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

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Abstract

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

and pitfalls.

1 Introduction

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

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

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

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

2 Material and Methods

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

2.1 The caves

Ursi is a famous repository of fossil remains of cave bears, hyenas and lions especially in its lower, hydrologically active, level (Constantin et al., 2014). It is developed within recrystallized Jurassic limestones from the foothills of Bihor Mountains (NW Romania) at 428 m.a.s.l. The lower level develops along a subterranean stream and includes alluvial terraces atop of which lie cave bear and cave lion remains. Sediment samples were taken at 10 cm resolution from a 170 cm long profile (Figs. 1 and 2). The age controls of the profile consists of radiocarbon ages of the fossil remains of a cave lion located atop a matching terrace surface in the vicinity and one OSL dating of the alluvial sediments at c. 0.75 m below.

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

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

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

2.2 Sampling

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

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

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

2.3 U-Th dating of speleothems

The U-Th dates reported in this paper were done in the late '990s by alpha spectrometry method, at that time the most widely used, at the U-series Geochronology Laboratory, Bergen University, Norway. Sub-samples were cut from what seemed to be, optically, the most suitable calcite (columnar or microcrystalline fabrics). The sub-samples were cut as closest as possible to the speleothem base and as thin as possible in order to incorporate correspondingly short stratigraphic intervals. However, the low uranium content required quite large amount of calcite (typically 20-25 g); for flowstones sub-samples as thin as 5 mm could be taken but for stalagmites they usually correspond to c. 1 cm axial extension. Analytical procedures for U-Th alpha spectrometric dating are described by Lauritzen and Onac (1999). Detrital ^{230}Th contamination was monitored using the ^{230}Th/^{232}Th index and corrected using an initial ^{230}Th/^{232}Th value of 1.5 (Schwarcz, 1986).

Although alpha-spectrometric U-Th dating method is largely considered obsolete nowadays, we consider that the age controls it offers are sufficiently adequate for the purpose of this study. First, the speleothems interbeded within or lying on top of alluvial sediment sequences are notoriously “dirty”. Alpha spectrometry allows for more robust chemical separation procedures to deal with both detrital ^{230}Th and contaminants such as phosphate that may affect chemical yields and dating reliability. Second, the dates are not used to construct a high-resolution time-series (as for typical speleothem records) but to provide broad chronological controls. Finally, in the absence of organic materials that could be radiocarbon dated (or beyond radiocarbon range), the typical dating uncertainties of ~10% are comparable or better than those of the OSL dates.

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

2.4 Radiocarbon datings

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

2.5 OSL datings

Sediment samples for OSL datings were collected by hammering 25 cm-long, opaque, plastic tubes (20 cm) into the sediment sections under no light. Sample preparation for luminescence measurements was performed under low intensity red light conditions. Only the central portion of the tubes was removed for dating. A three-day treatment with 10% HCl solution was used for carbonates removal, followed by another three-day H_2O_2 (30%) treatment for organic matter removal. The coarse grain fractions (>63-90 μm) were separated through wet sieving. For the extraction of the fine-grained quartz fraction (4-11 μm), the particles smaller than 11 μm were isolated from the fraction less than 63 μm by settling in Atterberg cylinders, according to the Stokes law. This fraction was treated with hexafluorosilicic acid (H_2SiF_6) for 10 days. Subsequently, the removal of the grains <4 μm was carried out by centrifugation.

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

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

2.6 Grain size analysis

Grain size measurements on fine samples were performed by treating ~5 g of the bulk sample, for 14 days, in a plastic box, with ~0.4 ml of a 1% solution of Na(PO3)n, n ≈ 25 - Graham's salt (Merck). A quantity of ~2.5 g sample was later extracted from the box and treated again with ~0.2 ml of 2% solution of Graham's salt. Each sample was analyzed on a HORIBA Partica LA-950V2 laser scattering particle size distribution analyzer to reveal grain size fractions. The coarser samples were analyzed by vibrating dry sieving of ~100 g of the bulk sample and weighing the sediment quantity retained on each sieve, on an OHAUS Scout digital balance, down to the 500 μm fraction, which was subjected to the same procedure as the fine samples.

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

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

2.7 Rockmagnetism

For rockmagnetism and paleomagnetism, samples were collected in plastic cylinders (11 cm^3) specially designed to avoid the rotation of the sample during the sampling or measurements. The moist sediment allowed us to press the cylinders into the clean face of the outcrop. Sampling interval was between 5 cm and 10 cm. The cylinders were then excavated, capped and packaged to avoid the loss of humidity during the transport. In the laboratory the samples were kept in a refrigerator between measurements, to preserve as much as possible the original humidity. Additional samples from the same locations were collected in plastic bags for granulometry and the stratigraphy was documented *in situ*.

In laboratory, the folowing rockmagnetic measurements were performed for all samples: frequency dependence of magnetic susceptibility, anhysteretic remanent magnetization, isothermal remanent magnetization acquired in a magnetic field of 2 T (IRM_{2T}) and the remaining isothermal remanent magnetization after applying a back field of 0.3 T ($IRM_{0.3T}$). Magnetic susceptibility (mass-specific) was measured using the AGICO MFK1-FA Multi-function Kappabridge at two frequencies of 976Hz (low frequency - lf) and 3904 Hz (high frequency - hf). The corresponding values are referred to χ_{lf} and and χ_{hf}, respectively. The frequency-dependent susceptibility was $fd=(\chi_{lf} - \chi_{hf})$, which is proportional to the concentration of the viscous superparamagnetic (SP) particles (Worm, 1998). Anhysteretic remanent magnetization (ARM) was imparted using an anhysteretic magnetizer AMU-1A (AGICO) coupled with the LDA-3A demagnetizer in an alternating field of 100 mT with a superimposed 50 μT bias field, and was then expressed by ARM susceptibility (χARM). The ARM is particularly sensitive to the content of stable single-domain (typically larger than 20–25 nm, but less than ~100 nm for magnetite) ferrimagnets (Dunlop and Özdemir, 1997). The isothermal remanent magnetizations were imparted using a pulse magnetizer MMPM10 (magnetic Measurements). Based on these isothermal remanent magnetizations S-ratio was calculated for 2T magnetizing fields (IRM_{2T}) and 0.3 T backfield ($IRM_{0.3T}$), following the procedures used by Bloemendal et al. (1988) (Eqn.1):

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

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

3 Results

3.1 Age controls

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

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

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

3.2 Sedimentological analysis

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

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

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

3.3 Rockmagnetism

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

3.4 Fossil invertebrates in cave sediments

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

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

One individual of *Zygoribatula frisiae* (Oudemans, 1916) was identified in Ursi, near the base of the profile (Fig. 3). In Ciur, oribatid mites were constantly present along the profile, even though in small number (Fig. 3). The mites were found only in the silt levels deposited under slow energy flow or stagnant water episodes. One individual belonging to *Oppiella* (Jacot, 1937) was identified in the sediments of Ciur, together with representatives of *Hypogeoppia* Subias, 1981, *Quadroppia* Jacot, 1939 and *Dissorhina ornata* (Oudemans, 1900). *Hypogeoppia* was found in several layers. This is a new species close to *Hypogeoppia belgicae* Wauthy and Ducarme, 2006 described from Belgian caves. The identified *Quadroppia*, similar to *Q. quadricarinata* (Michael, 1885), is a Holarctic distributed genus. One *Hypogeoppia* sp. and one collembolan *Entomobrya* sp. Rondani, 1861 were identified in Poleva sediments (Fig. 3).

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

4 Discussion

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

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

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

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

(Wauthy and Ducarme, 2006). *Oppiella* sp. is a common genus with broad range, including both more specialized species as well as euryoecous ones, so the interpretation remains difficult. The other taxon, *Dissorhina ornata,* that appear together with *Oppiella* sp.only in the upper part of the profile is also broadly distributed, but abundant in more open habitats, which might be associated with the flooding events during that time period and may explain the changes of species from one level to another. Flooding events in the upper part of the profile are also supported by the presence of *Cyclocypris* sp. that appears in a single level with gravels at -30 cm and could correspond to a rapid change of vegetation on the surface. *Dissorhina ornata* is found at the border between forest and open areas (Seniczak et al., 2006) and species of the genus *Opiella* (sensu lato) can be considered one of the most common arthropod groups on Earth (Norton and Palmer, 1991), with high diversity and abundance in forest litter, also present in shrublands, ecotone zones and grasslands. Some authors (Lotter et al., 1997; Taylor and Wolters, 2005) mentioned the tolerance of the genus *Dissorhina* to drought. *Alona guttata* found in the middle part of the profile (-60 cm) is generally associated with benthic, warmer conditions, with increasing density of vegetation, and more acidic waters (Szeroczyñska and Sarmaja-Korjonen, 2007; Nováková et al., 2013). The profile includes, thus, different communities indicating different environmental conditions at surface. The *Hypogeoppia* sp. from lowest part of the sediments indicate the presence of moist forrested/grassland areas. The middle part of the sediment layers suggest a warm climate, while the upper part is indicative for a mixture of open and forrested habitats.

The middle to low part of the profile in Ciur is associated with coarser sediments indicating a high-energy hydraulic regime and frequent flooding episodes. Within the middle and upper parts of the profile, the low values of magnetic susceptibility and minor frequency dependence suggest that the soils were not significantly eroded and transported in the cave. The estimated age (Late Holocene) explains the strong pedogenesis suggested by magnetic measurements. Pollen data in the region (Feurdean et al., 2013) document the onset of a rise in diversity and large-scale forest clearance (with *Fagus sylvatica* and *Abies alba* recording the greatest decline), burning, pastoral activities and arable farming at lower elevation in the same period. The invertebrates in Ciur are documenting fast changes and alternation of forest and more open habitats at the surface. The magnetic susceptibility variations do not support the hypothesis of significant changes in temperature; however, the changes in fossil invertebrates fauna suggest that vegetation changed at ~ 2000 years ago. The sudden changes in fauna communities and the massive input of sediments may be due to floodings during the

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

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

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

5 Conclusions

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

(i) rapid pluviometric/hydrological oscillations along relatively short periods during the Subatlantic and the Late Pleistocene, sometimes accompanied by floodings, as suggested in Ciur by the alternation of terrestrial and aquatic species;

(ii) rapid changes of the vegetation/temperature at the surface, with the alternance of forests and more open habitats as in Ciur, or with short cold episodes during a stadial as in the case of Lesu;

(iii) deposition of sediments in caves during both warm (Ursi) and cold (Lesu) stages of the Late Pleistocene, with no apparent relationship to the altitudinal position of the cave, which points to the importance of local conditions;

(iv) the different hydraulic regimes indicated by the presence of mites during slow flow or stagnant water, and the presence of cladocerans and ostracods during faster flow.

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

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

6 Perspectives

Caves sediments and their invertebrates may often be older than many other continental deposits, and are usually found in a relatively good state of preservation. The oldest cave invertebrate fossils found so far date from Carboniferous (Plotnick et al., 2009) and Middle Pliocene (Moldovan et al., 2011; Miko et al., 2012, 2013). Some of the clastic sediments cover long time intervals, thus potentially archiving valuable continuum history of the environment and karst evolution at the surface. Caves are numerous in one area and at all latitudes and many have sediments that may include invertebrate species and provide paleoenvironmental information to complement or cross-validate other paleoclimatic records, at least at regional scale.

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

Authors contributions

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

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

Lab. no.	Sample name/ position	U conc. (ppm)	$^{234}U/^{238}U$	$^{230}Th/^{234}U$	$^{230}Th/^{232}Th$	Calculated age (ka)	Corrected age (ka)
1810	PP 97-3 Base 1.5 cm	0.102 ±0.003	1.26±0.04	0.70±0.03	9.2	121.9 (+9.14; –8.5)	**109.9** **(+9.7; –9.1)**
2169	PP 98-11/1 Base 1 cm	0.09 ±0.003	1.13±0.05	0.26±0.02	2.6	32.5± 3	**<32.5± 3**
1805	PP97-4, base	0.076 ±0.004	1.25±0.07	0.33±0.03	11.4	42.38 (+4.2; -4.1)	**37.7** **(+4.6;-4.5)**
2309	PP99-11/1 base 1 cm	0.26 ±0.01	1.452 ±0.07	0.15 ±0.01	13.2	17.16±1.3	**15.4±1.4**
2348	PP99-10 base	0.035 ±0.002	1.05 ±0.1	0.13 ±0.02	>1000	**14.5±2.9**	

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

Cave	Depth (cm)	^{14}C (cal ka BP)	U-Th (ka)	OSL (ka)	Derived age of sediments (ka)	Inferrred 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

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

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

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

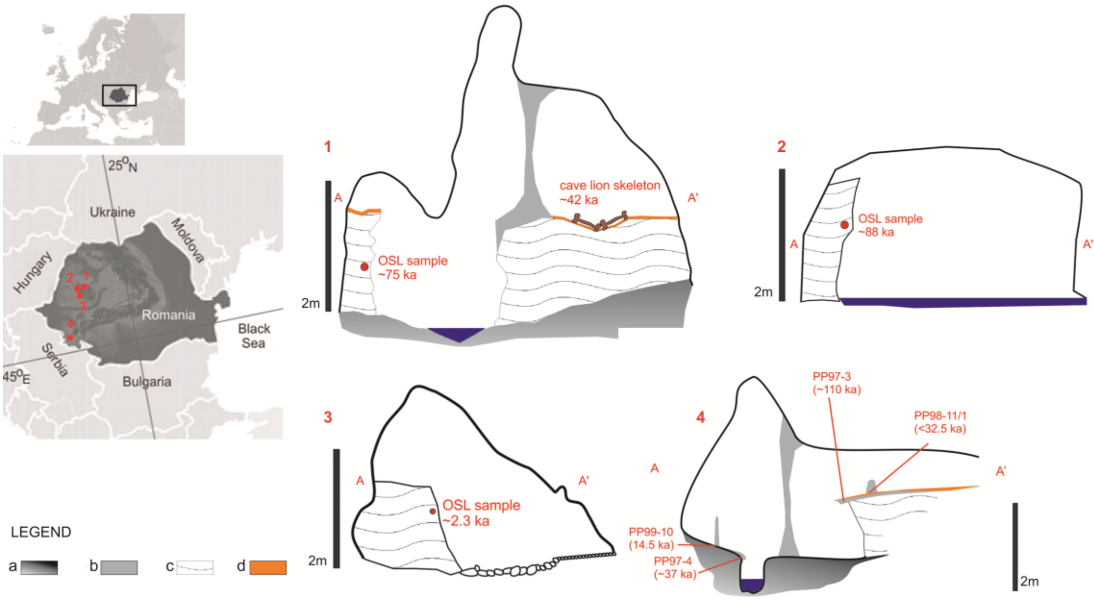

Figure 1. Map of Europe with the location of the studied caves in Romania (left), and the idealized cross-section of the profiles (right). 1. Ursi, 2. Lesu, 3. Ciur, 4. Poleva. Legend: a. Limestone, b. flowstone and stalagmites, c. Silt sediments, d. Fine, red clay. Note that speleothem dimensions were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

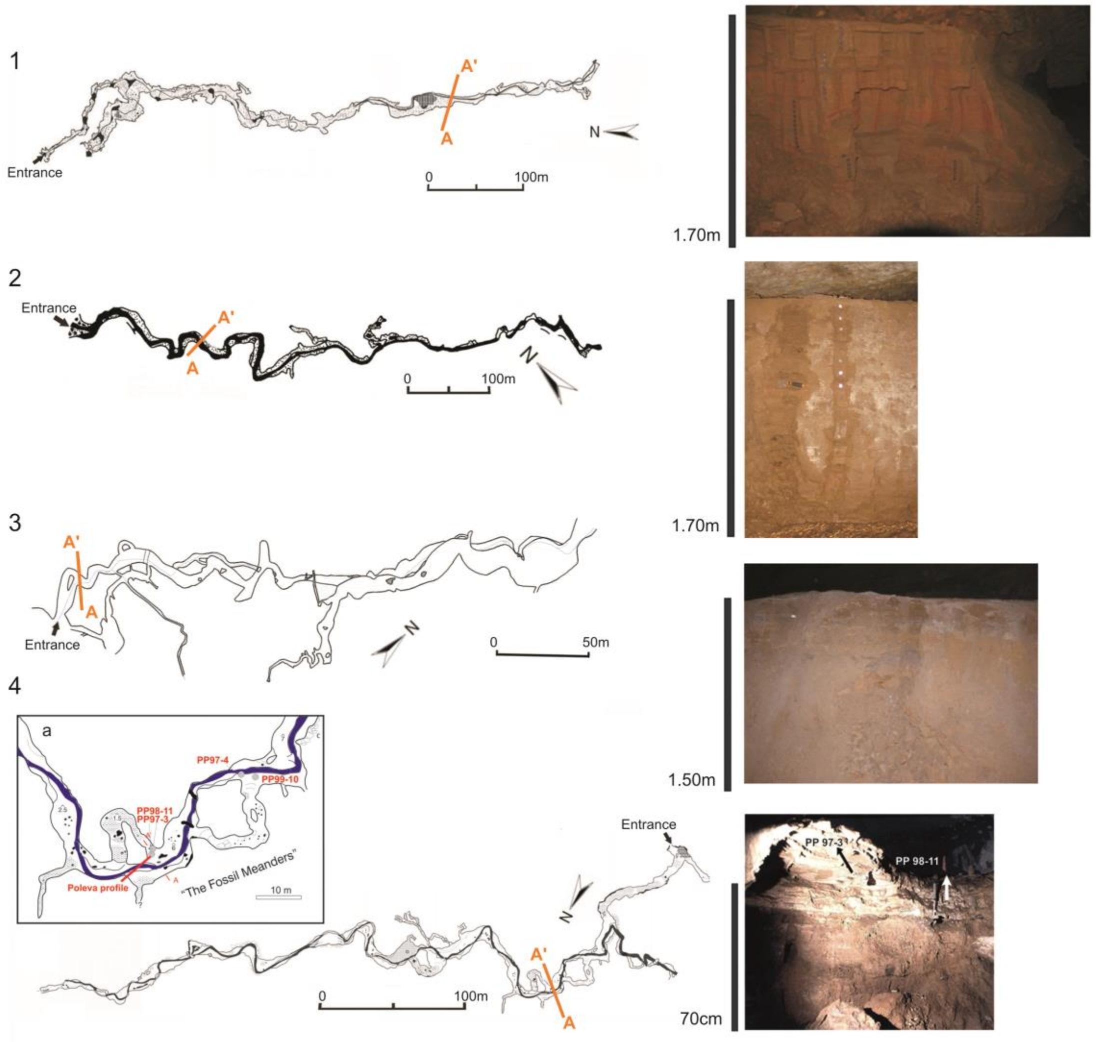

Figure 2. Maps of the selected caves with the location of the sampled profiles (A-A') and their photos. 1. Ursi (modified after Rusu, T. & Racoviţă, 1981), 2. Lesu (modified after Rusu, 1988), 3. Poleva (modified after Constantin et al., 2007) with the dated speleothem discussed in text (a), 4. Ciur (modified after Webb et al., 2014).

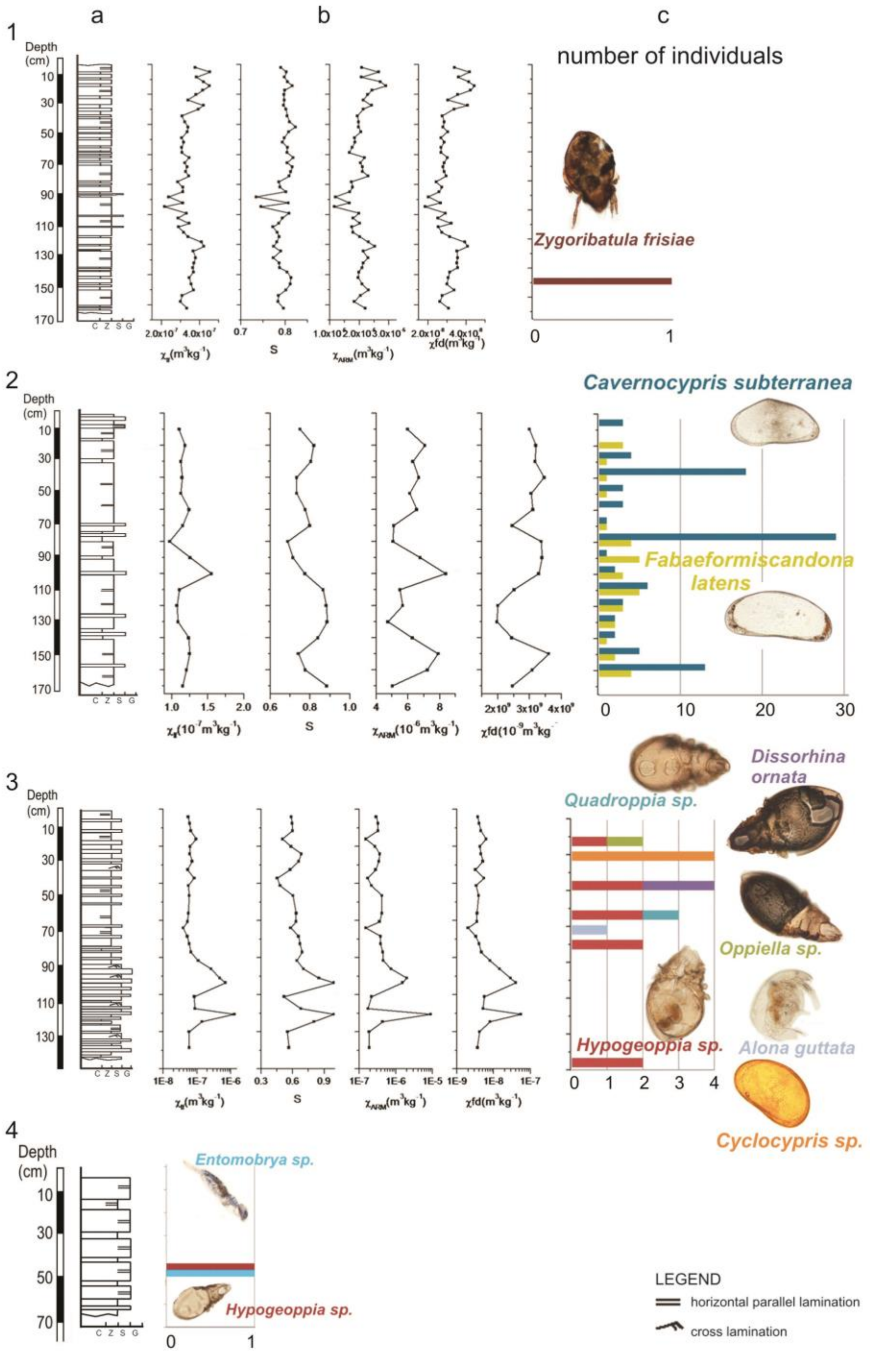

Figure 3. Multi-proxy analysis of the profiles in the four studied caves. a. Granulometry and type of lamination, b. Rockmagnetic parameters: χlf - low frequency magnetic susceptibility, S ratio, χARM - anhysteretic remanent magnetization susceptibility, χfd - frequency dependence of magnetic susceptibility; c. variation of the number of identified taxa; 1. Ursi, 2. Lesu, 3. Ciur, 4. Poleva, C = clay, Z = silt, S = sand, G = gravel.

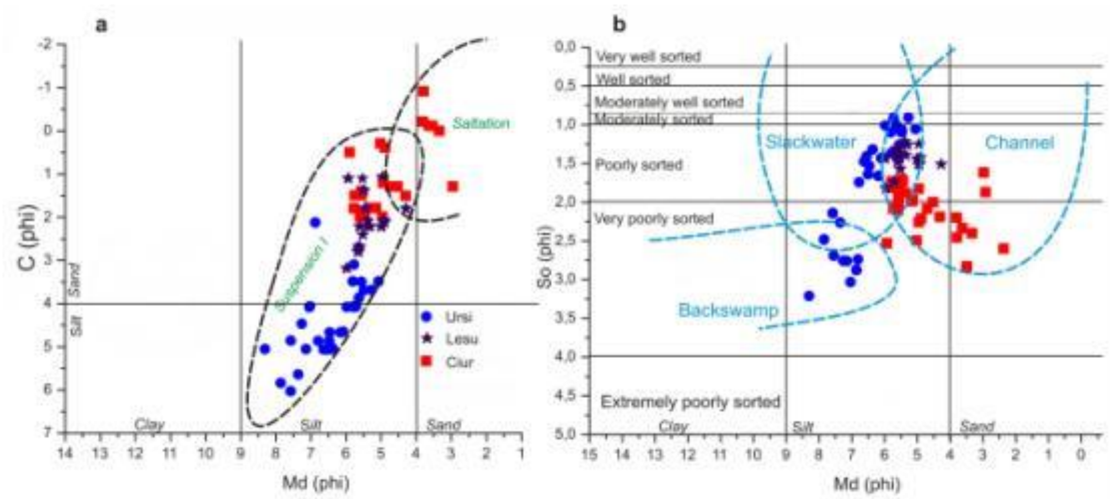

Figure 4. The plotting Median (Md) vs clasticity index (C) (a) and the plotting of *median* (Md) vs. *standard deviation* (So) (b) of the fraction finer than 2 mm, from the Ursi, Ciur and Lesu. Grain size is in *phi* units [-log$_2$ (dimension in mm)]. The data are grouped and suggest the transport and accumulations processes, typical for the fluidal, unidirectional flows, as saltation and suspension; the channel, slackwater and backswamp facies were recognized.

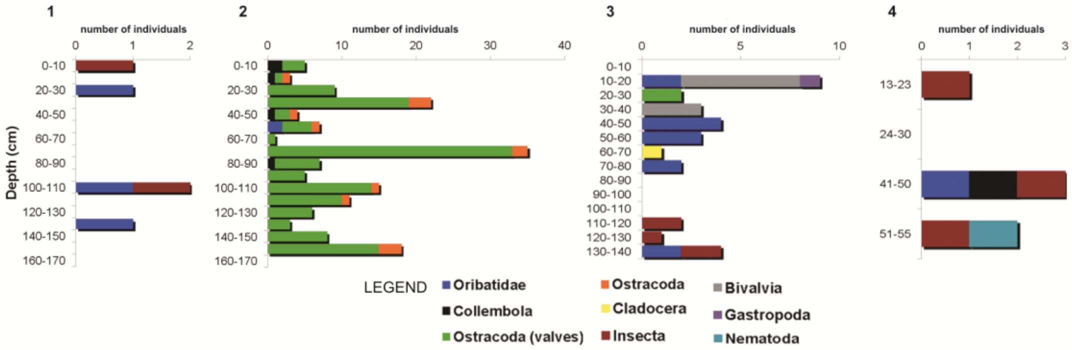

Figure 5. Identified fossil invertebrate groups from cave sediments of the Carpathian region: 1. Ursi, 2. Lesu, 3. Ciur, 4. Poleva.

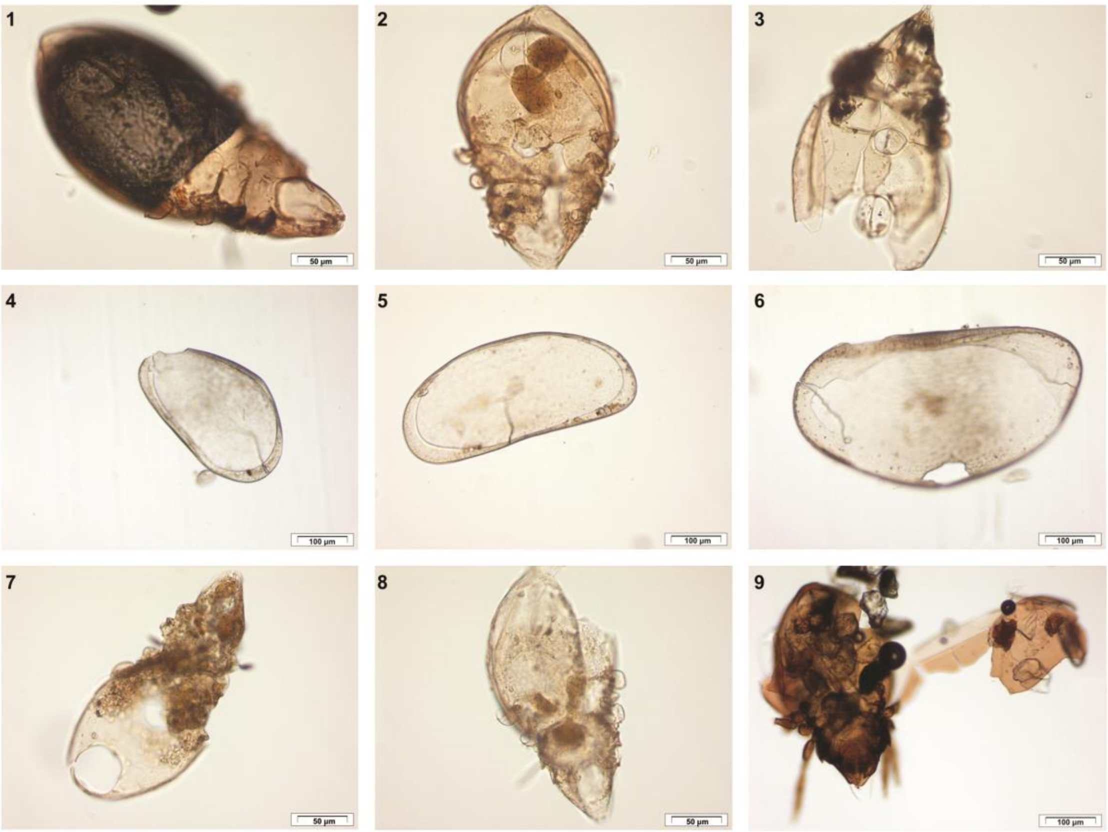

Figure 6. Some examples of fossil invertebrates in different states of preservation found in Ciur (1-3), Les (4-6), Poleva (7-8) and Ursi (9): 1-SR0038, 2- SR0032, 3-SR0038, 4-SR0154, 5-SR0162, 6-SR0165, 7-SR0128, 8-SR0129, 9 – *Zygoribatula frisiae*.

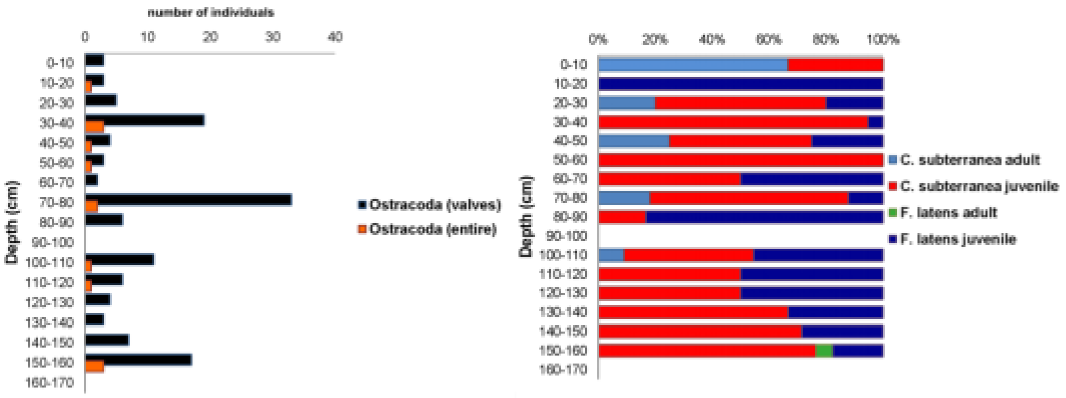

Figure 7. Analysis of the identified fossil ostracods in Lesu: the relationship between entire individuals and valves (left) and the absolute abundance of adults and juveniles of the two species (right).

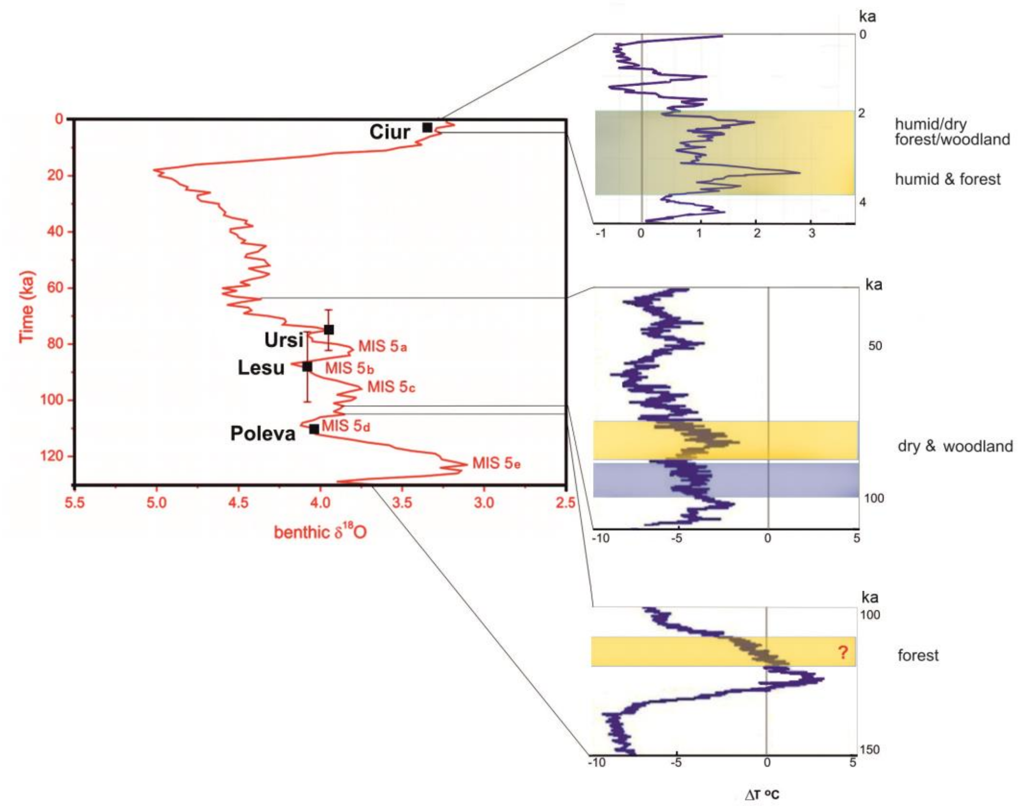

Figure 8. Benthic δ^{18}O record with the identified environmental parameters and corresponding temperature variation range during the last 150 ka (benthic δ^{18}O curve and temperatures were taken from Lisiecki and Raymo, 2005 and www.dandebat.dk/eng-klima5.htm): blue = cold; yellow = warm.