



# New trends in Biology: *from molecules to complex systems*

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# ABSTRACT BOOK



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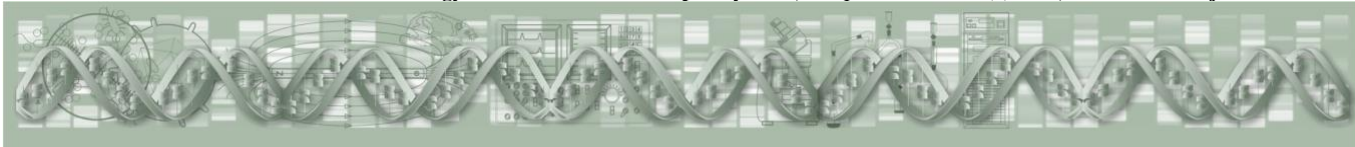
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## ***FOREWORD***

This volume contains abstracts of scientific work that have been communicated in the Scientific Session of the Faculty of Biology (SSFB), *New Trends in Biology: from Molecules to Complex Systems*, held as second edition on October 27th-28th, 2022. The Faculty of Biology of the “Alexandru Ioan Cuza” University of Iași, in partnership with “Anastasie Fătu” Botanical Garden, Museum of Natural History, and Romanian Academy – Iași Branch, organised a diversity of scientific events (i.e., oral communications, poster sessions, workshops, roundtable, and exhibition), summing 72 presentations with 180 authors from Romania and abroad (i.e., Canada, France, Germany, Italy, Republic of Moldova, Spain, Tunisia, USA). The scientific contributions have been grouped in three major themes named *Taxonomy and Ecology*, *Experimental and Molecular Biology*, and *Educational Resources in Biology*. This volume includes the abstracts of 62 papers presented within the SSFB 2022, disseminating new research results on a large variety of topics: bioarchaeology, ornithology, entomology, ecology, herpetology, plant diversity, biochemistry, molecular biology, genomics, genetics, neurobiology and physiology, pharmacology, microbiology and histology.

The scientific events occasioned by SSFB 2022 strengthened again the collegial and professional links between the participants. The 2022 edition of SSFB was a success as a result of the common effort and enthusiasm given by the organizing committee members, the moderators, as well as all the authors, whom we thank once again!

We wish that this Session will be developed through the next editions, and we are looking forward to debating relevant issues, challenges, opportunities, and new discoveries in Biology.

On behalf of the organizers, Luminița Bejenaru, Mihaela Danu, and Marius Mihășan



## THEME: TAXONOMY AND ECOLOGY

### SECTION: *HUMAN – ENVIRONMENT INTERACTIONS: CONTRIBUTION OF BIOARCHEOLOGICAL DISCIPLINES*

#### THE FIRST SEAL (*MONACHUS MONACHUS*) DISCOVERY IN THE ENEOLITHIC IN ROMANIA

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Between February and August 2020, specialists from the Constanța Museum of National History and Archaeology carried out a rescue archaeological research in the former Oituz Park, in the city of Techirghiol, where an accommodation and treatment centre is currently being built. The terrain is located in the eastern part of the town of Techirghiol, at a distance of approximately 50 m north of the shore of Lake Techirghiol, in the south-eastern extremity of the Techirghiol archaeological site. In the researched area of about 5000 square meters, 181 archaeological features were identified, which were chronologically framed as follows: 148 features dated to the Eneolithic (Hamangia), 19 features from the early Roman period, one pit dated to the late Roman period (4<sup>th</sup>- 5<sup>th</sup> centuries A.D.) and 13 features from the modern-contemporary period.

From the archaeological features of the Hamangia culture, in addition to the collected artefacts, an impressive amount of faunal remains (more than 8000 fragments weighing about 189 kg) were collected during the archaeological excavation. The state of preservation of the bone remains is relatively good. They show all the characteristics of household remains: strong fragmentation, rare traces of burning and sometimes tooth marks - especially from carnivores.

From feature 102, an oval Hamangia pit measuring 1.20 m x 1.25 m, 289 faunal remains were collected, of which 241 are mammals (83.4%). Among the latter, a distal humerus of a monk seal (*Monachus monachus*) was identified, which was dated by the radiocarbon method confirming its attribution to the Hamangia culture period.

In this presentation we will review the discoveries of seal remains in archaeological contexts during the Holocene period on the western coast of the Black Sea.

**Aknowlegdments:** This scientific contribution is made in the context of the Waterscape project - PN-III-P4-ID- PCE-2020-2369 (PNCDI III). The radiocarbon analysis was financed from the funds dedicated to Installations and Objectives of National Interest (IOSIN), as well as from the Core Project 19 06 02 02.





**ARCHAEOZOOLOGICAL DATA REGARDING THE CHALCOLITHIC SITE OF  
STOLNICENI (EDINEȚ DISTRICT, REPUBLIC OF MOLDOVA):  
CAMPAIGNS OF 2015-2016**

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The present study refers to the faunal remains discovered in the Chalcolithic site of Cucuteni culture from Stolniceni (Edineț District, Republic of Moldova), during the 2015-2016 archaeological campaigns. The animal remains are of domestic origin, as it is indicated by the butchery traces identified on them; some skeletal fragments also show gnawing marks produced by the teeth of bone-eating animals (especially dogs). Material sampling considered the year of the archaeological campaign, as well as the archaeological contexts/structures in which the faunal remains were discovered. Anatomical and taxonomic identifications, taphonomic evaluation, age estimation, osteometry and data quantification were performed.



## ARCHAEOZOOLOGICAL DATA REGARDING THE PREHISTORIC SETTLEMENT OF CUCUTENI CULTURE FROM COSTEȘTI-*CIER* (IAȘI COUNTY, ROMANIA)

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The analyzed faunal remains come from the archaeological site of Costești-*Cier* (Iași County), being discovered and collected during the excavation campaigns of 2015-2016. The archaeozoological analysis was carried out on 993 faunal remains belonging to the Cucuteni cultural level, phase A3 (4350-4200 cal BC). The identified faunal groups are represented by mammals (i.e., *Bos taurus*, *Ovis aries/Capra hircus*, *Sus domesticus*, *Canis familiaris*, *Bos primigenius*, *Capreolus capreolus*, *Cervus elaphus*, *Equus ferus*, *Meles meles*, *Lepus europaeus*, *Sus scrofa*, *Vulpes vulpes*, *Canis lupus*, Rodentia), birds and molluscs (i.e., *Cepeae* sp., *Helix* sp., and *Unio* sp.). Most of the analyzed remains belong to Mammalia (95.27% of the total identified remains), the domestic ones being predominant (54.88%). A significant number of skeletal remains (29%) could not be identified by species due to the high degree of fragmentation, therefore being classified only to class level. The preliminary archaeozoological analysis highlights the main animal resources associated with economic practices of the prehistoric community of Cucuteni culture from Costești-*Cier* (Iași County, Romania): animal husbandry, hunting, and mollusc gathering.



## **PALAEOENVIRONMENT ANALYSIS BASED ON FAUNAL REMAINS FROM SOME PREHISTORIC SETTLEMENTS IN THE MUREȘ VALLEY (HUNEDOARA COUNTY, ROMANIA)**

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Some wild animals identified during the study of archaeozoological material from an ancient settlement could have a limited ecological tolerance, they could only be found in certain biotopes (i.e., stenobiont species). Thus, their presence in a settlement can provide information on its surrounding environment, during its period of functioning.

Four multicultural sites dating from Neolithic and Bronze Age (i.e., Șoimuș-*Teleghi*, Șoimuș-*Lângă sat*, Uroi-*Sighet*, and Vețel-*Luncă*), from the Mureș Valley (Hunedoara County, Romania), were palaeoecologically analyzed, by evaluating the environment in terms of wild animal species.

The results of the study indicate dense and extensive forests, representative species being identified, such as red deer (*Cervus elaphus*), wild boar (*Sus scrofa*), brown bear (*Ursus arctos*), and lynx (*Lynx lynx*). Skeletal remains of fish and, in particular, mollusc valves that were identified in large proportions in some cultural levels (i.e., Late Neolithic, Middle and Late Bronze Age from Șoimuș-*Teleghi*; Middle Bronze Age from Uroi-*Sighet*; Late Bronze Age from Vețel-*Luncă*), indicate a rich hydrographic network in the area.

The Mureș Valley had also open field areas in prehistoric times, as it is indicated by the remains of aurochs (*Bos primigenius*), hare (*Lepus europaeus*), as well as the remains of wild horse (*Equus ferus*) and wild donkey (*Equus hydruntinus*) identified in the Late Neolithic of Șoimuș-*Teleghi*.



## SACIDAVA (2014-2021). ARCHAEOZOOLOGICAL RECORDS IN ARCHAEOLOGICAL CONTEXT

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During the Principate (1st-3rd centuries AD), Sacidava represented an important military outpost that served mainly the city of Tropaeum Traiani, and controlled the supply and transport in the Moesian sector of the Danube *limes*. This role will determine the development of the fortress and the emergence of *extramuros* civil structures, thus becoming an important landmark in the area in the 5th-6th centuries AD. and a depiction of the power of the Roman Empire in an area of ongoing conflict. Archaeological research carried out until 1979 identified the main characteristics of the fortress and proposed a stratigraphy. In 2014, archaeological research was resumed, to clarify the situation of levels of *intramuros* habitation and to verify the previously proposed chronology. Up to now, 5 sections have been excavated, through which the identification of a presumptive access route that connected the east and west gates of the citadel was sought, the research of the identified complexes, the eventual identification of the enclosure wall, the completion of the research at the East Gate. Archaeological research has led to the identification of a series of complexes, including two late Roman dwellings (one with internal paving), a pit used for the production of construction binder, a section of the *via* (?), which probably corresponds to the eastern access axis, as well as a part of the perimeter walls of some large buildings. In the 2019-2021 archaeological campaigns, 447 faunal remains of domestic origin were collected, coming from three taxonomic groups - fish, birds and mammals. Seven fragments were identified from fish (originating from carp, catfish and pike). Two fragments were identified from the birds, one from a hen and the second from a large (probably aquatic) bird. Most of the fragments are from mammals, which account for 97.98% of the total analyzed sample. 89.28% of the mammal remains identified belong to domestic mammals, and 10.72% to wild ones, this being an indicator of the increased importance of animal husbandry. The group of domestic mammals includes seven species (cattle, sheep, goat, pig, horse, donkey, dog). Most remains belong to cattle (46.65%), followed by sheep/goat (20.64%) and pig (17.16%). Hunting and fishing had low importance compared to animal husbandry for the procurement of animal protein. Four species of wild mammals were identified: red deer, wild boar, roe deer and auroch; the highest share consists of red deer and wild boar remains.



## HISTOMORPHOLOGY OF BONE LESIONS: EVIDENCE OF A SKELETAL TREPONEMATOSIS FROM 15<sup>TH</sup> -19<sup>TH</sup> CENTURIES, IN IASI, ROMANIA

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The analysis of archaeological human bone tissue can provide valuable information on cellular activity related to diseases from the past. The application of histology has become an essential and integral part in the study of human dry bone pathologies. Although paleopathology of human remains has been intensely studied, particularly by a macroscopical approach, the microscopical analysis of the affected archaeological bone tissue is an issue that has not been addressed at the same scale. This study represents the first paleohistological approach of some pathological lesions suggestive for syphilis from Romania. The aim of this study is to describe the paleohistological features (i.e., periosteal new bone formation and adjacent compact bone tissue morphology) of typical syphilitic changes in the long bones of a human skeleton (R30) from the 15th -19th centuries, discovered at the Roman Catholic Cathedral from Iași. The tissue material was subjected to the methodology for histological examination of dry bone samples. Samples were cleaned in multiple sonic baths and embedded in Buehler EpoThin 2 epoxy resin. The sections were ground and polished using carbide paper and visualized through a Confocal Laser Scanning Microscope CLSM—Leica TCS SPE DM. The preservation of the archaeological samples was assessed using the Oxford Histological Index stages. The taphonomic evaluation showed that the bone samples were very well preserved in archaeological context. During the microscopic analysis periosteal, new bone formation (e.g., polsters) and grenzlinie or remnants of these structures were identified, suggesting an inflammatory process. Sinuous lacunae were also observed in the compact bone tissue, indicating an osteolytic activity.

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## PHENETIC RELATIONSHIPS AMONG ARCHAEOLOGICAL HUMAN POPULATIONS FROM NORTH-EASTERN ROMANIA

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This study of dental morphology focuses the assessment of the biodistance relationships among archaeological human populations of Chalcolithic, Bronze Ages and the Late Middle Ages from North-Eastern of Romania. In this purpose, the lower second molar ( $M_2$ ) was used as phenotypic marker. The dental material comes from skeletons discovered in more archaeological sites from mention region and 2D Geometric Morphometrics techniques were applied. Bidimensional data were subjected to univariate and multivariate statistical tests to evaluate the significance of the obtained morphological patterns.

Our findings are presented in terms of size and shape of  $M_2$ , separately. Models of phenotypic variation of  $M_2$  were found, and both similarities and differences between them depending on the period, sex or archaeological site were highlighted.

As a general view, the  $M_2$  patterns are defined by the mobility of central landmarks and are in strong association with nonmetric traits occurrence in this molar (*4- and 5-cusped* configuration, *x* and *y* fissure patterns).

The discriminatory dental features observed on second molars support the idea of a high dental phenotypic variability and consequently the dynamic of phenotypes within human groups from the past. This study contributes to a better understanding of the history of populations of Romanian territory and their biological interconnections.

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## **ARCHAEOZOOLOGICAL RESEARCH REGARDING TRANSYLVANIA'S PRE AND PROTOHISTORY. PRESENT STATE OF RESEARCH**

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In the last few decades, in order to gain a better overview of the past, one of the main objectives of Romania's archeology consisted in its attempts of trying to join together with the exact, natural and humanistic sciences. Through this multidisciplinary has emerged a new domain that deals with the study of osteological remains belonging to animals that lived in archeological sites. The domain is now known as archaeozoology. With a rather recent history in our country, this discipline comes up with a new approach that merges the knowledge of the biology, ecology and archeology spectrum, so that, we can provide answers regarding our ancestors and the manner in which they have interacted with their environment.

The present paper is intended to be a historiographical exercise based on the analysis of the main scientific publications which cover archeozoological studies regarding prehistoric paleofauna of Transylvania. The given approach is a useful tool in showing us the actual state of archeozoological research in this area and, particularly, in providing us an overall picture regarding the main methods of management and means of subsistence that the prehistoric man has improved in time. Furthermore, by using the interpretation of the data provided from fauna samples, we could identify the main species of domestic and wild animals that compounded the paleofauna of each pre- and protohistoric age.



## TREPANNED HUMAN SKULL DISCOVERED IN A NECROPOLIS OF 17<sup>TH</sup>-19<sup>TH</sup> CENTURIES FROM IAȘI (ROMANIA)

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In this study, a case of double trepanation is reported, found in a sample of human skeletons dated for 17th and 19th centuries, from the necropolis of “Vulpe” Church in Iași (Romania), excavated during 2019-2020 archaeological campaign.

Cranial trepanation is an invasive procedure, performed intra-vitam or postmortem with specific tools and techniques, resulting in the opening of skull and, most often, the detachment of a bone fragment. Trepanations were discovered as far back as the Late Paleolithic to this century, in locations widespread in every part of the world. Being an extremely aggressive intervention on the human body, the trepanation (called craniotomy, in medical terms) is made with different purposes: medical, symbolic, or magical-religious.

The case of trepanation, described in this work, was identified in a skull belonging to an adult male of 35-40 years old. Two perforations were identified on the parietal bones, diagnosed as trepanations. The craniotomy was realized intra-vitam, most probably for medical/therapeutic purpose. Both openings of the skull are approximately oval (one is located on the right parietal near the temporal, and having 78/49 mm; other one is located on the left parietal near the sagittal suture, and having 52/26 mm); they were probably made by the scraping method with a flat implement used in a rotary movement on the bone surface, which was eroded to the formation of a complete holes.

Our analysis shows that in the case of the right perforation there was an osseous regeneration which means that the individual survived for a time after the operation. In the case of the left perforation, there is no evidence for a tendency to closure of it.

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## ANALYSIS OF THE FAUNAL REMAINS FROM THE ULMETUM FORTRESS (5TH - 6TH CENTURIES AD). PRELIMINARY DATA

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The archaeological site Ulmetum fortress (located in Pantelimon Commune, Constanța county) was declared a historical monument of national importance (class A), being entered in the List of Historical Monuments - LMI (CT-I-s-A-02726.01) and National Archaeological Register - RAN (62618.01). The first stage of the research dates from the years 1911-1914 (Vasile Pârvan), when the walls of the fortress were uncovered (resulting in an area of about 2 ha) and the towers, of various shapes (circular at the corners, rectangular on the route of the curtains, in the form of U in the area of the two main gates), were investigated. The second stage of research (starting in 2004, and continuing today) allowed establishing a relative chronology of the entire archaeological site. The fortress seems to have been built at the end of the 4th century, during the reign of Emperor Theodosius I (379-395), over the remains of a mixed settlement (civil and military) from the 2nd-4th century AD. An intense habitation is found in the 5th century AD, then a horizon of abandonment (between the last quarter of the 5th century and the middle of the 6th century AD due to Hunnic attacks), being then identified from an archaeological point of view a partial restoration in the 6th century AD, during the reign of Justinian (527-565). The Avar-Slavic attacks from the end of the 6th century and the beginning of the 7th century AD. deteriorated any form of continuity of living in the fortification. Only after almost three centuries the area seems to be populated again, during the entire period between the 9th-11th centuries (medieval-early period), perhaps even more with the return of Byzantine rule in the north of Dobrogea (after 971 AD). In the south-western sector (extramuros), an Ottoman-period hovel were discovered, dated on the basis of the discovered ceramics, to the 17th-18th centuries. In the specialized activity, a series of multidisciplinary researches were carried out. Thus, a PIXE analysis of the ceramic material paste was carried out, as well as anthropological studies on the material from the graves identified in the *intramuros* space (which dates from the beginning phase of the fortress and illustrates an important event in the history of late Roman Dobrogea - the entry of migratory populations after 376).

Thus, this work presents preliminary results of the study of faunal remains recovered from the archaeological complexes dated V-VIth centuries AD. Remains from molluscs (*Unio* sp.), fish (the identified species is common carp), reptiles (a dermal plate from a turtle's shell), birds (most from the hen), and mammals were identified in the faunal sample. Most of the remains come from domestic mammals, but fragments from wild ones have also been identified. The identified mammal species are: cattle, sheep, goat, pig, horse, donkey, dog, camel, red deer, roe deer, wild boar, auroch, hare, fox. For domestic mammals, the highest proportion have the remains from cattle and sheep/goats; for wild mammals, red deer and roe deer have the highest proportion.



## SECTION: *GENERAL 1*

### **BIRD MIGRATION AT THE BLACK SEA COAST OR AGIGEA BIRD OBSERVATORY**

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Bird migration is one of the most interesting nature phenomena, covering annually thousands of kilometers between the breeding and the wintering grounds. For medium and large sized bird species the technology developed nowadays, tracking systems through satellite or GSM networks can cover very well this journey, but for those with smaller sizes the puzzle still remains to be solved. For those species the solution is still a traditional one, based on capture and marking (ringing) and recapture. Therefore, in 2018 the first station with a permanent activity from Romania was born inside of the Marine Biology Station „Prof. Dr. Ioan Borcea” in Agigea. This station covers spring migration, nesting season and autumn migration of birds at the Black Sea coast.

Within this study more than 100 bird species are captured annually with an effective range between 13.000 – 16.000 individuals per year. The most abundant three species are the Robin, the Common chiffchaff and the Blackcap. The species presence for the two migration seasons (spring and autumn) is different, varying depending on the migration strategy of each species. Some have a circular migration (e.g. the red-breasted flycatcher – or the common redstart, subspecies *samamisticus*). Periods with maximum migration intensity are April – May for the spring season and September – October for the autumn season. In these periods, more than 400 – 500 individuals ringed per day can be reached.

Regarding this intense and long activity, even the rare species have appeared. Among these we mention the Eastern Subalpine warbler, the Sardinian warbler, the Red-flanked bluetail, the Dusky warbler, the Eastern Bonelli's warbler and the Pallas's Leaf warbler. Also, international recaptures are recorded annually therefore individuals ringed by us were recaptured in: Sweden, Finland, Russia, Bulgaria, Turkey, Israel, Cyprus, Lebanon, Ethiopia and Malta, thus covering a part of the East European migration route.



## HOT SPOTS AND PRIORITY AREAS IN ROMANIA FOR ALIEN PLANT SPECIES OF UNION CONCERN

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In order to fulfil the obligations of Romania, a Member State of the European Union, derived from Regulation (EU) 1143/2014, an important aspect is represented by the intensive inventory and mapping in the hot spots and priority points of introduction of alien plant species. This activity has been taking place since 2019 within the "Adequate management of invasive species in Romania, in accordance with EU Regulation 1143/2014 regarding the prevention and management of the introduction and spread of invasive alien species", SMIS code 2014+ 120008 and mainly targets 130 plant species considered invasive and potentially invasive for Romania. The data collection was carried out according to a methodology established within the project, which aimed to obtain information on the location of the species, the size of the populations, the abundance, the phenology, the type of invaded habitat, and the accompanying species. The data collected during 2019 were the basis for the adjustment of the work protocol, and those from the years 2020, 2021 and 2022 constituted a database that serves to analyse and interpret the information obtained. Among the invasive alien plant species of Union concern inventoried in hot spots and priority areas we mention the following: *Ailanthus altissima*, *Asclepias syriaca*, *Elodea nuttallii*, *Impatiens glandulifera* and *Ludwigia peploides*. In addition to these, other alien plants could also be inventoried, some of which have very large populations: *Ambrosia artemisiifolia*, *Robinia pseudoacacia*, *Erigeron canadensis*, *Xanthium orientale* subsp. *italicum*, *Amaranthus retroflexus*. High concentrations of invasive alien species are confirmed along roads and railways, in harbours, in border areas or along the Danube. In the Danube's entrance area of the to the country (Iron Gates Natural Park) and in the Danube Delta (Danube Delta Biosphere Reserve) are present most invasive and potentially invasive alien species. The data obtained by us regarding the presence of invasive and potentially invasive alien plant species will serve to implement the National Action and Control Plan for invasive species.



**THE ANALYSIS OF A HYBRID INDIVIDUAL BETWEEN THE *DORCADION LINEATOCOLLE* KRAATZ, 1873 AND *DORCADION LUGUBRE* KRAATZ, 1873 (COLEOPTERA: CERAMBYCIDAE)**

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Dorcadionini is a tribe within Cerambycidae, characterized by a large number of taxa and intraspecific variability or similarity between species. These longhorn beetles are flightless because they have the hind wings very reduced, and this feature makes their populations more or less isolated. *Dorcadion lugubre* is a species from the *etruscum* group that is found in Thesalia and in the N-E region of Greece, with a debated taxonomy and its area of distribution overlaps partially with that of *Dorcadion lineatocolle*. Because we have identified a hybrid between *D. lugubre* and *D. lineatocolle* in a previous study, we began to investigate how common is hybridisation between these species, and if it occurs in other populations. For this, we compared the morphology of our collected specimens and also, used molecular mitochondrial and nuclear markers to compare their DNA to comprise a broader picture of this phenomenon.



## **DINAMIC OF BIRD FAUNA IN THE PERIMETER OF THE ROSPA0049 PONDS FROM THE VALLEY OF IBĂNEASA – BAȘEU – PODRIGA (BOTOȘANI, ROMANIA)**

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Our ornithological study presents aspects on the diversity and dynamic of bird species present on the territory of ROSPA0049 Ponds from the Valley of Ibăneasa – Bașeu – Podriga. In our analysis, we use our recorded data during the '90s, respectively, during the period June 2019 – May 2022 but also the official data included in the standard dataforms and in the current management plan for the Natura 2000 site. We focus on the present status of the bird species from the Annex 1 of the Birds' Directive, species that need special conservation measures on this territory, presenting quantitative data for observed bird species during the principal periods in the annual biological cycle of the birds. We mention also the presence of bird species that appear in the Romanian Red Book of Vertebrates. We identified the present threatening factors for the bird fauna in the investigated area.



## METAGENOME-INFERRED DIVERSITY AND ECOLOGICAL ROLES OF UNCULTURED MICROORGANISMS INHABITING HYPERSALINE STRATIFIED LAKES

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Hypersaline lakes (i.e., >20% w/v total salts) are extreme habitats densely populated by obligate halophiles (or `salt-loving` organisms). Functional and taxonomic profiling of microbial communities along the water column in hypersaline stratified lakes may provide insights into the niche-based diversity and vertically-linked biogeochemical cycling. The aim of this study was to explore the metagenome-inferred diversity and metabolic roles of uncultured fraction of microbial communities parted along the density-stratified water column of Fără Fund and Ursu hypersaline lakes in Transylvanian Basin, Central Romania. A total of 2457 metagenome-assembled genomes (MAGs) with good quality (>30% completeness, <10% contamination) were recovered from both lakes. MAGs have been grouped into 798 genomospecies (gspp) out of which 117 (in Fără Fund Lake) and 118 gspp (in Ursu Lake) were classified as related to candidate bacterial and archaeal lineages. The uncultured fraction of microbial communities increased with depth and salinity along the water column in both sampled lakes. Most recovered candidate lineages were Hadarchaeota, Iainarchaeota, Nanohaloarchaeota, PWEA01 and Thermoplasmatota within Archaea, and Bipolaricaulota, Patescibacteria, Marinisomatota, Omnitrophota and Lindowbacteria within Bacteria. Metabolic marker gene analysis indicated that uncultured lineages apparently drive fermentation (Bipolaricaulota, Brocadiaceae and Patescibacteria), anaerobic carbon fixation (Bipolaricaulota, Brocadiaceae, Nanoarchaeota, Nanohaloarchaeota and Hadarchaeota), nitrogen mineralization (Bipolaricaulota, Brocadiaceae, Nanoarchaeota and Nanohaloarchaeota), and sulfate reduction (Bipolaricaulota and Nanohaloarchaeota). In conclusion, uncultured diversity is enriched in high salt concentrations along the water columns of saline stratified lakes and it appears to play key roles in the final steps of organic matter degradation and turnover of small molecules.



## THE DIVERSITY OF CERAMBYCIDES (COLEOPTERA: CERAMBYCIDAE) FROM ROSCI0076 DEALUL MARE – HÂRLĂU

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In 2021, we studied the biodiversity of Cerambycidae species from March to October in ROSCI0076 Dealul Mare-Hârlău. We identified 70 species belonging to 49 genera, 5 subfamilies, including 2 invasive species (*Hylotrupes bajulus* Linnaeus and *Trichoferus campestris* Falderman) and 9 Natura 2000 species, 3 of which belong to Cerambycidae family.



**THE COMPOSITION OF THE STOMACHAL CONTENT OF THE *PELOPHYLAX ESCULENTUS* COMPLEX FROM THE URBAN AND PERI-URBAN AREA OF THE MUNICIPALITY OF IAȘI**

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Urban and peri-urban aquatic ecosystems often represent a damper against pollution from big cities, being an alternative protection for different species of animals, which would hardly survive in urban conditions. In this study we tried to evaluate the populations of the *Pelophylax esculentus* complex, also managing to establish their trophic regime in the current environmental conditions. Two ecosystems were investigated - Lake Ciric III (Veneția), respectively Lake Ezăreni, being analysed over 40 individuals, belonging to the three species of the genus *Pelophylax* characteristic for these area (*Pelophylax ridibundus*, *Pelophylax esculentus* and *Pelophylax lessonae*). The climatic conditions of the year 2022 and the high level of pollution changed the numerical abundance of amphibian populations in the two studied areas, especially in Lake Ciric III (Veneția), where only 2 individuals were sampled. As for their trophic spectrum, it mainly consisted of individuals belonging to the Class Insecta (Orders Heteroptera, Hymenoptera, Diptera, Odonata and Coleoptera) and Oligocheta. In addition to the previously stated elements, vegetable remains, mineral elements and scales were also identified in the analysed stomachal contents. The cannibalism phenomena was observed in one of the individuals. Plastic microfibres, identified in very large numbers in the stomachal contents of the genus *Pelophylax*, constitute conclusive evidence of the manifestation of pollution in the studied areas.





## **CACTACEAE FAMILY - CONSIDERATIONS ON BOTANICAL TERMINOLOGY FROM ROMANIAN LITERATURE IN A MULTILINGUAL CONTEXT**

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The cactus family (Cactaceae) is recognized for its representatives with bizarre aspects, due the structural changes determined by adaptation to the environmental conditions. The description of the species of cacti involves the use of appropriate botanical terms, some of them specific to the family (areola, cephalium) or restricted to a group of plants with similar adaptations (podarium). In the Romanian specialty literature some of these terms are not known or used incorrectly.

Frequently the terms were taken from French literature, although the most numerous articles on cacti were published in English and German. In the academics volumes and journals they appear sporadically or not at all terms highlighting the peculiarities of cactus' structure.



## DINAMIC OF BIRD POPULATIONS OF THE PRUNDU RESERVOIR (ARGEȘ, ROMANIA) BETWEEN OCTOBER 2019 AND FEBRUARY 2020

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Between October 19th 2019 and February 15th 2020 there were identified 68 species of birds on and around the Prundu lake, belonging to 30 families and 14 orders. Out of these, the order with the most species observed was the order *Passeriformes* (33 species), while the order with the most individuals recorded was the order *Charadriiformes* (3422 individuals observed on January 5th). Both the number of species and the number of individuals fluctuated due to the autumn migration and the presence of winter visitors. The lake, as well as the rest of the reservoirs on the Argeș river, is an important location for the waterbirds that winter in the area, providing habitat for high numbers of individuals during this season. For this reason, the number of individuals goes up considerably during the month of January. The autumn migration brings numerous species of birds that use the lake as a stopover site during their journey. This is the reason why the most diverse species were recorded during the months of October and November, especially those belonging to the order *Passeriformes*. The species with the most individuals observed was *Chroicocephalus ridibundus*, followed by the species belonging to the genus *Larus*, *Aythya ferina*, *Anas crecca* and so on. I identified 6 species of birds from annex I of the Birds Directive, 24 species from annex II and 9 species from annex III of the same directive, 32 species of birds from annex II of the Bern Convention and 27 species from annex III of the same convention, 19 species of birds from annex II of the Bonn Convention and 6 species of birds included in the The Red Book of Romanian Vertebrates. The Prundu lake offers favorable life conditions to birds, especially during the winter months, some of them belonging to nationally and internationally protected species.



## **THE HERPETOFAUNA OF THE URBAN AND PERI-URBAN HABITATS FROM PITEȘTI, SOUTHERN ROMANIA**

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The first step in understanding the way reptiles and amphibians are adapting to anthropogenic factors is identifying urban and peri-urban areas in which they occur, especially in regions where sampling bias has left gaps in our understanding of fauna distribution. Here I present preliminary observations on the herpetofauna and its use of habitats in the urban and peri-urban environments from the city of Pitești, Argeș county, situated in southern Romania. I identified 9 species of amphibians and 9 species of reptiles. Their distribution is discussed in the presentation hereby, with some species recorded for the first time in the central area of the Argeș county. Despite the fact that most species thrived in their respective habitat, anthropogenic threats were identified, such as habitat loss, deliberate killing, road mortality, invasive species and poaching.



## STRUCTURE AND DIVERSITY OF MAMMAL COMMUNITIES IN FOREST ECOSYSTEMS

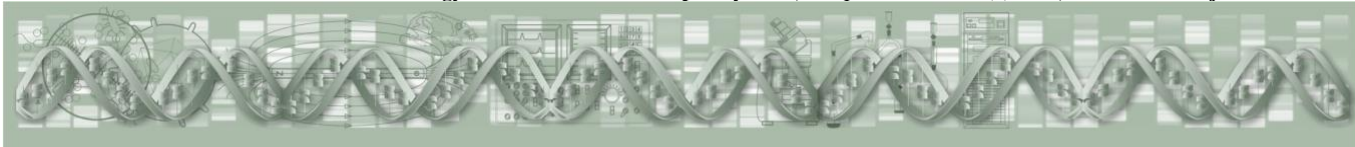
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The research was carried out in the period 2015-2022, selecting the test plots in different types of biotopes with different degrees of heterogeneity of the forest ecosystems. The determination of the specific composition and the dominance of the species was carried out by the methods of relative appreciation of the numerical population. It was determined that of the 21 species of the order Rodentia depending on the habitat, the largest number of species (33.3%) was recorded in the ecotone, and the smallest (4.8%) - in the meadow. During the research, the small mammal species were in the depression phase and the population growth phase. The capture rate varied between 2–10%. The dominant species is *Apodemus flavicollis* (50-60%). The presence of the following mammal species was recorded, among which the characteristic ones: the mole (*Talpa europaea*), the common shrew (*Sorex araneus*), the forest shrew (*Dryomys nitedula*), the field rat (*Apodemus agrarius*), the collared mouse (*A. flavicollis*), the mouse (*Clethrionomys glareolus*), larger species - wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), fox (*Vulpes vulpes*), forest ferret (*Mustela putorius*). Among the rare species we mention the otter (*Lutra lutra*), the wild cat (*Felis silvestris*), the forest marten (*Martes martes*). The highest number of species was recorded in the ecotone (33.3%), and the lowest – in the meadow (4.8%). It was determined, that a floristic diversity and the length of the internal linear borders, condition higher values of the edge effect, especially, in the ecotone (2.53). A positive correlation was established between the diversity index and the aridity index in the forest ecosystem ( $r=0.98$ ) and ecotone ( $r=0.97$ ) and a negative correlation in the meadow ecosystem ( $r=-0.96$ ). This is explained by the stronger impact of the drought in the meadow.

**Aknowlegdments:** The work was carried out within the project 20.80009.7007.02



**'SO CLOSE, NO MATTER HOW FAR': A REMARKABLE NEW RECORD EXTENDS THE DISTRIBUTION, BUT NOT THE GENETIC DIVERSITY OF THE MOLDAVIAN MEADOW VIPER (*VIPERA URSINII MOLDAVICA*)**

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Reptiles are amongst the vertebrates most threatened with extinction. Many snake species are affected by climatic changes as a result of their physiological characteristics and by the loss of habitat. In Central and Eastern Europe, the conversion of lowland habitats like grasslands and meadows into arable land had a major impact on species linked to such habitats. One such species is the meadow viper (*Vipera ursinii*). In Romania two subspecies have been identified (*V. u. rakosiensis* and *V. u. moldavica*), both of them being classified in a high risk category for extinction in the IUCN Red List. The Moldavian meadow viper (*V. u. moldavica*) is the easternmost subspecies of the *Vipera ursinii* complex, and it is also the most threatened (CR – critically endangered). Historically, it was recorded from Bulgaria, the Republic of Moldova, and five counties in eastern Romania, and for a long time it was only known to have survived in two counties from Romania: Iași in the north-east, and Tulcea (the Danube Delta). Here we update the current knowledge of the distribution and conservation status of the Moldavian meadow viper in Romania by presenting a remarkable new occurrence record from the Carpathian Bend area (Buzău county) and utilize species distribution modelling to identify new potentially suitable areas for the species. Finally, we ran phylogenetic analyses using mitochondrial and nuclear DNA markers sequenced from all currently known regions of occurrence, together with previously published sequences. A very low genetic variation was found for *V. u. moldavica*, suggesting a very recent dispersal and/ or fragmentation.



## **FACTOR IN FEAR? DISENTANGLING THE ROLES OF EXPLOITATIVE VERSUS INTERFERENCE COMPETITION IN DISRUPTIVE SELECTION**

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Disruptive selection arises when extreme phenotypes have a greater fitness advantage compared to the intermediate phenotypes between them. Disruptive selection is regarded as an important source of variation in natural populations, having the potential to initiate speciation. Current theory and recent empirical evidence suggest that by causing negative frequency dependent interactions, intraspecific resource competition is one of the key drivers of disruptive selection. However, intraspecific competition for resources can take different forms and be either indirect (exploitative competition) or direct (interference competition). To our knowledge, no previous study has investigated the role of interference competition in disruptive selection, and most general models of disruptive selection assume competition is exploitative.

We experimentally investigated whether the type of competition is relevant in competitive interactions using a system where disruptive selection is common: the Mexican spadefoot toads (*Spea multiplicata*). Tadpoles of this species develop into one of two extreme phenotypes; carnivore morphs consume mainly fairy shrimp, while omnivores feed mainly on algae and detritus. Previous studies have shown that intermediate morphs generally have much lower fitness when competition is intense, as they are outcompeted by the specialized tadpoles for both resources.

Our experiments revealed that (i) the presence of carnivores significantly modified the foraging behaviour of the focal (intermediate) tadpoles, and (ii) intermediate tadpoles exhibited a significantly lower growth rates in direct (interference) competition treatments in comparison to exploitative competition treatments, when in competition with carnivores but not omnivores. These results suggest that interference competition may play a greater role than currently believed in driving disruptive selection. As carnivorous tadpoles are also cannibalistic, the 'fear' effect may have a greater impact on intermediate tadpoles than exploitative competition or consumption alone for their shared resource, similarly to how nonconsumptive effects can alter prey ecology and evolution more than consumptive effects in predator-prey or intraguild relations.



## **DO DIFFERENTIAL ACTIVITY PATTERNS HELP MAINTAIN COLOUR POLYMORPHISM IN VIPERS FROM WARMER ENVIRONMENTS?**

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Animal colorations represent adaptations to different biotic or abiotic environmental factors and play crucial roles in predator avoidance (via crypsis, aposematism, or mimicry), inter – and intraspecific communication and sexual selection. In ectothermic animals, coloration may also be important for thermoregulation. Colour polymorphism (i.e. the occurrence of two or more phenotypic morphs in the same population) is present along numerous animal lineages, and melanism is probably the most studied type. In several Eurasian viper species of the genus *Vipera*, populations greatly vary with regards to the frequency of melanistic individuals, and the maintenance of polymorphism have been attributed to either adaptive or non-adaptive processes. Current theory would predict that melanistic vipers should be more frequent in colder environments (normally higher latitudes or altitudes), and this is mostly confirmed for the Asp Viper (*Vipera aspis*). Here we tested the hypothesis that differential habitat use and activity patterns could explain the maintenance of colour polymorphism in a viper population from warmer environment. In accordance with the thermal melanism hypothesis, we would expect melanistic vipers to predominantly use less open habitats and/or be more active at cooler temperatures, as they should be thermoregulatory superior to patterned (zigzag) individuals. Overall, our results show a weak support for the differential habitat use hypothesis, with only one habitat characteristic (microhabitat exposure) being significantly associated with morph, but only for females ( $\chi^2=13.475$ ,  $df = 6$ ,  $p = 0.036$ ). However, observational data does suggest that activity patterns do differ, with melanistic vipers being especially active during overcast and rainy periods, although no differences were observed between basking site temperatures. Other adaptive as well as non-adaptive hypotheses require testing before we can gain a deeper understanding of maintenance of melanism in vipers from warmer environments.



**THE FIRST RECORDS OF THE INVASIVE FRESHWATER PRAWN  
*MACROBRACHIUM NIPPONENSE* (DECAPODA: PALAEMONIDAE) IN ROMANIA**

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The present study reports the first occurrence of the oriental river prawn *Macrobrachium nipponense* (De Haan, 1849) in Romania. This is a subtropical freshwater decapod native to South-East Asia. Because the oriental river prawn is a common aquaculture species worldwide, it was intentionally introduced into cooling water outlets of several power stations in Belarus, Russia, and Moldova to increase their biological productivity. Feral populations were also reported in the Philippines, Singapore, Kazakhstan, Uzbekistan, Iran, Iraq, and more recently in the southern regions of Ukraine and Russia. In Romania the species was recorded for the first time on Chilia arm of the Danube Delta in November 2021. However, on social media (Facebook) there are reports of its presence in the lower reaches of the Danube and the Pruth rivers as early as August 2021. Already in July-August 2022 the oriental river prawn has become a common species in many lakes and canals of the Danube Delta and had reached upstream Brăila on the Lower Danube.

Romanian specimens range in total length (from the tip of rostrum to the end of telson) from 58.7 mm to 89.3 mm. Males are larger than females and have the second pereopods significantly longer than other walking legs. The proportion of ovigerous females is 20–86 %.

*Macrobrachium nipponense* is a voracious omnivore that feeds on aquatic invertebrates, but also consumes filamentous algae, decomposing plants and dead fishes. The species can also prey upon small fish or even its congeners.

The oriental river prawn was most likely introduced into the Danube River Basin from the neighbouring Dniester River Basin either through the stocking of ornamental organisms, either through the deliberate release or accidental escape from aquaculture facilities. Due to the global warming, at lower latitudes *M. nipponense* can spread through natural range expansion.





## THE CHEMICAL CAMOUFLAGE OF THE MYRMECOPHILOUS *MACULINEA ALCON* LARVAE (LEPIDOPTERA: LYCAENIDAE). PRELIMINARY RESULTS

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The ants' cuticle is covered by a waxy lipid layer that mostly consists of cuticular hydrocarbons. Besides the function of maintaining water balance, the cuticular hydrocarbon mixture is the main agent in chemical communication. Variations in the specific cuticular hydrocarbon composition can indicate intersexual differences, caste, developmental stage, and social status. The sum of all chemical profiles of individuals contributes to the specific odour of the colony (*gestalt odour*), being essential in discrimination between nestmate and foe.

The larvae of *Maculinea alcon* butterflies are highly specialized social parasites that can break the chemical code to enter and integrate into the social system of *Myrmica* ants. With extremely sophisticated adaptations, the larvae are not only adopted by ants and carried inside the nest but can achieve high social status in the host-ant system, being cared and fed preferentially by the workers, until pupation.

To identify what are the chemical mechanisms to integrate and how they function inside the social parasite-host system, we analysed the cuticular hydrocarbons of the two syntopic ecotypes of *M. alcon* larvae (*'cruciata'* and *'pneumonante'*) and the chemical profiles of their host-ant species *Myrmica scabrinodis* and *Myrmica sabuleti* from Natura 2000 Site „Eastern Cluj Hills” (Cluj County, România). The preliminary results show that despite radical differences (75% dissimilarity) found in the chemical profiles of *Myrmica scabrinodis* and *Myrmica sabuleti* species, the *Maculinea alcon* *'cruciata'* ecotype larvae were successfully adopted by the ants of both host species. Moreover, after just 10 days spent inside host-ant nests, the cuticular hydrocarbon composition of *M. alcon* *'cruciata'* larvae was qualitatively and quantitatively changed, becoming closer to the chemical profile of the ant species that adopted them.



## LARVAL POLYMORPHISM OF TWO SYNTOPIC *MACULINEA ALCON* (LEPIDOPTERA: LYCAENIDAE) ECOTYPES

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The *Maculinea* = (*Phengaris*) butterflies are extremely vulnerable species with a complex life cycle that implies specific host-plant species and *Myrmica* host ant species. In the first three instars, the caterpillars are phytophagous, but in the fourth instar, they leave the host plant and descend to the ground, where for their survival they will need to be adopted by *Myrmica* ants and be carried into their underground nest. The adopted larvae will develop by exploiting the host-ant colony's resources for 11 months (annual larvae) or 23 months (biannual larvae). The presence of two groups of larvae in the same population (polymorphic larvae) is a rare phenomenon and it is a strategy to reduce the risk of local extinction caused by unfavorable events (*bet-hedging*).

Our studies from Natura 2000 Site „Eastern Cluj Hills” (Cluj County, România) are showing the presence and the effects of larval polymorphism as a survival strategy of the local populations of two syntopic *Maculineaalcon* ecotypes (“*cruciata*” and “*pneumonanthe*”). For *M.alcon* “*cruciata*” larvae reared in laboratory conditions by three different *Myrmica* species, the results show a clear bimodal growth pattern, where two weight classes are distinguished: larvae with fast development that pupated after a year and slow-growing larvae that needed two years to pupate. Also, *M. a. “pneumonanthe”* and *M. a. “cruciata”* larvae reared by the same host-ant species, *Myrmica scabrinodis*, show a specific growth pattern well defined. There are differences between the two ecotypes in the bimodal growth pattern with annual and biannual larvae. The differences in larval development between the two syntopic *M.alcon* ecotypes is a mechanism to synchronize their flight period with the specific host-plant phenology, which can lead to intraspecific segregation, indicating a possible ongoing ecological divergence.



## **DOES DORSAL COLORATION AFFECT PREDATION RATES IN A POLYMORPHIC VIPER (*VIPERA NIKOLSKII*) POPULATION FROM A WARM, LOWLAND, ENVIRONMENT? PRELIMINARY RESULTS**

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Animal colorations represent adaptations to different biotic or abiotic environmental factors and play crucial roles in predator avoidance, communication and sexual selection. In ectothermic animals, coloration also plays important roles in thermoregulation. The thermal melanism hypothesis states that darker individuals belonging to ectothermic species will be superior to lighter individuals with regards to thermoregulation. However, individuals that are completely or almost completely black (melanistic) often lose the cryptic or aposematic signaling function of the patterned coloration, and may thus be more vulnerable to predation. While these statements appear to be valid for some species and populations, there are cases with peculiar characteristics, such as the lowland southern clades of the *Vipera berus* complex, where melanism is either common in polymorphic populations, or where entire populations comprise only melanistic individuals.

We aimed to experimentally test whether the type of coloration is relevant for predator-prey interactions (i.e. vipers being the prey) in a polymorphic population of *Vipera nikolskii* from North-Eastern Romania. Employing methods proven successful in previous studies, we used 90 viper replicas made of plasticine, that we installed in the field, in a habitat known to be inhabited by vipers. 50% of the replicas were patterned (typical zigzag pattern) while 50% were completely black (resembling melanistic vipers). 50% of all models were placed on a white sheet of cardboard, in order to eliminate the role of crypsis from any of the colorations. Replicas were inspected for predation events every 48 hours, and were retrieved after a total of 10 days in the field.

Our preliminary results indicate a significantly higher predation rate on melanistic individuals, confirming previous studies. We thus show that the high frequency of melanism in these populations is not a result of relaxed predation upon them. Future work will also be conducted in a purely melanistic population.



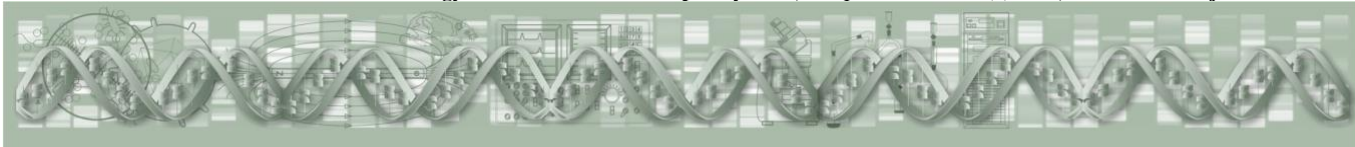
## **A NEW RECORD OF THE PROTECTED HABITAT “ALPINE RIVERS AND THEIR LIGNEOUS VEGETATION WITH *MYRICARIA GERMANICA*” FROM THE VALLEY OF MOLDOVA RIVER**

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The habitat “Alpine rivers and their ligneous vegetation with *Myricaria germanica*” (code 3230) is listed by the Habitats Directive and Romanian correspondent law, as natural habitat type of community interest whose conservation requires the designation of special areas of conservation. Such a habitat should be targeted by management plans of Natura 2000 sites, provided the habitat appears in the site’s standard data form. Unfortunately, sometimes this is not the case. Our study, carried out in 2021, aimed to investigate the plant communities of the site ROSCI0363 (Râul Moldova între Oniceni și Mitești). There, we found several phycosenoses with *Myricaria germanica* as dominant species, accompanied by several less abundant species (*Salix purpurea*, *Ranunculus repens*, *Lotus corniculatus*, *Elymus repens*, *Mentha longifolia*), which prove the presence of the habitat 3230 in site. As the habitat is not mentioned by the standard data form, its revision is required for site to fulfil its protective role.



## REPTILES FROM THE NATURA 2000 SITE “SĂRĂTURILE JIJIA INFERIOARĂ – PRUT” (ROSCI0222)

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European reptiles are among the most threatened groups of animals. For most of them are protected, reptiles may act as umbrella and flagship species in certain areas. The Natura 2000 site “Sărăturile Jijia inferioară – Prut” (ROSCI0222) is such an area that, despite its conservation status, is largely exposed to human activities and alterations. Thus, the aim of this study is to update the knowledge on local reptiles. Therefore, we carried out field surveys in the site perimeter, from April to August, 2018 and from March to July, 2019. We identified five reptile species (*Emys orbicularis*, *Lacerta agilis*, *Lacerta viridis*, *Coronella austriaca*, and *Natrix natrix*) that are typical for the major relief forms of the area. Four of the identified species are strictly protected according to European and national regulations, whereas the standard data form of the area lists only one species. Hence, the management plan of the site should consider all the strictly protected species as the standard data form fails to reflect the actual richness of the reptile assemblages.



## DISTRIBUTION AND OSMOADAPTIVE TRAITS OF ASGARD ARCHAEA IN SALINE ENVIRONMENTS

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Recently described Asgardarchaeota superphylum comprises representatives with eukaryotic-like attributes including cytoskeletal structures, vesicle trafficking and endomembrane formation potential. While most studies have been focused on the evolutionary relationship between Asgard archaea and Eukarya, their metabolism and ecology are scarcely explored. The majority of the unearthed Asgard archaeal metagenome-assembled genomes (MAGs) share preferences for low oxic sedimentary environments and organic carbon sources. Mixotrophy and phototrophy were also inferred in Asgard archaea suggesting metabolic versatility and ability to inhabit diverse milieus. While most of Asgard archaea were described in marine sediments, their occurrence in hydrothermal vents, saline microbial mats, coastal and inland brackish or saline lake sediments was also reported. To date, the available information on diversity and potential biogeochemical roles of Asgardarchaeota mostly sourced from marine habitats and to a much lesser extend from true saline environments (i.e., > 3% w/v total salinity). Here we provide an overview on diversity and ecological implications of Asgard archaea distributed across saline environments and briefly explore their encoded potential for osmoadaptation. To date, the prevalent Asgard clades detected in saline habitats are Loki-, Thor- and Heimdallarchaeota where they might employ anaerobic/microaerophilic organic matter degradation and autotrophic carbon fixation. Homologs of primary solute uptake ABC transporters seemingly prevail in Thorarchaeota whereas those putatively involved in trehalose and ectoine biosynthesis were mostly inferred in Lokiarchaeota. Additionally, our attempt to evaluate the proteome pI profiles predicted from the publicly available Asgard archaeal MAGs revealed an average pI of 6.9 with slightly lower value for Thorarchaeota. Based on the available data, we speculate that Asgard archaea are readily dwelling habitats up to 6-8% salinity and might adopt energy costly compatible solute-accumulating (‘salt-out’) strategy as response to salt stress.

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## ZOOBENTHOS OF THE LOWER PRUT RIVER DURING 2015-2019 ON THE TERRITORY OF THE REPUBLIC OF MOLDOVA

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The Prut River is the second largest river in Moldova (the length on the territory of Moldova is 695 km) and the last largest left tributary of the Danube. The Lower Prut includes the section of the river from the mouth of the right tributary of the Prut Jijia to the mouth of the Prut River. The benthic invertebrates have been collected at 5 sampling stations in the Lower Prut basin: Leuşeni, Leova, Cahul, Cîşliţa-Prut and Giurgiuleşti. The standard methods were applied, i.e. dredge, Petersen and Ekman grabs and hand sampling. Sampling was carried out seasonally during 2015-2019. A total of 152 taxa of benthic invertebrates were identified, including: Gastropoda - 11, Bivalvia - 14, Polychaeta - 1, Oligochaeta - 17, Crustacea - 20, Ephemeroptera - 12, Trichoptera - 12, Chironomidae - 41 and other groups - 24. The most numerous group is Chironomidae, numbering 41 taxa.

The rare species like us: *Theodoxus transversalis* (Pfeiffer, 1828), *Unio crassus* Philipsson in Retzius 1788 (IUCN Red List), *Gomphus flavipes* (Carpentier, 1825) and *Palingenia longicauda* (Olivier, 1791) (Ap.II, Bern Convention, 1998) have been registered.

Invasive species such as *Corbicula fluminea* (Müller, 1774), *Sinanodonta woodiana* (Lea, 1834), *Dreissena rostriformis bugensis* Andrusov, 1897 and *Branchiura sowerbyi* Beddard, 1892 were also found. These species formed stable populations in the study area.

The main negative factors affecting macroinvertebrate populations in the Prut River are changes in the quality of substrates (riverbed amelioration in the area of the port of Giurgiulesti), domestic wastewater runoff (Leuşeni, Leova and Cahul) and invasive species (Cîşliţa-Prut).



## ZOOPLANKTON COMMUNITY DYNAMICS OF TWO RESTORED WETLANDS FROM DANUBE DELTA BIOSPHERE RESERVE, ROMANIA

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The present contribution explores the usefulness of zooplankton as a tool for assessing the water quality and trophic status of two wetlands, situated in Danube Delta Biosphere Reserve (45°24'30"N 28°10'50"E), Romania, that recently have undergone ecological restoration projects. The water was sampled seasonally and investigated for environmental parameters and zooplankton community for a period of one year (March 2021 to October 2021). Regarding zooplankton species composition, a total of 46, respectively 29 species belonging to Rotifera, Cladocera and Copepoda were recorded. Variation of zooplankton species in respect of dominance, diversity, evenness and richness were calculated. Presence of certain species was considered to be a biological indicator for water quality status and trophic condition of the lake. Q Brachionus/Trichocerca (QB/T) index was used for the determination of trophic level of the wetlands. Overall, results showed that limnological parameters and zooplankton assemblages' have effects on the water quality and trophic condition and can potentially affect the functioning of ecosystems.

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## **TORYMUS SINENSIS AND CLOSE NATIVE SPECIES**

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*Torymus sinensis* Kamijo, has been used for the past 30 years to control the population of the cynipid gall wasp of the chestnut, *Dryocosmus kuriphilus* Yasumatsu. It was first introduced in Japan (1975) with a visible control of the pest population 8-10 years after its introduction. Shortly after the introduction, the first cases of hybridization with the native species *Torymus beneficus* Yasumatsu and Kamijo were reported. A few years later *T. sinensis* eliminated *T. beneficus* by hybridization and introgression.

The specimens used came from several European countries. In order to carry out the study, several molecular markers were amplified and analyzed: (i) the mitochondrial protein-coding gene Cytochrome c oxidase subunit I (COI); (ii) the wingless (Wg) nuclear gene, (iii) the Enolase (Eno) nuclear gene including introns and exons; (iv) the ribosomal internal transcribed spacer 2 (ITS2). The phylogenetic reconstructions for each marker were congruent enough to perform a multi-locus phylogeny. After concatenation, numerous evolutionary models and various partitioning schemes were tested. Phylogenetic analyses based on maximum likelihood (ML) unambiguously separate (bootstrap > 90%) most species except *T. auratus* with 82% bootstrap, the other European *Torymus* species as well the "*beneficus-sinensis*", providing a reliable basis for delimitation of species, morphological characterization and identification of diagnostic characters.

The resulting phylogeny with this method confirms that *T. notatus* and, to a lesser extent, *T. cyaneus* are the species closest phylogenetically to the complex "*beneficus-sinensis*". Phylogenetic data shows that only *T. notatus*, which can parasitize *D. kuriphilus*, may have the potential for hybridization with the biological control agent *T. sinensis*. The two species are part of the same group of species (the *cyaneus* group) and are the closest genetically on the basis of all three nuclear markers: Enolase (1.5%), Wingless (2%) and ITS2 (14.1%), but less on the mitochondrial marker COI (11.5%).

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## COMPARATIVE ANATOMY OF THE SHOOT FROM SOME HORTICULTURAL VARIETIES OF *SYRINGA VULGARIS*

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The biological material is represented by four horticultural varieties of the ornamental species *Syringa vulgaris*, namely var. *Mimetifolia*, var. *Jose*, var. *Primrose*, var. *Sensation* and the wild species. The material was collected from the "Anastasiu Fătu" Botanical Garden from Iași. From each species leaves with petioles and one-year-old stem fragments have been preserved. The material was processed through the classic techniques used in the plant histo-anatomy laboratory, the double staining being done with iodine green and ruthenium red.

The analyzed stems have a secondary structure; the presence of both secondary meristems (cambium and phellogen) was observed. Secondary conducting tissues have rings shapes and an interrupted band of sclerenchymatous elements is present at the periphery of the secondary phloem; the secondary xylem is much better developed in the *Jose* variety and the *Sensation* variety than in the *Primrose* variety. The pith is compact. The phellem (cork) has varying thicknesses, depending on the species or variety analyzed.

The transversal sections through the petiole have different shapes in the investigated taxa, these as well as the variations in the structure and the number of conducting bundles that enter into its structure can serve as identification criteria. In *Jose* variety numerous uncinat unicellular trichomes are present on the epidermis; in the other varieties they are absent.

The structure of the leaf lamina varies, both in thickness and in terms of the mesophyll composition (the number of layers of palisade and lacunar tissue cells, their sizes).

Although the general structure plan of the vegetative organs is the same in the analyzed varieties, there are significant differences in terms of various histo-anatomical characters that can be used in their differentiation.



## ***ECHIMUM ITALICUM* L. (FAMILY BORAGINACEAE): BIOLOGICAL CHARACTERISTICS AND POTENTIAL USES**

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The aim of this paper is to highlight the biological characteristics of the species, potential uses and identified biologically active compounds based on the analysis of data reported in the literature.

*Echium italicum* L. syn *E. altissimum* Jacq. (cowtail) is a biennial species, hispidly hairy, gray in appearance; with erect stem, rigid and sometimes very branched from the bottom, 40-100 (150) cm high; it blooms between June and August. The species is widespread in Europe, Western Asia, Asia Minor.

Different parts of the plant (root, aerial parts) are used in traditional medicine in some countries for the treatment of skin conditions, of the respiratory system, of rheumatic pains, as a diuretic, emollient, depurative, diaphoretic.

The chemical compounds identified in different parts of the plant are represented by flavonoids, phenolic acids, tannins, terpenoids, naphthoquinone pigments, volatile oils, fatty acids, etc.

Studies show that extracts from different parts of the plant and some isolated compounds show promising biological activities (analgesic, sedative, anti-inflammatory, antioxidant, antimicrobial, wound healing, insecticidal) and nutritional properties. The species represents an important material for future research and a potential source for obtaining some biologically active compounds.

**THEME: EXPERIMENTAL AND MOLECULAR BIOLOGY****SECTION: *EXPLORING THE BIO-MOLECULAR WORLD FOR A BETTER LIFE*****THE COPA2-TYPE CU(I) -ATPASE ENABLES CUPROPROTEIN MATURATION****ANDREEA ANDREI<sup>1,2,\*</sup>, HANS-GEORG KOCH<sup>1</sup>**<sup>1</sup>Institute for Biochemistry and Molecular Biologie, ZBMZ, Faculty of Medicine, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany<sup>2</sup>Faculty of Biology, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany\*Corresponding author: [andrei.c.andreea@gmail.com](mailto:andrei.c.andreea@gmail.com)

Cu(I)-ATPases are ubiquitous copper and silver transporters. There are two types of Cu(I)-ATPases: the well characterized CopA1-type ATPases, involved in Cu detoxification, and the less characterized CopA2-type ATPases, required for providing Cu(I) for the maturation of cuproproteins, like the cbb3-type cytochrome c oxidase (cbb3-Cox). In particular, the biochemical mechanisms by which CopA2-like ATPases coordinate copper trafficking from the cytosol into the periplasm for maturation of cuproproteins are poorly understood. In this work, we report on the acquisition, translocation and delivery of Cu by CcoI, a predicted CopA2-like ATPase of the phototrophic Gram-negative bacterium *Rhodobacter capsulatus*. We demonstrated the ATP-dependent Cu(I)-transport in reconstituted CcoI-proteoliposomes using solid-supported membrane electrophysiology. In vitro, Cu(I) translocation occurs independently of CopZ, a cytosolic Cu-chaperone also identified as an in vivo interaction partner of CcoI. The physiological relevance of three putative Cu-binding sites in CcoI was investigated. Mutating the two N-terminal Cu-binding sites reduced cbb3-Cox activity. However, cbb3-Cox activity and its steady-state levels were restored by increasing the Cu concentration in the medium. This suggests that the N-terminal Cu-binding sites are important at low Cu concentrations. In particular, the inactivation of the distal N-terminal metal binding site (N-MBS1), with a glutaredoxin-like CPxC motif decreased the ATPase activity. The inactivation of the proximal metal binding site (N-MBS2), with a CopZ-like CxxC motif, increased the Cu(I)-induced inhibition of the CcoI ATPase activity, indicative of its involvement in Cu acquisition. Mutating the membrane-integral Cu binding site completely diminishes Cu transport and cbb3-Cox activity. This mutation cannot be compensated by increased Cu concentrations. Furthermore, the periplasmic Cu chaperone SenC was fused with CcoI and their interaction was found to compensate for the cbb3-Cox activity in  $\Delta$ ccoI and  $\Delta$ senC strains. In summary, our data reveal that CcoI is the link between the cytosolic and periplasmic Cu chaperone networks for cbb3-Cox maturation.



## THE INFLUENCE OF *FALCARIA VULGARIS* EXTRACT ON NEURODEGENERATE DISEASES IN THE ZEBRAFISH MODEL

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Considering the physical and ethical problems of experiments on human patients, biomedical research has focused, in recent years, on the use of model organisms to better understand the pathogenesis of human and animal diseases, but also on the development and testing of new therapies, with animal studies having an important role in scientific evolution. Also, the use of animals in scientific research has generated many discussions in recent times, with increasing concerns related to bioethics and animal welfare.

The most common model organisms are small mammals, usually mice or rats. Although these models have significant advantages, they are expensive to maintain and difficult to manipulate embryonically, and large-scale genetic studies are limited. The zebrafish (*Danio rerio*) makes up for these deficiencies in mammalian experimental models, its low cost, small size, and external development making it an excellent model for vertebrate developmental biology. The use of adult and larval zebrafish in neuroscience has increased significantly in recent decades, the mammalian and zebrafish brain morphology being similarly.

In the present study, we used the zebra fish as an experimental animal model, the symptoms characteristic of Alzheimer's disease being induced by administration of scopolamine. The substance of interest used was the essential oil of *Falcaria vulgaris*, which has antioxidant effects, but also medicinal properties, the aim of our research being the evaluation of the influence of the administration of the essential oil of *Falcaria vulgaris* on cognitive processes in zebrafish.

Thus, the results of the Y-maze test showed that the administration of *Falcaria vulgaris* essential oil improves the memory of zebrafish, the most obvious effects being observed in the groups treated with the highest concentration of extract (100  $\mu$ l/L).

Also, the results of the novel object recognition test (NOR) demonstrate the ability of *Falcaria vulgaris* oil to improve the effects induced by the muscarinic antagonist - scopolamine. Thus, in the three groups to which the differential treatment with essential oil of *Falcaria vulgaris* was applied, longer exploration of the two objects, especially the new object, was found.

Consequently, we can state that the essential oil of *Falcaria vulgaris*, in concentrations of 50  $\mu$ l/L, 75  $\mu$ l/L and 100  $\mu$ l/L water, ameliorates cognitive deficiencies, but also the antagonistic effects that scopolamine has on the memory of zebrafish, however, further, more research is needed in the future.



## THE COMPLETE GENOME ASSEMBLY OF THE NICOTINE-DEGRADING *PAENARTHROBACTER NICOTINOVORANS* ATCC 49919

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*Paenarthrobacter nicotinovorans* ATCC 49919 was first isolated from soil on which tobacco was cultivated. A nicotine-degrading microorganism, *P. nicotinovorans* ATCC 49919 uses the pyridine pathway for nicotine catabolism. The strain has potential applications towards the conversion of nicotine-contaminated waste and natural resources into valuable precursors for drug synthesis (6-hydroxy-L-nicotine) and green chemicals ( $\gamma$ -aminobutyric acid). Until recently, the lack of complete genomic information impeded the strain's genetic and metabolic engineering. Here, *Paenarthrobacter nicotinovorans* ATCC 49919 was sequenced using two complementary technologies: Illumina NovaSeq 6000 and Oxford Nanopore Technology MinION. Hybrid assembly using Unicycler yielded the complete genome comprising of two circular replicons. The bacterial chromosome is represented by the largest replicon of 4 316 184 bp, with an overall GC content of 63.2%, and contains 3953 coding sequences. The second replicon represents the pAO1 megaplasmid of 165 141 bp and with an overall GC content of 59.7%. Gene Ontology (GO) analysis assigned 2421 GOs to 626 genes. COGs were assigned to 3383 genes (93%), most of which are involved in amino acid transport, carbohydrate transport and metabolism, and transcription. Comparisons between the complete genome of *P. nicotinovorans* ATCC 49919 and the genomes of other *Paenarthrobacter* species or different microorganisms that also use the pyridine pathway for nicotine catabolism revealed low identity at the nucleotide level. Therefore, the fully annotated complete genome sequence of *P. nicotinovorans* ATCC 49919 can be used as a reference genome for related species or for nicotine-degrading microorganisms which also use the pyridine pathway. Moreover, the complete genome enables precise and efficient genetic engineering of *P. nicotinovorans* ATCC 49919 for biotechnological applications.

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## 6-HYDROXY-L-NICOTINE AND ANTIAGGREGATING POTENTIAL AGAINST AMYLOID PEPTIDE: FROM NATURAL SOURCES TO THE BRAIN

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Alzheimer's disease (AD) is characterized by progressive degradation of memory processes, being associated with three major changes that occur in the brain: i) the formation of intra- and extracellular beta-amyloid deposits; ii) the appearance of neurofibrillary tangles and iii) the death of cholinergic neurons and a significant decrease in acetylcholine level. The identification of neuroprotective therapies for AD was dominated by the hypothesis of amyloid cascade and tau proteins. Unfortunately, both approaches have so far failed to provide an effective therapeutic strategy. The involvement of  $\alpha 7$  and  $\alpha 4\beta 2$  subtypes of nicotinic receptors (nAChRs) in the pathogenesis of AD has led to the proposal of a new therapeutic approach. Immunohistochemical studies in the brains of patients with sporadic AD have shown that A $\beta$ 1-42 and  $\alpha 7$ nAChR are present in neuritic plaques and this interaction may be inhibited by  $\alpha 7$ nAChRs ligands. Nicotine, through its ability to bind to nAChRs is the ideal molecule for the development of new derivatives with therapeutic applications. The project aims to test the antiaggregating potential against A $\beta$ 1-42 of 6-hydroxy-L-nicotine (6HLN), derived from the metabolism of nicotine in the microorganism *Paenarthrobacter nicotinovorans*. To achieve this goal, the project activities will focus on evaluating the action of 6HLN in vitro on cell lines and in vivo on 5xFAD transgenic mice, in order to identify its therapeutic potential.

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## EVALUATION OF THE COGNITIVE EFFECTS OF *EQUISETUM PRATENSE* EXTRACT IN *DANIO RERIO*

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*Danio rerio* is an important model organism in developmental genetics, neurophysiology and biomedicine, becoming, in recent years, a widely accepted model organism in the study of vertebrate developmental biology, yet little being known about its natural ecology and behavior.

Laboratory studies of zebrafish behavior have included learning and memory, sensory perception, anxiety, feeding, and reproduction. In recent years, more and more studies have shown that the zebrafish is a promising model organism in the study of various disorders of the central nervous system, including Alzheimer's disease - the main cause of dementia in the human population, which causes the progressive shrinkage of the brain and the death of its cells. Dementia results in loss of memory and thinking ability that interferes with daily functioning, and is universally fatal, usually within 10 years of onset. Thus, the development of new animal models to study the neurodegenerative mechanisms underlying Alzheimer's disease becomes an increasingly pressing issue.

The aim of this study was to evaluate the effects of *Equisetum pratense* extract on a model of Alzheimer's disease, the symptoms being induced by the administration of scopolamine - a substance that inhibits the central nervous system.

Our results indicated that scopolamine administration in zebrafish induced short-term memory defects, impaired motor activity and increased stress levels, specific signs of Alzheimer's disease.

The groups of fish that were treated with *Equisetum pratense* showed improvements in cognitive parameters depending on the amount of extract administered, so that the group treated with the highest concentration of extract (150 μl/L) recorded results close to those detected in the group control.

*Equisetum pratense* extract considerably improved and combated memory deficits, increased stress level and malfunctions in the locomotor system, in close relation to the dose of administered extract.





## **REVISITING THE CELLULAR PHYSIOLOGY OF THE BRAIN: OXIDATIVE STRESS, NEUROGLOBIN AND HIF1A DYNAMICS IN REPEATED RESTRAINT STRESS CONDITIONS**

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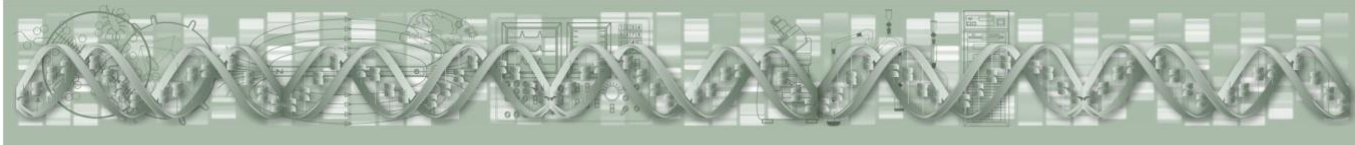
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Exposure of the body to stress has different effects on it, depending on the period of stress it was subjected to. While exposure for a short period of time can give the body certain benefits, chronic stress has mostly harmful effects. To prove this fact, Wistar male rats were subjected to repeated restraint stress for 3 and 7 days, 3 hrs/day. These procedures were followed by a series of biochemical, histochemical, and immunohistochemical analyzes to express the hypoxic background produced after the stress exposure. Therefore, the applied techniques and biochemical analyzes for brain tissue or brain lysates (cortical-thalamic area) are as follows: superoxide dismutase, catalase, TBARS, free leucine-like levels, total proteins, glucose, expression of the neuroglobin, and HIF1 $\alpha$  as well as glial cells markers GFAP and CNP. As the data shows, the hypoxic background after 7 days of repeated stress exposure was a more severe one, due to the inability of the system to face oxidative stress. These data showed a stress balance reaction of the brain via oxygen sensing reactions and proliferating glial cells, in a time-dependent manner.



## THE EFFECT OF BROOMRAPE (*O. CUMANA*) INFECTION ON THE YIELDS OF SUNFLOWER HYBRIDS

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The most common pathogen of the sunflower in the Republic of Moldova is the broomrape (*Orobanche cumana* Wallr.). The incidence of this pathogen varies yearly depending on environmental conditions, but it is constantly detected in the central and southern part of the country. Thus, our previous research indicates that approximately 60% of sunflower seeded areas in the southern region and approximately 47% in the central part of the country are affected by *O. cumana*. The effect of the infection on sunflower productivity varies greatly, from insignificant yield reductions to losses of up to 90%, depending on the intensity of the attack.

The data were collected, during the years 2015-2020, from the fields of the Testing Stations of the State Commission for Plant Variety Testing, located in various pedoclimatic areas of the Republic of Moldova, as follows: North (Visoca, Pelinia); Center (Băcioi); South (Grigorievca, Svetlîi).

The effect of *O. cumana* on the seed yields of sunflower hybrids, depends on hybrid tolerance to the weed and environmental conditions, The incidence of infection can vary for the same susceptible genotype from 37 to 1047 broomrape shoots per 100 host plants. The highest incidence of *O. cumana* was found in the localities from the south region, Grigorievca and Svetlîi with maximum values in 2016 and 2017.

To estimate the effect of broomrape on sunflower seed yield, we compared the yield of six sensitive, most strongly affected hybrids, with the harvest of the same genotypes grown under similar climatic conditions, but in the absence of infection or insignificant degree of infection. Thus we can see that the strong affected hybrids with broomrape can cause a decrease in seed yield of up to 52%. The rate of decline is not necessarily correlated with the number of broomrape shoots per 100 host plants.



## DIFFERENTIAL GENE EXPRESSION BETWEEN SUNFLOWER HYBRID AND ITS PARENTS IN MICROSPOROGENESIS

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Most heterosis research has been focused on growth vigor or increased yield and there is little research on heterosis and stress tolerance. The yield traits of plants depend of the male reproductive development including microsporogenesis and microgametogenesis, that is extremely sensitive to adverse climatic environments and abiotic stresses typically resulting in male sterility. The transcriptomic studies often suggest a complex genetic architecture underlying heterosis, being documented non-additive gene expression patterns in hybrids. Thus, transcripts analysis by RT-qPCR was employed to identify the differences in expression profiles of some meiosis and microsporogenesis related genes in sunflower F1 hybrid relative to its parents, thus underlying heterotic responses. The florets from inflorescence buds (R1-R3) were subjected to microscopical, physiological and molecular analyses. The model of additive/nonadditive genes expression was used to compare transcripts profiles between F1 hybrid and its parents. All studied sunflower transcripts (associated with mitochondrial stability, DNA repair and recombination) are differentially expressed. Also, the transcriptomic data showed more non-additive gene expression patterns in hybrids than additive revealing, often, microsporogenesis stage specificity. It is suggested that the genes with expression changes in F1 hybrids (non-additively) can be associated with heterosis and those involved in specific signaling pathways could be targeted regulated.

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## CURRENT STATUS OF THE OPENFLEXURE@BIOIASI PROJECT

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The OpenFlexure project (<https://openflexure.org>) aims to provide an open-source, 3D-printed microscope with applications in both medical and teaching environment. OpenFlexure@BioIASI is a student-based project that kicked off in 2021 with the goal of building and evaluating the real costs of a 3D printed OpenFlexure microscope and its applications in the practicals taking place at the Department of Biology, Alexandru Ioan Cuza University of Iași. All parts required were sourced from local and overseas distributors (Aliexpress and alike) at a total cost of about 200 euros. All objectives were sourced from old or not working microscopes. All printed parts were fabricated from PLA using an Creality Ender-5 printer at a total cost of 5 euro. Following the instructions available, a fully motorized, single objective and high-resolution microscope was built which uses a Raspberry Pi camera to acquire images and a Raspberry Pi single board computer running the specialized Raspbian OpenFlexure Lite OS to control the motors. The microscope was configured to generate its own Wi-Fi network and hence a simple laptop with a browser is all that is required to control it. Its optical performance was so far tested in different settings and it was shown that it provides images that are comparable with other microscopes such as: Leica DM 1000 LED, Lacerta BIM 151 T-LED, Optika B-157R-PL, Optika B-350 PL and Olympus BX41. Some drawbacks were nevertheless recorded, such as a low power light supply, poor condenser lenses and low overall robustness of the build. In a controlled environment, the 3D printed microscope could replace professional microscopes and provide good-enough images.



## GENETIC DIVERSITY AND RELATIONSHIPS IN *OROBANCHE CUMANA* GERMPLASM COLLECTION FROM THE BLACK SEA BASIN DETECTED BY ISSR MARKERS

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In the present study, the different origin broomrape populations from the Black Sea basin were selected in order to acquire a deeper insight into their population structure and genetic variability at the species level. Since it is in those sunflower producing countries has been observed the emergence of more aggressive populations (races) in the past 20 years that can rapidly spread to new areas, many breeders and geneticists are interested in operational detection various interactive genetical processes within species for constant monitoring to broomrape resistance.

On the basis of the descriptive population genetic statistics (observed number of alleles  $N_a=2.000$ , effective number of alleles  $N_e=1.3745$ , Nei's gene diversity  $H=0.2343$ , Shannon's Information index  $I=0.3707$ , total gene diversity  $H_t=0.2423$ , gene diversity within populations  $H_s=0.175$ , coefficient of gene differentiation among populations  $G_{st}=0.2779$ , gene flow among populations  $N_m=1.2991$ ) was revealed a significant level of the intrapopulation diversity at species level. These data were also corroborated by AMOVA analysis, demonstrating the higher genetic differences within populations (38%), lower level (34%) among the populations and the lowest level among countries (28%).

As a whole, all findings of this study showed that there is high intrapopulation diversity level of broomrape. Basing on these results, it is possible to conclude the existence of one main gene pool of *O. cumana* in the Black Sea Basin, which is supported by regular gene flow. However, it must be noted method of clustering and PCA revealed that Moldavian, Bulgarian and Romanian broomrapes share more genetical traits each other than with Turkish populations within one main gene pool. Evaluation of genetic diversity by molecular markers is important tool in germplasm characterization of *O. cumana* and the information obtained from this study may be highly relevant to contribute for the development of sustainable control strategies of pathogen and breeding programs of sunflower resistance to broomrape.



## THE EVOLUTION OF ANTIDEPRESSIVE THERAPY UNDER THE ACTION OF THE PSD-95 PROTEIN

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PSD-95 is a key scaffolding protein that is localized to the postsynaptic density of excitatory synapses where it regulates a wide variety of receptors, channels, and signaling molecules. PSD-95 specifically accumulates at the excitatory postsynaptic membranes and mediates cell surface ion channel clustering, such as AMPARs/TARPs. Cortical spreading depression is associated with the activation of NMDA receptors, which interact with the postsynaptic density protein 95 (PSD-95) that binds to nitric oxide synthase. Escitalopram is a type of antidepressant known as a selective serotonin reuptake inhibitor (SSRI). It is often used to treat depression and is sometimes used for anxiety, obsessive-compulsive disorder (OCD), or panic attacks. Our study was organized in 4 cohorts (Control, Depression, Escitalopram 5mg/kg, Escitalopram 10 mg/kg) Immunohistochemical detection of PSD-95 was performed with PSD-95 monoclonal antibody, HRP-biotinylated secondary antibody, and DAB final reaction on 5  $\mu$ m thickness coronal brain sections. Interestingly, escitalopram has induced high expression of PSD-95 in thalamic neurons in a dose-dependent manner. In conclusion, we saw a connection between the PSD-95, escitalopram, and thalamus, which we would like to research deeply and see what other effects might appear.



## THE HORTEGA-CAJAL METHODS AND IMMUNOHISTOCHEMISTRY FOR STUDY OF THE NEUROGLIAL CLUSTER-UNITS IN THE CENTRAL NERVOUS SYSTEM OF THE CORVIDS

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Nowadays, comparative neurobiology is in a shadow cone as compared to other aspects of the neurosciences. Studies with experimental models or representative organisms are exposed to erroneous generalizations as the diversity of the class of organisms increases. Thus, the structure of the pigeon's brain is different from the brain of a crow or the brain of a zebra finch, even though they are all birds. The whole brain of *Corvus frugilegus* was analyzed with a wide variety of histological methods such as Golgi, Golgi-Cox, Hortega, Nissl, H&E, Kluver-Barera, Bielschowsky, and Jones with tissue fixed in neutral formalin, Bouin's liquid, potassium dichromate, silver nitrate, dichromate-potassium chromate-HgCl. Positive immunohistochemical detection was observed for NeuN, CNP, DCX, and PCNA, while other markers were not clearly detected. The ultrastructural examination was performed using transmission electron microscopy. Confocal microscopy was performed with a Nikon AX1 microscope. Data gathered to date suggest that neurons and gliocytes are organized into structural and perhaps functional clusters with a central young motile neuron and 5–12 peripheral glial cells, perhaps some type of oligodendrocyte. The study is still ongoing and more studies will be conducted on the phenotype of neuroglial clusters and their function in the brain of *C. frugilegus*.



## CLONAL MICROPROPAGATION OF *LYTHRUM SALICARIA* L.

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The cultivation of *Lythrum salicaria* L. through unconventional techniques offers many opportunities to improve, perpetuate and conserve an important plant, not only for pharmaceutical value, but also used to detect pollution by heavy metals. The major phytochemicals of *Lythrum salicaria* L. include tannins, flavones and anthocyanins with a wide range of pharmacological activities. The regenerative potential of meristematic explants from *Lythrum salicaria* L. was evaluated for the establishment of a clonal propagation protocol. Plant regeneration has been achieved from meristem cultures, using shoot tips, taken from seedlings germinated in aseptic conditions. The procedure involved shoot tip cultures, followed by shoot multiplication, rooting and finally establishment of plantlets in soil. The cultures were established on 6 variants of MS medium, using two types of cytokinins: benzylaminopurine (1 mg/l, 0,5 mg/l, 0,1 mg/l) and kinetine (1 mg/l, 0,5 mg/l, 0,1 mg/l). The samples were kept in growth chamber at 24°C, under a 16 h light and 8 h dark photoperiod. The evaluation of explant reactivity was the number of developed shoots encountered on initial explant. Benzylaminopurine, in a concentration of 1 mg/l was optimum for shoots multiplication. The regeneration of whole plants was obtained in two steps: the shoots were excised and transferred to fresh medium and then rooting of these shoots was achieved on the same medium without growth regulators. The whole plants "reconstruction" protocol lasted for 10 weeks. For acclimatization „ex vitro” plants showed vigorous root system were transferred to pots with sterile soil.





## **A GOLGI-COX STUDY ABOUT THE MORPHOLOGICAL REACTION OF THE NEURONS AFTER HEMORRHAGE-INDUCED BRAIN HYPOXIA**

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The Golgi-Cox method implies labeling neurons of interest so that the finished product provides sparsely distributed neurons for easy access to a clear view of the complete dendritic arbors of every individual neuron. The protocol requires the study materials to be strained in a free-floating whole brain, or sections of it in a solution composed of potassium chromate and potassium dichromate in the presence of mercury chloride. The experimental design has involved 3 cohorts, Control, Hemorrhage, and Hemorrhage + sheep hemoglobin-based blood substitute (ShHbBS). After 12 hrs, the whole rat's brains were analyzed after processing by the Golgi-Cox method. Dendrites distribution area, neuronal body morphology, and the number of dendritic spines were evaluated by Image J software. The efficiency of the sheep hemoglobin and the effect of hemorrhage were compared to Control samples. Hypoxia has induced canonic changes in neuronal morphology whereas ShHbBS administration has maintained the neuronal hypoxia reaction near the Control aspect. In conclusion, this study is aiming to show the morphological and functional issues that hemorrhage-induced hypoxia, as well as an artificial O<sub>2</sub> carrier such as ShHbBS, brings to the morphology of brain tissue.



## SECTION: *GENERAL 2*

### BIOACTIVITY OF OAK (*QUERCUS* SP.) BARK EXTRACTS

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Recent studies highlight the great potential of the plants from the *Quercus* genus to comprise high contents of bioactive compounds with beneficial effects on human health. The aim of our work is to screen the potential biological effects of the bark of two oak species, namely *Quercus petraea* and *Q. pubescens*. The vegetal material was extracted using two different solvents (water and ethanol solution) and extraction methods, namely ultrasonic assisted extraction (UAE) and microwave assisted extraction (MAE). The total polyphenolic and tannin contents were quantified using the Folin-Ciocalteu method. The antioxidant capacity of the extracts was tested using two complementary methods based on the 1,1-diphenyl-2-picrylhydrazyl – DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid – ABTS reagents. The antimicrobial activity was evaluated on five different bacterial strains (*Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and three fungi from the *Candida* genus (*C. albicans*, *C. parapsilosis*, *C. krusei*). Moreover, three enzymes were tested to evaluate the potential inhibitory effects of the extracts ( $\alpha$ -glucosidase, tyrosinase and acetylcholinesterase). Higher total polyphenols and tannin yields were observed in the extracts obtained by MAE, compared to the UAE bark extracts. Also, the ethanolic solution was the better solvent for the extraction of bioactive compounds. Antioxidant activities were highlighted for all the tested extracts. A better antibacterial activity was observed against the Gram positive bacterial strains and *Klebsiella pneumoniae*, extracts from the bark of *Quercus petraea* being more efficient. The antifungal activity was highlighted against *C. krusei*. The tested extracts inhibited the activity of  $\alpha$ -glucosidase, tyrosinase and acetylcholinesterase. These results highlight biological effects of the bioactive compounds found in the oak bark, that may be used in future pharmaceutical formulations, due to their beneficial effects on human health.

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## THE ANTIMICROBIAL ACTIVITY OF P3.3S STRAIN AGAINST *AGROBACTERIUM TUMEFACIENS* AND *RHIZOCTONIA SOLANI*

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Microbial diseases cause considerable losses in fruit and vegetable production both during the cultivation of plants and their manipulation, transport and storage. In order to sustain crops yield, farmers use synthetic pesticides that have a negative effect on the environment and human health. For this reason, antimicrobial research is mainly focused on the development of efficient and eco-friendly technologies involving the elimination or reduction of synthetic pesticides in agriculture. One solution is represented by the use of soil bacteria that produce antimicrobial substances to inhibit the growth of phytopathogenic microorganisms. In this context, the main goal of this study was to evaluate the antimicrobial activity of P3.3S strain in order to be used as a biocontrol agent. Thus, the antimicrobial activity of P3.3S strain was assessed using agar diffusion method. Two bacterial strains - *Agrobacterium tumefaciens* C58, *Agrobacterium tumefaciens* GV220 and also *Rhizoctonia solani* were used as test phytopathogenic microorganisms. The results indicate that P3.3S strain inhibits the growth of tested phytopathogens, with a higher inhibitory activity against strain GV220 compared with C58. The largest zone of inhibition was recorded at 72 hours. Strain P3.3S showed better inhibitory activity than kanamycin (100 µg/ml) which was used as positive control. Regarding antifungal activity, the culture of P3.3S showed the best inhibition of mycelial growth, followed by the supernatant and filtrate. The inhibitory activity of the filtrate may indicate that the strain P3.3S produces antimicrobial substances that are released in the medium. In conclusion, strain P3.3S has potential to be used as a biocontrol agent against the tested phytopathogens, but further studies are needed.



## THE EFFECT OF USING DDGS AS AN INGREDIENT IN THE PRODUCTION OF LOW-COST FEED FOR COMMON CARP (*CYPRINUS CARPIO* LINNEUS)

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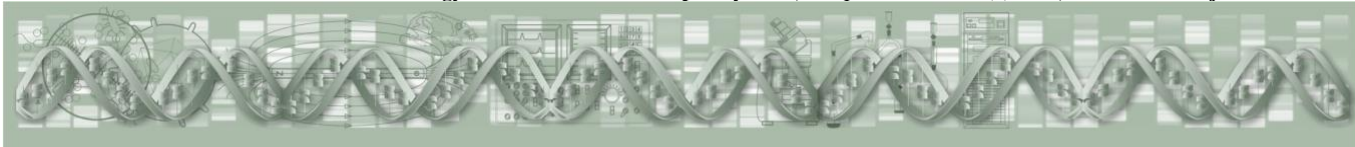
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The aim of this study was to evaluate the effect of distillers dried grains with solubles (DDGS), a co-product of the ethanol production industry, on growth and some physiological parameters in common carp. The experiment was carried out on fish with an initial weight of 86 g while the duration was seven weeks. The experimental diets contained DDGS 0% (D0), 25% (D1), and 35% (D2). Through FT-NIR analysis, it was established that DDGS had a protein content of 27.56% and an oil content of 6.75%. No differences were recorded in terms of growth, flesh quality and blood biochemical parameters. The analysis of some parameters of the oxidative status (SOD, GSH, CAT, GPX, MDA, carbonylated proteins) revealed changes depending on the content of DDGS. The analysis of the intestinal microbiota revealed a proliferative effect of DDGS. In conclusion, DDGS can be used in the carp diet, without producing negative changes on growth. At the same time, due to the reduced price compared to that of cereals, the use of DDGS in feed can contribute to reducing costs and ensuring the sustainability of production.



## THE EFFECTS OF SALINE STRESS INDUCED IN EXPERIMENTAL CULTIVATION CONDITIONS ON SOME VARIETIES OF BASIL (*OCIMUM BASILICUM* L.)

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Saline stress is an important factor that affects plant growth and limits the production of agricultural crops. The increased concentration of salts in saline soils produce nutrient imbalances and deficiencies. In order to identify plants capable of withstanding and developing under conditions of increased salinity, the present study aims at the adaptation and resistance of two varieties of basil to different degrees of salinity.

*Ocimum basilicum* L., a species of the genus *Ocimum*, belongs to the Lamiaceae family and is known as an aromatic, cultivated plant, originating in India, often used in traditional medicine, the food industry and in the cosmetic industry due to its rich content of volatile oils. The biological material used in the experiment was obtained from basil seeds, the variety "Genovese" and the variety "Aromat de Buzău" on which 3 concentrations of NaCl, 100, 150 and 200 mM, were applied, the saline solutions being administered in 3 repetitions. At the end of the cultivation interval, physiological and biochemical determinations were made to identify the degree of tolerance to salt stress of the two varieties.

Salinity produced biochemical and physiological effects in both basil varieties tested, showing a certain degree of tolerance to the stress induced by the application of NaCl solutions in the cultivation substrate.



## DO DIFFERENT WAVELENGTHS OF LIGHT IMPROVE DUCKWEED (*LEMNA MINOR* L.) GROWTH AND BIOMASS PRODUCTION?

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*Lemna minor* L. (popular, duckweed) belongs to the category of aquatic macrophytes, having small dimensions. It forms extensive populations on the surface of stagnant waters (lakes, marshes), but it is also found on slow-flowing riverbeds. It is generally used for phytotoxicity testing and has a great capacity of removing various substances from the water (heavy metals, pesticides, colorants, etc.).

Light quantity and quality are essential factors that influence plant development and composition, therefore it can be used in a convenient manner to obtain vegetal material with composition tailored to specific needs. Chlorophyll pigments absorb mostly the red (663 nm and 642 nm) and blue (430 nm and 453 nm) light, and these wavelengths can influence plant development.

For the current experiment, the response of the duckweed to different light treatments was observed in order to determine if it can accelerate growth and biomass production.

Each light treatment was provided by 6 (2 × 3) 1 W LEDs (produced by OSRAM), soldered on aluminium radiators to allow heat dissipation. The 4 light treatments were 100% white (White) and various red (R) to blue (B) ratios, as follows: RB (1:1), R and B. The light was supplied at a rate of 120 μmol m<sup>-2</sup> s<sup>-1</sup> for 12 h/day for 14 days.

Chlorophyll fluorescence was measured using a FMS2 fluorometer (HansaTech, Norfolk, UK) for 15 plants/treatment. Morphometric analysis was performed using ImageJ software.

The results indicated variations in total frond area and biomass production in all variants.



**ASPECTS REGARDING THE CONTENT OF ASSIMILATORY PIGMENTS IN *ECHIUM VULGARE* L. SPECIES FROM NATURAL AND ANTHROPOIZED HABITATS (NE REGION OF ROMANIA)**

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The paper brings to attention data on the content of assimilatory pigments in the species *Echium vulgare* L. (Boraginaceae family), originating from 7 locations in the NE region of Romania (Valea lui David, Vlădeni, Pădurea Repedea-Bârnova, Releu - Iași county; Borca - Neamț county; Băiceni – Botoșani county; Suceava – Suceava county; Bacău – Bacău county), in the period 2021 - 2022.

The assimilatory pigments were extracted and dosed from leaves of plants in the phenophase of flowering and fruiting using acetone (80%) as a solvent according to the method described by Lichtenthaler and Wellburn (1983).

The analyzed parameters were chlorophyll a content, chlorophyll b content, carotenoid pigments content, chlorophyll a/b ratio, chlorophyll a+b / carotenoid pigments ratio.

For the flowering phenophase, the analysis of the content of assimilatory pigments for the two consecutive years revealed lower average values in the first year compared to the average values in the second year (with a few exceptions).

The comparative analysis of the data obtained for the flowering and fruiting phenophases, the year 2022 (Vlădeni and Suceava locations) revealed lower values for the content of chlorophyll b and carotenoid pigments in the fruiting phenophase compared to the flowering one.



## EXPRESSION OF TWO GENES INVOLVED IN THE SYNTHESIS OF VOLATILE OILS IN FOUR VARIETIES OF *LAVANDULA ANGUSTIFOLIA* MILL.

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The *Lavandula* species are economically important plants cultivated for their essential oils with many therapeutic properties resulting from the biological activity of certain oil constituents.

The study aims to characterize the 1-Deoxy-D-xylulose-5-phosphate synthase (DXS) which catalyzes the first step of the plastidial methylerythritol phosphate (MEP) pathway for the production of most essential oil constituents and 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGR), a key enzyme in the mevalonate (MVA) pathway in the cytoplasm by expression as genes involved in terpene biosynthesis, in four different lavender cultivars (‘Provence Blue’, ‘Sevtopolis’, ‘Vera’ and ‘Codreanca’) of the *Lavandula angustifolia* species by quantitative Real-Time PCR.

The experiment was carried out in a protected (unheated greenhouse) and unprotected (field) space. To achieve the objectives proposed in this experiment, four varieties of the species *Lavandula angustifolia* Mill. were used, and four experimental variants, respectively: watered with H<sub>2</sub>O (v1); watered with standard Hoagland nutrient solution (v2); watered with Hoagland nutrient solution containing a double amount of K (v3); watered with Hoagland nutrient solution containing a double amount of P (v4). The plant material was collected, in the flowering period, in June 2019.

Total RNA was isolated from leaf and flower tissue using SV Total RNA Isolation System kit (Promega). The transcriptional activity of DXS and HMGR was analyzed by absolute quantification, based on ”in house” standards and specific primers targeting a 120 – 300 bp fragment size. The quantitative RT-PCR was performed in a Rotor-Gene 6000 5 Plex HRM Real-Time PCR system (Corbett).

The results indicate that the HMGR gene was the foremost expressed gene, with high values in both flowers and leaves, followed, by the DXS gene, which showed higher values in flowers compared to leaves. Reporting the results obtained from the gene expression analysis of the reference gene ( $\beta$  - actin) revealed overexpression of both genes.





## INITIATION AND GROWTH OF THE AEADES AND DROSOPHILA CELL LINE CULTURES TO OBTAIN THE BIOMASS NECESSARY IN TRANSFECTION TECHNIQUE

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The aim of this study is obtaining of cellular mass from two insect species using the following cell lines: Schneider's Drosophila Line 2 (CRL-1963<sup>TM</sup>) and Aedes albopictus (CCL-126<sup>TM</sup>) purchased from ATCC (American Type Culture Collection), each of them having particularly growth condition.

(1) Schneider's Drosophila Line 2 has epithelial origin, is derived from fruit fly (*Drosophila melanogaster*) embryos, was cultivated in Schneider medium supplemented with 10% heat-inactivated fetal bovine serum and 1% antibiotic solution (penicillin, 100 IU/ml and streptomycin, 100 µg/ml). The culture plates were placed in the incubator without CO<sub>2</sub>, at 23°C, the media being renewed every 2-3 days. After numerous subcultivations, during 1-2 month, the obtained cell mass was placed in a mixture of heat-inactivated fetal bovine serum (90%) and DMSO (10%), distributed in 1.0 ml cryotubes (1x10<sup>6</sup> cells/vial) and stored in an ultra-low temperature freezer.

(2) Aedes albopictus cell line has epithelial origin from Asian tiger mosquito (*Aedes albopictus*) larvae. For cultivation, Eagle's Minimum Essential Medium (EMEM) containing Earle's Balanced Salt Solution (Earle's BSS), 0.1 mM non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate was used, supplemented with 20% heat-inactivated fetal bovine serum and 1% antibiotic solution (penicillin, 100 IU/ml and streptomycin, 100 µg/ml). The culture flasks were placed in an incubator at 28°C, in high humidity condition, 95% air and 5% CO<sub>2</sub>, the medium was renewed 1 to 2 times per week. After reaching 95% confluence the cells were detached using Trypsin-0.53mM EDTA solution using 1:3 subcultivation ratio.

Both cell lines are considered suitable for transfection techniques. For this purpose, the insect cells were placed in contact with crude Wolbachia extracts from *Diplolepis rosae* larvae and centrifugated together at 2500 g, for 60 min, at 15°C followed by cultivation using the specific conditions of the respective cell line.



## **WORKSHOP: TRICYCLIC DITHIOLIUM FLAVONOIDS - NEW WEAPONS TO COMBAT ANTIBIOTIC RESISTANCE**

### **ANTIBACTERIAL ACTIVITY OF SYNTHETIC BrCl-FLAVONOID**

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Antibiotic resistance is one of the biggest threats to global health, that occurs when microorganisms like bacteria or fungi develop the ability to defeat the drugs designed to kill them. Because the number of new efficient antibiotics decreased in the last years, several alternative solutions are being explored to fight antibiotic resistance. For this purpose, a potential solution would be the use of synthetic flavonoids, well known for their antimicrobial properties. In this context, we investigated the antimicrobial activity of a new tricyclic flavonoid with bromine and chlorine as halogen substituents (BrCl), against several multidrug resistant (MDR) bacterial strains. The antibacterial activity was investigated using minimum inhibitory and minimum bactericidal concentrations (MIC/MBC). The influence of BrCl-flavonoid on cell viability was also assessed. The mechanism of action was evaluated using cell membrane permeability tests as well as scanning electron microscopy (SEM). Checkerboard assay was used to study the effect of the tested compound in combination with different antibiotics. The BrCl-flavonoid showed a very good antimicrobial activity with MICs varying between 0.24-125  $\mu\text{g}/\text{mL}$ . The mechanism of action is related to impairment of cell membrane integrity. The checkerboard method revealed a synergistic effect caused by different combinations of BrCl-flavonoid ( $1/4$  and  $1/8 \times \text{MIC}$ ) and penicillin ( $1/4 \text{ MIC}$ ). A significant bactericidal activity was evidenced when the two agents were used in combination, with a total kill effect recorded after 24 h. Our results suggested that BrCl-flavonoid has a high potential to be used as an efficient antibacterial agent.



## ACTIVITY OF BRCL FLAVONOID ON THE BIOFILM FORMATION BY *CANDIDA KRUSEI*

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*Candida* pathogenic species represent a serious public health problem due to increasing resistance to antifungal agents. Resistance to traditional drugs such as fluconazole and amphotericin B is largely due to their ability to form biofilms. Biofilm cells are up to 1000 times more resistant to antifungal agents than planktonic cells. Identifying new effective molecules represents a priority in medical research. Therefore, the aim of this study was to evaluate the activity of a synthetic flavonoid (BrCl) on biofilm formation by *Candida* species. In this context, the minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), biomass of the formed biofilm, cell surface hydrophobicity in the presence of hydrocarbons and cell adhesion time were assessed. The effect of BrCl flavonoid on biofilm formation produced by *C. krusei* was also evaluated. Investigation showed that only *C. krusei* and *C. glabrata* strains produced the highest amount of biofilm and showed a high affinity for hydrocarbons. The optimal adhesion time of *C. krusei* cells was 8 hours. The MIC value determined for BrCl was 15.62  $\mu\text{g/ml}$  and MFC was 31.25  $\mu\text{g/ml}$ . In the presence of BrCl flavonoid subinhibitory concentration (equivalent to  $\frac{1}{2}$  MIC and  $\frac{1}{4}$  MIC), *C. krusei* biofilm formation was inhibited 77.2% and 66.49%. The results suggest that BrCl flavonoid has an important activity on *C. krusei* biofilm formation.



## THEME: EDUCATIONAL RESOURCES IN BIOLOGY

### **THE LEPIDOPTERA COLLECTION OF PROF. DR. HONORIS CAUSE VASILE COȚOFAN, A NEW CONTRIBUTION TO THE HERITAGE OF THE NATURAL HISTORY MUSEUM OF IAȘI**

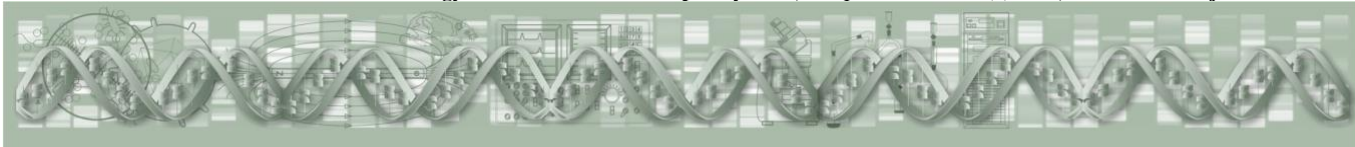
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In 2019, through a generous gesture, Mr. Alexandru Ioan Coțofan donated the Lepidoptera collection of his father, a university professor, to the Museum of Natural History in Iași.

Dr. Vasile Gheorghe Coțofan, was a veterinarian, professor of Comparative Anatomy at the Faculty of Veterinary Medicine of the "Ion Ionescu de la Brad" Agronomic University, Iași, honorary member of the Academy of Agricultural and Forestry Sciences and Doctor Honoris Causa of the universities of Timișoara, Cluj, Chisinau. The collection includes more than 1000 specimens of indigenous and exotic lepidoptera from the families Papilionidae, Nymphalidae, Saturniidae, Morphidae, Hesperidae, with scientific and aesthetic value. The donated material is being processed by the museum specialists and is kept in the new storage system purchased by the Museum.



## **THE ETHICS OF THE HUMAN-NATURE RELATIONSHIP – A THEME APPLIED IN MUSEUM EDUCATION AT THE OLTENIA MUSEUM CRAIOVA**

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The Department of Natural Sciences of the Oltenia Museum Craiova has a rich museum heritage, but also modern museum technologies that provide pupils and students with an informal and adequate framework for the development of knowledge and learning through interactivity.

Among the services and cultural-educational products of the Oltenia Museum Craiova, we mention the project "Museum - a School Otherwise", started in 2013, with the aim of serving the national program School Otherwise, the motto of this program being: "To know more, to be better". Therefore, the activities included in our museum education project (conferences, photo exhibitions, thematic workshops, etc.) aimed and continue to aim at contributing to the development of persons/public in all aspects (personal, social and professional).

The ethics of the human-nature relationship was one of the themes frequently addressed in the activities of the "Museum - a School Otherwise" project, either as an independent theme or as a "background" theme, a response to the problems generated by the current, technologized and excessively consuming of resources world.

The public conferences and a series of interdisciplinary/trans-disciplinary didactic activities carried out within the project represented, above all, the means of communication and transfer of ecological ethics norms and ideas from the academic sphere to the media sphere. As an example, we mention the events organized on the occasion of the World Environment Day (June 5): 1. The public conference "Ethics of the human-nature relationship" with two topics of interest to naturalists: "Ecological ethics - a new moral system?" and "Biodiversity Management: case study Romania – Canada – India" (2016); 2. The didactic activity "Experience the beauties of nature and you will learn something about yourself!" including presentations of good practices, respectively "10 golden rules for protecting the natural environment" and an ecological didactic workshop with the theme "Live Eco! Live Healthy!" (2022).