diets as shown in the Table 1. The treatments were as control (basal) diet with no savory powder; basal diet + 0.2% of savory powder (S1), basal diet + 0.4% of savory powder (S2), basal diet+ 0.6% savory powder (S3) respectively. All diets of each period were prepared with the same composition and they were both iso-nitrogenous and iso-caloric. Diets were formulated to exceed the requirements of NRC, 2007 recommendations. Feed and fresh water was provided ad-libitum during this experiment.

Data collection

The data on growth performance were collected for the following parameters: body weight gain (g), feed intake (g), feed conversion ratio (g), carcass yield (%),

and edible part weight (g). The body weight of individual bird was recorded on a weekly basis. Daily feed intake was calculated for weekly recorded and then calculated feed conversion ratio (FCR) on base daily feed intake. At the end of growth period, four birds from each replicate (i.e. 24 replicate) were randomly slaughtered for determination of carcass traits and the other parameters. Body sections were separated and weighed as percentage weight. Dressing percentage was calculated free from giblets and the organs were weighed separately as percentage of carcass weight. The point spread, performance index and production efficiency factor were also calculated for evaluating the growth performance. The blood samples were taken from the brachial vein from four birds per replicate and stored at 4°C in refrigerator. The blood serum samples were subjected to biochemical analysis by Pars Azmoon commercial kits. Samples from small intestine tissue were collected for determination intestinal characteristics such as villus height, crypt depth, villus width, epithelium layer and goblet cells. The histomorphometric investigation was performed by light microscopy, and the measurement was done using public domain image analysis software Image J version xx (National Institute of Mental Health, Bethesda, MD, USA).

Statistical Analysis

Data were collected and analyzed using as a completely randomized design using the General Linear Models procedure of SAS 9.2. The significance of the differences among least square means of treatments main effects was tested by Duncan's multiple-range test.

Table 1

Composition of the experimental diets for Cobb 500 broiler chicks

Ingredients %	0–7 (days old)	7–14 (days old)	15–29 (days old)	29–42 (days old)
Corn grain	52.22	53.30	49.25	43.20
Soybean meal	37.5	33	27	23.5
Wheat	6	10	20	30
Calcium Carbonate	1.60	1.50	1.55	1.40
NaCl	0.18	0.20	0.20	0.20
Vitamin Premix*	1.25	1	1	0.85
Mineral Premix*	1.25	1	1	0.85
Calculated nutrient content				
ME(Kcal/Kgr)	2830	2870	2920	2960
CP (%)	22	20.5	18.5	17.5
Ca (%)	1	0.95	0.85	0.85
Available Phosphorus (%)	0.50	0.45	0.45	0.40
Lysine (%)	1.34	1.20	1.05	0.95
Methionine+Cystine (%)	0.92	0.85	0.80	0.75

Supplied per kilogram of feed: 7,500 IU of vitamin A, 2000IU vitamin D3, 30 Mg vitamin E, 1.5 µg vitamin B1, 2Mg B6, 5 Mg Vitamin K, 5 Mg vitamin B2, 1 Mg vitamin B1, 40 Mg nicotinic acide, 160µg vitamin Biothine, 12 Mg Calcium pantothenate, 1MgFolic acid 20 Mg Fe, 71 Mg Mn, 100µg Se, 37Mg Zn, 6 Mg Cu, 1.14 Mg I, 400 µg Cu.

RESULTS AND DISCUSSION

Performance

The results obtained from performance of broiler chickens fed by savory powder are shown in the Table 2. Data showed that use of S1, S2 and S3 experimental feed treatments increased feed intake (FI) significantly ($P<0.05$) compared to control group. Body weight gain and final live weight were also significantly higher in S2 and S3 experimental feed treatments ($P<0.05$). Amiriandi (2015) reported that adding savory essential oil to the drinking water of chicken in 1–42 days period, this result showed that the live weight gain of chicks was significantly decreased in comparison to the than control group. Additionally, Kamel (2001) and Dibner & Richards (2005) also mentioned that the herbal powder and their essential oil mixture may be considered as a potential growth promoter.

Table 2

The effects of savory powder levels added as experimental diets on broilers performance

Treatments	FI (g.d)	BWG (g.d)	FCR	Live weight (g)	Carcass yield (g)
Control	103.20 ^b	48.94 ^b	2.00 ^a	2090.30 ^c	1440.11 ^b
S1	104.11 ^a	50.21 ^b	1.97 ^{ab}	2114.40 ^c	1495.00 ^a
S2	104.24 ^a	52.11 ^a	1.95 ^{ab}	2168.80 ^b	1516.25 ^a
S3	104.39 ^a	53.30 ^a	1.92 ^b	2199.41 ^a	1516.14 ^a
SEM	0.028	0.500	0.023	9.700	7.400

^{a,b} and ^c means within columns with different superscripts are different ($P<0.05$).

Overall means in the Table 2 showed the significant difference in body weight among treatments ($P\leq 0.05$). Ahmadian-Attari *et al.* (2011) showed that the herbal can improve growth and have beneficial effect on broilers. Cross *et al.* (2007) by studying on savory, rosemary, marjoram and their essential oil on growth and digestibility in 7 to 28 days broiler chicks, were suggested that the improvement of body weight gain and feed conversion ration may be due to the active compounds such as cinnamaldehyde and ugenol that they found in this herbal. Lee *et al.* (2003) showed that the herbal active compounds were caused better efficiency in the utilization of nutrients and better growth and performance. In the present study FCR was at the lowest in broilers fed by the highest savory powder. These results proved that savory though being more effective performed to certain extent and have a great potential to be utilized as an alternative. Data from this study also showed that significant differences for live weight (g) between treatments, and the higher live weight was for S2 and the lesser was for control group (Table 2).

The results of other studied showed that body weight gain, feed conversion ratio and dressing percentages increased ($P\leq 0.05$) in birds that supplemented with herb extract than control group birds, also mortalities and sudden deaths were minimized via used of herbal supplementation in birds diets (Kamel, 2001).

Zhang *et al.* (2005) reported that some plants or specific combinations of herbs in formulations may act as antioxidants by exerting superoxide scavenging activity or by increasing superoxide dismutase activity in various tissue sites (Khosravinia *et al.*, 2013). These results are in agreement with researchers who indicated that, addition of medicinal herbal plants had significant effects on improving digestibility coefficient and nutritive values (Shahin *et al.*, 2003).

Edible Organs

According to the Table 3 data, using of different levels of savory powder were decreased abdominal fat statistically ($p \leq 0.05$). The liver weight was higher when broilers fed by S2 ($p \leq 0.05$) and intestine weight were at the highest for S3 group than others.

Table 3

The effects of savory powder levels added as experimental diets
on some edible organs of broilers

Treatments	Liver (g)	Abdominal Fat (g)	Spleen (g)	Intestine (g)
Control	39.77 ^c	48.45 ^a	1.70 ^b	76.16 ^c
S1	41.73 ^b	44.71 ^b	1.83 ^a	78.35 ^{bc}
S2	43.32 ^a	42.43 ^b	1.80 ^a	80.44 ^b
S3	44.17 ^a	39.21 ^c	1.89 ^a	83.67 ^a
SEM	0.500	1.061	0.031	0.980

^{a,b} and ^c means within columns with different superscripts are different ($P < 0.05$).

Blood Biochemical Assay

Blood biochemical assay showed that the blood triglyceride, cholesterol and LDL content tended to decrease by using savory powder and HDL increased significantly ($p \leq 0.05$). The supplemented diets with different levels of savory powder had a beneficial effect on broiler performance and blood plasma cholesterol and glucose. Result of Ebrahimi *et al.* (2013) study showed that lowest cholesterol content was for savory powder and the highest of cholesterol was related to the control ($P \leq 0.05$). Inconsistent with the results of this study results (Ghalamkari *et al.*, 2011) showed that the use of savory powder had no significant effect on blood serum biochemical of broilers. The result of our finding are in agreement with those obtained by Lee *et al.* (2003), who found that dietary carvacrol, but not thymol, reduces plasma triglycerides and suggested that carvacrol may have more impact on lipogenesis than on cholesterol biosynthesis. In parallel with other investigation by (Ebrahimi *et al.*, 2013) they noted that the different levels of savory essential oil in drinking water had no effects on blood metabolites. Khalaf *et al.* (2008) showed that the phenolic compounds in some herbals may inhibit lipid peroxidation, scavenge and have the superoxide anion and hydroxyl radical, so they could enhance the activities of detoxifying enzymes such as glutathione-S-transferase (Ultee *et al.*, 1998). For example, Kurucuet *et al.* (2013) mentioned

that the D-limonene (1-methyl-4-(1-methylethenyl)-cyclohexane) is a monocyclic monoterpene component of some herbals have hypo-cholesterolemic effects. The hypo-cholesterolaemic action of herbals is possibly related to its poly-phenolic components (Shahin *et al.*, 2003; Seidavi *et al.*, 2017). Polyphenols have been shown to depress the reverse cholesterol transport, reduce the intestinal cholesterol absorption and even increase bile acid excretion (Faghani *et al.*, 2014).

Table 4

The effects of savory powder levels added as experimental diets on several blood biochemical of broilers (mg. dl)

Treatments	Triglyceride	Cholesterol	LDL	HDL
Control	73.26 ^a	136.02 ^a	60.15 ^a	66.14 ^c
S1	70.15 ^b	134.10 ^a	57.12 ^b	68.21 ^{bc}
S2	68.15 ^{bc}	130.26 ^b	56.12 ^b	70.02 ^b
S3	66.21 ^c	128.14 ^b	54.10 ^c	73.65 ^a
SEM	1.060	1.075	1.017	0.850

^{a,b} and ^c means within columns with different superscripts are different (P<0.05).

According to the Table 5 data, the savory powder affects villus height, crypt depth, villus width, epithelium layer and goblet cells significantly (P≤0.05). The villus height was decreased by using S1 and S2 respectively and the highest crypt depth was related to the S1 and S2 groups. According to our data the villus widths, epithelium layer and goblet cells were increased by using savory powder significantly (P≤0.05).

Intestinal Morphology

Table 5

The effect of savory powder levels added as experimental diets on intestinal characteristics of broilers (Micrometers)

Treatments	Villus height	Crypt depth	Villus width	Epithelium
Control	57.70 ^b	18.65 ^b	5.76 ^b	2.21 ^b
S1	58.17 ^b	19.20 ^b	6.65 ^{ab}	2.84 ^a
S2	60.21 ^b	20.44 ^{ab}	7.68 ^a	2.94 ^a
S2	63.50 ^a	22.55 ^a	7.87 ^a	3.10 ^a
SEM	1.091	1.076	0.840	0.120

^{a,b} and ^c means within columns with different superscripts are different (P<0.05).

Yeganeparast *et al.* (2016) showed that most active principles of plant extracts and powders are absorbed in the intestine by enterocytes and readily metabolized by the body and the products of this metabolism are transformed into polar compounds

by conjugation with glucuronate and excreted in the urine. As the active compounds are readily metabolized and have short half-lives, the risk of tissue accumulation is probably minimal.

CONCLUSIONS

According to the results of this study, using of different levels of Savory powder have beneficial effects on performance of Cobb 500 broiler chicks. The savory powder could reduce the blood serum cholesterol and LDL content, but did not affect on HDL levels. In conclusion, we could conclude that the basal diet with 0.6% of savory powder have beneficial effect on performance, some blood biochemical and intestinal characteristics on experimental broiler chicks.

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REFERENCES

- AMIRIANDI M., 2015, *Influence of Satureja hortensis L. essential oil in drinking water on broiler production and some blood biochemical parameters*. International Journal of Advanced Biological and Biomedical Research, **3** (4): 391–396.
- BUNYAPRAPHATSARA N., 2007, *Utilization of medicinal plants in animal production*. Pp.: 157–165. In: 1th Int. Cong., Leiden, Netherland Phytopharmacology.
- CROSS D. E., MCDEVITT R. M., HILLMAN K., ACAMOVIC T., 2007, *The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age*. British Poultry Science, **48** (4): 496–506.
- DIBNER J. J., RICHARDS J. D., 2005, *Antibiotic growth promoters in agriculture: History and mode of action*. Poultry Sciences, **84**: 634–643.
- EBRAHIMI A., ALAWQOTBI A. A., ZOHREH P., 2013, *The effect of different levels of savory (Satureja hortensis L.) on blood parameters and gastrointestinal microbial population of broiler chickens*. Annals of Biological Research, **4**: 332–336.
- GHANNADI A., 2002, *Composition of the essential oil of Satureja hortensis L. seeds from Iran*. Journal of Essential Oil Research, **14**: 35–36.
- GHALAMKARI G., TOGHYANI M., TAVALAEIAN E., LANDY N., GHALAMKARI Z., RADNEZHAD H., 2011, *Efficiency of different levels of Satureja hortensis L. (Savory) in comparison with an antibiotic growth promoter on performance, carcass traits, immune responses and serum biochemical parameters in broiler chickens*. African Journal of Biotechnology, **10**: 13318–13323.
- HAJHASHEMI V., SADRAEI H., GHAAADI A. R., MOHSENI M., 2000, *Antispasmodic and antidiarrheal effects of Satureja hortensis L. essential oil*. Journal of Ethnopharmacology, **71**: 187–192.
- KAMEL C., 2001, *Tracing modes of action and the roles of plant extracts in non-ruminants*. Pp.: 135–150. In: GARNSWORTHY P. C., WEISEMAN J. (Eds.), Recent Advances in Animal Nutrition. Nottingham University Press, Nottingham, UK.

- KHOSRAVINIA H., GHASEMI SAND, RAFIEI ALAVI E., 2013, *The effect of savory (Satureja khuzistanica) essential oils on performance, liver and kidney functions in broiler chickens*. Journal of Animal and Feed Sciences, **22**: 50–55.
- LEE K. W., EVERTS H., KAPPERT H. J., YEOM K. H., BEYNEN A. C., 2003, *Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens*. Journal of Applied Poultry Research, **12**: 394–399.
- LEUNG A. Y., FOSTER S., 1996, *Encyclopedia of common natural ingredients used in foods, drugs, and cosmetics*. 2nd ed. New York: Wiley: 465–466.
- SAHIN F., KARAMAN I., GÜLLÜCE M., ÖGÜTÇÜ H., ŞENGÜL M., ADIGÜZEL A., KOTAN R., 2003, *Evaluation of antimicrobial activities of Satureja hortensis L.* Journal of Ethnopharmacology, **87**: 61–65.
- SEIDAVI A. R., DADASHBEIKI M., ASADPOUR L., VAN DEN HOVEN R., ALIMOHAMMADI SARA EI M. H., ALISE M., SANTINI A., 2017, *Dietary green tea powder affects the immunologic parameters of broiler chicks*. Italian Journal of Animal Sciences, **16** (1): 108–114.
- ULTEE A., GORRIS L. G., SMID E. J., 1998, *Bactericidal activity of carvacrol towards the food-borne pathogen Bacillus cereus*. Journal of Applied Microbiology, **85**: 211–218.
- YEGANEPARAST M., KHALILI M., SALARI J., 2016, *Effect of different levels of Satureja hortensis essential oil on performance, carcass characteristics, acidity and intestinal microflora population in broilers*. Journal of Cell and Animal Biology, **10** (1): 1–8.
- ZAMANI MOGHADDAM A. K., GHANADI A. R., GAFARIAN A., SHOJADOOST B., 2007, *The effect of Satureja hortensis on performance of broiler chickens and NDHI titers*. 16th European symposium on poultry nutrition, Strasburg, France.
- ZARGARI A., 1997, *Medicinal Plants*, 3 Vol. 1. 6th Edit. Tehran University Publication, Tehran.
- ZHANG K. Y., YAN F., KEEN C. A., WALDROUP P. W., 2005, *Evaluation of micro encapsulated essential oils and organic acids in diets for broiler chickens*. International Journal of Poultry Science, **4** (9): 612–619.
- *** NRC, 2007, National Research Council, Nutrient requirements of small ruminant. Sixth edition, National Academy Press, Washington D.C., USA., 384 pp..
- *** SAS software, 2008, SAS User's Guide: Statistics, Version 9.2, SAS Institute, North Carolina, USA.

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GERMAN SCIENTIFIC PERSONALITIES SETTLED DOWN IN ROMANIA - WILHELM KARL (WILHELM CAROL) KNECHTEL, FATHER, BOTANIST AND WILHELM KARL W. KNECHTEL, SON, ZOOLOGIST

SANDA MAICAN*, MARIAN-TRAIAN GOMOIU**

The paper presents aspects of the life and scientific work of two German biological scientists established in Romania, along with other local people of German origin, thanks to Prince Carol de Hohenzollern-Sigmaringen, brought to lead the country, first as Prince and then King (May 10, 1866) of Romanian Principalities. It also approaches the dynasty of biologists WILHELM KARL (WILHELM CAROL) KNECHTEL, a botanist, specialist in designing and arranging gardens and parks, who had an extensive international experience in the field, but also as an entomologist, as well as his son, WILHELM KARL W. KNECHTEL, born in Romania, the founder of the Romanian school of agricultural entomology. He mainly researched Thysanoptera insects, was a professor of entomology in Bucharest and Chişinău and member of the Romanian Academy.

The thought of venerating prominent people of Romanian biology first led us to a remarkable zoological personality – the entomologist Wilhelm Knechtel. The history of the German origin of this scientist takes us back in time to the story of his father, an equally remarkable biologist of his time, which we are trying to synthesize today from the tangles of information that have emerged over the years.

Preamble

At the end of the 19th century, Romania was a poor, undeveloped and divided country, mainly agricultural, with a rudimentary agriculture, of subsistence and at the will of nature. With the bringing of Prince Carol of Hohenzollern-Sigmaringen (1839–1914) as leader of the country, first as Prince of the Romanian Principalities and then King (May 10, 1866), a number of specialists and businessmen of German origin also came in the Romanian Principalities in order to modernize the different areas of Romania.

The King brought a number of German families into the country [Physician C.F. Witting, who founded vascular surgery in Romania, the great manufacturer Erhard Luther, who built the Romanian Steam Brewery (later the Griviţa Brewery), in 1869], all Protestants, who had commercial interests, but were also in the King's entourage and they came as friends. Among them there was the young botanist Wilhelm Karl (Wilhelm Carol) Knechtel, Austrian-German, who was a passionate

lover of natural sciences, specialist in designing and arranging gardens and parks, with a vast international experience in the field (Fig. 1). As he said, “*no pleasure is more lasting than the pleasure that nature gives us; because it is above all*” (“*Landscaping Gardens*”, 1899).

He was born on August 13th, 1837, from Austrian parents, in a village in Bohemia (Czech Republic), in Burgstein commune, which now belongs to the town of Novy Bor, located between the Lužické Mountains (Horní Lužické) and the Central Bohemian Plateau (České středohoří), a region with a generous nature and a rich biodiversity. He attended courses at the Carolina University in Prague, where he was Professor Kosteletzki’s favorite student, a well-known botanist of the time. At first, Knechtel was hired as part of the maintenance team of the park of Schönbrunn Palace in Vienna.

At the court of Emperor Maximilian

Between 1859 and 1860, Archduke Ferdinand Maximilian, a member of the Habsburg-Lorraine Imperial House, hired for the first time the botanist Wilhelm Knechtel as a gardener for his small palace in Lokrum Island (Lacroma) in the Adriatic Sea. Later, for about five years, Knechtel worked at Castle Miramar (in Italian = *sea view*), the main residence of Maximilian from Trieste.

When the Archduke was crowned Emperor of Mexico (1864), Wilhelm Knechtel accompanied him to the American continent, being appointed the “gardener of the court”. An interesting detail is that, from the expeditionary team that accompanied Maximilian to Mexico, there were also two Romanians: Ilarie Mitrea, military physician in the Austrian army and Prince George Bibescu, the son of the former Prince of Wallachia (Gheorghe Bibescu), an officer in French army.



Fig. 1. Botanist Wilhelm Karl (Wilhelm Carol) KNECHTEL
(13.08.1837, Pihlerbaustellen – 22.10.1924, Bucharest)
(photo made at the beginning of 20th century, in a Viennese workshop).

In Mexico City, W. Knechtel designed various gardens, including the magnificent garden on the roof of Chapultepec Castle, the imperial residence of Maximilian, located on Chapultepec Hill (in Aztec *chapoltepec* = *locust hill*). This garden is still admired all over the world.

It is not known whether the time spent there was productive in gardening, but the period was certainly beneficial in terms of ... writing! He wrote by hand, in German, the book *Handschriftliche Aufzeichnungen meiner persönlichen Eindrücke und Erlebnisse in Mexiko in den Jahren 1864–1867* / *Personal notes and impressions on the events in Mexico between 1864–1867*, printed in 1905, at the expense of the author, in Karl Bellmann's printshop from Prague (Fig. 2). This is a unique document, in which the descriptions of nature are intertwined with numerous events of the tumultuous history of Mexico. The volume represents an European testimony of the different aspects of landscape architecture and daily life at the Mexican Court of the 19th century.

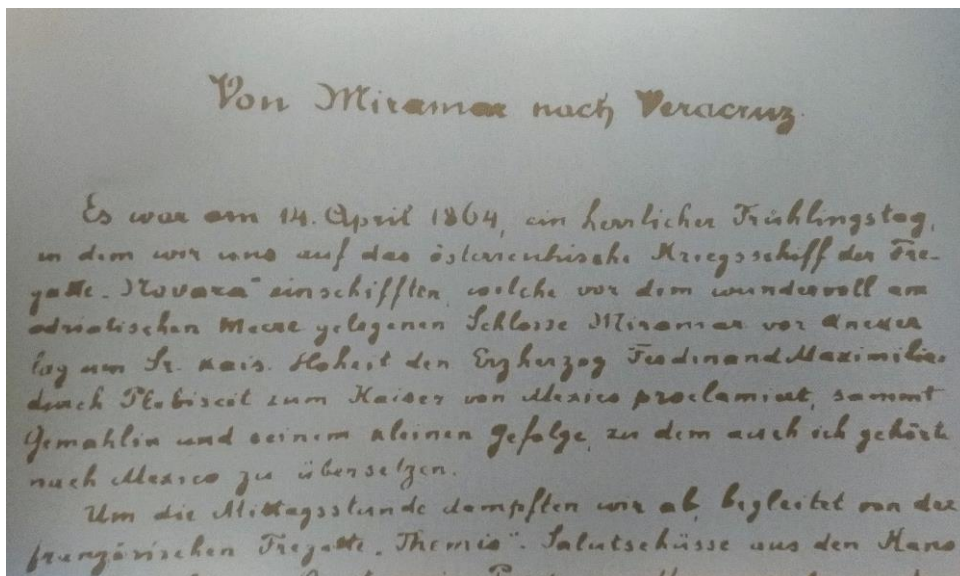


Fig. 2. Excerpt from the original manuscript in the German language of W. Knechtel's book *Handschriftliche Aufzeichnungen meiner persönlichen Eindrücke und Erlebnisse in Mexiko in den Jahren 1864–1867*.

The book was translated into Spanish (*Las memorias del jardinero de Maximiliano. Apuntes manuscritos de mis impresiones y experiencias personales en México entre 1864 y 1867*) and was published in 2012 at the National Institute of Anthropology and History of Mexico (Fig. 3a). The edition also includes a collection of rare photographs, business cards, colour post cards, maps and an extended bibliography.

In 2007 the book was translated into Romanian (Fig. 3b) and published after the copy with a dedication in German, offered by W. Knechtel to Dr. Grigore

Antipa. This precious volume was found thanks to the efforts of Dr. Alexandru Marinescu – historian of biological sciences, who discovered the book among the volumes of Grigore Antipa’s library, which had escaped censorship of the authorities after 1947 directed against the so-called “subversive” literature.



Fig. 3. Cover of Wilhelm Knechtel’s book, published in Spanish (a) and in Romanian (b).

The memoirs of the “royal gardener” include scientific data on plants, vegetation and weather in Mexico and references on plants that the emperor sent from Chapultepec to Miramar and vice versa. During his stay in Mexico, Knechtel discovered new species of plants, conducted speleological research, exploring the Cacahuamilpa cave, located in Sierra Madre del Sur, where he collected insects.

In the pages of the book there are numerous references to curious incidents and anecdotes occurred around the Mexican sovereign, Knechtel witnessing the disintegration of the Mexican Empire. “*This book is the testimony of a brave and honest man, who, in the whirlwind of horrific events, was supported by his great passion for nature*”, as Alexandru Marinescu writes.

The importance of the notes written by the botanist Knechtel lies in the fact that in his pages we discover the taste and passion that the emperor Maximilian has always shown for “beauty”, architecture and gardening.

Knechtel considered the garden as the recreation of Eden and, at the same time, the expression of power. Maximilian himself considered his gardens a public exhibition of elegance, order and education, and in almost all his residences he worked closely with Knechtel.

But the trip to Mexico ended tragically. Emperor Maximilian was imprisoned and executed by the revolutionaries (1867), Knechtel being forced to return to Europe. His return trip, which he describes in his memoirs, included the stops in Havana, Puerto Rico, Thomas Island, unshipping in Southampton, then the visits to the cities of London, Paris and Strasbourg.

Upon his return to Vienna, Knechtel presented himself as “*the one who came back from Mexico*”. After his return, Wilhelm Knechtel is rewarded with a pension and sent to the small island of Lacroma, to take care for the imperial property of Franz Josef I of Austria, for two years (Knechtel, 2012).

In the service of King Carol I of Romania

After the Mexican experience, at the age of 30, the skilful gardener Wilhelm Knechtel was invited to Romania by Carol I de Hohenzollern, in order to work “*in the service of the gardens*” of the King, being named “head” of the Royal Gardens, then of the Public Gardens.

He becomes a lecturer in the disciplines Viticulture, Horticulture and Entomology at the Central School of Agriculture in Herăstrău – Bucharest (the future Faculty of Agronomy).

On January 17th 1883, he was appointed *Knight of the Romanian Crown Order* by Carol I of Romania [Pralong (Coord.), 2013] for “*special merits within the Special Commission for the fight against phylloxera*”. In 1884, he received the Silver Cup of Honour from King Carol I as a gift.

In Romania, Wilhelm Knechtel had a significant contribution to the modernization of public spaces, having a remarkable activity in the field of landscaping. His achievements include: the arrangement of the Posada-Comarnic Park (for Prince George Bibescu), in 1898, Princess Maria’s tomb, the Botanical Garden, the Cișmigiu and Kiseleff parks, the garden of the Royal Palace in Calea Victoriei Bucharest, the garden of Peleş Palace in Sinaia, etc. Wilhelm Knechtel is one of the most prolific gardeners in Romania in the 19th century, carrying out numerous works (Mexi, 2017, cf. Peleş Museum Archives).

The history of the Peleş Castle garden begins with the construction of the castle in 1873. From the archives it appears that the first gardener of the Royal House of Romania, at the same time the leader of all the arrangements in the royal fields, was Wilhelm Knechtel [(<http://arhivadearhitectura.ro/architects/wilhelm-knechtel/>; cf. Dr. Elisabeta Dobrescu, from the Faculty of Horticulture at the University of Agronomic Sciences and Veterinary Medicine of Bucharest) (Mexi, 2017; Soroș, 2016)]. The history of Peleş Castle’s gardens is almost 150 years old and begins with the gardener of King Carol I - Wilhelm Knechtel (Soroș, 2016).

Wilhelm Knechtel, an excellent botanist and a remarkable organizer, was one of the foreign personalities who contributed substantially to the modernization of public spaces in Bucharest. He has performed a remarkable activity not only in the field of landscaping, but also in the one of education.

On March 15, 1888, the Austrian-German gardener born in Bohemia, Wilhelm Knechtel, who had accumulated a great deal of life experience traveling many places around the world, came to Bucharest and permanently settled in Romania, obtaining Romanian citizenship by royal decree.

In the latter part of his life he devoted himself to numismatic study (being one of the founding members of the Romanian Numismatic Society) and published several specialized courses and works, such as:

- Epidemiile insectelor [Insect epidemics]. Tipografia Voința Națională, București, 1894;
- Grădinile peisajere [Landscaping gardens]. Tipografia Gutemberg Joseph Gobel, București, 1899;
- Trandafirul [The Rose]. Tipografia Ziarului Cronica – Thoma Basilescu, București, 1905;
- Curs de Entomologie și Viticultură [Entomology and Viticulture course]. Școala Superioară de Agricultură, Herăstrău, 1905;
- Insectele vătămătoare din România și mijloacele de combatere a lor [Harmful insects in Romania and means to control them]. Editura Albert Baer, București, 1909 (together with his son).

The life and work of this wizard of floral architecture is beautifully synthesized by the title of Erhard F. Knechtel's paper (2012), Wilhelm Knechtel's great-grandson - *From an apprentice of a Bohemian gardener to the Romanian director of the Royal Garden, Wilhelm Knechtel - a European destiny between the West and East*.

He died at the age of 87. His son, the entomologist Wilhelm Knechtel, succeeded in showing him the monograph on the Thysanoptera in Romania, a few days before his death.

Wilhelm Knechtel, the son, zoologist. Biographical incursions

On October 10, 1884, spouses Wilhelm Knechtel and Helene Dierke's joy was great. Their eldest son, Wilhelm K. Knechtel was born. Here he attended the school. The son could not be less than his father. He was urged by his father to attend the same school – the Higher School of Agriculture in Herăstrău, now the University of Agronomic Sciences of Bucharest.

Ever since his childhood, the young Knechtel loved nature's beauties (Fig. 4). He remembered with emotion the times when he crossed the paths of the Bucegi Mountains with his father, the guidance received from him leading to his increasing love for the environment, learning from him the mountain flora, habitats, plant associations, insects. He confessed: "*My room of my family home was a small museum, on one side the herbarium containing the mountain and*

alpine flora and from Bucharest surroundings, on the other side stuffed birds and animals, an insectarium, a collection of minerals and a small beginner's library” (from the speech delivered in the Auditorium of the Romanian Academy, on the 75th anniversary).

Since there was no upper agricultural school in the country at that time, in 1904, W.K. Knechtel was sent to Germany, at the Higher Agricultural School at Stuttgart - Hohenheim (Stanciu, 2016) in Wurttemberg, in order to deepen his studies in this field, agriculture, school which he graduated in 1907. Here he had the privilege of attending lectures in the fields of botany, entomology and pathophysiology, being captivated by the courses held by famous professors of that time: Valentin Haecker, Kirchner, Pompetsky, Schule. They had an overwhelming influence on the preparation and later development of young Knechtel, arousing his interest, passion and love for entomology.

Returning to the country, in 1909, W. Knechtel started to work at the Experimental Station for Tobacco Cultivation, the Department of Phytopathology, where he began research on the thrips (Insecta, Thysanoptera), especially on the species *Thrips tabaci*, known as an important pest in the crops of tobacco.



Fig. 4. Entomologist Wilhelm Karl W. KNECHTEL
(28.09/10.10.1884, Bucharest – 8.03.1967, Bucharest).

Between 1912–1914 he attended a specialization course in the field of plant protection in Italy, at Naples, Scafati, then at the Institute of Biology of Berlin - Dahlem, where he developed his knowledge on experimental methods of pest control. Within the period 1916–1918, he held the position of sub-inspector in the field of tobacco culture (Fărcaș & Soran, 1982).

The call of the reunited Bessarabia

After the First World War and the joining of Bessarabia to the mother country, General Ernest Broșteanu, who ruled Chișinău with authority, called for volunteers to occupy the vacant chairs left by the Russian withdrawal. To his appeal, professors I.C. Teodorescu and Wilhelm Knechtel replied, among many others, who go there to teach temporarily, within the period 1918–1920, being professors of entomology and phytopathology at the Viticulture School of Chișinău. Here, Professor Knechtel was entrusted with the management of the Bioentomology Station where he continued his research on the ecology and systematics of Thysanoptera. Also, here he met Matilda, Lucia's sister, Prof. I.C. Teodorescu's wife, who accompanied them and whom he married; also in Chișinău, their son Ekkerhardt (Hardy) was born.

Entomologist of the Ministry of Agriculture and Domains

He returned to the country in 1921, when he was called to Bucharest, to the Ministry of Agriculture and Domains, as an entomologist, in a time when, in our country, the research and knowledge on pest biology and the application of the control methods was very low. In the neighboring countries (Hungary, Russia) there was already a tradition of agricultural entomology research. During almost the entire period of activity, W. Knechtel was a consultant in this ministry.

Unlike other scientists, Professor Knechtel was not only limited to theoretical concerns, but also a man of action, an excellent organizer and coordinator, a true mentor. In a difficult historical period, after many difficulties that he overcame only through hard work and devotion, W. Knechtel managed to establish the first research center of the Agricultural Entomology Station in Romania. This station, with a single employee and a single manager, Knechtel himself, initially worked in a shed in Libertății Park of Bucharest. Professor Andrei Popovici-Bâznoșanu, who was involved in the beginning of entomological research in Romania (Ciubuc, 2002), was the one who brought Knechtel as an assistant to the Faculty of Natural Sciences in Bucharest. Prof. C. Manolache considered that “*We cannot talk about entomological research at the Faculty of Natural Sciences except with the academic Knechtel [...]*”. In 1924, W. Knechtel was appointed head of the Entomology Department of the University's Descriptive Zoology Laboratory, position which he held until the foundation of the Institute of Agronomic Research.

Professor M. Ionescu wrote in 1959: “*It is not a coincidence that all the staff of assistants of the Laboratory of descriptive zoology went to entomology researches, becoming appreciated entomologists*”. In the Entomology Laboratory, eight entomologists worked, doctoral theses were elaborated here, five fascicles of P.R.R. Fauna Series were published – all being related to the beginnings of agricultural entomology research in Romania and to Wilhelm Knechtel's name.

Hard work and devotion to science

In 1926, W. Knechtel obtained the title of Full Professor at the School of Horticulture, where he taught entomology, for 14 years, without being paid! After the foundation of the Institute of Agronomic Research, in 1929, his professor, Gheorghe Ionescu-Șișești, a colleague of studies at Hohenheim, appointed him coordinator of the Department of Phytopathology, Entomology and Agricultural Parasitology.

In 1940, he was appointed lecturer in the agricultural entomology discipline at the Faculty of Agronomy in Bucharest, where he remained until 1944, when he retired and moved to Sinaia, temporarily retiring from his activity.

After two years, Academician Traian Săvulescu summoned him to Bucharest to work in the newly established teams of the Academy, becoming senior researcher in the Fauna of the P.R.R. group, dealing with thysanopteran insects. Referring to the activity within the group, in the speech given on the occasion of Professor Knechtel's 75th anniversary, Nicolae Botnariuc said: "*Academician Knechtel is a model of discipline and order in zoological work [...]. Through his entire complex activity in Fauna R.P.R. team, the academician Knechtel made an essential contribution to the success of this work of national interest*".

The professional prestige amplified by Professor Knechtel's election as a full member of the Academy, within the Department of Biology and Agricultural Sciences, in 1955, at the proposal of Traian Săvulescu.

Academician Gheorghe Ionescu-Șișești considered him "*one of his colleagues of great scientific value and high moral authority*".

Highlights of the scientific activity

Professor Knechtel's scientific activity was carried out both in the field of applied entomology and theoretical entomology. The main research directions consisted of:

- Faunal, systematic and zoogeographic studies on some groups of insects: Thysanoptera, Vespidae, Bombinae, Coccidae, Formicidae, Aphididae, Orthoptera;
- Research on the biology, ecology and control of some species of insect pests in cereal crops, technical plants, orchards and greenhouses;
- Activities having a technical character and popularization, with an impact in the agricultural field: involvement in effective actions to combat the locust – *Locusta migratoria* in the Danube Delta; combating orchard pests, mainly turtle lice (Lecanidae); biological control of the woolly louse (*Eriosoma lanigerum*) using the parasite *Aphelinus mali*;
- An important objective of the faunistic and systematic researches developed by Professor Knechtel was represented by the study of thysanopteran insects in Romania.

Why did Knechtel choose to study Thysanoptera (thrips)?

Initially, Knechtel published together with his father the work “Harmful insects in Romania and means to control them”, continuing the preoccupations already existing in the family with regard to insects that can cause damage. Although he could have chosen a group of insects easier to work with, Knechtel chose Thysanoptera (thrips), a group of insects difficult to study because of their small size and large number of species. Due to their very small size (usually 0.5–5 mm), their identification requires microscope slides preparation, in a sequence of steps, which requires a lot of time, increased attention and careful observations. However, Knechtel preferred Thysanoptera, which are bioeconomically important insects. His research on thrips was not only theoretical, but also especially practical, applied. Under favorable environmental conditions, thysanopterans have a strong mass development, producing significant damage in cereal crops, in greenhouses, orchards, etc. These thrips can cause atrophy of the spike in grasses, destruction of floral organs and leaves, appearance of stains on leaves and flowers, decreased concentration of substances (cellulose, starch). The appearance of stains on different organs of ornamental plants produced by species of thrips leads to a decrease in their commercial value. It also causes vectorization of tospoviruses (the most harmful plant viruses in the world).

The activity of Prof. W. Knechtel in support of agriculture reflects the spirit of the century on ecology, a spirit triggered by the great scholar Ernst Haeckel (1834–1919) - father of ecology, professor of the brilliant Romanian student Grigore Antipa in Jena. In Romania, Dr. Grigore Antipa carried on the teachings received from his master Haeckel, developing some issues (ichthyology applied to fisheries in Romania, hydrobiology applied to the management of the Danube Delta and the Black Sea, etc.). This encyclopedic biologist initiated the research of the economic aspects of biological productivity, thus being in fact the creator of a new science, which he called bioeconomics, at the interference between ecology and economy. Later, in 1971, the illustrious American economist of Romanian origin Georges Georgescu-Roegen (1906–1994) minutely developed the science of bioeconomy on the basis of thermodynamics.

Probably, the ecological paradigm of bioeconomy was taken over by Prof. Knechtel and applied to the study of Thysanoptera, insects harmful to agriculture, which cause great economic losses by destroying crops. Besides direct damage, thysanopterans can create favorable conditions for further colonization with pathogenic microorganisms. For example, alternariosa (the disease of tobacco seedlings) is caused by the action of several factors: different developmental conditions offered to seedlings, *Thrips tabaci*, *Alternaria* fungus and climatic conditions. Necrosis of plant tissues initially produced by *T. tabaci* constitutes a favorable environment for the further development of *Alternaria* fungus (Knechtel, 1951).

In short, what are thrips

Around 6.000 species of Thysanoptera have been described worldwide, they can be found in all regions, preferably in warm areas. They can reach alpine areas, at the upper limit of the vegetation. In Romania's fauna 215 species, classified into four families, were reported (Vasiliu-Oromulu, 1998).

The body of these insects consists of three distinct regions: head, chest and abdomen. The head carries a pair of antennae, the mouth cone, two compound eyes with large facets, the ocelli being present only at adults with wings. The buccal apparatus is adapted for stinging and sucking, an interesting feature being the absence of the right mandible. The wings are membranous, elongated, but there are also species in which the wings are reduced or completely missing. As a rule, the wings are bordered with fringes of bristles (Figs. 5–6). The color is pale and transparent at immature forms, while adults are variously colored, in all shades of yellow, brown and black.

From the biological point of view, most thysanopterans are phytophagous. About 40% of the species feed exclusively on fungi, mostly with hyphae, and some with spores. A small number of species are obligatory predators on other small arthropods. Thrips are food for a lot of insects (including other thrips), mites, birds, salamanders and lizards.

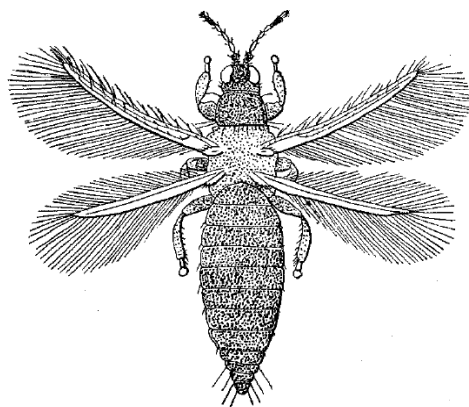


Fig. 5. *Frankliniella intonsa*
(Knechtel orig.).

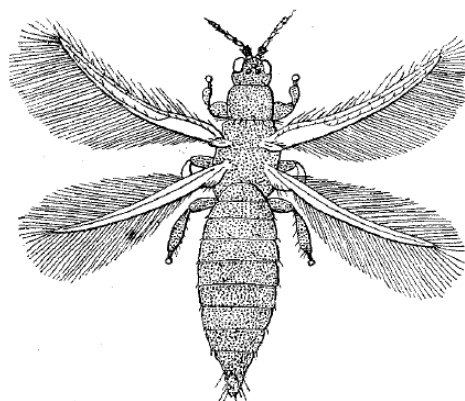


Fig. 6. *Thrips tabaci*
(Knechtel orig.).

In 1937, Knechtel obtained the title of Doctor of Science with the doctoral thesis entitled *Study on the distribution of Thysanoptera in Romania*. As a result of an intense and meticulous activity, in 1951, Professor Knechtel published the volume *Thysanoptera* in the series Fauna of P.R.R, in which 152 species from the fauna of Romania are described. 18 species are new to science (Table 1). Subsequently, the complex study of these insects from a taxonomic, biological, ecological point of view and methods of control became his basic concern. The

papers published in the thysanopterological field present descriptions of the species, diagnoses, research on the spread of the species according to the vegetation floors, the influence of the environmental factors on their ecology, the relations of interdependence between thrips and plants.

Table 1

New Thysanoptera species described by Wilhelm Knechtel
(according to Vasiliu-Oromulu, 1998)

Family Aeolothripidae
<i>Aeolothrips priesneri</i> Knechtel, 1922
<i>Aeolothrips verbasci</i> Knechtel, 1955
Family Thripidae
<i>Ereikethrips calcaratus</i> Knechtel, 1960
<i>Eremiothrips manolachei</i> (Knechtel, 1955)
<i>Kakothrips dentatus</i> Knechtel, 1939
<i>Mycterothrips albidicomis</i> (Knechtel, 1923)
<i>Oxythrips cannabensis</i> Knechtel, 1923
<i>Oxythrips dentatus</i> Knechtel, 1923
<i>Oxythrips euxinus</i> Knechtel, 1932
<i>Thrips euphorbiae</i> Knechtel, 1923
Family Phlaeothripidae
<i>Haplothrips floricae</i> Knechtel, 1960
<i>Haplothrips scyticus</i> Knechtel, 1961
<i>Hoplothrips absimilis</i> Knechtel, 1954
<i>Hoplothrips lichenis</i> Knechtel, 1954
<i>Hoplothrips muscicola</i> Knechtel, 1954
<i>Hoplothrips quercinus</i> Knechtel, 1935
<i>Phlaeothrips bacauensis</i> Knechtel, 1948
<i>Priesneriella clavicornis</i> (Knechtel, 1935)

Professor W. Knechtel worked on thysanopterans for almost 50 years, collected rich material from Romania and other corners of the world, the results obtained recommending him as one of the most famous specialists in the world in this group, being appreciated and consulted by many scientists outside the country's borders. The evidence is both the rich material he received from them, to be identified or verified, and the fact that many researchers dedicated a number of species to him, in honour of his name (*Melanthrips knechteli* Priesner 1936, *Haplothrips knechteli* Priesner 1923, *Amphibolothrips knechteli* Priesner, 1936, etc.).

Scientific collection of thysanopterans made by Acad. Knechtel was in the custody of the Institute of Biology Bucharest of the Romanian Academy, in 2010 being transferred, in its original boxes, at "Grigore Antipa" National Museum of Natural History of Bucharest.

This collection of reference for the Romanian entomofauna of Romania was revised by Dr. Liliana Vasiliu-Oromulu, a successor of Prof. Knechtel's thysanopterological studies (Fig. 7). The collection includes blade-lamella scientific

preparations belonging to the suborders Terebrantia (families Aeolothripidae, Fauriellidae and Thripidae – including 128 species) and Tubulifera (family Phlaeothripidae, represented by 66 species). Out of the total of 3.275 specimens, 177 are type-specimens (3 holotypes, 2 paratypes, 164 syntypes, 8 cotypes), of special scientific value (Stan, 2017).

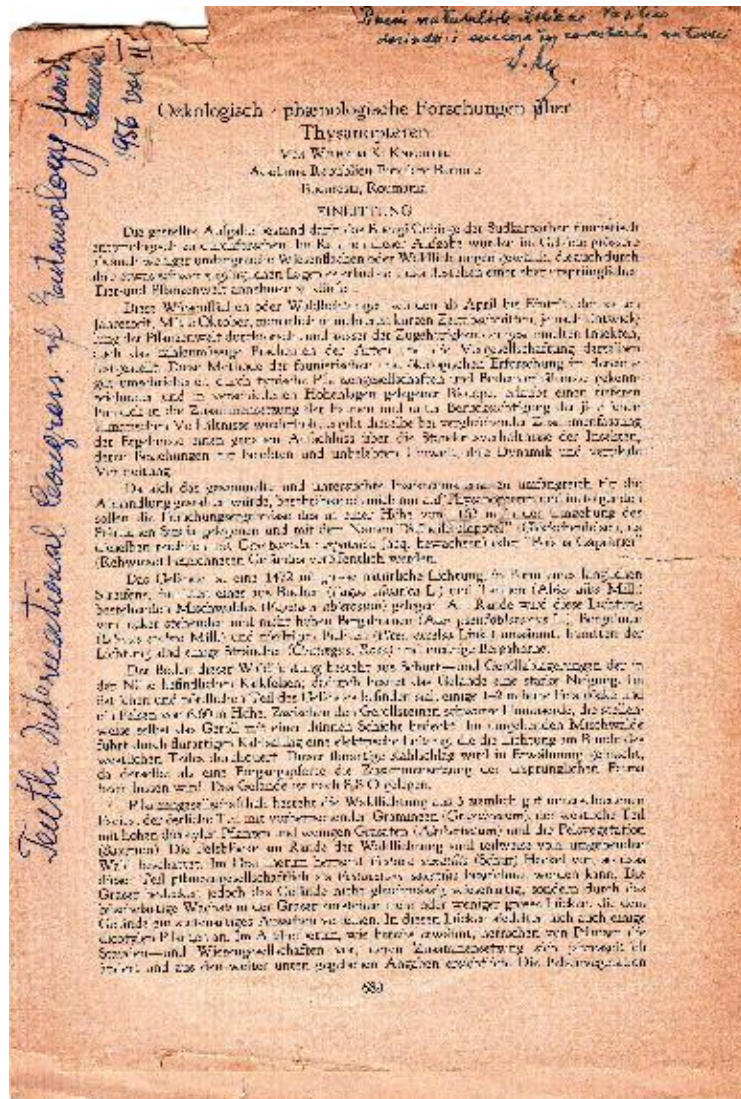


Fig. 7. The first page of the paper *Oekologisch-phaenologische Forschungen Thysanopteren* Von WILHELM K. KNECHTEL, with the Professor's holographic dedication in Romanian for Liliana Vasiliu-Oromulu: *Young naturalist Liliana Vasiliu, wishing her success in nature research.* W. Knechtel.

In addition to Thysanoptera, Prof. Knechtel also dealt with the study of other groups of insects, such as coccids. He highlighted the species present in the Romanian fauna and researched host plants, ecology, biology and control methods.

He also initiated the systematic study of the bumblebee species (Bombinae family) of our fauna, which included research on the intraspecific variability depending on the climatic conditions, the geographical spread influenced by the zonation of the woody vegetation, the results obtained being published in a monograph.

In 1940, in collaboration with Prof. Constantin Manolache, W. Knechtel began the study of aphids, insects with a pronounced polymorphism and which had been researched by Prof. Ioan Borcea. These investigations resulted in the establishment of the systematic position of some species present in the fauna of Romania, the description of new species, information on the ecology and control methods.

Along his long career, W. Knechtel created an impressive collection of formicides (Hymenoptera), and researched chewinglice and orthopterans, on the latter, publishing in collaboration with Prof. A. Popovici-Bâznoșanu a volume in Fauna of P.R.R., in which all the species of Orders Saltatoria, Dermaptera, Blattodea and Mantodea are treated. Professor Knechtel contributed to the taxonomic and systematic knowledge of the species from Vespidae family (*Vespa germanica* and *Vespa vulgaris*).

Prof. Knechtel and his collaborators' (Mihail Ionescu, Ecaterina Dobreanu, Constantin Manolache) second major research direction was represented by the studies on the morphology, biology, ecology and control of some pest species. In this respect he carried out a series of researches on the species *Thrips tabaci*, *Phytodecta fornicata*, *Locusta migratoria*, *L. caudata*, *Phlyctaenodes sticticallis*, *Eulecanium corni*, *Aspidiotus perniciosus*, *Claudius pectinicornis*, *Lema melanopus*, etc. bringing valuable contributions to the knowledge of the development cycle, post-embryo stages and number of generations. These investigations were based on careful microscope observations and experiments performed in laboratory and field conditions.

In the papers on the species *Phytodecta fornicata* (pest of alfalfa crops), published in collaboration with Cornelia Hrisafi, information on the diapause phenomenon is presented. Important contributions are made by Knechtel, along with Prof. Mihail Ionescu, another distinguished Romanian entomologist, on the insect *Pyrausta nubilalis*, a pest species intensively studied between 1929 and 1932, in Europe and America, within the "International Corn Borer Investigation Association".

As a representative of Romania, Acad. W. Knechtel has participated in numerous international conferences, congresses and symposiums, among which we mention: The Entomology Congresses from Ithaca, U.S.A. (1929), Paris (1930), Warsaw (1932), Prague (1958). In the summer of 1956, he participated, together with M. Ionescu, in the International Congress of Entomology in Montreal. On this occasion he met eminent Prof. Soren L. Tuxen, director of the Museum of Zoology in Copenhagen and a member of the Standing Committee of the Entomology

Congresses. At the invitation of the two Romanian entomologists, Prof. Soren Tuxen paid a visit to Romania, delighted by our country where he met many scientists and culture, among whom Prof. Constantin Motaş was present (Oprîş, 2009).

Prof. Knechtel's scientific achievements have been materialized in more than 120 papers, among which there are:

- Insectele vătămătoare din România și mijloacele de combatere a lor [Harmful insects in Romania and means to control them] (together with his father) (1909);
- *Phlyctaenoles strictalis*. Un vătămător al tutunului [*Phlyctaenoles strictalis*. A Tobacco pest] (1915);
- Rolul stațiilor entomologice [The role of entomological stations] (1928);
- Oekologischzoogeographisches Studium an Coleopteren des Rumänischen Faunengebiets (1944);
- Oekologisch-faunistische Untersuchungen an Thysanopteren Rumäniens (1947);
- Thysanoptera, in the Fauna P.R.R. (1951);
- Hymenoptera, Apinae, in the Fauna P.R.R. (1955);
- Orthoptera (Orders Saltatoria, Dermaptera, Blattodea, Mantodea), in the Fauna P.R.R. (in collaboration with Andrei Popovici-Bîznoşanu, 1959).

As a recognition of the value of his scientific activity in the field of entomology, for the contribution made to the development of biological sciences in Romania, in 1951, Prof. W. Knechtel was awarded the "State Prize", being decorated with different Orders.

Wilhelm K. Knechtel lived in Sinaia, in a building built in 1939, located on Calea Codrului street, no. 34. After retirement, he lived in the silence of the woods of Sinaia, continuing his work, according to his own confessions "*in order to finish my work, to leave everything in full order, for the sake of education and discipline in activity received from my parents*". The building was used as a home for his family and as an Entomological Research Laboratory that belonged to the Institute of Biology Bucharest of the Romanian Academy. In 1950, the building was nationalized, but Acad. W. Knechtel continued to live in this building until the end of his life (1967).

He was the first director of the Center for Biological Research in Bucharest, founded in 1957 and transformed into the current Institute of Biology (1960). Even during the three years in which he held the position of Director, Professor Knechtel's working laboratory was in Sinaia's house. After his death, in order to continue and develop the scientific activity initiated by him, the house from Sinaia (Fig. 8) became an Ecological Stationary, here carrying out a series of research activities. Until 2006, it in the custody of the Institute of Biology Bucharest.



Fig. 8. Professor Wilhelm Knechtel's house from Sinaia.
(www.ibiol.ro/posada/Posada.pdf)

In 1944, the Knechtel family was struck by terrible news; they lost their son, Ekkehardt, a lieutenant in the Mountain Troops, a tragedy that marked family members for the rest of their lives. Who knows? If this misfortune had not happened, Ekkehardt might have followed his grandfather's and his father's career and skills, benefiting from the impressive library left by them, inaugurating the third generation of the German biology dynasty in Romania.

W. Knechtel loved very much Romania and Romanians. However, from the testimonies of those who knew him closely, after 1944, he suffered because of his German origin, being fired, having no income for a period of time I found out late Professor's sufferings created by the injustices of the communist regime established after the war in Romania by the Soviet winners.

In 1959, one of the authors of this article (Marian - Traian Gomoiu) was freshly employed at the Center for Biological Research of the Romanian Academy, located in Locotenent Ștefan Lemnea street, in some rooms of the former "royal stables", as it was said at that time. In one of the larger rooms on the floor of the building, there were several tables, usually for young researchers ("the colts of the Academy" – as Prof. Theodor Bușniță called them). A table was more isolated, in front of a window. The occupant of the table was a slightly older gentleman, with a normal height, silent, sober, concerned about his work, imperturbable at the hum of

the room. It was Professor Knechtel, about whose life I would find out little by little, and almost conspiratorially over time. The entire outfit of this scholar was imposing, and we, the youngest, addressed him with the name "Mr. Professor" ... and not using the expression, common that time "Comrade Professor".

Besides the scientific activity of recognized value, W. Knechtel was also a great admirer of the Romanian folk art and a passionate bibliophile, over the years collecting a rich literature, not only in the scientific field, but also in the artistic one. A large part was donated to the Library of the Romanian Academy and the Central Library of the Institute of Agronomic Research.

On November 2, 1959, Acad. Wilhelm Knechtel was celebrated at the age of 75, in the Auditorium of the Romanian Academy, on which occasion honored speeches were given by Acad. Gh. Ionescu-Șișești, Prof. C. Manolache, Prof. M. Ionescu and Conf. N. Botnariuc.

In the speech given by Prof. C. Manolache on this occasion, he said: "*You were near the dear microscope and you analyzed diligently and tested tens of thousands of micro and macro-entomofauna preparations from Romania and abroad. You loved a number of other groups of insects with a still mysterious life, the ants and bumblebees once sung so beautifully by Dante in the Divine Comedy*".

In his reply, warmly thanking his colleagues for the words of appreciation addressed to a "*modest worker in the field of natural sciences*", Acad. W. Knechtel confessed: "*I have received a severe education, directed to work, discipline and order. I led a sober life. I created myself a special world - the world of insects - in which I felt thankful and happy and where I found the peace of the soul in times of restraint [...]. I fulfilled my dream from a young age. I created a research institution, I trained the scientific staff, I trained working people*".

In 1967, the year in which Acad. W. Knechtel passed away, after a deep suffering, there were almost 60 years since the appearance of his first synthesis paper in the field of agricultural entomology, "Harmful insects in Romania and means to control them", having as authors W. Knechtel, the father, and W. Knechtel, the son. This paper represented the touchstone for generations of students, pupils, specialists in the field of agricultural entomology.

Over time, detailed appraisals of Professor Knechtel's scientific achievements were made by those entitled to do so, personalities of Romanian science and culture who have expressed their appreciation and acknowledged the value and merits of the beginnings and development of entomology in our country.

For over half a century dedicated to entomological research, Acad. W. K. Knechtel, the founder of the school of agricultural entomology in our country, served with modesty, devotion and high competence the biological

sciences in Romania. His personality and work represent an example of tenacity, hard work, love for science and will remain forever inscribed in the national scientific heritage.

Acknowledgements: The authors express kindly thanks to Dr. Liliana Vasiliu-Oromulu for information on the life and scientific work of Professor Wilhelm Karl Knechtel.

Publications of Wilhelm Karl Knechtel

1909

1. *Insectele vătămătoare din România și mijloacele de combatere a lor*. Editura Albert Baer, București, 124 pag. (in collaboration with Wilhelm Knechtel father) [Harmful insects in Romania and means to control them]

1910

2. *Thrips tabaci* (Lind.) *Thrips communis* (Uzel). *Buletinul cultivei tutunului*, An V, nr. 24/25: 51–54.
3. Alte trei specii de Thysanoptere găsite în România pe răsadul de tutun. *Buletinul cultivei tutunului*, An V, nr. 28/29: 291–295. [Another three species of Thysanoptera found in Romania on tobacco seedlings]

1911

4. Studiu asupra unor specii din ordinul Thysanoptera. *Viața Agricolă*, An II, nr. 6: 434–442 [Study on some species from order Thysanoptera]
5. Studiu asupra unor specii din ordinul Thysanoptera. *Viața Agricolă*, An II, nr. 7–8: 522–528 [Study on some species from order Thysanoptera]

1914

6. Pythium de Baryanum Hesse, ca provocator al unei boale de răsad de tutun. *Supliment la Buletinul Regiei Monopolurilor Statului*, București, 48 pag. [Pythium de Baryanum Hesse, damage at the tobacco seedling]

1915

7. *Phlyctaenodes stiticalis*, un vătămător al tutunului. *Buletinul Regiei Monopolurilor Statului*, București, pp.: 3–18. [*Phlyctaenodes stiticalis*, a pest of tobacco]

1919

8. Alternariosa, o maladie a răsadului de tutun. *Basarabia Agricolă*, Anul I, nr. 7, Chișinău: 3–21. [Alternariosa, a tobacco disease]
9. Purecele lînos (*Schizoneura lanigera* Hausm.). *Revista de Viticultură, Oenologie și Horticultură*, nr. 3: 71–74; nr. 4: 118–123.

1920

10. Combaterea fluturașului *Conchylis ambiguella*. *Viața Agricolă*, 11, nr. 22: 729–731.
11. Combaterea purecelui lînos (*Schizoneura lanigera*). *Viața Agricolă*, 11, nr. 23–24: 773–775.
12. Notițe asupra fluturașului *Conchylis ambiguella* Hb. *Buletinul stațiunii experimentale viticole și oenologice Chișinău*, An I, nr. 2: 6–9.

1921

13. Contribution á la faune des Coleoptères d'eau douce de Roumanie. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Année VII-ème, nr. 4/6: 106–107 (in collaboration with Constantin Motaș)

1922

14. *Phytodecta fornicata* Brüggen. *Editura Serviciului Publicațiunilor și al Statisticii Agricole*, București, nr. 25: 3–32.

1923

15. Einige neue Thysanopteren aus Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Année VIII-ème, nr. 5–6: 71–76.

16. *Oxythrips dentatus* nov. spec. eine neue Thysanopteren spezie aus Roumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Année VIII-ème, nr. 7/8: 122–125.
17. Thysanoptere din România. Studiu sistematic și fitopatologic, București. *Buletinul Agriculturii și Domeniilor*, 235 pag., Vol. II, nr. 4–6: 84–127; Vol. III, nr. 7–9: 49–171; Vol. IV, nr. 10–12: 181–216. [Thysanoptera from Romania]
- 1924**
18. Thysanoptere din România. Studiu sistematic și fitopatologic. București, *Buletinul Agriculturii și Domeniilor*, Vol. I, nr. 1–3: 23–63. [Thysanoptera from Romania]
- 1925**
19. Un vrăjmaș de moarte al prunului. Păduchele țestos. *Revista Horticola*, 3 (32): 588–589.
- 1927**
20. Saramuratul grâului. *Viața agricolă. Revista bilunară a Societății Agronomilor*, Extras din nr. 15–16: 1–17. [Salt solution of Corn]
- 1928**
21. Observations on the Corn Borer in Romania. *Scientific Reports*, pp.: 194–200.
22. Rolul stațiilor entomologice. *Societatea Agronomilor, Congresul Agricol*, 6 pag. [The role of entomological stations]
- 1929**
23. Zur Kenntnis der Coccidenfauna Rumäniens. Premier Congres des Naturalistes de Roumanie (Cluj, 1928). 1930 (Pt. 2): 230–237.
24. Neuer Beitrag zur Kenntnis der Thysanopteren Fauna von Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Tom. XII, nr. 3: 61–63.
- 1931**
25. *Thrips tabaci* Lind. ein Feind der an der Schwertlilie vorkommenden Blattwespe *Rhadinoceraea reitteri* Kon., Sonderabdruck aus der "Zeitschrift für wissenschaftliche Insektenbiologie". *Allgemeine Zeitschrift für Entomologie*, Bd. XXVI, nr. 4/6: 148–151.
- 1932**
26. Beitrag zur Kenntnis der individuellen zeichnungsvariation bei *Vespa germanica*. *Publicația Societății Naturaliștilor din România*, nr. 11, 30 pag.
27. Dritter Beitrag zur Kenntnis der Thysanopterenfauna von Rumänien. *Publicația Societății Naturaliștilor din România*, nr. 10: 1–6.
28. *Oxythrips euxinus* sp. n. Rumania. *Publicația Societății Naturaliștilor din România*, nr. 10, 92 pag.
29. *Phlaeothrips bispinosus*. *Publicația Societății Naturaliștilor din România*, nr. 10, 94 pag.
30. *Thysanoptere din România*. București, 235 pag. [Thysanoptera from Romania]
- 1933**
31. Notizen über das erste Larvenstadium der Wanderheuschrecke *Locusta migratoria* L. *Notationes Biologicae*, Vol. I, nr. 1: 35–36.
32. O mare primejdie pentru pomicultura țării. *Revista horticola*, 11, 130: 184–185.
- 1934**
33. Studiu asupra biologiei Tenthredinidului *Rhadinoceraea reitteri* Kon. *Analele Institutului de Cercetări Agronomice al României*, Vol. VI, Anul V: 3–19.
34. Thysanopterele din Romania. *Studiu monografic, Buletinul Agriculturii*, nr. 24 [Thysanoptera from Romania]
- 1935**
35. Malofage parazite pe păsările din România. *Revista vânătoarei*.
36. Zur Kenntnis der Thysanopterenfauna Rumäniens. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Année XVII-ème, Tom. XVIII, nr. 7: 1–4.
37. Observațiuni asupra biologiei curculionidului *Rhynchites bacchus*. *Revista pomicola*, București.
38. Constatări asupra păduchelui țestos de San José. *Progresul horticol* 1, 2, 24 pag. (in collaboration with C. I. Manolache)
- 1936**
39. Observații biologice asupra gândacului ovăzului (*Lema melanopus* L. în România). *Analele Institutului de Cercetări Agronomice*, vol. VII.

40. Contribuțiuni noi la studiul faunei de pe Insula Șerpilor. *Buletinul Societății Naturaliștilor din România*, nr. 9: 1–7.

1937

41. Studiu asupra repartiției Thysanopterenelor din România. *Teza de doctorat*, 87 pag.

1938

42. Über die Zeichungsvariabilität bei *Phytodecta fornicata* Brüggen. *Sonderdruck aus der Jubiläumsschrift "Grigore Antipa"*, Monitorul Oficial și Imprimeriile Statului, pp.: 1–12.
43. *Taenithrips dianthi* Pr. un dușman al garoafelor, nou pentru România. *Progresul horticol*, An IV, nr. 7–8: 127–129.
44. Über die Wonderheuschrecke in Rumänien. *Bulletin of Entomological Research*, 29 (2): 175–183.
45. Thripsul grîului. Animale dăunătoare și folositoare agriculturii I.C.A.R., *Biblioteca de Agricultură practică*, Seria III, nr. 4: 3–5.
46. Inamicii porumbului – *Pyrausta nubilalis*. Animale dăunătoare și folositoare agriculturii. I.C.A.R., *Biblioteca de Agricultură practică*, Seria III, nr. 4: 5–13.
47. Beitrag zur Kenntnis der Molliphen der Vogelwelt Rumänien. București, Imprimeria Națională, Depozitul General, Cartea Românească. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Tom. XIX, nr. 6–7 (in collaboration with Ion I. Cătuneanu)
48. *Păduchii țestoși* (Coccidele). Animale dăunătoare și folositoare agriculturii. I.C.A.R., *Biblioteca de Agricultură practică*, Seria III, nr. 4: 55–66.
49. Viespea neagră a stânjeneului (*Rhadinoceraea reitteri*). Animale dăunătoare și folositoare agriculturii. I.C.A.R., *Biblioteca de Agricultură practică*, Seria III, nr. 4: 52–54.

1939

50. *Kakothrips dentatus*. *Comptes rendus des séances de la Institut des Sciences de Roumanie*, Vol. III, 322 pag.
51. Hummelin des Bucegi. București. *Buletinul Societății Naturaliștilor din România*, nr. 14: 56–69.
52. Coccine dăunătoare plantelor horticole. *Progresul horticol*, 5 (2): 29–31.
53. *Emphytus cinctus* L. un dăunător al culturilor de căpșuni. *Horticultura românească*, An 17, nr. 8: 1–3.

1940

54. Neue Blattlausarten für Rumänien. Beitrag I-IX. *Bulletin de la Section Scientifique*, Tom. XXII, nr. 3, 5; XXIV, nr. 4, 8; XXV, nr. 5; XXVI, nr. 6, 10; XXVII, nr.7; XXIX, nr. 7 (in collaboration with Constantin Manolache)

1940

55. Observațiuni asupra sistematicii unor specii de afide din România. *Analele I.C.A.R.*, 12 (XI): 289–307 (in collaboration with Constantin Manolache)
56. *Pemphigus bursarius* L. *Horticultura Românească*, 18, nr. 9–10: 130–134 (in collaboration with Constantin Manolache)
57. Păduchele negru de frunze (*Doralis fabae*). *Viața Agricolă*, București, 31, nr. 8: 241–247 (in collaboration with Constantin Manolache)
58. Neue Blattläuse für Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 24, nr. 4: 247–254.

1941

59. Observații asupra sistematicii unor specii de Aphide din România (a doua contribuție). *Analele I.C.A.R.*, 13 (XII): 217–267.
60. Observații asupra afidului *Doralina pomi* De Geer în România. *Viața Agricolă*, 32, nr. 8, 3 pag. (in collaboration with Constantin Manolache) [*Investigations on aphid Doralina pomi de Geer in Romania*].

1942

61. *Heliothrips femoralis*. Reuter eine neue Thysanopteren-Art für Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 24, nr. 8: 559–561.
62. *Thrips lini* Ladureau. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 25, nr. 1: 19–21.

63. Neue Blattläuse für Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 24, 8: 547–558.

64. Afide din sere găsite în România. *Horticultura Română*, 20, nr. 1–2: 1–5

1943

65. Neue Blattläuse für Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 25, nr. 5: 261–275.

66. Specii de Thysanoptere dăunătoare plantelor de seră. *Horticultura românească*, An 21, nr. 1–2: 1–2.

67. Observațiuni asupra sistematicii unor specii de afide din România. *Analele I.C.A.R.*, B: 217–267 (in collaboration with Constantin Manolache) [Investigation on the systematic of aphids species from Romania]

68. Observații sistematice, ecologice și de combatere la afidele din sere. *Viața Agricolă*, 34, nr. 5: 136–144.

1944

69. Neue Blattläuse für Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 26, nr. 6: 382–392.

70. Neue Blattläuse für Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 26, nr. 10: 690–702.

71. Beitrag zur Kenntnis der individuellen Zeichnungsvariation bei *Vespa vulgaris* L. *Académie Roumaine, Section Scientifique*, Tom. XXVI, nr. 8, 21 pag.

72. *Ökologisch - zoogeographisches Studium an Coleopteren des rumänischen Faunengebietes*. București, 219 pag. (in collaboration with Sergiu A. Panin)

73. *Dușmanii viței de vie*. Ediția I, București, Editura Universul, 36 pag. (in collaboration with Ioan Martin) [The enemy of vine]

1945

74. Neue Blattläuse für Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 27, nr. 7: 475–485.

75. Ökologisch-faunistische Forschungen an Thysanopteren Rumäniens. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Tom. XXVII, nr. 7: 1–22.

76. Beitrag zur Kenntnis der Biologie der Blattwespe *Claudius pectinicornis* Geoffroy. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Tom. XXVII, nr. 8: 539–566. (in collaboration with Hrisafi Cornelia)

1946

77. Ökologisch-faunistische Forschungen an Thysanopteren Rumäniens. II. *Bulletin de la Section Scientifique de l'Académie Roumaine*, Tom. XXVIII, nr. 8: 562–584.

1947

78. Neue Blattläuse für Rumänien. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 29, nr. 7: 459–470.

79. Ökologisch-faunistische Untersuchungen an Thysanopteren Rumäniens. III. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 29, nr. 6: 392–404.

1948

80. Ökologisch-faunistische Forschungen an Thysanopteren Rumäniens. IV. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 30, nr. 6: 377–390.

81. Zur Systematik einiger Thysanopteren Arten. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 30, nr. 8: 480–483.

1951

82. Thysanoptera. *Fauna R.P.R., Insecta*, vol. VIII, Fasc. 1, Editura Academiei Române, 260 pag.

1953

83. Constatări asupra păduchelui țestos din San José (*Aspidiotus perniciosus* Comst.). *Revista pomicolă* (in collaboration with Constantin I. Manolache)

1954

84. Étude zoogéographie et écologique sur les Bombines de la Republique Populaire Roumaine. *Buletinul Științific al Academiei R.P.R., Secțiunea de Științe Biologice, Agronomice, Geologice și Geografice*, 6 (3): 757–775.
85. Specii noi de Thysanoptere, *Hoplothrips muscicola*, *H. lichenis*, *H. absimilis*. *Buletin Științific, Secțiunea de Științe Biologice, Agronomice, Geologice și Geografice*, Tom. VI, nr. 4.

1955

86. Trei specii noi de Thysanoptera. *Comunicările Academiei R.P.R.*, Tom. V, nr. 12: 1713–1716.
87. Hymenoptera, Subfam. Apinae. *Fauna R.P.R., Insecta*, vol. IX, fasc. 1, Editura Academiei Române, 111 pag.

1956

88. Contribuții la studiul formicidelor din Valea Prahovei. *Buletinul Științific al Academiei R.P.R., Secția de Biologie și Științe Agricole*, Tom. VIII, nr. 4.
89. Neubeschreibung einiger Thysanopterenarten. *Revue Roumaine de Biologie*, Tom. I, nr. 2.
90. Ökologisch-phaenologische Forschungen über Thysanopteren. Tenth International Congress of Entomology, Montreal, Vol. II: 689–695.

1957

91. Dritter Beitrag zur Kenntnis der Thysanopterenfauna von Rumänien. *Publicația Societății Naturaliștilor din România*. Bucharest, nr. 10: 90–95.

1959

92. Sistematica unor specii de Thysanoptere. *Comunicările Academiei R.P.R.*, Tom. IX, nr. 4: 349–354.
93. O nouă specie de Thysanoptere. *Comunicările Academiei R.P.R.*, Tom IX, nr. 11: 1147–1149. [A new species of Thysanoptera]
94. Variația individuală la unele specii ale genului *Formica*. În: *Omagiu lui Traian Săvulescu, București* [Individual variation of some species of the genus *Formica*]
95. Câteva Coccide noi pentru fauna R.P.R. *Comunicările Academiei R.P.R.*, 9, 2: 115–122.
96. Einige für die Rumänische Volkrepublik neue schildlaunsarten. *Revue Roumaine de Biologie*, 4, 1: 87–93.
97. Orthoptera (Ord. Saltatoria, Dermaptera, Blattodea, Mantodea). *Fauna P.R.R., Insecta*, vol. VII, fasc. 4, Editura Academiei Române, 337 pag. (in collaboration with Andrei Popovici-Bîznoșanu).

1960

98. Gen și specii noi de Thysanoptere. *Comunicările Academiei R.P.R.*, Tom. X, nr. 11: 985–990.
99. Thysanoptere din stepa dobrogeană. *Probleme actuale de Biologie și Științe agricole*. Editura Academiei R.P.R., pp.: 109–112.
100. Neue Thysanopterengattung und Arten. *Comunicările Academiei Române, Bucharest*, 10: 985–990.
101. Phaenologische Forschungen über Thysanopteren (Die Thysanopteren der Dobrogeasteppe). *XI Internationale Kongress für Entomologie*, Wien, Bd. I: 489–493.
102. Eine neue Thysanopterenart. *Revue Roumaine de Biologie*, Tom V, nr. 1–2: 143–144.

1961

103. O nouă specie de Thysanoptere. *Comunicările Academiei R.P.R.*, 9, nr. 11: 1325–1328.

1962

104. Zur Kenntnis der Geographischen verbreitung der Ameisen in der Rumänischen Volkrepublik. *Revue Roumaine de Biologie*, Tom. VII, nr. 2: 243–254 (in collaboration with Dinu Paraschivescu)
105. Legături trofice între furnici și plante. *Studii și Cercetări de Biologie, Seria Biologie Animală*, Tom XIV, nr. 3: 315–330.
106. Hummeln aus Singeorz – Băi (r. Năsăud) und ihre Färbungsvariationen. *Comunicările Academiei R.P.R.*, 12 (7): 843–853. (in collaboration with Dinu Paraschivescu)
107. Hummeln der Dobrudscha (Bombini Michener, Ord. Hymenoptera, Fam. Apidae Leach). *Studii și Cercetări de Biologie, Seria Biologie Animală*, 14 (2): 181–195.

1963

108. Thysanoptera din regiunea superioară a Văii Teleajănelui. *Comunicările Academiei R.P.R.*, Tom XIII, nr. 12: 1055–1062.

109. Studiu ecologic și fenologic asupra Thysanopterelor din Dobrogea. Thysanoptere din regiunea Babadag. *Studii și Cercetări de Biologie, Seria Biologie Animală*, XV (3): 281–318.
110. Zur Systematik einiger Thysanopteren. *Comunicările Academiei R.P.R.*, 9 (1959): 343–345.
111. Thysanopteren der oberen Gegend des Teleajan-Tales. *Comunicările Academiei R.P.R.*, 13: 1055–1061.
112. Ökologisch-phaenologische Forschungen über Thysanopteren. Zur Kenntnis der Thysanopterenfauna der Karpathen. *Sonderdruck aus Beiträge zur Entomologie*, Band 13, nr. 3/4: 369–377.
113. Hummeln aus der umgebung des Pețea-sees (Region Crișana). *Comunicările Academiei R.P.R.*, Tom XIII, 8: 711–715.
- 1964**
114. Contribuții la studiul ecologic al Thysanopterelor. *Studii și Cercetări de Biologie, Seria Zoologie*, 16, nr. 5: 443–451 (in collaboration with Liliana Vasiliu).
115. Contribution à l'étude écologique des Thysanopteres. *Revue Roumaine de Biologie, Série de Zoologie*, 9, nr. 5: 355–363 (in collaboration with Liliana Vasiliu).
116. Eine neue Thrips-art aus Rumänien (Thysanoptera). *Revue Roumaine de Biologie, Série de Zoologie*, 9, nr. 6: 375–376.
117. O nouă specie dobrogeană din genul *Thrips* (Thysanoptera). *Studii și Cercetări de Biologie, Seria Zoologie*, 16, nr. 6: 479–480.
- 1965**
118. Zwei neue Thysanopterenarten. *Revue Roumaine de Biologie, Série de Zoologie*, 10, nr. 3: 133–135.
- 1969**
119. Evoluția sezonală a unor specii de Thysanoptere. *Cercetări de Ecologie animală (Lucrările primei sesiuni științifice de Ecologie animală)*, 23–28 mai 1966), pp.: 395–340.
120. Aspecte ale faunei tisanopterologice dobrogene. *Cercetări de Ecologie animală*, pp.: 401–403.
121. Aspecte asupra faunei tisanopterologice carpatine. *Societatea de Științe Biologice, Comunicări de Zoologie*, pp.: 71–73.
122. Aspecte asupra faunei tisanopterologice carpatine. *Comunicări de Zoologie (prima Consfătuire Națională de Entomologie)*, pp.: 43–46.

REFERENCES

- CIUBUC C., 2002, *80 years of activity of Sinaia Zoological Research Station (Bucegi Mountains, Romania)*. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa", **XLIV**: 497–511.
- FĂRCAȘ S., SORAN V., 1982, *Knechtel Wilhelm Karl*. Pp: 207–208. În: *Personalități românești ale științelor naturii și tehnicii. Dicționar. Agricultură, Arhitectură, Astronomie, Biologie, Chimie, Fizică, Geografie, Geologie, Matematică, Medicină, Tehnică*. Editura Științifică și Enciclopedică, București, 420 pag.
- IONESCU M. A., 1959, *Sărbătorirea Acad. Wilhelm Knechtel*. Analele Academiei R.P.R., nr. 9: 205–221.
- IONESCU M. A., 1967, *Wilhelm Karl Knechtel (1884–1967)*. *Revue Roumaine de Biologie, Série de Zoologie*, **12** (4): 211–213.
- KNECHTEL W. (CAROL), 1894, *Epidemiile insectelor* [Insect epidemics]. Tipografia Voința Națională), 12 pp.
- KNECHTEL W. (CAROL), 1899, *Grădinile peisajere* [Landscaping Gardens]. București, Tipografia Gutenberg Joseph Göb, 48 pag.
- KNECHTEL W. (CAROL), 1905, *Handschriftliche Aufzeichnungen meiner persönlichen Eindrücke und Erlebnisse in Mexiko in den Jahren 1864–1867 / Memorias del jardinero de Maximiliano de México (Written notes of my personal impressions and experiences in Mexico in the years between 1864–1867 / Memoires of the gardener to Maximilian of Mexico)*. Translation by Susanne Iglar, Prologue by Amparo Gómez Tepexicuapan, Edition by Jean-Gerard Sidaner. Published in Mexico City by The National Museum of History, Castillo de Chapultepec, Mexico (2012). German original published in approx. 1905 by Karl Bellmann (Prague).

- KNECHTEL W. (CAROL), 1905, *Trandafirul* [The Rose]. Tipografia Ziarului Cronica Thoma Basilescu, 28 pag.
- KNECHTEL W. (CAROL), 1905, *Curs de entomologie și Viticultură* [Entomology and viticulture course]. Școala Superioară de Agricultură, Herăstrău, 123 pag., 40 pag.
- KNECHTEL W. (CAROL), 1909, *Insectele vătămătoare din România și mijloacele de combatere a lor* [Harmful insects in Romania and means to control them]. Editura Albert Baer, București, (together with his son).
- KNECHTEL W. (CAROL), 2007, *Întâmplări și impresii personale despre întâmplările din Mexic între anii 1864–1867*. Colecția “Călătorii uitate”. Traducere de Matilda Pfeiffer și Traian Jacotă Bârgău. Editura CD Press, București, 171 pag.
- KNECHTEL W. (KARL), 1951, *Thysanoptera*. Fauna R.P.R., Insecta, vol. VIII, Fasc. 1, Editura Academiei Române, 260 pag.
- KNECHTEL E. F., 2009, *Wilhelm Knechtel von Nordböh en über Mexiko nach Rumäni n Vom Gärtnerlehrling zum Königlichen Gartendirektor in Bukarest*. Wiesbaden, 133 pag. (in German)
- KNECHTEL E., 2012, *Vom Gärtnerlehrling in Böhmen zum königlich rumänischen Gartendirektor Wilhelm Knechtel – ein europäisches Schicksal zwischen West und Ost*. Deutsch Rumaenische Hefte, an XV, nr. 1, pp.: 9–11 (in German)
- MEXI AL., 2017, *The gardens of Peleş Castle - History, intentions and interventions*. ARA Reports 8, pp.: 205–214.
- OPRIȘ I., 2009, *A danish zoologist and his romanian colleagues*. Travaux du Muséum National d’Histoire Naturelle “Grigore Antipa”, **LII**: 567–572.
- PRALONG S. (Coord.), 2013, *Mai români decât românii? De ce se îndrăgostesc străinii de România*. Editura Polirom, 496 pp.
- ROSETI R., 2009, *Discretul grădinar al Regelui Carol I*. Jurnalul.ro Special (<https://jurnalul.antena3.ro/special-jurnalul/discretul-gradinar-al-regelui-carol-i-530639.html>).
- STAN M., 2017, *The development of „Grigore Antipa” National Museum of Natural History collections by aquisitions and donations within the period 2007-2016*. MARISIA, XXXVII, pp.: 49–58.
- STANCIU M.-D., 2016, *Deutsche Persönlichkeiten aus Rumänien, Mitglieder der Rumänischen Akademie (1882–2014)*. Transylvanian Review, **XXV** (1): 33–41.
- VASILIU-OROMULU L., 1998, *Lista revizuită a speciilor de thysanoptere (Insecta: Thysanoptera) din România*. Studii și Cercetări de Biologie, Seria Biologie Animală, **50** (2): 77–83.
- *** *Academicianul Wilhelm Karl Knechtel, 1884–1967. Necrolog*. Natura, Revista Societății de Științe Naturale și Geografie din R.S.R., **XIX** (2): 94-95.
- *** <https://arhivadearhitectura.ro/arhitecti/wilhelm-knechtel/>
- *** www.ibiol.ro/posada/Posada.pdf
- *** <http://decoratiiflorale.blogspot.com/2016/05/ce-putem-admira-in-gradinile-castelului.html> (consemnat de Dana Soroș, 2016)
- *** <https://thrips.info/wiki/Knechtel>

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