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Arboretele de stejar cu molid din zona Tulgheș (județul Harghita)

Adrian OPREA^{1*}, Culiță SÎRBU²

¹Grădina Botanică "Anastase Fătu", Universitatea "Alexandru Ioan Cuza", Iași, România

²Facultatea de Agricultură, Universitatea de Științele Vieții "Ion Ionescu de la Brad", Iași, România

* Corresponding author: Adrian OPREA, a_aoprea@yahoo.co.uk

Lucrarea prezintă vegetația arboretelor de amestec de la Tulgheș (jud. Harghita), situate pe versantul sudic al munților Bistriței, pe partea stângă a râului Bistricioara, în bazinetele pâraielor Jgheabu Mare, Jgheabu lui Tudor, Jgheabu lui Năstase și Prisăcani. Arboretele sunt considerate relictar-cuaternare, fiind instalate pe substrat de fliș grezo-calcaros, cu șisturi cristaline, acoperite de marne cu șisturi argiloase, gresii, dolomite, calcare recifale masive și conglomerate calcaroase, de vârstă Cretacic Mijlociu și pe soluri brun eumezobazice. Vegetația este edificată de fitocenoză dominate de stejar (*Quercus robur*) și molid (*Picea abies*), însoțite de o serie de specii mai mult sau mai puțin termofile. Aceste arborete sunt situate sub limitele actuale ale rezervației naturale „Pietrele Roșii”, ocupând părțile inferioare și mijlocii ale versanților, la altitudini cuprinse între 650 și 1000 m altitudine, în fondul forestier de stat UP VIII Prisăcani.

Keywords: Tulgheș, Pietrele Roșii, vegetație, arborete de amestec

Prevalence and distribution of the canine heartworm, *Dirofilaria immitis* in dogs from Galápagos

Carla A. Cuda¹, Nathalie G. Tejena Rodriguez², Puleo Fortunato³, Georgiana Deak¹, Rafael M. Polit Sosa⁴, Jaime Grijalva⁴, Andra C. Barbu¹, Andrada S. Carstolovean¹, Romane Dionnet¹, Teodora Dan¹, Renato Leon⁵, Lenin R. Vinuesa^{4,5}, Diego Páez-Rosas^{6,7}, Andrei D. Mihalca^{1*}

¹Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

²Universidad Regional Amazonica Ikiam

³Faculty of Veterinary Medicine, University of Life Sciences "King Mihai I" of Timișoara.

⁴Universidad San Francisco de Quito, Escuela de Medicina Veterinaria, Cumbayá, Quito, 150157, Ecuador

⁵Universidad San Francisco de Quito, Laboratorio de Entomología Médica & Medicina Tropical LEMMT, Cumbayá, Quito, 150157, Ecuador.

⁶Universidad San Francisco de Quito, School of Biological and Environmental Sciences and Galapagos Science Center

⁷Dirección del Parque Nacional Galápagos, Unidad Técnica Operativa San Cristóbal, Isla San Cristóbal, Islas Galápagos 200150, Ecuador.

*Corresponding author: Andrei D. Mihalca; e-mail: amihalca@usamvcluj.ro

It all begins with a thought and a story of species evolution. On 17th September 1835, Charles Darwin noted his first observations about the Galapagos in his journal after taking the first steps on San Cristobal Island. Moving ahead in time by nearly 200 years, on 1st July 2021 until 2022, our team arrived to the same biodiversity hotspot: the Galápagos Islands. The project revolved around the concept of conservation medicine about the risk and impact of a multi-host parasite, the canine heartworm, *Dirofilaria immitis* on the endemic and endangered species, the Galapagos sea lion, *Zalophus wollebaeki*. Infectious and parasitic diseases were previously suggested as a possible cause of the decline of sea lions. Significant mortalities were observed in colonies located close to human settlements. Owned and feral dogs are present on all inhabited islands. The parasites of dogs in Galapagos were investigated only on a few occasions. They revealed a high diversity of pathogens. The canine heartworm is particularly interesting as it is transmitted via mosquitoes. Blood samples (n=1221) were randomly collected from owned dogs on the inhabited islands of San Cristobal, Isabela, Santa Cruz and Floreana. Ten samples from San Cristobal (prevalence 10%) and four samples from Isabela (2.01%) were found positive for *D. immitis* by Knott's test. Blood samples were negative by Knott's test on Floreana and Santa Cruz Islands.

Keywords: *Dirofilaria immitis*; domestic dogs, inhabited islands, zoonoses

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The taxonomical consequences of morphological variation the circum-Carpathian populations of *Phytoecia tigrina* (Coleoptera: Cerambycidae) -

Andrew M. Zamoroka, **Adrian Ruicănescu**, Cosmin O. Manc

Phytoecia (Pilemia) tigrina Mulsant, 1851 is a Ponto-Pannonian subendemic species, distributed mainly in the basins of Danube and Dnister and Aegean Sea. Its current range is disjunctive, separated for several centers by mountain systems in South-East Europe. Additional center of *Ph. tigrina* is probably occupies Maritime Alps, which we discussed in this paper. We studied morphology of *Ph. tigrina* from circum-Carpathian populations. Resultantly, we found crucial morphological differences which distinguishing populations of *Ph. tigrina* in Danube and Dnister basins. On the basis of these features and geographical disjunctions, we subdivided *Ph. tigrina* on subspecies: *Ph. tigrina tigrina*, *Ph. tigrina anchusae* **nom. res. & stat. nov.** and *Ph. tigrina podillica* **ssp. nov.** We redescribed *Ph. tigrina anchusae* **nom. res. & stat. nov.** and described *Ph. tigrina podillica* **ssp. nov.** All these subspecies evolved in separate isolated last glacial maximum (LGM) refugia and expended their ranges in postglacial time.

Distribution and diversity of fishes and lampreys in Transylvania (Romania): a complete survey and suggestions of new protected areas

András Attila Nagy^{1,2*}, Nándor Erős^{1,3}, István Imecs⁴, Gábor Bóné^{2,5,6}, Attila Fülöp^{1,7,8}, Péter László Pap¹

¹Evolutionary Ecology Group, 3B Centre for Systems Biology, Biodiversity and Bioresources, Hungarian Department of Biology and Ecology, Babeş-Bolyai University, Clinicilor street 5–7, RO–400006 Cluj-Napoca, Romania

²Milvus Group Bird and Nature Protection Association, Crinului street 22, RO–540343 Târgu Mureş, Romania

³Institute of Aquatic Ecology, Centre for Ecological Research, Bem Square 18/C, H-4026 Debrecen, Hungary

⁴Department of Freshwater Fish Ecology, Hungarian University of Agricultural and Life Sciences, 2100 Gödöllő, Hungary

⁵Department of Evolutionary Zoology and Human Biology, University of Debrecen, Egyetem tér 1, H–4032 Debrecen, Hungary

⁶Juhász-Nagy Pál Doctoral School, University of Debrecen, Egyetem tér 1, H–4032 Debrecen, Hungary

⁷STAR-UBB Institute of Advanced Studies in Science and Technology, Babeş-Bolyai University, Mihail Kogălniceanu street 1, RO– 400084 Cluj-Napoca, Romania

⁸ELKH-DE Behavioural Ecology Research Group, Department of Evolutionary Zoology and Human Biology, University of Debrecen, Egyetem tér 1, H–4032 Debrecen, Hungary

* Corresponding author: András Attila Nagy, nagyandrasattila@yahoo.com

Freshwater fishes are in a serious state of decline across the world, making them one of the most threatened groups of vertebrates. The Danube River catchment area in Europe holds the richest freshwater fish community, but our knowledge of the current distribution of these species is limited. Transylvania is one of the important tributaries of the Danube, from where 77 fish and 2 lamprey species were recorded until now. Despite of this large diversity of freshwater fishes, there is a lack of systematic survey for the past 50 years. We present data on the occurrence and distribution of fishes and lampreys collected between 2007-2022. We recorded 65 species of fish and 3 species of lampreys, and we also report an additional 9 species based on information from competent people. Of the 77 species recorded 19 (24.7%) are non-native, although their relative abundance was low (5.1%) compared to other similar regions in Europe. We present the first records of *Eudontomyzon mariae*, *Neogobius melanostomus*, *Piaractus brachypomus*, *Pygocentrus nattereri* and *Salvelinus alpinus* in Transylvanian rivers, the first record of *Cobitis elongata* outside the Nera River basin (from the Caraş River), and the detection of three new populations of the endangered *Umbra krameri*. We also provide data on changes in distribution that have occurred since the last comprehensive survey 50 years ago. We discuss the importance of our results in conservation planning, including the designation of new protected areas for freshwater bodies, and the compilation of the Romanian Red List of fishes.

Keywords: conservation, fish distribution, freshwater ichthyofauna, Natura 2000, non-native species

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The CarpaTherm soil temperature monitoring network: a unique resource for alpine ecosystem research

Pavel Dan Turtureanu^{1, 2, 3, 4 *}, Mihai Pușcaș^{1, 2, 3, 4}, Tudor-Mihai Ursu⁵, Philippe Choler⁶

¹ A. Borza Botanic Garden, Babeș-Bolyai University, 42 Republicii Street, 400015 Cluj-Napoca, Romania

² Faculty of Biology and Geology, Babeș-Bolyai University, 44 Republicii Street, 400015 Cluj-Napoca, Romania

³ Centre for Systems Biology, Biodiversity and Bioresources (3B), Babeș-Bolyai University, 3-5 Clinicilor Street, 400006 Cluj-Napoca, Romania

⁴ Emil G. Racoviță Institute, Babeș-Bolyai University, 5-7 Clinicilor Street, 400006 Cluj-Napoca, Romania

⁵ Institute of Biological Research, National Institute of Research and Development for Biological Sciences, 48 Republicii Street, 400015, Cluj-Napoca, Romania

⁶ Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, LECA, F-38000 Grenoble, France

* Corresponding author: Pavel Dan Turtureanu, pavel.turtureanu@ubbcluj.ro

Microclimatic conditions, particularly in the alpine zone, play a vital role in the functioning of plants and ecosystems. In this presentation, we provide a comprehensive overview of an extensive long-term soil temperature monitoring network in the Carpathian Mountains. The network comprises 70 permanent sites, spanning a range of elevations from mountain meadows at 1000m to the highest summits as high as 2400m. We used autonomous miniaturized loggers to record near-surface soil temperatures at hourly or bi-hourly intervals. The dataset, which now includes over 155,000 days of measurements and more than 3.6 billion total recordings, has been derived from three major monitoring initiatives: GLORIA (since the early 2000s), ODYSSEE (since 2014), and MEMOIRE (since 2018), and is continuously expanding. In order to ensure the integrity of the data collection, summer field campaigns are carried out on an annual basis. Scientific goals involve characterizing thermal niches of alpine biodiversity components, linking local temperatures to regional climate, examining the effect of soil temperature regime on ecosystem phenology and ecosystem functioning. CarpaTherm is a prime monitoring case for practical disciplines of ecology and biogeography, and can be integrated with advanced geospatial data analysis tools such as Google Earth Engine for educational purposes at Babeș-Bolyai University. The integration of this extensive collection of soil temperature time series enables a thorough understanding of microclimatic variability in the Carpathian Mountains. This far-reaching monitoring initiative serves as an invaluable resource for conservation management and ecological research, as we adapt to changing climatic conditions.

Keywords: alpine, Carpathians, long-term monitoring, microclimate, soil temperatures

Funding: Temperature data was acquired through these monitoring initiatives / projects: GLORIA (Global Observation Research Initiative in Alpine Environments), MICROCLIM (European Research Council; No. 883669), ODYSSEE (UEFISCDI; PN-II-ID-JRP-RO-FR-2012), MEMOIRE (UEFISCDI; PN-III-P1-1.1-PD2016-0925), BIODIVMOUNT (UEFISCDI; PN-II-CT-ROFR-2014-2-0011 and ANR France; ANR-13-ISV7-0004).

Distribution of Cladonia species in Romania and their ecological preferences: an exploratory relationship analysis

Florin Crişan, Dan Gafta

The objectives of the study were: 1) establishing the optimal number and component of groups of Cladonia species based on their distribution in ecoregions or their preferences for substrate; 2) establishing significant differences between ecoregions or between preferential substrate types in terms of indicator values of edaphic humidity and continentality of Cladonia species; 3) highlighting the association or dissociation relations between various ecoregions and substrate types in terms of presence and preferences of Cladonia species.

The obtained results show that Cladonia species are optimally grouped into three groups both in relation to their distribution in ecoregions (alpine + continental — the largest group, exclusively alpine and ubiquitous), and their preferences for substrate (terricolous, corticolous/lignicolous/muscicolous and saxicolous/terricolous). The indicator values for substrate humidity are on average significantly lower in Cladonia species present in the continental eco-region and, above all, steppe compared to all others. The indicator values for substrate moisture are not on average different between Cladonia species that prefer different substrates. The indicator values for continentality do not differ on average significantly between Cladonia species present in an ecoregion compared to all others or preference for one substrate type over all others.

The unicorns of wild roses: do *Liebelia* really exist in Europe?

Zoltán László^{1,2*}, Lilla Szócs³

¹Hungarian Department of Biology and Ecology, Faculty of Biology and Geology, UBB Cluj-Napoca, Romania

²Centre for Systems Biology, Biodiversity and Bioresources (3B), UBB Cluj-Napoca, Romania

³Faculty of Biology and Geology, UBB Cluj-Napoca, Romania

*Corresponding author: Zoltán László, str. Clinicilor 5-7, 400006 Cluj-Napoca, Romania,

zoltan.laszlo@ubbcluj.ro

Rose galls are abnormal growths developing on wild roses caused by gall wasps, including *Diplolepis*. These wasps lay eggs on host rose leaves or buds, and the larvae cause the plant to form protective tissues around them. *Diplolepis* species are distributed throughout the world, including Europe, Asia, Northern Africa, and North America. *Liebelia*, the sister genus of *Diplolepis* is distributed in Middle Asia, causing mostly globular galls on rose buds. Currently there are known nine species of *Liebelia* from which only one has a distribution outside of Asia: *Liebelia cavarae*. Based on its description it reminds of a small bedeguar as it consists of a large number of tiny galls, but each has an unusual erect, circular lamella. Since its first description much has not been written on this species even though its distribution is really outstanding being found only on an island in the Mediterranean: Sardinia. Sardinia has a dynamic geological history, being connected then detached from the European mainland. To check the presence of the species *L. cavarae* we collected galls occurring on *Rosa seraphinii* in mount Genargentu, Sardinia in 2021. Since we have found the common European *Diplolepis*, but no *Liebelia* galls the existence or availability of the species remains without new proofs. However, we present proofs for the potential validity of the species *L. cavarae* in Sardinia and also the insect community found in the collected galls of *Diplolepis*. But the question still persists: European *Liebelia* are just like or unicorns, or some really existing species?

Keywords: rose galls, sister genera, *Liebelia*, Sardinia,

Molecular biotechnologies (health, environment)

Screening spontaneous native flora for phytomanagement purposes at a heavily mercury contaminated polymetallic site

Emanuela D. Tiodar^{1,2}, Cecilia Chiriac³, Filip Pošćić⁴, Cristina L. Văcar^{1,2}, Zoltan R. Balazs^{1,2}, Cristian Coman⁵, David C. Weindorf⁶, Manuela Banciu^{2,7}, Ute Krämer⁸, Dorina Podar^{2,7,*}

¹ Doctoral School of Integrative Biology, Babeş-Bolyai University, Cluj-Napoca, Romania;

² Centre for Systems Biology, Biodiversity and Bioresources (3B), Babeş-Bolyai University, Cluj-Napoca, Romania;

³ Institute of Hydrobiology, Biology Centre CAS, České Budějovice, Czech Republic;

⁴ Department of Environmental Science, University of Arizona, Tucson, USA;

⁵ NIRDBS, Institute of Biological Research, Cluj-Napoca, Romania;

⁶ Department of Earth and Atmospheric Science, Central Michigan University, Mt Pleasant, MI, USA;

⁷ Department of Molecular Biology and Biotechnology, Babeş-Bolyai University, Cluj-Napoca, Romania.

⁸ Faculty of Biology and Biotechnology, Ruhr University Bochum, Germany

* Corresponding author: Dorina Podar, dorina.podar@ubbcluj.ro

Mercury pollution of soil, air and water is a global multifaceted problem, due to its extreme toxicity to biota and its biomagnification properties. Globally, Hg hotspot sites advance its transfer from soil to plants, local atmosphere and human communities. Here, we conducted a floristic survey of the native flora colonizing the grounds of a former chlor-alkali plant, presently characterized by a median topsoil Hg concentration of 962 mg kg⁻¹. The aim of the study was to assess the suitability of an *in situ* phytomanagement approach of the site, by determining the potential plant metal accumulation of local populations. Metal concentrations in root and shoot were measured with a pXRF. Trace metal substrate concentrations explained 35% of the total observed variance between plant species distribution and plant elemental content, with Hg as the main community driver, based on a redundancy analysis. Overall, the plant species were separated into two groups, driven by Zn-Mn or Hg-Pb-Cu. Out of the six plant species growing in more than one location at the site, *Diplotaxis muralis* was the dominant one. *Lotus tenuis* accumulated the highest concentrations of Hg, up to 10869 mg Hg kg⁻¹ dry weight in roots and 1070 mg kg⁻¹ in shoots, followed by *D. muralis* with up to 6049 and 1204 mg Hg kg⁻¹ roots and shoots, respectively. Therefore, our data suggests that both plant species are suitable Hg indicator species, and we propose both as candidate species for *in situ* Hg phytomanagement approaches, mainly fit for immobilizing Hg at the root level.

Keywords: contamination, *Diplotaxis muralis*, *Lotus tenuis*, mercury, phytoremediation

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Development of a 3D biomimetic model to unravel the intricacies of tumor microenvironment chemoresistance

Giorgiana Negrea¹, Szilvia Meszaros², Stefan Drăgan², Vlad-Alexandru Toma^{2,3}, Bogdan Dume¹, Valentin-Florian Rauca², Emilia Licărete², Manuela Banciu², Alina Sesărman²

¹ Doctoral School in Integrative Biology, Faculty of Biology and Geology, “Babes-Bolyai” University, Cluj-Napoca, Romania,

² Department of Molecular Biology and Biotechnology, Center of Systems Biology, Biodiversity and Bioresources, Faculty of Biology and Geology, “Babes-Bolyai” University, Cluj-Napoca, Romania,

³ Department of Experimental Biology and Biochemistry, Institute of Biological Research, Branch of NIRDBS Bucharest, Cluj-Napoca, Romania

* Corresponding author: Alina Sesărman, alina.sesarman@ubbcluj.ro

Recent advancements in oncology have brought to light the crucial role of the tumor microenvironment (TME) in drug resistance mechanisms. Consequently, accurate models must be developed to reflect the complexity of the TME, which necessitate significant effort. 3D tumor models offer a revolutionary solution to the limitations of 2D cell culture models in accurately representing the complex tumor microenvironment (TME). These models capture the true heterogeneity of a tumor and the intricate interactions between the cancer cells and the resident or infiltrated cells in TME, that shape drug response. With the potential to drastically reduce animal experimentation, ethical concerns, and costs, 3D models are a groundbreaking tool for cancer research, paving the way for innovative and effective therapies. In this study we keened to establish a highly refined co-culture 3D model consisting of B16.F10 murine melanoma cells and endothelial cells, and macrophages, with the aim of accurately mimicking TME interactions and replicating chemoresistance developed in response to treatments. Our initial findings reveal that the model has successfully demonstrated chemoresistance to doxorubicin (DOX), further indicating its potential as a robust tool for investigating TME interactions. However, additional studies are required to gain a complete and in-depth understanding of these complex interactions.

Keywords: 3D co-culture model; tumor microenvironment; endothelial cells; chemoresistance model; extracellular matrix.

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Protective effects of chrysin in calixarene 0118 (OTX008) and sulfobutylether- β -cyclodextrin (SBE β CD) nanoformulation on diabetes-associated cardiac fibrosis – *in vitro* and *in vivo* studies

Anca Hermenean^{1,2}, Maria Consiglia Trotta³, Cornel Balta¹, Alina Ciceu¹, Herman Hildegard¹, Caterina Lepre^{1,3}, Judit Váradi⁴, Ferenc Fenyvesi⁴, Michele D'Amico³

¹“Aurel Ardelean” Institute of Life Sciences, Vasile Goldiș Western University of Arad

²Faculty of Medicine, Vasile Goldiș Western University of Arad, Romania

³Department of Experimental Medicine, University of Campania “Luigi Vanvitelli”, Naples, Italy;

⁴Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen, Hungary;

* Corresponding author: Hermenean, Anca, anca.hermenean@gmail.com

Cardiac fibrosis is a pathophysiological consequence of chronic hyperglycemia in diabetes. There are still many knowledge gaps in understanding the processes involved in cardiac fibrosis.

Galectin-1 (Gal-1) is a β galactoside-binding lectin that emerged as a regulator of cardiac inflammation, hypertrophy and neovascularization. However, Gal-1's role in diabetes-induced cardiac fibrosis has never been explored.

Therefore, we aimed to investigate the protective effects of a new nanoformulation of chrysin (CHR) in calixarene 0118 (OTX008) and sulfobutylether- β -cyclodextrin (SBE β CD) on cardiac fibrosis, as a consequence of chronic diabetes, by *in vitro* and *in vivo* studies. Through complexation, we intended to increase the solubility and bioavailability of chrysin, known for its anti-fibrotic activity, and to add a new molecular target, given by OTX008, an inhibitor of galectin-1, a key player of fibrogenesis.

In our experimental design, H9c2 cells were exposed to normal/ high glucose for 48 hours and then treated for 6 days with chrysin, SBE β CD, OTX008, and complexes. The *in vivo* experiment was performed on streptozotocin-induced chronic diabetes in mice. Treatments were administered 2 times/week for 2 weeks to chronic diabetes animals by i.p. injections.

Our *in vitro* and *in vivo* data showed a significant increase in Gal-1 expression in cardiomyocytes exposed to high glucose and cardiac tissue. The CHR/OTX- SBE β CD treatments induced inhibition of Gal-1 and reduced cell death and cardiac fibrosis, respectively. These effects were associated with the downregulation of TGF β pathway.

The new proposed nanocomplex could be further investigated as a possible candidate to prevent and manage diabetes-induced cardiac fibrosis.

Keywords: calixarene OTX, SBE β CD, cardiac fibrosis, chronic diabetes

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The impact of HIF-1 α knockdown on doxorubicin sensitivity in B16.F10 murine melanoma cells

Bogdan Dume^{1*}, Alina Sesărman², Manuela Banciu², Emilia Licărete²

¹Doctoral School in Integrative Biology, Faculty of Biology and Geology, "Babes-Bolyai" University, Cluj-Napoca, Romania;

²Department of Molecular Biology and Biotechnology, Center of Systems Biology, Biodiversity and Bioresources, Faculty of Biology and Geology, "Babes-Bolyai" University, Cluj-Napoca, Romania;

* Corresponding author: Bogdan Dume, bogdan.dume@ubbcluj.ro

The aggressiveness of malignant melanoma has been linked to its increased invasiveness and resistance to treatment. Hypoxic conditions have been known to amplify the aggressive phenotype of these cancer cells *via* the activation of hypoxia-inducible factor-1 (HIF-1). HIF-1 is a heterodimer protein that comprises of two subunits, HIF-1 α and HIF-1 β , which are involved in tumor progression by stimulating angiogenesis, cell proliferation, and suppressing apoptotic pathways. Melanoma cells have been observed to possess persistent HIF-1 activity under normoxic conditions, which has been correlated with decreased sensitivity to doxorubicin's cytotoxicity. To address this issue, the current study aimed to develop an effective combined therapy for B16.F10 murine melanoma cells by simultaneously administering doxorubicin and HIF-1 α specific siRNA *in vitro* to sensitize melanoma cells to doxorubicin. Under hypoxic conditions, B16.F10 murine melanoma cells were transfected with HIF-1 α specific siRNA and scrambled siRNA as a negative control. Proliferation and migration assays were then performed. Western blot analysis was used to measure the levels of HIF-1 α and other pro-survival proteins. A mouse array kit was also used to determine angiogenesis related proteins. The results of our study indicated that transfecting B16.F10 murine melanoma cells with HIF-1 α specific siRNA had a significant effect, reducing the expression levels of HIF-1 α protein by 95%. Furthermore, co-administration of doxorubicin and HIF-1 α siRNA showed a reduction of angiogenesis, cell proliferation and migration, along with the induction of apoptosis. The research concluded that the combination of treatments could potentially provide notable benefits for treating melanoma in the future. It was observed that this approach could counteract cells resistance to doxorubicin, and stimulate apoptosis, as well as inhibit cell proliferation, migration, and angiogenesis.

Keywords: melanoma, HIF-1 α , siRNA, doxorubicin, resistance.

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Geology

**Palaeoenvironmental conditions reconstruction of the last two millenia in
NW Transylvania**

Ruskal Agnes^{1*}, Dumitrița Tiution², Andrei Cosmin Diaconu¹, Andrei Panait¹, Ioan Tanțău¹

¹Babeș-Bolyai University, Department of Geology, M. Kogălniceanu Street 1, 400084 Cluj-Napoca, Romania;

²University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Mănăștur Street 3-5, 400372, Cluj-Napoca, Romania;

* **Corresponding author: Ruskal Agnes, agnes.ruskal@gmail.com**

In the present study, we analyzed a peat sequence from Pădureni-Țop (NW Transylvania, Romania) using a multi-proxy approach (lithology, radiocarbon dating, loss on ignition, magnetic susceptibility, testate amoebae, microcharcoal) to reconstruct the evolution of the peat bog and the changes of the environmental conditions in the area during the last 2000 years.

The abiotic proxies (lithology, radiocarbon dating, loss on ignition, magnetic susceptibility) gave us insight about the evolution phases of the studied peat bog, the variation of the organic and minerogenic matter ration and the amount of the ferromagnetic minerals.

We reconstructed the depth to water table values by using a testate amoebae-based transfer function thus identifying drier and wetter periods.

By counting the microcharcoal particles throughout our sequence, we gained insight into the past fire history.

Our sequence recorded the local responses to the European climate change events such as the Roman Climatic Optimum, the Dark Ages Cold Period, the Medieval Warm Period and the Little Ice Age.

Keywords: testate amoebae, palaeoecology, palaeoenvironment, hydrology, microcharcoal

Palynological analysis of archaeological samples from the Mureş Valley

Grindean Roxana^{1,2*}, Tanţău Ioan², Egri Mariana¹, Rustoiu Aurel¹

¹Institute for Archaeology and History of Arts, Romanian Academy, Cluj-Napoca, Romania

²Department of Geology, Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania

* Corresponding author: Grindean Roxana, roxana.grindean@yahoo.com

The study aims at presenting aspects of environmental reconstruction through pollen and non-pollen palynomorphs' (NPPs) analyses from two archaeological contexts sites pertaining to the Iron Age. We look at the anthropogenic indicators and the different types of human activity that can be revealed by the studied plant and fungal taxa.

The pollen of trees and shrubs can help identify the relative composition of the surrounding forests and/or disturbances in the forest cover that could be linked to human agro–pastoral activities (expansion of the farmland through fire and deforestation and/or wood exploitation). We identified conifers (*Pinus*, *Picea abies*, *Abies alba*) and deciduous tree taxa, with the latter compiled into three categories: i) local broadleaved tree species (*Quercus* sp., *Carpinus betulus*, *Tilia*), ii) typical secondary tree species (*Alnus*, *Betula*), and iii) grapevine (*Vitis*) and fruit trees (*Juglans*, *Castanea*).

The pollen of herbs/grasses was divided into categories of pollen associated with: i) crops – cultivated plants (*Cerealia*, *Cannabis sativa*, *Vicia faba*) and weeds growing on the farmed land (e.g., *Centaurea cyanus*) or as fodder (*Medicago*, *Trifolium*); ii) ruderals – weeds colonizing areas that have been disturbed, as by fire or cultivation and human settlement (e.g., *Scabiosa*, *Arctium lappa*, *Carduus nutans*, *Polygonum aviculare*); iii) pastures – herbs growing on pasturelands and dry/wet meadows (e.g., *Poaceae*, *Valeriana*, *Plantago* sp., *Artemisia*, *Cardaminopsis arenosa*, *Chenopodiaceae*).

The NPPs selected for this study include spores of: i) fungi growing on animal dung and associated with grazing livestock (*Sordaria*), ii) smut fungi (*Nigrospora*, *Tilletia*), and eggs of intestinal parasites (*Ascaris*, *Taenia*).

Keywords: palynology, archaeology, anthropogenic indicators, cultural landscape, Iron Age.

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Paleoclimatic records of the Quaternary in Saïis Basin using clay mineral assemblages

Ayman Agharabi^{1*}, Nicolae Har², Lahcen Gourari¹, Lhoucine Karrat¹, Anasse Benslimane¹, Cristian Victor Mircescu²

¹First affiliation: Department of Geology, Faculty of Sciences Dhar Mahraz, Sidi Mohamed Ben Abdellah University, Fez, Morocco.

² Second affiliation: Department of Geology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania.

* Corresponding author: Ayman Agharabi, email address: ayman.agharabi@usmba.ac.ma

The Saïis Basin is located in north-central Morocco and is surrounded by the Middle-Atlas and Rif mountains. It formed during the Miocene due to marine transgression and later regressed during the Middle Pliocene, leading to the formation of continental deposits such as limestones, tufas, conglomerates, and clay deposits.

The continental sediments within the Saïis Basin were formed in fluvial, fluvial-lacustrine, and lacustrine depositional environments. The clay deposits in the Saïis plain are characterized by the presence of lenticular conglomerates.

Thirty-three clay samples were collected from four major areas (Northern, Central, Southern, and South-western) for clay minerals analysis. X-ray diffraction was performed on both oriented and glycolated specimens in order to examine the mineral composition of clay deposits in the four areas. The initial challenge involved eliminating the carbonate material (chalk and powder) from the samples by subjecting them to a 24-hour treatment with 30% acetic acid. This was followed by the extraction of the silt and clay-sized fractions from the top 5 centimeters of the suspension.

The extracted silt and clay were used for preparing oriented specimens. These specimens were air-dried before undergoing XRD measurement, after which they were solvated with ethylene glycol.

The objective of this study is to compare and contrast the clay mineral composition between the Early Pleistocene, Middle Pleistocene, and Holocene, highlighting the differences between them and thus interpret the paleoclimatic records of the studied area.

Keywords: Clay minerals, Fluvial deposits, Quaternary, Saïis Basin.

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The Talzast volcano-plutonic complex (Middle Atlas, Morocco): Petrography and geochemistry.

Mali Brahim¹, Dahire Mohamed¹, Ntarmouchant Ahmed¹, Driouch youssef¹, M. Bento dos Santos Telmo^{2,3}, Har Nicolae⁴, Jeddi El Mehdi¹, Elabouyi Mustapha⁵, Agharabi Ayman¹.

¹: GERA – Laboratory of Geosciences, Environment and Associated Resources, University Sidi Mohammed Ben Abdellah - Faculty of Sciences - Dhar El Mehraz - Fez, Morocco.

²: Instituto Dom Luiz (IDL), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016, Lisboa, Portugal.

³: DG-FCUL – Departamento de Geologia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, Edifício C6, 1749-016 Lisboa, Portugal.

⁴: Department of Geology, Babeş-Bolyai University, Cluj-Napoca, Romania

⁵: Department of Geology, Faculty of Sciences, Moulay Ismail University, Meknes, Morocco.

* Corresponding author: MALI Brahim, brahim.mali@usmba.ac.ma

The Talzast undersaturated alkaline complex is the Plio-Quaternary volcano-plutonic apparatus unique of its kind in the tabular Middle Atlas. This annular complex, is piercing Liasic limestones of the Ain Leuh cause, and it is different of the other Plio-Quaternary volcanic apparatuses of the Middle Atlas. The difference consist in the its texture, mineralogy, petrography and geochemistry as well as of the facies of the alkaline rocks. The rocks are developed in three main facies and the magmatic bodies are elliptic in shape with a NE-SW orientation. The innermost rocks are composed of ijolite with a grainy to pegmatitic texture called "talzastite", surrounded by a facies of medium-grained fasinites. The outermost facies in contact with the limestones is represented by a lava of ankaratritic nature encompassing the talzastite-fasinite assemblage.

The macroscopic study of the outcrops shows the complexity of the relationships between the different facies composing the Talzast volcano-plutonic complex. Petrographic analysis shows that the main paragenesis of the talzastites is composed of nepheline, orthoclase, and titaniferous augite, to which apatite, hematite, and magnetite are added as accessory phases. Fasinites and ankaratrites are distinguished from talzastites by the presence of olivine.

Geochemically, the Talzast rocks are classified mainly in the foidite field, derived from similar OIB source regions (Th/Yb vs. Nb/Yb). They are enriched in the most incompatible elements, with a steady increase from Yb to La, accompanied by a strong downward anomaly in K and Hf. The clinopyroxene-melt equilibrium pairs of the Talzast rocks allowed us to estimate clinopyroxene crystallization temperatures and pressures between 0.14 to 5.07 kbar 1144°C and 1263°C.

Keywords: Petrography, Geochemistry, Plio-quaternary, Talzast, Middle Atlas.

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POSTERS: Ecology, biodiversity, bioconservation

TranSuMan - Advanced training on sustainable management of pastoral systems

Irina Goia^{1*}, Michail Vrachnakis², Pablo Vidal³, Federico Maria Tardella⁴, Yannis Kazoglou², Maria Mortan⁵, Sara Moscatelli⁶, Paola Scocco⁶, Victor Sachez³, Marios Trigkas², Joel Bueso³, Vincențiu Veres⁵, Alexandru Stermin¹, Andrea Catorci⁴

¹Dept. of Taxonomy and Ecology, Faculty of Biology and Geology, Babes,-Bolyai University, Cluj-Napoca, Romania

² Dept. of Forestry, University of Thessaly, Karditsa, Greece

³ Anthropology Research Institute, Catholic University of Valencia, Spain

⁴ School of Biosciences and Veterinary Medicine, University of Camerino, Italy

⁵ Dept. of Taxonomy and Ecology, Faculty of Biology and Geology, Babes,-Bolyai University, Cluj-Napoca, Romania

⁶School of Biosciences and Veterinary Medicine, University of Camerino, Italy

* Corresponding author: Irina Goia, irina.goia@ubbcluj.ro

TranSuMan project highlights the significance of conserving pastoral systems in the European Union due to their ecological, cultural, and economic value. However, achieving this conservation requires a multidisciplinary approach to governance, which includes economic and environmental aspects.

The project is introduced as an innovative educational approach that aims to develop transdisciplinary professionals with green skills in sustainable management of pastoral systems. The partnership comprises four higher education institutions and brings together HE students, researchers, teachers, and local stakeholders in a partnership.

The project's activities include teacher training workshop, methodology training workshop, and a joint intensive course. The intellectual results of the project include a methodology for sustainable grassland management, a guide for stakeholder engagement in grassland management, and a methodology for teaching grassland management. Transnational project meetings and national multiplier events will also be held to discuss progress, share results, and disseminate findings. Overall, this project aims to increase job opportunities and prepare professionals with the necessary skills to manage pastoral systems sustainably.

Keywords: transdisciplinary education, grasslands management.

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Low variability in the floristic composition of *Nardus stricta* assemblages from two different floristic regions

Mardari Constantin^{1*}, Vassilev Kiril², Bîrsan Ciprian¹

¹ A. Fătu Botanical Garden, Alexandru Ioan Cuza University, Iași, Romania

² Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

³ Faculty of Biology, Alexandru Ioan Cuza University, Iași, Romania

* Corresponding author: Mardari Constantin, constantin.mardari@uaic.ro

Nardus stricta establish monotone assemblages, with diagnostic species transgressive from the plant communities they replace. While some communities are species-poor due to biotope, competition and land management, other are characterized by high species diversity. In this study we intended to observe the variability, and if there are major differences in the floristic composition of these communities in two floristic regions: central European (Romanian Carpathians) and sub-Mediterranean (Bulgarian Mountains). We performed a numerical classification based on an initial dataset of 4860 relevés using the agglomerative hierarchical clustering. For the optimum number of clusters the corrected Rand index and the Silhouette index were employed. The diagnostic species were identified based on indicator value. We used ordination methods to describe the vegetation - environment relationships. Vegetation was classified into 5 classes and 10 alliances. Floristic composition was mainly shaped by mean annual temperature highlighting that, in sites with low temperatures are distributed communities of species with increased frost hardness, which can tolerate little or no snow cover and strong winds. In contrast, the composition of communities from lower elevations can be explained by the narrower thermal niche of plants, shifted toward the higher temperatures, impeded by severe soil frosts. An analysis of the floristic elements highlighted the Eurasian and European background of the floristic composition and, at higher altitudes the increased frequency of circumpolar elements. *Nardus* communities are not especially rich in endemic species, but they include a significant number of Carpathian - Balkan elements, which offer a regional character to these communities.

Keywords: classification, vegetation, comparison, floristic region.

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Macromycetes assemblages in the beech forests from Eastern Romania

Bîrsan Ciprian^{1*}, Mardari Constantin¹, Tănase Cătălin²

¹ A. Fătu Botanical Garden, Alexandru Ioan Cuza University, Iași, Romania

² Faculty of Biology, Alexandru Ioan Cuza University, Iași, Romania

* Corresponding author: Bîrsan Ciprian, ciprian.birsan@uaic.ro

Beech forests occupy the largest area of Romania's forest fund and are distributed in different vegetation belts. Certain species of fungi tend to be closely connected with certain species of plants (especially mycorrhizal ones). The study area included beech forests from NE and E Romania, including mountain forests (Borca, Gosman, Nemtisor, Tarcau) as well as hill forests (Arsura, Barnova, Dalhauti, Dragomirna, Gadinti, Homita, Humosu, Magura Ocnei, Runc, Valea Fagilor). The collection of the mycological material was carried out over three successive years (2020-2022), from April to November, in 40 permanent plots with a size of 1000 square meters, installed in areas with vegetation dominated by *Fagus sylvatica*. The main purpose of the study was to observe if there are some fungal assemblages for a particular beech forest type, what are the main drivers of the fungal composition, and if some indicator species can be identified. A hierarchical agglomerative clustering procedure was used, diagnostic species were determined based on their indicator value, while the relationship between fungal composition and environmental variables was detected using detrended correspondence analysis. A total of 270 fungal taxa were registered in investigated plots. The fungi species aggregated into two main groups: a group specific to the mixed forest communities (including coniferous trees), and another group typical for deciduous forests in lower altitudes. Thus, the composition of the fungal communities in the studied forests preponderantly depended on altitude (in fact an underlying effect of climate conditions and tree species composition of the forests).

Keywords: mycobiota, diversity, assemblages, beech forest.

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What's on the plate of the critically endangered fish *Romanichthys valsanicola* (asprete)?

Karina P. Battes^{1,2}, Anca-Mihaela Şuteu^{1,2,*}, István Falka³, Mircea Mărginean⁴, Alex Găvan⁵, Mirela Cîmpean^{1,2}

¹ Department of Taxonomy and Ecology, Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania

² Center of Systems Biology, Biodiversity and Bioresources "3B", Advanced Hydrobiology and Biomonitoring Laboratory (LabHAB), Babeş-Bolyai University, Cluj-Napoca, Romania

³ S.C. Limnades S.R.L., Târgu Mureş, Romania

⁴ Fauna & Flora International, Deva, Romania

⁵ Alex Găvan Foundation, Romania

* Corresponding author: Anca-Mihaela Şuteu, anca.ciorca@ubbcluj.ro

The conservation efforts for the critically endangered species *Romanichthys valsanicola* Dumitrescu, Bănărescu & Stoica, 1957 must employ a holistic approach, including all the elements essential to its survival, like food, habitat, competition etc. In this context, the present paper aims to characterize the algal and invertebrate communities from the Vâlsan River, the fish native area. Described in the literature as an invertebrate feeder, *R. valsanicola* needs a sustainable food supply for its future survival.

Algal, invertebrate and fish samples were collected between 10 and 16 October 2022, from 15 sampling points located on the main course of the Vâlsan River, using standard methods for each target community. *R. valsanicola* was found in 7 locations, with a total of 58 individuals on a 15 km river stretch. Before this survey, official estimates of its population from the Ministry of Environment were 10-15 individuals over a 1-7 km river stretch.

Phytobenthic communities showed relatively high species richness. The number of diatom taxa increased from upstream on going downstream, probably parallel with an increase in substrate heterogeneity. Even if the Diatom Biotic Index indicated very good water quality in most of the sampling sites, other biotic indices indicated the presence of undecomposed organic matter in the system and an increase in the river trophic state towards the lowland sampling sites, where eutrophic conditions were met.

Zoobenthic communities also included a high diversity of taxa, not only groups living in relatively clean waters like Ephemeroptera, Plecoptera and Trichoptera, but also tolerant taxa like Oligochaeta and Diptera-Chironomidae. Herbivores, detritivores, and carnivores were well represented in the samples. The biomass estimated for the benthic invertebrate communities showed high values for the groups known as dietary preferences of the *R. valsanicola*, like Ephemeroptera.

Keywords: *Romanichthys valsanicola* (asprete), phytobenthos, zoobenthos, conservation.

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Structural and morphological characterization of two *Allium* species dependent on their phytochemical profile

Ioana Andreea Barbu^{1,2,3*}, Alexandra Ciorîță^{1,4}, Rahela Carpa^{1,3,5}, Lucian Barbu-Tudoran^{1,4}, Cristina Mircea¹, Marcel Pârnu^{1,3}, Anca Butiuc-Keul^{1,2,3} and Augustin Moț⁶

¹ Faculty of Biology and Geology, Babeş-Bolyai University, 1, M. Kogălniceanu Street, 400084 Cluj-Napoca, Romania;

² Doctoral School of Integrative Biology, Babeş-Bolyai University, 400015 Cluj-Napoca, Romania

³ Centre for Systems Biology, Biodiversity and Bioresource, Babes-Bolyai University, 5-7 Clinicilor Street, 400006 Cluj-Napoca, Romania

⁴ National Institute for Research and Development of Isotopic and Molecular Technologies, 400293 Cluj-Napoca, Cluj, Romania

⁵ Institute for Research-Development-Innovation in Applied Natural Sciences, Babeş-Bolyai University, 30 Fântânele St., Cluj-Napoca, 400294 Romania

⁶ Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, 11 Arany Janos Street, 400028 Cluj-Napoca, Romania;

* Corresponding author: Alexandra Ciorîță, alexandra.ciorita@ubbcluj.ro;
Contact at: ioana.barbu@ubbcluj.ro

In recent years, many studies have shown that garlic and its bioactive constituents, especially allicin, exhibit pharmacological properties. In previous studies, the phytochemical analyses of two *Allium* species showed that *Allium obliquum* L. contains higher concentrations of allicin than *Allium fistulosum* L., while the former is richer in polyphenols. The present study focuses on the structural characterisation of the leaves of these two species and how it might influence the chemical composition, using light (LM) and scanning-transmission electron microscopy (S/TEM) techniques. SEM revealed major differences in the morphological aspect of the leaves. Through LM the upper, lower epidermises, and the mesophyll were observed. The leaves of both species have an epidermis with thick cell walls, cuticular layers, and epicuticular waxes. However, a major difference in the structure of the two leaves was the lack of the lower epidermis in *A. fistulosum*. In *A. fistulosum* the chloroplasts were present only under the outer epidermis, compared to *A. obliquum* which had chloroplast under both epidermises. Several similarities were seen at an ultrastructural level through TEM. The cell walls of the epidermal cells had visible cuticular layers and epicuticular waxes, while the form and distribution of the chloroplasts were similar in both species. Since some of the components might be stored in the epidermal cells, the presence of both epidermises in *A. obliquum* might explain why this plant extract had higher concentrations of allicin, compared to *A. fistulosum*, which had only one layer of epidermal cells. Further studies are required to confirm these findings.

Keywords: *Allium fistulosum*, *Allium obliquum*, leaf morphology, phytochemical composition

Development and characterization of the first set of microsatellite markers for *Carex curvula*

Zoltán Robert Balázs^{1,2,3}, Mihai Puşcaş^{1,3,4,5*}, Dana Şuteu⁶, Ioan Băcilă⁶, Mihai Miclăuş^{6,7}, Philippe Choler⁸

¹ Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania

² Doctoral School of Integrative Biology, Babeş-Bolyai University, Cluj-Napoca, Romania

³ Center for Systematic Biology, Biodiversity and Bioresources—3B, Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania

⁴ A. Borza Botanic Garden, Babeş-Bolyai University, Cluj-Napoca, Romania

⁵ Emil G. Racoviţă Institute, Babeş-Bolyai University, Cluj-Napoca, Romania

⁶ Department of Experimental Biology, National Institute of Research and Development for Biological Sciences, Cluj-Napoca, Romania

⁷ Babeş-Bolyai University, Cluj-Napoca, Romania

⁸ University Grenoble Alpes, University Savoie Mont Blanc, CNRS, LECA, Grenoble, France

* Corresponding author: Mihai Puşcaş, mihai.puscas@ubbcluj.ro

Carex curvula (Cyperaceae) is a dominant endemic species distributed in alpine grasslands across the European Alpine System, with two described subspecies: *C. curvula* subsp. *curvula* and *C. curvula* subsp. *rosae*.

In this study, we developed a set of simple sequence repeat (SSR; or microsatellite) markers with the aim of inferring the phylogeographic relationships of the populations within species and the boundaries between the two subspecies. We used next-generation sequencing and identified a large number of potential SSR markers. We selected a candidate subset of 18 microsatellites of which we validated 13 that exhibited high polymorphism and high amplification success rates for both subspecies. Genotyping results showed high levels of genetic diversity within populations of *C. curvula*, but also significant genetic differentiation among populations. The total number of alleles per locus varied from four to 23 (including both infrataxa). Moreover, we found evidence for two distinct genetic clusters that correspond to the two described subspecies of *C. curvula*.

Due to the highly informative content, the first set of microsatellite markers developed for the alpine species *Carex curvula* represents a promising tool for evolutionary studies in Cariceae section. These SSR markers could be successfully used to distinguish between the two varieties of *C. curvula*, as well to refine the phylogeographic history of *C. curvula* within the European Alpine System.

Keywords: Alpine plants · Cariceae · Microsatellites · Phylogeny · Phylogeography

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Genome-inferred metabolic reconstruction of *Patescibacteria* from two hypersaline meromictic lakes from Romania

Sturzu-Cosma Simina¹, Gridan Ionuț-Mădălin², Banciu Horia Leonard^{1,*}

¹ Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeș-Bolyai University, Cluj-Napoca, Romania

² Doctoral School of Integrative Biology, Faculty of Biology and Geology, Babeș-Bolyai University, Cluj-Napoca, Romania

* Corresponding author: Banciu Horia Leonard, horia.banciu@ubbcluj.ro

Patescibacteria (Candidate Phyla Radiation) is a candidate bacterial lineage recovered from a broad range of habitats including marine water, freshwater, hydrothermal vents, plant rhizosphere, and alpine permafrost. These yet uncultured bacteria have small genomes (~1 Mbp), peculiar ribosomal structures, reduced metabolic capacities, retaining only the essential functions for growth and reproduction, such as genetic information processing. Moreover, *Patescibacteria* are postulated to feature ultra-small cells, high metabolic rates and truncated membrane complexes including flagellar assembly, ABC transporters, and two-component regulatory systems. Considering the wide habitat range of *Patescibacteria*, there is a scarce knowledge of their phylogeny and physiology especially of their adaptive traits enabling to withstand a variety of conditions and possibly driving their evolution. Thus, the aim of this research is to find whether *Patescibacteria* are part of microbial communities populating hypersaline systems and to reveal their encoded metabolic peculiarities enabling resilience to high salinity. To achieve this aim, the water columns and sediments of two hypersaline and permanently stratified (meromictic) salt lakes located in Transylvanian Basin (Central Romania), namely Fără Fund and Ursu lakes, were sampled. Following biomass separation, total environmental DNA extraction and shotgun-sequencing, the resulted metagenomic datasets were computationally processed and analyzed for metagenomic-assembled genomes (MAGs)-inferred taxonomy and metabolic potentiality. In both sampled lakes we recovered 70 high-quality MAGs affiliated to *ABY1* (20 MAGs) and *Paceibacteria* classes (50 MAGs) within *Patescibacteria*. Unlike earlier reported *Patescibacteri*, we detected genes encoding ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) involved in autotrophic CO₂ fixation pathways. This metabolic trait was apparent in a few *Paceibacteria* found in the deep water mass (6 and 9 m depth) and sediments of Fără Fund Lake and the transition water layer (3.5 m depth) of Ursu Lake. Additionally, despite other scientific reports claiming the absence of genes for citrate cycle and oxidative phosphorylation, except for the ATPase complex, there were found genes for NADH-quinone oxidoreductase, succinate dehydrogenase, cytochrome *c* oxidase, suggesting the possibility of aerobic respiration. Genes encoding transporters for osmoprotective compounds likely involved in salt adaption were also inferred in a few *Paceibacteria* -affiliated MAGs recovered from Fără Fund Lake.

In conclusion, the particular features of *Patescibacteria* detected in Transylvanian hypersaline meromictic lakes expand our knowledge on the habitats and lifestyles among this lineage and suggest that this uncultured bacterial group may be an overlooked contributors to ecosystem functioning. Besides, our findings outline the relevance of the rare microbial biosphere in nutrient cycling in hypersaline aquatic ecosystems.

Keywords: carbon fixation, hypersaline lakes, metagenomics, *Paceibacteria*, salt adaption

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POSTERS: Molecular biotechnologies (health, environment)

The Hortega-Cajal methods and immunohistochemistry for study of the neurological cluster-units in the cervical nervous system of the corvids

Maria-Alexandra Nuțu^{1*}, Bogdan Dume¹, Lucian Barbu¹, Ioana Roman², Vlad-Alexandru Toma^{1,2}

¹Babes-Bolyai University, Department of Molecular Biology and Biotechnology, Cluj-Napoca, RO

²Institute of Biological Research, Cluj-Napoca, RO

*maria.nutu@stud.ubbcluj.ro

Nowadays, comparative neurobiology is in a shadow cone as compared to other aspects of the neurosciences. Studies with experimental models or representative organisms are exposed to erroneous generalizations as the diversity of the class of organisms increases. Thus, the structure of the pigeon's brain is different from the brain of a crow or the brain of a zebra finch, even though they are all birds. The whole brain of *Corvus frugilegus* was analyzed with a wide variety of histological methods such as **Golgi, Golgi-Cox, Hortega, Nissl, H&E, Kluver-Barera, Bielschowsky, and Jones** with tissue fixed in neutral formalin, Bouin's liquid, potassium dichromate, silver nitrate, dichromate-potassium chromate-HgCl.

Positive **immunohistochemical detection** was observed for NeuN, CNP, DCX, and PCNA, while other markers were not clearly detected. The ultrastructural examination was performed using transmission electron microscopy. **Confocal microscopy** was performed with a Nikon AX1 microscope.

Data gathered to date suggest that neurons and gliocytes are organized into structural and perhaps functional clusters with a central young motile neuron and 5–12 peripheral glial cells, perhaps some type of oligodendrocyte. **The study is still ongoing** and more studies will be conducted on the phenotype of neuroglial clusters and their function in the brain of *Corvus frugilegus*.

Keywords: immunohistochemistry, *Corvus frugilegus*.

Novel strategies for diminishing the oxidative side effects of ibuprofen – a computational study

Szilvia Toth¹, Augustin C. Moț², Anca D. Stoica^{1*}

¹ Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeș-Bolyai University, Cluj-Napoca, Romania

² Department of Chemistry, Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University, Cluj-Napoca, Romania

*Corresponding author: Anca D. Stoica, anca.stoica@ubbcluj.ro

Ibuprofen is one of the most frequently administered NSAIDs, available worldwide since 1983 as an OTC drug, with several indications, including fever and different types of pain. Ibuprofen is a monocarboxylic acid, functionally related to propionic acid. Its mechanism of action consists of rapidly and reversibly binding to cyclooxygenases (COX), thus inhibiting prostaglandin (PG) synthesis. The reactive carboxylic group causes several side effects by generating ROS and altering cellular membrane function. The adverse effects appear mainly in the gastro-intestinal (GI) tract, causing ulcerations, hemorrhages, perforations, in the cardiovascular and renal systems. Over the years, ibuprofen has been conjugated with numerous compounds to alleviate side effects. The aim of our study was to produce an ibuprofen-BSA conjugate, with covalent binding. BSA is preferred in pharmacology due to biocompatibility and biodegradability, improving drug absorption and bioavailability. *In silico* investigations were performed using SwissADME free web tool to predict the pharmacokinetic properties, GI interaction and possible human molecular targets. Results showed variations in GI absorption, depending on protein structure. The absorbed fragments efficiently interacted with COX-2. Thus, the conjugate maintains ibuprofen anti-inflammatory and analgesic activity.

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A technique for evaluation of the main resistance strategies to Cu, Hg, and Zn in a filamentous fungus

Lorena Văcar^{1, 2*}, Karen Viacava³, Adrien Mestrot³, Marcel Pârvu^{2, 4}, Dorina Podar^{2, 5}

¹ Doctoral School of Integrative Biology, Babeş-Bolyai University, Cluj-Napoca Romania

² Centre for Systems Biology, Biodiversity and Bioresources (3B), Babeş-Bolyai University, Cluj-Napoca, Romania

³ Institute of Geography, University of Bern, Bern, Switzerland

⁴ Department of Taxonomy and Ecology, Babeş-Bolyai University, Cluj-Napoca, Romania

⁵ Department of Molecular Biology and Biotechnology, Babeş-Bolyai University, Cluj-Napoca, Romania

* Corresponding author: Lorena Văcar, cristina.vacar@ubbcluj.ro

Pollution with trace or toxic elements is threatening environmental security due to their persistent nature and widespread exploitation, while their remediation is a challenging task. Recently, mycoremediation has been considered an appealing strategy. Fungi present interesting properties: may provide significant biomass quantities for large-scale applications, branched growth which allows penetration of the porous matrix of soil, high surface area of the cell with cation binding properties due to the presence of various functional groups on the surface of the cell wall. However, mycoremediation of toxic elements did not take the technological transfer leap, due to insufficient understanding of the mechanisms employed. A *Fusarium oxysporum* isolate was previously recovered from soil of a polymetallic contaminated site, especially with Hg. In pure culture, the fungal isolate exhibited elevated degrees of resistance to Cu, Hg, and Zn. Moreover, preliminary tests indicated that Hg biovolatilization might occur. The aim of this study was to develop a technique to discern between the main resistance strategies in this filamentous fungus: intracellular uptake, cell wall-binding (for Cu, Hg, and Zn), and biovolatilization (for Hg). A system of aerated liquid cultures was developed. The biovolatilized Hg was trapped using Anasorb C300 bulk material, connected to the outlet of the Hg-spiked liquid cultures. At the end of the experiment, filtrates, biomasses and traps were recovered for analysis. Parallel biomass samples were washed with 0.1 M HCl to desorb the toxic elements bound to the cell wall. The processed samples were analyzed by ICP-MS for Cu and Zn content and by DMA-80 for the Hg content. The success rate of the technique is to be established after complete analysis of the samples and data processing.

Keywords: filamentous fungi, toxic elements, bioremediation

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Advancing Melanoma Treatment Through 3D Models of Tumor Microenvironment

Márta-Szilvia Mézáros¹; Giorgiana-Gabriela Negrea²; Vlad-Alexandru Toma^{1,3}; Valentin-Florian Rauca¹; Emilia Licarete¹; Laura Pătraș¹; Manuela Banciu¹; Alina Sesarman^{1*}.

¹Department of Molecular Biology and Biotechnology, Center of Systems Biology, Biodiversity and Bioresources, Faculty of Biology and Geology, "Babes-Bolyai" University, Cluj-Napoca, Romania,

²Doctoral School in Integrative Biology, Faculty of Biology and Geology, "Babes-Bolyai" University, Cluj-Napoca, Romania,

³Department of Experimental Biology and Biochemistry, Institute of Biological Research, Branch of NIRDBS Bucharest, Cluj-Napoca, Romania

* Corresponding author: Alina Sesarman (alina.sesarman@ubbcluj.ro)

Immunotherapies, have emerged in the past decade as promising treatments for various types of cancers, including melanoma, the most aggressive skin cancer. However, not all patients respond to these treatments, and there is a need to develop better models for testing new therapies. One key aspect of introducing immunotherapies as an option for patients is the preclinical testing of their efficacy on models that represent the tumor microenvironment as accurately as possible. In particular, 3D models have shown promise in mimicking the tumor microenvironment more precisely, with the addition of dendritic cells providing a more accurate representation of the immune response to melanoma. Furthermore, the use of 3D models may reduce the need for animal models in drug testing, which is both ethical and cost-effective. Our aim was to develop an experimental model for melanoma microenvironment, utilizing 3D co-culture (spheroids) of B16.F10 murine melanoma cells and bone marrow-derived dendritic cells. The model was tested using two compounds, one with a cytotoxic effect and another with an immunomodulatory profile, revealing promising results. This model offers a promising opportunity to explore the complex interactions between cancer and immune cells, providing a valuable approach to test novel and effective therapies for melanoma and other type of solid tumors.

Keywords: melanoma, 3D models, spheroids, dendritic cells.

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The *in vitro* anti-tumor effects of anthocyanin extracts from aronia, red grapes and red cabbage on colorectal cancer cells, before and after a simulated gastrointestinal digestion

Chilian Simona-Bianca¹, Drețcanu Georgiana^{1,2}, Diaconeasa Zorița², Cruceriu Daniel^{1,3*}

¹ Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, “Babes-Bolyai” University, Cluj-Napoca, Romania

² Department of Chemistry, Biochemistry and Molecular Biology, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

³ Department of Genetics, Genomics and Experimental Pathology, “Prof. Dr. Ion Chiricuta” Oncology Institute, Cluj-Napoca, Romania

*Corresponding author: Cruceriu Daniel, daniel.cruceiru@ubbcluj.ro

The quality of the food we eat is critical in preventing various types of cancer, including colorectal cancer (CRC). Thus, consumption of fruit and vegetables, which are rich in bioactive compounds, is known to contribute to the prevention of different environmentally influenced cancers. Anthocyanins, a class of polyphenols abundantly found in different plants, might have many applications in cancer prevention and therapy due to their anti-tumor properties. However, studying their efficacy as cancer prevention agents is a key issue, especially as their biological activity after the gastrointestinal digestion is questionable. Therefore, the present study aims to compare the anti-tumor activity on CRC cells of anthocyanin extracts from aronia fruits, red grapes or red cabbage, plants known to be rich in these compounds, before and after an *in vitro* static simulated gastrointestinal digestion. The anthocyanin extracts were obtained by purification on SepPack C18 columns of previously obtained polyphenol extracts derived from all three plants species. Part of the anthocyanin extracts were further subjected to an *in vitro* digestion, simulating all buccal, gastric, and intestinal digestions. All extracts were characterized in terms of their biochemical profile, both before and after digestion, by LC-MS, whereas their *in vitro* anti-tumor activity was evaluated by the MTT assay, at 24h after administration, on the CRC cell line DLD-1. As expected, in all three extracts, the simulated digestion strongly decreased the overall quantity of anthocyanins, but significant quantities of anthocyanins metabolites were found. In both aronia and red cabbage extracts the anthocyanins identified were cyanidin-derivates, whereas in the red grapes extract a higher diversity of anthocyanins was found. Comparing the three plant species, the anthocyanins extract obtained from aronia fruits had the strongest anti-tumor activity *in vitro*, both before (IC₅₀=10.06 µg/mL) and after (IC₅₀=5.89 µg/mL) digestion. Interestingly, even though the overall quantities of anthocyanins decreased after digestion, the anti-tumor activity significantly increased (by 70%, 89% and more than 400% for the aronia, red grapes and red cabbage extracts respectively). Therefore, we hypothesize that at least for anthocyanins, the gastrointestinal digestion actually increases the anti-tumor activity of these compounds.

Keywords: anthocyanins, *in vitro* gastrointestinal digestion simulation, colorectal cancer

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Peroxisomes identification in melanoma tumor cells – a modified protocol for electron and light microscopy

Vlad-Alexandru Toma^{1,2}, Alina-Viorica Sesărman¹, Alexandra Ciorăță^{1,3}, Lucian Barbu^{1,3}, Septimiu Tripon^{1,3}, Manuela Banciu¹

¹ Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, Babeş-Bolyai University, Clinicilor Street No. 5-7, Cluj-Napoca, RO

² Institute of Biological Research, Republicii Street No. 48, Cluj-Napoca, RO

³ NIRD of Isotopic and Molecular Technologies, Donat Street No. 67-103, Cluj-Napoca, RO

* Corresponding author: **Vlad-Alexandru TOMA**, vlad.toma@ubbcluj.ro

Peroxisomes perform crucial functions related to lipid metabolism (lipids > 22 carbon atoms before the mitochondrial beta-oxidation, e.g., bile acids with 24 C) and redox homeostasis, among others. Our previous results have revealed that liposomal simvastatin treatment significantly reduced cancer cell invasion and re-educated the tumor-associated macrophages. Based on cytochemistry principles, the current research question is about peroxisome identification in melanoma tissue. One experimental group with mice (8 mice/group) was inoculated with B16F10 cells in order to develop malignant melanoma in ~ 10 days. The animals were used as the Control group. The sampling was done as follows: (i) Tumor tissue for ultrastructural examination; transmission electron microscopy was performed with samples fixed in glutaraldehyde and contrasted with osmium tetroxide; (ii) Tumor tissue incubated in modified diaminobenzidine reagent for peroxisome staining and then, examination of the samples in light and electron microscopy. Ultrastructural investigations revealed that our modified protocol for peroxisomes staining was effective and dark microbodies - peroxisomes - were noticed besides mitochondria without any structural alterations. Further, light microscopy investigation revealed a reproducible method for peroxisome identification in cryosections.

Keywords: peroxisome, melanoma, ultrastructure, cryosections, diaminobenzidine

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The potential of curcumin in reversing irinotecan-acquired resistance in colorectal cancer cells

Ivăncuță Andrei¹, Baldașici Oana², Gavrilaş Laura³, Cruceriu Daniel^{1,2*}

¹ Department of Molecular Biology and Biotechnology, "Babes-Bolyai" University, Cluj-Napoca, Romania

² Department of Genetics, Genomics and Experimental Pathology, "Prof. Dr. Ion Chiricuta" Oncology Institute, Cluj-Napoca, Romania

³ Department of Bromatology, Hygiene, Nutrition, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

* Corresponding author: Cruceriu Daniel, daniel.cruceiru@ubbcluj.ro

When dealing with colorectal cancer (CRC) under current clinical settings, a major problem is represented by the cancer acquired resistance to conventional chemotherapy, including resistance to irinotecan, a compound often used as a first- or second-line chemotherapeutic drug. Plants represent a rich source of bioactive constituents, specific compounds having the potential to resensitize chemoresistant cancer cells to certain drugs. Curcumin, the major bioactive compound found in turmeric (*Curcuma longa*) is one such promising compound. Therefore, the purpose of this study is to assess the curcumin's capacity to reverse the acquired resistance to irinotecan of CRC cells, *in vitro*. An irinotecan-resistant cell line (DLD-1_IRI-R) was obtained through successive exposures of DLD-1 cells to increasing concentrations of irinotecan, until cells would survive and proliferate in culture medium containing irinotecan at a concentration of 20 μM , the concentration at which irinotecan is found in the blood stream of CRC patients. Both cell lines were evaluated for their sensitivity to irinotecan by the MTT assay at 24h after exposure, DLD-1_IRI-R cells being 1,78X more resistant than the parental cells (DLD-1 IC₅₀= 212.5 μM ; DLD-1_IRI-R IC₅₀=378.8 μM). The sensitivity to curcumin remained the same between the two cell lines. When a combinatorial treatment of irinotecan in successive concentration and curcumin at a constant low concentration (IC₁₀) was administered on DLD-1_IRI-R cells, a significant decrease in the irinotecan specific IC₅₀ was observed (118,3 μM), demonstrating a resistance reversal fold of 2,96X. Furthermore, the combined treatment was characterized by synergistic effects on the resistant cell line according to the Chou-Talalay analysis (CI<1) for every tested combination of concentrations, both in experiments with a constant-dose ratio and in combinations of irinotecan with curcumin at IC₁₀. Therefore, curcumin is capable of reversing the acquired resistance to irinotecan in CRC cells, whereas the combinatorial therapy might increase the treatment efficacy.

Keywords: colorectal cancer, curcumin, irinotecan, acquired resistance

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The effect of *Berberis vulgaris*, *Mahonia aquifolium* and *Phellodendron amurense* plant extracts over cell viability in different types of tumor cell lines

Sabina Emanuela Pojar^{1,2*}, Alexandra Ciorîță^{1,2}, Marcel Pârnu¹, Cătălin Augustin Moț^{1,3}

¹ Doctoral School of Integrative Biology, Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania

² National Institute for Research and Development of isotopic and Molecular Technologies, Cluj-Napoca, Romania

³ Department of Chemistry, Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj-Napoca, Romania

* Corresponding author: Sabina Emanuela Pojar, sabina.pojar@ubbcluj.ro; sabina.e.erhan@gmail.com

In recent years, plant extracts are used increasingly worldwide in the allopathic treatment of different types of disease-associated symptoms. Several studies showed that plant extracts containing a certain alkaloid, such as berberine, have various beneficial effects (Andreicuț et al., 2019; Milata et al., 2019).

Melanoma is described as a malignant tumor that originates from melanocytes (Avram et al., 2017). Non-small cell lung cancer (NSCLC) is one of major subtypes of lung cancer, and it accounts for 80% of lung cancers and has a low 5-year survival rate. Despite the advancements made in anticancer medicine for NSCLC, the side effects of first-line chemotherapy remain one of the barriers to sustainable therapy (Hu et al., 2022).

Taking this information into consideration, *Berberis vulgaris*, *Mahonia aquifolium* and *Phellodendron amurense* extracts were chosen for analysis on A375 melanoma cells and A549 non-small cell lung cancer cells. The chemical composition of these extracts was studied by HPLC, through which it was possible to identify different types of polyphenols. One of the most important alkaloids that is found in considerable amount in every one of the plant extracts is berberine. Berberine is one of the most important compounds, due to its various benefits such as anti-inflammatory activity, antitumoral and antioxidant activity. The viability and membrane integrity were investigated for the cells treated with all three plant extracts. The A375 cells were more affected by the plant extracts compared to the A549 cells. Of all three plant extracts used, *M. aquifolium* was proven most efficient in cancer inhibition in vitro, which highlights it as a promising adjuvant.

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Keywords: MTT, A375, A549, plant extracts, cancer