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Molecular Biology and Biotechnology

Genomics of the Viking world

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The Viking Age was a short period in northern European history, spanning roughly from 750 (793) to 1050 CE (1066), corresponding to little more than twelve human generations. Within this brief timeframe, Scandinavia underwent profound cultural transformation, beginning as a predominantly pagan society and gradually becoming part of Christian Europe. At the same time, people from Scandinavia expanded across an extraordinary geographic range, reaching four continents through trade, migration, and raiding. This outward movement has long captured both scholarly and popular imagination. Viking mobility connected Scandinavia to regions as distant as North America, the Byzantine Empire, and the Islamic world, leaving archaeological and historical traces of far-reaching networks. However, recent advances in archaeogenomics allow us to complement this outward looking narrative with a more nuanced question: who came to Scandinavia, and why? Genomic data now reveal that the Viking world was not only defined by expansion, but also by substantial inward mobility. Urban centers such as Birka and Sigtuna show considerable genetic diversity, reflecting individuals of local, regional, and even continental origins. These findings suggest that Viking Age Scandinavia was embedded in wider networks of interaction, attracting people through trade, political connections, and emerging urbanization. Thus, the Viking Age can be understood not only as a period of Scandinavian expansion, but also as a time when Scandinavia itself became a destination. By integrating genetic data with archaeology and history, we gain a more balanced view of the Viking world as a dynamic and interconnected system shaped by both outward and inward mobility.

Keywords: Viking Age; archaeogenetics; mobility; population demographics.

Funding: Riksbankens Jubileumsfond; Sweden; Vetenskapsrådet; Sweden

Like fish out of water? Elucidating the ecophysiology of upland-soil methanogens through cultivation and 'omics methods

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Methane is a potent greenhouse gas produced almost exclusively by anaerobic methanogenic archaea. Accordingly, methanogenesis was thought to occur only in anoxic environments such as waterlogged soils, aquatic sediments, and animal guts. However, our work over the past years has progressively challenged this notion. We first showed that methanogens belonging to *Methanosarcina*, *Methanobacterium*, and *Methanocella* are globally ubiquitous in aerated upland soils and can be readily activated under wet anoxic conditions. In arid biological soil crusts (biocrusts) from the Negev Desert, we demonstrated active methanogenesis even under oxic conditions, with both genera transcribing the key *mcrA* gene as well as oxygen-detoxifying catalase genes. Importantly, while in situ measurements showed that undisturbed arid soils harbour active methanotrophs belonging to uncultured *pmoA* lineages, these were absent from the biocrust layer itself—pointing to a previously unrecognized role of desert soils in the global methane cycle. Using RNA-stable isotope probing we further revealed complex microbial resuscitation dynamics in biocrusts following simulated rainfall, with distinct aerobic and anaerobic communities developing rapidly upon wetting. Building on this foundation, we recently enriched and genomically characterized seven methanogenic cultures from Israeli and Namibian desert biocrusts, affiliated with *Methanosarcina*, *Methanocella*, and *Methanobacterium*—the first methanogen isolates from oxic soils, six of which represent new species. Comparative genomics revealed diverse repertoires of antioxidant and desiccation-resistance genes, yet no evolutionary distinction from relatives in anoxic habitats. Notably, *Methanobacterium*—a Class I methanogen expected to be oxygen-sensitive—possessed the fewest stress-tolerance genes, challenging assumptions about Class I methanogens' oxygen sensitivity. Through controlled experiments coupled with comparative (meta)genomics, transcriptomics, and physiological assays, our data provide first insights into the unique adaptation mechanisms of these organisms and expand the known ecological breadth of methanogenic archaea.

Keywords: Archaea; biocrusts; drylands; methane; stable isotopes.

Mitochondrial dynamic redistribution regulates phenotype-specific migration during confinement-induced mesenchymal-to-amoeboid transition

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Metastatic dissemination relies on pronounced phenotypic plasticity, particularly the epithelial-to-mesenchymal transition that enhances tumor cell motility. Under increased physical confinement, mesenchymal cells can undergo a further switch to an amoeboid phenotype through the mesenchymal-to-amoeboid transition (MAT). Although amoeboid cells are considered highly metastatic, they remain poorly characterized due to limited experimental models. Mitochondrial redistribution to the leading edge is known to sustain mesenchymal migration by supplying localized energy, yet its contribution to amoeboid motility remains unclear. Here, we investigated how mitochondrial positioning regulates MAT during confined melanoma cell migration in a microfluidic approach. Microfluidic devices comprising a central reservoir connected to 600 µm-long microchannels of varying widths (6–50 µm) were used to model physical confinement. A375 melanoma cells were monitored during 3D migration by live-cell microscopy and classified as mesenchymal or amoeboid based on actin organization (LifeAct-Ruby), leading-edge protrusions, and focal adhesion distribution (Paxillin-GFP). Mitochondrial positioning (mito-YFP) was quantified using a Mitochondrial Localization Index. Migratory capacity was assessed by penetrance and single-cell velocity along the channels. Amoeboid cells exhibited cortical actin enrichment, membrane blebbing, and absence of focal adhesions, whereas mesenchymal cells displayed actin stress fibers spanning the cell body and lamellipodia or filopodia at the leading edge. Mesenchymal cells were further subdivided into M1, retaining trailing-edge focal adhesions and M2, lacking a defined trailing edge. Functionally, amoeboid cells showed the greatest migratory capacity, followed by M2 and M1 cells. Mitochondrial distribution strongly correlated with phenotype: posterior localization predominated in amoeboid cells, anterior localization in M2 cells, and unpolarized distribution in M1 cells. Increasing confinement enhanced the frequency of amoeboid cells, reduced mesenchymal subpopulations, promoted posterior mitochondrial redistribution, and selectively increased the migratory capacity of M2 and amoeboid cells. Under strong confinement, amoeboid cells with posterior mitochondria and M2 cells with anterior mitochondria displayed the greatest motility. Overall, spatial mitochondrial redistribution emerges as a key determinant of phenotype-specific migratory capacity under confinement and might facilitate melanoma MAT.

Keywords: mesenchymal-to-amoeboid transition; metastasis; microfluidics; mitochondria.

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Characterization of a novel primordial germ cell-specific monoclonal antibody

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Primordial germ cells (PGCs) are the precursors of the germline, playing a fundamental role in gametogenesis and the transmission of genetic information across generations. Precise identification and characterization of PGCs are essential for advances in developmental biology, germline preservation, and reproductive science. This study aims to characterize a novel mouse monoclonal antibody (clone name: 30B6) originally generated against bursa of Fabricius cell suspension. Preliminary immunocytochemical analyses indicate that 30B6 labels PGCs during early developmental stages. In avian species, identification of PGCs requires multiple markers, as no single marker consistently labels PGC populations throughout development. We hypothesize that 30B6 exhibits greater sensitivity and specificity for PGC detection than currently available markers and have broader applicability across various species and developmental stages. We examined the staining characteristics and specificity of the antibody in different developmental stages of chicken embryos and adult lymphoid organs using immunohistochemical and immunofluorescent staining techniques. Our findings indicate that the 30B6 antibody specifically marks PGCs in birds from early developmental stages, showing intense immunoreactivity in the germinal crescent of a 20-hour-old (Hamburger-Hamilton stage 4) chicken embryo. 30B6 provides more distinct labelling of PGCs in the gonadal ridges than commercially available markers (DAZL, EMA1, SSEA1). Additionally, 30B6 recognizes goblet cells lining the intestinal epithelium in birds, reptiles, and mammals, suggesting broader cross-species specificity. Taken together, these findings establish 30B6 as a novel and versatile monoclonal antibody for the detection of developing PGCs, with broad utility in comparative, developmental, and reproductive biology.

Keywords: avian; primordial germ cells; monoclonal antibody.

Cell-free immunomodulation using curcumin-loaded dendritic cell EVs to enhance CD8⁺ T-cell activation in an *in vitro* model

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Malignant melanoma is a highly aggressive cancer with strong metastatic potential and limited responsiveness to conventional therapies. Although immune checkpoint inhibitors such as PD-L1 blockade have improved clinical outcomes, therapeutic resistance remains a major challenge, highlighting the need for combinatorial immunomodulatory strategies. Extracellular vesicles (EVs) derived from immune cells have emerged as promising cell-free platforms for antigen delivery and immune modulation due to their ability to transfer bioactive molecules involved in antigen presentation and immune signaling. Here, we developed an immunostimulatory nanoformulation based on curcumin-loaded EVs (EVs-CURC) derived from CpG-ODN-activated and Trp-2-pulsed dendritic cells, designed to enhance anti-tumor immunity in combination with PD-L1 blockade. EVs were isolated using ultrafiltration coupled with size-exclusion chromatography (UF-SEC) and characterized by dynamic light scattering (DLS), Western blotting, and LC-MS/MS proteomics. They exhibited a predominant size of ~160 nm, consistent with small EVs, and expressed canonical EV markers (TSG101, LGALS3-BP, Stomatin), while lacking the Golgi marker GM130. Proteomic analysis revealed the presence of proteins associated with immune regulation, antigen presentation, and CD8⁺ T-cell activation in EVs isolated from activated dendritic cells compared to EVs enriched from non-activated dendritic cells. Functionally, EV-CURC significantly enhanced CD8⁺ T-cell activation, as demonstrated by increased CD69 expression observed via flow cytometry and elevated secretion of Granzyme B and TNF- α , as shown by ELISA assays. Importantly, curcumin encapsulation improved the immunomodulatory efficacy compared to free curcumin. Therapeutic efficacy was evaluated in a 3D heterocellular spheroid model mimicking the immunosuppressive melanoma microenvironment. Combination treatment with EVs-CURC and anti-PD-L1 antibody reduced spheroid viability by 50% ($p < 0.0001$) and volume by two-fold ($p < 0.01$) after 48 h. Collectively, these findings demonstrate that curcumin-loaded dendritic cell-derived EVs enhance CD8⁺ T-cell responses and represent a promising cell-free adjuvant strategy to potentiate PD-L1 blockade in melanoma.

Keywords: extracellular vesicles; curcumin; melanoma; immunomodulation.

Funding: This work was funded from UEFISCDI project PN-III-P1-1_1-TE-2021-0366 "Targeted therapy for the treatment of melanoma based on co-administration of anti-PD-L1 antibodies and curcumin-loaded extracellular vesicles", granted to Alina Sesarman.

Acknowledgements: The DC2.4 cell line was kindly provided by Dr. Loredana Saveanu from the Centre de Recherche sur l'Inflammation, Faculté de Médecine X Bichat, Paris, France.

Immunomodulatory therapy for melanoma based on co-administration of PD-L1-specific antibodies and curcumin-loaded extracellular vesicles from activated dendritic cells

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Despite the clinical success of immune checkpoint inhibitors (ICIs) in advanced melanoma, primary and acquired resistance remain major barriers, and durable responses are confined to a subset of patients. Inhibition of Programmed cell death protein 1 (PD-1) can promote compensatory upregulation of Cytotoxic T-lymphocyte associated protein 4 (CTLA-4), further limiting antitumor T-cell activity. These challenges underscore the need for novel strategies that reprogram the tumor immune microenvironment and enhance ICI efficacy. In this context, the aim of our study was to improve the response rate of melanoma to an anti-PD-L1 antibody based ICI through an adjuvant therapeutic approach based on curcumin-loaded extracellular vesicles (EV-CURC) derived from activated dendritic cells. Therapeutic efficacy was assessed in 3D heterocellular melanoma spheroids generated by liquid overlay and in C57BL/6 melanoma models. Spheroid viability and volume, as well as tumor growth, were measured. Treatment-induced molecular and mechanistic changes were analyzed by transcriptomics, proteomics, fluorescence microscopy, HPLC, ELISA, Western blotting, protein arrays, gel zymography, and enzymatic assays. Our results showed tumor growth reduction *via* fine-tuning immunomodulatory effects of the combined therapeutic strategy. Antitumor immune regulation was induced by an overall oxidative stress decrease, by overexpression of tumor necrosis factor ligand (TNF- α) and cytotoxic T-cell costimulator receptor TNFR2, as well as concomitant upregulation of TNF superfamily members – CD 27, a known costimulatory receptor for T-Cell expansion and CD 40, involved in adaptive immune priming. Compensatory mechanisms of resistance were hindered by inhibition of invasion-related effector matrix metalloproteinase 2 (MMP-2), sustained by simultaneous upregulation of its direct inhibitor, tissue inhibitor of matrix metalloproteinase 2 (TIMP-2). Inhibitory action on CTLA-4 co-stimulatory molecule CD-80 and downregulation of Fas-FasL-mediated immune cell apoptosis in the tumor microenvironment confirmed EV-CURC as an enabler of ICI efficiency, by abrogating acquired resistance responses.

Keywords: immunotherapy; extracellular vesicles; adjuvant; immunomodulation; melanoma.

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Acknowledgements: The DC2.4 dendritic cell line was kindly provided by Dr. Loredana Saveanu from the Centre de Recherche sur l'Inflammation, Faculté de Médecine X Bichat, Paris

Understanding fungal heavy-metal resistance: mechanisms underlying remediation opportunities

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Soils affected by mining and industrial activities are frequently contaminated with hazardous concentrations of heavy metals, among which mercury (Hg) is particularly persistent and toxic due to its high mobility, bioaccumulation potential, and long-term ecological risks. Emphasizing indigenous, site-adapted organisms and microorganisms is crucial to exploit naturally evolved resistance traits for effective and sustainable soil remediation. A collection of over 150 metal-resistant micromycetes was established with a culture-based approach, using soil from a severely Hg-contaminated site. Biomass developed by five fungal species performed 47-97% Hg²⁺ removal from aqueous solution in 48 h (100 mg L⁻¹ initial). Screening for tolerance to Cd, Cu, Hg, Pb, and Zn identified several highly resistant isolates, including a highly tolerant *Fusarium oxysporum* (minimum inhibitory concentrations in mg kg⁻¹: 240 – Cu, 140 - Hg, 2092 - Zn). Microbiological assays combined with ICP-MS analysis revealed multiple resistance strategies in this isolate to Cu, Hg, or Zn: cell wall sorption, intracellular accumulation, and indications of Hg volatilization. Bacterial mercuric reductase (MerA) is known to reduce the reactive Hg²⁺ to the volatile Hg⁰, which readily escapes the cell, enabling detoxification. However, the mechanism of Hg²⁺ reduction in Fungi remains unclear. Genomic analysis of *F. oxysporum* identified a MerA-like gene. Transcript detection under both Hg-exposed and non-exposed conditions suggested constitutive expression, contrasting with the inducible regulation typical of bacteria. Phylogenetic analysis was further employed to infer evolutionary relationships of the MerA across life Domains: Archaea, Bacteria, and Eukarya (Fungi). These findings link Hg removal efficiency with the presence of a MerA-like system in *F. oxysporum*, with a possible constitutive expression. Ongoing work focuses on functional characterization of the fungal MerA-like and broader Hg stress responses, with the aim of elucidating fungal Hg detoxification mechanisms to support the development of targeted Hg bioremediation strategies.

Keywords: heavy metals; mercury; micromycetes; mycoremediation; resistance mechanisms.

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Twenty years later: how remediation strategy shapes soil bacterial recovery at two metal-contaminated sites

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Long-term exposure to pollutants such as heavy metals and organic compounds, threatens soil ecosystem integrity and public health, necessitating effective intervention strategies. However, the long-term ecological effect of these strategies remains to be understood. This study examines how contrasting remediation strategies shaped soil bacterial communities two decades after implementation at two contaminated sites. At the Homécourt (France) site, a coking plant operated for many years, inadvertently introducing polycyclic aromatic hydrocarbons (PAH) alongside Cd and Hg into the soil. Restoration followed an engineered approach: thermal desorption was deployed to eliminate contaminants, complemented by pedological reconstruction and establishment of grassland vegetation cover. The Moldova Nouă site (Romania) has a long history of copper mining which lead to elevated Cu, Zn, Pb, and As levels in soils. No contaminant removal was attempted; instead, interventions focused on establishing perennial grassland species and afforestation. Soil microbial diversity and community composition were profiled using environmental DNA metabarcoding. Comparing treated plots against untreated contaminated controls and unimpacted reference ecosystems, we evaluated both alpha and beta diversity to determine whether interventions promoted ecological restoration, the emergence of novel communities, or persistent degradation. The biodiversity at the two sites exhibited different trends. At Homécourt, engineered remediation drove significantly elevated species richness relative to reference soils, while community composition remained strongly differentiated from reference conditions. Treated communities were structured primarily by nutrient availability and soil physical properties, whereas untreated controls continued to reflect PAH contamination gradients. At Moldova Nouă, richness did not differ significantly across groups, and the treated and reference communities were more similar to one another than at Homécourt, suggesting that nature-based approaches-maintained trajectories more aligned with natural succession, although persistent metal contamination continued to constrain recovery. These results highlight a fundamental trade-off in remediation ecology: engineered strategies can decisively improve soil conditions and eliminate pollutant pressure, yet their application remains largely confined to smaller, well-defined areas and risk assembling microbial communities with no historical analog, while nature-based solutions may better conserve the directionality of natural recovery at the cost of pace.

Keywords: eDNA; metals; pollution; remediation; soil microbiota.

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Distinct structuring of the rhizosphere microbiome in a legume growing across various degraded soils

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Soils provide approximately 95% of global food production and represent a major carbon sink (~0.4 – 1.2 gigatons of carbon sequestered per year). However, nearly 33% of the world's soils are currently degraded because of salinisation, acidification, contamination, alkalisation, erosion, etc. The EU Soil Directive highlights the urgent need for soil protection and restoration strategies, as soil is a vital and non-renewable resource. Severely degraded habitats represent valuable natural systems for identifying plant species and associated microorganisms that have adapted to extreme conditions and developed mechanisms that can be exploited for environmental remediation. Yet, such environments remain poorly documented, limiting the development of targeted soil restoration strategies. This study assessed the structure and diversity of bacterial and fungal communities associated with the rhizosphere and nodules of a legume species naturally growing in four degraded sites, impacted by different dominant stressors—mercury (Hg), hexachlorocyclohexane (HCH), copper (Cu), and moderate salinity (NaCl). The selected plant species is consistently found – often as a dominant species – at degraded sites and, through its capacity to establish symbiosis with rhizobia and perform nitrogen fixation, plays a key role in improving soil nutrient status and facilitating the establishment of subsequent plant communities for remediation purposes. QIIME2 pipeline was used for sequencing data processing, with downstream analysis conducted in R using *vegan* and *phyloseq* packages. The HCH-contaminated site exhibited the most distinctive microbial composition, with higher abundances of *Acetobacteraceae*, *Frankiaceae*, *Trichocomaceae*, and *Herpotrichiellaceae*. Redundancy analysis (RDA), integrating soil physico-chemical parameters and nitrogen-cycle genes (*amoA*, *amoB*, *nirS*, *nirK*), revealed environmental drivers shaping microbiome composition. The nodule compartment was dominated by *Rhizobiales*, although non-rhizobial taxa were also detected (e.g., *Pseudomonas*). Identification of the rhizosphere microbiome represents the foundation for designing microbial synthetic communities for phytomanagement strategies. Future assessment of plant molecular responses to Hg and HCH will improve our understanding of the legume's resilience and of its use for soil remediation.

Keywords: degraded sites; legumes; microbiome; nodule; rhizosphere.

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Isotopic insights into specialized husbandry practices in medieval south-eastern Romania

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Stable carbon and nitrogen isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) provide critical insights into past subsistence and livestock management. This study investigates animal husbandry practices in the proximity of a 10th century fortress in south-eastern Romania, a region currently underrepresented in the isotopic record. Bone collagen analysis was conducted on local fauna (n = 39 domestic; n = 1 wild). Results indicate a predominantly C3 based diet for most species, however, cattle values reveal significant C4 supplementation. This unexpected discovery suggests specialized differential management strategies at this medieval site. Further evidence for complex husbandry is seen in the elevated $\delta^{15}\text{N}$ signatures of caprids, likely reflecting grazing on crop residues from manured fields. These findings demonstrate that local populations possessed an advanced understanding of species-specific dietary ecology. Beyond reconstructing past husbandry strategies, this study establishes an indispensable environmental baseline for interpreting the isotopic signals of the associated human population.

Keywords: animal management; husbandry; local baseline; medieval; stable isotopes.

Funding: Doctoral research grant.

Development and Validation of the Academic Stress Resilience Index (ASRI) in Undergraduate Medical Students

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Academic stress resilience in undergraduate medical students represents a critical determinant of their capacity to sustain engagement in the learning process and acquire the knowledge and competencies essential for future professional practice. In this context, the present study aims to develop and validate a composite index, termed the Academic Stress Resilience Index (ASRI), to assess stress adaptation in undergraduate medical students. A cross-sectional study was conducted among 55 first- and second-year medical students aged 18–20 years. Four variables were assessed: circadian rhythm (MEQ-SA questionnaire), heart rate variability (HRV), derived from resting ECG recordings (BIOPAC MP100) and analyzed using Kubios HRV with FFT-based parameters, academic performance measured by GPA on a 10-point scale, and academic burnout (MBI-SS questionnaire). Selected variables were combined to derive a composite index termed the Academic Stress Resilience Index (ASRI). Statistical analysis was performed using SPSS version 26 (IBM Corp.). The ASRI ranged from –2 to +2 and was categorized into three levels: high (–2 to –0.5), moderate (–0.5 to 0.5), and low resilience (0.5 to 2). ROC analysis demonstrated a strong discriminative ability for academic burnout (AUC = 0.892). A strong positive correlation was observed between ASRI and academic burnout ($r = 0.838$, $p < 0.001$). Binary logistic regression confirmed the statistical significance of the model ($\chi^2 = 29.415$, $p < 0.001$), explaining 41.4% to 55.2% of the variance in academic burnout, with an overall classification accuracy of 87.3%. Based on the findings of this study, it can be concluded that the Academic Stress Resilience Index (ASRI) demonstrates strong validity and predictive performance, as evidenced by its high discriminative ability, significant correlation with academic burnout, and robust regression model. Therefore, ASRI may be considered a reliable and effective measure for determining stress adaptation in undergraduate medical students.

Keywords: academic burnout; circadian rhythm; heart rate variability; medical students; stress resilience.

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Development of an enzymatic technology for the synthesis of 3(4-hydroxy-3-iodophenyl) propanoic acid and its application in the iodine biofortification of *Lactuca sativa* crops

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Iodine deficiency represents a persistent global health concern, necessitating innovative strategies for micronutrient enrichment in human diets.^[1] Plant biofortification has gained prominence as a sustainable solution, with iodo-cumaric acid emerging as a key vehicle for enhancing iodine uptake in leafy vegetables such as *Lactuca sativa*. This study focuses on the biocatalytic production of iodo-cumaric acid leveraging the catalytic prowess of phenylalanine ammonia-lyase from *Rhodospiridium toruloides* (RtPAL). Wild-type PAL from *Rhodospiridium toruloides* (RtPAL) was expressed in *E. coli* Rosetta (DE3) pLysS host cells using the pET-19b vector.^[2] Following purification via Ni-NTA affinity chromatography, the enzyme's homogeneity was verified through SDS-PAGE analysis. Detailed kinetic characterization was performed spectrophotometrically at 310 nm to compare the transformation of the natural substrate, L-phenylalanine, with the non-natural analogue, 3-iodo-L-tyrosine. Our findings reveal a remarkable catalytic preference: although RtPAL maintains a higher affinity for its native substrate, the conversion of 3-iodo-L-tyrosine proceeded 15 times faster. Furthermore, the catalytic efficiency (K_M , V_{max} , k_{cat} , k_{cat}/K_M) for the iodinated substrate was 10-fold higher than that of the natural one. The synthesized iodo-cumaric acid was successfully internalized by *Lactuca sativa* plants. Following harvest and analysis, the data confirmed the plants' capacity for compound uptake, suggesting a significant potential for this method in agrochemical biofortification strategies. To enhance the scalability and efficiency of this biocatalytic process, preparative-scale transformations have already been successfully conducted using whole-cell biocatalysts. While whole-cell systems offer a practical approach for initial production, the presence of cellular debris often complicates the subsequent purification and downstream processing. Therefore, the immobilization of the purified enzyme or whole-cells is proposed as a crucial step to enhance both purity and process efficiency.

Keywords: biocatalysis; biotechnology; enzyme immobilization; phenylalanine ammonia-lyase; iodine biofortification.

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Deciphering symbiotic efficiency: genotypic and phenotypic characterization of *eds1a*, *eds1b*, *bir1-like*, *bir2-like*, and *cdki1* *Lotus japonicus* mutants

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The symbiotic interaction between legume plants and nitrogen-fixing bacteria (*Rhizobia*) requires the host plant's immune system to efficiently differentiate between symbionts and potential pathogens. However, while genes associated with immune defense (such as *eds1a*, *eds1b*, *bir1-like*, and *bir2-like*) and cell cycle regulation (*cdki1*) are well-studied in pathogenic contexts, their specific regulatory roles during beneficial nodulation remain missing from current literature. Yet, mapping this genetic crosstalk is essential to figure out how plants balance protecting themselves from disease while simultaneously allowing beneficial root infections to occur. This study evaluates the genotypic and phenotypic characteristics of *Lotus japonicus* knocked-out mutants for the *eds1a*, *eds1b*, *bir1-like*, *bir2-like*, and *cdki1* genes to determine their impact on nitrogen fixation efficiency. Genotypic characterization was performed via PCR analysis and sequencing, while the phenotypic evaluation was conducted using the Acetylene Reduction Assay (ARA), alongside measurements of nodule weight and viable nodule counts. The collected data are currently being analysed to assess the symbiotic performance of the mutant lines compared to the wild-type. Specifically, the comparative analysis aims to reveal how the disruption of Effector-Triggered Immunity (ETI) signalling pathway (*eds1a*, *eds1b*), the potential constitutive activation of basal immunity (*bir1-like*, *bir2-like*), and the alteration of cell cycle regulation (*cdki1*) influence nodular biomass and overall nitrogenase activity, the rhizobial enzyme system responsible for reducing atmospheric nitrogen (N₂) to ammonia, which can be assimilated by plants. Ultimately, these findings will provide deeper insights into the complex molecular mechanisms by which *L. japonicus* balances immune defense and root organogenesis to facilitate the symbiotic infection.

Keywords: Acetylene Reduction Assay (ARA); Effector-Triggered Immunity (ETI); *Lotus japonicus*; nitrogen fixation; symbiosis.

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Platinum(IV) - NSAID conjugates: A promising therapeutic strategy for Triple-Negative Breast Cancer

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Breast cancer is the leading cause of female cancer diagnosis and the second in mortality. Triple-negative breast cancer (TNBC) is particularly aggressive with limited options. While Cisplatin, a Platinum(II) complex, is a standard therapy, its use is hindered by toxicity and resistance. To overcome these limitations, Platinum (IV) complexes have emerged as superior alternatives. These prodrugs have a higher chemical inertness and are selectively activated within the hypoxic, reductive tumor microenvironment, where they undergo hydrolysis to release active Cisplatin and their respective axial ligands. This study investigated the antitumor potential of two novel Platinum(IV) prodrugs - MonoOxaprozin ((Oxaprozin)(OH)-Pt(IV)cis) and Ibu-NHS-Mono ((IBU)(OH)-Pt(IV)cis) - which incorporate oxaprozin and ibuprofen (non-steroidal anti-inflammatory drugs) as ligands. The compounds were synthesized and evaluated against two TNBC cell lines: BT-549 and MDA-231. Cytotoxicity was assessed via MTT assay to determine IC₅₀ values. Furthermore, the mechanisms of action were explored through apoptosis assays, cell cycle distribution analysis, reactive oxygen species (ROS) formation, and DNA damage assessment using the Comet assay. In vitro analyses revealed that both MonoOxaprozin and Ibu-NHS-Mono exhibited significantly higher antitumor activity compared to Cisplatin in both TNBC cell lines. Notably, the free NSAID ligands alone showed no cytotoxic activity, suggesting that the enhanced efficacy is unique to the Platinum(IV) conjugate. Mechanistically, the compounds induced apoptosis and triggered S-phase cell cycle arrest in a higher proportion compared to Cisplatin. Additionally, they promoted cellular stress, as evidenced by increased reactive oxygen species (ROS) formation, and significant DNA degradation, as shown by the Comet assay. The Platinum(IV) prodrugs MonoOxaprozin and Ibu-NHS-Mono demonstrate potent anticancer activity and superior efficacy over Cisplatin in TNBC models. These complexes represent a promising therapeutic strategy to improve outcomes and reduce the toxicity associated with traditional breast cancer treatments.

Keywords: breast cancer; cytotoxicity; IBU-NHS-Mono; MonoOxaprozin; Platinum (IV) complexes.

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Determination of biological activities of *Stachys byzantina* K. Koch

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The genus *Stachys* L. is considered one of the largest in the family Lamiaceae, highlighting its remarkable diversity and adaptability. The genus *Stachys* includes 467 taxa worldwide and 131 taxa in Türkiye. 52 of the taxa in Türkiye are endemic. *S. byzantina* K. Koch. is noted for anti-inflammatory, sedative, and wound-healing properties. *Stachys* species consist of terpenoids, saponins, polyphenols, phenolic acids, and flavonoids. This study aims to screen and evaluate the antioxidant and cell-culture activities of *S. byzantina* to confirm its potential as a natural therapeutic agent and to explore its applications across various fields. The in vitro effects of *S. byzantina* extract on cell viability in the L929, HUVEC, and U87MG cell lines were evaluated using the MTT assay. Treatments were applied for 24, 48, and 72 hours. The extract demonstrated mild proliferative effects in fibroblasts and endothelial cells, particularly at lower concentrations, supporting its traditional use in wound healing. U87MG cells were largely unaffected, indicating low neurotoxicity. The observed increase in HUVEC viability suggests potential pro-angiogenic activity, which may be advantageous in regenerative applications but warrants caution in cancer-related contexts. The study also used four standard analyses to investigate the antioxidant activities (DPPH, CUPRAC, FRAP, and TPC). The results showed that the plant extract possesses concentration-dependent antioxidant activity in all tests. Furthermore, *S. byzantina* has high phenolic content, especially at higher extract concentrations. Overall, the study confirms the plant's chemical and biological diversity, supporting its potential as a natural therapeutic agent. *S. byzantina* is a promising candidate for further research in regenerative skin applications. Also, it may be effective in preventing illnesses related to oxidative stress, including cardiovascular conditions and neurodegenerative diseases, though further studies are needed to elucidate its mechanisms of action and safety profile.

Keywords: Stachys; MTT; U87MG; HUVEC; L929

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In vitro virucidal efficacy of *Lilium* spp.

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Natural *Lilium* extracts represent a biocompatible pharmacological frontier, utilizing polyphenols and flavonoids to inhibit viral activity by disrupting microbial membranes and metabolic pathways. In this study, 70% ethanolic extracts obtained from *Lilium asiaticum* 'White', *L.* 'Dark Secret', *L.* 'Sunset Boulevard', *L. candidum* 'Madonna Lily', and *L. robina* were comparatively characterized for total phenolic content (TPC) and DPPH radical scavenging activity, and subsequently evaluated for cytotoxicity and in vitro virucidal efficacy. Adenovirus type 5 (DNA virus, *Adenoviridae*) and Echovirus (RNA virus, *Picornaviridae*) were used—non-enveloped viruses selected for their high environmental stability and ability to survive on surfaces. The initial phase of the study evaluated the cytotoxicity of the *Lilium* extracts on the HeLa cell line to determine half maximal inhibitory concentration (IC₅₀), using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay. To assess the virucidal efficacy of the *Lilium* extracts, viral infectivity was quantified using a parallel titration on HeLa cell monolayers incubated with or without the compound-virus mixture (pre-incubated together for 30 minutes). The half-maximal inhibitory concentration (IC₅₀) for the tested extracts was consistently identified within the concentration range of 1.72% to 3.95%. The reduction in viral titer was calculated by comparing the logarithmic TCID₅₀ values—determined via the Spearman-Kärber method—before and after exposure to the non-cytotoxic dilutions of the macerates. Among the tested cultivars, *Lilium asiaticum* 'White' emerged as the most effective antiviral agent, significantly reducing viral titers from -8.5 to -2.5 log TCID₅₀ for Adenovirus and from -7.5 to -3.5 log TCID₅₀ for Echovirus. A general positive association was observed between phenolic content, antioxidant capacity, and biological activity, suggesting that phenolic compounds contribute to both the virucidal effect and the modulation of cytotoxicity. The observed reduction in viral titer suggests a robust virucidal effect, even against non-enveloped viruses known for their increased resistance to chemical agents.

Keywords: adenovirus; Echovirus; HeLa cells; *Lilium* spp.; virucidal activity.

Functional reorganization of nitrogen-cycling microbial processes across degraded soils

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Heavy metals, organic pollutants, and salinity are major environmental stressors that degrade soils and impair ecosystem functioning, including nitrogen cycling processes. Plant productivity largely depends on microbially mediated nitrogen (N) transformations that generate ammonium (NH₄⁺) and nitrate (NO₃⁻) for plant uptake from soil. Consequently, plant nutrition may be compromised when environmental stressors disrupt microbial metabolism, functional gene expression, and enzymatic activities involved in nitrogen cycling. However, the extent to which distinct stressors differentially reshape microbial nitrogen cycling, and how this influences plants, particularly legumes that can acquire nitrogen through symbiosis with rhizobia, remains insufficiently understood. This study aimed to comparatively assess nitrogen cycling processes and the abundance of key microbial functional genes across four degraded soils affected by mercury (Hg), hexachlorocyclohexane (HCH), copper (Cu), and moderate salinity (NaCl), relative to a control site naturally colonized by a legume species. The results indicate that these stressors exert distinct effects on microbial nitrogen cycling. Hg and HCH strongly reduced denitrification activity (<1 μg N-N₂O g⁻¹ dry soil h⁻¹), while Cu showed a moderate reduction (~1.5 μg), compared to the control (~7 μg) and the NaCl-affected soil (~9 μg), suggesting inhibition of denitrifying processes under organic and metal stress. Hg contamination was associated with the highest abundance of ammonia-oxidizing bacteria (AOB), as indicated by *amoA* gene copy numbers (~4.8 × 10⁵ copies g⁻¹ dry soil). Concordant increases in comammox *amoA* (clade A), a marker of complete ammonia oxidizers capable of performing full nitrification (ammonia to nitrate), further suggest a shift toward nitrifying communities under metal stress. In contrast, denitrification-related genes *nirS* and *nirK* remained relatively stable across sites (10⁶-10⁷ copies g⁻¹). Further analyses of functional gene variability will provide additional insight into nitrogen cycling dynamics across these degraded soils. Overall, these findings demonstrate that environmental stressors drive distinct functional reorganization of microbial nitrogen cycling, with important implications for nitrogen availability and ecosystem functioning, and provide a foundation for future soil restoration strategies.

Keywords: denitrification (*nirK*, *nirS*); nitrification (comammox *amoA* (clade A)); soil contamination; heavy metals.

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Continuous-flow synthesis of L-Phenylalanine *via* metal affinity-based immobilized phenylalanine ammonia-lyase

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Phenylalanine ammonia-lyases (PAL, E.C 4.3.1.24), which occur predominantly in plants, some fungi, yeasts and bacteria, catalyze the non-oxidative ammonia elimination from L-phenylalanine to *trans*-cinnamic acid. However, PAL enzymes serve as efficient catalysts for the reverse reaction, the stereospecific addition of ammonia to substituted *trans*-acrylic acids, yielding high-value aromatic L-amino acids. The transition of PALs from bench-scale biocatalysis to robust industrial applications requires efficient immobilization strategies to overcome the instability of the free enzyme. Among various techniques, metal affinity-based immobilization offers distinct advantages, such as easy carrier regeneration, high selectivity, and the ability to immobilize the poly-histidine tag (His-tag) engineered enzymes directly from crude lysates without prior purification. By this approach, a site-specific attachment of His-tagged enzymes, ensuring favorable biocatalyst orientation, can be achieved, minimizing steric hindrance and activity loss compared to aggressive covalent methods. In this study, a screening of various commercial supports from Chiral Vision was conducted to evaluate their suitability for the immobilization of recombinant PAL from *Arabidopsis thaliana* (*At*PAL). The biocatalytic performance was assessed based on the conversion rates achieved in the ammonia elimination and addition reactions. The HIS-22 support bearing iminodiacetic acid (IDA) chelating group and charged with Ni²⁺ yielded the highest conversion in both reactions and was further applied for the *At*PAL immobilization directly from cell lysate. After optimization of the immobilization procedure the best performing biocatalyst was implemented in a continuous-flow packed-bed micro-reactor fabricated from urethane for the ammonia addition reaction to produce L-phenylalanine. Initial catalytic activity was remarkably high at room temperature, with conversions reaching 88% at 20 µL/min flow rate. However, a progressive decline in catalytic performance (61%) was observed throughout three days of continuous operation, although several washing procedures were applied to restore the biocatalyst activity. Nevertheless, after five days the biocatalyst retained 65% of its initial activity.

Keywords: continuous-flow; coordinative binding; L-phenylalanine; phenylalanine ammonia-lyases.

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Bioarchaeological analysis of a female with potential non-local traits from the Capidava Necropolis

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The Capidava fortress, situated in Constanța, is a site with complex history, where archaeological excavations are conducted regularly to gather information about the populations that once inhabited this area. Among the individuals recovered during the 2025 campaign, one skeleton exhibited notable morphological differences compared to the rest of the assemblage, prompting further analysis. This work aims to conduct a non-destructive anthropological analysis to gain insights on the individual's biological profile and potential origins. The results reveal that the individual was female, with an estimated age between 35 and 39 years. Osteometric analysis suggests possible ancestry-related traits consistent with African populations, a finding that contrasts with the local population. Additionally, osteomyelitis was identified in the right humerus. While the cause of death could not be determined, the results offer preliminary information about this individual and contribute to our understanding of the diverse populations inhabiting the region.

Keywords: biological profile; Capidava; Middle Ages; osteometry.

Glycoprotein Profiling in MPTP-Induced Experimental Parkinson's Disease


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Parkinson's disease is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss and complex molecular alterations. Emerging evidence suggests a link between metabolic dysfunction and neurodegeneration, highlighting protein glycation as a potential contributing factor. This study aims to analyze glycation-derived glycoprotein profiles in an MPTP-induced mouse model of Parkinson's disease and to evaluate associated changes in protein abundance and distribution. Two groups of mice (control and MPTP-treated) were used, with protein extracts obtained from cortex and striatum. Glycoprotein profiling was performed using SDS-PAGE and native electrophoresis, followed by Periodic Acid-Schiff (PAS) staining and silver staining for enhanced sensitivity. Additionally, complementary *in vitro* glycation assays were conducted using model proteins (BSA and hemoglobin) incubated with glucose to validate glycation-related modifications. PAS staining revealed weak bands, particularly in the low molecular weight region, suggesting a predominance of glycation-derived proteins rather than glycosylated glycoproteins. A weak band in MPTP-treated cortex samples may indicate early glycation of small proteins. Bioinformatic analysis further suggested that α -synuclein, a low molecular weight protein, is susceptible to glycation, potentially even more than hemoglobin, supporting its possible involvement in glycation-driven mechanisms in Parkinson's disease. Silver staining showed subtle differences in band intensity under denaturing conditions, pointing to changes in protein abundance. Overall, the results suggest that glycation-related alterations in glycoprotein profiles may occur in early stages of Parkinson's disease but remain difficult to detect using conventional methods. These findings support the potential involvement of protein glycation in Parkinson's disease pathology and highlight the need for more sensitive analytical approaches to identify glycation-specific biomarkers.

Keywords: α -synuclein; glycation; glycoproteins; MPTP; Parkinson.

Functional Gene *merA* in Rhizosphere Communities: Implications for Phytoremediation Potential in Mercury-Contaminated Soils

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Mercury (Hg) contamination poses significant threats to ecosystem functioning and human health. Microbial Hg resistance, largely mediated by the *merA* gene encoding mercuric reductase, plays crucial role in inorganic Hg detoxification. Understanding how plant-microbe interactions influence *merA* abundance in Hg-contaminated soils remains poorly understood. Such knowledge is essential for designing effective phytoremediation or phytomanagement strategies. Objective: This research aims to quantify the *merA* gene in the rhizosphere microbial communities of two plant species native to a highly Hg-polluted site, to evaluate whether *merA*-enriched rhizosphere correlates with microbial Hg-resistance and therefore could be used to tailor the future remediation approaches. Total DNA was extracted from rhizosphere soils of *Lotus tenuis* and *Diplotaxis muralis* collected at Hg-contaminated and control locations. Quantification of *merA* was performed through qPCR and gene copy numbers will be normalized to soil mass and compared between the selected model plant species. The *merA* gene was detected in 83% of *D. muralis* rhizosphere samples and in all *L. tenuis* from Hg-contaminated sites. On the contrast, the *merA* gene was not detected in control samples except for one *L. tenuis* control sample. This work provides baseline evidence that plant species differ in their capacity to mediate enrichment of *merA*-carrying microbial communities in Hg-contaminated soils. Expected outcomes include (1) identification of plant species with high capacity to enrich mercury-resistant bacterial communities and (2) development of criteria for selecting optimal plant candidates for phytoremediation attempts or phytomanagement. Further work will integrate transcriptomic profiling of *merA* expression and assessment of Hg bioavailability to refine phytoremediation strategies.

Keywords: *Diplotaxis muralis*; *Lotus tenuis*; *merA*; microbiome; soil.

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The microbiota as a potential reservoir and vehicle for the dissemination of antibiotic resistance genes

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Carbapenems, last-line antibiotics, are used in the treatment of severe infections caused by multidrug-resistant Gram-negative bacteria. To investigate the potential transfer of carbapenem resistance genes (CRG) to strains from the intestinal microbiota, the present study used bacterial strains isolated from the experimental model *Drosophila melanogaster* (Dm) and a multidrug-resistant *Enterobacter kobei* strain. Ten *D. melanogaster* individuals (Oregon line) were homogenized in physiological saline, then inoculated onto CHROMagar medium. Subsequently, 20 bacterial colonies were selected. The plasmid transfer potential was investigated using conjugation assay, chemical transformation, and electroporation. Plasmid DNA was isolated from blaKPC producing *E. kobei* strain, and the presence of the CRG was evaluated by PCR. Plasmid DNA was introduced into recipient bacteria by electroporation. Electrocompetent cells were prepared from the strains isolated from *D. melanogaster* and from the *E. coli* J53 and DH5 α strains. The transformed bacteria were inoculated onto medium supplemented with imipenem, and transfer was confirmed by PCR. Repeated conjugation experiments, which remained inconclusive, and chemical transformation assays, which were negative, failed to demonstrate the transferability of the plasmid carrying the blaKPC gene. In contrast, electroporation led to the emergence of cells that had acquired resistance to imipenem, while PCR confirmed the presence of the blaKPC gene, thereby indicating the capacity of strains from the *D. melanogaster* microbiota to express genes encoded by this plasmid. The study shows that bacterial strains from the normal microbiota of *D. melanogaster* were able to acquire and express a blaKPC-carrying plasmid following experimental electroporation in vitro.

Keywords: carbapenem resistance; electroporation; intestinal microbiota; plasmid transfer.

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Transmembrane phosphatase YejM, the gate for LPS regulation

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Escherichia coli is a gram-negative bacteria containing two membrane layers, this characteristic represent a challenge for antibiotics accessibility. The outer membrane is made of lipopolysaccharides (LPS), a flexible, diverse, and highly antigenic compound, considered a promising starting point for the development of a new generation of antibiotics. The regulation mechanism for LPS synthesis is based on a protein-protein interaction cascade situated in the inner membrane. The complex of the membrane proteins YejM and LapB is disrupted by a high concentration of LPS in the periplasmic domain. LPS attached to YejM, and LapB forms a complex with the membrane protease FtsH. This new complex activates the degradation of LpxC, a key enzyme in LPS synthesis system. YejM interactions controls the regulation mechanism. This study has the purpose of optimizing the isolation, analyzing the properties and the interactions of the transmembrane protein YejM, in order to develop a high throughput assay for antibiotic collections. YejM was extracted from cell membrane and isolated in DDM detergent micelles, the stability during concentration was tested with fast protein liquid chromatography and the influence of LPS on the thermic stability using NanoDSF method. YejM was co-expressed with LapB and the complex was isolated based on the interaction between them. An unexplored feature of YejM, the phosphatase activity was tested and confirmed using a fluorogenic compound 6,8-Difluoro-4-Methylumbelliferyl Phosphate (DiFMUP) as substrate. The measurements were optimized and adapted for an inhibition assay. The conversion is 50%, after 1.5 h of reaction. According to phosphatase activity in presence of LPS, it influences the process proportionally to the concentration, thus confirming the YejM-LPS interaction.


Keywords: lipopolysaccharides; membrane, phosphatase, YejM.

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Phytochemical Characterisation And Antioxidant Activity Of Anthocyanins From Edible Flowers

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Introduction: Anthocyanins, a subclass of flavonoids, are water-soluble pigments responsible for the characteristic red, purple, and blue hues of many plants, including edible flowers. Beyond their role in pigmentation and plant defense mechanisms, anthocyanins are increasingly recognized for their significant biological activities in humans, including potent antioxidant properties and therapeutic potential. Their structural diversity—shaped by different glycosylation and acylation patterns—strongly influences both their color stability and bioavailability, as well as their antioxidant efficacy. Consequently, comprehensive phytochemical characterization of anthocyanins in edible flowers combined with antioxidant activity assessment is essential to identify novel natural sources of bioactive pigments for use in functional foods, pharmaceuticals, and cosmetic applications. This study aimed to comprehensively characterize the anthocyanin profiles and evaluate the antioxidant activity of petal extracts from diverse edible flower species using advanced analytical techniques. The research sought to establish correlations between anthocyanin composition and antioxidant capacity to provide insights for selecting botanical sources with enhanced bioactive properties. Petal extracts from edible flowers including violet (*Viola* sp.), passionflower (*Passiflora* sp.), tulip (*Tulipa* sp.), and rose (*Rosa* sp.) were analyzed for phytochemical composition and anthocyanin profiling using liquid chromatography—mass spectrometry (LC-MS). Antioxidant activity was assessed using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay. LC-MS analysis revealed the presence of diverse anthocyanin derivatives across all flower species examined, including delphinidin-, pelargonidin-, cyanidin-, petunidin-, peonidin-, and malvidin-based glycosides and acylated conjugates. Significant inter-species variations were observed in both anthocyanin composition and structural diversity, reflecting distinct biosynthetic patterns and enzymatic regulation of pigment synthesis. Antioxidant capacity determined by ABTS assay demonstrated substantial radical scavenging activity in flower petal extracts, with notable differences in antioxidant efficacy among the various botanical sources. Strong correlations were established between the presence of specific anthocyanin structures and their corresponding antioxidant potential. The integration of structural identification with antioxidant activity assessment demonstrates the potential of edible flowers as valuable sources of bioactive natural compounds with significant therapeutic and industrial applications.

Keywords: antioxidant activity; anthocyanins; edible flowers; LC-MS; phenolic compounds.

Metal affinity-based immobilization of phenylalanine ammonia-lyase on single-walled carbon nanotubes

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Enzyme immobilization plays a pivotal role in synthetic biocatalysis, providing enhanced enzyme stability and improved resistance to various environmental conditions, including high temperatures, extreme values of pH levels, and organic solvents. In line with green chemistry principles, immobilization makes enzymes easily reusable, facilitating their recovery and recycling. However, classical immobilization methods require preliminary enzyme purification, which incurs additional costs. Therefore, key findings underscore the importance of developing specific immobilization techniques that enable simultaneous enrichment and immobilization. Immobilized metal affinity chromatography (IMAC) demonstrates considerable promise in this context, being one of the most popular strategies for purifying recombinant proteins. This involves attaching a six- to ten-histidine sequence, known as a His-tag, to the *N*- or *C*-terminus of the target protein. The carrier must also be derivatized with chelating moieties like nitrilotriacetic or iminodiacetic acid, bearing divalent or trivalent metal ions, such as Ni²⁺, Co²⁺, or Fe³⁺. The present study aims to develop robust, efficient and sustainable biocatalytic systems through the affinity-based immobilization of phenylalanine ammonia-lyase from *Petroselinum crispum* (*PcPAL*) on carbon nanotubes. PAL is one of the most intensively studied enzymes in plant secondary metabolism. It catalyzes the reversible elimination of ammonia from L-phenylalanine yielding *trans*-cinnamic acid, as well as the reverse ammonia addition reaction. Single-walled carbon nanotubes (SWCNTs) functionalized with carboxyl- and amino- terminal groups, modified with an EDTA dianhydride (EDTAda)-derived chelator and charged with Co²⁺ were tested as immobilization carriers. In order to achieve this, 1,4-cyclohexanedimethanol diglycidyl ether (CHX), glycerol diglycidyl ether (GDE), 1,4-butanediol diglycidyl ether (BDE) and neopentyl glycol diglycidyl ether (NEO) were used as bisepoxy-type linkers. The activity of *PcPAL* immobilized on the metal-charged supports was measured and compared with that of the free enzyme in the ammonia elimination reaction of L-phenylalanine, and three of the immobilized *PcPAL* biocatalysts were also tested in the reverse reaction.

Keywords: carbon nanotubes; metal affinity-based immobilization; phenylalanine ammonia-lyase; selective enzyme immobilization; support regeneration.

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Potential applications of red grape extract as adjunctive treatment in colorectal cancer

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Colorectal cancer (CRC) ranks 3rd in incidence and 2nd in mortality among cancers worldwide. The standard treatment for advanced CRC is oxaliplatin-based chemotherapy, but its efficacy is hindered by acquired tumor resistance. In this context, the aim of this study was to identify adjunctive agents capable of enhancing the activity of oxaliplatin. Given the well-documented antitumor, antioxidant and anti-inflammatory activities of flavonoids, polyphenols rich dietary sources were evaluated for their antitumor activity, as potential adjuvants in the treatment of CRC. Polyphenols were isolated from red grapes by mechanical processing, followed by methanol extraction, centrifugation, filtration, and solvent evaporation. Methanol reconstituted extracts were used to test their tumor cytotoxic activity, in DLD-1 CRC cell line. Also, the sensitivity of DLD-1 cells to the standard oxaliplatin chemotherapy was assessed, using MTT assays. Briefly, cells were seeded in 96-well plates, and after 24 hours, a concentration range of 25-250 μM of oxaliplatin or a concentration range of 5-120 μM of red grape extract was administered. After 24 hours, MTT was added and incubated for 1 hour. Cells were then lysed in DMSO and absorbance was measured by spectrophotometry. Cell viability percentages were calculated based on these values. All experiments were performed in biological triplicates, yielding an IC_{50} of 159,9 μM for oxaliplatin and an IC_{50} of 64,75 μM for red grape extract. Subsequently, an apoptosis assay using flow cytometry was performed to evaluate the mechanisms of cell death. Cells were seeded in a 6-well plate, and after 24 hours, oxaliplatin and red grape extract were administered at their previously determined IC_{50} concentrations. After further 24 hours, cells were stained with propidium iodide and Annexin V. The results of this assay indicated that both treatments induce apoptosis, and that red grape treatment may represent a promising adjunct to oxaliplatin, which is to be evaluated.

Keywords: apoptosis assay; colorectal cancer; MTT assay; oxaliplatin; red grape extract.

Characterization and Genetic Diversity Assessment of *Pyrus* Germplasm in Romania Using a Multi-Locus SSR Marker Panel

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The conservation of *Pyrus* genetic resources is a fundamental pillar for sustainable horticulture and successful breeding programs, particularly in response to climate changes and phytosanitary challenges. Romania's rich reservoir of local pear varieties and wild populations represents a critical genetic heritage that requires precise molecular characterization to develop programs to prevent genetic erosion and ensure future crop improvement. This study aims to establish high-resolution molecular profiles for a selection of *Pyrus* germplasm using Simple Sequence Repeat (SSR) markers. The primary focus was to evaluate genetic diversity, identify unique genotypes, and validate the transferability of a diverse set of microsatellite markers within local collections. Genomic DNA was extracted from leaf tissue and screened using a comprehensive panel of 21 SSR primers, integrating species-specific and universal markers. The primer selection integrated both species-specific and universal markers for Rosaceae to ensure high transferability and discriminative power. The set included the Pycom series (S5, S7, S8), specifically optimized for *Pyrus*, alongside highly polymorphic markers from the CH, GD and KA (KA_14, KA_16) series. A key technical feature was the analysis of the S5 PycomC1 locus, utilizing a multi-primer approach (1 Forward and 6 Reverse primers) to detect complex allelic variations. Genetic parameters were calculated to assess the informativeness and discriminative power of each locus. Molecular characterization confirmed high levels of polymorphism and allowed for a clear differentiation among the analyzed genotypes. The combined use of Pycom, CH, and GD primer series provided the resolution necessary to distinguish closely related local cultivars from international commercial cultivars. These results validate the efficiency of the 21 SSR marker panel for cultivar identification and genetic diversity assessment in pear germplasm. This robust "fingerprinting" approach provides an essential tool for the management of Romanian germplasm, facilitating the certification of authentic plant material and the protection of indigenous genetic resources.

Keywords: genetic diversity; Pycom primers; *Pyrus communis*; SSR markers.

Funding: This work was supported by a doctoral research grant provided by the Doctoral School of Integrative Biology of Babeş-Bolyai University Romania.

Protein engineering of bacterial HALs for therapeutic applications

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Histidine ammonia-lyases (HALs) belong to the MIO-dependent enzyme family, alongside phenylalanine ammonia-lyases (PALs) and tyrosine ammonia-lyases (TALs). Particularly, HALs catalyze the non-oxidative deamination reaction of L-histidine with the formation of *trans*-urocanic acid. These enzymes have attracted significant interest due to their biocatalytic versatility and therapeutic potential. In particular, PALs are currently employed in the treatment of phenylketonuria (PKU), a metabolic disorder characterized by the inability to convert phenylalanine, leading to toxic buildup and severe brain damage. While PAL-based enzyme substitution therapy has shown clinical success, it relies on a bacterial enzyme that can trigger immune responses, highlighting the need for improved alternatives. Notably, no human PAL exists; however, a human histidine ammonia-lyase (hHAL) is present. Despite its structural and mechanistic similarity to PALs, hHAL faces significant challenges when expressed recombinantly in *Escherichia coli*, including poor solubility and low yield, limiting its direct therapeutic application. To address this, we employed data mining approaches to identify novel bacterial HALs sharing a conserved catalytic fingerprint with hHAL. One such candidate, identified through metagenomic sampling was selected for further study. The selected bacterial HAL (bHAL) was successfully expressed and purified, and its enzymatic activity was characterized, confirming its functionality. Building on this scaffold, protein engineering strategies targeting the catalytic site are proposed to shift substrate specificity from histidine toward phenylalanine, effectively converting HAL activity into PAL-like activity. Finally, the insights gained from bHAL engineering may be translated to the human enzyme, aiming the design of a human-compatible biotherapeutic enzyme for PKU treatment, potentially overcoming the immunogenicity associated with current bacterial PAL therapies.

Keywords: histidine ammonia-lyase; phenylketonuria; protein engineering.

Funding: This work was financed by the National Authority for Scientific Research and Innovation (ANCSI), PNRR-III-C9-2023 – I8, Project code: CF 92/31.07.2023

Optimizing compost application to enhance the growth of a leguminous plant species in contaminated soil

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Phytoremediation is an ecological and cost-effective method making use of plants for removal or stabilization of toxic elements or compounds from contaminated soils. However, several physical and chemical properties of soil, like low organic matter (OM) content, sandy texture, and type and concentration of contaminants, can significantly alter plants growth in such substrates. The objective of this research is to identify suitable nature-based solutions for remediating a heavily contaminated soil with hexachlorocyclohexane (HCH) and dichlorodiphenyltrichloroethane (DDT), located at a former chemical plant site in Turda, Cluj County, Romania. The most prevalent leguminous plant species that has naturally colonized the site was selected as a potential candidate for phytoremediation. The soil at the site has a sandy composition, with only 3% OM, and contaminated with 13.3 mg kg⁻¹ Hg, and the following organic pollutants DDD o,p', DDD p,p', DDE o,p', DDE p,p', DDT o,p', DDT p,p', and lindane (γ -HVH) in average concentrations of 0.4, 0.6, 0.1, 0.9, 4.5, 11.7, and respectively 0.2 mg kg⁻¹. To support plant growth and improve the effectiveness of nature-based solutions, soil amendment with a minimum level of organic matter is considered necessary to enhance both soil structure and nutrient availability. In the current preliminary study, we investigated the optimum compost concentration addition to the *in situ* soil to support growth until flowering stage of the plants. Seven treatments, 0%, 10%, 20%, 30%, 40%, 50%, and 100% compost, were tested over an 18-week period, with 6 pot replicates per treatment. At the end of the experiment, plant fresh and dry biomass weight were measured. The results demonstrated that compost amendment significantly improved plant growth, even at the lowest concentration (10%). Similar growth responses were observed at 20%, 30%, and 40% compost levels, while the highest biomass production was recorded in the 50% treatment. Higher compost concentrations also determined faster flowering compared to the lower ones, however increase significantly the remediation costs. These findings indicate that even low compost additions could significantly sustain plant growth and represents an effective additive strategy for phytoremediation in sandy, contaminated soils.

Keywords: phytoremediation; compost; contaminated soil; hexachlorocyclohexane (HCH).

Funding: The research is funded by the Horizon Europe project ARAGORN (Achieving Remediation And Governing Restoration of contaminated soils Now) under grant agreement 101112723).

Telomeres and type 2 diabetes

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Diabetes mellitus is a metabolic disorder characterized by elevated blood sugar levels resulting from impaired insulin production or action. Type 2 diabetes is a widespread chronic disease affecting more than 400 million people worldwide, with complex causes. Telomere shortening, influenced by oxidative stress, chronic inflammation, insulin resistance, and hyperglycemia, is implicated in the development of diabetes. Thus, understanding the involvement of telomeres may help identify new therapeutic targets. This study compared telomere length in patients with type 2 diabetes and healthy controls using a case-control design and quantitative PCR technique. Fourteen participants were included: 7 patients and 7 age- and sex-matched controls, recruited from the National Institute of Diabetes, Nutrition and Metabolic Diseases - Prof. Dr. N. Paulescu, Bucharest. DNA was extracted from peripheral blood using the GeneJET Genomic DNA Purification Kit (ThermoFisher) following the manufacturer's protocol. Relative telomere length was measured by qPCR using GAPDH as the reference gene, according to the Cawthon (2002) protocol ($t/s = [2^{Ct(\text{telomere})}/2^{Ct(\text{reference gene})}] - 1 = 2^{-\Delta Ct}$), with all samples analyzed in duplicate. The analysis revealed that the mean telomere length in the control group was 3.416 times higher than in patients with type 2 diabetes, consistent with the data obtained from similar studies to date. However, detailed analysis of diabetes complications and their influence on telomere length was not possible, as patients were recently diagnosed, despite their relatively advanced age (mean being 59,7 years). The small sample size limited statistical analysis and the ability to establish strong correlations between telomere length and diabetes. Nevertheless, the promising preliminary results and the efficiency of the applied methodology underscore its strong potential for further research with larger study groups and pave the way for the development of new therapeutic strategies.

Keywords: type 2 diabetes; reference gene; telomeres; qPCR.

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Comparing the Antioxidant Activities of Ylang_Ylang Essential Oils From Different Commercial Brands

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Ylang ylang essential oil is obtained from the flower of *Cananga odorata*, an evergreen perennial tropical tree that belongs to the Annonaceae family. *Cananga odorata* is a medium-sized tree that may reach heights of 10-20 meters. It is characterized by long, drooping branches in its native Indo-Pacific. This plant has a unique shape and fragrant yellow or greenish-yellow flowers. Ylang ylang has a distinctive, rich fragrance that has led to its use in many cultures for various medicinal purposes. Ylang ylang essential oil has recently garnered significant interest due to its pharmacological potential and biological activity. The oil contains significant concentrations of volatile compounds, including monoterpenes, sesquiterpenes, and phenylpropanoids, which are responsible for its biological activities. In this study, the antioxidant activities of five different commercial brands of ylang ylang essential oil were assessed for their potential to mitigate oxidative stress and protect neurons from damage induced by reactive oxygen species, which are associated with the development of neurodegenerative disorders such as Alzheimer's disease. For this, DPPH and ABTS radical scavenging assays, as well as a total phenolic content assay, were done. The five brands of essential oil are categorized as: oil A, oil B, oil C, oil D, and oil E. The results show that oil C has the highest total phenolic concentration and the highest antioxidant activity in both the DPPH and ABTS tests. Oil E had the second most activity, followed by oil B and oil D. Oil A had the lowest activity. In the conclusion, the results show that ylang ylang essential oils, especially oil C, are important natural antioxidants that may represent a promising natural precursor for the development of therapeutic agents against neurodegenerative disorders such as Alzheimer's disease.

Keywords: ABTS; Alzheimer's disease; antioxidant activity; DPPH; neurodegenerative diseases; Ylang ylang essential oil.

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Characterization of the secretory dendritic cells of the bursa of Fabricius using novel monoclonal antibodies

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The bursa of Fabricius (BF) is a primary lymphoid organ unique to birds, playing an essential role in B-lymphocyte differentiation. The BF is a walnut-sized organ composed of 10–12 folds, with its lumen communicating with the cloacal cavity. These folds contain lymphoid follicles, which are histologically distinguishable into a mesodermal cortex and an ectodermal medulla. The medulla harbors reticular epithelial cells and secretory dendritic cells (BSDCs), which create a bursa-specific microenvironment crucial for B-lymphocyte precursor maturation. Unlike the supporting epithelial cells, the ontogeny and immunocytochemical properties of BSDCs are only partially understood. To characterize the lymphoid and myeloid cells composing the follicles of the adult chicken bursa of Fabricius using immunocytochemical methods. To characterize the secretory dendritic cells of the bursa using novel monoclonal antibodies recognizing membrane proteins. BF isolation from embryos (n = 9) and adult animals (n = 12), tissue fixation, gelatin embedding, dual immunocytochemistry, fluorescence, confocal, and electron microscopy, as well as in vitro cell culture. Using immunocytochemical methods, we searched for markers that reliably identify BSDCs. We demonstrated that, in addition to E-cadherin+ epithelial cells, chB6+ B- lymphocytes, and TIM4+ macrophages, CSF1R selectively labels BSDCs in both embryonic and adult BF. Dual immunofluorescence staining confirmed that CSF1R+ BSDCs express CD11d, p75-neurotrophin receptor, and alphaVbeta3 integrin. These surface molecules have not been previously published on avian dendritic cells. The identification of BSDC-specific membrane molecules enables the efficient isolation of these cells using fluorescent cell sorting, which is essential for their molecular characterization and in vitro culture.

Keywords: B-lymphocyte; bursa of Fabricius; BSDC; CSF1R; immunocytochemistry.

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Ecology and biodiversity conservation

A systems view on ecological communities: experimentally testing model predictions

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Ecological interactions and indirect effects make multi-species communities complex. A variety of models exist for complex ecosystems, including food webs, and these provide predictions. Yet, our knowledge and understanding are seriously limited because these predictions are only very rarely tested, especially in complex ecosystem models. I present a food web model of a freshwater microbial community that is studied by several modelling approaches, and some predictions were also tested in parallel in vivo and in silico perturbation experiments. The first, preliminary results of model competition will be presented.

Keywords: ecological interactions; food web modeling; multi-species communities; perturbation experiments; model validation.

Learning from failure: how mistakes shape a career in science

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We live in an increasingly competitive and changing world, in which having a career, including in science, is equally challenging. While many students, and even young researchers, believe that a scientific career is mostly driven by passion and requires a clear set of steps to be followed, the reality is that the path to becoming a scientist is often not linear and depends on many factors beyond passion. In this talk, I discuss some of these aspects, emphasizing the importance of good mentorship, accepting that mistakes are inevitable, and recognizing that the most effective approach is to learn from them. Failure is not only unavoidable, but also necessary in order to improve a scientist's performance. Taken together, if failed (but honest) scientific efforts are guided in the right direction, they can promote ambition, resilience, and ultimately a strong mindset that is increasingly needed in a highly competitive environment. Here, I will use some personal examples of unsuccessful experiences, both from the beginning of my career and from more recent work, using them as case studies for early-career researchers and, hopefully, helping them to better adjust their expectations of a career in science. Ultimately, the goal is not to avoid failure, but to understand it, use it, and grow from it as part of a realistic and sustainable scientific career.

Keywords: career development; early-career researchers; failure; mentorship; scientific career.

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Red Wood Ants of Romania – Diversity, distribution and conservation

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Red wood ants (the *Formica rufa* group) comprise a complex of species native to the coniferous, deciduous, and mixed forests of Europe. Widely recognized as ecosystem engineers, they construct large, dome-shaped mounds from spruce needles, twigs, and grass. These structures function as sophisticated solar collectors and climate-controlled nurseries. Red wood ants play a keystone role in forest ecology through their aggressive predation of invertebrates, mutualistic tending of aphids for honeydew, and contributions to nutrient cycling and seed dispersal. Seven red wood ant species are currently recognized in Europe: *Formica rufa*, *F. polyctena*, *F. lugubris*, *F. aquilonia*, *F. pratensis*, *F. paralugubris*, and *F. truncorum*. This study discusses the distribution of the four species present in Romania (*F. rufa*, *F. polyctena*, *F. pratensis* and *F. truncorum*) and highlights key protocols for their monitoring and conservation.

Keywords: *Formica rufa* group; forests; MonitAnt.

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Impacts of long-term fire and grazing on *Rhopalomyia* spp. galls in Idaho

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Fire and grazing are common in western North American sagebrush steppe. Following these events, plants often regrow vigorously, providing new vegetation that can support herbivorous insects, including gallers, enabling higher colonization rates, increased feeding sites for larvae, and improving survival. The idea of herbivores favoring rapidly growing plants is called the plant vigor hypothesis. Insect galls induced by *Rhopalomyia* spp. are found on *Artemisia* spp. (sagebrush) leaves, stems, and buds. Sagebrush populations in the western U.S. are declining due to threats from increasing fire severity/frequency and spread of invasive grass species. As a result, *Rhopalomyia* spp. and other insect and vertebrate species may be affected. We surveyed long-term fire and grazing sagebrush plots at the U.S. Sheep Experiment Station in southeastern Idaho to determine the impact of fire and grazing on insect gall populations. Per the plant vigor hypothesis, we expected more galls in grazed pastures; we also anticipated a higher plant volume in burned pastures. In the spring, summer, and fall we measured gall diversity and vegetation characteristics of sagebrush within six pastures subjected to either fire, grazing, a combination of fire and grazing, or controls with no burning or grazing. We measured sagebrush plant architecture and gall communities. Our results suggest that pastures exposed to fire have a higher average plant volume. We also observed a significantly lower number of galls in unburned pastures—noting a substantially higher number of galls in ungrazed, burned pastures compared to ungrazed, unburned pastures.

Keywords: fire and grazing; plant vigor hypothesis; sagebrush ecosystems; insect galls; *Rhopalomyia* spp.

Linking microclimate and satellite phenology to understand vegetation dynamics in the Carpathian Mountains

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Fine-scale thermal heterogeneity is a key, yet still underexplored, driver of mountain vegetation dynamics. We compiled an original dataset of in situ soil temperature time series collected between 2014 and 2025 across multiple sites in the Carpathian Mountains. The network spans broad elevational and topographic gradients and includes mountain meadows, subalpine pastures, high-elevation grasslands, and late snowmelt sites. Using this dataset, we explore how near-ground thermal conditions relate to vegetation dynamics. This presentation focuses on exploratory analyses linking satellite-derived vegetation phenological indices at 30 m spatial resolution with temperature conditions. Preliminary results describe variation in thermal regimes among sites and within years (seasonal variation) in relation to vegetation spectral indices. By combining these field-based microclimatic measurements with remote sensing products, our study aims to improve understanding of how fine-scale temperature heterogeneity influences the functioning of Carpathian grasslands.


Keywords: Alpine; Carpathians; microclimate; vegetation.

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Diversity of planktonic microcrustaceans in the Somova-Parcheş Lake Complex (the Danube Delta Biosphere Reserve, Romania)

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In the context of the global biodiversity crisis, freshwater ecosystems represent a conservation priority, encompassing some of the most threatened ecosystems on the planet and being exposed to a wide array of both natural and anthropogenic stressors. The present study focuses on the analysis of zooplankton community diversity within a region of globally recognized ecological, social, and geopolitical importance, namely the Danube Delta Biosphere Reserve. Planktonic microcrustacean samples were collected during four sampling campaigns conducted in the summers and autumns of 2023 and 2024, from nine aquatic ecosystems located in the Somova-Parcheş Lake Complex, a “mini-delta” situated upstream of the city of Tulcea. Despite its smaller spatial extent, this area plays a biodiversity conservation role comparable in importance to that of its larger counterpart, the Danube Delta, located downstream of Tulcea. A total of 22 cladoceran species (Crustacea, Cladocera) and 12 copepod species (Crustacea, Copepoda) were identified, along with immature copepod stages, resting eggs, various rotifer genera, and other taxa that were incidentally present in the plankton samples. The species diversity of planktonic microcrustacean communities was assessed from both taxonomic and functional perspectives.

Keywords: cladocerans; copepods; diversity; species richness.

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Assessing dog's aggressive and avoidance behaviour when interacting with a quadruped robot and a waiter-assistant robot

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Social robots (SR) are required to operate effectively in human environments, without eliciting avoidance in their social partners. While SRs may interact not only with humans but also with animal species sharing human environments, their effects on potential non-human social partners are unknown. The dog is one of the most common non-human species living in close association with humans, making it a relevant model for studying animal–robot interactions. As SRs become more widespread, family dogs accompanying their owners to public places are increasingly likely to encounter and interact with such agents. This study is the first to examine the impact of different SRs on family dogs from a behavioural perspective. We tested N=90 companion dogs that accompany their owners to restaurants, offices or malls at least monthly. Each dog encountered two agents separately in the following pairings: (1) an animal-like quadruped robot (QR) and a medium-sized dog; (2) a QR and a waiter-assistant robot (WR); and (3) a WR and a small woman. Each agent performed a one-minute behavioural sequence twice, during which the dogs' behaviour was recorded. We expected more frequent aggression and avoidance responses toward the robots in every experimental group. Linear regression analyses showed that dogs displayed aggression more frequently toward the QR than toward both the unfamiliar dog and the WR. However, there were no significant differences in avoidance responses between the encounters with the two robots, nor were there differences in aggression or avoidance between the human and the WR. A potential contributing factor to the measured aggression towards QR may also have been the noise emitted by the robot. Our findings suggest that while WRs may function effectively in public spaces, the design of QRs requires reconsideration before their practical deployment. Further research is needed to evaluate QR–dog interactions more thoroughly.

Keywords: animal-robot interactions; dog-robot interactions; social robots; quadruped robot; waiter-assistant robot.

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Bridging the gap between occurrence data and conservation action: automated workflows, temporal awareness, and ai-ready evidence

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Biodiversity databases are essential for conservation assessment yet remain optimised for human browsing rather than structured machine consumption, creating critical bottlenecks of fragmented, poorly documented data disconnected from temporal reality. Using global freshwater crayfish as a model system, we present three integrated innovations delivered through cheCkOVER, an open-source R pipeline that automates the transformation of occurrence records into standardised species knowledge packages. Applied to 465 species across 97 countries, cheCkOVER harmonises taxonomy, derives IUCN-aligned spatial metrics (E00/A00), and performs geospatial contextualisation across administrative units, hydrographic basins, ecoregions, and protected areas, generating publication-ready maps and canonical geo-narratives with full source traceability. We further demonstrate that current databases cannot natively represent documented local extinction, producing temporal inflation whereby legacy presences obscure real decline in conservation metrics. Implementing a loss_event model that links extinctions to historical localities enables time-aware range mapping; application to European crayfish data reveals measurable IUCN metric inflation. Finally, we evaluate how structured, machine-readable outputs transform AI accessibility through controlled large language model (LLM) query tests across nine poorly documented endemic species and one well-documented control, comparing closed-book, web search, and manifest-directed retrieval strategies. Closed-book queries failed catastrophically (mean score 1.72/8; 50% failure rate), web search improved performance inconsistently (4.25/8; 19% failures), while manifest-directed retrieval achieved consistent success (6.88/8; zero failures; 56% perfect scores), with gains scaling inversely with existing species documentation. Together, these advances establish practical infrastructure for reproducible, AI-assisted conservation workflows, making occurrence data simultaneously human-interpretable, machine-consumable, and temporally explicit — directly addressing accessibility gaps where conservation needs are greatest.

Keywords: biodiversity informatics; conservation assessment; freshwater crayfish; large language models; temporal dynamics.

The assessment of the impact of check-dams on biodiversity of riparian vegetation of torrential watercourses

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At the European level, the 2030 Biodiversity Strategy promotes, among other things, the restoration of aquatic ecosystems, the removal of “barriers” and the maintenance of free-flowing rivers. On the other hand, the intensification of extreme climate events requires active measures to prevent and mitigate the negative effects of floods on economic and social objectives, as well as on the stability of natural ecosystems. In this context, the present study aims to assess the impact of check dams on biodiversity, taking into account the state, dynamics and specificity of riparian flora and vegetation. Replacing time with space, managed and unmanaged valley sectors with hydrotechnical works in the Baiului Mountains were inventoried. A systematic stratified sampling was used, within which inventories were carried out in blocks of 3 transversal transects on the riverbed, located upstream of the check dams, respectively in unmanaged sectors. On each transect, ten 1 m² surveys were placed, useful in capturing habitat diversity. An asymmetric beta-diversity index of species gain adapted for abundance data was tested and adjusted. Its testing on hypothetical data highlighted the properties of the index and its relationship with other diversity indicators. A total of 15 EUNIS habitats and 236 plant species were identified. For check dams over 40 years old, no significant differences were detected between the managed and unmanaged sectors for any biodiversity indicator. The synthetic multicriteria index allowed the identification of communities with a high contribution to the total alpha diversity, the characteristic one and the overall floristic heterogeneity. In the context where torrent control structures can generate both effects on biodiversity and socio-ecological benefits, the proposed index offers an integrative tool that can support the identification of important areas for biodiversity, the quantification of anthropogenic impact, the prioritization of interventions and informed management decision-making.

Key-words: anthropogenic impact; biodiversity; check dams; directional beta-diversity; EUNIS habitats.

Funding: This research was funded by the National Forestry Administration – Romsilva (Contract no. 4715/04.04.2024).

The Vărășoia Cave System (Bihar Mountains, Western Romania): an integrated biological approach

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The Vărășoia Cave System (VCS) in the Apuseni Mountains (North-Western Romania) represents one of the most complex karst systems in the country, marked by intricate interconnected underground conduits increasing with depth (ca. 28 km, –653 m depth). VCS displays a dynamic hydrological regime, ranging from diffuse infiltrations through the vadose zone with relative high permeability and porosity and reduced residence time of the percolating waters, to flooded channels with partially known flow directions, drainages and outflows. These conditions favor a dynamic allochthonous input of organic matter representing the primary food resource for troglofauna. Since the discovery of the deepest pothole of the system in 1986 (V5), the VCS has been a hotspot for cave exploration, however it remains a *cold spot* in biodiversity due to the lack of scientific investigations. We aim to fill this gap and provide an overview of the subterranean biodiversity, ecological and environmental conditions and trophic structure, with focus on the cave Peștera din Poiana Vărășoia (V11) based on a 1-year monitoring at 2-month intervals (2025). Additionally, we provide a preliminary trophic structure food web for terrestrial species, based on ¹³C and ¹⁵N stable isotopes analyses. Thermal measurements in the deep part of the cave point to a mean winter temperature of 6.7°C, higher than the mean annual temperature of the area (4°C). The invertebrates community includes 17 taxa, marked by the presence of endemic troglobionts and stygobionts belonging to beetles (*Drimeotus laevimarginatus*, *Pholeuon knirschi*, *Duvalius paroecus*), copepods (*Elaphoidella* sp., *Acanthocyclops* sp. *kieferi* gr.), and syncarids. The food web structure shows the dominance of opportunistic primary and secondary consumers like *D. laevimarginatus* and *Typhloiulus* aff. *strictus* and the spider *Carpathonesticus plesai* identified as top predator. The present study provides a preliminary cave-ecosystem based approach into one of the largest cave systems of Romania, as a prerequisite for forthcoming studies, essential when addressing monitoring, protection and conservation issues of subterranean systems.

Keywords: biomonitoring; cave; food web; invertebrate fauna.

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Acknowledgements: Special thanks to the Vărășoia Team, Cristal Speleology Club (Oradea, Romania) and the Politehnica Speleology Club (Cluj-Napoca, Romania) for the help in the field sampling campaigns.

Soil mesofauna of Bortig Ice Cave sinkhole (Apuseni Mountains, Northwest Romania)

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Ice caves are permanent cryosphere unique ecosystems that store ancient genetic diversity and serve as refuges for life surviving in cold environmental conditions. Ice caves and their corresponding sinkholes display distinct transition ecotones, where microclimatic inversion fosters a rich biological diversity. This study examines the soil mesofauna of Bortig Ice Cave (Apuseni Mountains), one of the largest cave with perennial glacier of Romania. We aim to assess the invertebrate's biodiversity and create a biological baseline for forthcoming environmental DNA (eDNA) research on the perennial ice block, where soil-dwelling taxa are expected to be sequestered. Soil sediment samples were collected from three sites along a vertical transect (November 2026). The community consists of a wide range of invertebrates, with Nematoda, Oligochaeta, and Acarina being the most common, followed by Collembola, Gasteropoda, and Chilopoda, and few Isopoda, Diplopoda, Harpacticoida, and insect larvae. The top-forested site has the highest invertebrate's biodiversity, dominated by Nematoda, dropping off at the sinkhole bottom, where only Collembola and Oligochaeta are present. The shift pattern of organism's distribution corresponds to a change in environmental conditions, from warm and dry deciduous leaf litter to cold and wet soil close to the ice block. Our preliminary results are consistent with researches conducted in other ice caves, where taxa such as Acarina and Collembola are essential indicators of cold and stable microclimates. The biotic assemblage provides clues regarding typical ice caves fauna adapted to cold environmental conditions and highlights the ecological gradient from glacial to periglacial zones of these caves. This study fills a big gap in our knowledge on ice caves fauna of Romania. As these frozen archives are in decline due to climate changes, it is important to record the current diversity of these hidden habitats to learn more about their resilience and evolutionary history in cryosphere environments.

Keywords: invertebrates, cold-stenotherm; soil sediment; diversity.

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Shall we let botanists go extinct? Comparing traditional plant survey and eDNA methods - a case study across habitats from the paradise of Nera Gorge-Beuşniţa National Park to the heavily contaminated Moldova Nouă

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Within biology studies, botany represents less than 1% of the curriculum. Consequently, there are fewer expert botanists whose skills extend beyond species identification and who weave together ecological, cultural, and social perspectives. As technology advances, alternative methods of species detection such as environmental DNA (eDNA) are becoming the golden standard. However, these approaches still require resources, tools and specialists to analyze the data. Despite the many advances, few soil eDNA studies have targeted vascular plants. Here, we compared a traditional plant survey with eDNA analysis across habitat types ranging from high-biodiversity grassland meadows and forests in Nera-Gorge-Beuşniţa National Park to heavily contaminated sites at the former copper mine in Moldova Nouă. Of the total species detected by both methods, 83% were recorded by the traditional survey and 36% by eDNA, and only 19% were detected by both methods. Therefore, the eDNA method added 17% more species that were not observed in the traditional surveys. However, eDNA method alone cannot encompass the full picture. Notably, 85% of species recorded in the traditional survey had representative sequences in the DNA reference libraries used, suggesting that reference databases are becoming less of a limiting factor. In conclusion, eDNA method can be used complementary to traditional plant survey, making the botanists essential.

Keywords: eDNA; plants; environment; biodiversity; ecology.

Funding: The research is funded by the Horizon Europe project ARAGORN (Achieving Remediation And Governing Restoration of contaminated soils Now) under grant agreement 101112723.

Study of bryophytes from alder forests in the Western Mountains

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Anthropogenic pressures are increasingly evident in Romania's forest ecosystems, leading to a measurable decline in bryophyte species richness and, consequently, affecting overall biodiversity. Bryophytes serve as reliable indicators of habitat ecological conditions and the extent of human disturbance. The present study focuses on three forest plots in Cluj County, each characterized by a distinct management regime: an unmanaged forest, a young even-aged stand, and a selectively thinned forest. The main objectives are: (i) to determine whether differences exist in bryophyte flora among these forest types; (ii) to identify the ecological characteristics of the studied stands; and (iii) to evaluate the effectiveness of bryophytes as indicators of anthropogenic impact. Within each 1-hectare plot, 10 living trees and 10 logs or stumps at various stages of decay were randomly selected to assess lichen communities. A species database was compiled for each plot and subsequently compared. Contrary to expectations, the unmanaged forest exhibited lower species richness than both the young even-aged stand and, in particular, the selectively thinned forest. From an ecological perspective, the studied forests display a weakly acidophilous, microthermic, and mesophilous to xeromesophilous character, ranging from sciaphilous to heliosciaphilous conditions. The dominant life forms are bryochamaephyta reptantia, while the geoelement spectrum reflects a temperate montane character. Analysis of habitat condition indicates a moderate level of disturbance.

Keywords: bryophyte species richness; bioindicators; forest management history.

Funding: This research is part of the *Experimental Implementation of Participatory Monitoring of Forest Quality and Biodiversity Based on the Cryptogamic Component (Mosses, Lichens) in the Montane Forests of the Apuseni Mountains (Muntele Mare & Bihor Mountains)* project funded by Tomorrow Forest Foundation.

Population size challenge in endemic freshwater species: Field and spatial modelling of the Idle Crayfish in Cambria

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Estimating population size remains one of the most challenging tasks in ecology, particularly for endemic freshwater species where restricted distributions and data scarcity compound uncertainty. Using the Idle Crayfish (*Austropotamobius bihariensis* Pârvulescu, 2019), endemic to Romania's Apuseni Mountains, as a case study, we develop and validate a practical framework for population size estimation in data-scarce taxa. Capture-mark-recapture (CMR) surveys were conducted and three estimators (Chapman, Chao, Jackknife) applied to obtain absolute abundance estimates, which were integrated into a zero-inflated negative binomial (ZINB) modelling framework projected across an optimum river network under three habitat suitability scenarios (permissive, balanced, restrictive). Models incorporating field-verified non-presences (RA strategy) were compared against presence-only approaches (PO strategy), and validated through intensive surveys at 15 randomly selected sites using near-complete census collections as benchmarks. The Chao estimator within the RA-ZINB framework yielded the best predictive accuracy (RMSE = 22.3, $R^2 = 0.98$), while presence-only models performed substantially worse across all metrics. The balanced habitat suitability scenario — integrating spatial predictions with independently derived genetic population structure — produced the most ecologically realistic total population estimate of approximately 170,000 individuals. This framework offers a repeatable methodological template for population monitoring and conservation planning in narrow-range freshwater species globally.

Keywords: conservation; endemic species; population size; spatial ecology; species distribution modelling.

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Ecological drivers of macrofungal diversity in the protected area Făgetul Clujului-Valea Morii

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Macrofungal conservation is an essential component of ecosystem functioning, yet it remains an underrepresented group in the monitoring and management strategies of protected areas. Current management practices and conservation objectives often do not explicitly include fungal species. This study aims to assess the influence of habitat characteristics and forest management practices on macrofungal diversity within the ROSCI0074 Făgetul Clujului–Valea Morii (Cluj-Napoca, Romania). The study was conducted between October 2024 and February 2026 and included 31 quantitative sampling events across three habitat types: (i) *Picea abies* and *Pseudotsuga* sp. stands, (ii) mixed deciduous stands (oak and beech), and (iii) pure beech stands. For each habitat, three sampling plots of 25 m² were established. Macrofungal fruiting bodies were recorded and, where necessary, collected for further identification using spore print analysis and microscopic examination. The samplings were taken 2 times per month, 2 weeks apart, only for summer was one sampling per month. The highest species richness was recorded in the *Picea abies* and *Pseudotsuga* plantation, where most taxa were symbiotic. Although, the other two habitats are also located within the Făget reservation, this habitat maintained a higher macrofungal diversity. In the pure beech stands, the constant species identified were predominantly saprotrophic, including *Daldinia concentrica* and *Stereum hirsutum*. The coniferous habitat was characterized by species such as *Strobilurus esculentus* and *Galerina marginata*, while the mixed forest supported species like *Mycetinis alliaceus* and *Exidia nigricans*. Across all three habitat types, macrofungal diversity peaked during the autumn season. These findings provide new insights into the ecological importance of macrofungi in forest ecosystems and highlight the influence of habitat characteristics and management practices on their diversity. The results emphasize the need to explicitly integrate fungal conservation into forest management strategies.

Keywords: macrofungi; biodiversity; forest management; conservation; protected area.

Acknowledgements: Sampling within the ROSCI0074 Făgetul Clujului–Valea Morii site was conducted with permission from the protected area administration.

Integrating food composting into sustainable food systems: implications for biodiversity, ecosystem conservation, climate resilience

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The global food system faces increasing pressure to balance affordable food production with environmental sustainability, biodiversity conservation, and climate resilience. This study aims to evaluate the role of food composting in reducing environmental impacts associated with agricultural systems and food supply chains, particularly within the context of the “cheap food” paradigm. The research is based on a qualitative synthesis of eight recent studies (2022–2025), including life-cycle assessment, experimental, modeling, and review papers. These studies were analyzed to assess the environmental, agronomic, and systemic implications of food waste composting within sustainable food systems. The results indicate that composting significantly contributes to nutrient recycling, improving soil structure, enhancing microbial diversity, and increasing water retention capacity. These effects support ecosystem restoration and strengthen the resilience of agricultural systems under climate stress. Additionally, composting reduces the amount of organic waste directed to landfills, thereby lowering methane emissions and contributing to climate change mitigation. However, the findings also show that composting alone is insufficient to reduce the environmental burden of the food system fully. Its effectiveness depends on proper implementation and integration with complementary strategies such as food waste prevention, circular resource management, and agroecological practices. In some cases, alternative waste treatment methods may provide greater environmental benefits depending on local conditions. In conclusion, food composting represents a valuable component of sustainable agriculture and circular food systems, but its impact is maximized when combined with systemic changes in food production and consumption. Bridging scientific knowledge with practical implementation is essential to enhance biodiversity conservation, ecosystem health, and long-term climate resilience.

Keywords: biodiversity; composting; food systems; sustainability; waste.

Determination of heavy metals in bee pollen as an indicator of environmental pollution

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Bee pollen is widely recognized as a potential bioindicator of heavy metal contamination, reflecting the level of environmental pollution in the areas where it is collected. Due to its ability to accumulate substances from air, soil, and water, bee pollen can provide valuable information regarding the presence of inorganic contaminants such as Pb, Cd and As. This study aims to highlight the importance of determining heavy metals in bee pollen as a tool for monitoring environmental pollution, as well as the relevance of such analyses for the safety of apicultural products intended for human consumption. In addition, there is a clear need to develop appropriate analytical methods and to set regulatory limits for inorganic contaminants in bee products. In this study, the determination of selected heavy metals in bee pollen samples was performed using flame atomic absorption spectrometry (FAAS) and graphite furnace atomic absorption spectrometry (GFAAS), following microwave-assisted acid digestion. In addition, fractionation of polyfloral pollen into distinct botanical types was employed which could enable the evaluation of species-dependent accumulation patterns and the identification of pollen types with highest potential of heavy metal bioindicator. Method validation included the assessment of precision, limits of detection (LOD), and accuracy, the latter verified through spike-recovery experiments. Typical concentrations in bee pollen range from 0.01–0.1 mg/kg for Cd, 0.1–1 mg/kg for Pb and 0.01–0.1 mg/kg for As. The results highlight the analytical procedures for the assessment of some heavy metals in bee pollen as a potential bioindicator of environmental pollution.

Keywords: bee pollen; environmental pollution; heavy metals; inorganic contaminants.

Assessment of risk exposure in pedestrian red-light crossing - a case study in human ethology

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Risk represents a fundamental component of the existence of living organisms, and risk-taking behaviors are widely observed within human populations. Understanding the mechanisms underlying these behaviors is essential for their prevention and management. In this context, the present study aims to evaluate the degree of risk exposure as a function of age group, sex, and time of day, as well as to quantify the level of risk in relation to the severity of the situations individuals are exposed to. Additionally, a sociocultural comparative analysis is conducted between two distinct urban populations. The methodology was based on direct observation of pedestrians in the cities of Grenoble (France) and Cluj-Napoca (Romania), at two different times of the day, over a period of four months for each location. Statistical analysis included the application of the χ^2 (chi-square) test to assess differences between groups, namely between the two cities (Grenoble and Cluj-Napoca), between sexes, and between times of day (morning and evening). The results indicate that women are more frequently represented among pedestrians who cross during the red signal in both populations analyzed. Regarding the time of day, illegal crossings were more frequent in the morning. From an age perspective, most risk-taking behaviors were observed among young adults (19–40 years), as well as among elderly individuals. Furthermore, a significantly higher frequency of illegal crossings was observed in Grenoble compared to Cluj-Napoca. The results are consistent with trends identified in the literature, with the exception of the sex distribution, suggesting a combined influence of biological and socio-cultural factors.

Ethnoiatric studies in Arcalia village (Bistrița-Năsăud County)

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Medicinal plants represented, since ancient times, a fundamental resource for treating various diseases in both humans and animals. In our country, their use has its roots in the practices of our ancestors, the Geto-Dacians. Despite all economical and social changes, the relationship between humans and plants continues to persist in rural communities of Transylvania. This study aims to highlight the ways in which the inhabitants of Arcalia village, use and preserve the tradition of healing through medicinal plants, based on a survey conducted among a group of 50 local residents of different ages. A total of 177 plant species belonging to 53 botanical families were identified, highlighting the rich ethnoiatric knowledge of the area. Based on the semi-structured questionnaire, the majority of respondents (80%) reported that they had acquired their knowledge about plants within the family, from parents and grandparents, confirming that intergenerational transmission is the primary means of preserving and passing on this knowledge. It was found that women are familiar with a greater number of plant species than men. Leaves, flowers, and aerial parts are the most commonly used, and they are predominantly prepared as infusions. Most plant species are used to treat digestive system diseases (52 species), followed by respiratory diseases (39 species). Key species such as *Mentha × piperita*, *Matricaria chamomilla*, and *Eryngium planum* are frequently associated with these conditions, reflecting both their widespread use in traditional practice and their importance within local knowledge systems. The use of plants extends beyond the medicinal sphere, as they also play a role in cultural manifestations such as customs, beliefs, and rituals. Despite advances in modern medicine, the results show that traditional remedies continue to be used within the community, highlighting the importance of documenting and preserving this knowledge as part of the local cultural heritage

Keywords: ethnoiatry; medicinal plants; cultural diversity.

Genome-resolved metagenomic analysis of microbial diversity and ARGs/BGCs across a salinity gradient in Romanian lake sediments

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Saline and hypersaline lake sediments constitute important long-term reservoirs of microbial communities adapted to steady elevated osmotic pressure. Surveying microbiome composition along large salinity gradients can provide insights into the mechanisms that drive the metabolic and ecological restructuring of resident microbial communities, processes that ultimately influence overall ecosystem functioning. Among the key molecular determinants modulating microbial interactions within ecological networks are antibiotic resistance genes (ARGs) and biosynthetic gene clusters (BGCs) responsible for the production of secondary metabolites. To assess the salinity-driven potential of antibiotic resistance genes (ARGs) and biosynthetic gene clusters (BGCs) in sediment-associated microbiomes, sediments from three Romanian lakes with distinct salinity levels were analyzed: Amara (~1%), Tekirghiol (6–8%), and Ursu (~27%). Metagenome-assembled genomes (MAGs) were reconstructed and subjected to taxonomic and functional annotation to evaluate microbial diversity and metabolic potential, with emphasis on ARG and BGC distribution along the salinity gradient. The bioinformatic analysis will be streamlined using Nextflow DSL2 pipelines from the nf-core framework to ensure high reproducibility and standardized processing. The nf-core/mag/taxprofiler/funcscan pipelines will be utilized for *de novo* assembly and hybrid binning, the taxonomic identification and profiling, and the functional screening (ARGs/BGCs/AMPs) with structural annotation of the recovered MAGs. These findings will improve understanding of how salinity influences microbial diversity and functional potential in aquatic sediments, particularly in the context of salinization driven by global climate warming.

Keywords: antibiotic resistance genes; bacterial gene clusters; sediments; metagenomics; salinity.

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Biological relatedness at Medieval Dăbâca: insights from nonmetric cranial variation

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Dăbâca Fortress (Cluj County) has been the subject of intense historiographical debate with regard to its origin since its initial excavation. Four necropolises were also discovered in the Fortress' proximity, which were excavated throughout the 1960s. Of these, Grădina lui Tămaş, which lies adjacent to a small church, is of special interest, as it covers a relatively small area and has been in use for a limited time during the medieval period. The premise of this study is that, given the location, size and chronology of this particular burial site, it may have been used by individuals with familial connections to one another. Among the individuals that could be identified through the archaeological record, four possible familial clusters were recognised through kinship analysis based on shared morphological cranial traits. Furthermore, this study identified individuals belonging to more than two possible generations based on heritable traits and the archaeological descriptions of the graves. Lastly, the current analysis aims to better understand medieval funerary practices by identifying the possible spatial distribution of the individuals in order to establish a pattern of funerary landscape use by familial groups.

Keywords: heritable traits; kinship analysis; medieval necropolis; nonmetric cranial traits.

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Air currents as orientation cues in underground environments for *Pantherophis guttatus* (Squamata: Colubridae)

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Underground spaces represent a vital dimension of a colubrid snake's life. Virtually all major moments (hatching, hibernation, nocturnal thermoregulation) happen within underground tunnels and galleries dug, principally, by rodents and moles. Naturally, this implies a great deal of understanding of the underground space, based on various cues, which may be mechanical, chemical, physical or thermic in nature, cues complemented by spatial learning. Orientation is poorly understood in reptiles and amphibians, and the present study investigated the use of air currents as mechanical cues used for orientation and navigation in underground environments. We used a three-chambers model to test innate behaviour in juvenile corn snakes (*Pantherophis guttatus*), confirming that colubrid snakes are capable of detecting air currents. Results also show that seventy-five percent of individuals reacted positively to air current stimulation, taking refuge in the shelter where it was present, though both shelters were identical in shape and colour. In conclusion, we present that airflow is a highly attractive cue for corn snakes, acting as the primary navigational signal for locating shelters through positive rheotaxis. From our observations and the relevant literature, we can infer that this cue is likely significant for other colubrid snakes as well.

Keywords: airflow; colubrids; orientation; underground; thermoregulation.

Historical status and recent breeding record of the lesser kestrel (*Falco naumanni*) in Romania

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The Lesser Kestrel (*Falco naumanni*), a species formerly classified as globally threatened, has undergone significant population declines across much of its historical range, including southeastern Europe. In Romania, the species was formerly a widespread breeder but became locally extinct as a nesting species by the mid-20th century. In recent decades, scattered records have suggested occasional presence during migration periods and the breeding season, yet no confirmed breeding had been documented. Here we report the first verified breeding record of *F. naumanni* in Romania after several decades of absence, based on observations from August 2025 from Istria (Constanța county). The breeding pair successfully raised four fledglings, providing direct evidence of recolonization in suitable habitats. We review the historical and current status of the species in Romania, summarize recent observations, and discuss possible factors influencing its reappearance, including changes in land use, conservation initiatives in neighbouring countries, and regional population trends. Our findings underscore the need for targeted conservation measures, including habitat management and nest-site provisioning, to support the re-establishment of *F. naumanni* as a regular breeder in Romania.

Keywords: *Falco naumanni*; Lesser Kestrel; breeding record; historical and current occurrences; Romania.

Lichens dwelling on wood: the importance of substrate properties and forest structure

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We aimed to disclose the relationships between macrolichen species occurrence/richness, on one hand, and wood substrate properties (species identity and decomposition degree)/forest management history (old-growth, recovered after clear-cutting, and recently thinned), on the other hand. All macrolichen species occurring on ten living trees and ten dead-wood fragments (including stumps when logs were not available) were recorded within nine 1 ha-plots that were delimited in pure/mixed beech and spruce forest stands from the Apuseni Mountains. Generalised linear mixed models were employed to test the effects of the three variables of interest, while controlling for the effects of several environmental and biometric variables. Mean species richness was significantly higher on spruce than on beech wood, but that was true only for dead wood. Macrolichen richness declined steadily from living wood toward late stages of decomposition. Macrolichen species richness on living trees was on average higher in old-growth forests compared to those recently recovered after clear-cutting, probably due to the higher diversity of substrates, the continuity of tree canopy and the higher light availability resulting from lower tree density. On the contrary, the mean number of macrolichen species on dead wood was significantly lower in old-growth forests compared to younger (recently recovered) or recently disturbed (thinned) forests, reflecting substrate availability, where old-growth living wood reduces macrolichen reliance on dead wood. *Parmelia sulcata* was more likely to occur in old-growth forests and thinned stands than in those recently recovered, the latter being denser and consequently, darker. The availability of dead wood was a limiting factor for *Cladonia digitata*, which showed a significantly increasing probability of occurrence toward more advanced stages of wood decomposition. These results highlight the importance of dead wood availability and forest continuity for the persistence of specialised corticolous and lignicolous macrolichen species, most of which being rare or threatened.


Keywords: macrolichen diversity; wood species identity; wood decomposition stage; management history.

Funding: This research is part of the Experimental Implementation of Participatory Monitoring of Forest Quality and Biodiversity Based on the Cryptogamic Component (Mosses, Lichens) in the Montane Forests of the Apuseni Mountains (Muntele Mare & Bihor Mountains) project funded by Tomorrow Forest Foundation.

Acknowledgements: We express our gratitude to the Natural Park Apuseni Administration and Central Hall Cluj-Napoca, as project partners, for their valuable assistance in the selection of forest sites.

Dwarf Pine (*Pinus mugo* Turra) encroachment in mountain Grasslands as the main contributor to Carpathian greening

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Alpine greening has been widely documented across mountain regions, yet the relative contribution of shrub expansion and land-use legacies remains poorly quantified at regional scales. Here, we combine declassified CORONA spy satellite imagery with contemporary high-resolution imagery and Landsat NDVI time series (1984–2023) to assess the contribution of dwarf pine to greening across the Carpathian Mountains. We reconstructed historical and present distributions of *Pinus mugo* across 74, above treeline sites (1 × 1 km) and quantified expansion, retreat, and stable cover in relation to topography, land-use potential, and visually detectable land use legacies. Greening was influenced primarily by changes in dwarf pine cover, followed by topography and land-use conditions. The strongest increases in greenness occurred in grassland environments where pine expanded, while more moderate increases were observed in scree environments. Notably, pine expansion was most evident in areas with clear signs of past shrub removal. Our results identify pine encroachment driven by secondary succession as a major contributor to alpine greening in the Carpathians and highlight the importance of accounting for land-use legacies when interpreting greening trends (1984 - onwards) in mountain ecosystems.

Keywords: CORONA spy satellite; abandonment; NDVI_{max}; remote sensing; secondary succession.

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Soil mesofauna of Bortig Ice Cave sinkhole (Apuseni Mountains, Northwest Romania)

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Ice caves are permanent cryosphere unique ecosystems that store ancient genetic diversity and serve as refuges for life surviving in cold environmental conditions. Ice caves and their corresponding sinkholes display distinct transition ecotones, where microclimatic inversion fosters a rich biological diversity. This study examines the soil mesofauna of Bortig Ice Cave (Apuseni Mountains), one of the largest caves with perennial glacier of Romania. We aim to assess the invertebrate's biodiversity and create a biological baseline for forthcoming environmental DNA (eDNA) research on the perennial ice block, where soil-dwelling taxa are expected to be sequestered. Soil sediment samples were collected from three sites along a vertical transect (November 2026). The community consists of a wide range of invertebrates, with Nematoda, Oligochaeta, and Acarina being the most common, followed by Collembola, Gasteropoda, and Chilopoda, and few Isopoda, Diplopoda, Harpacticoida, and insect larvae. The top-forested site has the highest invertebrate's biodiversity, dominated by Nematoda, dropping off at the sinkhole bottom, where only Collembola and Oligochaeta are present. The shift pattern of organism's distribution corresponds to a change in environmental conditions, from warm and dry deciduous leaf litter to cold and wet soil close to the ice block. Our preliminary results are consistent with researches conducted in other ice caves, where taxa such as Acarina and Collembola are essential indicators of cold and stable microclimates. The biotic assemblage provides clues regarding typical ice caves fauna adapted to cold environmental conditions and highlights the ecological gradient from glacial to periglacial zones of these caves. This study fills a big gap in our knowledge on ice caves fauna of Romania. As these frozen archives are in decline due to climate changes, it is important to record the current diversity of these hidden habitats to learn more about their resilience and evolutionary history in cryosphere environments.

Keywords: invertebrates; cold-stenotherm; soil sediment; diversity.

Funding: This research was funded by Biodiversa+, the European Biodiversity Partnership, in the context of the Sub-BioMon - Developing and testing approaches to monitor subterranean biodiversity in karst project under the 2022-2023 BiodivMon joint call. It was co-funded by the European Commission (GA № 101052342) and the following funding organizations: Ministry of Higher Education, Science and Innovation (Slovenia), The Belgian Science Policy (Belgium), Ministry of Universities and Research (Italy), National Research, Development and Innovation Office (Hungary), Executive Agency for Higher Education, Research, Development and Innovation Funding (Romania) and self-financing partner National Museum of Natural History Luxembourg (Luxembourg).

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Algae, invertebrates and fish communities from the Vâlsan River, Romania

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Freshwater ecosystems are central to the global water crisis, as they provide essential ecological services and biodiversity yet remain among the most threatened systems worldwide. Rivers play a central role in sustaining freshwater biodiversity, and those characterized by high ecological diversity often support a remarkable number of endemic species; among them is the extremely range-restricted Romanian fish *Romanichthys valsanicola*, considered one of the rarest freshwater fishes in Europe. In this context, studies investigating the diversity of different biotic compartments in areas critical for the conservation of these endemic species have a dual significance: they provide insights into current ecological conditions and generate data on the resources available to these species, and consequently on their long-term sustainability. The present study focused on the Vâlsan River, a tributary of the Argeş River, within the recognized distribution range of *Romanichthys valsanicola*. Sampling efforts targeted phyto-benthic and zoobenthic communities, considered key resources for the fish assemblages in the area. The sampling campaign was conducted in November 2023 and included eight sites along the river course, from upstream of the Vâlsan Waterfall to the locality of Vâlsăneşti. Phyto- and zoobenthic diversities are discussed in relation with ichthyofaunal characteristics. As samples from all these communities were collected simultaneously, the study provides an integrated perspective on the trophic resources available to fish assemblages in the study area.

Keywords: biotic diversity; benthic invertebrates; ichthyofauna; phyto-benthic communities; trophic resources.

When specimens break but data survive: the Gheorghe Pătraşcu Lepidoptera collection

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The collection was assembled between 1982 and 1989 by the entomologist Gheorghe Pătraşcu and comprises 2217 specimens representing 340 species. Donated after the collector's death, the collection was in poor condition, with many specimens severely damaged by *Anthrenus spp.* (*Dermestidae*), resulting in the loss of important body structures. To preserve both taxonomic information and collection data, the most deteriorated specimens were conserved using an unconventional but effective method: detached wings were placed together with the original labels between transparent plastic foils and then laminated. This allowed the preservation of diagnostic morphological characters alongside locality and date information. The collection was subsequently digitized and organized into a database. The material documents 38 collecting localities, most of them in Suceava County, and provides valuable faunistic data for a still insufficiently studied region of northeastern Romania. Its scientific relevance is enhanced by the presence of several rare or biogeographically important species, including *Pharmacis carna*, *Plusidia cheiranthi*, *Argynnis laodice*, *Hipparchia statilinus*, *Rheumaptera hastata*, and *Odezia atrata*. This collection expands knowledge of Lepidoptera diversity in Suceava County and demonstrates a workflow for conserving and digitizing heavily deteriorated entomological collections. Even severely damaged material retains irreplaceable scientific value; targeted preservation combined with systematic digitization can rescue data for future research. Our approach may serve as a model for institutions facing similar challenges with damaged natural history collections.

Keywords: Lepidoptera; natural history collections; digitization; conservation; faunistic data.

City nesting: Ecological consequences

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Understanding how urbanised areas affect wildlife is becoming more and more important as they grow and change around the world. For animals, city living can be both stressful and beneficial, and the combination of these impacts show up in reproductive success. The aim was to examine the influence of vegetation diversity, tree composition, clutch size, and air pollution on the reproductive failure of two cavity-nesting avian species, the great tit (*Parus major*) and Eurasian blue tit (*Cyanistes caeruleus*). The unhatched eggs were collected from 246 nestboxes in 5 urban parks in Malmö, Sweden from 2023 to 2024 in spring. This study examined the physiological traits of nestbox populations of the two passerines with regard to hatching failure. Using dissection and a fluorescence microscope to measure the amount of DNA-stained sperm cells, the embryo's malposition, deformity, and stage of death were all determined. The findings highlight that vegetation structure plays a key role in explaining both hatching failure and the number of unhatched eggs, whereas the effects of air pollution appear less pronounced. Across models, we found that lower vegetation diversity is consistently associated with higher hatching failure and a slight increase in the number of unhatched eggs. Tree composition also influenced outcomes: greater *Fagus sylvatica* cover increased failure rates, while higher *Quercus robur* cover tended to reduce them. Clutch size emerged as a strong driver in all models, with larger clutches showing lower hatching failure but naturally producing more unhatched eggs in absolute numbers. Interestingly, air pollution that was assessed via predicted NO₂ and PM_{2.5} levels and an urbanisation score did not show significant independent effects on either hatching failure or unhatched egg counts.

Keywords: *Cyanistes caeruleus*; hatching failure; *Parus major*; urbanization.

Acknowledgements: I express my gratitude to Zsófia Tóth for providing me with the opportunity to work and grow in this project as a research trainee, for her support, guidance and patience. Further, I want to thank Andrei Petrişor, my master's colleague, for sharing and allowing me to utilize his stunning images of both species.

Earth Sciences and Ecosystem Dynamics

Large benthic foraminifera of the Western Tethyan Eocene: case-studies on their evolution and biostratigraphy

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Fossils of large benthic foraminifera (LBF, giant unicellulars sizing over 1 mm and up to 15 cm) are widely distributed in shallow marine tropical and subtropical deposits of the Eocene in the Western Tethys, they occur frequently in rock-forming quantity (e.g., the Egyptian pyramids are full of them). Their large size is due to their symbiosis with low-ranked plants capable of photosynthesis, which served as nutrients for them. Due to their rapid evolution some LBF, especially the spirally coiled, lenticular genus *Nummulites* have long been used to determine the age of their host rocks, i.e., for biostratigraphic purposes. Some other groups, however, were believed to be worthless in this sense. Two case-studies of the presentation exhibit the studies, as a result of which other LBF groups were also made suitable for biostratigraphic purposes. These are (i) the orthophragmines (the second most widespread LBF of the Late Paleocene to terminal Eocene time-span in the W Tethys) with orbitoidal architecture, and (ii) the Late Eocene nummulitid genus *Heterostegina* with secondary chamberlets. By integrating these new results, in the third case-study the refined stratigraphic subdivision of the late Middle Eocene to terminal Eocene time-span is discussed.

Keywords: biostratigraphy; Eocene; evolution; large foraminifera; W Tethys.

When the reality confirms the hypothesis: a case study of a nummulitic accumulation in the NW Transylvanian Basin

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Nummulitic accumulations (banks) are peculiar sedimentary units of the Eocene period in the Neotethyan realm. This type of sedimentary record is widely observable at the top of the Eocene Căpuş Formation in the NW part of the Transylvanian Basin. Based on the study of 18 outcrops situated between Cluj-Napoca and Huedin, a hypothetical depositional and evolutionary model was proposed earlier. This model is mainly based on the observed distribution of *Nummulites* assemblages and associated sedimentary features. Due to the size, exposed area and spatial distribution of the studied outcrops, the transitional zones between the identified *Nummulites* assemblages were postulated hypothetically. Fortunately, the discovery of a new outcrop located close to the Călăţele locality allowed us to check the validity of the hypothetical depositional model and confirm the assumed features of the postulated transitional zones, enabling a better understanding of the evolution of the proposed depositional model through the studied nummulitic accumulation.

Keywords: depositional model; *Nummulites*; paleoecology; Romania; Transylvanian Basin.

Micropaleontological, biostratigraphic and paleoenvironmental analysis of the early Eocene (late Ypresian) nummulitic limestones of southern Dobrogea

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Abstract: The present study investigates the Eocene nummulitic limestones of southern Dobrogea. Despite a long record of paleontological and stratigraphical studies of this sedimentary unit since the discovery of the first outcrops at Cetatea (Azarlâc) in 1882, the age of these deposits remains highly debated. Furthermore, there were no attempts made to reconstruct the depositional environment of this carbonatic succession. The material analyzed in this study originates from a drill core recovered in Mangalia. In order to constrain the age of the studied limestones isolated specimens of larger benthic foraminiferas (LBF) were selected randomly from the residue resulted from the acetic acid treatment (99%) of the samples. The biometrical parameters of the LBF were measured, then they were identified on species level. The paleoenvironmental reconstruction is based on microfacies determined on thin-sections and described using the Extended Dunham Classification. The studied samples could be assigned to the Cetatea Formation based on the lithological characteristics and the main bioclasts. The taxonomic studies revealed the presence of six *Nummulites* taxa (*N. rotularius*, *N. distans*, *N. alexisi*, *N. archiaci*, *N. aff. pavloveci* and *N. nitidus*), two *Assilina* (*A. laxispira* and *A. placentula*) and two *Operculina* species (*O. escheri* and *O. karrieri*). The recovered LBF assemblage is typical for the SBZ11, which confers to the studied sedimentary record a late Ypresian (middle Cuisian) age. The microfacies and micropaleontological analysis indicates a warm, oligotrophic environment with moderate hydrodynamics. The foraminiferal association shows similarities to other LBF assemblages in the Peri-Tethys, indicating that they developed in the same biogeographical province.

Keywords: biostratigraphy; nummulitic limestone; Eocene; LBF; microfacies.

Preliminary data on a new Oligocene ichthyofauna occurrence from northwestern Transylvania

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Oligocene ichthyofaunas are relatively well-documented in Romania, primarily based on skeletal remains recovered from deep marine deposits of the Eastern Carpathian Flysch. In contrast, such occurrences remain poorly known or entirely undocumented in other regions. Here, we report a new Oligocene fish assemblage from north-western Transylvania. Located on the left bank of the Luncii Valley near its confluence with the the Someş River, the outcrop exposes two well-known stratigraphic units from the Transylvanian Basin: the Cozla Limestone (Priabonian–Rupelian) and the Cuciulat Formation (Rupelian). The latter yields a diverse ichthyofauna comprising batoids, sharks and bony fish. Associated faunal proxies (foraminifera, nannoplankton, and ostracods), together with microfacies analysis, indicate deposition in a normal marine, well-oxygenated environment. These data suggest a shallow shelf setting within the photic zone, characterized by relatively low nutrient flux.


Keywords: batoids; bony fish; new outcrop; Rupelian; sharks.

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Microfossils as key tools for reconstructing badenian biostratigraphy and paleoenvironments in the Southwestern Transylvania and Făget basin

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The present study analyzes diverse and well-preserved microfossil assemblages identified in samples collected from marine deposits, gathered from an outcrop located in the southwestern part of Hunedoara County and from a borehole drilled near the boundary with Timiș County. The research aims to reconstruct the paleoenvironmental conditions during the Badenian (Middle Miocene). Using standard micropaleontological processing, foraminiferal and calcareous nannofossil assemblages were integrated with sedimentological data for age determination and depositional environment correlation. The occurrence of *Orbulina suturalis* and *Sphenolithus heteromorphus*, together with the absence of *Helicosphaera ampliapertura*, indicates attribution to the M6 (planktonic foraminifera) and NN5 (calcareous nannoplankton) Biozones, corresponding to the middle Badenian. The foraminiferal assemblages from both locations include agglutinated benthic, calcareous benthic, and planktonic groups. Based on the distribution of benthic foraminifera, paleoecological factors such as oxygenation levels, nutrient availability, salinity, and substrate type were inferred. *Reticulofenestra minuta* dominates the calcareous nannofossil assemblage, indicating nutrient-rich, eutrophic, nearshore environments. These results highlight the marine dynamics in specific areas of the sedimentary basins during the Badenian.

Keywords: biostratigraphy; microfossils; paleoenvironments; statistics.

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Vegetation changes and ecosystem resilience in Southern Transylvania during the Holocene

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Palaeoecological studies provide valuable long-term records that can support the identification of suitable vegetation compositions for sustainable landscape restoration in the context of disturbances and climate change. Nevertheless, such historical data are often regarded as supplementary background information and are rarely fully integrated into forestry and conservation policies. In this study, we combine multiple palaeoecological proxies, using pollen as an indicator of past vegetation from a peat bog situated at the northern foothills of the Făgăraş Mountains, together with magnetic susceptibility data and published records of charcoal, trace elements, and stable isotopes. The pollen data, along with statistical analyses (DCCA, rate of change, and rarefaction), identify five distinct phases in historical community dynamics: (i) a period of moderate to high variability between 10,200 and 9,000 cal BP; (ii) a stable interval with minimal compositional change from 9,000 to 5,200 cal BP; (iii) a phase of moderate turnover between 5,200 and 3,800 cal BP; (iv) a period of low to moderate change between 3,800 and 500 cal BP; and (v) the last 500 years, characterized by moderate to high turnover and increased diversity. These phases are examined to better understand the underlying drivers—such as climate shifts, species interactions, and both natural and human disturbances—and to evaluate the resilience of ecosystems to environmental change throughout the Holocene.

Keywords: pollen; multi-proxy; landcover; disturbance; ecosystem stability.

Holocene climate variability and recent anthropogenic impacts recorded in a Romanian peatland

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Peatlands are highly sensitive archives of past environmental change, with their hydrology closely linked to climate variability. In this study, we investigate Holocene hydroclimatic dynamics in Central Romania using a multi-proxy record from the Mlaca Tătarilor peat bog (Făgăraş Depression). Depth-to-water-table (DWT) changes were quantitatively reconstructed based on testate amoebae assemblages and integrated with pollen data and peat physical properties (organic matter content, moisture, bulk density, and magnetic susceptibility). The studied sequence spans the last ~11,700 years and reveals pronounced hydrological variability. The early Holocene is characterized by large-amplitude fluctuations, including a marked drying phase, followed by a shift toward wetter conditions. During the mid-Holocene, relatively stable and predominantly wet conditions prevailed, although short-lived dry events are clearly expressed in the DWT record. The late Holocene shows increased variability, including a drying phase around the 4.2 ka event and subsequent oscillations linked to regional climate dynamics. Comparison with other palaeoclimate records from the Romanian Carpathians indicates that these hydrological shifts are broadly synchronous at the regional scale, although site-specific responses highlight the importance of local controls. The last millennium is marked by a long-term drying trend culminating in the mid-20th century, likely reflecting both climatic variability and increasing human impact (e.g., drainage and land use). Notably, the most recent part of the record suggests a partial recovery toward wetter conditions, which may be associated with restoration measures implemented in recent decades. These findings highlight the sensitivity of peatland hydrology to both natural and anthropogenic drivers and underscore the importance of long-term records for informing peatland conservation and management strategies.

Keywords: Holocene; hydroclimate; palaeohydroclimate; peatland; testate amoebae.

The mineralogy of kidney stones and their related bacterial cultures

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Kidney stones can generally form due to many factors: unbalanced diet, not enough liquid consumption, hormonal issues, urinary postponement etc. We can give a mineralogical insight in order to help prevent and treat these biological "concretions" analyzing their structure and formation conditions. We can gain fresh perspectives on these issues using thin section analysis, X-ray diffraction (XRDP), and electron microscopy images (SEM). The basic idea behind their formation is as follows: something precipitates or crystallizes out of the urine, which serves as a starting point for other crystals to grow and consolidate into a potentially dangerous kidney stone. Numerous minerals, including the calcium oxalates weddellite and whewellite, the phosphates apatite and struvite, and other minerals such as uricite, have been identified to be major components in kidney stones. In addition to identifying potential bacterial colonies that might flourish in the forming conditions of or within the stones, potentially leading to additional complications, the various properties and genesis of urinary tract associated minerals can be used to our advantage in determining different ways of eliminating, corroding, or completely avoiding these unpleasant outgrowths. It is possible to identify the bacterial colonies in biological materials by first incubating them and then seeding them on various culture media. Bacteria can thrive in the urinary tract due to the production of mineral precipitates. Differentiating between pathogenic and non-pathogenic bacteria is crucial because the latter can cause either mild or severe illnesses. Additionally, we have conducted statistical analysis of our results in order to properly determine the observed correlations and in order to find the infection potential of a kidney stone. In this presentation, we will discuss the results of mineralogical analyses performed on a set of 39 kidney stone samples, predominantly of oxalatic and phosphatic composition. These analyses will determine the stones composition and examine their structure, as well as their origin and the bacterial colonies that have been discovered to be developing inside or around them. We will also talk about possible connections between those different bacterial strains and the mineralogy of kidney stones.

Keywords: Bacterial cultures; biominerals; kidney stone mineralogy; oxalates; phosphates.

Hydro-chemical analysis and assessment of surface water quality for drinking and irrigation in Oued Fez watershed (Fez plain, Saïs Basin, Morocco)

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The Saïs Basin, whose northeastern area encompasses the city of Fez, is located in northern Morocco between the Middle Atlas and Pre-Rif mountain ranges. Its surface water network supports agricultural, urban, and industrial activities in this semi-arid region. However, increasing anthropogenic impact has contributed to the degradation of this water quality, highlighting the need for continuous monitoring and sustainable management of these resources. This study assesses the physicochemical characteristics, hydrochemical evolution, and suitability of surface waters for drinking and irrigation in the Oued Fez Basin (OFB). Hydrochemical analyses show that major ion concentrations (mg/L) decrease in the following order: $\text{HCO}_3^- > \text{Cl}^- > \text{Na}^+ > \text{Ca}^{2+} > \text{SO}_4^{2-} > \text{NO}_3^- > \text{Mg}^{2+} > \text{K}^+$. The spatial distribution of water facies reveals a transition from Ca-HCO₃ type waters in upstream areas to Na-HCO₃ in intermediate zones and Na-Cl downstream. These variations are primarily controlled by water-rock interaction processes and are further influenced by anthropogenic inputs in densely urbanized areas. Stable isotope signatures range from -7.09 to -5.68‰ for $\delta^{18}\text{O}$ and from -42.7 to -32.8‰ for $\delta^2\text{H}$, indicating a meteoric origin of the waters and recharge zone occurring in the Middle Atlas at elevations between 1011 and 1577 m. Water quality evaluation shows that most samples are suitable for drinking, except the samples located within urban areas (Fez city). Irrigation suitability indices (SAR, Na%, PI, KI, IWQI) indicate that downstream waters, located in the urban area of Fez, present significant sodium and salinity hazards, making them unsuitable for irrigation. This study provides a spatially integrated understanding of the processes controlling surface water chemistry and identifies the main anthropogenic sources of degradation. The results underline the combined influence of geological, climatic, and human factors on water quality and offer valuable information for the protection and sustainable management of water resources in the Oued Fez Basin.

Keywords: Fez-Morocco; surface waters; hydrochemistry; isotopic signatures; irrigation and drinking suitability.

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Foraminiferal study of the Oligocene sedimentary record of southern Albania

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We have investigated the foraminiferal assemblages recovered from the Oligocene Bozdovec Formation occurring in the Albanian–Thessalian intramountain basin, southern Albania using both qualitative and quantitative methods. The examined samples were prepared using standard micropaleontological procedures using a 63 µm sieve. The dried >63 µm residue was analyzed for planktonic and benthic foraminiferal tests using two different size-fractions: the larger than 125 µm, and that above 63 µm. The lack of prior foraminiferal studies forced us to use the age and paleoenvironmental results based on calcareous nannoplankton remains as the starting point of our investigation. According to these studies, the depositional environment of the examined formation during the Oligocene (possibly Chattian) was characterized by a neritic, shallow marine setting with warm-temperate waters and significant continental sedimentary input. These findings are assumed to be consistent with results based on planktonic foraminiferal analyses, while benthic foraminiferal assemblages help to clarify the environmental conditions of the seafloor during the deposition of the Bozdovec Formation.

Keywords: Albania; foraminifera; Oligocene; paleoenvironment.

Factors influencing the distribution and morphology of *Entzia macrescens* in an inland salt marsh

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Salt marshes are interesting habitats hosting many macroscopic species, but they might be also habited by microscopic unicellular organisms such as *Entzia macrescens* (Brady, 1870), an agglutinated foraminifera, which have been reported so far in Romania from Turda, Deva, and Sic. We assumed that this taxa should be widespread, therefore might occur in other salt marshes as well, we targeted the Cojocna area, from which we successfully collected living and subfossil specimens. The samples were collected from the uppermost, oxygen-rich layer of the soil at 10 sampling points along a transect, then stained in situ with Rose Bengal following FOBIMO guidelines. We measured the water's physicochemical parameters i.e., temperature, pH, electrical conductivity, derived the total dissolved solids from the electrical conductivity, and determined the redox potential Using the Orion Star A 324 multiparameter meter and Thermo Fisher Scientific electrodes on site. The stained sediment was sieved through a 63 µm sieve two weeks after the sampling, the resulted residue placed in distilled water, and whenever it was possible 100 foraminiferal tests were selected randomly from each using a brush. Residues having less than 100 individuals were checked and picked thoroughly. The picked tests were morphological examined: we measured the diameter of the first chamber (proloculus) and that of the test, counted the number of chambers and whorls, then investigated the possible relationship between the physical-chemical parameters of the water and the morphological parameters. The study of these unicellular organisms is important not only because of their role as bioindicators, but also because of their extremophilic nature.

Keywords: agglutinated foraminifera; Cojocna; salt marshes.

Mammoth findings from the Quaternary of Cluj-Napoca

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The Paleontological and Stratigraphical Museum of the Babeş-Bolyai University (PSMBBU) holds many molars and other skeletal elements belonging to mammoth species, which originate from all across the country and some even from outside of it. Over the course of last year, I have overviewed this collection and created a database containing every information found alongside each piece (place of origin, collector's name, date of collection etc.). The purpose of my research is to introduce a new mammoth remain consisting of several skeletal elements found at Cluj-Napoca into this collection. Radiocarbon dating was applied on the new remains in order to constrain its age. CT scanning and morphometrics was used to infer on the morphology of the skeletal elements found, and to constrain its taxonomical status. The sample collected at the site was studied using standard micropaleontological methods. So far shells of charophyta, gastropoda and ostracoda were recovered. These suggest the existence of a marsh environment at the time of the burial of the mammoth remains.

Keywords: gastropods; mammoth; Pleistocene.