STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Composition of the stonefly (Plecoptera) fauna of the Balkans and the Carpathians

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The stoneflies are well known as a neopteran insect order adopted to cold running waters. Due to their strict habitat demands and ancient origin, often used as a model group in zoogeography, both in wide and narrow scales. In the West Palaearctic, peaks of their diversity connected to Pleistocene refugee areas. In regards of this phenomenon, the composition and diversity of the Carpathians and the Balkans are rather different: the Carpathians have 134 described species with only 19 endemic or subendemic taxa, while the Balkans have 204 described species with nearly half of them are endemics. The Carpathian endemics are rarely narrow endemics, while many Balkan taxa are restricted to small areas, and their distribution outlines Illyrian, Moesian and Attikan centres. The Balkan-Carpathian species are mostly restricted to the Moesian ranges in the Balkans while widespread in the Carpathians. Alpine-Carpathian species are mostly restricted to the North Carpathians, while Alpine-Balkanian species are restricted to the Dinarids. The few Balkano-Anatolian species are restricted to the Aegean Isles or having a disjunct Balkanian area. Widespread (European or Central-South European) species are common in the whole Carpathians and most of the Balkans, but generally lacking from the southern extremes and from the isles. Hotspots of endemicity and/or diversity considered are the Oaş-Ţibleş range in the Eastern, and the high ranges of the Southern Carpathians, the central Balkanian high ranges (Prokletije, Korab, Ríla, Pirin, Lakmos), Balkanian hills with special climate (Epirus, SE Macedonia, Strandzha) and the Aegean Isles.

Keywords: distribution, endemic taxa, hotspots, Pleistocene.

In vivo assessment of a doxorubicin chemoresistance profile in melanoma

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Recent international guidelines recommend the development of technologies that contribute to the reduction, refinement, and replacement of animals for experiments (3Rs) [1]. Thus, the aim of our project was to develop and validate *in vitro* 3D biomimetic platforms composed of multicellular spheroids to replicate melanoma microenvironment chemoresistance. Doxorubicin (DOX), a well-known anthracycline antibiotic commonly used in cancer chemotherapy, to which melanoma cells are known to develop chemoresistance, was used to obtain our model's chemoresistance profile. DOX encapsulated in extracellular vesicles purified from B16.F10 melanoma cells, grown under metabolic stress (PEG-EV-DOX) was used to restore chemosensitivity based of the superior targeting capacity of this nanoformulation [2]. We investigated the molecular mechanisms of DOX chemoresistance profile by qPCR, protein array, WB, HPLC, spectrophotometry and ELISA. Our main findings suggest a DOX chemoresistance pro-angiogenic profile sustained by strong upregulation of IGF-II, IL-1α, IL-9, MCP1, TPO, TIMP1 and PF4 proteins. In addition, resistance to apoptosis was mediated by upregulation of anti-apoptotic factor FasL, while PEG-EV-DOX treatment restored the pro-apoptotic status via upregulation of Bax and Casp3 pro-apoptotic factors. While subtherapeutic dose of free DOX induced no significant changes in redox status, DOX induced gene expression upregulation of tumor cell survival factors HIF-1α and Akt, and an increase in invasion marker MMP-2 on both gene and protein levels. Regarding epigenetic changes, global methylation increased in DOX chemoresistant group compared to Control and PEG-EV-DOX-treated groups. Our data suggested that several features of chemoresistance were met by our model, regarding angiogenesis, apoptosis, survival, and epigenetic regulation. Further mechanistic studies and RNAseq are needed to elucidate more aspects of the model, as well as *in vivo* studies on mice inoculated with resistant tumor cells, required to compare the outcome with data from the present study.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Unlocking the past: Exploring the biocultural landscape of a pre-modern necropolis in Southeastern Romania

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Human skeletal remains from archaeological sites offer valuable insights into various aspects of ancient life, death, and social dynamics. Advances in ancient biomolecule analysis have enhanced our understanding of the past, yet new discoveries continually enrich the narrative as more unique osteological collections are studied. This study seeks to reconstruct the biological profile of a historical population discovered within a sprawling, multi-phase archaeological site in southeastern Romania (Mireasa necropolis, T38) by integrating multiple lines of evidence. A key aspect of this investigation involves the reconstruction of complete mitogenomes from human archaeological remains, employing Next-Generation Sequencing (NGS) technology, which has emerged as the gold standard in ancient DNA (aDNA) research. The preliminary results regarding maternal line genetic signatures of historical individuals from Mireasa indicated the presence of a mixture of mitochondrial haplogroups of West Eurasian origin and variants less closely associated with European regions. This observation may possibly reflect the demographic shifts within the historical province of Dobruja during the 15th-16th centuries AD.

Keywords: pre-modern necropolis, ancient mitogenome, NGS, radiocarbon dating

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The transcriptional landscape of cancer stem-like cell functionality in breast cancer

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Background: Cancer stem-like cells (CSCs) have been extensively researched as the primary drivers of therapy resistance and tumor relapse in patients with breast cancer. However, due to lack of specific molecular markers, increased phenotypic plasticity and no clear clinicopathological features, the assessment of CSCs presence and functionality in solid tumors is challenging. While several potential markers, such as CD24/CD44, have been proposed, the extent to which they truly represent the stem cell potential of tumors or merely provide static snapshots is still a subject of controversy. Recent studies have highlighted the crucial role of the tumor microenvironment (TME) in influencing the CSC phenotype in breast cancer cells. The interplay between the tumor and TME induces significant changes in the cancer cell phenotype, leading to the acquisition of CSC characteristics, therapeutic resistance, and metastatic spread. Simultaneously, CSCs actively shape their microenvironment by evading immune surveillance and attracting stromal cells that support tumor progression. Methods: In this study, we associated in vitro mammosphere formation assays with bulk tumor microarray profiling and deconvolution algorithms to map CSC functionality and the microenvironmental landscape in a large cohort of 125 breast tumors. Results: We found that the TME score was a significant factor associated with CSC functionality. CSC-rich tumors were characterized by an immune-suppressed TME, while tumors devoid of CSC potential exhibited high immune infiltration and activation of pathways involved in the immune response. Gene expression analysis revealed IFNG, CXCR5, CD40LG, TBX21 and IL2RG to be associated with the CSC phenotype and also displayed prognostic value for patients with breast cancer. Conclusion: These results suggest that the characterization of CSCs content and functionality in tumors can be used as an attractive strategy to fine-tune treatments and guide clinical decisions to improve patients therapy response.

Keywords: breast cancer, cancer stem cells, tumor microenvironment

Mycoremediation of mercury and metal resistance strategies of a micromycete

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Metal contaminants threaten environmental stability and human health, while their remediation is a challenging task. Mycoremediation, which employs fungi to mitigate metal toxicity, holds promise due to fungi's characteristics like significant biomass production, branched growth facilitating soil matrix penetration, and high surface area with cation binding properties. However, the progress of mycoremediation technologies for metal contaminants is hindered by a limited understanding of the underlying mechanisms. Terricolous micromycetes isolated from a mercury (Hg) contaminated environment, adapted to extreme metal toxicity, showed significant Hg removal efficacies (47-97%) and biosorption capacities (33.8-54.9 mg Hg g^{-1} dry weight) during 48 h exposure to 100 mg L^{-1} Hg^{2+} in aqueous solution. The highly resistant *Fusarium oxysporum* P2.7 isolate (GenBank ID: MT913528) was studied for its resistance strategies against toxic concentrations (mg L^{-1}) of Cu (250), Hg (10 and 100), and Zn (150) in liquid culture medium. Avoidance strategies against Cu and Zn stress were indicated by important proportions of these elements associated with extracellular fractions. Resistance strategies against Hg were, 14.55-47.57% extracellular immobilization, 30.4-48.93% intracellular accumulation, and 5.29-36.51% possible biovolatilization, depending on culture conditions. Removal efficacies were 6.84% for Cu, 90.67-93.73% and 81.53-83.09% for initial 10 and 100 mg L^{-1} Hg, respectively, and 4.67% for Zn. Biosorption capacities were (mg metal g^{-1} dry weight) 13.2 for Cu, 0.29-2.51 and 4.75-9.66 for 10 and 100 mg L^{-1} Hg, respectively, and 2.87 for Zn. A putative mercuric reductase protein sequence, from the genome of *F. oxysporum*, is likely the homologue for the well-described bacterial Hg^{2+}-reducing enzyme. Bioinformatic analyses suggested a possible early common origin of this enzyme for Cyanobacteria and Fungi phyla, and a distinct mechanism of Hg^{2+} reduction compared to bacteria. Research of metal-resistant micromycetes holds implications for the development of remediation applications and contributes to understanding their role in metal speciation in the environment.

Keywords: biosorption, bioaccumulation, biovolatilization, *Fusarium oxysporum*, metals

Acknowledgements. This work was supported by a Short Term Scientific Mission (STSM) grant through Cost Action 19116 – Trace metal metabolism in plants (PLANTMETALS) and the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2019-5254, within PNCDI III, contract no. 390PED/2020.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Insights into Plant Colonization in Mercury-Contaminated Sites: Trace Metals and Rhizosphere Microbiome Interactions

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Mercury soil pollution is a great threat to ecosystem and human health due to its acute toxicity and biomagnification processes. At the site of a decommissioned chlor-alkali plant where Hg cathodes were used for more than 30 years of activity, we conducted a trace metals site assessment, a floristic survey of the pioneer native flora and determined the taxonomical and functional diversity of the microbial communities in the rhizosphere of the dominant plant species. The aim of the study was to explore the plant metal accumulation potential of local populations that would be of use for an *in situ* remediation strategy of the site. Presently, the median rhizosphere Hg concentration at the site was 962 mg kg^{-1}, and the highest concentrations of Hg accumulated in plant tissues were 10869 mg Hg kg^{-1} dry weight in roots and 1070 mg kg^{-1} in shoots, by the legume plant *Lotus tenuis*. *Diplotaxis muralis* was the most abundant plant at the site and the associated microbial communities from its rhizosphere did not exhibit an alpha diversity decrease with the soil Hg concentration gradient. The most abundant bacterial phyla were Pseudomonadata, Actinomycetota, and Acidobacteriota. Moreover, most rhizosphere communities tested positive for the presence of *merA* in the total soil DNA. For these latter communities, an inferration-based PICRUSt2 analysis revealed an abundance of ABC transporters. All in all, the five rhizosphere soil trace metals investigated in this study explained 35% of the total variance observed at the site for plant population distribution and plant metal accumulation profile, and Hg was the main community driver. Based on the Hg accumulation pattern, *L. tenuis* and *D. muralis* are Hg indicator species of potential interest for future soil Hg phytomanagement approaches due to their abilities of immobilizing Hg at the root level.

Keywords: tolerance, phytoremediation, *Diplotaxis muralis*, *Lotus tenuis*, *mercuric reductase* (MerA)

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An *in vivo* pilot study on the effects of a combined immunotherapy on murine melanoma

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Over the past two decades, immunotherapies have gained a major interest as treatments for many types of cancers, showing promising results. One type of cancer that has been shown to be responsive to such treatments is melanoma, one of the most aggressive skin cancers. Melanoma is known to be highly immunogenic due to the elevated tumor mutational burden, hence the effectiveness of immunotherapies based on immune checkpoint inhibitors, targeting specific proteins present in the tumor microenvironment. Nonetheless, the heterogeneity found in patients still causes diminished results, proving that further optimization of therapies is needed. To address this, combinations of various treatments are tested, while considering the desired immunomodulatory effect to improve results. This study aimed to test a combined immunotherapy based on anti-PD-L1 antibodies and curcumin-loaded extracellular vesicles, derived from stressed and peptide-pulsed dendritic cells. This immunotherapy strategy was based on past published results of the anti-PD-L1-based therapy, the immunomodulatory effect of curcumin, and the specific targeting of the tumor microenvironment with extracellular vesicles.

To test this combined immunotherapy, a pilot *in vivo* study was conducted on B16.F10 tumor-bearing C57BL/6 mice. Throughout the investigation, tumors were measured and the mice were weighed. After the treatment administration, tumors were collected, measured, and analyzed through a set of molecular methods such as western blotting, protein array, and gelatin zymography.

Our results show an overall reduction of the expression of cytokines involved in angiogenesis in the combined therapy group, compared to the anti-PD-L1 treatment group. Proteins involved in the inflammatory pathways or immune regulation such as pNF-κB and iNOS also showed different expression patterns in the two treatment cohorts.

This combined immunotherapy presents potential that can be further improved through adjusting administration time points and dosages.

Keywords: melanoma, immunotherapy, inflammation

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Advancing the identification of novel direct gene targets of NODULE INCEPTION, a critical transcriptional regulator in legume-rhizobia symbiosis

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Plants form beneficial associations with microorganisms to enhance nutrient uptake essential for their growth. Legumes, particularly in nitrogen-depleted soils, establish symbiotic relationships with rhizobia, resulting in new root organs, called nodules. At this level, atmospheric nitrogen is converted by endosymbionts into ammonia, the latter being exchanged for plant-derived photosynthates. Nodule development involves intricate transcriptional reprogramming, orchestrated by major plant transcription factors, with one example being NIN (NODULE INCEPTION). While NIN Binding Site (NBS) *cis*-promoter motifs have been identified on NIN target promoters, further exploration is needed to understand their conservation among other potential NIN targets. Within this framework, the aim of the present study was to determine if two rhizobia-induced genes of *Medicago truncatula*, namely *MtSOKL* and *MtAnn1,* whose expression is regulated and dependent on NIN, could represent new direct targets of NIN. To this end, we combined *in silico cis*-motif search and transcription assays in *Nicotiana benthamiana* with mutated promoter *cis*-motifs, to identify and functionally validate motifs that would mediate NIN regulatory activity. Consequently, *in silico* analyses revealed several putative NIN binding sites (NBS) on promoters of the two genes (8 in the case of *MtAnn1*, and 6 for *MtSOKL*), consistent with the hypothesis that they may represent direct NIN targets. Furthermore, in the case of *pMtSOKL*, we identified a promising NBS, called NBS3, which when mutated, strongly affects the NIN activation of *pMtSOKL* transcription. This suggests that NBS3 represents a key *cis*-motif for NIN DNA transcriptional regulatory activity. Upcoming studies employing promoter deletion, gain-of-function assays, and DNA binding examination are warranted to consolidate the role of NBS3 in NIN-mediated transcriptional regulation.

Keywords: *MtAnn1, MtSOKL*, *Medicago truncatula*, NIN-binding site, NIN transcription factor, nodule development.

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Challenges in molecular barcoding analyses – case study of *Austropotamobius bihariensis*

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Austropotamobius bihariensis is a crayfish species discovered in Romania's Apuseni Mountains. Its origin is debated due to its phylogenetic link to *Austropotamobius torrentium* from Croatia's Dinaric Mountains. To hypothesize about *A. bihariensis's* origin, we focused on its ecological spread tendencies, whether cosmopolitan or endemic. This study aimed to create a time-calibrated phylogenetic tree for the three *Austropotamobius* species in Europe (*A. bihariensis, A. torrentium, A. pallipes*). By examining genetic variations of these species, we aimed to uncover evolutionary patterns and understand the factors behind their divergent distribution and ecological traits. The phylogenetic tree was built using mitochondrial cytochrome c oxidase subunit I (MT-CO1) gene sequences, obtained through sequencing for *A. bihariensis* and from GenBank for *A. torrentium* and *A. pallipes*. The tree was time-calibrated based on the divergence between the haplogroup from the Zeleni Vir Mountains, Croatia and *A. bihariensis*, an event from 15 million years ago. This analysis showed a reduced, spatially concentrated diversity for *A. bihariensis* and the Dinaric haplogroups, indicating endemism. Conversely, haplogroups from Central and South Europe and the Southern Balkans, as well as three haplogroups from *A. pallipes*, showed high haplotype diversity over a short period, suggesting cosmopolitan tendencies. In conclusion, this study supports the endemism of *A. bihariensis* and, on a larger scale, offers valuable insights for the conservation and management of *Austropotamobius* species.

Keywords: molecular phylogenetics, population genetics, endemictendencies, cosmopolitan tendencies

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Drug resistance in bacterial and fungal diversity in smokers and non-smokers

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The oral microbiome plays a crucial role in maintaining oral health, while oral dysbiosis is associated with numerous systemic diseases. Cigarette smoking is a notorious risk factor for a multitude of oral conditions like periodontal disease, tooth decay, and oral cancers, and exerts a significant influence on the oral microbiome. A profound understanding of the repercussions of smoking on the resident communities of oral bacteria and fungi is significant for elucidating the connection between smoking-induced oral conditions and their systemic implications. This study aims to investigate the changes in the oral microbiome caused by cigarette smoking and their correlation with individual dietary and oral hygiene habits. Moreover, the study shows that smoking affects the antidrug resistance in both bacteria and fungi. Ten participants, comprising five smokers with a minimum of one-year smoking history and five non-smokers, were recruited. Each subject self-collected oral samples using sterile cotton swabs, which were inoculated on LB and Czapek culture media to quantify bacteria and fungi. Morphologically distinct isolates were selected for DNA extraction, followed by the amplification of the 16S rRNA gene (for bacteria) and the ITS region (for fungi). The obtained PCR fragments were sequenced and used for species identification. Results revealed quantitative differences between the microbiome of smokers and non-smokers. Generally, the smokers presented higher bacterial and fungal colonies than the non-smokers. In addition, smoking was associated with an increase in the antibiotic resistance of oral bacteria against ampicillin and an increase in the antifungal resistance of oral fungi against naftifine hydrochloride. This paper emphasizes the impact of smoking on oral microorganisms, demonstrating quantitative changes between the two groups of subjects and its correlation with the lifestyle of each participant. Therefore, understanding the modifications found in this study may serve as a starting point for developing therapies that can reduce the effects of smoking on oral health and also for the elaboration of targeted treatment strategies.

Keywords: drug resistance, oral microbiome, cigarette smoking, ITS and 16S rRNA amplification, culture-based methods**.**

Cultivable microbial diversity associated with three Anuran species from Romania.

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The skin microbiome of Anura plays an important role in maintaining host health through its capacity to protect against fungal pathogens and viruses. By culture-dependent methods it has been shown that bacterial strains belonging to *Pseudomonas* sp., and *Janthinobacterium* sp., are colonizing the amphibian skin and are involved in the protection against chytridiomycosis. The aim of this study was to investigate the cultivable diversity of bacterial and fungal species inhabiting the skin of three Anuran species. The selected species were *Bufo bufo*, *Bufotes viridis*, and *Bombina variegata*. For each species, swab samples were collected, suspended in sterile phosphate-buffered saline (PBS), mixed thoroughly, and serially diluted before inoculation onto tryptic soy agar (TSA), Reasoner's 2A agar (R2A), Actinomycete isolation agar (AIA), whereas Sabouraud medium was used for fungal isolation. The plates were incubated at 22^{0}C. From pure cultures genomic DNA was extracted and the 16S rRNA gene for bacteria and ITS for fungi was amplified by PCR followed by Sanger sequencing. FASTA sequences were trimmed with MEGA 11 and strain identification was performed with BLASTn. *Pseudomonas* sp. was the most abundant bacteria across all three amphibian species. Notably, *B. bufo* additionally harbored *Janthinobacterium sp.*, *Flavobacterium* sp., and *Kocuria* sp., *B. viridis* displayed *Chryseobacterium* sp and *B. variegata* only *Pseudomonas* sp. Diverse skin-associated fungal strains were isolated including *Neocucurbitaria* and *Trichoderma* spp. from *B. bufo*; *Penicillium*, *Didymella*, and *Cadophora* spp. from *B. viridis* and *Hypocrea,* and *Beauveria* spp. from *B. variegata*, respectively. This study revealed the prevalence of *Pseudomonas* sp. among bacteria isolated from the skin of selected Anuran species and the apparent absence of skin-associated fungal pathogens. However future investigations are needed to understand the function of the microbiome in the studied amphibian species.

Keywords: *Anura***,** amphibian, culturable diversity, *Pseudomonas* sp.

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Iron oxide nanoparticles: How does the size and coating affect the interaction with cancer cells?

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Nanotoxicology research is paramount to nanoparticles research as their types and applications continue to increase, especially in biomedicine. To better understand their effect on human cells, the nanotoxicity of iron oxide nanoparticles (IONPs) functionalized with polyethylene glycol (PEG), polyethylene glycol-silica oxide (PEGSiO2) and (3- Aminopropyl) triethoxysilane (APTES) were investigated. As consulted in the literature IONPs functionalization improves the cytotoxic effects compared to bare IONPs. This could also result in higher nanoparticle-uptake in cells. Investigating the *in vitro* effects ofIONPs formulations for future use in experiments. The investigations were carried out on A549 (lung adenocarcinoma epithelial cells) and A375 (melanoma cells), using the MTT viability assay. The cells were cultured on supplemented culture media for 24 h in 96 well plates. The treatment was applied for 24 h as well, and the results were investigated spectrophotometrically. Ten different concentrations were tested. IONPs-PEG80 formulation showed significant cytotoxicity on A549 cells but not on A375 cells, while PEG80SiO2 had milder toxicity on A549 cells, and IONP-APTES had no cytotoxic effect on A375 cells. The results are all reported at 1 mg/mL nanoparticle concentration. IONPs-PEG80 has a toxic effect depending on the type of cell used, not having a toxic effect on A375s while being very toxic on A549, raising questions about the cellular factors that may be in play. PEG80SiO2 and APTES formulations have lower toxicity and thus may need to be loaded with a cytotoxic drug to achieve the same effects. All results are preliminary and further investigations are required.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Snapless wonders: Unravelling the molecular milestones behind the Venus flytrap's snap closure mechanism

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Within the plant kingdom, few species have evolved fast movement upon touch. One such exception is the charismatic Venus flytrap (*Dionaea muscipula*), a carnivorous plant which relies on a millisecond-range snap-trapping mechanism to catch its prey. The special tactile abilities are given by the trigger hairs, found on the trap surface, which upon bending elicit an action potential. In contrast to a functional Venus flytrap, which snaps upon the induction of two action potentials, the so-called 'ERROR' mutant – a cultivated variety of the Venus flytrap – is unable to snap its traps. To get a better understanding of the essential molecular milestones necessary for a successful trap closure, the transcriptome of the 'ERROR' phenotype was compared to the functional Venus flytrap phenotype before and after the application of mechanostimulation. The results show, for the first time, the importance of a special cell wall architecture that might confer the mechanical properties together with a certain geometry that assures a ready-to-snap configuration of the trap before stimulation. This might be crucial for a functional buckling system to amplify the speed of the trap closure, which is essential for a successful carnivorous lifestyle among green hunters.

New contributions to vegetation knowledge of Danube Delta

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New investigations on the flora, vegetation and natural habitats of the Danube Delta (Romania) were recently carried out. Thus, more than 1200 vascular plants species were inventoried, phytocoenoses of 254 plant communities were recorded on the ground, and 32 Natura 2000 habitats were recorded, all of them mapped on a large scale. Among these, the following phyto- and coenotaxa were registered as novelties:
- a newly identified taxon for the vascular flora of Romania: *Paspalum distichum* subsp. *Paucispicatum; and*
- two new phytocoenotaxa for science are proposed here, namely: 1) Ass. *Periploco graecae*-*Alnetum glutinosae* ass. nova, and 2) Ass. *Cypero flavescenti*-*Paspaletum distichi* Horv. 1954 subass. *paspaletosum paucispicati* subass. nova.

The Natura 2000 habitats in the Danube Delta that include the new coenotaxa proposed above are: 91E0* Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*); 3160 Natural dystrophic lakes and ponds; 3270 Rivers with muddy banks, with vegetation of *Chenopodion rubri* p. p. and *Bidention* p. p.

Deciphering above-treeline vegetation greenness trends: Insights from the Carpathian Mountains

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The long-term increase in vegetation greenness is a topic of considerable interest in ecology. This study aims at deciphering the spatial distribution patterns of greening and their relationships to land cover in the above-treeline ecosystems of the Carpathian Mountains, a range that has been understudied so far in greening studies. We calculated the greenness trends using Landsat imagery dating back to 1984 and employed a large training dataset for high-resolution land cover classification. The land cover types included screes, grasslands, low shrublands (mainly composed of Ericaceae and Juniperus communis), tall shrubs (composed of Pinus mugo) and woodlands dominated by Picea abies. Our analysis revealed widespread greening and high spatial variability associated with land cover types. Overall, the most pronounced greening signal was detected within Ericaceae-rich shrublands, with varying intensity observed across different mountain ranges and other land cover types. Our findings highlight that high-resolution distribution models of land cover types are pivotal to unravel the complexity of greening in mountains. In perspective, our research aims to integrate remote sensing imagery with dendroecology, with a specific focus on Pinus mugo (as part of the MUGO project) and Ericaceae species, to further elucidate the greening phenomenon in the Carpathian Mountains.

Acclimation ability, life history, and behavioral traits of an endemic copepod species from an Italian karst system

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Despite their prevalence in groundwater ecosystems, the functional ecology of groundwater-obligate copepods remains relatively understudied. In 2016, we embarked on a comprehensive investigation focusing on the life history and functional traits of *Moraria* sp., an endemic harpacticoid species inhabiting Antro del Corchia, a karst cave in the Apuan Alps (north-central Italy). We assessed the oxygen consumption rates of twenty adult females of *Moraria* sp. as a proxy of metabolic changes in a climate scenario with temperatures ranging from 8.0 °C (mean annual temperature of the Antro del Corchia cave) to 12.5 °C (maximum temperature of the cave according to climate change scenarios in 2100). In detail, we measured oxygen consumption after a 30-day acclimation period to varying temperatures to assess the species' ability to adjust its metabolic rates back to baseline levels. Our results highlighted the low metabolic activity of this species and its limited thermal adaptation ability, indicative of stenothermy. In particular, even minimal temperature variations (i.e., +1.5 °C) significantly influenced the metabolic rate of *Moraria* sp. over the long period, indicating its potential vulnerability to thermal fluctuations induced by global change. Furthermore, we assessed the life history and locomotion behavior of this species at its optimal temperature of 8.0 °C and compared them with its surface water counterpart *Bryocamptus zschokkei*. We found out that the low metabolic rates of *Moraria* sp. correlate with its longevity and low reproductive and locomotion rates, which seem to highly diverge from its epigean counterpart. These findings illuminate the ecological peculiarities of *Moraria* sp., enhancing our understanding of groundwater copepod ecology and highlighting the critical need for conservation strategies. This study emphasizes the importance of safeguarding subterranean biodiversity against the backdrop of global environmental change.

Insights into crustacean adaptations in the sulfidic mesothermal aquifer from Mangalia (Southern Dobrogea, Romania)

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Aquifers are crucial geological formations that store large quantities of groundwater and serve as vital habitats for diverse organisms like stygofauna, subterranean aquatic fauna. They also provide essential services such as water purification, biodegradation of pollutants, pathogen removal, and flood mitigation, highlighting their significance for both human well-being and biodiversity. In South-East Romania on the Black Sea coast, the presence of sulfide-rich overlapping aquifers hides an intriguing and complex biological community. The aquifers provide a high diversity of microhabitats for crustaceans (in their sulfidic vs. sulfide-free parts) and abundant food sources, enabling the coexistence of species with similar morphological and/or ecological adaptive traits. Here, the primary production is supplemented through chemosynthesis, supposedly supported by chemoautotrophic bacteria that derive their energy from chemical reactions with inorganic molecules. This study explores the most upper Sarmatian aquifer, which is accessible through the old hand-dug wells and the Movile Cave. These sites provide a suitable habitat for a diverse community of crustaceans, including cyclopoids, ostracods, harpacticoids, amphipods, and isopods, that face survival challenges due to environmental conditions. Apart from nutrient and energy limitations, darkness, oxygen scarcity, and geochemically variable conditions, that usually characterize groundwater ecosystems, sulfidic-rich groundwater poses additional pressure to these organisms, including elevated temperature (cca. 21 °C), and increased concentrations of H_2S, CH_4, NH_4, and other ions (e.g., heavy metals, arsenic). In this study, we will discuss some of the adaptations that the crustaceans have developed to cope with these challenging extreme conditions. This includes the presence of symbiosis with sulfur-oxidizing filamentous bacteria of the genus *Thiothrix* in the case of some microcrustacean species and the epibiotic ciliates of the genus *Lagenophrys*. Such associations have been previously identified in ecosystems with similar conditions, highlighting the versatility of such associations in sulfidic groundwaters. Additionally, we will discuss the presence of respiratory pigments that can contribute to the efficient utilization of oxygen in this hypoxic environment. Sulfidic groundwater communities are recognized as an example of "life at extremes". Investigating this particular ecosystem will enhance our knowledge of life forms, establish life's limits, and help us understand the capacity of life to withstand and adapt to change.

Keywords: crustaceans, sulfidic aquifer, adaptations, symbiosis, respiratory pigments

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Monitoring of groundwater fauna from Vârtop Cave (Apuseni Natural Park, Romania).

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Ice caves host a distinctive type of subterranean ecosystems with year-round negative temperatures, spanning a wide range of latitudes and latitudes across the Northern Hemisphere. These caves offer habitats for both localy-adapted species and glacial relicts that depend on the specific cold microclimate for their survival. This study explores the influence of environmental parameters on the subterranean fauna inhabiting Ghețarul de la Vârtop Cave, a small (300 m long) ice cave located in Romania's Apuseni Natural Park. The cave was selected for monitoring of groundwater fauna due to the presence of temporary ice formation near its entrance, persisting until spring, which could indicate the presence of cold-adapted fauna, as well. Our research aims to shed light on the relationship between temperature conditions within the cave and their effects on the subterranean fauna seasonal and spatial fluctuations. The monitoring has been performed between February 2022 until March 2023, from gours and percolating waters. Field measurements indicate a mean water temperature of 3.9°C (3.93°C for gour water and 3.92°C for percolation water). The lowest recorded temperature, 1.6°C, was observed at a percolation site near the entrance of the cave in early spring (April 2022). In contrast, the highest recorded temperature, 5.8°C, occurred at both gour and percolation sites in early summer (May and June, respectively). Our study indicates that the percolation water within the cave, along with its associated gours, contain diverse communities of aquatic invertebrates that shows a spatial distribution along the cave with slight seasonal fluctuations. These communities include nematodes, oligochaetes, and crustaceans, with the most prevalent groups belonging to the orders Harpacticoida and Bathynellacea (*Bathynella* cf. *motrensis* Serban, 1971). Additionally, Cyclopoida

(*Acanthocyclops reductus* Chappuis, 1925) and Oligocheta are also present, mostly found in the middle portion of the cave. Studying aquatic fauna in these caves with temporary ice formations can provide valuable insights into the understanding of the ecological dynamics of the subterranean fauna and further to establish the physiological mechanisms adaptation to low temperature.

Keywords: karst, stygofauna, percolating water, ice caves.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Morphological examination of green sea turtle (*Chelonia mydas*) hatchlings from original and relocated nests

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The protection of endangered marine species is essential nowadays due to global warming, sea level rise and unethical fishing. Green sea turtles (*Chelonia mydas*) are also suffering the consequences of anthropogenic effects. Relocation of nests is commonly used in conservation biology, and it occurs when the nests are too close to the tide line and there is a potential risk of flooding. Even if the parameters of the new nests are similar to the original, the morphological characteristics of the hatchlings can be affected during the process and by the environmental background variables. I dedicated three months to conducting comprehensive research on green sea turtle nest relocation effects in Northern Cyprus. I measured 380 individuals using a TRESNA 300 mm digital caliper from a total of 12 nests. In R statistical environment, the ANOSIM and NMDS methods showed significant differences in both morphological characteristics and environmental background variables between original and relocated hatchlings. Unfortunately, there are no other publications using the NMDS method for this species, which is why I think it is important to present my results. This research can provide information for future morphological studies and crucial insights into green sea turtle nest relocation. Additionally, it plays a pivotal role in raising awareness about the conservation of endangered species.

Keywords: *Chelonia mydas,* endangered, digital caliper, hatchling morphology

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

The reproduction and early development of *Mnemiopsis leidyi* (Ctenophora) in the Adriatic Sea

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Mnemiopsis leidyi is the only species in the genus Mnemiopsis. They are successful generalists to the point where they were misidentified as three different species, whereas they simply adapted to their environment by changing their morphology. Presumably, these ctenophores reached the Adriatic Sea by ballast waters and effortlessly earned their spot on the world's 100 most invasive species list. With practically no natural predators and no commercial value, this species has a negative effect on the local fishermen in addition to being an active threat against diversity. In order to have a better grasp of why they are strong competitors, we studied their reproductive rate and their early ontogenesis.

Keywords: Ctenophora, invasive species, reproductive rate, early ontogenesis

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Patterns of habitat use by the brown bear *Ursus arctos* in the Southern Carpathians revealed by occupancy modelling

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We live amid a global wave of anthropogenically driven biodiversity loss, especially targeting species from megafauna. Understanding the dynamics of large mammal species and their interaction with human activities is crucial for effective conservation efforts. Occupancy modeling provides an efficient framework for studying species distributions and habitat use. In this study, we employed a dynamic occupancy modeling approach to investigate the presence of brown bears *(Ursus arctos)* in the Ţarcu Mountains, located in the south-western region of Romania. The study area, encompassing the Natura 2000 site ROSCI0126, is characterized by diverse habitats, including forests, meadows, rocky areas and river systems supporting a rich assemblage of large mammals. We conducted systematic surveys using randomly selected transects covering a grid of 270 km^2. These transects were traversed monthly over two seasons from September 2022 to September 2023, recording signs of brown bear presence using GPS devices. Additionally, we extracted land cover data from the Pan-European land cover map of 2015 and assessed forest disturbance levels using the European forest disturbance map. Presence-absence data were analyzed at the grid cell level, with variables such as ruggedness and elevation also considered. Our analysis revealed insights into brown bear occupancy patterns, habitat preferences, and seasonal variations in detection probabilities. The results provide valuable information for conservation management strategies in the Ţarcu Mountains, emphasizing the importance of maintaining habitat connectivity and minimizing human-wildlife conflicts. This study underscores the utility of dynamic occupancy modeling coupled with spatial analysis techniques in informing biodiversity conservation efforts in complex landscapes.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Autum bird migration on Chituc Spit: the first ten years

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Bird migration remains one of the main study topics of ornithology. Although the study of this phenomenon is experiencing a renaissance in part due to the technological advance in the field of telemetry, traditional study methods still have a strong contribution in less studied areas. Birds cross Europe on three main migratory flyways, Romania being located on the least studied route: the Eastern Flyway, used by birds from West Asia and North-East Europe. During each Autumn in the past ten years, Milvus Group organized a ringing camp on Chituc Spit, to study the migratory phenology of birds crossing Europe on this latitude. We marked 145,000 individuals belonging to 190 species in the past ten years, thus collecting data both about the migration of common species and the presence of species rarely observed in Romania. The most abundant species marked are the red-breasted flycatcher (*Ficedula parva*, 13.704 individuals), the blue tit (*Cyanistes caeruleus*, 13592 ind.), the robin (*Erithacus rubecula*, 13.245 ind.), and the sedge warbler (*Acrocephalus schoenobaenus*, 12.696 ind.). This long-term dataset contributes to the identification of migration phenology of some species, and the permits the long-term monitoring of migratory- and breeding population dynamics of the species crossing this study site.

Keywords: bird migration, migratory phenology, Easter Flyway

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

The hidden world of wasp galls: Insights from wild rose research

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A wide range of organisms, including nematodes, arthropods, fungi, and bacteria, can produce characteristic growths on plants known as plant galls. These gall inducers force plants to develop new tissues and organs, which serve as food sources for the larvae of the inducers. Plant galls have long captivated ecologists and taxonomists, despite the fact that their formation mechanisms are still unknown. Since their discovery, the main objective of gall scholars has been to better understand the induction mechanisms. Advancements in genetic and molecular techniques now allow for a thorough investigation of the molecular mechanisms underlying gall formation. The different evolutionary origins of gall formation across the animal kingdom suggest different mechanisms in different groups, making it challenging to uncover these mechanisms. Although molecular techniques have advanced, finding appropriate plant and gall inducer models is still essential for this type of research.

Our research aimed to establish a sustainable laboratory ecosystem comprising wild roses (*Rosa* sp.) and gall-inducing insects, specifically rose gall wasps of the genus *Diplolepis*. We optimized controlled indoor conditions to facilitate plant growth. Following transplantation, the wild roses were subjected to exposure from gall inducers and underwent systematic monitoring. Our research has successfully established a new laboratory community to advance the study of gall formation mechanisms.

Keywords: plant gall induction, wild roses, mossy rose gall, plant vigor, *Diplolepis*

Interspecific interactions in water birds

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Analyzing the behavior of birds offers an insight into the conservation of biodiversity, a heavily discussed subject in the fields of ecology, as well as ornithology. Therefore, this study aims to explore the dynamic of interspecific interactions between water birds in the Afon Peța portion of the Crișul Repede river in Bihor, Romania. We collected data over the course of 8 months, from October 2022 to May 2023, summing 51 field observations. While there have been changes related to season regarding the behavior of the studies species, no correlation between the number of species and interactions has been found in birds with a constant presence in the study area. The results obtained through this study will help broaden the knowledge about common aquatic bird species in the area, as well as the dynamic of behaviors over the course of three seasons.

Keywords: Crișul Repede river, interspecific behavior, water birds, aggression, cooperation.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Comparative phylogenetic analyses of phosphofructokinase and hexokinase evolution in several Neognathae avian species

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Evolutionary insights soar thanks to phylogenetic analyses, shedding light on the origins and diversification of many vertebrates, including birds. Birds possess unique metabolic features, with high circulating glucose levels compared to other animals of similar size. However, despite this fact, the evolution and diversity of the key enzymes involved in glucose catabolism among birds remains elusive. Through these analyses we aimed to examine, compare and phylogenetically investigate the evolution of phosphofructokinase (PFK) and hexokinase (HK) among Neognathae avian species. The amino acid (aa) sequences from 50 bird species were collected from NCBI Genome database. A total of 208 sequences for HK and 322 for PFK were analysed. Sequences were currated manually for isoformes and aligned by MAFFT. Maximum Likelihood (ML) phylogenetic trees were constructed using IQ-TREE with the automatic model selection, ultrafast bootstrap for 1000 replicates and SH-aLRT branch test. We identified two different enzymes annotated as hexokinase (HK) which formed a distinct clade compared to the second enzyme the HK-domain containing protein 1 (HKDC1). A clear segregation between the two enzyme groups was observed and supported by robust statistical backing (99% for HK, 97% for HKDC1). In the case of PFK, the platelet-derived aa sequences are considerably more heterogeneous, and their clustering was not accurate as the statistical support was low. The trees reconstructed from the PFK aa sequences from muscle and liver, revealed that a clear cluster is formed by the Galloanseres (*Galliformes* and *Anseriformes)* with strong support (100% in both cases). Another cluster is formed by *Passeriformes* (95-99%) and between these two clusters, there is a highly diverse grouping of other avian members like *Strigiformes*, *Accipitriformes*, *Pelecaniformes* and *Falconiformes* among others. These findings indicate the presence of at least two classes of HK in birds, and a tissue-specific distribution of PFK. Moreover, it strongly supports the genome-scale family tree of modern birds.

Keywords: hexokinase, phosphofructokinase, genome comparison, sequence analysis, phylogenetic tree.

Immune cell concentrations and cancer mortality risk in mammals

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One of the current challenges in evolutionary biology is to answer why species differ in the incidence of cancer mortality. Some species appear to be resistant to tumours, while others are more susceptible to die from tumours. According to the immune surveillance hypothesis, the immune system plays a crucial role in shaping this variation. Moreover, it posits that cancer might have been a primary selective factor in the evolution of the immune system as immune cells prevent the uncontrolled cell proliferation in tumours. This contrasts with the immunopathology hypothesis, stating that elimination of intruding pathogens and parasites by the immune system comes at a cost, as chronically activated innate immunity contributes to the formation of tumours. However, these hypotheses have never been tested by cross-species comparisons. To this end, we estimated age-controlled cancer mortality risk (immunopathology hypothesis) for 249 mammalian species and we calculated a species-specific parameter expressing inherent cancer resistance in function of body mass and longevity (immune surveillance hypothesis) and using data provided by zoos. The association of both parameters with total and specific white blood cell concentrations was analysed using phylogenetic regressions. Our results indicate that cancer is more likely to be detected in species with low monocyte and neutrophil concentrations but high eosinophil concentrations. Cancer mortality risk is unrelated to both total or specific white blood cell concentrations. Inherent cancer resistance increases with higher monocyte and neutrophil concentrations. The results suggest that certain white blood cell types might play important roles in cancer protection and evolution might have shaped their abundance in parallel with the augmenting risk of tumours with the evolution of large body sizes and extended longevities.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

House sparrows on time-restricted diet: effects on body condition, blood glucose and ketone levels

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Time-restricted feeding is a dietary regimen in which access to food is limited by time and it is followed by a prolonged period of fasting. Previous studies have shown that time-restricted feeding has beneficial effects on various physiological parameters, it can postpone ageing and it can reduce the risk of pathologies linked to old age. However, like most dietary restriction studies conducted so far, the effects of time-restricted feeding were tested only on laboratory model organisms artificially selected and often inbreed, while its beneficial effects are yet to be tested on wild-living organism. We conducted an experiment in which house sparrows (*Passer domesticus*) were subjected to a time-restricted feeding regimen of 8 hr/day (i.e., 16 hr of fasting/day), while control birds had *ad libitum* access to food throughout the day. We sampled the birds prior to the treatment and 50 and 120 days after starting the treatment, and collected data regarding their body condition, body temperature, blood glucose and ketone levels. Elevated ketone levels in the experimental group show that the treatment has its proposed effects (i.e., being higher in the food-restricted group), however, we found no differences between the groups regarding body condition, body temperature and blood glucose levels. Future laboratory analyses will test if time-restricted feeding managed to alleviate telomere attrition rates and oxidative stress in experimental birds, compared to controls.

Keywords: time-restricted feeding, fasting, glucose, ketone

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Redescription of the larva of *Eurythyrea aurata* (Pallas, 1776) using microphotography and SEM technics

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The larval stage of *Eurythyrea aurata* was initially described by M. G. Volkovitsh in 1975, documented in the Russian language, primarily for comparative purposes vis-à-vis the larval form of *E. quercus*. This scholarly endeavor was supplemented with meticulously crafted illustrations of exemplary quality.

A comprehensive redescription of the mature larva of *Eurythyrea aurata* is presented herein, employing microphotography and scanning electron microscopy (SEM) images as demonstrative exemples. Furthermore, endeavors were made to establish correlations between these morphological attributes and the larval biology.

3D in vitro model development to mimick DOX-chemoresistant melanoma microenvironment

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Advances in immunological oncology emphasize tumor microenvironment (TME) as the primary source of drug resistance, proving the importance of 3D tumor models, which better reflect the TME, and reduce animal experimentation. Our objective was to develop a 3D-spheroid model containing B16.F10 murine melanoma cells, macrophages, endothelial cells, and fibroblasts to simulate the chemoresistant TME. We aimed to validate this model using doxorubicin (DOX), a frequently used chemotherapy drug; notably, patients with melanoma typically do not respond to DOX, which is precisely why it was selected in our investigation. To create the spheroids, B16.F10 cells were seeded in 1% commercial extracellular matrix, along with 2H11 endothelial cells, primary fibroblasts and macrophages at a ratio of 1:1:1:4 ratio, in ultra-low attachment plates. The viability of cells within spheroids was assessed by measuring acid phosphatase activity. DOX at concentration corresponding to IC30 was applied to induce DOX-chemoresitance in spheroids. The emergence of chemoresistance was assessed by various markers, such as of apoptotic proteins: Bid, Bax (western blot), of oxidative stress: total antioxidant capacity (TAC) and catalase activity (measured *via* spectrophotometry), malondialdehyde levels (HPLC technique) and of metastatic potential: matrix metalloproteinase activity (*via* zymography). Our model showed similar levels of oxidative stress markers, such as MDA, TAC and catalase post-DOX treatment compared to control group. The activity of matrix metalloproteinase-9 significantly increased in DOX-treated group, while similar expression degree was observed in the case of apoptotic proteins Bid and Bad between groups. Therefore, as an overall view, our model in which we incorporated a variety of cell types from TME showed some traits of DOX-chemoresistance demonstrated by higher invasion capabilities compared to control and similar oxidative stress levels.

This model serves as a valuable tool for investigation of TME induced drug resistance.

Keywords: 3D model; fibroblasts; endothelial cells; chemoresistance.

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Ancient biomolecules reveal the past of premodern individuals from southeastern Romania

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Ancient DNA is a valuable repository of information that enables scientists to reveal the past of different populations or individuals. Oftentimes, its study is complemented by those based on isotopes, providing further insights into the lifestyles of those analysed. This multidisciplinary approach can be of great help, especially when the lack of funerary inventory and historical context limits the classic archaeological analyses. The aim of this research is to determine the geographical origin and dietary habits of individuals discovered in a necropolis located near Mireasa village (Constanța, Romania), using ancient mitochondrial DNA, stable and radioactive isotope analyses. The methods followed in this study consisted in the extraction of genomic DNA from tooth samples, PCR amplification of the hypervariable regions of the human mitogenome, Sanger sequencing and haplogroup assignments, followed by computational analyses intended to discover the biogeographical origin of the subjects. The major components of the diet and the sample dating were subsequently determined based on the analysis of carbon and nitrogen isotopes. The results revealed the presence of both Asiatic and European mitochondrial DNA haplogroups within the population and a diet based possibly on a combination of plants and marine food. Regarding the time period of their existence, the resulting data indicated the individuals lived in the premodern era. These results complement the little information known so far about the populations of Dobruja in the 17th century, highlighting how dynamic the human migrations and interactions were in that region at that time.

Keywords: ancient mitochondrial DNA, isotope analysis, Dobruja.

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Comparative analysis of the *in vitro* effects of resveratrol and genistein on non-small cell lung cancer (NSCLC) cell line A549

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer mortality in men and the second cause of mortality in women. Exploring treatments is imperative for patient survival. We investigated how resveratrol/genistein affects a NSCLC cell line as a treatment option. The NSCLC cell line chosen was A549. To evaluate the resveratrol/genistein cytotoxic effects, we employed the MTT assay. We performed both an apoptosis assay and a cell cycle analysis to assess the treatments' ability to induce cell death or cell cycle arrest, using the Nexcelom's Celigo. The clonogenic assay was performed and scanned with the same Nexcelom's Celigo, while the scratch assay was used to investigate whether the treatments have a cell migration inhibitory effect. IC50s of 169,6 µM for resveratrol and 112,6 µM for genistein were obtained. Both compounds induced cell death by apoptosis in the A549 cell line. Resveratrol exhibited more promising effects than genistein– 15% of apoptotic cells compared to 12,5%, when cells were treated with the IC25 concentrations. Resveratrol-treated cells also exhibited a higher rate of cell cycle redistribution from G_2/M phase to G_0/G_1 compared to genistein-treated cells: 55% for resveratrol compared to 45% for genistein. The colony formation ability of the resveratrol-treated category was statistically the same as in the genistein-treated category: <300 colony count in untreated cells to 150 colony count for resveratrol and <300 to 140 colony count for genistein. Genistein seemed to show no effect on the migration rate of A549 cells; in this case, resveratrol exhibited a reduction of cell migration area by 35,15 % after 48h of treatment. This study underscores the potential of resveratrol as a promising alternative to conventional chemotherapy agents for NSCLC.

Dopaminergic striatal system lesioned with MPTP: Michaelis-Menten profile on oxidative stress and validation of kinetic data in mice.

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This study explores how exogenous antioxidants affect endogenous peroxidase activity. Using both in vitro and in vivo approaches, we simulated cellular conditions in a spectrophotometer cuvette with a citrate-phosphate buffer, hydrogen peroxide, and o-dianisidine, initiating the reaction with peroxidase (**EC 1.11.1.7**). Initial kinetics studies revealed enhanced enzyme affinity at 40 µM Fe^{2+}. Exposure to these conditions for 10 minutes increased peroxidase activity, with a 5-minute interaction with 40 µM Fe^{2+} notably boosting enzyme affinity. Similar effects were observed with Fe^{3+}. Free dopamine (DA 100 nM) decreased peroxidase activity, while ascorbate's effects varied based on concentration and iron redox state. Moving to *in vivo* analysis, we induced a Parkinson's disease model in male CD21 mice using MPTP, confirming pathology through elevated α-synuclein and decreased dopamine levels. The Turnbull staining correlated focal brain ferroptosis and peroxidase activity, illustrating dopamine's role in the Haber-Weiss reaction. Dopamine acted as a noncompetitive peroxidase inhibitor, decreasing K_{MM} and V_{max}. *In vivo*, peroxidase activity decreased with dopamine variations.

In summary, our study highlights complex interactions between exogenous antioxidants, endogenous enzymes, and dopamine. These findings offer insights into potential therapeutic strategies for managing oxidative damage in Parkinson's disease.

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Enhancing melanoma immune status: Utilizing extracellular vesicles loaded with curcumin in 3D models

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Immune checkpoint inhibitors (anti-PD-1/PD-L1antibodies and CTLA-4) have revolutionized cancer therapy. However, advanced melanoma has often been associated with immunotherapy resistance. To improve immunotherapy efficiency in melanoma, we aimed to develop an adjuvant immunomodulatory approach based on using extracellular vesicles derived from activated dendritic cells, loaded with curcumin and delivered into a three-dimensional model for melanoma, to mimic the *in vivo* immune status. For this, we cultured murine melanoma B16.F10, dendritic cells 2.4 and CD8$^+$ lymphocytes in a ratio of 1:1:5, in low-adherent plates to create spheroids, that were exposed to the curcumin-loaded extracellular vesicles purified from dendritic cells activated with CpG and pulsed with TRP-2 peptide. Spheroids were characterized based on size, morphology and presence of dendritic cells and lymphocytes, using histochemical and immunofluorescence techniques. Moreover, the effects of 48h treatment of free curcumin compared to extravesicular curcumin on the viability of spheroids was determined using acid phosphatase assay, and the differences in curcumin uptake were assessed after 2h of incubation of spheroids with the two therapies tested.

Our results showed that the treatment with EVs loaded with curcumin has a higher efficiency compared to the free administered curcumin with an IC_{50} of 23 μM compared to 77 μM, inhibiting the cell viability by two-fold ($p<0.001$), compromising the spheroid integrity and it's tumoral landscape. When compared to the free form, the loaded curcumin had a significant increase in uptake in the three-dimensional model ($p=0.0144$), which may account for the effect. In summary, this innovative formulation of extracellular vesicles containing curcumin may serve as a viable immunomodulatory therapy for concurrent administration with established immune checkpoint inhibitors, potentially enhancing their efficacy.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Expression of cytochrome P450 (CYP2A13) fused to MBP and SKIK

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The production of soluble recombinant proteins can often be challenging and require significant additional resources. Recombinant DNA technology techniques (fusion with solubilization tags, co-expression with protein chaperon) and modification of culture conditions (lowering temperature, changing inducer concentration, adding cofactors) are most commonly used to optimize expression of recombinant proteins in soluble form. Maltose binding protein (MBP) is frequently used to increase the solubility of recombinant proteins expressed in bacterial cells such as *Escherichia coli*. Meanwhile, human cytochrome P450 2A13 (CYP2A13) is an enzyme involved in the metabolism of some tobacco compounds, resulting in reactive metabolites with a carcinogenic effect. Recombinant CYP2A13 is expressed insoluble in *E. coli* cells, making its study challenging. The aim of this study was to illustrate and compare the efficiency of various methods (fusion with MBP and SKIK, lowering culture temperature) in producing a soluble CYP2A13 protein in *E. coli*. In this work, the CYP2A13 gene was fused separately at the N-terminus with MBP and SKIK tag, and with His6 affinity tag at the C-terminus by cloning them into the pET28a expression vector. The recombinant proteins (MBP-P450-His6 and SKIK-P450-His6) were expressed in *E. coli* BL21(DE3) cells. Analysis of recombinant gene expression was performed by denaturing polyacrylamide gel electrophoresis. Thus, it was found that both recombinant proteins are overexpressed, but a large part is soluble only when it is fused with MBP. SoluProt tool was also used for predicting the soluble expression by using the protein sequences of CYP2A13, MBP and of the recombinant proteins. In conclusion, recombinant cytochrome P450 2A13 protein can be expressed in soluble form by fusion with MBP at the N-terminus without other modifications that are commonly used to solubilize recombinant proteins.

Keywords: MBP, His6, SKIK, *E. coli*, CYP2A13.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Halloysite and Aerosil: The impact on melanoma cell cultures

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Halloysite and Aerosil are two types of Si-based nanoparticles and are usually used as precursors for bio-polymer nanocomposites. For their in vitro cytotoxic effects, the nanoparticles were left to interact for 24 hours with cancerous cells (A375 skin melanoma). The viability and membrane integrity were assayed through biochemical methods and the morphology was determined by light microscopy. Based on these results, the nanoparticles with higher biodisponibility (Halloysite) were functionalized with keratin extracted from chicken feathers. Two formulations were obtained (A and B) and tested again on the same cell line. The results showed that Halloysite has a medium cytotoxic effect at 195.34 µg/mL, while the IC50 for formulation A is 58.91 µg/mL, and 14.4 µg/mL for formulation B respectively. This indicates that a lower dose of nanoparticles is requested to induce cytotoxic effects in the A375 cell line. This could be associated with a higher nanoparticle uptake, however, further investigations are required to demonstrate this supposition.

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Keywords: Halloysite, Aerosil, chicken-feathers keratin, skin melanoma

Halloysite vs. fibroblasts: Is keratin a good functionalising agent?

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One kind of silicon-based nanoparticle used as a precursor for bio-polymer nanocomposites is called Halloysite. These nanoparticles were incubated for 24 hours on normal human fibroblast (BJ) cell lines in order to evaluate their in vitro cytotoxic effects. The nanoparticles were functionalized with keratin derived from chicken feathers and retested on the same cell lines following an assessment of cell viability and membrane integrity. Two formulations were produced as a result: A and B. The results showed that at 173.07 µg/mL, halloysite had a medium level of cytotoxicity. For formulation A, the IC50 values were 166.81 µg/mL, while for formulation B, they were 103.18 µg/mL. The results imply that, probably as a consequence of higher nanoparticle uptake from the organic functionalization, lower doses of the functionalized nanoparticles are required to impact BJ cell lines. Additional research is required to validate this hypothesis.

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Keywords: Halloysite, chicken-feathers keratin, fibroblasts

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

In vitro evaluation of oxidative stress induced by halloysite nanotubes in human lung cells line A549

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Halloysite nanotubes (NP) have been increasingly used in many industrial and biomedical fields. Therefore, assessing the risks and consequences of halloysite nanotubes exposure is crucial for improving human safety measures. In this study, we investegated the possible cytotoxic effects. 48,29 µl NP were left to interact for 24 hours with human lung adenocarcinoma A549 cell line. The morphology was investigated through light microscopy, while the stress oxidative was performed using two strategies: the enzymatic (CAT, SOD) determinations and the non-enzymatic pathways regarding the concentrations of TAC, TOS and oxidative stress index estimation. Therefore, the results showed that CAT activities decreased, presenting significant changes, and SOD activities increase. There were no significant changes in oxidative stress index level compared with control. A549 cells were resistant and did not present any particular changes in the oxidative status after exposure to NPs. This study demonstrates that nanoparticles do not induce significant toxic effects.

Keywords: halloysite nanotubes, oxidative stress, cytotoxicity, A549 cells.

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Investigating the phytoremediation potential of an indicator plant species in mercury-contaminated soils

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Mercury (Hg) contamination represents a significant hazard to both ecosystems and human health, given its elevated toxicity, current widespread presence in soils, and tendency to biomagnify within the food chain. Phytoremediation, an eco-friendly technology that makes use of plants and of microorganisms to reclaim environmental pollutants, has been shown promising in removing heavy-metals from soils. In this context, the present lab-scale experiment aimed to assess *Diplotaxis muralis* plants' resistance to various Hg concentrations, along with different bacterial and fungal inoculation treatments, for their potential phytoremediation usage. Plants were grown for 8 weeks in soils either artificially or naturally contaminated with Hg, with microorganism inoculation performed at 2 and 6 weeks using a non-pathogenic bacterium (*Pseudomonas chlororaphis)*, a fungus (*Sarocladium kiliense),* or their consortium. The concentrations of Hg in soil and plant samples were analyzed via ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometry). concentrations of chlorophyll, carotenoids, and ROS-scavenging enzymes, as well as enzyme activity, were determined using UV-VIS spectroscopy. Plants cultivated in artificially contaminated soils (22 ppm and 55 ppm $HgCl_2$, respectively) exhibited proportionally higher biomass compared to control plants. Upon replicating the experiment using naturally contaminated soil (with concentrations of 285 ppm and 759 ppm Hg), plant growth persisted but was reduced compared to the control group, obviously due to the particularly high Hg concentrations. These findings may suggest a notable Hg resistance of this indicator *Brassicaceae* plant species. Further, there was a significant reduction in both chlorophyll and carotenoid concentrations with increasing soil Hg levels, effect partially alleviated by the fungal inoculation. The activities of catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) exhibited a decline in the presence of microorganisms for plants cultivated in Hg-contaminated soils compared to the control group, indicating potential mechanisms aiding in the mitigation of Hg toxicity. Taken together, these outcomes indicate that *Diplotaxis muralis* inoculated with the aforementioned microorganisms may be used in phytoremediation to reduce soil Hg concentrations.

Keywords: mercury resistance, microorganisms, heavy-metals, phytoremediation, antioxidant enzymes.

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Macrophage/neutrophil dialogue in the presence of TNF-α affects the endothelium

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The innate inflammatory response, controlled by immune cells and with effects on the vasculature, is a fundamental process involved in various pathologies, including cardiovascular diseases and inflammatory disorders. Macrophages, particularly the M1 subtype, play critical roles in the initiation and maintenance of inflammation. Of particular interest is the impact of TNF-α concentration on the dialogue between macrophages and neutrophils, which are critical regulators of innate immunity. When activated by inflammatory mediators, endothelial cells promote leukocyte adhesion and vascular permeability by up-regulation of adhesion molecules, key events in tissue damage associated with inflammation. We hypothesize that soluble factors derived from macrophage-neutrophil interactions, particularly under conditions of TNF-α stimulation, will induce changes in endothelial cell adhesion promoting leukocyte-endothelial cell interactions and inflammatory cell recruitment. This study aimed to investigate the impact of macrophage-neutrophil interactions, under varying concentrations of TNF-α, on the secretion of soluble factors that modulate endothelial cell activation. Specifically, we aimed to characterize the effects of soluble mediators released during macrophage-neutrophil cross-talk on endothelial cell activation and barrier function.

To evaluate the effects of TNF-α on the macrophage-neutrophil dialogue, we used a co-culture system where we seeded macrophages obtained by treating monocytes with phorbol 12-myristate 13-acetate for 72h, on the basolateral side, subsequently neutrophils were seeded in the lumenal side of the porus trans-well. The cells interacted for 24h in the presence or absence of different concentrations of TNF-α. At the end of the incubation period, cultured media resulting from macrophage-neutrophil co-cultures was collected to further investigate the effects of the secreted soluble factors on endothelial cell monolayers.

Our results showed that SAPK/JNK presented a dose-dependent response specifically for the p56 isoform, while the p46 isoform did not exhibit the same pattern of activation. ERK2 demonstrated a dose-dependent relationship, whereas ERK1 did not. NFκB levels were elevated in macrophages co-cultured with neutrophils in the absence of TNF-alpha. Endothelial ICAM expression did not show significant changes under the experimental conditions tested, suggesting potential differences in responsiveness compared to other markers like VCAM and integrins, but we also noticed that endothelial cells treated with CM from the experimental groups exposed to high levels of TNF-alfa enter apoptosis via a mechanism involving caspase-3 activation.

In conclusion, the dialogue between macrophages and neutrophils can activate both cell types, in the presence or absence of TNF-alfa by downstream signaling and activation of NFkB, SAPK/JNK, and ERK, upon this interaction macrophages transition towards a pro-inflammatory phenotype characterized by elevated levels of iNOS, IL-1β and TNF-alfa. These soluble factors released after the dialogue between the two cell types present profound effects on endothelial cells by up-regulation of adhesion proteins like VCAM-1 and ITGA5. Conditioned media from TNF-alfa exposed leukocytes induced apoptosis in endothelial cells.

Keywords: macrophage, neutrophil, TNF-α, inflammation, endothelium

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

MMP-9 as a candidate prediagnostic parker in Parkinson's disease

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In searching for a new prediagnostic blood marker for Parkinson's disease we investigated this pathology using an MPTP dopaminergic lesion (25 mg/kg b.w. i.p.) in male CD21 mice (n=10). Subsequently, the whole brain was dissected, and cortical and striatal areas were prepared for immunohistochemistry (α-synuclein, GFAP) and cytokine analyses (IL-1α, IL-1β, IL-6, IL-10 and PGE2). Additionally, blood was collected for serum MMP analyses. The study revealed a functional relationship between cytokines and serum matrix metalloproteinases (MMP-2 and MMP-9).

The data showed that IL-10, which was increased in striatum after the MPTP exposure, acted as a blood MMP-9 inhibitor, while IL-6, IL-1β and PGE2 were identified as MMPs activators. IL-1β, IL-10 and PGE2 levels decreased in the cortical area, while IL-1α decreased in both cortical and striatal areas. In turn, IL-6, a canonical pro-inflammatory cyotokine, increased in cortical, as well as, striatal brain regions.

Overall, the clinically silent pathology induced at the cellular level was related to serum MMP-9 decreasing, suggesting its potential as a candidate blood marker for prediagnosis of Parkinson's disease.

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Revealing biogeochemical microbial actors in hypersaline, meromictic Fără Fund Lake by meta-omics

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Currently, the biogeochemical cycles in hypersaline and permanently stratified lakes are underexplored, mainly due to the uncultivability of the vast majority of prokaryotes. Fast advances in sequencing technologies and data analysis strategies allowed for high-resolution prediction of diversity, interactions, and ecological roles of cultivated and uncultivated members of microbial communities in a broad range of environments. We sought to unveil the main microbial actors involved in the cycling of main elements along the water column and sediments in the hypersaline (190 to 320 g/L total salts) and permanently stratified (meromictic) Fără Fund Lake in Central Romania. Metagenomics and metatranscriptomics were jointly employed to predict the spatial distribution of microbial communities inhabiting physico-chemically distinct water strata alongside their potential metabolic traits mediating the biogeochemical cycles of main elements (C, N, S), metals (Fe, Mn), and metalloids (As, Se). The taxonomic diversity of metagenome-assembled genomes (MAGs) increased with depth, with the large majority (>90%) having no cultured representatives. By scrutinizing transcript abundance of functional marker genes, active methylotrophy, anaerobic carbon fixation by the Wood-Ljungdahl pathway, thiosulfate disproportionation, Mn-oxidation, Fe reduction/oxidation, and As and Se-utilization were detected throughout different water strata. In conclusion, the studied hypersaline, meromictic lake seems inhabited by niche-partitioned microbial communities mostly consisting of uncultured representatives of Bacteria and Archaea. These findings underscore the significance of studying stratified aquatic ecosystems to gain insights into how biogeochemical cycles are driven, distributed, and interconnected across different physico-chemical gradients.

Keywords: Biogeochemical cycles, hypersaline, MAGs, meta-omics, microbial communities

Vascular response to sheep poly-Hb in hemorrhagic conditions. The big loser: Dextran 40.

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The study investigated the impact of poly-Hb on vascular homeostasis through an in vivo experiment using Wistar rats divided into three groups: Hemorrhage, Hemorrhage + Dextran 40, and Hemorrhage + sheep poly-Hb (8 rats per group). Exposure to sheep poly-Hb and Dextran 40 was limited to 24 hours. Subsequently, cervical dislocation was performed, and abdominal aorta and blood serum were sampled. The aorta underwent electron microscopy and iNOS immunohistochemistry, and inflammation status was assessed via prostaglandin E2, IL-1 alpha, IL-1 beta, IL-6, and IL-10. Catalase and peroxidase activities were measured kinetically, while colorimetric methods estimated levels of reduced and oxidized iron. Gelatin zymography was used to evaluate the serum MMP 1, 2, and 9.

Poly-Hb exposure resulted in a significant increase in oxidative stress, but ultrastructural examinations showed only superficial endothelial damage in the hemorrhage group. Both Dextran and poly-Hb had minimal detrimental effects on the vascular endothelium. Poly-Hb exposure led to decreased levels of proinflammatory cytokines and matrix metalloproteinases and an increase in IL-10, while Dextran 40 increased cytokine and MMPs levels compared to the control and poly-Hb groups.

In conclusion, poly-Hb demonstrated superiority over Dextran 40, a commonly used hemodynamic substitute in humans.

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2D GMM comparison of scute shape changes in green sea turtle (*Chelonia mydas*) hatchlings from original and relocated nests

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In recent decades, there has been a notable decrease in sea turtle populations, largely due to habitat destruction, including the conversion of nesting beaches for human use. Additionally, factors such as high predation rates on juveniles, illegal harvesting of eggs and adults, pollution, and unsustainable fishing methods have also played a significant role in the population decline. Conservation efforts for green sea turtles often involve relocating endangered nests, though this approach carries risks for the embryonic development and post-hatching characteristics of the hatchlings, prompting extensive research in this area. I studied the 2D GMM (Geometric Morphometric Methods) scute morphology of green sea turtle in Northern Cyprus. I performed Principal Component Analysis (PCA) to determine which environmental background variables explain the greatest effect on the nests and which traits explain the greatest proportion of the total phenotypic variance. By performing a Procrustes ANOVA test on the shape and size changes of the carapace showed statistically significant results between groups (relocated and non-relocated nests) for shape. For the data deriving from landmarks, we have created a Thin Plate figure for better understanding on the scute and carapace dimorphisms between the two groups. Based on our results, we got an explanation of the significant differences of scute and carapace morphology between the specimens from relocated and the original nests.

Keywords: green sea turtle, endangered species, hatchling, scute morphology, 2D GMM

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Aquatic invertebrate diversity in the Ţiganilor Rivulet from the "Alexandru Borza" Botanical Garden, Cluj-Napoca

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The present study aims to describe the benthic invertebrate communities found in the Ţiganilor Rivulet from the "Alexandru Borza" Botanical Garden, Cluj-Napoca, and to assess the water quality based on the presence of 16 systematic units. The three sites sampled in October 2023 were located within the botanical garden, in an area with relatively low human impacts. The samples were processed using standard methods for benthic invertebrates. Organisms belonging to phyla Nematoda, Annelida, Mollusca and Arthropoda were identified, to different taxonomic levels. Oligochaetes and chironomids dominated the benthic communities in all three samples. The water quality was assessed using the Extended Biotic Index (EBI), which converts the indicative values of the benthic groups into five classes of water quality (high, good, moderate, poor and bad). Because tolerant taxa were dominant in the Ţiganilor Rivulet, the EBI depicted moderate and poor water quality in the three sampling points selected for the present study.

Conserving hidden plant treasures: the living plant collections in "Alexandru Borza" Botanic Garden (Cluj Napoca, Romania)

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The preservation of plant diversity, encompassing all its aspects, has been one of the most important activities at "Alexandru Borza" Botanic Garden for quite a long time. On this basis, a special area has been designated to protect some of the rare or endangered plants found in Romanian flora. Although such plants are sheltered in different sections of the garden, a rockery was constructed to showcase rare plants from Romanian flora specifically. This rockery is located in the ornamental section of the garden, near the rose collection.

Plants that dwell in the rare plants rockery in "Alexandru Borza" Botanic Garden were obtained from seeds received through plant material exchanges with similar institutions worldwide, or collected from different habitats. The seeds were requested based on the seed catalogues issued annually by these institutions. The seeds underwent two procedures: 1) they were sown in a mixture of soil, sand and peat - according to the plants' specific needs - in small pots, watered and kept under observation; 2) they were germinated by *in vitro* culture. All the pots were labeled with the name of the plant species, the sowing date and the origin of the seeds. After germination, the seedlings were divided and transferred to larger pots. This repotting procedure was repeated several times. Once robust plants were achieved, they were transplanted into the specially designed area of the garden. These plants were monitored for at least one year, and if the acclimatization was successful, they would be marked with a standard label used for identifying plants in the botanic garden.

Following the above-mentioned procedures, several plant species were obtained. Thus, 53 plant species listed in different national red lists, with varying conservation status, are found in the rockery specifically designated for rare plants of Romanian flora. Among these, 41 plant species are considered rare (according to the Red list of plants from Romania – Oltean et al., 1994), such as: *Allium coloratum* Spreng., *A. obliquum* L., *Alyssoides utriculata* (L.) Medik., *Anchusa leptophylla* Roem. & Schult., *Delphinium simonkaianum* Pawl., *Draba* *aizoides* L., *Dracocephalum austriacum* L., *Phyteuma spicatum* L., *Scutellaria alpina* L., *Silene saxifraga* L., *Veronica fruticans* Jacq. etc. Also, 12 species are threatened, such as: *Achillea ptarmica* L., *Centaurea kotschyana* Heuff., *Dianthus nardiformis* Janka, *Sedum dasyphyllum* L. The section of the rare plants from Romanian flora also shelters some endemic and/or subendemic plant species, such as: *Dianthus callizonus* Schott & Kotschy, *D. giganteus* subsp. *banaticus* (Heuff.) Tutin, *D. henteri* Heuff. ex Griseb. & Schenk, *D. serotinus* Waldst. & Kit., *Silene dinarica* Spreng., *S. nivalis* (Kit.) Rohrb., *S. zawadzkii* Herbich, *Thymus comosus* Heuff. ex Griseb. & Schenk, *Viola jooi* Janka.

One of the multiple roles of botanic gardens is to conserve *ex situ* plant species that undergo any kind of threatening. Therefore, “Alexandru Borza” Botanic Garden implements several strategies of *ex situ* plants conservation. Living plant collections are a viable way of conserving important plant species from Romanian flora, in addition to *in vitro* plants collections and seed collections.

Differential effects of neutral genetic variation, temperature and drought on the transcriptome of *Lobaria pulmonaria* Hoffm (1796)

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Biodiversity comprises the variety of ecosystems, species and genes; it is crucial to ecosystem services being a measure of prosperity. However, climate change and human activities threaten global biodiversity, necessitating the understanding of species' responses. *Lobaria pulmonaria*, an epiphytic lichen, serves as a model organism for studying climate adaptation. We aimeed to elucidate its transcriptomic response to changing climates to unravel resilience mechanisms against climatic shifts. We conducted gene expression analyses using RNA-seq. Raw RNA-seq data underwent a standardized bioinformatic pipeline, including quality control (using FastQC and fastp), mapping to reference genome (utilizing HiSat2), counting reads per gene (with FeatureCount), testing for differential gene expression (using DESeq2), and visualization of results. The reference genome for Lobaria pulmonaria was obtained from the Joint Genome Institute (JGI). *L. pulmonaria* demonstrated resilience to high temperatures, both in a continuously wet state and when alternating between wet and desiccated states. The ascomycete symbiotic partner, *L. pulmonaria*, was categorized into two genepools: Continental and Mediterranean. The impact of climatic region on *L. pulmonaria* varied depending on the genepool. Temperature fluctuations exerted a stronger influence on the number of differentially expressed genes (DEGs) compared to humidity variations. Populations from the Mediterranean genepool exhibited fewer DEGs in response to temperature stress. For the Continental genepool, significant differences in DEGs were observed among populations under different treatments, indicating complex responses. This highlights the vulnerability of the continental gene pool to rising temperatures, emphasizing the urgent need to prioritize the protection of habitats that harbor it. Conversely, for the Mediterranean genepool, consistent differences in DEGs were observed between populations across treatments, suggesting a stable response regardless of climatic conditions.

Epizoic diatoms found on turtles from different freshwater ecosystems

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Diatoms are living in various aquatic ecosystems to which several cellular adaptions help them thrive. Macrophytes, stones, sand, sea shells or even the upper shell of turtles become the best microhabitats for the diatom species which prefer living attached to a surface. The aim of this study is to investigate epizoic freshwater diatoms on the turtle carapaces from three different anthropic aquatic habitats: a small pool, an anthropic pond from "Alexandru Borza" Botanic Garden and a greenhouse pool from "Vasile Fati" Botanic Garden. By scraping the entire surface of the turtle shells, samples were taken from a total of ten individuals living in these water bodies. A total number of 79 taxa were identified belonging to 30 genera. While 78% of these taxa were found in the three epizoic samples from the outside anthropic pond, the six samples from the greenhouse reached lower numbers of taxa. Moreover, at each sampling site a diatom bloom was observed: *Craticula subminuscula* (small pool), *Achnanthidium affine* (on the three turtles from the anthropic pond) and *Nitzschia inconspicua* (on almost all individuals from the greenhouse pool). A higher α-diversity and equitability was identified in the habitat found outside, thus the natural light, the higher organic and nutrient input have influenced the composition of the epizoic diatom community. The Jaccard similarity indices separates the natural environment from the controlled one in two distinct groups, with a higher similarity value observed in the greenhouse samples (0.55). Finally, the results indicate that an ecosystems diversity is indirectly proportional to the anthropic contribution to it.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

First record of *Atypophthalmus umbratus* (de Meijere, 1911) (Diptera, Limoniidae) from Central Europe, a species introduced accidentally throughout global trade of exotic plants

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Here we report the first data of the exotic *Atypophthalmus umbratus* (de Meijere, 1911) from two Central European countries, Romania and Hungary. This is the first time that the presence of an introduced exotic species of Limoniidae (Diptera) has been reported in the area. The above-mentioned species was first observed in Cluj, Romania, on tropical plant specimens (e.g. *Alocasia x mortfontanensis* 'Polly'), which we bought from a large store selling tropical plants, from which both male and female specimens were collected. After that, checking some citizen science platforms of entomological interest, we also noticed record of the species in Hungary, based on their conspicuous wing pattern and general habitus. The presence of this accidentally introduced species far from its native tropical environment highlights the growing intensity of the global exotic plant market and the importance of citizen science in early warning systems for biological invasions.

Imaginal feeding of twenty-two Japanese endemic winter stoneflies (Plecoptera: Capniidae)

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The winter stonefly genera *Apteroperla* Matsumura, 1931 and *Eocapnia* Kawai, 1955 are restricted to Honshu and Hokkaido of Japan. They are well known for their aptery and association with habitats having thick snow cover during the whole cold season. Recently, the taxonomy of both genera is under revision, and several new species will be added to the currently distinguished three *Eocapnia* Kawai, 1955 and six *Apteroperla* Matsumura, 1931 species. In the present study, we investigated the feeding of the adults of 16 *Apteroperla* and five *Eocapnia* species, by means of dissection and analysis of their gut content. *Takagripopteryx nigra* Okamoto, 1922, an additional Japanese winter stonefly was also studied, to compare the feeding of the small sized and apterous taxa with a cohabiting larger, winged species. Contrary to most other stonefly imagoes, the studied species proved to be actively feed. Their guts contained various pollen, plants and fungi, as well animal remnants. The diet of each species seems to be different, but differences probably refer on the food supply of different habitats, and these stoneflies are most probably opportunistic feeders.

Keywords: *Apteroperla*, *Eocapnia,* gut content, *Takagripopteryx*.

On the tracks of crayfish evolution: A multidisciplinary approach

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Aquatic environments pose unique challenges for organisms due to fluctuating oxygen levels. Crayfish were generally considered freshwater dwellers, often encountering hypoxic or anoxic conditions, particularly in aquatic burrow habitats due to low oxygen diffusion in water but there are some species which dig galleries in soil, away from any water sources, living in aerated burrows. This divergence in specialization prompts questions about the evolution and transmission of anoxia tolerance mechanisms among crayfish populations. To survive in such environments, crayfish rely on various physiological and behavioral adaptations, including increased ventilation, cardiovascular adjustments, and metabolic rate modulation. These responses, likely inherited from their lobster ancestors, are crucial for maintaining oxygen consumption and cellular function in hypoxic conditions. However, the presence of aerated burrows in certain crayfish species challenges the necessity of anoxic mechanisms. Advanced burrow structures optimize airflow, potentially reducing the reliance on these mechanisms by ensuring adequate oxygen supply. Recent discoveries of independent losses of HIF pathway components in certain crustacean groups further complicate our understanding of anoxia tolerance mechanisms in crayfish. This underscores the importance of considering evolutionary history in shaping an organism's ability to survive in oxygen-depleted environments. Future research should focus on elucidating the molecular and physiological mechanisms underlying anoxia endurance in crayfish, as well as exploring the adaptive significance of fossorial behavior and the evolutionary context of oxygen sensing pathways in crustaceans.

Keywords: anoxia, evolution, burrow, oxygen.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Possibilities of using herbaria, digital herbaria, and plant databases in botany and biodiversity teaching at Eszterházy Károly Catholic University

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In the past herbaria were almost exclusively visited by researchers and only a small percentage of those interested had insights into collections. Digitization opened up completely new perspectives on the collection's visibility. Nowadays herbaria could have an active role in the education of botany, biodiversity, and bioconservation especially in several secondary and higher educational institutions. The Herbarium of Eszterházy Károly Catholic University (EGR) has been involved in student education for many years. The combination of studying „real" and virtual herbaria together can be a much more interesting educational approach in botany. The digital collections can bring students as well as the public closer to the world of scientific research and help preserve herbaria for the next generation. Besides the traditional utilization of herbaria, a huge possibility is using virtual plant collections as well. The main target is to highlight more important Hungarian and worldwide-used plant online resources suitable for botany teaching to the students as well as the wide public at Herbarium of Eszterházy Károly Catholic University, Eger (EGR).

Keywords: herbarium, online resources, systematics.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Preliminary results in the reassessment of *Orchidaceae* L. family within the Iron Gates Natural Park

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According to previous research, 28 species of orchids have been documented within the Iron Gates Natural Park. This study aims to reassess and incorporate recent changes in the orchid flora of the park. Field surveys were conducted in the spring of 2024, relying largely on maps where these species had been confirmed. Determination and individual counting were performed *in situ*, while geographic coordinates were collected using GPS. The surveyed areas included the southern slopes near Divici and Coronini and the Baziaș and Cracul Găioara nature reserves. Four species have been identified: *Neotinea tridentata* (Scop.) R.M.Bateman, Pridgeon & M.W.Chase (1997), *Orchis simia* Lam., 1779, *Cephalanthera damasonium* (Mill.) Druce (1906), and *Limodorum abortivum* (L.) Sw. (1799). It is noteworthy that the high temperatures during the spring growing season of 2024 hastened the flowering process of these orchids by several weeks compared to literature records.

New discoveries include the confirmation of *Limodorum abortivum* in the Cracul Găioara region and *Orchis simia* on a slope near Coronini. The study will persist throughout the entire vegetation period of orchids.

Keywords: Iron Gates Natural Park, orchids, biodiversity

Preserving biodiversity: The role of museum collections. A case study on moths from the Marg Wladimir Manoliu Lepidoptera collection at the Zoological Museum of Babeș-Bolyai University

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Our study presents an overview of the Marg - Wladimir Manoliu moth collection, currently archived at the Zoological Museum of Babeș-Bolyai University in Cluj-Napoca, Romania. M. W. Manoliu, born in 1940 in Solca, Suceava County, initiated his moth collecting pursuits in 1951, with his most prolific period spanning from 1980 to 2008. The assemblage encompasses 4858 specimens representing 464 distinct species, predominantly sourced from Cluj Napoca and Solca, supplemented by specimens acquired through exchanges or expeditions to other locales.

In Solca, specimens were procured from both local courtyards and a nearby water treatment station, utilizing a 400W mercury vapor lamp for attraction. In Cluj Napoca, collection efforts were concentrated on the balcony of Manoliu's third-floor apartment, where he deployed light traps equipped with mercury vapor lamps of varying wattages. Additionally, a 400W light trap was positioned at the Institute of Plant Protection in Cluj.

Despite its modest size and localized focus, Manoliu's collection holds significant value for biodiversity documentation and research. It offers valuable insights into species distribution and dynamics, thereby contributing to biodiversity conservation efforts. The meticulous documentation accompanying Manoliu's collection provides researchers with concrete data on species occurrences and trends, emphasizing the pivotal role of individual contributions in advancing scientific knowledge and promoting conservation endeavors.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Recent advances in the taxonomic revision of the *Dendrobaena alpina* (Rosa, 1884) species group (Oligochaeta, Lumbricidae)

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The *Dendrobaena alpina* species group, as originally defined, contained some fifteen nominal species characterized by a clitellar position on segments 26, 27–33, 34, tubercles around 30–32. It was thought that a characteristic synapomorphy of this group was the reduction of the number of hearts with the last pair in segment 10 or 11 and lacking extraoesophageals in 12, and the presence of calciferous diverticula in 11–12. On the other hand, these species show a great variability in the case of several other characteristics e.g., the number of vesicles, position of the spermathecal openings, pigmentation. In order to reveal the relationships and patterns within this species group, we applied an integrative taxonomic approach. Thorough morphological examinations were carried out on several specimens collected from the Alps, Carpathians, Balkans and Anatolia. In addition, our specimens were implemented in a wider context of the genus *Dendrobaena*, and a molecular phylogenetic reconstruction was performed using COI, 16S rDNA and ITS2 sequences. Our results revealed the presence of two well-separated clades, an Alpine-Carpathian-Balkan and a Balkan-Anatolian-Levantine group. The deeply pigmented *D. alpina alpina* from the Alps formed the first clade together with the also pigmented Northeastern Carpathian *D. alpina alteclitellata*, the Dacian *D. clujensis* and a *D. alpina* ssp. from North Macedonia. However, our study pointed out that the original *D. alpina* species group was polyphyletic, because the unpigmented Bulgarian *D. alpina* specimens joined the Anatolian and Levantine species such as *D. orientalis*, *D. pentheri*, *D. orientaloides* and *D. semitica*. Consequently, the unpigmented population from Bulgaria was described as a new species, *Dendrobaena misirlioglui*. Two slightly pigmented specimens collected in the Retezat Mts, the Southern Carpathians, Romania, identified as *D. alpina alpina* in a former study, together with the Balkan-Southern Carpathian *D. alpina popi* joined the Balkan-Anatolian-Levantine clade of *Dendrobaena* as well. The genetic and morphological characteristics support the description of these Retezat specimens as a new species. The study also revealed that the subspecies *D. alpina armeniaca* and *D. alpina popi* have no connection with *D. alpina*, thus their elevation to species rank is suggested.

Keywords: Balkans, Carpathians, earthworms, new species, Retezat Mts.

Skin-associated microbiome of the yellow-bellied toad (*Bombina variegata*) in a population from Transylvania

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Microbial symbionts play a vital role in host health. The amphibian skin crucial for ion, gas and water exchange harbors a diverse microbiome studied mainly using short-read amplicon sequencing. Here we propose to explore the diversity of skin microbiome in *Bombina variegata* by using ONT long-read sequencing technology followed by taxonomic identification by One Codex platform. The toads (n=5) were captured in small ponds located near Micești (Cluj), Romania, then swabed on the dorsal, ventral, and lateral sides. From the swab samples (n=10), DNA was extracted using the Quick-DNA Faecal/Soil Microbe MiniPrep kit (ZR, USA). For long-read sequencing, libraries were prepared using Rapid Barcoding 24 V14 kit (ONT, UK) and the sequencing was performed using R10.4.1 chemistry flow cells and a MinION Mk1B sequencer from Oxford Nanopore Technologies. The sequences were analised using One Codex. A total of 9308 reads were generated from the samples. The analysis in the One Codex revealed that *Pseudomonadota* (64.44%), *Actinomycetota* (14.5%), *Bacteroidota* (3.53%) and *Cyanomicrobiota* (1,89%) are the most abundant phyla associated with the skin microbiome of the yellow-bellied toad. The analyses also indicated that the sample was contaminated with host-specific DNA (59%). While host DNA contamination highlights the need for method optimization, this study demonstrates the potential of long-read sequencing combined with the easy-to-use One Codex platform, for comprehensive characterization of amphibian skin microbiomes.

Keywords: *Bombina variegata*, long-read sequencing, microbiome.

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Snapshot on large and medium-sized mammals in Ceahlău National Park

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Knowledge of the diversity, relative abundance and activity pattern of large and medium-sized mammal species is of major importance for management planning. With an area of 8,396 ha, Ceahlău National Park is the third smallest park in Romania, the entire area having the status of a Natura 2000 site. Until now, no qualitative and quantitative information has been published regarding large and medium-sized mammals in the park. Therefor, the aim of this study was to evaluate the diversity of large and medium-sized mammal species, their relative abundance and the circadian pattern of activity. The study was carried out between October 2022 and November 2023, using a number of 16 Moultrie and Bushnell camera traps. No scent lure or another attractant was applied. Since activation, the camera traps have been in the working mode throughout the research period. During the 1-year period, 3460 trap nights were processed, 557 images with large and medium-sized mammal were obtained and 14 mammal species were recorded, five of them being herbivores and nine carnivores. The herbivore species identified were *Cervus elaphus, Capreolus capreolus, Sus scrofa, Rupicapra rupicapra* and *Lepus europaeus*, and the carnivorous species were *Canis lupus, Felis silvestris, Lynx lynx, Martes foina, Martes martes, Meles meles, Mustela putorius, Ursus arctos* and *Vulpes vulpes*. With 243 records, *Capreolus capreolus* was the most abundant species, followed by *Cervus elaphus* with 92 records. Out of a total of 24 records, the combined share of photos featuring large carnivores like wolves, lynxes, and bears amounts to just 4.31% of the overall count. Most likely, for the large carnivores, which inhabit large territories, of the order of tens of square kilometers, the surface of the Ceahlău National Park is relatively small, so they cross National Park boundaries, expanding outside the park, while for herbivores the habitat is optimal. Our findings provide the first qualitative and quantitative data on the fauna of large and medium-sized mammals in Ceahlău National Park.

Keywords: camera traps, carnivores, herbivores, large mammals.

Acknowledgements. Our study was carried out with the help of the Cehlău National Park Administration

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 69, 1, 2024, ABSTRACT

Spatial occupancy estimation and modeling of grey wolf (*Canis lupus*) of the Bükk Mountain in Hungary

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The wolves inhabiting the Bükk Mountain in Hungary play a vital role in maintaining the region's biodiversity and ecosystem balance. Understanding their behaviour and ecology is crucial for developing effective conservation strategies that ensure their long-term survival in the Bükk mountain landscape. As apex predators, they help regulate the population of herbivores like deer, roe deer and boars, thereby prevents forest vegetation from overgrazing. Despite facing various challenges such as habitat loss and human-wildlife conflicts, efforts are being made to study and conserve the Bükk mountain wolf population. The utilization of camera traps in studying the wolves of the Bükk Mountain in Hungary has renewed: revolutionized our understanding of these elusive predators. In the present study, we employed network of camera traps to observe the key mammal species in the area, to assess their occupancy and detection probabilities and their interactions with wolves. Our results reveal the dominant territorial presence of wolves compared to other species. Remarkably, wolves demonstrate resilience in the face of primary roads and forest trails but exhibit avoidance behaviour towards populated settlements and open areas. By harnessing the capabilities of a camera trap network conservation efforts can be enhanced by monitoring wolf populations, identifying threats, and developing targeted protection strategies to ensure the long-term viability of these majestic species in the Bükk mountain ecosystem.

Keywords: *Canis lupus*, Bükk Mountain, occupancy models.

Study of the spiders (Arachnida: Araneae) in the Cheile Baciului Reservation

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Cheile Baciului is a geological reservation, situated North-West of the Hoia Forest, close to Cluj-Napoca, comprising of habitats with clear anthropogenic influence. The area is lacking in information on the present arachno-fauna thus this study aims to expand and contribute to the knowledge of Romania's spider fauna, with Cheile Baciului as a target by realizing a list of species. The spiders were collected through different methods, excluding traps*, from the 10th of May 2023 to the 14th of August 2023. The study area was split into 7 points of collecting based on the differences in habitat vegetation composition. The specimens have been conserved in 75% isopropyl alcohol and identified in the laboratory, using a stereoscopic microscope and various identification keys and lists. Juveniles, males and females have been collected, numbering to a total of 80 species from 18 families. A remarcable diversity considering these habitats are clearly affected by human activity. Some dominant species of note are *Mangora acalypha, Neriene radiata, Synema globosum and Evarcha falcata*. Some rare species also have been identified as well as species present on red lists of other countries of the European Union, such as *Nematogmus sanguinolentus, Leptorchestes berolinensis* and *Uloborus walckenaerius*. These results show a rich diversity of spiders in this reservation. This could prompt the upgrading of this area into a mixed natural reservation on top of its geological status, with the conservation of the arachno-fauna in mind. Moreover, the rare species could be added to the red list of Romania's spiders. This would be a useful first move for conservation efforts concerning spiders, something that is lacking in Romania. If possible, a more comprehensive faunistic study should follow, to have an even more complete image of the ensemble of Araneae from Cheile Baciului. **Collecting inside Cheile Baciului was realized with access from the reservation overseers. Only manual collecting was done, no traps were used.*

Keywords: spider, conservation, biodiversity, habitat, rare species.

The effect of different light treatments of chamomile (*Matricaria chamomilla* L.)

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Chamomile occurs naturally in Hungary and also it is a cultivated medicinal plant species. The seeds germinate in direct light, and we do not know the limits of environmental conditions of the early period of germination. The basic research question was whether the early short light treatments of chamomile seeds and seedlings have further effect on the development of the plant individuals. Previous experiments showed that seeds can germinate well in distilled water without soil. Climate chamber experiments were elaborated with two different light spectra and continuous light exposition with parallel control observations with chamomile seeds. The treated individuals have been observed in the greenhouse and roof garden until they reached their maximal vegetative size. The results show that the early light treatments have effect on the further development stages of plant individuals. It is also a new result that all the groups successfully germinated and grew in distilled water for two weeks with low mortality rate.

Keywords: chamomile, light treatment, seedling observation, germination

The egg collection of the Zoological Museum of Babeș-Bolyai University

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An important oological collection is held in the Zoological Museum of Babeș-Bolyai University, which is unique in many ways: it covers a long-time span, it contains a variety of species belonging to different families and orders of both invertebrates and vertebrates, and it is composed of the work of several naturalists. It is one of the oldest collections of the museum. To date, however, no research has been conducted on this collection. The specimens had only been partially catalogued and no updates or revision had been carried out. Hence, our aim was to systematically check the egg specimens in the oological collection in order to identify the species to which they belong, thus providing a catalogue of these specimens.

Up to date, we identified a total of 2801 eggs, attributed to 170 species, 48 families and 19 orders. A significant proportion of the egg collection comes from Romania, mainly from the region of Cluj, but there are also collections from other areas of Transylvania. In addition, many specimens were collected in Hungary, Macedonia, Greece, Italy and the United Kingdom. The egg collection held by the Zoological Museum of the BBU spans the period between 1848 and 1960.

Considering its historical background and the presence of rare species, this collection can be viewed as one of the most valuable oological collections of Eastern-Europe, which could serve as good basis for further studies. In addition, the maps that we created represent ‘hotspots’ of faunistic information concerning the distribution of different species in the Carpathian Basin from the 19th and 20th century.

The revision of the ornithological collection of the Zoological Museum of Babeș-Bolyai University, Cluj-Napoca, Romania

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The collections at the Zoological Museum of Babeș-Bolyai University, Cluj-Napoca, Romania are among the oldest, most diverse, and historically most interesting natural history collections. The museum houses a large ornithological collection consisting of skins, full taxidermic mounts of birds, eggs, nests and birds' skeletons, which have never been revisited. Here we present the catalogue of the skins and taxidermic mounts of birds deposited or exhibited at the Zoological Museum of Babeș-Bolyai University, Cluj-Napoca, Romania. We identified 2876 specimens, belonging to 489 species from 105 families and 32 orders. The collection includes numerous local and exotic rarities. The information held in this collection can be used as a basis for many valuable ornithological studies. This collection also represents a source of information for the status of the avifauna of the Carpathian basin in the 19th and 20th centuries.