Dietary shift of Late Pleistocene bears from the Romanian Carpathians as a response to climate variability (PN-III-P1-1.1-PD-2021-0262)

● **Phase 3 - Interdisciplinary analysis of cave deposits and data integration**

► During the final stage of the project (January -March 2024), we focused on integrating data from published research and the data generated by our project into a Geographic Information System (GIS) database. This involved combining and organizing all the relevant data into a single, accessible platform for further analysis and interpretation (*Fig. 1 and 2*).
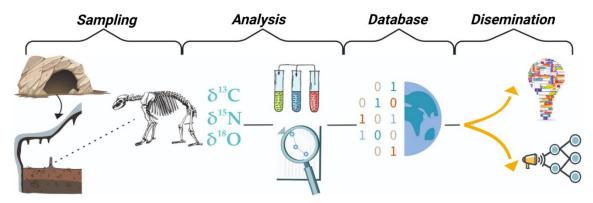

Figure 1. Workflow sketch of the project

► The PaleoTrace project, initiated by PD 95/2022, successfully accomplished its objectives (O1, O2, O3, and O4) within a two-year span (1 April 2022 to 31 March 2024). The project's activities encompassed ten tasks aligned with the four primary objectives. **The project's objectives were fulfilled 100%.** Throughout this period, the project conducted sampling activities of fossil remains and speleothems from various areas of the Romanian Carpathians and from paleontological collections. These samples, after being sent to laboratories, underwent analysis, leading to new findings concerning the chronology and diet of bears from the Romanian Carpathians (*Fig. 3*) and associated fauna from the Upper Pleistocene, the diagenesis of speleothems and the role of microbiological communities in caves on fossil remains (Table 1 and 2).
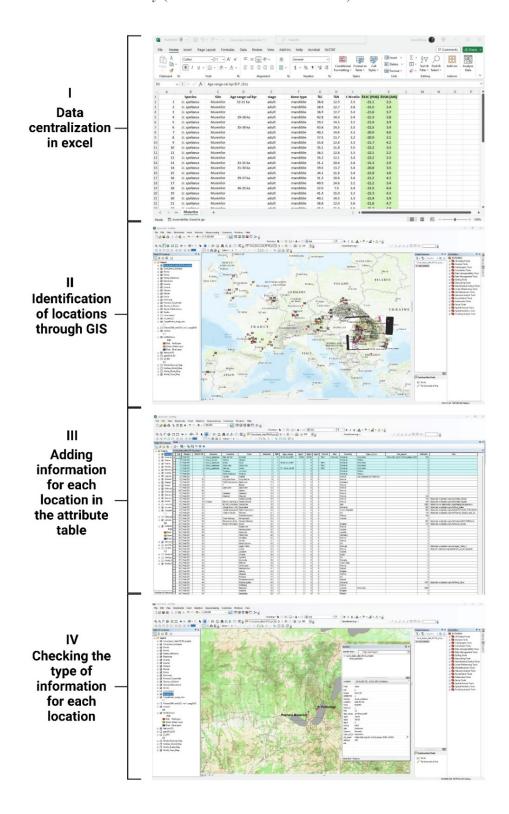
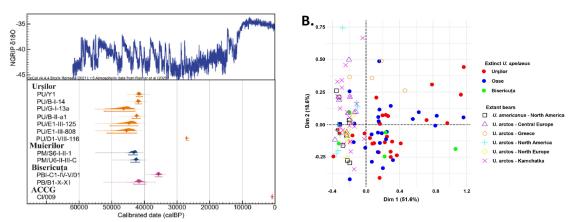**Figure 2.** Data integration in GIS database

Figure 3. A. Radiocarbon data from three bear population from Apuseni Mountains (Urșilor Cave – *Ursus spelaeus*), Parâng Mountains (Muierilor Cave-*Ursus spelaeus*) and Mehedinți Mountains (Cloșani area – *Ursus arctos*); **B.** Comparison of the microwear variables fossil populations of *U. spelaeus* from Ursilor, Oase and Bisericuța with the extant bears (Duno-Iglesias et al., 2024).

Research impact of the project

► The study's findings revealed the following new information: (i) *The chronological intervals for bears populations from Peștera Urșilor (Apuseni) - between ~27.1 and 46.5 cal ka BP (Marine Isotopic Stage 3) and between ~29.1 and 42.2 cal ka BP (Muierilor Cave - Southern Carpathians); (ii) The diet of the bears and their response to climatic variability in the Upper Pleistocene (Ursus spelaeus vs Ursus arctos) reflects the competition for resources in a changing environment; (iii) The processes involved in the preservation of fossil remains in caves, including the implications of diagenesis in collagen preservation; (iv) The diversity and role of microbiological communities in cave sediments on fossil remains and speleothems.*

Results

► The PaleoTrace project (PD 95/2022) analyzed approximately 300 fossil remains of *Ursus spelaeus* and *Ursus arctos* for stable isotopes (δ^{13}C and δ^{15}N) to determine their diet. An additional 200 samples were analyzed for δ^{18}O and δ^{13}C to capture seasonal feeding patterns and water intake in herbivores. Furthermore, around 100 bear lower molars were analyzed for tooth wear. To establish the chronology, 20 fossil remains were radiocarbon dated in Vilnius and Poznan laboratories. The results align with the project's work plan, fulfilling all proposed activities. The data were integrated into a GIS database, and the findings were disseminated through **three published scientific articles (ISI), five international conference** presentations, and three

submitted articles (under review). Two more articles are being prepared for submission. A project website (www.paleotrace.com) was created, and three training courses in paleontology, archaeozoology, and GIS (Hayonim Cave - Israel, Atapuerca - Spain, Kosice - Slovakia) were attended. The project also involved two volunteers from the University of Zagreb and University of Bucharest, and four collaborators in field activities, laboratory work, and dissemination. Additionally, two working visits were made to the Institute of Zoology in Chisinau (Rep. Moldova) and the National Museum of History in Sofia (Bulgaria) for future collaborations (Table 1 and 2).

► All scheduled tasks within the project have been successfully accomplished, meeting or surpassing the set targets. The extent of completion for each activity and its outcomes are elaborated in the tables provided below.

Table 1. Project tasks and level of accomplishment (April 2022-March 2024)

Indicators	Planned	Implemented	Percentage of achievement
Project website	YES	**Yes** https://www.paleotrace.com/	100%✓
Scientific reports	3	**3**	100%✓
Scientific papers	2 ISI papers	**3 ISI published, 3 in review and 2 in preparation.** Papers published in: ♦ *Geomicrobiology Journal* -I.F. 2.3 (2023) ♦ *Microbial Ecology*- I.F. 3.6 (2023) ♦ *Palaeogeography, Palaeoclimatology, Palaeoecology* – I.F. 3 (2024) Three papers in review: ♦ *2 papers in Quaternary Science Reviews -I.F. 4* ♦ *1 paper in Tetonophysics -I.F. 2.9* ♦ Two papers in preparation	100%✓
Conferences	2	**5**	100%✓
Work visits/ training courses	2	**2** (+3 from other sources) ♦ training course at Hayonim Cave, Israel (2022) ♦ training course at Atapuerca, Spania (2023) ♦ working visit at Chisinau, Rep. Moldova (2023) ♦ working visit Sofia, Bulgaria (2023) ♦ training course at -GIS- Kosice, Slovakia (2024)	100%✓
Volunteers	1	**2**	100%✓

Tabel 2. Project deliverables and activities (April 2022-March 2024)

Activities	Results
● *Phase 1 - Assessment of work methodology, gathering samples from field sites and/or paleontological collections*	
Tasks for Phase 1: Sampling; Stable isotope analyses and microwear analyses.	**Project website:** **https://www.paleotrace.com/**
	♦ **Conference 1:** **Ionuț-Cornel Mirea**, Marius Robu, Alexandru Petculescu, Marius Kenesz, Luchiana Faur, Răzvan Arghir, Vlad Codrea, Silviu Constantin. *Reconstructing the evolution of Muierilor cave system (Romanian Carpathians) during the last ~120 ka.* Interdisciplinary Archaeology: Methods, Studies, Results, 15-17 August 2022, Orheiul Vechi, Republic of Moldova.
	♦ **Conference 2:** **Ionuț-Cornel Mirea**, Marius Robu, Alexandru Petculescu, Luchiana Faur, Marius Kenesz, Laura Tîrlă, Silviu Constantin. *Late Pleistocene climate variability in the Southern Carpathians: Insights from Muierilor Cave (Romania).* Geographical Perspectives on Global Changes, 18-19 November 2022, Bucharest, Romania.

● Phase 2 - Interdisciplinary analysis of cave deposits *Tasks for Phase 2: Sampling fossil remains, radiocarbon dating and stable isotope analysis.*	♦ **Paper 1:** Cătălina Haidău, Paul Adrian Bulzu, **Ionuț Cornel Mirea,** Ruxandra Bucur & **Oana Teodora Moldovan** (2023). *Potential Environmental Drivers of Fossil Bones Degradation—A Metabarcoding Approach in Two Carpathian Caves.* Geomicrobiology Journal, 40:7, 654-666. https://doi.org/10.1080/01490451.2023.2227625 (F.I. 2.3).✓ ♦ **Paper 2:** Theodorescu, M., Bucur, R., Bulzu, PA., Faur, L., Levei EA., **Mirea I.-C.,** Cadar O., Lopes Ferreira R., Souza-Silva M. and **Moldovan OT** (2023). *Environmental Drivers of the Moonmilk Microbiome Diversity in Some Temperate and Tropical Caves.* Microbial Ecology (2023). https://doi.org/10.1007/s00248-023-02286-8 (F.I. 3.6).✓ ♦ **Conference 3:** Cătălina Haidău, **Ionuț-Cornel Mirea**, Luchiana Maria Faur, **Oana Teodora Moldovan**. *Cave sediment bacteria as possible proxy for paleoenvironments?* SedDNA Meeting, 06-09 June 2023, Potsdam, Germany (https://pastglobalchanges.org/calendar/129294). ✓ ♦ **Conference 4:** **Ionuț-Cornel Mirea**, Marius Robu, Alexandru Petculescu, Luchiana Faur, Silviu Constantin. *Deciphering climate variability by studying Late Pleistocene cave infillings. Case study: Muierilor Cave, Romania*. XXI INQUA Congress 2023, July 14th-20th 2023, Sapienza University of Rome, Italy (https://inquaroma2023.org/). ✓ ♦ **Conference 5:** Marius Robu, **Ionuț-Cornel Mirea**, Theodor Obadă, Vitalie Burlacu. *The taphonomy of the MIS 3 cave bear bone assemblages from several key sites of the Republic of Moldova.* Conferința Științifică - Istorie - Arheologie - Muzeologie - a Muzeului Național De Istorie a Moldovei (ediția a XXXIII-a), 26-27 octombrie 2023, Chișinău (Republica Moldova). ✓

● **Phase 3 - Interdisciplinary analysis of cave deposits and data integration**	
Tasks for Phase 3: Integrating the results from the project in GIS database	♦ **Paper 3 (Open Access):** Duno-Iglesias P., Ramirez-Pedraza I., Rivals F., **Mirea I.-C.,** Faur L.M., Constantin S., Robu M. (2024). *Palaeodiet during the pre-dormancy period of MIS 3 Romanian cave bears as inferred from dental microwear analysis.* Palaeogeography, Palaeoclimatology, Palaeoecology, 636, 1119888. https://doi.org/10.1016/j.palaeo.2023.111988. ✓ ♦ **Papers in review:** ● *Underrated ambush specialists: wolf predation on cave bears in Muierilor Cave, Romania* -**QSR** ●*Warm versus cold climate oscillations: evidence of MIS 3-2 flash flood events in the Carpathians*-**QSR** ●*From nappe stacking to strike-slip deformations: Alpine structural overprints in the Danubian thin-skinned units refined by cave geology (Isverna, Southern Carpathians)* -**Tectonophysics**

● **References**

♦ Catalina Haidău, Paul Adrian Bulzu, **Ionuț Cornel Mirea**, Ruxandra Bucur & O**ana Teodora Moldovan** (2023). *Potential Environmental Drivers of Fossil Bones Degradation—A Metabarcoding Approach in Two Carpathian Caves*. Geomicrobiology Journal, 40:7, 654-666, DOI: 10.1080/01490451.2023.2227625

♦ Duno-Iglesias P., Ramirez-Pedraza I., Rivals F., **Mirea I.-C.,** Faur L.M., Constantin S., Robu M. (2024). *Palaeodiet during the pre-dormancy period of MIS 3 Romanian cave bears as inferred from dental microwear analysis.* Palaeogeography, Palaeoclimatology, Palaeoecology, 636, 1119888. https://doi.org/10.1016/j.palaeo.2023.111988.

♦ Theodorescu, M., Bucur, R., Bulzu, PA., Faur, L., Levei EA., **Mirea I.-C.,** Cadar O., Lopes Ferreira R., Souza-Silva M., and **Moldovan OT** (2023). Environmental Drivers of the Moonmilk Microbiome Diversity in Some Temperate and Tropical Caves. Microbial Ecology (2023). https://doi.org/10.1007/s00248-023-02286-8