

1 **Fossil invertebrates records in cave sediments and**
2 **paleoenvironmental assessments. A study of four cave**
3 **sites from Romanian Carpathians**

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5 **Oana Teodora Moldovan^{1*}, Silviu Constantin^{2*}, Cristian Panaiotu³, Relu Dumitru**
6 **Roban³, Peter Frenzel⁴, Ladislav Miko⁵**

7
8 [1] (“Emil Racovitza” Institute of Speleology, Romanian Academy, Cluj-Napoca, Romania)

9 [2] (“Emil Racovitza” Institute of Speleology, Romanian Academy, Bucuresti, Romania)

10 [3] (University of Bucuresti, Faculty of Geology and Geophysics, Bucuresti, Romania)

11 [4] (Institut fuer Geowissenschaften, Friedrich-Schiller-Universität Jena, Jena, Germany)

12 [5] Faculty of Environmental Sciences, Czech University of Life Sciences, Prague,

13 Czech Republic

14 [6] Institute for Environmental Studies, Charles University, Prague, Czech Republic

15 * These authors contributed equally to this work.

16
17 Correspondence to: O.T. Moldovan (oanamol35@gmail.com)

18
19 **Abstract**

20 Fossil invertebrates from cave sediments have been recently described as a potential new
21 proxy for paleoenvironment and used in cross-correlations with alternate proxy records from
22 cave deposits. Here we present the results of a fossil invertebrates study in four caves from
23 two climatically different regions of the Romanian Carpathians, to complement
24 paleoenvironmental data previously reported. Oribatid mites and ostracods are the most
25 common invertebrates in the studied cave sediments. Some of the identified taxa are new to
26 science, and most of them are indicative for either warm/cold stages or dry/wetter oscillations.
27 In two caves the fossil invertebrates records indicate rapid climate oscillations during times
28 known for a relatively stable climate. By corroborating the fossil invertebrates’ record with
29 the information given by magnetic properties and sediment structures, complementary data on
30 past vegetation, temperatures, and hydraulic regimes could be gathered. This paper analyses
31 the potential of fossil invertebrate records as a paleoenvironmental proxy, potential problems

1 and pitfalls.

2

3 **1 Introduction**

4 Karst areas account for only c. 20% of the planet's ice-free land but they are already known as
5 repositories for well-dated, high-resolution paleoclimate and paleoenvironmental proxies such
6 as speleothems (Ford and Williams, 2007). In addition, cave sediments preserve geological,
7 environmental and biological information on the past that may allow paleoenvironmental and
8 paleoclimatic reconstructions (Bosák et al., 1989; Sasowsky and Mylroie, 2004). Clastic
9 sediments transported from the surface through the caves and sometimes intercalated with
10 chemical precipitates are frequently preserved unaltered for millions of years. They provide
11 climate and environmental proxies such as environmental magnetism, sedimentary structures
12 and stratigraphic indicators, and fossil remains and they may be directly or indirectly dated
13 (Bosák et al., 1989; Bosák et al., 2003; Sasowsky, 2007; Zupan Hajna et al., 2008). Specific
14 **microclimatic** features of the cave environments delay the degradation processes of fossil
15 remains (Sasowsky and Mylroie, 2004; Polk et al., 2007; Plotnick et al., 2015). It has been
16 shown (Willerslev et al., 2003; Haouchar et al., 2014; Epure et al., 2014, 2015) that cave
17 sediments provide a buffer environment where even ancient DNA can be preserved under
18 non-frozen conditions. Lack of light, stable environment and oligotrophy are typical for the
19 majority of the subterranean environments (low energy caves) and explain the low number of
20 permanent cave inhabitants, the low diversity of microorganisms and the slow biochemical
21 processes. Under optimal conditions, degradation processes may slow down thus ensuring the
22 preservation of fossil remains.

23 First investigations on fossil invertebrates from cave clastic sediments were undertaken in the
24 Classical Karst of Slovenia (Moldovan et al., 2011). They revealed that Pliocene/Pleistocene
25 invertebrate remains can be found in a relatively good state of preservation in cave sediments
26 and that they can be used for the assessment of paleoclimatic and paleoenvironmental
27 conditions of the past 2 Ma or even more. The next step to this approach would be to look
28 into how abrupt climate oscillations of the Quaternary may be reflected in the invertebrate
29 record from caves sediments and what is the potential of this new proxy. With this in mind,
30 we have investigated clastic sequences of four caves from the Carpathian Mountains of
31 Romania in an attempt to develop the use of cave fossil invertebrates and to complete the
32 scarce and fragmentary data on the paleoclimate and paleoenvironment of the region.

1 From a paleoclimate perspective, the Romanian Carpathians are an interesting region being
2 situated in the transitional zone between the oceanic climates of Western Europe and the arid
3 regions of interior Asia, and connecting the Alpine and the Balkan **mountain ranges** (Reuther
4 et al., 2007). The palaeoenvironmental history of the Carpathians responded to the relative
5 influence of the European continental, Atlantic and Mediterranean synoptic systems, as well
6 as to those of central Asia (Stevens et al., 2011).

7 Four caves from karst areas in western Romania were investigated for fossil invertebrates
8 (Fig. 1): Pestera Urşilor de la Chişcau (further abbreviated as Ursi), Pestera cu Apă din Valea
9 Leşului (Lesu), Pestera Ciur-Izbuc (Ciur) (north-western Carpathians), and Pestera Poleva
10 (Poleva, south-western Carpathians). The topography of all these caves includes a main
11 passage **formed by** small subterranean streams with fluvial sediments deposited as
12 underground terraces. **All caves are resurgences of sinking streams originating from surface**
13 **ponors**. Sediment samples were taken from the exposed faces, in places marked on Fig. 2. In
14 both regions, the climate broadly reflects a distal influence of the North-Atlantic Oscillation
15 (NAO) with mild summers and temperate winters. However, in the case of SW Carpathians
16 (Poleva), the current climate is also influenced by the Mediterranean, with more arid summers
17 and warmer winters than in the northwestern regions.

18

19 **2 Material and Methods**

20 As a general rule, split sediment samples were taken for the analysis of invertebrates and
21 rock-magnetic properties, respectively. Sediment structures were analysed in situ, and
22 granulometry analyses were done in the laboratory. The variation of rock-magnetic properties
23 within sediments was used as a proxy for climatic oscillations (Evans and Heller, 2003) with
24 chronological tie-points represented by direct optically stimulated luminescence (OSL) or
25 indirect U-series or radiocarbon datings. We further compared the rock-magnetic record and
26 sedimentological features with the known environment for the biological finds to check if the
27 latter may be used as an independent paleoenvironmental proxy.

28 **2.1 The caves**

29 Ursi is a famous repository of fossil remains of cave bears, hyenas and lions especially in its
30 lower, hydrologically active, level (Constantin et al., 2014). It is developed within

1 recrystallized Jurassic limestones from the foothills of Bihor Mountains (NW Romania) at
2 428 m.a.s.l. The lower level develops along a subterranean stream and includes alluvial
3 terraces atop of which lie cave bear and cave lion remains. Sediment samples were taken at 10
4 cm resolution from a 170 cm long profile (Figs. 1 and 2). The age controls of the profile
5 consists of radiocarbon ages of the fossil remains of a cave lion located atop a matching
6 terrace surface in the vicinity and one OSL dating of the alluvial sediments at c. 0.75 m
7 below.

8 Lesu is also located in the Pădurea Craiului Mountains, at c. 650 m.a.s.l. and it is carved in
9 Jurassic limestones. It is a sub-horizontal stream cave formed along an 800 m-long passage
10 that displays meanders and remnants of alluvial clay-sandy terraces. One profile of 170 cm in
11 length (Fig. 1) was sampled at a resolution of 10 cm from an alluvial terrace located at c. 200
12 m from the water emergence (Fig. 2). The age control of the profile consists of one OSL date
13 of the alluvial sediments at c. 0.6 m below the surface.

14 Ciur is located in the Pădurea Craiului Mountains of NW Romania at 530 m.a.s.l. and it is
15 carved in Triassic limestone along two levels. The upper, hydrologically inactive, level has a
16 total length of 650 m of a phreatic origin. Along the lower, hydrologically active level
17 remnants of alluvial terraces are well preserved. From one such profile, 150 cm-high, located
18 close to the stream emergence (Fig. 2), samples were taken every 10 cm (Fig. 1). The age
19 control of the profile consists of one OSL dating of the alluvial sediments at c. 0.4 m below
20 the surface.

21 Poleva is located in SW Romania, at c. 20 km north of the Danube Gorge (390 m.a.s.l.) and
22 was formed in massive Cretaceous reef limestones. The stream **in the cave passage** currently
23 flows through a small (1-1.5 m deep) canyon incised in rock or older **cave** sediments. A
24 flowstone sample that grew atop the rocky rim of the canyon and two stalagmites from the
25 alluvial terraces were taken for U-Th dating of the most recent phase of stream entrenching.
26 Sediment samples were collected from a 55 cm long profile of eroded alluvial deposits from
27 the cut-bank of an abandoned meander of the underground stream (Fig. 2). Here sediments are
28 capped by massive flowstone ca. 25 cm thick overlaid by thin **layers of silty clay** on top of
29 which stalagmites have grown (Fig. 1). The sediments were indirectly dated by using the U-
30 Th ages of speleothems from stratigraphically relevant positions.

31 **2.2 Sampling**

1 All sediment profiles were chosen so that they do not show any visible depositional hiatuses,
2 therefore we reasonably assume that they accumulated continuously under relatively constant
3 hydraulic conditions during extended time-periods of the Late Pleistocene and/or the
4 Holocene.

5 The sediments from the subterranean terraces were collected along vertical trenches created
6 by removing ~5 cm of the outer sediment layers, except for Poleva where only small amount
7 of sediments were available at different depths in the profile. Duplicate sediment samples
8 (typically 0.5 kg each) were taken for: (1) measurements of granulometry, geochemistry, and
9 rock-magnetic properties, and (2) screening of invertebrate fossil fauna, respectively.

10 A total of 59 samples for fossil invertebrates were analyzed. Approximately 1 kg of sediment
11 was taken from each sampling point and placed in sealed and labeled plastic bags. In the
12 laboratory, the samples were kept in 10% KOH for 30 min and washed successively through
13 sieves of 250 μm , 125 μm and 40 μm with filtered water. Sub-samples for each sieve
14 dimension were examined separately under an Olympus SZX2 stereomicroscope in 90°
15 alcohol and each specimen was identified under an Olympus BX51 microscope. Identification
16 of the species was carried out following the specific methods for each group.

17 **2.3 U-Th dating of speleothems**

18 The U-Th dates reported in this paper were done in the late '90s by alpha spectrometry
19 method, at that time the most widely used, at the U-series Geochronology Laboratory, Bergen
20 University, Norway. Sub-samples were cut from what seemed to be, optically, the most
21 suitable calcite (columnar or microcrystalline fabrics). The sub-samples were cut as closest as
22 possible to the speleothem base and as thin as possible in order to incorporate
23 correspondingly short stratigraphic intervals. However, the low uranium content required
24 quite large amount of calcite (typically 20-25 g); for flowstones sub-samples as thin as 5 mm
25 could be taken but for stalagmites they usually correspond to c. 1 cm axial extension.

26 Analytical procedures for U-Th alpha spectrometric dating are described by Lauritzen and
27 Onac (1999). Detrital ^{230}Th contamination was monitored using the $^{230}\text{Th}/^{232}\text{Th}$ index and
28 corrected using an initial $^{230}\text{Th}/^{232}\text{Th}$ value of 1.5 (Schwarcz, 1986).

29 Although alpha-spectrometric U-Th dating method is largely considered obsolete nowadays,
30 we consider that the age controls it offers are sufficiently adequate for the purpose of this
31 study. First, the speleothems interbedded within or lying on top of alluvial sediment sequences

1 are notoriously “dirty”. Alpha spectrometry allows for more robust chemical separation
2 procedures to deal with both detrital ^{230}Th and contaminants such as phosphate that may
3 affect chemical yields and dating reliability. Second, the dates are not used to construct a
4 high-resolution time-series (as for typical speleothem records) but to provide broad
5 chronological controls. Finally, in the absence of organic materials that could be radiocarbon
6 dated (or beyond radiocarbon range), the typical dating uncertainties of ~10% are comparable
7 or better than those of the OSL dates.

8 In case of Poleva we have used a combination of several U-Th dates to infer the age of the
9 sediment stack (Table 1). The age of the sediment is considered to be older than c. 110 ka,
10 which is the age of PP97-3 flowstone (see also Fig. 1). Subsequent floodings in the cave have
11 deposited finer layers of sediments, such as those overlying the PP97-3 flowstone. Their
12 timing is not well constrained but it is believed to be anywhere younger than c. 32 ka which is
13 the maximum age of stalagmite PP98-11. Other indirect age controls are represented by
14 several U-Th dated stalagmites and flowstone from the matching terraces surfaces in the
15 vicinity of the profile. In a nearby suspended meander, stalagmite PP98-10 started to grow at
16 c. 62 ka being subsequently covered by a clay deposit at some time before c. 42 ka
17 (Constantin, 2003). A flowstone collected from the rim of the small underground canyon
18 (PP97-4) yielded a corrected age of c. 37.7 (+/- 4.5) ka indicating a minimal age for canyon
19 incision. Two stalagmites (PP99-10 and 11) were dated from the terraces at c. 1.2 m above the
20 current stream bed and yielded reliable and consistent ages of c. 15 ka.

21 **2.4 Radiocarbon datings**

22 The AMS ^{14}C dating of two samples from Ursi were dated at the Oxford Radiocarbon
23 Accelerator Unit (ORAU) and reported by Stuart and Lister (2011).

24 **2.5 OSL datings**

25 Sediment samples for OSL datings were collected by hammering 25 cm-long, opaque, plastic
26 tubes (20 cm) into the sediment sections under no light. Sample preparation for luminescence
27 measurements was performed under low intensity red light conditions. Only the central
28 portion of the tubes was removed for dating. A three-day treatment with 10% HCl solution
29 was used for carbonates removal, followed by another three-day H_2O_2 (30%) treatment for
30 organic matter removal. The coarse grain fractions (>63-90 μm) were separated through wet

1 sieving. For the extraction of the fine-grained quartz fraction (4-11 μm), the particles smaller
2 than 11 μm were isolated from the fraction less than 63 μm by settling in Atterberg cylinders,
3 according to the Stokes law. This fraction was treated with hexafluorosilicic acid (H_2SiF_6) for
4 10 days. Subsequently, the removal of the grains $<4 \mu\text{m}$ was carried out by centrifugation.

5 For the annual dose determination, radionuclide specific activities (U-238/Ra-226, Th-232, K-
6 40) were measured using high resolution gamma-ray spectrometry, and the dose rates were
7 calculated using the conversion factors tabulated by Adamiec and Aitken (1998). Equivalent
8 dose (De) measurements were undertaken on the standard Risø TL/OSL-DA-20 reader,
9 equipped with blue LEDs ($470 \pm 30 \text{ nm}$). IR ($875 \pm 80 \text{ nm}$) LEDs were used for infrared
10 stimulation. Blue light stimulated OSL signal was detected through a 7.5 mm thick Hoya U-
11 340 UV filter. Irradiations were carried out using the incorporated ^{90}Sr - ^{90}Y radioactive source,
12 calibrated against gamma dosed calibration quartz supplied by the Risø National Laboratory,
13 Denmark. A dose rate of 0.123 Gy/s for the fine grains mounted on aluminium disks was
14 obtained at the time of measurement.

15 The measurement protocol was the Single Aliquot Regeneration (SAR) applied on quartz
16 (Murray and Wintle, 2003; Wintle and Murray, 2006). The OSL dating was done at the
17 Dating and Luminescence Dosimetry Laboratory, Babes-Bolyai University, Cluj-Napoca,
18 Romania.

19 **2.6 Grain size analysis**

20 Grain size measurements on fine samples were performed by treating $\sim 5 \text{ g}$ of the bulk sample,
21 for 14 days, in a plastic box, with $\sim 0.4 \text{ ml}$ of a 1% solution of $\text{Na}(\text{PO}_3)_n$, $n \approx 25$ - Graham's
22 salt (Merck). A quantity of $\sim 2.5 \text{ g}$ sample was later extracted from the box and treated again
23 with $\sim 0.2 \text{ ml}$ of 2% solution of Graham's salt. Each sample was analyzed on a HORIBA
24 Partica LA-950V2 laser scattering particle size distribution analyzer to reveal grain size
25 fractions. The coarser samples were analyzed by vibrating dry sieving of $\sim 100 \text{ g}$ of the bulk
26 sample and weighing the sediment quantity retained on each sieve, on an OHAUS Scout
27 digital balance, down to the 500 μm fraction, which was subjected to the same procedure as
28 the fine samples.

29 Calculations and plots were done using the GRADISTAT 8 software; we applied the method
30 of Folk and Ward (1957), and logarithmic statistics.

1 The four caves have been sedimentologically analyzed in terms of thickness, grain size and
2 internal structures of depositional units. Grain size analysis was done macroscopically for the
3 fraction >2 mm and with a HORIBA laser machine for the fraction <2 mm. The granulometric
4 scale uses the typical ranges as follows: gravel > 2 mm, sand (2-0.063 mm), silt (0.063-0.002
5 mm) and clay <0.002 mm. Grain size parameters such as *standard deviations* and *medians*
6 were calculated and plotted for environmental interpretations.

7 **2.7 Rockmagnetism**

8 For rockmagnetism and paleomagnetism, samples were collected in plastic cylinders (11 cm³)
9 specially designed to avoid the rotation of the sample during the sampling or measurements.
10 The moist sediment allowed us to press the cylinders into the clean face of the outcrop.
11 Sampling interval was between 5 cm and 10 cm. The cylinders were then excavated, capped
12 and packaged to avoid the loss of humidity during the transport. In the laboratory the samples
13 were kept in a refrigerator between measurements, to preserve as much as possible the
14 original humidity. Additional samples from the same locations were collected in plastic bags
15 for granulometry and the stratigraphy was documented *in situ*.

16 In laboratory, the following rockmagnetic measurements were performed for all samples:
17 frequency dependence of magnetic susceptibility, anhysteretic remanent magnetization,
18 isothermal remanent magnetization acquired in a magnetic field of 2 T (IRM_{2T}) and the
19 remaining isothermal remanent magnetization after applying a back field of 0.3 T (IRM_{0.3T}).
20 Magnetic susceptibility (mass-specific) was measured using the AGICO MFK1-FA Multi-
21 function Kappabridge at two frequencies of 976Hz (low frequency - lf) and 3904 Hz (high
22 frequency - hf). The corresponding values are referred to χ_{lf} and χ_{hf} , respectively. The
23 frequency-dependent susceptibility was $fd = (\chi_{lf} - \chi_{hf})$, which is proportional to the
24 concentration of the viscous superparamagnetic (SP) particles (Worm, 1998). Anhysteretic
25 remanent magnetization (ARM) was imparted using an anhysteretic magnetizer AMU-1A
26 (AGICO) coupled with the LDA-3A demagnetizer in an alternating field of 100 mT with a
27 superimposed 50 μ T bias field, and was then expressed by ARM susceptibility (χ_{ARM}). The
28 ARM is particularly sensitive to the content of stable single-domain (typically larger than
29 20–25 nm, but less than ~100 nm for magnetite) ferrimagnets (Dunlop and Özdemir, 1997).
30 The isothermal remanent magnetizations were imparted using a pulse magnetizer MMPM10
31 (magnetic Measurements). Based on these isothermal remanent magnetizations S-ratio was

1 calculated for 2T magnetizing fields (IRM_{2T}) and 0.3 T backfield ($IRM_{0.3T}$), following the
2 procedures used by Bloemendal et al. (1988) (Eqn.1):

$$3 \quad S = 0.5 \left(1 - \frac{IRM_{0.3T}}{IRM_{2T}} \right) \quad (1)$$

4 S values close to 1 show that the dominant presence of low coercivity minerals (magnetite
5 and/or maghemite) and lower values indicate the presence of high coercivity minerals
6 (goethite and/or hematite). All remanent magnetization were measured using JR5
7 magnetometer. To identify the high coercivity minerals in the presence of more magnetic low
8 coercivity minerals selected specimens from each section were subjected to high field IRM
9 acquisition curves (between 0.3 T and 7 T). We applied the protocol of Maher et al. (2004):
10 the specimen packed in caps gel (~ 0.6 g) was first magnetized using the MMPM10 pulse
11 magnetizer, then the sample was AF demagnetized in 100 mT and the remaining remanence
12 was measured.

13

14 **3 Results**

15 **3.1 Age controls**

16 Although age control is crucial in paleoclimate/paleoenvironment interpretation, cave
17 sediments are difficult to date at high-resolution. Our approach was to compare the relative
18 changes in rockmagnetic properties with the faunal ones and use available dating methods to
19 broadly assess the corresponding Pleistocene time-periods of their deposition.

20 In the case of Ursi, the age of the sediments is constrained by a radiocarbon date of a cave
21 lion skeleton on top of the sediment and was assessed to roughly correspond to Marine
22 Isotope Stages (MIS) 3-5c based on one OSL date at a depth of 75 cm. Additional proof for
23 this age was obtained by a combination of OSL dating on correlated terraces and overlying U-
24 Th dating flowstone as shown by Constantin et al. (2014). For Ciur and Lesu, the age of the
25 depositional events were assessed using one OSL dating for each profile, to Late Holocene
26 and MIS 5a-5c, respectively. In Poleva, the sediments are considered to be older than c. 110
27 ka and to correspond, most probably to the Eemian. This assumption is sustained by U-Th
28 datings of several other speleothems grown atop correlated terrace sediments from the same
29 cave.

1 Under these circumstances, the age controls are only rough estimates of the depositional
2 periods. A synthesis of these estimates is presented in Table 2.

3 **3.2 Sedimentological analysis**

4 The thickness of sediment stacks varies between 70 cm for Poleva and 150-170 cm for Ciur,
5 Ursi and Lesu (Figs. 1 and 3). All sediments show parallel lamination (Fig. 3). Both Ursi and
6 Lesu are formed by alternating silts and clays, with Lesu showing several centimetre levels of
7 fine sand in the middle part. The Ciur and Poleva profiles are formed of coarser sediments
8 with fine sand being dominant. The Ciur profile starts with an intercalation of silt and sand
9 showing cross laminations with loose gravel levels at c. 50 cm from base. In Poleva, the
10 profile includes medium-sized sand in the first part (c. 30 cm) followed by fine sand towards
11 the top. These are covered by a c. 5 cm-thick layer of flowstone that marks the end of
12 sediment deposition.

13 The plot of the clasticity index (C) (the diameter of the bigger clast) with respect to Median
14 (Md) is relevant for the hydrodynamics of the streams and indicates a transport through
15 intermittent suspension (Suspension I) for Ursi, Lesu and part of Ciur sediments. In the latter
16 case, part of the clasts was also transported by saltation (Fig. 4a).

17 The plot of the Standard deviation (S_0) versus Median (Md) (Fig. 4b) is indicative for the
18 depositional conditions. The Ursi profile shows a combination of slackwater and backswamp
19 facies, and the terrace sediments were deposited at high water levels, from a low-energy
20 stream. In Lesu and especially in Ciur the sediments were deposited from higher energy
21 waters and channel facieses are present. No samples were available for Poleva, but the field
22 observation indicates a channel facies in this case too.

23 **3.3 Rockmagnetism**

24 In all measured sediments from the Ursi, Leşu and Ciur the ferromagnetic minerals are a
25 combination of low **coercivity** minerals (magnetite and/or maghemite) and high **coercivity**
26 minerals (goethite or hematite) as it is indicated by most of the values of S ration (Fig. 3).
27 High field isothermal remanent magnetization curves acquisition curves (between 0.3 T and 7
28 T) showed that the high coercivity mineral is hematite in case of the Urşi and Leşu and
29 goethite in the Ciur. Despite this complex mineralogy, the oscillations of the magnetic

1 susceptibility are mainly controlled by input of single domain and superparamagnetic grains
2 of low coercivity minerals.

3 **3.4 Fossil invertebrates in cave sediments**

4 The following terrestrial and aquatic fauna groups were identified in the sediments of the four
5 studied caves: Bivalvia, Gastropoda, Ostracoda, Cladocera, Oribatidae and Insecta (mainly
6 Collembola) (Fig. 5). All taxa were introduced into the caves together with surface sediments.
7 They are in relatively good state of preservation allowing their identification at species or
8 genus level for most of the specimens (Fig. 6).

9 There were differences between caves both in number of fauna groups and dominant group.
10 In Ursi and Poleva the number of both groups and individuals was very low, while in the
11 other two caves the number of groups (6 in Ciur) and individuals (131 ostracod valves in
12 Lesu) was higher. Most of the identified species belong to oribatid mites (Acarina, Oribatida)
13 and ostracods.

14 One individual of *Zygoribatula frisiae* (Oudemans, 1916) was identified in Ursi, near the base
15 of the profile (Fig. 3). In Ciur, oribatid mites were constantly present along the profile, even
16 though in small number (Fig. 3). The mites were found only in the silt levels deposited under
17 slow energy flow or stagnant water episodes. One individual belonging to *Oppiella* (Jacot,
18 1937) was identified in the sediments of Ciur, together with representatives of *Hypogeoppia*
19 Subias, 1981, *Quadroppia* Jacot, 1939 and *Dissorhina ornata* (Oudemans, 1900).

20 *Hypogeoppia* was found in several layers. This is a new species close to *Hypogeoppia*
21 *belgicae* Wauthy and Ducarme, 2006 described from Belgian caves. The identified
22 *Quadroppia*, similar to *Q. quadricarinata* (Michael, 1885), is a Holarctic distributed genus.
23 One *Hypogeoppia* sp. and one collembolan *Entomobrya* sp. Rondani, 1861 were identified in
24 Poleva sediments (Fig. 3).

25 Aquatic representatives were identified in Ciur and Lesu. In Ciur, there were two layers
26 containing only aquatic representatives. At -30 cm four individuals of the ostracod
27 *Cyclocypris* sp. Brady and Norman, 1889 and at -60 cm one individual of the cladoceran
28 *Alona guttata* G.O. Sars, 1862 were found. Lesu was dominated by the ostracods,
29 *Cavernocypris subterranea* (Wolf, 1920) and *Fabaeformiscandona latens?* (Klie, 1940) (Fig.
30 5). None of these two species was identified in present-day cave fauna. Most ostracods were
31 single valves, with only 13 entire individuals (Fig. 7). The ratio between juvenile and adults is
32 high, of 89.3% (Fig. 7).

1

2 **4 Discussion**

3 Direct dating of invertebrate fauna from cave sediments is impossible due to its smallness and
4 scarcity. On the other hand, there are only a few absolute dating methods that may be applied
5 to such sediments, such as OSL, radiocarbon (for fossil remains), or U-Th (for speleothems),
6 each having their limitations. One method that may be routinely used in order to generate
7 records that may be interpreted paleoclimatically is the measurement of rockmagnetic
8 properties of sediments.

9 Recent soil and paleosols from the loess deposits in surrounding areas of the Carpathians
10 Mountains have shown a strong enhancement of the magnetic susceptibility due to the
11 production of superparamagnetic and single domain magnetite and/or maghemite during
12 pedogenesis (e.g. Necula et al., 2013; Buggle et al., 2014). Taking this into account we
13 interpret that the presence of superparamagnetic grains indicates the transport of soils in the
14 studied caves by underground rivers (Ellwood et al., 2001). The frequency dependence and
15 the amplitude of magnetic susceptibility suggest stronger pedogenesis outside the cave for
16 Ursi and Ciur than in the case of Leșu. This is consistent with the MIS 5b age tentatively
17 assigned to the Lesu profile, and with our interpretation of the paleoenvironmental conditions
18 as derived from the faunal spectra in this cave (see below).

19 *Zygoribatula frisiae* found in Ursi is living today in more arid settings (Shepherd et al., 2002).
20 This mite is xero-tolerant, living in drying-out mosses and lichens and often in arboricole
21 microhabitats. The presence of the xero-tolerant *Z. frisiae* at -140 cm in the studied profile is
22 indicative for the deposition of the sediments in one of the MIS5 inter-stadials, at least for the
23 lower part of the profile (Fig. 8). This is further supported by the backswamp-type
24 depositional facies that include silts and laminated clays pointing towards a deposition during
25 a climatic optimum (Constantin et al., 2014), and explained by the strong pedogenesis outside
26 the cave also apparent in the magnetic susceptibility profile.

27 *Hypogeoppia* representatives are present at different levels in the Ciur sediment profile (at -20
28 cm, -40 cm, 50 cm, -70 cm, and -140 cm), alone or in association with *Quadroppia* sp. This
29 last taxon is indicative for a forest habitat (Seniczak et al., 2006) above the cave, while
30 *Hypogeoppia* species are euedaphic found in moist nutrient-poor soils of grasslands and
31 forests (Subías and Rodríguez, 1987; Siepel and Dimmers, 2010) but also in extant cave fauna

1 (Wauthy and Ducarme, 2006). *Oppiella* sp. is a common genus with broad range, including
2 both more specialized species as well as euryoecous ones, so the interpretation remains
3 difficult. The other taxon, *Dissorhina ornata*, that appear together with *Oppiella* sp. only in
4 the upper part of the profile is also broadly distributed, but abundant in more open habitats,
5 which might be associated with the flooding events during that time period and may explain
6 the changes of species from one level to another. Flooding events in the upper part of the
7 profile are also supported by the presence of *Cyclocypris* sp. that appears in a single level
8 with gravels at -30 cm and could correspond to a rapid change of vegetation on the surface.
9 *Dissorhina ornata* is found at the border between forest and open areas (Seniczak et al., 2006)
10 and species of the genus *Opiella* (sensu lato) can be considered one of the most common
11 arthropod groups on Earth (Norton and Palmer, 1991), with high diversity and abundance in
12 forest litter, also present in shrublands, ecotone zones and grasslands. Some authors (Lotter et
13 al., 1997; Taylor and Wolters, 2005) mentioned the tolerance of the genus *Dissorhina* to
14 drought. *Alona guttata* found in the middle part of the profile (-60 cm) is generally associated
15 with benthic, warmer conditions, with increasing density of vegetation, and more acidic
16 waters (Szeroczyńska and Sarmaja-Korjonen, 2007; Nováková et al., 2013). The profile
17 includes, thus, different communities indicating different environmental conditions at surface.
18 The *Hypogeoppia* sp. from lowest part of the sediments indicate the presence of moist
19 forrested/grassland areas. The middle part of the sediment layers suggest a warm climate,
20 while the upper part is indicative for a mixture of open and forrested habitats.
21 The middle to low part of the profile in Ciur is associated with coarser sediments indicating a
22 high-energy hydraulic regime and frequent flooding episodes. Within the middle and upper
23 parts of the profile, the low values of magnetic susceptibility and minor frequency
24 dependence suggest that the soils were not significantly eroded and transported in the cave.
25 The estimated age (Late Holocene) explains the strong pedogenesis suggested by magnetic
26 measurements. Pollen data in the region (Feurdean et al., 2013) document the onset of a rise
27 in diversity and large-scale forest clearance (with *Fagus sylvatica* and *Abies alba* recording
28 the greatest decline), burning, pastoral activities and arable farming at lower elevation in the
29 same period. The invertebrates in Ciur are documenting fast changes and alternation of forest
30 and more open habitats at the surface. The magnetic susceptibility variations do not support
31 the hypothesis of significant changes in temperature; however, the changes in fossil
32 invertebrates fauna suggest that vegetation changed at ~ 2000 years ago. The sudden changes
33 in fauna communities and the massive input of sediments may be due to floodings during the

1 Subatlantic, a period characterized by a climatic cooling and rainfall increase.

2 The aquatic species in Lesu are different in their ecological requirements and indicate the
3 deposition of sediments in a broadly cold period, with variations towards even lower
4 temperatures when *Cavernocypris subterranea* was dominant. *C. subterranea* prefers springs
5 and is coldstenothermal, polyrheophilic, polytitanophilic, stygophilic, while
6 *Fabaeformiscandona latens* prefers groundwater and is oligothermophilic, mesorheophilic,
7 oligotitanophilic, stygobitic (Meisch, 2000). The presence of only a few entire individuals and
8 the high juveniles/adults ratio suggest that the species were not typical cave dwellers but they
9 were transported from surface. We estimated that sediments were deposited during the MIS
10 5b (Fig. 8). This was a period of significant changes in climate, including northward
11 expansion of grassland and dry shrubland in Eurasia (Herold et al., 2012). The low values of
12 both magnetic susceptibility and its frequency dependence come into agreement with the
13 deposition of sediments during a cold period when the pedogenesis was probably less intense
14 outside the cave.

15 The identified fossil invertebrates in Poleva belong to soil or grassland/forest litter fauna and
16 their ecology indicate an above-cave environment dominated by moist deciduous forest. The
17 *Hypogeoppia* species that was found in this cave is the same as the one found in Ciur,
18 suggesting that the vegetation at surface was similar during the sediment deposition in both
19 caves (i.e. during the Late Holocene and Eemian, respectively). All identified fossil elements
20 are describing a situation similar to the present, of a sub-Mediterranean forest, with dry and
21 wet elements. This comes into agreement with the assigned Eemian age, an interstadial that is
22 considered to closely resemble the current climate. The beginning of the Eemian is identified
23 in the vegetational sequence by a simultaneous drop in steppic elements and a rise in
24 Eurosiberian and Mediterranean trees (Shackleton et al., 2003). It is also interesting that the
25 same species was identified in sediment stacks from caves located in different topoclimatic
26 regions of the Carpathians, corresponding to different, yet similarly warm major climatic
27 epochs.

28

29 **5 Conclusions**

30 Although invertebrate fossils from cave sediments cannot be designated as an ideal biological
31 proxy, as shown by Elias (2007), the study of fossil invertebrates in cave sediments from the
32 four Carpathian caves indicates their potential as environmental indicators (Table 3) for:

- 1 (i) rapid pluviometric/hydrological oscillations along relatively short periods during the
2 Subatlantic and the Late Pleistocene, sometimes accompanied by floodings, as suggested in
3 Ciur by the alternation of terrestrial and aquatic species;
- 4 (ii) rapid changes of the vegetation/temperature at the surface, with the alternance of forests
5 and more open habitats as in Ciur, or with short cold episodes during a stadial as in the case
6 of Lesu;
- 7 (iii) deposition of sediments in caves during both warm (Ursi) and cold (Lesu) stages of the
8 Late Pleistocene, with no apparent relationship to the altitudinal position of the cave, which
9 points to the importance of local conditions;
- 10 (iv) the different hydraulic regimes indicated by the presence of mites during slow flow or
11 stagnant water, and the presence of cladocerans and ostracods during faster flow.

12 Two of the most abundant fauna representatives, ostracods and mites, were identified in caves
13 from both Carpathians and Dinarids (Moldovan et al., 2011). While ostracods are more often
14 used in paleoenvironmental research, only a few authors advocated the use of mites in such
15 studies (e.g. Solhøy and Solhøy, 2000; Moldovan et al., 2011). Owing to their hard cover
16 (exoskeleton, valves), the preservation of both groups is generally good even in pre-Holocene
17 cave sediments and both are systematically diversified, with various ecological requirements
18 to allow for paleoclimatic or paleoenvironmental assessments.

19 The presence of common species, such as *Hypogeoppia* sp., both at different periods and
20 different Carpathian locations (Ciur and Poleva), suggests similar surface ecosystems and
21 points on the need for a regional vs. local approach. The identification of similar taxa in a
22 larger region, such as the Carpathians, and at different periods also emphasize the possible
23 designation of some species as regional indicator for vegetation across a larger time scale.

24

25 **6 Perspectives**

26 Caves sediments and their invertebrates may often be older than many other continental
27 deposits, and are usually found in a relatively good state of preservation. The oldest cave
28 invertebrate fossils found so far date from Carboniferous (Plotnick et al., 2009) and Middle
29 Pliocene (Moldovan et al., 2011; Miko et al., 2012, 2013). Some of the clastic sediments
30 cover long time intervals, thus potentially archiving valuable continuum history of the
31 environment and karst evolution at the surface. Caves are numerous in one area and at all
32 latitudes and many have sediments that may include invertebrate species and provide

1 paleoenvironmental information to complement or cross-validate other paleoclimatic records,
2 at least at regional scale.

3 The greatest potential of the cave sediments in paleoenvironmental studies is given by the fact
4 that multiple proxies can be extracted from them even if, in this case, the multi-proxy
5 approach is rather a theoretical than statistical inference. To counterbalance the low density of
6 fossil invertebrates in caves' clastic sediments there are few rules that may enhance the
7 probability of findings: (a) Sedimentary profiles should be long enough to have been
8 deposited in different climatic situations; (b) Sediments should be fine-grained, from silty-
9 sandy to clay, the best to preserve the fossil invertebrates, owing to the anoxic conditions that
10 block the development of microorganisms involved in biodegradation; (c) The sediments
11 should have been deposited by fluvial flow with surface origin or subterranean lakes fed by
12 streams with surface origin since most taxa we found were of surface origin.

13

14 **Authors contributions**

15 O.T.M. designed the research and performed the sampling. C.P. and R.D.R. performed the
16 sedimentological and rockmagnetic measurements. P.F. and L.M. identified the ostracods and
17 mites, respectively. **All authors** contributed to data interpretation. O.T.M. and S.C. wrote the
18 manuscript.

19

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29

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- 1 Table 1. Alpha-spectrometry U-series dating results from Poleva; all ratios are activity ratios,
 2 and all uncertainties are 1σ (only ages in bold were considered in this study).

Lab. no.	Sample name/U conc. (ppm)	$^{234}\text{U}/^{238}\text{U}$	$^{230}\text{Th}/^{234}\text{U}$	$^{230}\text{Th}/^{232}\text{Th}$	Calculated age (ka)	Corrected age (ka)
1810	PP 97-3	0.102	1.26 ± 0.04	0.70 ± 0.03	121.9	109.9
	Base 1.5 cm	± 0.003			(+9.14; -8.5)	(+9.7; -9.1)
2169	PP 98-11/1	0.09	1.13 ± 0.05	0.26 ± 0.02	32.5 ± 3	<32.5\pm 3
	Base 1 cm	± 0.003				
1805	PP97-4, base	0.076	1.25 ± 0.07	0.33 ± 0.03	42.38	37.7
		± 0.004			(+4.2; -4.1)	(+4.6; -4.5)
2309	PP99-11/1	0.26	1.452	0.15	13.2	15.4\pm1.4
	base 1 cm	± 0.01	± 0.07	± 0.01		
2348	PP99-10	0.035	1.05	0.13	>1000	14.5\pm2.9
	base	± 0.002	± 0.1	± 0.02		

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4

1 Table 2. Age controls of the studied profile in the four Carpathian caves and inferred Marine
 2 Isotope Stages (MIS) of their deposition

Cave	Depth (cm)	¹⁴ C (cal ka BP)	U-Th (ka)	OSL (ka)	Derived age of sediments (ka)	Inferred climatic period
Ursi	0	42.03 (± 1.52; -0.8)*			41 - 43	MIS 3 to MIS 5a
	- 75			75.0 ± 7.2***	68-83	
Ciur	- 50			2.29 ± 0.26	2 - 3	SubAtlantic
Lesu	- 60			88.1 ± 12.5****	80 - 111	MIS 5b
Poleva	overlying flowstone		109.9 (+9.7; -9.1) **		>110	Eemian

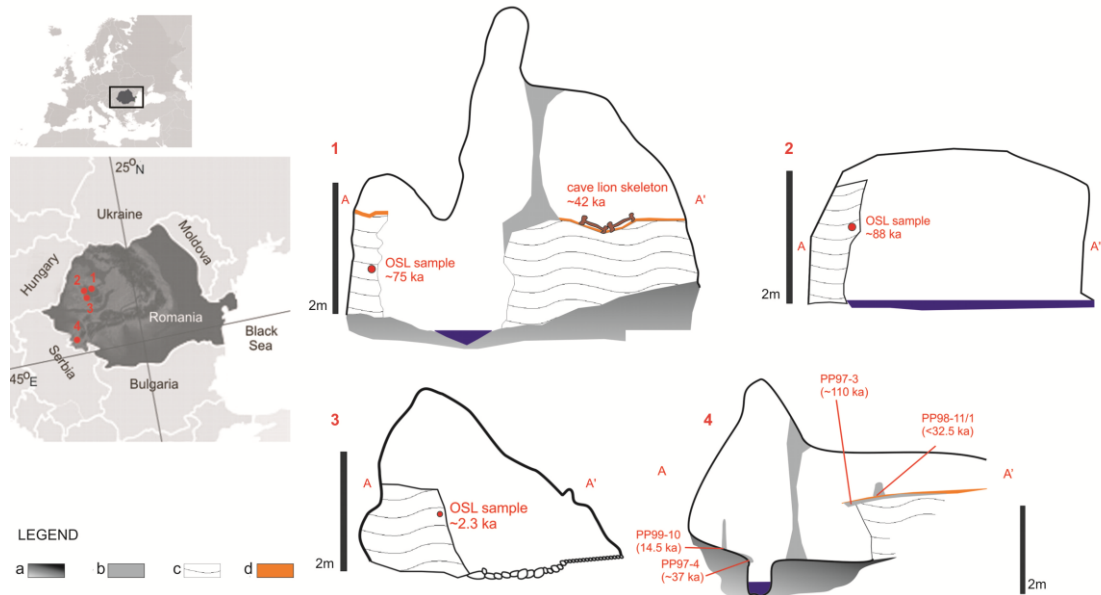
3 * from Stuart and Lister (2011), ** from Constantin (2003), ***from Constantin et al. (2014), ****from Epure
 4 et al. (2015)

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1 Table 3. Taxa found in cave sediments of Romanian caves with the corresponding vegetation,
 2 sediment type and origin.

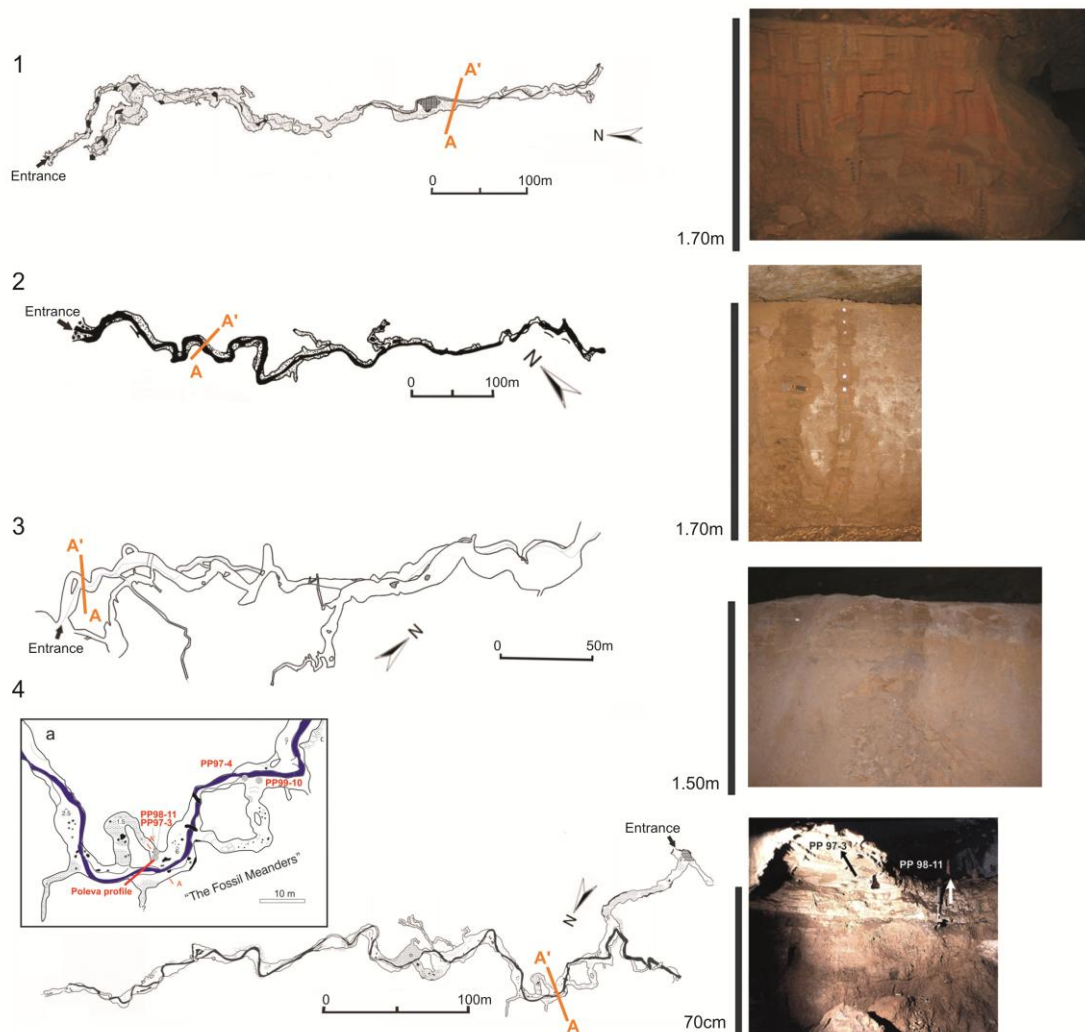
Stage	TAXA				Environment
	Ursi	Ciur	Lesu	Poleva	
SubAtlantic		<i>Oppiella</i> sp.			open habitats
		<i>Dissorhina ornata</i>			
		<i>Cyclocypris</i> sp.			
		<i>Hypogeoppia</i> sp.			moist
		<i>Quadroppia</i> sp.			forest/grassland
		<i>Alona guttata</i>			warm, dense vegetation
MIS 3 –	<i>Zygoribatula</i>				arid habitats
MIS 5a	<i>frisiae</i>				with trees
MIS 5b			<i>Fabaeformiscandona</i>		cold waters
			<i>latens</i>		
			<i>Cavernocypris</i>		colder waters
Eemian				<i>Hypogeoppia</i>	moist
				sp.	forest/grassland

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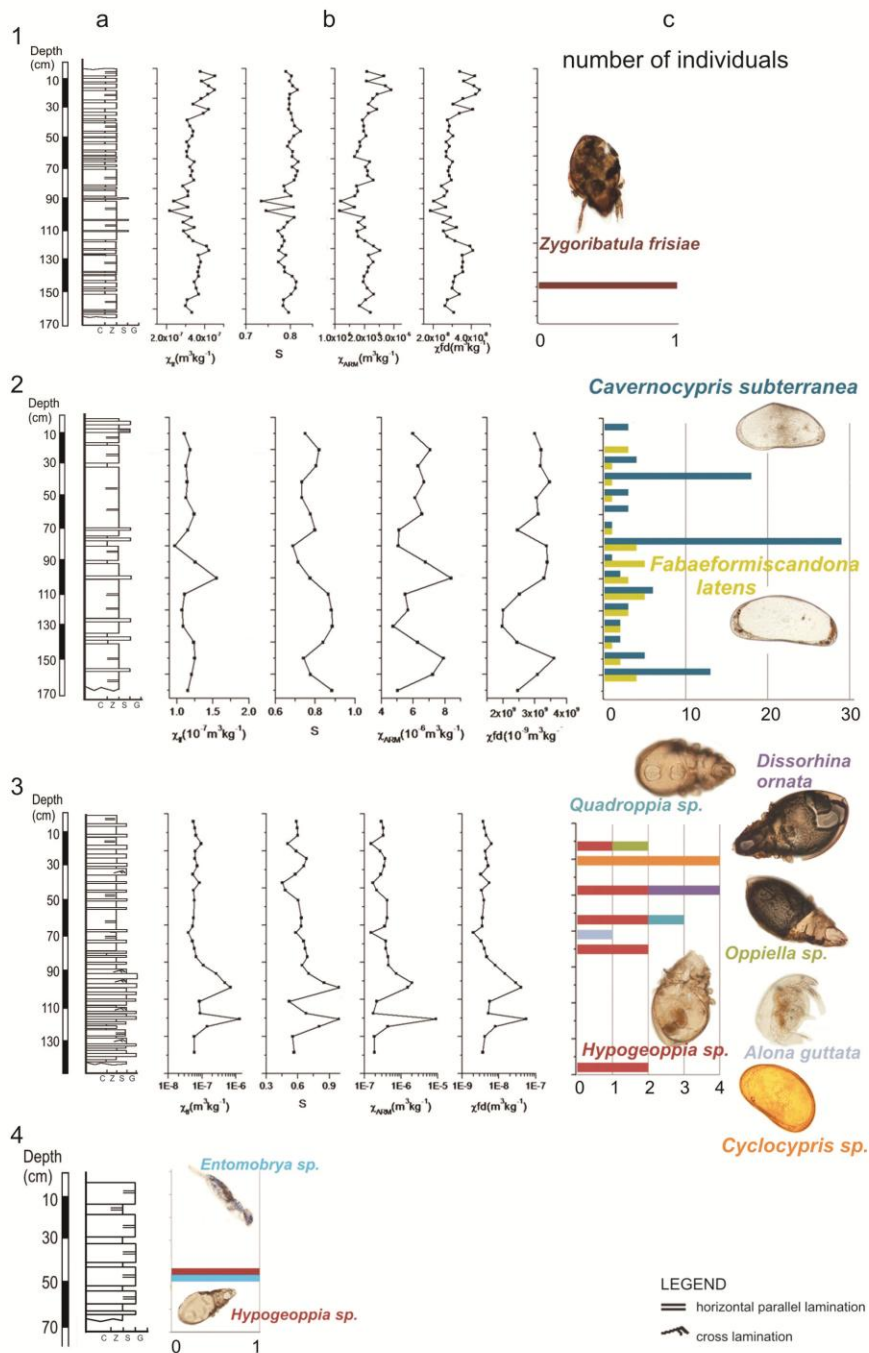


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 2 Figure 1. Map of Europe with the location of the studied caves in Romania (left), and the
 3 idealized cross-section of the profiles (right). 1. Ursi, 2. Lesu, 3. Ciur, 4. Poleva. Legend: a.
 4 Limestone, b. flowstone and stalagmites, c. Silt sediments, d. Fine, red clay. Note that
 5 speleothem dimensions were exaggerated for clarity.

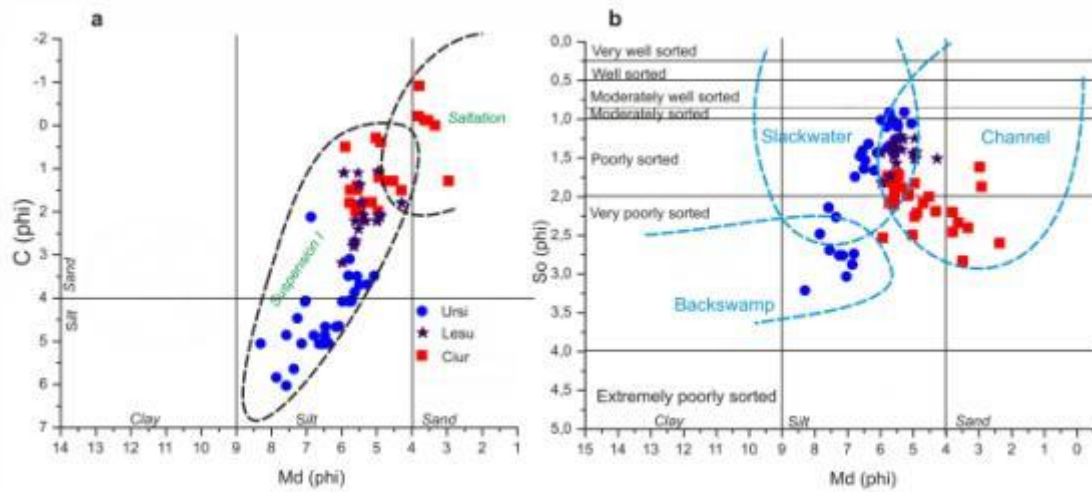
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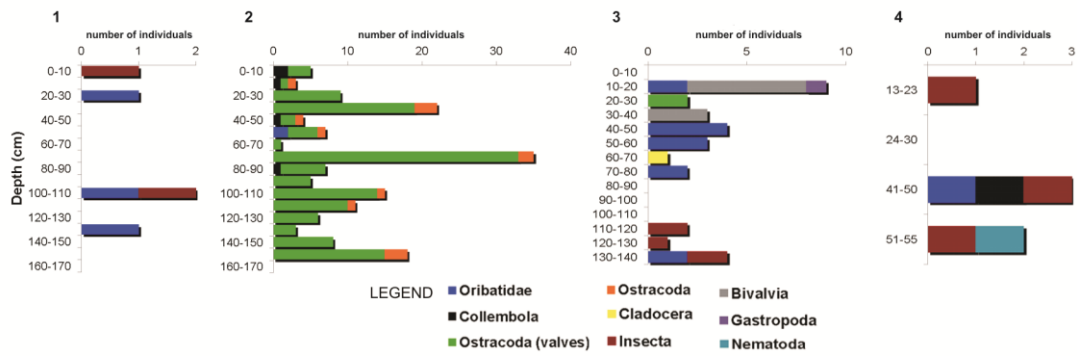
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 2 Figure 2. Maps of the selected caves with the location of the sampled profiles (A-A') and
 3 their photos. 1. Ursi (modified after Rusu, T. & Racoviță, 1981), 2. Lesu (modified after
 4 Rusu, 1988), 3. Poleva (modified after Constantin et al., 2007) with the dated speleothem
 5 discussed in text (a), 4. Ciur (modified after Webb et al., 2014).
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 2 Figure 3. Multi-proxy analysis of the profiles in the four studied caves. a. Granulometry and
 3 type of lamination, b. Rockmagnetic parameters: χ_{lf} - low frequency magnetic susceptibility,
 4 S ratio, χ_{ARM} - anhysteretic remanent magnetization susceptibility, χ_{fd} - frequency
 5 dependence of magnetic susceptibility; c. variation of the number of identified taxa; 1. Ursi, 2.
 6 Lesu, 3. Ciur, 4. Poleva, C = clay, Z = silt, S = sand, G = gravel.
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 2 Figure 4. The plotting Median (Md) vs clasticity index (C) (a) and the plotting of *median*
 3 (Md) vs. *standard deviation* (So) (b) of the fraction finer than 2 mm, from the Ursi, Ciur and
 4 Lesu. Grain size is in *phi* units [$-\log_2$ (dimension in mm)]. The data are grouped and suggest
 5 the transport and accumulations processes, typical for the fluidal, unidirectional flows, as
 6 saltation and suspension; the channel, slackwater and backswamp facies were recognized.
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2 Figure 5. Identified fossil invertebrate groups from cave sediments of the Carpathian region:

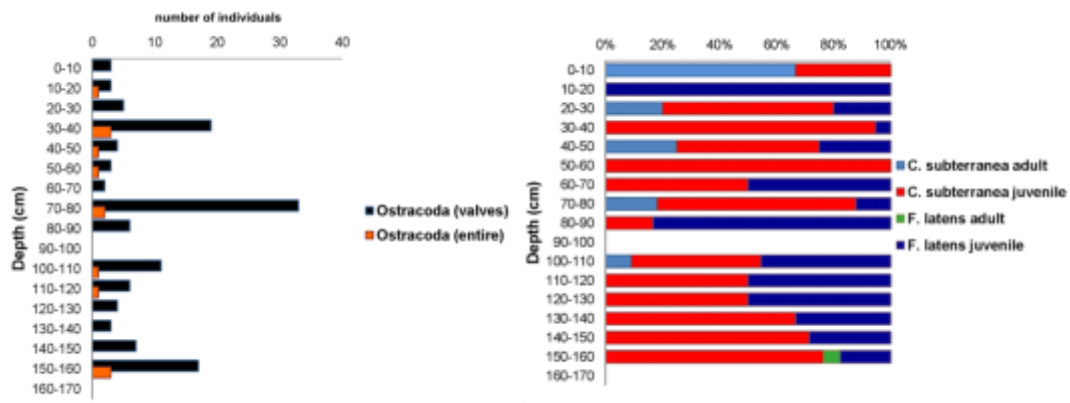
3 1. Ursi, 2. Lesu, 3. Ciur, 4. Poleva.

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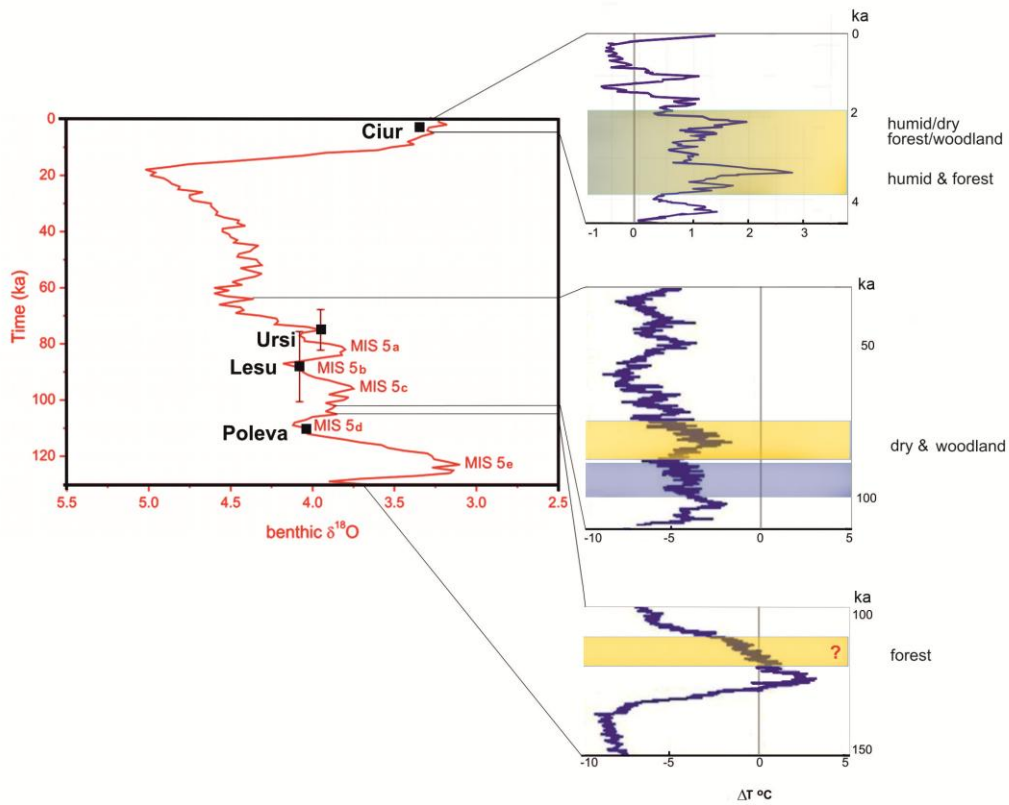


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 2 Figure 6. Some examples of fossil invertebrates in different states of preservation found in
 3 Ciur (1-3), Les (4-6), Poleva (7-8) and Ursi (9): 1-SR0038, 2- SR0032, 3-SR0038, 4-SR0154,
 4 5-SR0162, 6-SR0165, 7-SR0128, 8-SR0129, 9 – *Zygoribatula frisiae*.

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 2 Figure 7. Analysis of the identified fossil ostracods in Lesu: the relationship between entire
 3 individuals and valves (left) and the absolute abundance of adults and juveniles of the two
 4 species (right).
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 2 Figure 8. Benthic $\delta^{18}O$ record with the identified environmental parameters and
 3 corresponding temperature variation range during the last 150 ka (benthic $\delta^{18}O$ curve and
 4 temperatures were taken from Lisiecki and Raymo, 2005 and [www.dandebat.dk/eng-](http://www.dandebat.dk/eng-klima5.htm)
 5 [klima5.htm](http://www.dandebat.dk/eng-klima5.htm)): blue = cold; yellow = warm.

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