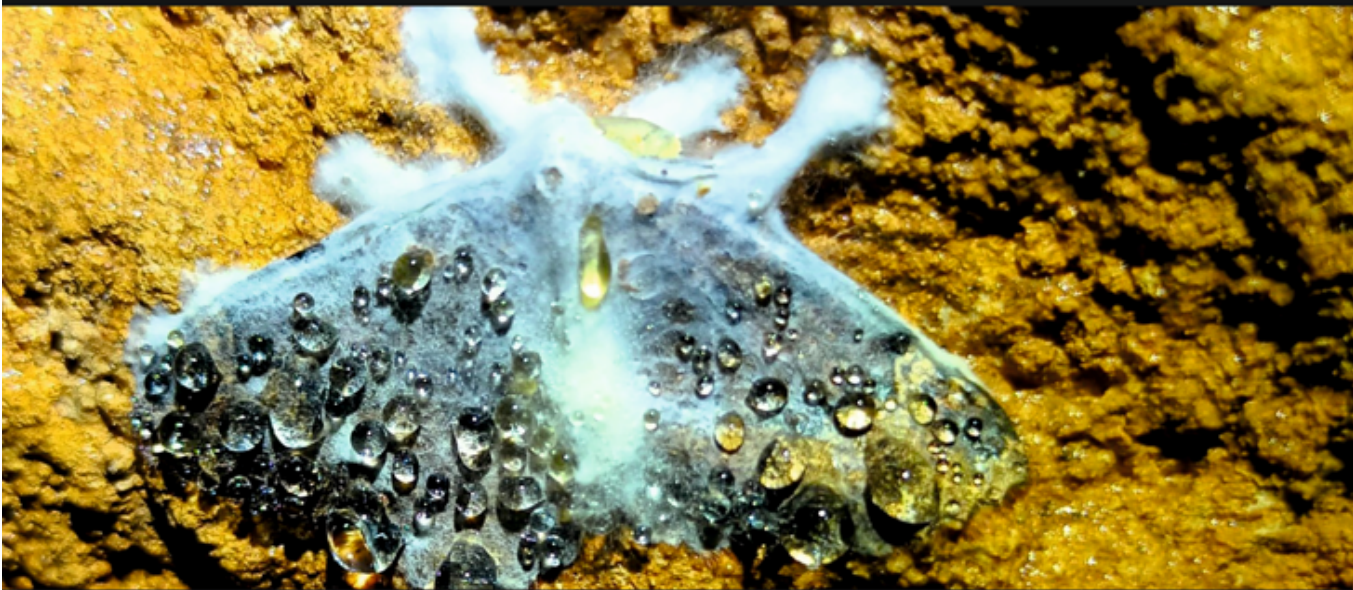


Karst Science Days Symposium Proceedings



Editor-in-Chief: Acad. Dumitru Morariu
Guest Editor: Virgil Drăgușin

1 / 2023



**"Emil Racovita" Institute of Speleology
of the Romanian Academy**



Karst Science Days Symposium Proceedings
2023

Published and distributed by:

**“EMIL RACOVIȚĂ” INSTITUTE OF SPELEOLOGY
OF THE ROMANIAN ACADEMY**

Calea 13 Septembrie, Nr. 13 (Casa Academiei), Cod 050711,
Sector 5, București. Telefon/Fax: 021 318 81 32
<https://www.iser.ro>

How to cite:

Murariu D., Drăgușin V. (Eds.). 2023. Karst Science Days Symposium Proceedings, Vol. 1. “Emil Racoviță” Institute of Speleology of the Romanian Academy.

ISSN 2972-127x

ISSN-L 2972-1261 (electronic)

Printed at SMART PRINT - ARTIX PLUS SRL, www.smartprint.ro.

Cover: Nicolae Cruceru

Cover Photos:

Up. Image showing the excavation site in Toplița de Vida Cave (photo: Irinel Cîrlănaru)

Down. Lepidoptera from Biserica Scochii Cave (Photo: Sanda Iepure)

The abstracts in this book are published on the basis of the information submitted by the respective authors. The editor cannot be held responsible for errors or any consequences arising from the use of information contained in this book of abstracts. Reproduction is authorized, provided that appropriate mention is made of the source.



Karst Science Days Symposium Proceedings

1/2023

Editor in Chief:

DUMITRU MURARIU

Guest Editor:

VIRGIL DRĂGUȘIN

“EMIL RACOVIȚĂ” INSTITUTE OF SPELEOLOGY
OF THE ROMANIAN ACADEMY

“EMIL RACOVIȚĂ” INSTITUTE OF SPELEOLOGY
&
DEPARTMENT OF BIOLOGICAL SCIENCES OF THE ROMANIAN ACADEMY

Karst Science Day Symposium

**March 31, 2023
Bucharest, Romania**

ORGANIZING COMMITTEE

Chairman

Acad. Dumitru Murariu

Secretary

Virgil Drăgușin

Members

Sanda Iepure

Nicolae Cruceru

Cristian-Mihai Munteanu

Andrei Giurginca

Cătălina Haidău

Robert Opran

Technical support & IT

Mihnea Cristian Vasile

Logo

Maria-Mirabela Pop

Orest A. Sambor

Contents

| | |
|---|-----------|
| Insights into the cultivable microbiota of Isverna cave guano deposits | 1 |
| Lavinia V. Manolachi, Georgiana A. Grigore, Irina G. Barbu, Ionuț Pecete, Virgil Drăgușin, Carmen M. Chifiriuc | |
| Solving the puzzle of the colonisation pattern of <i>Mesoniscus graniger</i> (Frivaldsky, 1865) in Romania using population genetics | 3 |
| Robert F. Opran | |
| Cold-adapted phototrophic microorganisms from Scărișoara Ice Cave: Suitable models for developing electrochemical biosensors for herbicides | 5 |
| Robert Ruginescu, Alina Vasilescu, Roberta Banciu, Cristina Purcărea | |
| A laboratory experiment of carbon stable isotope fractionation by several fungal species | 7 |
| Ștefan Surugiu, Georgiana A. Grigore, Virgil Drăgușin, Irina G. Barbu, Bogdan Ispas, Viorel Atudorei, Ionuț Pecete, Carmen M. Chifiriuc | |
| Exploring the crustaceans world in the sulfidic mesothermal aquifer from Mangalia, Romania (Southern Dobrogea, Romania) | 9 |
| Maria M. Pop, Filip P. Boancă, Andrei Ștefan, Sanda Iepure | |
| The response of aquatic fauna to variable environmental conditions in Ghețarul de la Vârtop cave (Apuseni Natural Park, Romania) | 11 |
| Orest A. Sambor, Bianca Șarcani, Aurel Perșoiu, Constantin Marin, Ana I. Camacho, Karina P. Battes, Mirela Cîmpean, Alin Tudorache, Sanda Iepure | |
| Inferring spatio-temporal palaeodynamics: from ancient biomolecules to organismal (overviews on cave sediment sequences | 13 |
| Adrien Lörincz, Aritina Haliuc, Andrea Demjén, Călin Ghemiș, Răzvan Dumbravă, Adriana Vulpoi, Beatrice S. Kelemen, Augustin Nae, Ioana Rusu, Viorel T. Lascu, Florin Gogâltan, Bogdan P. Onac, Ioana N. Meleg | |
| Carbon and nitrogen stable isotope values of Late Pleistocene fauna from Stoieni Cave, Romania | 15 |
| George Mirea, Virgil Drăgușin, Bogdan Ispas, Georgiana A. Grigore, Ștefan Vasile, Ionuț Mirea, Tiberiu Sava, Carmen M. Chifiriuc, Viorel Atudorei, Bogdan-Alexandru Torcărescu, Cătălin Lazăr | |
| Wolves and dogs – A stable isotopes analysis perspective | 17 |
| Bogdan Ispas, Virgil Drăgușin, George Mirea, Georgiana A. Grigore, Viorel Atudorei, Ștefan Vasile, Cătălin Lazăr, Tiberiu Sava, Gabriela Sava, Carmen M. Chifiriuc | |
| Identification of <i>Vulpes</i> species from La Adam Cave (Dobrogea) based on morphology and morphometry of the first lower molar | 19 |
| Mihai Caminschi, Virgil Drăgușin, Ștefan Vasile | |

| | |
|--|-----------|
| Carbon and nitrogen stable isotope measurements of bulk organic matter in sediments from Obanul Mare paleolake, Mangalia, Romania | 21 |
| Georgiana A. Grigore, Virgil Drăgușin, Bogdan Ispas, Viorel Atudorei, Carmen M. Chifiriuc | |
| Polyaromatic hydrocarbons (PAHs) in a paleolake sediment core from eastern Romania | 23 |
| Denisa Igescu, Virgil Drăgușin, Sorin Avramescu, Alina E. Olaru | |
| The morpho-osteometry of the mandibular ramus and lower dentition – <i>Ursus spelaeus</i>, Romanian Carpathians | 25 |
| Andra-Maria Ilie | |
| Contributors | 27 |



Insights into the cultivable microbiota of Isverna cave guano deposits

Lavinia V. Manolachi^{*1,2}, Georgiana A. Grigore^{1,2,3}, Irina G. Barbu^{1,4}, Ionuț Pecete⁵, Virgil Drăgușin^{2,4},
Carmen M. Chifiriuc^{1,4,6}

1. Faculty of Biology, University of Bucharest, Splaiul Independenței 91-95, 050095 Bucharest, Romania
2. "Emil Racoviță" Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania
3. National Institute Research and Development for Biological Sciences, Splaiul Independenței 296, 060031 Bucharest, Romania
4. Research Institute of the University of Bucharest, University of Bucharest, Șoseaua Panduri 90, 050663, Bucharest, Romania
5. Central Reference Synevo-Medicover Laboratory, B-dul. Pache Protopopescu 81, 021408 Bucharest, Romania
6. The Romanian Academy, Calea 13 Septembrie 13, 010071 Bucharest, Romania

Caves are unique, extreme ecosystems, hosting diverse microbial communities adapted to these ecological niches that exhibit important roles in supporting underground ecosystems. One of the sources of caves microbiome is represented by bat guano deposits. The very diverse diet of Chiroptera (mainly insectivory, but also frugivory, nectarivory, hematophagy, and carnivory) (Graham, 1994) influences the diversity of their microbiota.

Besides the ecological, biotechnological, and economic importance that could arise from the investigation of bat microbiome, the presence of these small mammals and their capacity to migrate and adapt to a range of habitats can serve as a source for the spread of potentially powerful pathogens.

The purpose of this study was to get an insight into the richness and bacterial diversity of cultivable microbiota from the guano deposits from Isverna Cave, located in the Mehedinți Mountains, Romania. Three distinct sampling points were chosen based on the location of the bat colonies. According to Baciuc *et al.* (2019), the first portion of the cave serves as a shelter for maternity and hibernation colonies formed by *Myotis myotis*, *M. emarginatus*, *Rhinolophus ferrumequinum*, and *R. hipposideros insectivorous* species.

The samples were collected in the winter and summer in optimal conditions to prevent contaminations caused by the cave's aeromicrobiota and microbiota of human tegument and further submitted to microbiological analyses.

Each sample (1 g and 10 g) was diluted with 9 mL and 40 mL, respectively, of sterile water solution, then five ten-fold dilutions were obtained, and 100 μ L of each dilution were spread on two non-selective media: TSA (Tryptic Soy Agar) and Blood Agar. All plates were incubated under aerobic conditions at 37 °C. From each sampling point, colonies with different morphologies were randomly selected and subcultured on the same media, and bacterial strains were identified by MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry).

We isolated 41 bacterial species, predominantly representatives of the *Paenibacillaceae* family (26.82%), followed by the *Bacillaceae* family (17.07%), *Enterobacteriaceae*, and *Lactobacillaceae* families (9.75%), *Yersiniaceae*, *Staphylococcaceae*, and *Pseudomonadaceae* families (4.87%), *Corynebacteriaceae*, *Comamonadaceae*, *Micrococcaceae*, *Fusobacteriaceae*, *Planococcaceae*, *Promicromonosporaceae*, *Morganellaceae*, *Streptococcaceae*, and *Alcaligenaceae* families (2.43%). The highest richness of the bacterial species was recorded for the *Bacillaceae* family (53.73%), with the representatives: *Viridibacillus neidei* (32.83%), followed by *Bacillus simplex* (8.59%), and *Bacillus muralis* (7.46%). Similar results were obtained by Newman *et al.* (2018) from the layer of bat guano accumulations using dependent and non-dependent cultivation techniques. Depending on the depth of analysis, a decline in the frequency of the *Bacilli* class was observed from fresh to subsurface guano. However, the persis-

*Corresponding author. E-mail address: l.manolachi20@bio.unibuc.ro (L. V. Manolachi).

tence of these species throughout the deposit may suggest that they are specialized to adapt to environmental changes regarding oxygen availability or the nutritional composition of guano subject to decay.

The Gram-positive bacteria were isolated from the majority of the cultivable bacterial taxa, while the Gram-negative bacteria, including pathogenic or opportunistic species, were less frequent, being present only in the fresher faeces, probably because they are vulnerable to chemical decomposition processes (Newman *et al.*, 2018). Among these, *Raoultella ornithinolytica*, which causes histamine poisoning (Kanki *et al.*, 2002) was isolated in both sampling seasons. Another clinically relevant Gram-negative bacterial species was *Morganella morganii*, which causes surgical wound infections, urinary tract infections, abscesses, cellulitis, chorioamnionitis, sepsis, and bacteremia, even if this taxon is a normal inhabitant of the intestinal microbiota. *Morganella morganii* is known for its rapid development of antibiotic resistance to many antibiotics, including ampicillin, cephalosporins, fosfomycin, colistin, and lincosamides (Liu *et al.*, 2016).

Acknowledgments. Part of this work was financially supported by a grant of the Roma-

nian Ministry of Education and Research, CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2020-2282 (ECHOES) to V.D, C1.2.PFE-CDI.2021-587/Contract no. 41PFE/30.12. 2021 awarded to M.C.C and PN-III-P2-2.1-PED-2021-2526 (736 PED/2022).

References

- Baciu, M., Mogyorosi, G., Virgil Drăgușin, V., Gyurka, Z., Storozynski, S., 2019. Mehedinti Mountains: Isverna Cave, in: Ponta, G.M.L., Onac, B.P. (Eds.), Cave and Karst Systems of Romania. Springer International Publishing, pp. 165–171. 1
- Graham, G.L., 1994. Bats of the World, first ed. Saint Martin's Press, New York. 1
- Kanki, M., Yoda, T., Tsukamoto, T., Shibata, T., 2002. *Klebsiella pneumoniae* produces no histamine: *Raoultella planticola* and *Raoultella ornithinolytica* strains are histamine producers. Appl. Environ. Microbiol., 68, 3462–3466. 2
- Liu, H., Zhu, J., Hu, Q., Rao, X., 2016. *Morganella morganii*, a non-negligent opportunistic pathogen. Int. J. Infect. Dis. 50, 10–17. 2
- Newman, M.M., Kloepper, L.N., Duncan, M., McInroy, J.A., Kloepper, J.W., 2018. Variation in Bat Guano Bacterial Community Composition With Depth. Front Microbiol. 9, 914. 2



Solving the puzzle of the colonisation pattern of *Mesoniscus graniger* (Frivaldsky, 1865) in Romania using population genetics

Robert F. Opran^{*1,2}

1. "Emil Racoviță" Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania
2. University of Bucharest, Faculty of Biology, Bucharest, Romania

Mesoniscus Carl, 1906 is a genus of terrestrial isopods (Isopoda: Oniscidea), from the family *Mesoniscidae*, section *Microcheta*. The genus is the only member of the family and contains two cavernicolous species, with a distribution strictly limited to the Alpino-Carpathian chain.

M. alpicola is recorded from the karst regions of the Alps (Italy, Austria, Germany - Bavaria) and lives exclusively at high altitudes, while the more common *M. graniger* has a wider distribution, along the Carpathian Mountains (Slovakia, Poland, Ukraine, Romania, Serbia) and the Dinaric Alps (Serbia, Bosnia and Herzegovina, Croatia, Slovenia). *M. graniger* can also be found in the deeper layers of the soil, not only in caves (Giurginca, 2009). In rare instances, the species *M. graniger* was recorded at low altitudes, on the plains, at a relative proximity to the mountainous areas in which it is usually found (Ferenți & Covaciu-Marcov, 2018).

The objective of this study is to determine the direction from which the species colonised the Carpathians, using population genetics techniques. In his book, "Aspects concerning the genus *Mesoniscus*", Giurginca (2009) provided two hypothesis regarding the origin of the genus *Mesoniscus*, described by him as "the Northern Hypothesis" and "the Southern Hypothesis".

According to the Northern Hypothesis the populations would have an Alpine or, perhaps, a Bohemian origin, and they would have migrated southward, through the Carpathians, towards the Dinaric Alps. On the other hand, the Southern Hypothesis suggests the populations would have an Alpine origin, and migrated southwards through the Dinaric Alps and then northward through the

Carpathian mountains.

Two independent events - the recurring opening and closing of the seaway from the Mediterranean and the Central Paratethys in Slovenia and the ice cap covering the Bohemian Massif - would block the gene flow between the populations from the Alps and the Carpathians, pushing the two populations towards speciation (Giurginca & Munteanu, 2007).

Based on morphological characters, Giurginca (2009) suggests that *M. alpicola* is a more basal taxon compared to *M. graniger*, so he argues that it is more likely that the origin of the genus is somewhere around the current distribution area of *M. alpicola*: the Alps (Giurginca & Munteanu, 2007).

In Romania, as stated earlier, the only present species from this genus is *M. graniger*. The species has three subspecies identified on the Romanian territory: *M. graniger graniger*, by far the most common subspecies, *M. graniger dragani*, endemic to the Sighiștelului Valley, Bihor Mountains and *M. graniger moldavicus*, present in the Eastern Carpathians (Fig. 1). *M. graniger dragani* has only been found in caves, while *M. graniger moldavicus* has only been found on the surface. *M. graniger graniger* was found both on the surface and underground (Giurginca, 2022).

The populations of *M. graniger* on the Romanian territory are spread following an island model, the populations being limited to the karst regions of the Carpathians, with some exceptions. Fig1 shows where the species has been identified so far in Romania.

A population will be considered a cluster of sites not divided by any natural boundary. In this study, we intend to use the populations with

*Corresponding author. E-mail address: robert.opran.7@gmail.com (R. F. Opran).

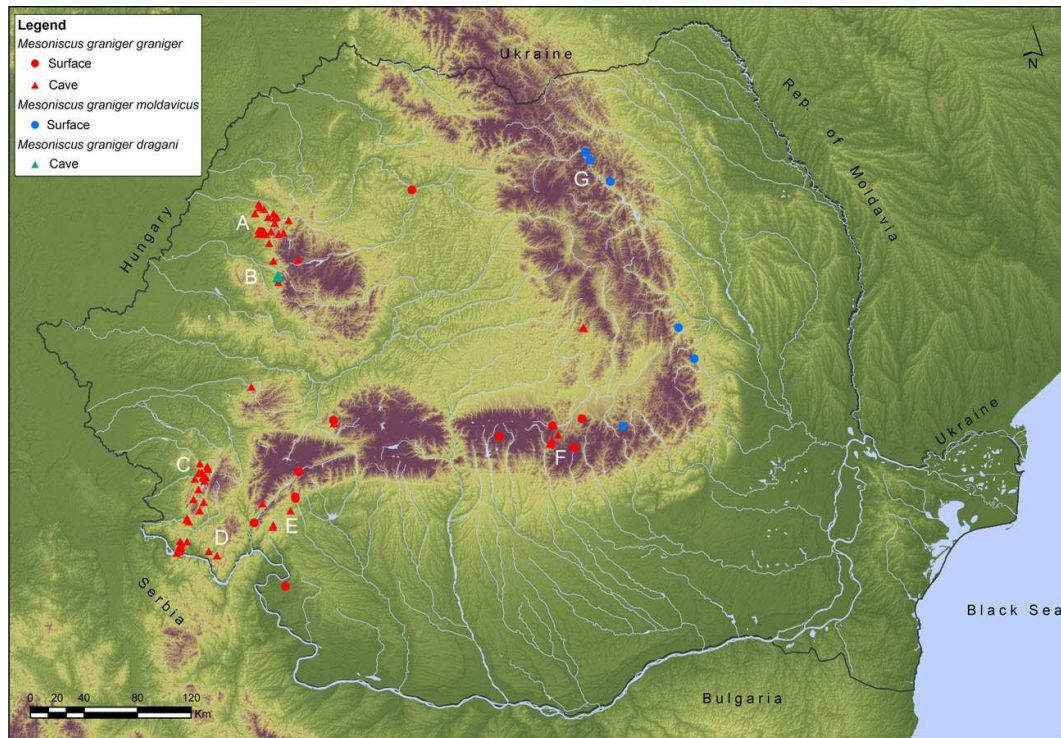


Fig. 1 – Population clusters of *M. graniger* and its subspecies in Romania: A - Apuseni Mountains cluster; B - Bihor Mountains cluster; C - Aninei Mountains cluster; D - Danube Gorge cluster; E - Oltenia cluster; F - Bucegi Mountains cluster; G - Bistriței Valley cluster.

the largest area and the largest number of individuals, for each subspecies: Population A - *M. graniger graniger* from the Apuseni Mountains, Population B - *M. graniger dragani* from the Bihor Mountains, Sighiștelului Valley, Population C - *M. graniger graniger* from Aninei Mountains (the northern part of Banatului Mountains), Population D - *M. graniger graniger* from the Danube Gorge (the southern part of Banatului Mountains), Population E - *M. graniger graniger* from North-West Oltenia, Population F - *M. graniger graniger* from Bucegi Mountains and Population G - *M. graniger moldavicus* from Bistrița Valley (Fig. 1).

It is expected that the genetic diversity in the populations furthest from the origin to decrease due to founder effect. The population with the highest genetic diversity is the earliest population to have colonised the Romanian territory and the source population for the others (Schmitt *et al.*, 2002).

The genetic diversity is measured using the frequency of heterozygosity (H), the number of alleles and percentage of polymorphic loci. A population with a high genetic diversity is characterised

by a high value of H , a high number of alleles for every locus and a high percentage in polymorphic loci (Schmitt *et al.*, 2002). To avoid using loci influenced by natural selection, microsatellites will be used. Ideally, the number of loci chosen and the number of individuals collected are to be as high as possible, for a more accurate description.

References

- Ferenți, S., Covaciu-Marcov, S. D. 2018. Beyond the rule: A mountainous cave species, *Mesoniscus graniger* (Isopoda, Oniscidea) on a plain of South-Western Romania. Muzeul Olteniei Craiova. Oltenia. Studii CTMi comunicări. Științele Naturii, 34 (1), 89–92. 3
- Giurginca, A., Munteanu, C.M., 2007. Historical biogeography of the genus *Mesoniscus* Carl, 1906. "Time in Karst" Symposium, Postojna (Slovenia). 3
- Giurginca, A., 2009. Aspects concerning the genus *Mesoniscus*. Politehnica Press, București p. 9–108. 3
- Giurginca, A. 2022. Oniscidea of Romania., Transversal, Târgoviște. p. 32–37. 3
- Schmitt, T., Gießl, A., Seitz, A., 2002. Postglacial colonisation of western Central Europe by *Polyommatus coridon* (Poda 1761) (Lepidoptera: Lycaenidae): evidence from population genetics. Heredity, 88, p. 26–34. 4



Cold-adapted phototrophic microorganisms from Scărișoara Ice Cave: Suitable models for developing electrochemical biosensors for herbicides

Robert Ruginescu^{*1}, Alina Vasilescu², Roberta Banciu², Cristina Purcărea¹

1. Department of Microbiology, Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independenței, 060031 Bucharest, Romania
2. International Centre of Biodynamics, 1B Intrarea Portocalelor, 060101 Bucharest, Romania

The excessive use of herbicides has led to their accumulation in the environment, resulting in severe ecological and health consequences. Thus, the development of efficient on-site methods to detect herbicides is becoming increasingly important. Electrochemical biosensors that rely on the inhibition of photosynthesis of microorganisms by herbicides have emerged as an attractive alternative to conventional analytical techniques due to their high level of specificity, sensitivity, speed, and relatively low cost (Tucci *et al.*, 2019). Phototrophic microorganisms adapted to cold environments may serve as ideal models for developing biosensors that can operate effectively in low-temperature environments, such as seawater. In this context, our study aimed to explore the potential of microorganisms isolated from Scărișoara Ice Cave for developing cold-active electrochemical biosensors for herbicides.

In order to isolate phototrophic microorganisms, ice samples previously collected from the top of an ice block located in a sun-exposed site (Hillebrand-Voiculescu *et al.*, 2014) were inoculated in BG-11 growth medium (Stanier *et al.*, 1971) and incubated at 15 °C for 50 days under constant illumination. Green-pigmented colonies were purified by re-streaking. The phototrophic strain LT4 was identified among the obtained isolates by sequencing the plastidial 16S rRNA gene, which showed 100% identity (1319 bp) with the Antarctic microalga *Coccomyxa subellipsoidea* C-169. Cells and subcellular components, including thylakoids and PS II complexes, were isolated from the LT4 strain using previously described methods (Vavilin

, 2011). These components were then either tested in solution or immobilized on screen printed electrodes as part of the development of herbicide electrochemical biosensors.

The photosynthetic activity of whole cells, thylakoids and PS II from *Coccomyxa subellipsoidea* TL4 was evaluated via amperometry measurements. The experimental system included a portable potentiostat, an electrochemical cell containing a screen-printed carbon nanotubes electrode (type DRP110D CNT, Metrohm Dropsens, Spain) and a white light lamp and adjustable light intensity for controlled lighting and a laptop with appropriate software for data acquisition and interpretation. The measurements were performed by applying a potential of 0.65 V in the electrochemical cell containing biological material (whole cells, thylakoids or PS II) in 20 mM phosphate buffer pH 7.1 with 0.15 mM NaCl, 5 mM MgCl₂ and 0.1 mM to 0.5 mM 2,6, diclorobenzoquinone (DCBQ) - as electrochemical mediator. The tests were done by applying 3 consecutive cycles of 1 minute light / 15 minutes dark and the intensity of the photocurrent generated in the electrochemical cell was expressed as average of the 3 measurements. The measurements were repeated with fresh biological material in the presence of diuron (0.26 μM-2.57 μM). The inhibition degree of the photosynthetic activity due to diuron was calculated from the decrease in photocurrent intensity (I_1), compared to the value recorded in the absence of the herbicide (I_0): Inhibition (%) = $(I_0 - I_1) / I_0 \times 100$.

The amperometry tests have emphasized that the photosynthetic activity of thylakoids is inhibited

^{*}Corresponding author. E-mail address: robert.ruginescu@ibiol.ro (R. Ruginescu).

ited by $40.2 \pm 7.3\%$ in the presence of $60 \mu\text{g kg}^{-1}$ diuron. By comparison, the same concentration of herbicide induced only $10.2 \pm 5.3\%$ inhibition of the photosynthetic activity of the whole cells.

Stability tests have shown that the best results after 6 days at -20°C were obtained for the samples that contained 2 M sucrose as additive. In these conditions the photosynthetic activity of thylakoids decreased to 55.6% of the initial value and the inhibition caused by $60 \mu\text{g kg}^{-1}$ diuron was $14.9 \pm 2.5\%$. Consequently, a more extensive study is needed to identify efficient additives to preserve the photosynthetic activity and functionality (i.e., sensitivity to diuron) of thylakoids kept at -20°C . PS II preparations had low chlorophyll concentration (0.1 mg/mL), low photosynthetic activity and lack of sensitivity to $60 \mu\text{g kg}^{-1}$. This leads to the conclusion that the stability of PS II must be improved and, related to this, the protocol for obtaining the PS II must be revisited. For the electrochemical sensing of photosynthesis-inhibiting herbicides, thylakoids appear as the most promising in terms of combined stability and sensitivity to diuron.

Acknowledgments. The authors acknowledge

the financial support from the Romanian Executive Agency for Higher Education, Research, Development and Innovation Funding (UEFISCDI) through ERANET-MARTERA-MOBILTOX project, contract 224/04.01.2021.

References

- Tucci, M., Bombelli, P., Howe, C.J., Vignolini, S., Bocchi, S., Schievano, A., 2019. A Storable Mediatorless Electrochemical Biosensor for Herbicide Detection. *Microorganisms*, 7, 630. 5
- Hillebrand-Voiculescu, A., Iltus, C., Ardelean, I., Pascu, D., Perşoiu, A., Rusu, A.; Brad, T., Popa, E., Onac, B.P., Purcărea, C., 2014. Searching for Cold-Adapted Microorganisms in the Underground Glacier of Scărişoara Ice Cave, Romania. *Acta Carsologica*, 43, 319–329. 5
- Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue-green algae (order *Chroococcales*). *Bacteriol. Rev.*, 35, 171–205. 5
- Vavilin, D.V., 2011. Methods for the Isolation of Functional Photosystem II Core Particles from the Cyanobacterium *Synechocystis* sp. PCC 6803, in: Carpentier, R. (Eds), *Photosynthesis Research Protocols. Methods in Molecular Biology*, vol. 684. Humana Press, Totowa, NJ, pp. 29–40. 5



A laboratory experiment of carbon stable isotope fractionation by several fungal species

Ștefan Surugiu^{*1,2}, Georgiana A. Grigore^{1,2,3}, Virgil Drăgușin^{2,4}, Irina G. Barbu^{1,4}, Bogdan Ispas^{1,2,4}, Viorel Atudorei⁵, Ionuț Pecete⁶, Carmen M. Chifiriuc⁷

1. Faculty of Biology, University of Bucharest, Splaiul Independenței 91-95, 050095 Bucharest, Romania
2. "Emil Racoviță" Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania
3. National Institute Research and Development for Biological Sciences, Splaiul Independenței 296, 060031 Bucharest, Romania
4. Research Institute of the University of Bucharest, University of Bucharest, Șoseaua Panduri 90, 050663, Bucharest, Romania
5. Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, United States
6. Central Reference Synevo-Medicover Laboratory, B-dul Pache Protopopescu 81, 021408 Bucharest, Romania
7. The Romanian Academy, Calea Victoriei 125, 010071 Bucharest, Romania

Fungi are a critical component of the carbon cycle, having an important role in the decomposition of soil organic matter. During the decomposition process, CO₂ is released in the soil where it dissolves into infiltrating water, forming carbonic acid. In carbonate settings, the resulting carbonic acid is the main driver behind karst formation and speleothem deposition. The isotopic composition of carbon in CO₂ is one of the main factors affecting the δ¹³C in speleothems, an important paleoenvironmental proxy.

In this study, we investigate the extent to which several fungi species are fractionating carbon isotopes during their development, as compared to the growth medium. For this experiment, we took four soil samples under grassland and forest cover in the Mehedinți Mountains (SW Romania), where speleothem δ¹³C records were published (Drăgușin *et al.*, 2014; Staubwasser *et al.*, 2018) and a cave monitoring program is ongoing (Drăgușin *et al.*, 2017, 2020). Soil samples were taken from 10 cm and 30 cm depths, aliquots of 1 g from each sample were mixed with 9 mL of sterile water solution, then three-fold dilutions were obtained and 100 μL aliquots of each dilution were spread on Sabouraud dextrose agar (this medium contains glucose, meat peptone, casein peptone, and agar). The fungal colonies were observed after 5–7 days after incubation at room temperature and colonies with different morphologies were randomly selected from each sampling point to be

subcultured on the same media in order to obtain pure cultures. Examination of macroscopic characters of culture and colony, together with examination of mycelium structure under the microscope allowed a presumptive phenotypic identification, followed by the confirmation of fungal species / genera by MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time of Flight) mass spectrometry.

All strains of isolated microfungi belong to the *Ascomycota* phylum: *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma*, *Cladosporium*, and *Scopulariopsis* genera.

For this study, we have selected some of the filamentous fungal strains: *Fusarium oxysporum* isolated from both grassland and forest ecosystems, *Trichoderma* sp. that has been dominant in the grassland, *Fusarium proliferatum* isolated from grassland soil, and *Scopulariopsis brevicaulis* identified in forest soil. All the microfungi were individually cultured for 13 days on Sabouraud agar, as well as on maize (*Zea mays*), a C₄ plant. After incubation, the next step consisted of sampling from maize together with the mycelium developed and separating the fungal biomass, respectively, followed by freezing at –20 °C and then, freeze-drying.

An amount of 0.5 mg–0.6 mg from each sample, as well as growth medium samples, were analyzed for δ¹³C at the Stable Isotopes Laboratory of the Research Institute of the University of Bucharest

*Corresponding author. E-mail address: s.surugiu20@s.bio.unibuc.ro (Ș. Surugiu).

(ICUB-SIL) using a Nu Instruments Horizon 2 Isotope Ratio Mass Spectrometer (IRMS) coupled to a EuroVector 3024 elemental analyzer. The samples were corrected using an in-house standard, a C4 plant (Blue Grama), and two international standards, IAEA-CH3 and IAEA-600. Within-run standard deviation values for these standards were 0.15‰ for Blue Grama, 0.08‰ for IAEA-CH3, and 0.06‰ for IAEA-600.

Initial growth medium values were -12.95‰ for maize and -20.00‰ for Sabouraud. After 4 days since cultivation, maize had average values of -12.66‰ (SD=0.04‰, n=6), while after 7 days the average $\delta^{13}\text{C}$ value was -12.52 (SD=0.3, n=6). Sabouraud medium was sampled only on the 13th day after cultivation and had an average value of -18.40‰ (SD=1.26, n=6).

Fungal biomass recovered from Sabouraud agar after 13 days of cultivation exhibited in all cases an enrichment in ^{13}C between 2.44‰ and 5.34‰. This is in accordance with other similar studies that found ^{13}C enrichment between fungi and their growth substrate (Henn *et al.*, 2002; Zeller *et al.*, 2007).

Acknowledgments. Part of this work was financially supported by a grant of the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2020-2282 (ECHOES) to V.D, C1.2.PFE-CDI.2021-587/Contract no.41PFE/30.12.2021 awarded to

M.C.C. and PN-III-P2-2.1-PED-2021-2526 (736 PED/2022).

References

- Drăgușin, V., Staubwasser, M., Hoffmann, D.L., Ersek, V., Onac, B.P., Vereș, D., 2014. Constraining Holocene hydrological changes in the Carpathian-Balkan region using speleothem $\delta^{18}\text{O}$ and pollen-based temperature reconstructions. *Clim. Past.*, 10, 1363–1380. 7
- Drăgușin, V., Balan, S., Blamart, D., Forray, F.L., Marin, C., Mirea, I., Nagavciuc, V., Orășeanu, I., Perșoiu, A., Tîrlă, L., Tudorache, A., Vlaicu, M., 2017. Transfer of environmental signals from the surface to the underground at Ascunsă Cave, Romania. *Hydrol. Earth Syst. Sci.*, 21, 5357–5373. 7
- Drăgușin, V., Mirea, I.C., Ersek, V., Tîrlă, L., 2020. Farmed calcite $\delta^{13}\text{C}$ at Ascunsă cave, Romania, and its relation with CO_2 outgassing and drip rate. *Quaternaire*, 31/2, 165–174. 7
- Henn, M.R., Gleixner, G., Chapela, I.H., 2002. Growth-dependent stable carbon isotope fractionation by basidiomycete fungi: $\delta^{13}\text{C}$ pattern and physiological process. *Appl. Environ. Microbiol.*, 68, 4956–4964. 8
- Staubwasser, M., Drăgușin, V., Onac, B.P., Assonov, S., Ersek, V., Hoffmann, D.L., Vereș, D., 2018. Impact of climate change on the transition of Neanderthals to modern humans in Europe. *Proc. Natl Acad. Sci. USA*, 115, 9116–9121. 7
- Zeller, B., Brechet, C., Maurice, J.P., Le Tacon, F., 2007. ^{13}C and ^{15}N isotopic fractionation in trees, soils and fungi in a natural forest stand and a Norway spruce plantation. *Ann. For. Sci.*, 64, 419–429. 8



Exploring the crustaceans world in the sulfidic mesothermal aquifer from Mangalia, Romania (Southern Dobrogea, Romania)

Maria M. Pop^{*1}, Filip P. Boancă², Andrei Ștefan^{1,3}, Sanda Iepure^{1,4}

1. “Emil Racoviță” Institute of Speleology of the Romanian Academy, Cluj-Napoca Department, Clinicilor 5, 400006 Cluj Napoca, Romania
2. Doctoral school of Ecology and Evolution, Limnology Department, University of Vienna, Vienna, Austria
3. National Museum of Natural History “Grigore Antipa”, Pavel D. Kiseleff 1, 011341, Bucharest, Romania
4. Department of Taxonomy and Ecology, University Babeș-Bolyai, Cluj Napoca, Romania

The Mangalia town region in Southern Dobrogea, Romania, conceals dark, isolated, and sulfide-rich overlapping aquifers hiding intriguing and complex hydrogeological characteristics (Marin & Nicolescu, 1993; Sârbu, 2000). This unique ecosystem is sustained by a high level of primary production through chemosynthesis, mainly supported by chemoautotrophic bacteria and protozoa that derive their energy from chemical reactions with inorganic molecules such as hydrogen sulfide, elemental sulfur, and ammonia under low oxygen or anaerobic conditions (Sârbu et al., 1994, 1996; Engel, 2007; Kumaresan et al., 2014; Sârbu et al., 2019). The Upper Sarmatian sulfidic mesothermal aquifer, the focus of the current study, which is accessible through the old hand-dug wells (sulfidic and non-sulfidic water), and the Movile Cave provide a suitable habitat for a diverse community of cyclopoid crustaceans (eight species) with a preponderance of stygobites (75%), from the following genera: *Acanthocyclops* Kiefer, 1927, *Diacyclops* Kiefer, 1927, *Tropocyclops* Kiefer, 1927, and *Eucyclops* Claus, 1893. The spatial distribution of the identified species does not indicate a distinct affinity for a specific type of water, sulfidic or non-sulfidic.

The primary production in Movile Cave is mainly dependent on the activity of sulfur-oxidizing bacteria, particularly sulfur-oxidizing filamentous gammaproteobacterial species of the genus *Thiothrix* (Sârbu et al., 1994; Chen et al., 2009).

Thiothrix bacteria can be found either as free-living bacteria or as epibionts or ectosymbionts attached to other animals, such as amphipods,

in marine or continental sulfidic habitats (Gillan & Dubilier, 2004; Bauermeister et al., 2012; Flot et al., 2014). The first *Thiothrix*-amphipod symbiosis in a sulfidic groundwater habitat was reported in the Frassasi Caves system, Italy, involving three *Niphargus* amphipod species (Bauermeister et al., 2012). Later, the same *Niphargus*-*Thiothrix* association was also discovered in aquifers located 1200 kilometers apart, in Movile Cave and the surrounding aquifer in Mangalia, as well as in other groundwaters in the Dobrogea region (Limanu, Dulcești, Doi Mai, Vama Veche) (Flot et al., 2014). Thus, this suggests that they could be widespread in continental sulfidic and non-sulfidic aquifers in Europe, especially since a similar association was also observed in Melissotrypa Cave from Greece, where *Niphargus gammariformis* seems to have established an interaction with *Thiothrix* filamentous bacteria (Flot et al., 2014; Borke et al., 2019). Furthermore, *Thiothrix* bacteria has been found to be also present on cyclopoid or ostracod microcrustaceans from both the Movile Cave and the top layer mezothermal aquifer in Mangalia, especially since this association could potentially provide advantages for the species involved.

Applying the sequencing of 16S ribosomal DNA *Thiothrix* genes, we show that bacteria of the genus *Thiothrix* are present on *Eucyclops graeteri scythicus*, *Tropocyclops prasinus* (Cyclopoida, Copepoda) and *Pseudocandona movilaensis* (Ostracoda) thriving in the aquifer of the Mangalia town and Movile Cave, highlighting the versatility of such *Thiothrix*-crustacean associations in sulfidic groundwaters. This study signals the first

*Corresponding author. E-mail address: mariamirabelapop@gmail.com (M. M. Pop).

discovery of such associations between ostracod and cyclopoid crustaceans and *Thiothrix* in sulfidic groundwater and a cave ecosystem worldwide, expanding our understanding of symbiotic relationships between crustaceans and *Thiothrix*.

Acknowledgments. This work was supported by grant of the Romanian Ministry of Education and Research, CNCS-UEFISCDI project number PN-III-P4-PCE-2020-2843 (EVO-DEVO-CAVE), within PNCDI III.

References

- Bauermeister, J., Ramette, A., Dattagupta, S., 2012. Repeatedly evolved host-specific ectosymbiosis between sulfur-oxidizing bacteria and amphipods living in a cave ecosystem. *PLoS ONE* 7(11), e50254. [9](#)
- Borko, Š., Collette, M., Brad, T., Zakšek, V., Flot, J.-F., Vaxevanopoulos, M. *et al.* 2019. Amphipods in a Greek cave with sulphidic and non-sulphidic water: phylogenetically clustered and ecologically divergent. *Syst. Biodivers.*, 17 (6), 558–572. [9](#)
- Chen, Y., Wu, L., Boden, R., Hillebrand, A., Kumaresan, D., Moussard, H., Baciu, M., Lu, Y., Colin Murrell, J., 2009. Life without light: microbial diversity and evidence of sulfur- and ammonium-based chemolithotrophy in Movile Cave. *ISME J.*, 3 (9), 1093–1104. [9](#)
- Engel, A.S., 2007. Observations on the biodiversity of sulfidic karst habitats. *J. Caves Karst. Stud.*, 69 (1), 187–206. [9](#)
- Flot, J.-F., Bauermeister, J., Brad, T., Hillebrand-Voiculescu, A., Sârbu, S.M., Dattagupta, S., 2014. Niphargus-Thiothrix associations may be widespread in sulphidic groundwater ecosystems: evidence from southeastern Romania. *Mol. Ecol.*, 23 (6), 1405–1417. [9](#)
- Gillan, D. C., Dubilier, N., 2004. Novel epibiotic Thiothrix bacterium on a marine amphipod. *Appl. Environ. Microbiol.*, 70 (6), 3772–3775. [9](#)
- Kumaresan, D., Wischer, D., Stephenson, J., Hillebrand-Voiculescu, A., Murrell, J.C., 2014. Microbiology of Movile Cave – A chemolithoautotrophic ecosystem. *Geomicrobiol. J.*, 31 (3), 186–193. [9](#)
- Marin, C., Nicolescu, T., 1993. The geochemistry of groundwater from southeastern Dobrogea, Romania. *Travaux de l'Institut de Speologie "Emile Racovita"* 32, 229–247. [9](#)
- Sârbu, S.M., Kinkle, B. K., Vlasceanu, L., Kane, T. C., Popa, R., 1994. Microbiological characterization of a sulfide-rich groundwater ecosystem. *Geomicrobiol. J.*, 12 (3), 175–182. [9](#)
- Sârbu, S.M., Lascu, C., Brad, T., 2019. Dobrogea: Movile cave, *in*: Ponta, G.M.L., Onac, B.P. (Eds.) *Cave and karst systems of Romania*. Springer, Cham, 429–436. [9, 23](#)
- Sârbu, S.M., 2000. Movile Cave: a chemoautotrophically based groundwater ecosystem, *in*: Wilkens, H, Culver, D.C., Humphreys, W.F. (Eds.) *Subterranean Ecosystems*. Elsevier, Amsterdam, 319–343. [9](#)
- Sârbu, S.M., Kane, T.C., Kinkle, B.K., 1996. A chemoautotrophically based cave ecosystem. *Science* 272 (5270), 1953–1955. [9, 22](#)



The response of aquatic fauna to variable environmental conditions in Ghețarul de la Vârtop cave (Apuseni Natural Park, Romania)

Orest A. Sambor^{*1}, Bianca Șarcani¹, Aurel Perșoiu^{2,3}, Constantin Marin⁴, Ana I. Camacho⁵, Karina P. Battes¹, Mirela Cîmpean¹, Alin Tudorache⁴, Sanda Iepure^{1,2}

1. Department of Taxonomy and Ecology, Faculty of Biology and Geology, Babeș-Bolyai University, Clinicilor 5-7, Cluj-Napoca, 400006, Romania
2. "Emil Racoviță" Institute of Speleology of the Romanian Academy, Cluj-Napoca Department, Clinicilor 5, 400006 Cluj Napoca, Romania
3. Ștefan cel Mare University, Universității 13, Suceava, 720229, Romania
4. "Emil Racoviță" Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania
5. Department of Biodiversity and Evolutive Biology, National Museum of Natural History, C/ José Gutiérrez Abascal 2, Madrid, 28006, Spain

Ice caves can be found at mid-to-high northern latitudes, with elevations ranging from 0 to over 3000 meters above sea level (Perșoiu *et al.*, 2021). These caves are home to rare and endemic species, as well as glacial relicts that require cold microclimates to survive (Iepure, 2018). The extremely low temperatures in these caves have played a significant role in shaping the structure of both terrestrial and aquatic communities (Iepure, 2018).

Aquatic organisms that live in ice caves have adapted to cope with the constantly low air and water temperatures. One such cave is Ghețarul de la Vârtop, which is located in the Apuseni Natural Park in Romania. This short cave hosts temporary ice formations near its entrance and was the subject of a study initiated in 2021. The study aims to understand how the environmental parameters in the cave affect the structure of underground fauna by monitoring the air temperature, water characteristics, and aquatic fauna communities on a monthly basis.

The measurements made on site reveal that the temperature remains at an average value of 3.93 °C for the rimstone and 3.92 °C for the percolation sites. The minimum recorded temperature value found was 1.6 °C at a percolation site at the entrance of the cave in spring (April). The highest value recorded was 5.8 °C in both percolation and

rimstone sites at the beginning of summer (May for rimstone sites and June for percolation sites).

Our data reveals from the study reveals that the cave's percolation water and associated rimstones contain a variety of aquatic invertebrate communities, including nematodes, oligochaetes, and crustaceans (i.e., harpacticoids, Bathynellacea, cyclopoids, and oligochaets), mostly found from the middle sector of the cave. Most of these species are stygobites and new to science, indicating the importance of the cave and prone to special measures of protection and conservation.

Studying groundwater fauna in caves with permanent and temporary ice formations can help us better understand how these organisms are distributed in certain habitats, and later, what are their adaptations to constantly low-water temperatures. This knowledge can provide insight into the ecology of the fauna and the mechanisms involved in their adaptations.

References

- Iepure, S., 2018. Ice cave fauna, *in*: A. Perșoiu, S.-E. Lauritzen (Eds.), Ice caves. Elsevier, pp. 163–171. 11
- Perșoiu, A., Buzjak, N., Onaca, A., Pennos, C., Sotiriadis, Y., Ionita, M., Butorac, V., 2021. Record summer rains in 2019 led to massive loss of surface and cave ice in SE Europe. *The Cryosphere*, 15(5), 2383–2399. 11

*Corresponding author. E-mail address: orestus745@gmail.com (O. A. Sambor).



Inferring spatio-temporal palaeodynamics: from ancient biomolecules to organismal (overviews on cave sediment sequences)

Adrien Lörincz^{*1,2}, Aritina Haliuc^{2,3,4}, Andrea Demjén^{2,4,5}, Călin Ghemiş^{2,4,6}, Răzvan Dumbravă^{2,4,7}, Adriana Vulpoi^{2,4,8}, Beatrice S. Kelemen^{4,9,10}, Augustin Nae², Ioana Rusu^{4,9,10}, Viorel T. Lascu¹¹, Florin Gogâltan^{2,12}, Bogdan P. Onac^{4,13}, Ioana N. Meleg^{2,4}

1. University of Debrecen, Egyetem tér 1, 4032, Debrecen, Hungary
2. "Emil Racoviță" Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania
3. University of Suceava, Department of Geography, Universitatii 13, 720229, Suceava
4. "Emil G. Racoviță" Institute, Babeş-Bolyai University, Clinicilor 5-7, 400006 Cluj-Napoca, Romania
5. National Museum of Transylvanian History, C. Daicoviciu 2, 400020 Cluj-Napoca, Romania
6. Criş Country Museum - Oradea, str. Calea Armatei Române nr. 1A. Oradea, Romania
7. University of Oradea, Faculty of Geography, Tourism and Sport, 1st University Street, 410087, Oradea, Romania
8. Interdisciplinary Research Institute on Bio-Nano-Sciences, Babeş-Bolyai University, 400271 Cluj-Napoca, Romania
9. Molecular Biology Center, Interdisciplinary Research Institute on Bio-Nano-Sciences, Babeş-Bolyai University, 400271 Cluj-Napoca, Romania
10. Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeş-Bolyai University, 400006 Cluj-Napoca, Romania
11. National Institute for Research and Development in Environmental Protection (INCDPM), Bucharest 060031, Romania
12. Institute of Archaeology and Art History, M. Kogălniceanu 12-14, Cluj-Napoca, Romania
13. Karst Research Group, School of Geosciences, University of South Florida, Tampa, FL, USA

Cave sediments are natural repositories that store geochemical and palaeobiological information and sometimes archaeological materials, thus providing evidence of past changes in the environment and clues of human activities over long temporal scales (Springer, 2019). Here we aimed to reconstruct the Holocene ecosystem dynamics using poorly studied, non-disturbed cave sediments from important sites with known hominin occupation (Ciur Izbug and Toplița de Vida) located in the Apuseni Mountains (western Romania, Fig. 1).

In both caves, an interdisciplinary approach that combines chronology, lithostratigraphy, geochemistry, ancient DNA, and a detailed archaeological survey, has been undertaken to: *i*) identify and document the potential zones for excavations; *ii*) evaluate the sediment accumulations and prepare digital databases using last generation surveying tools – the 3D scanning; *iii*) collect sediments and other materials (organic material, seeds) for detailed archaeological, palaeogenomics, palaeobiological and sedimentological analysis (Fig. 2).

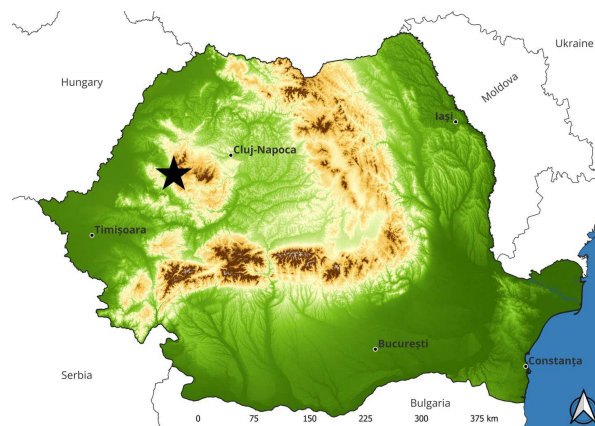


Fig. 1 – Study area (black star) on the topographic map of Romania.

In Ciur Izbug Cave, one sedimentary profile of 128 cm was opened following the standard procedure: cleaning the oxidation layer, lithological and archaeological description, 3D scanning and acquiring photos followed by sequential sampling at 2 cm resolution for more detailed sedimentological and mineralogical analysis and lower resolution sampling for ancient DNA analysis.

*Corresponding author. E-mail address: lorinczadrika@gmail.com (A. Lörincz).



Fig. 2 – Image showing the excavation site in Toplița de Vida Cave (photo: Irinel Cîrlănaru).

The preliminary results of the radiocarbon dating show that the bottom of the sedimentary sequence is of mid-Holocene age and the depositional environment is characterized by different phases of sedimentation in response to climate and environmental changes.

In Toplița de Vida Cave, a box grid excavation with the following dimensions has been carried: C1_CX006 of 2.00 m x 1.60 m, to remove two of the skeletons found inside the cave for further anthropological analysis. Both skeletons were radiocarbon-dated to the Early Bronze Age (ca. 2,400–2,300 BC). Furthermore, Middle Neolithic Age ceramic fragments, grain and charred wood fragments were found. This level of occupation yielded dates belonging to the late 6th-early 5th millennium BC.

Our work explores the faunal, human, climate,

and environmental perspectives, being of cultural and scientific significance especially in SE Europe where early and unique human records are found. Ultimately, it is critical for advancing our understanding of past human dynamics and changes in the environments.

Acknowledgments. We are grateful to cavers from Speodava Caving Club in Ștei for their help during the fieldwork. We would also like to thank Horea Meleg for providing the map.

References

Springer, G.S., 2019. Clastic sediments in caves, *in*: White W.B., Culver, D.C., Pipan T. (Eds.), *Encyclopedia of Caves* (Third Edition). Academic Press, Cambridge, pp. 277–284. [13](#)



Carbon and nitrogen stable isotope values of Late Pleistocene fauna from Stoieni Cave, Romania

George Mirea^{*1,2}, Virgil Drăgușin^{1,3}, Bogdan Ispas^{1,2,3}, Georgiana A. Grigore^{1,2,7}, Ștefan Vasile^{1,4}, Ionuț Mirea¹, Tiberiu Sava⁵, Carmen M. Chifiriuc³, Viorel Atudorei⁹, Bogdan-Alexandru Torcărescu^{10,11}, Cătălin Lazăr^{3,8}

1. “Emil Racoviță” Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania
2. University of Bucharest, Faculty of Biology, Bucharest, Romania
3. Research Institute of the University of Bucharest (ICUB), Bucharest, Romania
4. University of Bucharest, Faculty of Geology, 1 Nicolae Bălcescu Avenue, 010041 Bucharest, Romania
5. National Institute of Research and Development for Physics and Nuclear Engineering “Horia Hulubei”, Măgurele, Romania
6. Romanian Academy, Bucharest, Romania
7. National Institute for Research and development for Biological Sciences, Bucharest, Romania
8. University of Bucharest, Faculty of History, Bucharest, Romania
9. University of New Mexico, Department of Earth and Planetary Sciences, Albuquerque, U.S.A.
10. Geological Institute of Romania, 1st Caransebeș Street, 012271 Bucharest, Romania
11. University of Bucharest, Faculty of Geology and Geophysics, Doctoral School of Geology, 6 Traian Vuia Street, 020956 Bucharest, Romania.

The diet of large mammals can be studied based on the carbon and nitrogen isotopic composition of bone collagen. The stable isotope composition of these elements undergoes fractionation along the food chain and one can distinguish the trophic level and food source of mammals based on their isotopic signature (Bocherens & Drucker, 2003).

In this study, we aim at identifying the trophic relationships between herbivores, omnivores, and carnivores at Stoieni Cave, southwestern Romania, using the C and N stable isotope signature of collagen extracted from fossil remains. At the same time, we intend to use the isotopic signature to assign unidentified fossil remains to their respective trophic level.

Following recent paleontological excavations at Stoieni Cave, a series of bones, bone fragments, and teeth were used to build the chronostratigraphic context of the sediment infill of the cave using ¹⁴C dating. The samples were dated at the RoAMS Laboratory (Măgurele, Romania) following the method described by (Sava *et al.*, 2018). The remaining, unused, collagen was analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ composition at the Stable Isotope Laboratory of the Research Institute of the University

of Bucharest (ICUB-SIL). Samples weighing 0.5 mg were measured on a NuInstruments Horizon 2 IRMS in continuous flow mode, coupled to a EuroVector elemental analyzer. Two in-house protein standards were used, namely casein and tuna fish, whose within-run standard deviation was 0.3‰ for $\delta^{15}\text{N}$ and 0.09‰ for $\delta^{13}\text{C}$, respectively.

Calibrated radiocarbon ages place the analyzed materials between 32,000 and 11,700 years before present. Stable isotope values vary between 2.53‰ and 10.33‰ (vs. AIR) for $\delta^{15}\text{N}$, and between -21.58‰ and -18.50‰ (vs. VPDB) for $\delta^{13}\text{C}$. The average C/N ratio of the samples presented here is 3.3.

In Fig. 1, a cluster of values can be observed, between $\delta^{13}\text{C}$ values of -20.17‰ and -19.22‰, and between $\delta^{15}\text{N}$ values of 2.53‰ and 3.72‰, a range consistent with values reported for herbivore taxa. Among these, we identified two artiodactyl metapodials and a mandible, as well as a tibia belonging to a sub-adult *Capra ibex*. The highest values in our dataset, consistent with a carnivorous diet, were obtained on a *Canis lupus* mandible fragment (10.33‰ and -18.50‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively).

A small cluster of three samples that could be

*Corresponding author. E-mail address: g.mirea20@s.bio.unibuc.ro (G. Mirea).

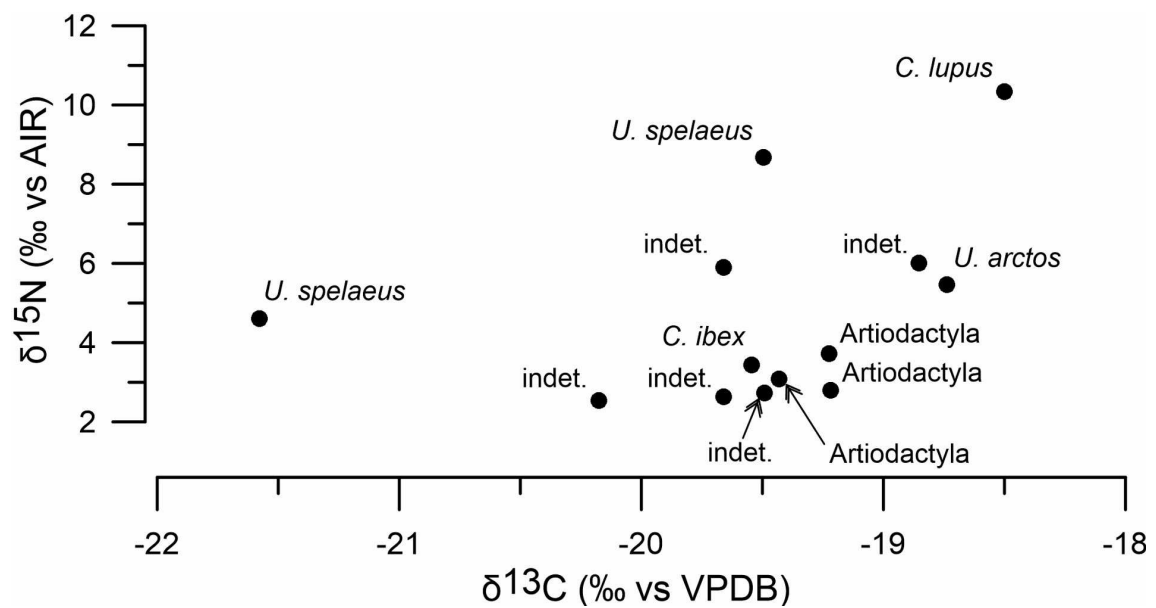


Fig. 1 – Nitrogen and carbon isotopic composition of collagen sampled from vertebrate fossil remains of Stoieni Cave. Dentine samples are not corrected as proposed by [Bocherens *et al.* \(2015\)](#).

assigned to omnivores has $\delta^{15}\text{N}$ values of $\sim 5\text{‰}$, while $\delta^{13}\text{C}$ values range between -19.66‰ and -18.74‰ . The highest $\delta^{13}\text{C}$ value was obtained from an *Ursus arctos* mandible, while the other two specimens in the omnivorous group are unidentified.

A fourth apparent population is given by two *Ursus spelaeus* individuals, as they plot above the general trend in herbivore-to-carnivore values. A radius fragment yielded values of 8.68‰ and -19.50‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively, while a premolar root had values of 4.60‰ and -21.58‰ . While dentine is known to have higher $\delta^{15}\text{N}$ values compared to adult bone collagen ([Bocherens *et al.*, 2015](#); [Robu *et al.*, 2018](#)), the difference between the two specimens from Stoieni Cave is 4‰ , exceeding this effect. The isotope values of *U. spelaeus* from Stoieni Cave are comparable to those from other sites in Romania, but the reduced dataset precludes a more thorough comparison.

Acknowledgments. Part of this work was financially supported by a grant of the Romanian Ministry of Education and Research,

CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2020-2282 (ECHOES) to V.D.

References

- Bocherens, H., Drucker, D., 2003. Trophic level isotopic enrichments for carbon and nitrogen in collagen: case studies from recent and ancient terrestrial ecosystems. *Int. J. Osteoarchaeol.* 13, 46–53. [15](#)
- Bocherens, H., 2015. Isotopic tracking of large carnivore palaeoecology in the mammoth steppe. *Quat. Sci. Rev.* 117, 42–71. [16, 18](#)
- Robu, M., Wynn, J.G., Mirea, I.C., Petculescu, A., Kenes, M., Pușcaș, C.M., Vlaicu, M., Trinkaus, E., Constantin, S., 2018. The diverse dietary profiles of MIS 3 cave bears from the Romanian Carpathians: insights from stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analysis. *Palaeontology* 61, 209–219. [16, 18](#)
- Sava, T.B., Simion, C.A., Gâza, O., Stanciu, I.M., Păceșilă, D.G., Sava, G.O., Wacker, L., Ștefan, B., Moșu, V.D., Ghiță, D.G., Vasiliu, A., 2018. Status report on the sample preparation laboratory for radiocarbon dating at the New Bucharest Roams Center. *Radiocarbon*, 61, 649–658. [15](#)



Wolves and dogs – A stable isotopes analysis perspective

Bogdan Ispas^{*1,2,3}, Virgil Drăgușin^{1,3}, George Mirea^{1,2}, Georgiana A. Grigore^{1,2,9}, Viorel Atudorei⁵, Ștefan Vasile^{1,6}, Cătălin Lazăr^{3,7}, Tiberiu Sava⁸, Gabriela Sava⁸, Carmen M. Chifiriuc^{2,3,4}

1. “Emil Racoviță” Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania.
2. University of Bucharest, Faculty of Biology, Splaiul Independentei 91-95, Bucharest, R-050095, Romania.
3. The Research Institute of the University of Bucharest (ICUB), Bucharest, Romania.
4. Romanian Academy, Bucharest, Romania.
5. University of New Mexico, Department of Earth and Planetary Sciences, Albuquerque, U.S.A.
6. University of Bucharest, Faculty of Geology and Geophysics, 1 Nicolae Bălcescu Avenue, 010041 Bucharest, Romania.
7. University of Bucharest, Faculty of History, Bucharest, Romania.
8. National Institute of Research and Development for Physics and Nuclear Engineering “Horia Hulubei”, Măgurele, Romania.
9. National Research Institute for Biological Sciences, Bucharest, Romania.

This study aims to compare stable isotopes of carbon and nitrogen in wolves and domesticated dogs populations from various paleontological and archaeological sites from Romania.

Stable isotopes are a powerful tool which can provide us with valuable insights into the dietary and ecological changes that occurred during the domestication of dogs and can help to understand the evolutionary relationship between humans and dogs.

In this study, we analyzed collagen $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ from 6 samples retrieved from Pleistocene *Canis lupus* individuals from Romania, as well as from 3 domesticated *C. lupus* from the archaeological site of Cuina Turcului, a neolithic locality.

Collagen is the most abundant type of protein from the mammalian organisms. It is a fibrous protein that is formed from three polypeptide chains, which are made up from repeating triplets of Gly and two other amino acids, where proline and hydroxyproline are the most abundant (Schwarcz & Nahal, 2021; Zhang *et al.*, 2015). Because bones are one of the most numerous remains which archaeologists usually find in the field, collagen extracted from archaeological bones is very useful for paleodietary studies, the type one collagen representing 90% of the mammalian bone (Talamo & Fewlass, 2021).

For collagen extraction, fragments of bone were cut with a diamond disc, then washed in an ultrasonic bath with distilled water and acetone in order

to remove any contaminants. After drying at room temperature, they were ground to a fine powder. About 350 mg of bone powder was demineralized with HCl 1 M for 20 minutes at room temperature and then filtered using a 5 μm filter. The next step was to treat the residue with a 0.125 M NaOH solution for 20 h at room temperature. These steps were followed by rinsing and after that the collagen was heated in a weak acidic solution of 0.01 M HCl at 90 °C to 95 °C for 17 h in order to gelatinize. After filtration, the resulting solution (collagen) was freeze-dried (Bocherens *et al.*, 1997).

An aliquot of 0.5 mg to 0.6 mg of collagen was weighed and encapsulated in tin capsules and the samples were measured on a Nu Instruments Horizon 2 IRMS coupled to an EuroVector 3024 elemental analyser at the Stable Isotopes Laboratory of the Research Institute of the University of Bucharest (ICUB-SIL). Stable carbon isotope ratios are expressed relative to VPDB (Vienna PeeDee Belemnite), and stable nitrogen isotope ratios are measured relative to AIR (atmospheric N_2). For calibration, two internal protein standards were used, casein and tuna, with isotopic values of 6.43‰ (casein) and 13.32‰ (tuna) for the $\delta^{15}\text{N}$ and -26.52‰ (casein) and -16.7‰ (tuna) for $\delta^{13}\text{C}$.

The $\delta^{15}\text{N}$ values of wolf specimens vary between 7.8‰ and 11.7‰ with a mean of 10.3 ± 0.3 ‰. In the case of $\delta^{13}\text{C}$, the values are ranging from -17.6‰ to -20.2‰ with a mean of -18.7 ± 0.1 ‰. We compared our results with the values from the

*Corresponding author. E-mail address: ispas.bogdan@s.bio.unibuc.ro (B. Ispas).

Gravettian Předmostí site in the Czech Republic, a site that could be very important in sustaining the idea that the domestication of the wolf started long before the end of the Last Glacial Period (Germonpré *et al.*, 2012). The $\delta^{15}\text{N}$ values vary between 8.9‰ and 10.7‰ and the $\delta^{13}\text{C}$ between -18.7‰ and -19.5‰. For Palaeolithic dogs from Předmostí, the values ranged from 7.8‰ to 10.4‰ for $\delta^{15}\text{N}$, and from -18.3‰ to -19.3‰ for $\delta^{13}\text{C}$ (Bocherens *et al.*, 2015). Moreover, Robu *et al.* (2018) reported similar values for the wolves from Muierilor Cave, namely between -17.4‰ and -18.0‰ for $\delta^{13}\text{C}$, but higher values, from 12.1‰ to 14.0‰, for $\delta^{15}\text{N}$. In Oase Cave, the values for $\delta^{13}\text{C}$ range from -18.3‰ to -19.8‰, and $\delta^{15}\text{N}$ varies between 7.9‰ and 11.5‰ (Robu *et al.*, 2018).

Acknowledgments. This work was financially supported by grants of the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project numbers PN-III-P4-ID-PCE-2020-2282, PN-III-P4-ID-PCE-2020-2369, and PN-III-1.2PDI-PFE-C1-2021.

References

- Bocherens, H., Billiou, D., Patou-Mathis, M., Bonjean, D., Otte, M., Mariotti, A., 1997. Paleobiological Implications of the Isotopic Signatures (^{13}C , ^{15}N) of Fossil Mammal Collagen in Scladina Cave (Sclayn, Belgium). *Quat. Res.* 48, 370–380. 17
- Bocherens, H., Drucker, D.G., Germonpré, M., Laznickova-Galetova, M., Naito, Y.I., Wissing, C., Brůžek, J., Oliva, M., 2015. Reconstruction of the Gravettian food-web at Předmostí I using multi-isotopic tracking (^{13}C , ^{15}N , ^{34}S) of bone collagen. *Quat. Int.* 359, 211–228. 16, 18
- Germonpré, M., Láznicková-Galetová, M., Sablin, M. V., 2012. Palaeolithic dog skulls at the Gravettian Předmostí site, the Czech Republic. *J. Archaeol. Sci.*, 39(1), 184–202. 18
- Robu, M., Wynn, J.G., Mirea, I.C., Petculescu, A., Keneszi, M., Pușcaș, C.M., Vlaicu, M., Trinkaus, E., Constantin, S., 2018. The diverse dietary profiles of MIS 3 cave bears from the Romanian Carpathians: insights from stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analysis. *Palaeontology* 61, 209–219. 16, 18
- Schwarcz, H.P., Nahal, H., 2021. Theoretical and observed C/N ratios in human bone collagen. *J. Archaeol. Sci.* 131, 1–7. 17
- Talamo, S., Fewlass, H., 2021. “Here we go again”: the inspection of collagen extraction protocols for dating and palaeodietary analysis. *STAR* 7, 62–77. 17
- Zhang, Y.Z., Ran, L.Y., Li, C.Y., Chen, X.L., 2015. Diversity, structures, and collagen-degrading mechanisms of bacterial collagenolytic proteases. *AEM* 81, 6098–6107. 17



Identification of *Vulpes* species from La Adam Cave (Dobrogea) based on morphology and morphometry of the first lower molar

Mihai Caminschi^{*1,2}, Virgil Drăgușin^{1,3}, Ștefan Vasile^{1,2}

1. "Emil Racoviță" Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania
2. Faculty of Geology and Geophysics, University of Bucharest, N. Bălcescu 1, 010041 Bucharest, Romania
3. Research Institute of the University of Bucharest, Panduri 60, Bucharest, Romania

The Quaternary fauna of Romania contained several species belonging to the *Vulpes* genus (family *Canidae*, subfamily *Caninae*, tribe *Vulpini*), namely *Vulpes vulpes*, *Vulpes corsac*, and *Vulpes lagopus*. The most diverse *Vulpes* fauna reported so far from Romania was discovered at La Adam Cave and contains all three species mentioned above (Dumitrescu *et al.*, 1963, 1965). Nevertheless, the fox specimens from this site were not systematically studied. We propose here to identify the individuals belonging to these three species, using the morphology of the first lower molar (m1), following the approach of Gimranov *et al.* (2015).

Gimranov *et al.* (2015) proposed the identification of some m1 morphotypes that have a higher probability of occurrence among each species. These traits are characterized in particular by the dimensional variation of the entoconide, but also by the presence or absence of the entoconulide and the hypoconulide. The proposed morphotypes are: A, B1, B2, B3, B4, C1, C2, C3, C4, C5, C6, C7, D1, D2, E, each describing the m1 by a unique combination of features. For example, the B2 morphotype is expressed by the presence of the entoconulide and a small entoconide, while the C1 morphotype is defined only by the presence of a massive entoconide.

Further, we present the morphometric parameters of m1, namely the greatest length (GL) and the greatest breadth (GB), based on the method of von Driesch (1976).

We assigned a predominant morphotype for each of the 44 lower carnassials available from La Adam Cave (B1, n=9; B2, n=2; B3, n=1; B4, n=4; C1, n=3; C3, n=8; C6, n=17). In addition to determin-

ing the predominant morphotype of the lower carnassial, the method proposed by Gimranov *et al.* (2015) can also be supported by the morphological analysis of the second lower molar (m2), whenever this approach is applicable (Lanoë, 2012).

The length of m1 from our samples ranges between 12.70 mm and 18.19 mm whereas the greatest breadth ranges from 4.40 mm to 7.43 mm (Fig. 1). The B1-B4 morphotypes are assigned more often to *V. lagopus* individuals (whose m1 size at our site varies from 12.70 mm to 16.30 mm for GL, and from 4.40 mm to 6.29 mm for GB). In the case of morphotypes C1-C3, which are more often attributed to *V. corsac*, our specimens vary between 12.85 mm and 16.58 mm for GL, and between 5.02 mm and 6.42 mm for GB. For populations of *V. vulpes*, the morphotype with the highest frequency of occurrence is C6 and the resulting dimensions fit between 14.81 mm and 18.19 mm for GL, and between 5.63 mm and 7.43 mm for GB in our dataset.

We can observe in Fig. 1 an obvious separation in m1 size between *V. lagopus* and *V. vulpes*, where the arctic fox individuals are well delimited with the exception of two individuals, that overlap with *V. corsac* or *V. vulpes*. Considering that morphotypes B1 and B4 have an occurrence rate of only 0.5% in *V. vulpes* and 4.7% in *V. corsac*, it is possible these two individuals belong, in fact, to *V. corsac* or they may be exceptions from the dimensional average of *V. lagopus*. At La Adam Cave, *V. vulpes* has a well-defined morphometric distribution, with m1s being notably larger than *V. lagopus*. We can see an overlap between the measurements of *V. vulpes* from our site and Pleistocene red foxes from France (Bonifay, 1971) and Middle Pleistocene individu-

*Corresponding author. E-mail address: mihai.caminschi@s.unibuc.ro (M. Caminschi).

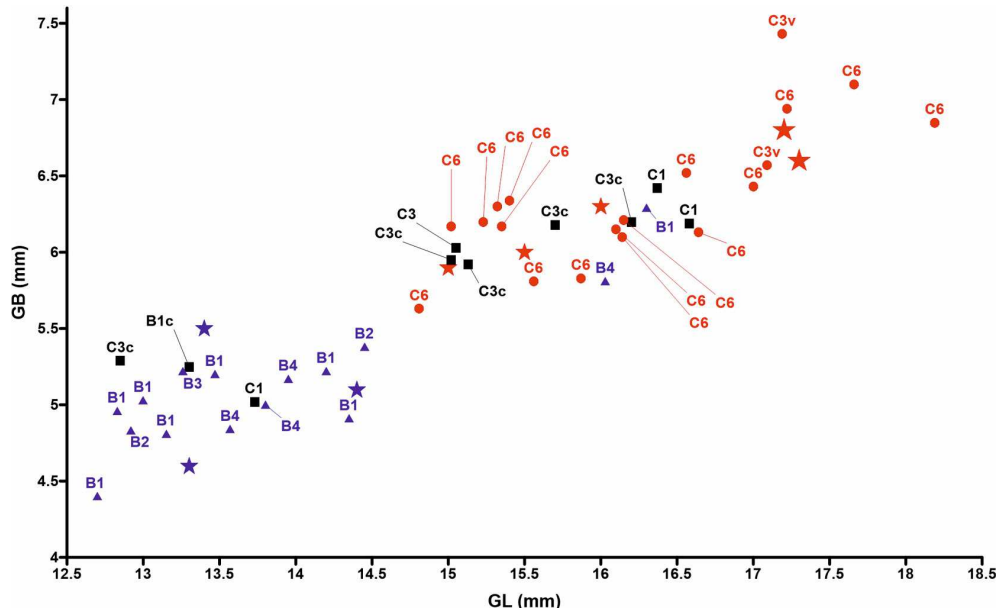


Fig. 1 – Morphometric distribution of lower first molar belonging to genus *Vulpes* individuals from La Adam Cave. Blue triangles: *V. lagopus*; black squares: *V. corsac*; red circles: *V. vulpes*; blue stars: Pleistocene *V. lagopus* from France (Bonifay, 1971); red stars: Pleistocene *V. vulpes* from France (Bonifay, 1971); larger red stars: middle Pleistocene *V. vulpes* from Spain (Madurell-Malapeira *et al.*, 2021). The labels indicate the morphotypes according to Gimranov *et al.* (2015).

als from Spain (Madurell-Malapeira *et al.*, 2021). In the case of *V. corsac* the m1 size has a higher variability, with dimensional overlaps with the other two species. Other exceptions include one individ-

ual that displays the B1c morphotype, in which the size of the m1 fits better among arctic foxes, but the morphological analysis of the m2, showed that it is, in fact closer to *V. corsac*.

Therefore, the morphological analysis of m1 proposed by Gimranov *et al.* (2015), supported by the morphological analysis on the m2 (Lanoë, 2012), and further combined with morphometric analysis, helped us identify the individuals belonging to the three species of the genus *Vulpes* reported by Dumitrescu *et al.* (1963, 1965) from La Adam Cave. Having demonstrated all three fox species are present in this fossil assemblage, similar methodologies can be further applied for identifying other specimens, such as those that preserve the upper dentition.

Acknowledgments. Part of this work was financially supported by a grant of the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2020-2282 (ECHOES) to V.D.

References

- Bonifay, M.-F., 1971. Carnivores quaternaires du Sud-Ouest de la France. Mém. Mus. Natl. Hist. Nat., Nouv. Sér. C, Sci. Terre., 21 (2), 1–334. 19, 20
- Dumitrescu, M., Samson, P., Terzea, E., Rădulescu, C., Ghica, M., 1963. Peștera “La Adam”, stațiune pleistocenă. Lucr. Inst. Speol. “Emil Racoviță”, 1–2, 230–284. 19, 20
- Dumitrescu, M., Samson, P., Terzea, E., Radulescu C., Ghica, M., 1965. Sur l’état actuel des fouilles paléontologiques dans la station pleistocene de la grotte “La Adam”, in Trimel, H. (Ed.), Third International Congress of Speleology, vol. IV, 15–19. 19, 20
- Gimranov, D.O., Kosintsev, P.A., Gasilin, V.V., 2015. Species diagnostic of corsac (*Vulpes corsac*), fox (*Vulpes vulpes*), and arctic fox (*Vulpes lagopus*) according to ontological characteristics of mandible teeth. Zool. Zhurnal, 94 (11), 1338–1350. 19, 20
- Lanoë, F., 2012. Détermination de *Vulpes corsac* (L., 1768) au sein d’assemblages fossiles du Pléistocène supérieur: données ostéométriques et clef morphologique dentaire. Bull. de la Soc. Prehist. Française., 109 (2), 331–334. 19, 20
- Madurell-Malapeira, J., Bartolini Lucenti, S., Alba, D.M., 2021. Middle Pleistocene fox from the Vallparadès Section (Vallès-Penedès Basin, NE Iberian Peninsula) and the earliest records of the extant red fox. Riv. It. Paleontol. Strat., 127 (1), 179–187. 20
- von den Driesch, A., 1976. A guide to the measurement of animal bones from archaeological sites. Peabody Mus. Bull., 1, 1–137. 19



Carbon and nitrogen stable isotope measurements of bulk organic matter in sediments from Obantul Mare paleolake, Mangalia, Romania

Georgiana A. Grigore^{1,2,3}, Virgil Drăgușin^{2,4}, Bogdan Ispas^{1,2,4}, Viorel Atudorei⁵, Carmen M. Chifiriuc^{1,4,6}

1. Faculty of Biology, University of Bucharest, Splaiul Independenței 91-95, 050095 Bucharest, Romania
2. "Emil Racoviță" Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania
3. National Institute Research and Development for Biological Sciences, Splaiul Independenței 296, 060031 Bucharest, Romania
4. Research Institute of the University of Bucharest, University of Bucharest, Șoseaua Panduri 90, 050663, Bucharest, Romania
5. Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, United States
6. The Romanian Academy, Calea Victoriei 125, 010071 Bucharest, Romania

Pleistocene climate changes have led to vegetation type and distribution changes. In Central-Eastern Europe, long paleoenvironmental archives are scarce, thus for this region environmental reconstructions on glacial/interglacial time scales are not available.

In eastern Romania, near the Black Sea, a series of deep collapse dolines functioned as lakes and accumulated thick sedimentary sequences (Constantinescu & Constantin, 2001) that could have deposited during the Pleistocene. Recently, a 60 meters long core was retrieved from the Obantul Mare paleolake in order to study biogeochemical environmental proxies throughout the existence of the lake. In this study, we present preliminary $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements of bulk organic matter and bulk carbon content of these sediments.

The samples were chosen along the entire length of the sediment core in order to investigate if there are significant changes regarding the $\delta^{13}\text{C}$. The sections of the sedimentary sequence are well defined, starting with the recent soil and brown clays and continuing with yellowish and yellowish-reddish deposits, a segment with dark gray clays, to the last unit of the core characterized by gravels and limestone blocks.

The samples were analyzed for total carbon isotopes ($\delta^{13}\text{C}_{\text{total}}$), organic carbon isotopes ($\delta^{13}\text{C}_{\text{org}}$), total nitrogen isotopes ($\delta^{15}\text{N}$), and C/N. For the measurement of $\delta^{13}\text{C}$, bulk sediment samples from various depths were collected and processed using a decalcification method by wet chemical treat-

ment, slightly modified after Pötter *et al.* (2021). Before acid treatment, 5 g of each sample were dried in a drying oven at 60 °C for 24 h and in order to obtain a homogenized sample, they were ground thoroughly in a mortar with pestle. Approximately 150 mg were weighed from each sample in centrifuge tubes, treated with 25 mL of 1 M hydrochloric acid (HCl) which corresponds to a concentration of 5% HCl and kept in water bath for 4 hours at a 50 °C. After digestion, the sediment samples were sequentially rinsed twice with 40 mL of ultrapure water and centrifuged between rinses, in order to remove any remaining HCl. The final preparation step consists in drying the samples in a drying-oven at 60 °C overnight.

Samples were analyzed at the Stable Isotopes Laboratory of the Research Institute of the University of Bucharest (ICUB-SIL) using a Nu Instruments Horizon 2 IRMS coupled to a EuroVector 3024 elemental analyzer.

Calibrations were performed using three organic reference standards: two internal standards, Blue Grama, a C4 plant, and a C3 soil standard, whose standard deviation within-sequence was 0.09 ‰, 0.09 ‰ for $\delta^{13}\text{C}$ and 0.85 ‰, 0.4 ‰ for $\delta^{15}\text{N}$, respectively. We also used international standards, IAEA-CH3, a pure substance with known values and with a standard deviation of 0.09 ‰ for $\delta^{13}\text{C}$.

The organic carbon content is highest in the present-day soil (~2%) but decreases sharply to 0.2% in the underlying sediments. The N content of the topsoil is 0.2% and decreases to an average of

*Corresponding author. E-mail address: grigore.georgiana-alexandra@s.bio.unibuc.ro (G.A. Grigore).

0.03%, with a resulting organic C/N ratio varying between 7 and 11. The sedimentary organic matter origin can be evaluated due to the C/N ratio and for C/N values lower than 10, we can assert that the organic material can be derived from lacustrine phytoplankton compared with terrestrial organic components that point to a ratio higher than 20 (Leng *et al.*, 2005).

$\delta^{15}\text{N}_{\text{total}}$ values vary between 6‰ and 10‰ (vs AIR), while $\delta^{13}\text{C}_{\text{org}}$ is relatively stable throughout the dataset, varying very little around an average of -25.5‰ (vs VPDB), thus showing that the main contributor to sediment organic carbon was C3 terrestrial plants.

The existence of a sulphidic aquifer extending in the region around Obantul Mare promoted the presence of a chemoautotrophic ecosystem, whose isotopic signature is different from its surroundings (Sârbu *et al.*, 1996). Its $\delta^{15}\text{N}$ values vary between ~-10‰ and ~0‰, while $\delta^{13}\text{C}$ values do not surpass -30‰. Thus, we can state that from our preliminary measurements we do not discern the sulphidic ecosystem as a source of organic content into the lake.

Acknowledgments. Part of this work was

financially supported by a grant of the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2020-2282 (ECHOES) to V.D and C1.2.PFE-CDI.2021-587/Contract no.41PFE/30.12.2021.

References

- Constantinescu, T., Constantin, S., 2001. La genèse et l'évolution des grandes dolines (obans) de la zone karstique de Mangalia (Dobrudja du Sud, Roumanie). *Theor. Appl. Karstol.* 13–14, 87–92. [21](#)
- Leng, M.J., Lamb, A.L., Heaton, T.H.E., Marshall, J.D., Wolfe, B.B., Jones, M.D., Holmes, J.A., Arrowsmith, C., 2005. Isotopes in lake sediments, *in*: Leng, M.J. (Eds.), *Isotopes in Palaeoenvironmental Research*, Springer, Netherlands, pp. 147–176. [22](#)
- Pötter, S., Schmitz, A., Lucke, A., Schulte, P., Obrecht, I., Zech, M., Wissel, H., Markovic, S.B., Lehmkuhl, F., 2021. Middle to Late Pleistocene environments based on stable organic carbon and nitrogen isotopes of loess-palaeosol sequences from the Carpathian Basin. *Boreas.* 50, 184–204. [21](#)
- Sârbu, M.S., Kane, T.C., Kinkle, B.K., 1996. A chemoautotrophically based cave ecosystem. *Science*, 272, 1953–1955. [9, 22](#)



Polyaromatic hydrocarbons (PAHs) in a paleolake sediment core from eastern Romania

Denisa Igescu^{*1,2}, Virgil Drăgușin^{1,3}, Sorin Avramescu⁴, Alina E. Olaru^{1,2,4}

1. "Emil Racoviță" Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania
2. Faculty of Biology, University of Bucharest, Bucharest, Romania
3. Research Institute of the University of Bucharest, University of Bucharest, Romania
4. PROTMED Research Centre, University of Bucharest, Bucharest

In southern Dobrogea, around the town of Mangalia, there is an important karst area where large dolines are dominating the landscape. The presence of mesothermal groundwater rich in H₂S and CH₄ created conditions for the development of hypogene karst (Sârbu & Lascu, 1997; Onac & Drăgușin, 2017; Drăgușin *et al.*, 2021), while also providing the basis of a chemoautotrophic ecosystem (Sârbu & Lascu, 1997; Sârbu *et al.*, 2019; Brad *et al.*, 2021).

Large collapse dolines present in this area functioned as lakes during the Pleistocene and into the present, allowing for the accumulation of lake sediments for extensive periods of time. Such a doline, Obantul Mare, was recently cored throughout its sediment infill and we present here data on the content of polyaromatic hydrocarbons (PAHs) in these sediments.

PAHs are a class of chemical compounds consisting of two or more condensed aromatic rings, which can be used as proxies for the intensity and frequency of biomass burning. Under certain circumstances, several PAHs can be created by geogenic processes and are frequently found in hydrocarbon formations (Stogiannidis & Laane, 2015).

By studying PAHs from the Obantul Mare paleolake, we intend to reconstruct local fire activity in parallel with paleo-fire reconstructions based on microcharcoal from the same sediment core. Thus, we focused on measuring 15 PAHs from 26 samples that were selected in order to cover the entire sediment sequence. While the level of the mesothermal sulphidic aquifer is controlled by that

of the neighboring Black Sea, we intend to identify periods with geogenic PAH input that would help us identify sea level high stands.

Sediment samples were allowed to dry at ambient temperature in order to prevent the loss of low molecular weight PAHs (e.g. naphthalene) by sublimation. Prior to the extraction step, the sediment was ground into a fine powder using an agate mortar and pestle, in order to enhance the contact between the sample and the solvent [acetone-dichloromethane (1:1)] and to insure a high extraction yield. Extraction was performed using the accelerated solvent extraction method in a Dionex ASE 200 system at 100 °C and 1500 psi.

The accelerated solvent extraction method takes advantage of the fact that solubility increases with temperature and, at the same time, liquids can penetrate more effectively into different matrices under higher pressure. Higher temperatures allow for more effective extraction of analytes that have been encapsulated in the matrix, as well as of chemicals that have been adsorbed on the surface of some matrices.

After extraction, sample concentration was performed using a Cole Parmer Type 28100 Dri-Bath Thermolyne concentrator in a stream of nitrogen, until the sample dried. Afterwards, a fixed volume of 2 mL of acetonitrile was added in order to retrieve the sample from the vial. Samples were stored in 2 mL vials at -20 °C prior to chromatographic analyses.

Samples were analyzed on a Varian ProStar HPLC fitted with a fluorescence detector, as it is favorable in the detection of PAHs at trace level due

*Corresponding author. E-mail address: d.igescu20@s.bio.unibuc.ro (D. Igescu).

to its high sensitivity and selectivity. A significant challenge was to obtain a good separation of these PAHs taking into account that matrices of samples were not always similar, and that other compounds present in the sample were overlapping with the signal of the PAHs.

Our preliminary results show that low molecular weight PAHs (LWPAHs) had concentrations of less than 0.1 ng g^{-1} , significantly less than high molecular PAHs (HWPAHs), that showed concentrations between 1 ng g^{-1} and 10 ng g^{-1} . Dibenz(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3-C,D)pyrene were the three HWPAHs that were most frequently found in our samples. However, the distribution of HWPAHs varied considerably and in order to better understand the variety of pyrogenic PAHs and their geogenic delimitation, we will use paleofire data inferred from microcharcoal studied from the same sediment samples.

Acknowledgments. Part of this work was financially supported by a grant of the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2020-2282 (ECHOES) to V.D.

References

- Brad, T., Iepure, S., Sârbu, S.M., 2021. The chemoautotrophically based Movile Cave groundwater ecosystem, a hotspot of subterranean biodiversity. *Diversity* 13, 128. [23](#)
- Drăgușin, V., Tîrlă, L., Covaliov, S., Cruceru, N., Mirea, I.C., Șandric I., 2021. The unique topography from Obanul Mare (Mangalia, SE Romania): remnant of a maze cave. *Géomorphol., Relief Process. Environ.*, 27 (3), 221–229. [23](#)
- Onac, B.P., Drăgușin, V., 2017. Hypogene Caves of Romania, in Klimchouk, A., Palmer, A.N., DeWaele, J., Auler, A.S., Audra, P. (Eds.), *Hypogene Karst Regions and Caves of the World, Cave and Karst Systems of the World*, 257–265. [23](#)
- Sârbu, S.M., Lascu, C., 1997. Condensation corrosion in Movile cave, Romania. *J. Cave Karst Stud.*, 59 (3), 99–102. [23](#)
- Sârbu, S.M., Lascu, C., Brad, T., 2019. Dobrogea: Movile Cave, in Ponta, G.M.L., Onac, B.P. (Eds), *Cave and Karst Systems of Romania*, Springer International Publishing, 429–436. [9, 23](#)
- Stogiannidis, E., Laane, R., 2015. Source characterization of polycyclic aromatic hydrocarbons by using their molecular indices: an overview of possibilities, in Whitacre, D.M. (Ed.), *Reviews of Environmental Contamination and Toxicology*, 234. Springer, 49–133 [23](#)



The morpho-osteometry of the mandibular ramus and lower dentition – *Ursus spelaeus*, Romanian Carpathians

Andra-Maria Ilie^{*1,2}

1. "Emil Racoviță" Institute of Speleology of the Romanian Academy, Calea 13 Septembrie 13, 050711 Bucharest, Romania
2. University of Bucharest, Faculty of Geology and Geophysics, Str. Traian Vuia 6, 020956 Bucharest, Romania

The aim of the osteometric analyses is to assess the limit of the dimensional variation for a certain species during a certain period. In Quaternary palaeontology, where the fossils have better occurrences (i.e. when comparing with the Mesozoic era), especially in cave bears - whose bones are the most encountered among all the mammals found in cave deposits - the osteometric comparisons between populations from different sites (either contemporaneous or with different age) may indirectly provide information about the palaeobiology of this species and climate changes of the ecosystems where these animals lived (Tsoukala & Grandal-d'Anglade, 2002).

In the Romanian Carpathians, although one of the most abundant area of Europe with Marine Isotopic Stage 3 (MIS 3; 57 ka – 29 ka BP) cave bear fossils, few sites were properly excavated and therefore, few data of significantly large populations were generated on morpho-osteometry. More than 80 (N = 84) mature cave bear mandibles and their teeth from Urșilor (N = 43) (Robu, 2015), Biseriçuța (N = 13), Muierilor (N = 9) and Oase (N = 19) (Quilès *et al.*, 2006) caves were measured, plotted and compared among them. Our aim was to

calculate the sex ratio of the studied populations and to detect if dimensional variations occurred between both males and females and between analyzed populations. Such as, between the two sexes, a obvious sexual dimorphism was identified, with the males being much larger than females. Moreover, females exceed in numbers the males at all sites. Among the studied sites, there is no significant difference of average values between the lengths of the mandibles or the dental parameters.

References

- Quilès, J., Petrea, C., Moldovan, O., Zilhao, J., Rodrigo, R., Rougier, H., Constantin, S., Milota, Ș., Gherase, M., Sarcină, L., Trinkhaus, E., 2006. Cave bears (*Ursus spelaeus*) from Peștera cu Oase (Banat, Romania): Palaeobiology and taphonomy. *Comptes Rendus Palevol*, 5. 25
- Robu, M., 2015. The palaeontology of the cave bear bone assemblage from Urșilor Cave of Chișcău – Osteometry, palaeoichnology, taphonomy, and stable isotopes. Editura Universitară, Bucharest, 248 p. 25
- Tsoukala E., Grandal-d'Anglade, A., 2002. Système de mesures sur le squelette d'Ursidae. Actes du Colloque d'Auberives-en-Royans, Études et recherche archéologiques de l'Université de Liège. 25

*Corresponding author. E-mail address: andra.ilie1999@gmail.com (A.-M Ilie).

Contributors

- Atudorei, Viorel, 7, 15, 17, 21
Avramescu, Sorin, 23
- Banciu, Roberta, 5
Barbu, G. Irina, 1, 7
Battes, P. Karina, 11
Boancă, P. Filip, 9
- Cîmpean, Mirela, 11
Camacho, I. Ana, 11
Caminschi, Mihai, 19
Chifiriuc, M. Carmen, 1, 7, 15, 17, 21
- Demjén, Andrea, 13
Drăgușin, Virgil, 1, 7, 15, 17, 19, 21, 23
Dumbravă, Răzvan, 13
- Ghemiș, Călin, 13
Gogâltan, Florin, 13
Grigore, A. Georgiana, 1, 7, 15, 17, 21
- Haliuc, Aritina, 13
- Iepure, Sanda, 9, 11
Igescu, Denisa, 23
Ilie, Andra-Maria, 25
Ispas, Bogdan, 7, 15, 17, 21
- Kelemen, S. Beatrice, 13
- Lörincz, Adrien, 13
Lascu, T. Viorel, 13
Lazăr, Cătălin, 15, 17
- Manolachi, V. Lavinia, 1
Marin, Constantin, 11
Meleg, N. Ioana, 13
Mîrea, George, 15, 17
Mîrea, Ionuț, 15
- Nae, Augustin, 13
- Olaru, E. Alina, 23
Onac, P. Bogdan, 13
Opran, F. Robert, 3
- Pecete, Ionuț, 1, 7
Perșoiu, Aurel, 11
Pop, M. Maria, 9
Purcărea, Cristina, 5
- Ruginescu, Robert, 5
Rusu, Ioana, 13
- Sambor, A. Orest, 11
Sarcani, Bianca, 11
Sava, Gabriela, 17
Sava, Tiberiu, 15, 17
Stefan, Andrei, 9
Surugiu, Ștefan, 7
- Torcărescu, Bogdan-Alexandru, 15
Tudorache, Alin, 11
- Vasile, Ștefan, 15, 17, 19
Vasilescu, Alina, 5
Vulpoi, Adriana, 13



Emil G. Racoviță (1868–1947)

The Institute of Emil Racoviță – A Century of High Scientific Research

“Emil Racoviță” Institute of Speleology, the first of its kind in the world, is the result of the genius of the great scientist Emil Racoviță (1868-1947) - biologist, polar explorer and founder of Biospeleology-its founding being consecrated by a special law issued by King Ferdinand Ist.



Fig. 1 – Emil Racoviță, together with several collaborators, in one of the laboratories of the Institute of Speleology in Cluj (courtesy of T. Brad).

In the beginning, the institute functioned as a part of the Faculty of Sciences from Cluj, the official framework where the founder of the institute also moved the headquarters of the “Biospeologica” Scientific Society, founded by him in 1907, as an international body designed to coordinate the activity of all zoologists involved in the study of the subterranean fauna.

Together with Emil Racoviță, two famous biologists developed their research: the French entomologist and biogeographer Rene Jeannel and the Swiss specialist in the subterranean fauna Pierre-Alfred Chappuis.

In 1940, following the Vienna Diktat, Racoviță was forced into refuge at Timișoara taking with him the archives of the institute while the Biospeologica collection remained in Cluj under the supervision of P.A. Chappuis. Only in 1945, Racoviță was able to return in Cluj, enduring along with his institute all the hardships of the post-war poverty and inflation. After the death of Racoviță, in 1947, the activity of the institute diminished slowly, especially after Chappuis left in 1949.

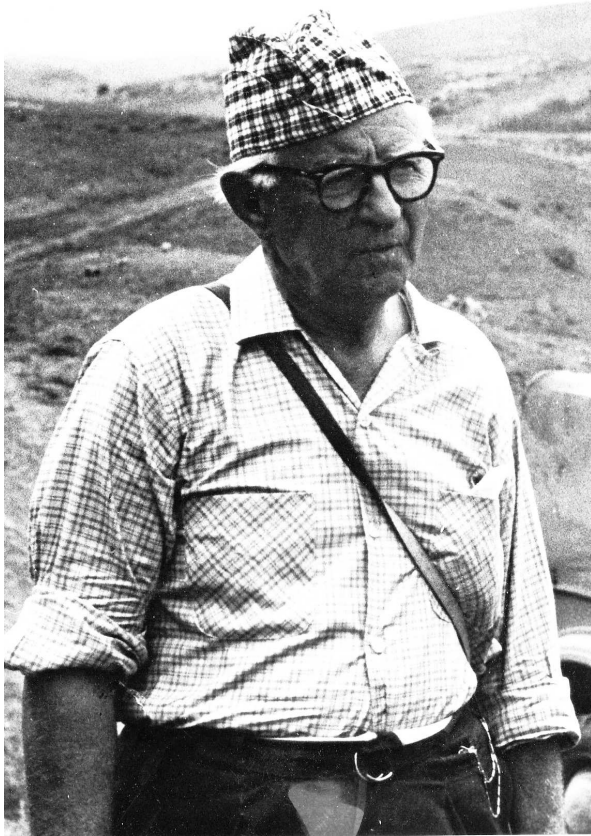


Fig. 2 – Professor Constantin Motaș (courtesy of A. Giurginca).

In 1956, following the initiative of the Professors Traian Orghidan, Margareta Dumitrescu and Valeriu Pușcariu, the institute was reorganized with the headquarters in Bucharest and a research compartment in Cluj. The management of the institute was entrusted to Professor Constantin Motaș (1891–1980), an internationally renowned systematician and taxonomist, the founder of *Phreatobiology*. A year later, the Institute received the name of its initiator, Emil Racoviță, and three years later passed under the aegis of the Romanian Academy.

From 1973 to 1990, the Institute functioned, as a self-contained unit and keeping its name, with the Central Institute of Biology. In 1990 the “Emil Racoviță” Institute of Speleology returned to the Romanian Academy, maintaining the previous structure, with research compartments in Bucharest and, respectively, in Cluj-Napoca.

In present “Emil Racoviță” Institute of Speleology (ERIS) is dedicated to the interdisciplinary research of the physical and biological elements of the karst environment and related domains, aiming to describe and analyze the current state

of the environments, palaeoevolutive reconstitution, evolutive tendencies, and conservation of protected or strategic natural areas.

Currently, ERIS comprises four research compartments and a laboratory with specific research programs:

(1) Biospeleology and Karst Edaphobiology Compartment focusing on the systematics, phylogeny, diversity and distribution of the subterranean fauna, the evolution and adaptations of the fauna, biotic and abiotic networks of the subterranean and edaphic environments;

(2) Geospeleology and Paleontology Compartment focusing on the geology of caves and karst regions, sedimentology and mineralogy of the endokarst deposits and ascertaining their absolute age, biostratigraphy, paleogeography, morphology and genetics of the fossil remains from karst deposits, defining Quaternary climatic shifts based on cave deposits;

(3) Karstology, Karst Inventory and Protection Compartment focusing on classification, inventory and registration of the exo- and endokarst structures, identification of karst heritage and biodiversity assets in need of a special protection status, management norms issuance in order to assign protected area status to karst sites;

(4) Cluj-Napoca Compartment focusing on interdisciplinary studies in all the domains of the other three compartments;

(5) Hydrogeochemistry Laboratory focusing on geochemical processes from karst-prone areas and related terrains, the transfer mechanisms in karst-prone environments of priority dangerous chemical compounds, chemical-analytic studies on natural matrix specific to the Institute domain of research. The laboratory is accredited by RENAR for investigations on various types of natural waters.

In the last two decades, the ERIS researchers published over 400 ISI indexed papers, including Nature and Science, over 200 BDI indexed papers and 48 books in prestigious international, university and academic publishing houses and actively participated in numerous international scientific events. Within the same time frame, ERIS obtained 60 research contracts, 9 being international contracts. Also, a part of the ERIS researchers acquired prestigious research scholarships within projects financed by the European Commission. In the same time, ERIS maintains a large network of collaboration with research institutes and universities from the entire world. Consequently, the ERIS researchers follow with abnegation, enthusiasm and loyalty the idea of the great visionary Emil Racoviță a century after the founding of the Institute.

Karst Science Days Symposium Proceedings

1 / 2023

- Lavinia Vasilica MANOLACHI, Georgiana Alexandra GRIGORE, Irina GHEORGHE BARBU, Ionuț PECETE, Virgil DRĂGUȘIN, Mariana Carmen CHIFIRIUC - *Insights into the cultivable microbiota of Isverna cave guano deposits.*
- Robert F. OPRAN - *Solving the puzzle of the colonisation pattern of Mesoniscus graniger (Frivaldsky, 1865) in Romania using population genetics.*
- Robert RUGINESCU, Alina VASILESCU, Roberta BANCIU, Cristina PURCĂREA - *Cold-adapted Phototrophic Microorganisms from Scărișoara Ice Cave: Suitable Models for Developing Electrochemical Biosensors for Herbicides.*
- Ștefan SURUGIU, Georgiana Alexandra GRIGORE, Virgil DRĂGUȘIN, Irina GHEORGHE BARBU, Bogdan ISPAS, Viorel ATUDOREI, Ionuț PECETE, Mariana Carmen CHIFIRIUC - *A laboratory experiment of carbon stable isotope fractionation by several fungal species.*
- Maria Mirabela POP, Filip Paul BOANCĂ, Andrei ȘTEFAN, Sanda IEPURE - *Exploring the crustaceans world in the sulfidic mesothermal aquifer from Mangalia, Romania (Southern Dobrogea, Romania).*
- Alexandru Orest SAMBOR, Bianca ȘARCANI, Aurel PERȘOIU, Constantin MARIN, Ana I. CAMACHO, Karina P. BATTES, Mirela CÎMPEAN, Alin TUDORACHE, Sanda IEPURE - *The response of aquatic fauna to variable environmental conditions in Ghețarul de la Vârtop cave (Apuseni Natural Park, Romania).*
- Adrienn LŐRINCZ, Aritina HALIUC, Andrea DEMJÉN, Călin GHEMIȘ, Răzvan DUMBRAVĂ, Adriana VULPOI, Beatrice S. KELEMEN, Augustin NAE, Ioana RUSU, Viorel T. LASCU, Florin GOGĂLTAN, Bogdan P. ONAC, Ioana Nicoleta MELEG - *Inferring spatio-temporal palaeodynamics: from ancient biomolecules to organismal (overviews on cave sediment sequences).*
- George MIREA, Virgil DRĂGUȘIN, Bogdan ISPAS, Georgiana Alexandra GRIGORE, Ștefan VASILE, Ionuț MIREA, Tiberiu SAVA, Gabriela SAVA, Carmen Mariana CHIFIRIUC, Viorel ATUDOREI, Bogdan-Alexandru TORCĂRESCU, Cătălin LAZĂR - *Carbon and nitrogen stable isotope values of Late Pleistocene fauna from Stoieni Cave, Romania.*
- Bogdan ISPAS, Virgil DRĂGUȘIN, George MIREA, Georgiana Alexandra GRIGORE, Viorel ATUDOREI, Ștefan VASILE, Cătălin LAZĂR, Tiberiu SAVA, Gabriela SAVA, Carmen Mariana CHIFIRIUC - *Wolves and dogs – A stable isotopes analysis perspective.*
- Mihai CAMINSCHI, Virgil DRĂGUȘIN, Ștefan VASILE - *Identification of Vulpes species from La Adam Cave (Dobrogea) based on morphology and morphometry of the first lower molar.*
- Georgiana Alexandra GRIGORE, Virgil DRĂGUȘIN, Bogdan ISPAS, Viorel ATUDOREI, Mariana Carmen CHIFIRIUC - *Carbon and nitrogen stable isotope measurements of bulk organic matter in sediments from Obantul Mare paleolake, Mangalia, Romania.*
- Denisa IGESCU, Virgil DRAGUSIN, Sorin AVRAMESCU, Alina E. OLARU - *Polyaromatic hydrocarbons (PAHs) in a paleolake sediment core from eastern Romania.*
- Andra-Maria ILIE - *The morpho-osteometry of the mandibular ramus and lower dentition – Ursus spelaeus, Romanian Carpathians.*

ISSN 2972-1261

ISSN -L 2972-1261