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► To cite this version:

Marius Robu, Jonathan Wynn, Cristina Montana Puşcaş, Jérémy Martin, Silviu Constantin. Palaeoecology and palaeoclimatic context of Romanian Carpathian MIS 3 cave bears using stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 2019, 534, pp.109288. 10.1016/j.palaeo.2019.109288 . hal-02264278

HAL Id: hal-02264278

<https://hal.science/hal-02264278>

Submitted on 10 May 2020

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1 **Palaeoclimatic context and palaeoecology of Romanian Carpathian MIS 3 cave bears**
2 **using stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$)**

3

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17 **ABSTRACT**

18 Millimeter-scale growth rings in canine dentine of MIS 3 cave bears have been interpreted as
19 annual growth bands produced, in part, by seasonal variation in growth rate. We present new
20 intra-tooth stable carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotope profiles in dentine hydroxyapatite
21 of early forming permanent teeth from three famous Late Pleistocene cave bear sites from
22 Romanian Carpathians. We measured $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of the CO_3 fraction of dentine
23 hydroxyapatite from samples covering consecutive inferred years of growth in canines. The
24 $\delta^{13}\text{C}$ values retrieved from the dentine layers – from the oldest to the newest – of the same
25 individual, record an increase throughout immature life of bears as has been shown
26 previously, with lower precision, using age categories. For the first time, based on $\delta^{13}\text{C}$ data
27 analysis, the weaning process in cave bears was identified. The $\delta^{18}\text{O}$ values show substantial
28 variations related, most probably, to seasonal growth of the dentine. Finally, the CO_3 of
29 dentine apatite extracted from cave bear canines proves to be reliable for geochemical
30 analyses, reflecting palaeoclimate conditions, physiology and behavior.

31 **Keywords:** Geochemistry, dentine, Mammalia, Ursidae, Late Pleistocene

32 **INTRODUCTION**

33 The discovery of measurable natural variation in the stable isotopic composition of vertebrate
34 fossil remains brought palaeontologists a valuable tool for studying fossil mammals from
35 ancient marine and terrestrial communities (e.g. Longinelli, 1984; Clementz, 2012; Matthews

36 et al., 2016; Martin et al., 2017). Carbon and oxygen isotopic abundances (reflected in $\delta^{13}\text{C}$
37 and $\delta^{18}\text{O}$ values) measured from fossil vertebrate teeth have been used for a long time to
38 decipher the dietary habits and ecology of extinct species (Sullivan and Krueger, 1981;
39 Krueger and Sullivan, 1984; Ambrose and Norr, 1993; Longinelli, 1984; Koch et al., 1989;
40 Reinhard et al., 1996). Carbon isotopic abundances in carbonate hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4$,
41 $\text{CO}_3)_6(\text{OH}_2)$] reflect the average of the whole diet components (amino acids, carbohydrates
42 and lipids). The oxygen isotopic abundances recorded from the same tissue reflect the
43 variation in the $\delta^{18}\text{O}$ value of body fluids controlled mainly by the isotopic composition of the
44 ingested water. As the ingested water tracks the local precipitation, $\delta^{18}\text{O}$ values obtained from
45 dentine are used to reconstruct palaeoclimatic conditions and sources of water used by
46 animals (Longinelli, 1984; Koch et al., 1989; Reinhard et al., 1996; Dotsika et al., 2011;
47 Bocherens et al., 2011; Clementz, 2012; Krajcarz and Krajcarz, 2014; Krajcarz et al., 2014).
48 Late Pleistocene cave bear fossils are the most abundant remains to be found in the caves of
49 Europe, this extinct species being one of the best studied in Quaternary palaeontology.
50 Nonetheless, there are still many debates within the scientific community regarding the diet,
51 physiology and behavior of these ursids. The permanent canine teeth of adult bears are useful
52 for tracing age-related changes, mostly due to their resilience to weathering. The
53 chronological age of individuals can be accurately determined (Debeljak, 1996) from the
54 annual layers of dentine or cementum, emphasized when odontogenesis is slowed during the
55 annual period of denning (nutritional stress). Although the cementum layers record a longer
56 history of the bear's life, dentine layers are better suited for geochemical analyses as they are
57 thicker and can provide sufficient sampling material. It has been shown that the annual canine
58 dentine layers in adult individuals may vary between five and eight, with the first five layers
59 belonging to the primary dentine, and the others to the secondary dentine. Each dentine layer
60 consists of a thick, light-stained inner zone, deposited during the annual period of activity, and
61 a very thin, dark-stained outer zone related to the period of denning (Rausch, 1961, 1967,
62 1969; Mundy and Fuller, 1964; Stoneberg and Jonkel, 1966; Sauer, 1966; Sauer et al., 1966;
63 Koch et al., 1989). Knowing that the cyclicity of dentine growth layers in bear canines ends
64 when adulthood is reached (Rausch, 1969), one can trace back both the ecological conditions
65 and the climatic evolution by using a geochemical approach (e.g., using $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values)
66 for every identified dentine layer.

67 The carbon isotope analysis of organic tissues of such tooth dentine provides information
68 reflective of the protein portion (collagen) of an individual's diet (Sullivan and Krueger, 1981;
69 Krueger and Sullivan, 1984; Ambrose and Norr, 1993; Harrison and Katzenberg, 2003),

70 whereas stable isotope values from bone, dentine, and enamel apatite carbonate largely reflect
71 the whole diet (*i.e.*, proteins, carbohydrates, and lipids; Ambrose and Norr, 1993; Harrison
72 and Katzenberg, 2003). In general, apatite is more ^{13}C -enriched compared to collagen (by
73 +9,6-13‰; DeNiro and Epstein, 1978a, b; Sullivan and Krueger, 1981; Krueger and Sullivan,
74 1984; Lee-Thorp and Van der Merwe, 1987; Fahy et al., 2015), their relationship being
75 affected by the trophic level. The utility of bone/tooth apatite for stable carbon isotope
76 analysis in palaeodietary studies stems from the fact that previous results indicate that
77 diagenesis of biogenic carbonates in the mineral over time is unexpectedly limited, and that
78 chemical pretreatment further removes the effects of diagenetic alteration of the biogenic
79 signal (Lee-Thorp et al., 1989). Therefore, palaeodietary inferences could be carried out using
80 this method for dentine apatite for fossil remains up to $\sim 10^5$ years old (Clementz, 2012). Thus,
81 apatite $\delta^{13}\text{C}$ measurements should not be regarded as an alternative to $\delta^{13}\text{C}$ collagen
82 measurements, but may provide additional useful information about dietary trends.

83 Previous studies have focused on cave bear physiology (e.g. Nelson et al., 1998; Bocherens,
84 2015; Robu et al., 2018) using $\delta^{13}\text{C}$ values from bone collagen and have shown that the
85 isotopic profiles of the four age groups (neonates, juveniles, sub-adults and adults) provide
86 patterns of transfer of stable isotope signatures throughout the immature life of bears.

87 Studying the $\delta^{13}\text{C}$ transfer signature throughout the first five to eight years of life, using $\delta^{13}\text{C}$
88 data from apatite of each individual, could bring a better understanding of MIS 3 cave bear
89 physiology and a better resolution of this process.

90 Sequential sampling of distinct dentine layers within a single tooth can provide information
91 on dietary and habitat changes over the course of an individual development from juvenile to
92 adult as well as seasonal variations in these ecological parameters later in the animal's life.

93 Previous studies of fossil proboscideans – elephants and their extinct relatives (Koch et al.,
94 1989; Fox and Fisher, 2004; Hope et al., 1999; Rountrey et al., 2007; Clementz, 2012)
95 highlighted how serial sampling of both tooth enamel and dentine can be used to answer
96 different questions about the ecology and life history of these animals. Koch et al. (1989)
97 analyzing the $\delta^{18}\text{O}$ values from dentine layers, have shown that CO_3 fraction of apatite retains
98 a signal in molars (proboscideans and bears), reflecting seasonal changes in composition of
99 drinking water.

100 The Romanian Carpathians host numerous, complex, and rich Late Pleistocene cave bear
101 sites, suitable for palaeoecological and palaeoclimatological investigations. Three already
102 famous Late Pleistocene cave bear sites from Romanian Carpathians were chosen for this
103 study – the Urşilor, Oase and Cioclovina caves – known to the international scientific

104 community for their impressive fossil bonebeds, human and faunal remains, and for their
105 taphonomic and palaeoichnologic features (e.g. Richards et al., 2008; Robu et al., 2018).
106 Analyzing both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, retrieved from dentine apatite serial samples, has not been
107 applied systematically to any MIS 3 species as yet. Thanks to their large dimensions,
108 analyzing a large set of MIS 3 cave bear canines provides an excellent record of seasonal
109 variations in diet and climate during tooth growth and strengthen our understanding of the
110 palaeoclimatic and palaeoecological significance of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values retrieved from
111 dentine apatite.

112 **MATERIALS AND METHODS**

113 *Carpathian site-specific samples*

114 The Carpathian cave bear specimens derive from excavations in three extensive karst systems:
115 Peștera Urșilor de la Chișcău, Peștera Cioclovina Uscată, and Peștera cu Oase (Fig. 1).

116 The Peștera Urșilor de la Chișcău (further on: Urșilor; 46° 55' 37" N, 22° 56' 92" E) is
117 situated in the northwestern part of the Romanian Carpathians (the Apuseni Mountains). The
118 analyzed cave bear material was sampled from the palaeontological excavation, situated
119 within the Scientific Reserve (lower level of the cave). The analyzed cave bear population
120 was AMS ^{14}C dated between *c.*47 and 39 kyr cal BP (Constantin et al., 2014; Fig. 1A).

121 The Peștera Cioclovina Uscată (*Dry Cioclovina Cave*; further on: Cioclovina; 45° 34' 28"
122 N, 23° 08' 04" E) (Fig. 1B) is located in the northwestern part of the Southern Carpathians
123 (the Șureanu Mountains) (Soficaru et al., 2007; Onac et al., 2011). Results of AMS ^{14}C dating
124 indicate that the cave bear population inhabited the cave between *c.*44.2 and 31.4 kyr cal BP
125 (Soficaru et al., 2007; Meleg et al., 2018; this study).

126 The Peștera cu Oase (*Ponor Plopa Cave*; further on: Oase; 45° 05' 21" N, 21° 51' 21" E)
127 is situated in the southwestern part of the Southern Carpathians (the Anina Mountains) (Fig.
128 1C). The analyzed cave bear samples were excavated from the upper level of the cave (Sala
129 Mandibulei and Panta Strămoșilor). The cave bear population of the bone assemblage was
130 dated between *c.*50 and 40.4 kyr cal BP (Higham and Wild, 2013; this study).

131 <Insert Figure 1>

132 *Cave bear samples*

133 Teeth of the analyzed cave bears were identified by their morphology (according to Torres *et*
134 *al.*, 1978; Tsoukala and Grandal-d'Anglade, 2002). DNA analyses have not been completed
135 for all studied sites, although some populations were attributed to *U. ingressus* either on
136 mtDNA or nuclear DNA basis or dental and metacarpal morphology (Richards et al., 2008;
137 Robu, 2016; Meleg et al., 2018).

138 A number of 18 fused cave bear lower canines ($N = 18$) were analyzed to obtain carbon and
139 oxygen isotopic composition from the structural carbonate of their dentine. The canines were
140 selected from the analyzed sites ($N = 9$ females and $N = 9$ males; Table S1), with six canines
141 being sampled from each collection. Hydroxyapatite samples ($N = 112$) were drilled from
142 series of holes aligned parallel to incremental dentine layers (Fig. 2); the powder from each
143 row of holes formed a single sample (*c.* 2 mg). We obtained five to nine non-overlapping
144 samples within each inferred year. The samples were analyzed following the method
145 described by Koch et al. (1997).

146 <Insert Figure 2>

147 The isotopic ratios are expressed as $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values according to calculation: $\delta^{\text{A}}\text{X} =$
148 $(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$, where X is an element (C or O), A is the atomic mass number and
149 R is the isotopic ratio $^{13}\text{C}/^{12}\text{C}$ or $^{18}\text{O}/^{16}\text{O}$. Values are reported in relation to Vienna-PDB
150 (VPDB) standard, with $\delta^{18}\text{O}$ values being converted to VSMOW using the formula
151 $\delta^{18}\text{O}_{\text{VSMOW}} = 1.03091 \times \delta^{18}\text{O}_{\text{VPDB}} + 30.91$ (Skrzypek et al., 2011). Laboratory standards used
152 were CO_2 :He for $\delta^{13}\text{C}$, and NBS18 and CARRARA 11 for $\delta^{18}\text{O}$. Both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values
153 were measured using a Delta V+ (Thermo-Finnigan, Bremen, Germany) isotope ratio mass
154 spectrometer (IRMS), in continuous flow mode (Fry et al., 1992) coupled to an ECS410
155 elemental analyzer (Costech Analytical, Valencia, CA, USA) in the Department of Geology,
156 University of South Florida.

157 *Dating method*

158 Ten cave bear samples ($N = 10$) derived from the analyzed canines were AMS ^{14}C dated at
159 Poznan Radiocarbon Laboratory (Poland; Tab. S1). At the Poznan Laboratory, collagen
160 extraction was performed using the method described by Pietrowska and Goslar (2002) and
161 the extracted collagen was then purified on Elkay 8 mm filters, and ultrafiltered on Vivaspin
162 15 MWCO 30 kD filters (Bronk Ramsey et al., 2004). The content of ^{14}C in carbon samples
163 was measured following the protocol described by Goslar et al. (2004).

164 *Statistical analyses*

165 Statistical analyses were performed using the software PAST (Paleontological Statistics),
166 version 3.20 (Hammer *et al.* 2001). Hierarchical clustering was used to explore relationship
167 patterns of $\delta^{18}\text{O}$ variability among the studied samples. Euclidean distances were used to
168 calculate a similarity matrix based on the average values of $\delta^{18}\text{O}$ for 18 canines from Urşilor,
169 Oase and Cioclovina caves, and Ward's method was selected to define the distance between
170 identified classes. To visualize the similarity among studied samples, multidimensional

171 scaling and minimum-spanning tree linking samples were computed based on average values
172 of $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and median ^{14}C date for 18 canines from Urşilor, Oase and Cioclovina caves.

173 RESULTS

174 The results of radiocarbon dating are given in Table S1 ($N = 10$ samples). All ages were
175 calibrated using the OxCal 4.3.2 program (Bronk Ramsey, 2017) and the IntCal13 calibration
176 curve (Reimer et al., 2013). Radiocarbon ages (Fig. 3) of the 10 cave bear bone samples
177 collected from the analyzed sites range between *c.* 45 and 31 kyr cal BP (median values) for
178 both surface and excavated finds. The majority of the dated samples of bears belong to 39-45
179 kyr cal BP interval – known for its faunal richness across caves from both the Carpathians and
180 the rest of Eurasia – and to a last pulsation of this species, around 31 kyr cal BP ($N = 2$; from
181 Cioclovina Cave) at a time believed to be close to its extinction (Pacher and Stuart, 2009).

182 <Insert Figure 3>

183 $\delta^{13}\text{C}$ values

184 The obtained $\delta^{13}\text{C}$ values of all 112 dentine apatite samples range from -17.5 to -9‰ (average
185 of all samples -12.8‰; Fig. 4A-C). All $\delta^{13}\text{C}$ profiles are recording an increment of carbon
186 values throughout the deposition of cave bear dentine until adulthood. Urşilor samples ($N =$
187 36 dentine layers) have extreme values between -16.7 and -9.2‰, Oase samples ($N = 38$
188 dentine layers) spread between -14.9 and 9.0‰ and, Cioclovina samples ($N = 38$ dentine
189 layers) spread between -17.5 and -9.9‰.

190 <Insert Figure 4>

191 Comparing the $\delta^{13}\text{C}$ values of the oldest dentine layers of the analyzed canines (precluding
192 adulthood in bears), with the average of the $\delta^{13}\text{C}$ collagen values obtained for the same
193 populations (e.g. Robu et al., 2013, 2018), we found that the lowest $\Delta\delta^{13}\text{C}_{\text{collagen-apatite}}$
194 difference ($\delta^{13}\text{C}_{\text{collagen}} - \delta^{13}\text{C}_{\text{apatite}}$) was recorded for Cioclovina, +7.9‰ ($\delta^{13}\text{C}_{\text{collagen}} = -21.1$
195 and $\delta^{13}\text{C}_{\text{apatite}} = -13.2\text{‰}$), while the Urşilor population had a $\Delta\delta^{13}\text{C}_{\text{collagen-apatite}}$ value of +9.6‰
196 ($\delta^{13}\text{C}_{\text{collagen}} = -21.6$ and $\delta^{13}\text{C}_{\text{apatite}} = -12.0\text{‰}$). The highest average difference recorded in the
197 Oase population between the two parameters $\Delta\delta^{13}\text{C}_{\text{collagen-apatite}}$ was +11.3‰ ($\delta^{13}\text{C}_{\text{collagen}} = -$
198 21.4 and $\delta^{13}\text{C}_{\text{apatite}} = -10.1\text{‰}$, respectively).

199 Figure 5 shows the variation of $\delta^{13}\text{C}$ values between sexes among the studied Carpathian
200 populations: for females, it appears that the highest average is recorded for the Oase and
201 Urşilor females (-11.9‰, and -12‰, respectively), and the lowest for Cioclovina, -14.6‰
202 (Fig. 5A). For males, the highest average value was measured in the Oase population: -
203 11.9‰, and lowest values in Urşilor (-13.9‰) and Cioclovina (-13.7‰) (Fig. 5B). Both
204 graphs suggest differences among sexes within all three cave bear populations: for Urşilor,

205 females show $\delta^{13}\text{C}$ values enriched by +1.9‰ when compared with males, while for Oase and
206 Cioclovina, the situation is opposite, males have enriched $\delta^{13}\text{C}$ values by +0.5 and +0.9‰,
207 respectively. The differences in $\delta^{13}\text{C}$ values among sexes could be due to either random and
208 size sampling (only three males and females analyzed/site), to different intra-population
209 dietary habits, or a combination of both.

210 <Insert Figure 5>

211 *$\delta^{18}\text{O}$ values*

212 The obtained $\delta^{18}\text{O}$ values of the 112 dentine apatite samples range from 16.4 to 29.5‰
213 (average of all samples 23.1‰; Fig. 4D-F) and are in agreement with data obtained elsewhere
214 for MIS 3 cave bears (e.g. Krajcarz et al., 2014). All $\delta^{18}\text{O}$ profiles show high variability
215 throughout the deposition of cave bear dentine until adulthood. Urşilor samples ($N = 36$
216 layers) have extreme values ranging between 19.3 and 29.5‰ (average 23.4‰), Oase ($N = 38$
217 layers), range between 16.4 and 25.8‰ (average 23.1‰) and Cioclovina ($N = 38$ layers),
218 range between 17.9 and 26.6‰ (average 22.7‰).

219 The intra-tooth $\delta^{18}\text{O}$ variability of the analyzed bears ranges from 1 to 8.2‰. Among the three
220 populations, Oase has the highest intra-tooth variability for all $\delta^{18}\text{O}$ profiles, while Cioclovina
221 has the lowest. Nonetheless, all sites have bears with both types of $\delta^{18}\text{O}$ profiles, suggesting
222 intra-population variability.

223 <Insert Figure 6>

224 **DISCUSSION**

225 *Physiology and behavior using $\delta^{13}\text{C}$ data*

226 The large range of $\delta^{13}\text{C}$ values measured for the MIS 3 Carpathian cave bears span all dietary
227 range, from herbivores to carnivores, taking into account the values for dentine apatite
228 published so far (e.g. Bocherens et al., 1994). While the few data obtained from dentine
229 apatite for Pleistocene bears (adults) were recorded around -16 to -14‰ (Bocherens et al.,
230 1991), our mean values of the $\delta^{13}\text{C}$ show a large inter-population variation (-16 to -9,0‰, for
231 the last layer of the analyzed specimens), with values typical for both carnivores and
232 herbivores (Fig. 7). The large variation in $\delta^{13}\text{C}$ values of the Carpathian cave bears may
233 indicate ecological differences, linked to different dietary and/or habitat preferences as
234 previously shown by Robu et al. (2013, 2018).

235 <Insert Figure 7>

236 The $\delta^{13}\text{C}$ records of studied cave bears show a similar pattern among them, in which $\delta^{13}\text{C}$
237 values are increasing from juveniles to adults, in accordance to previous research (e.g. Nelson
238 et al., 1998; Robu et al., 2018), indicating a change of diet across time (Fig. 4A-C).

239 For all studied cave bears, a negative shift of $\delta^{13}\text{C}$ profiles was recorded during the second
240 (mainly at Urşilor) or the third (mainly at Oase and Cioclovina caves) year of life.
241 Apparently, the „second year drop” profile is present as well at Oase and Cioclovina caves,
242 while the „third year drop” profile was recorded at Urşilor as well, to a lesser extent.
243 Although it seems that these records are site-characteristic, in fact, it is a matter of random
244 sampling and it is very likely for the $\delta^{13}\text{C}$ negative shifts to be related to the weaning
245 completion in cave bears, a critical period for the future adults, reflected in low lipids and
246 energy input in their diet. It was shown in some studies that the positive trend in $\delta^{13}\text{C}$
247 associated with weaning could be the result of decreasing proportion of lipids in the diet
248 brought about by the transition from high to low dependence on milk (e.g. Hobson and Sease,
249 1998). Thus, when fat represents an important component in the animal diet, carbonate $\delta^{13}\text{C}$
250 should have more negative values than when fat is less important (Rountrey et al., 2007). In
251 modern brown bear, *Ursus arctos*, it was documented that the weaning period takes place
252 during the first or second year of life, while the separation of cubs from their mothers occurs
253 at 1–3 (occasionally 4) years of age (McLellan, 1994; Dahle and Swenson, 2003), a fact
254 supported as well by our results. The same $\delta^{13}\text{C}$ variation related to the weaning process have
255 been reported for human canines retrieved from archaeological context, by Sandberg et al.
256 (2014).

257

258 *Paleoclimatic context using $\delta^{18}\text{O}$ data*

259 The similarity of $\delta^{18}\text{O}$ patterns between our fossil data and modern bears published by Koch
260 et al. (1989), in both amplitude and relation to growth layers, indicate that the isotopic signal
261 recorded in our fossil samples has no diagenetic influence. The lower values corroborated
262 with the dark bands, could be, as in the case of modern black bears (Stoneberg and Jonkel,
263 1966) the isotopic expression of the late fall/winter denning (Fig. 4D-F).
264 The $\delta^{18}\text{O}$ values of dentine retrieved from the structural carbonate for the MIS 3 Carpathian
265 cave bears are in agreement with the results published elsewhere on dental enamel apatite
266 (e.g. Bocherens et al., 1994; Krajcarz et al., 2014). The amplitude of isotopic variation of $\delta^{18}\text{O}$
267 values (max. 8.2‰) is the same as the one observed for the Younger Dryas North American
268 mammoths and bears (Koch et al., 1989). Large $\delta^{18}\text{O}$ intra-population variations have been
269 recorded as well for modern mammal population, ranging from 5.2‰ for wild horses, to
270 8.5‰ for yaks and 10.8‰ for horses from the Tibetan Plateau (Wang et al., 2008). On the
271 other hand, no sex-related differences in $\delta^{18}\text{O}$ values among the analyzed cave bear

272 populations was recorded, with high and low variation patterns being obtained for both
273 females and males.

274 With some exceptions it appears that bears, during the first two dentine growth layers (first
275 two years of life) have similar $\delta^{18}\text{O}$ values suggesting a low mobility whereas starting with
276 the third growing layers, the values may show significant shifts, sometimes up to 6‰. This
277 pattern may indicate a growing mobility and could be related with the $\delta^{13}\text{C}$ data that show a
278 significant change in bears physiology between the second and the third year of life. Modern
279 brown bears, the closest relative to the MIS 3 cave bears, have a home-range size according to
280 recent studies (Krofel et al., 2010, 2012) between 300 and 1600 km, with an average of 600-
281 700 km. It is therefore plausible that during the feeding and mating period, bears could drink
282 water with different $\delta^{18}\text{O}$ signature than usual, a notion reflected in the $\delta^{18}\text{O}$ isotopic record.
283 Thus, the high variations could be a reflection of a broader territorial range. On the other
284 hand, the large intra-species $\delta^{18}\text{O}$ variations seen in cave bears could be related as well to the
285 fact that a large part of their body water was obtained from plant sources (*e.g.*, fruits) in the
286 context of the unstable climate of MIS 3. Both males and females show high variability of
287 $\delta^{18}\text{O}$ intra-tooth values. However, it must be kept in mind as well that during the period
288 between 30 and 50 kyr BP many short-term climatic changes have occurred and the $\delta^{18}\text{O}$
289 signal could be biased (Shackleton and Hall, 2001; Van Meerbeeck et al., 2009; Bocherens et
290 al., 2011; Bocherens, 2015).

291 Bocherens et al. (2011) suggested that warmer time periods led to more positive $\delta^{18}\text{O}$ values
292 retrieved from cave bear tissues, due to temperature effects on $\delta^{18}\text{O}$ values of meteoric water.
293 Therefore, values $>22\text{‰}$ would correspond to warmer periods, values $<21.3\text{‰}$ correspond to
294 a colder period, and those between 21.3 - 22‰ indicate intermediate climatic periods. Figure
295 8 shows how the average $\delta^{18}\text{O}$ values of Romanian bears cluster, indicating two main sets of
296 values: first one, with highest values, situated between 25.4 and 25.5‰ (two individuals from
297 Urșilor and Cioclovina, a male and a female, respectively; $N = 2$), and the second one,
298 situated between 21.3 and 24‰ ($N = 16$). The second set of data has two subsets, one that
299 ranges between 22.6 and 24‰ ($N = 12$), while the second one is situated between 21.3 and
300 22.1‰ ($N = 4$). According to Bocherens et al. (2011) criteria, the vast majority of the studied
301 bears lived during a warmer climate ($N = 14$ bears; 22.6 - 25.5‰), while only four of them
302 during a colder stage (21.3 - 22.1‰; $N = 4$).

303 <Insert Figure 8>

304 Corroborating the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of the studied bears (Fig. 9A), it appears that there is a
305 high variability and affinity among the three sites. Nonetheless, the core of the data shows a

306 slightly stronger similarity between Urşilor and Oase. When adding an additional parameter
307 to this analysis, *i.e.*, the absolute age of bears (Fig. 9B), two „different” cave bear populations
308 emerge: (i) one dated at around 45-39 kyr cal BP, with numerous individuals from all
309 analyzed sites, and with similar high $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values among the bears and (ii) a second
310 one, derived only from Cioclovina (two individuals), dated at 34.5-31.5 kyr cal BP.
311 Interestingly, within the first population, two individuals, a male (PO/42) and a female
312 (PU/072), from Oase and Urşilor, respectively, show similar data in terms of age and $\delta^{13}\text{C}$
313 and $\delta^{18}\text{O}$ variation, suggesting a similar environment and physiology.

314 <Insert Figure 9>

315 **CONCLUSIONS**

316 The CO_3 component of dentine apatite retrieved from cave bear canines proves to be a reliable
317 proxy for reconstructing paleoclimatic conditions, physiology and behavior.

318 Based on $\delta^{13}\text{C}$ data profiles, our study shows that the weaning process in cave bears can be
319 identified and attributed to the first and second year of life, while the mother-offspring
320 separation takes place during the second and the third year. Serial dentine samples display
321 large intra-tooth $\delta^{18}\text{O}$ variation of up to 8,2 ‰, reflecting seasonal variations in the $\delta^{18}\text{O}$ of
322 meteoric water and a variable mobility of bears, regardless of sex. This study shows that bear
323 ontogeny – when analyzing the dentine layers – encompasses the neonate period and
324 adulthood, thus providing more data of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ for a single individual and
325 palaeoecological insights than before. Corroborating the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values with
326 radiocarbon data may lead to correlations with past climatic and habitat changes during the
327 lifetime of the cave bears.

328 **ACKNOWLEDGEMENTS**

329 Thirty years ago, Paul L. Koch, Daniel C. Fisher and David Dettman published in *Geology*
330 (1989) a study where they highlighted, for the first time, how dentine serial sampling of bear’s
331 canine can reveal paleoecological and paleoenvironmental insights from the past. Their study
332 was an inspiration to us and therefore, this paper is dedicated to them. The authors are
333 indebted to the Apuseni Mountains Natural Park, to dr. I.N. Meleg, and to prof. dr. B.P. Onac
334 (‘Emil Racoviţă’ Institute of Speleology) for their field assistance and scientific support.
335 This work was financed by the Romanian National Authority for Scientific Research and
336 Innovation, through grants PCE 197/2016 and PCCF 16/2018.

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543 **Figure captions**

544 Figure 1. The studied Romanian MIS 3 cave bears sites: A – Urşilor Cave, B – Cioclovina
545 Cave, C – Oase Cave (cave maps redrawn after Vălena, 1979; Onac et al., 2011; Constantin
546 et al., 2013).

547 Figure 2. Sampling methodology within a cross-section of a cave bear canine. Dentine layers
548 1→6 layers were sampled for stable isotope analysis; the 1st layer is the earliest to form while
549 the 6st is the last to form.

550 Figure 3. Calibrated ¹⁴C AMS ages of the cave bear samples (*N* = 10).

551 Figure 4. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ data obtained from the dentine layers of MIS 3 cave bears from
552 analyzed sites (six canines analyzed/each site). A – C: $\delta^{13}\text{C}$ results from Urşilor (*N* = 36
553 layers), Oase (*N* = 38) and Cioclovina caves (*N* = 38), respectively; D – E: $\delta^{18}\text{O}$ results from
554 Urşilor (*N* = 36), Oase (*N* = 38) and Cioclovina caves (*N* = 38), respectively. X axis 1 → 8:
555 dentine layers.

556 Figure 5: Boxplots of $\delta^{13}\text{C}$ data from Urşilor, Oase and Cioclovina caves. A: females (*N* = 9
557 canines, 52 layers); B: males (*N* = 9 canines, 60 layers).

558 Figure 6: Boxplots of $\delta^{18}\text{O}$ data from Urşilor, Oase and Cioclovina caves. A: females (*N* = 9
559 canines, 52 layers); B: males (*N* = 9 canines, 60 layers).

560 Figure 7: Jitter plot of $\delta^{13}\text{C}$ values from dentine apatite from various MIS 3 mammals across
561 Europe. Cave bears: CBUR – Urşilor (*N* = 6); CBOA – Oase (*N* = 6); CBCI – Cioclovina (*N*
562 = 6); CBAL – Aldéne (*N* = 5); CBMI – Mialet (*N* = 4). Herbivores: CAMI – Mialet (*N* = 3);
563 CEES – Escale (*N* = 2) (data for Aldéne, Mialet and Escale from Bocherens et al., 1994).

564 Figure 8: Hierarchical clustering of average values of $\delta^{18}\text{O}$ of all dentine layers of each
565 analyzed canine (*N* = 18) data from Urşilor, Oase and Cioclovina caves. Red lines indicate
566 warm periods, blue lines indicate colder periods.

567 Figure 9: Multidimensional scaling and minimum spanning tree in a two-dimensional visual
568 representation constructed for the MIS cave bears from the analyzed sites based on: A –
569 average values of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ data for each canine (*N* = 18) (first two components explain 95%
570 of the variance; stress = 0,002); B – $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ (average value for each canine) and ¹⁴C AMS
571 data (*N* = 10) (first two components 88% of the variance; stress = 0,05). Red dots – Oase,
572 yellow dots – Cioclovina, blue dots – Urşilor.

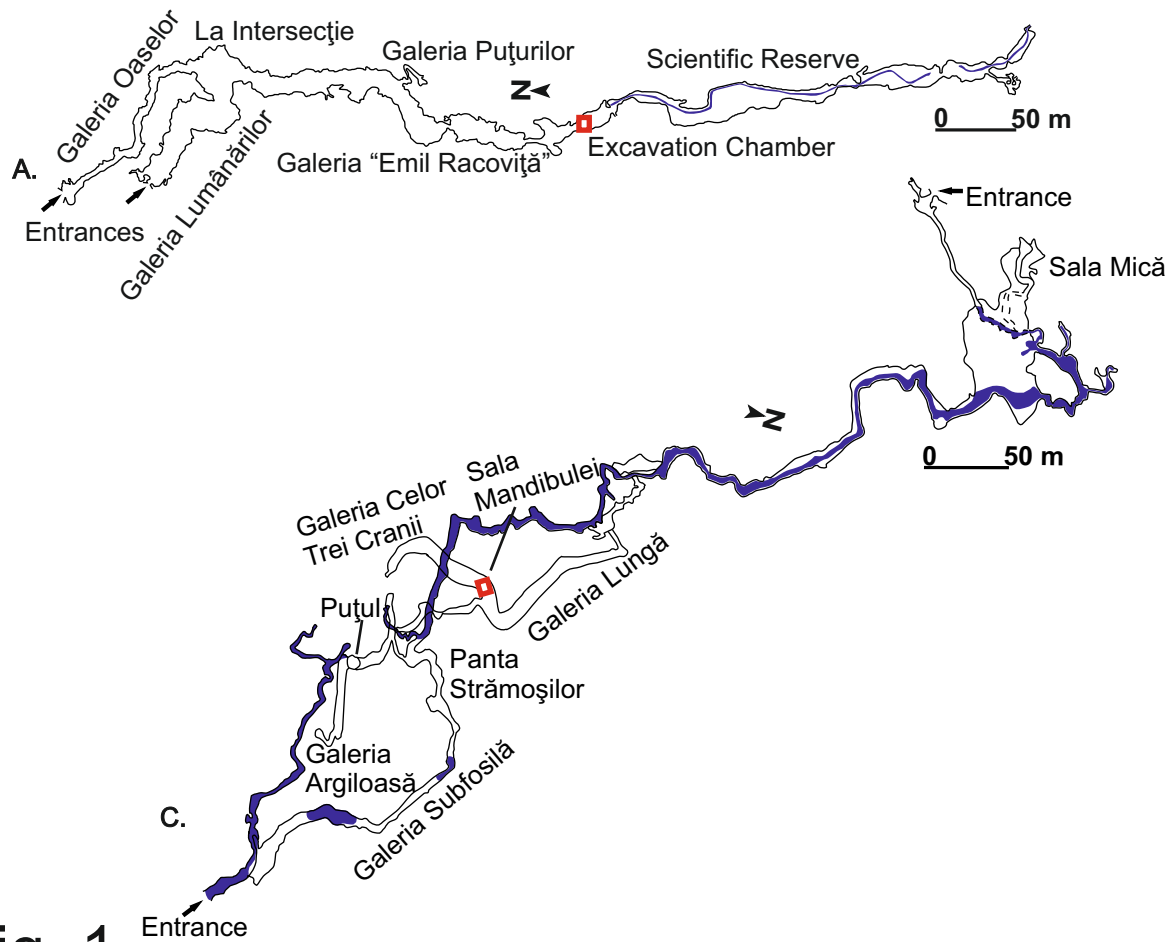


Fig. 1

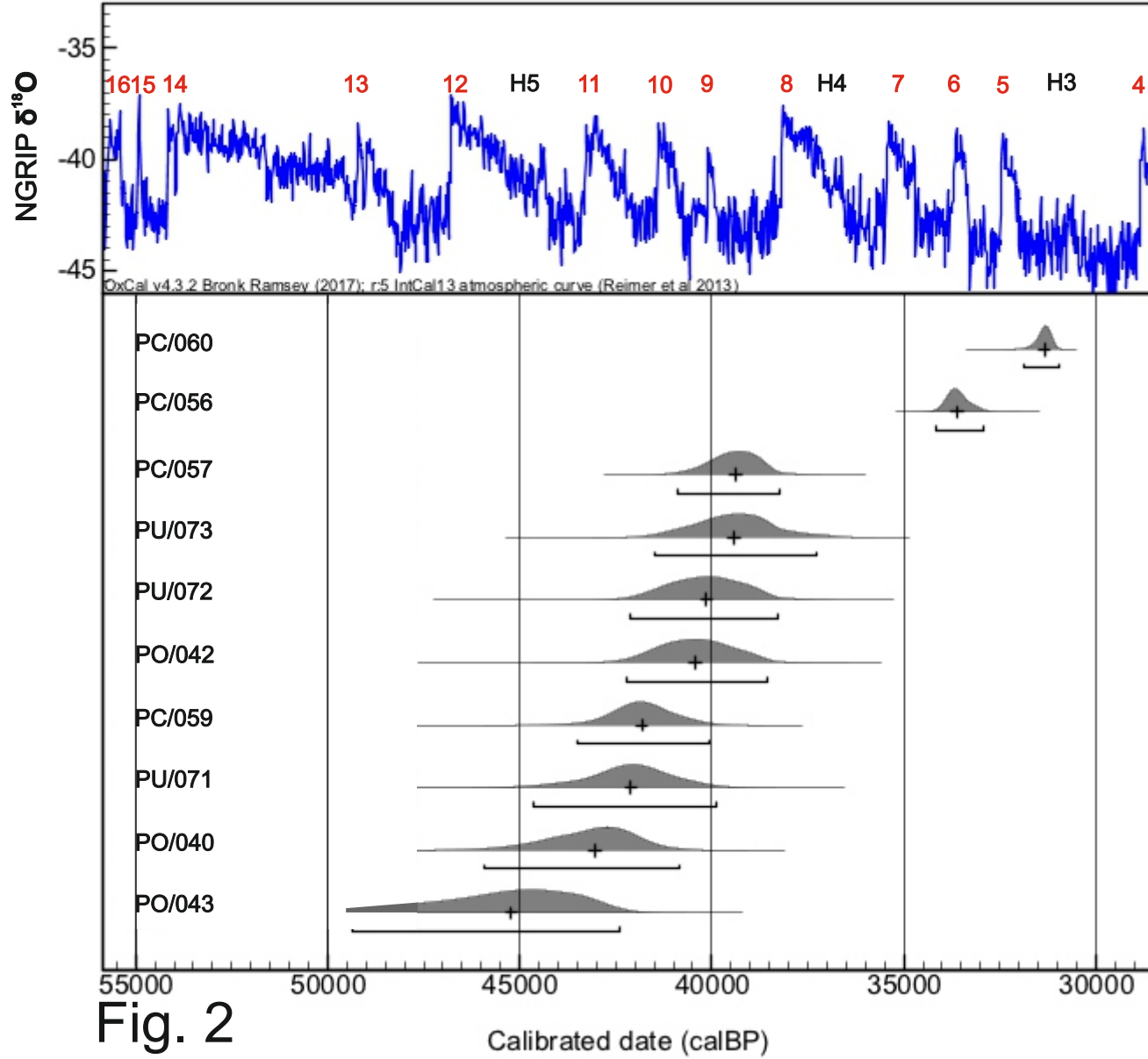


Fig. 2

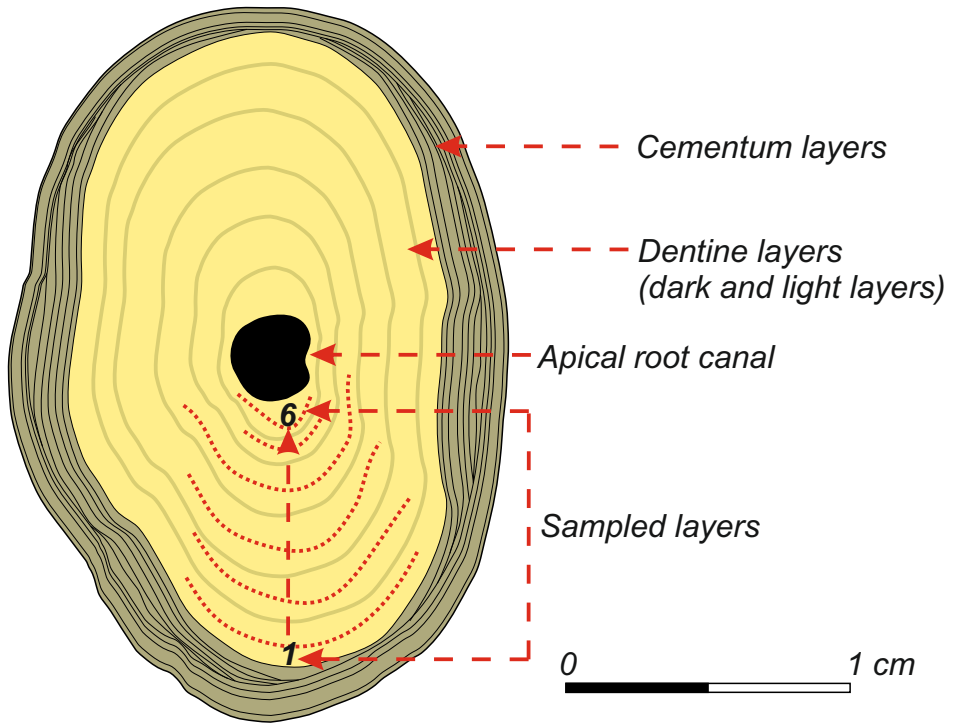
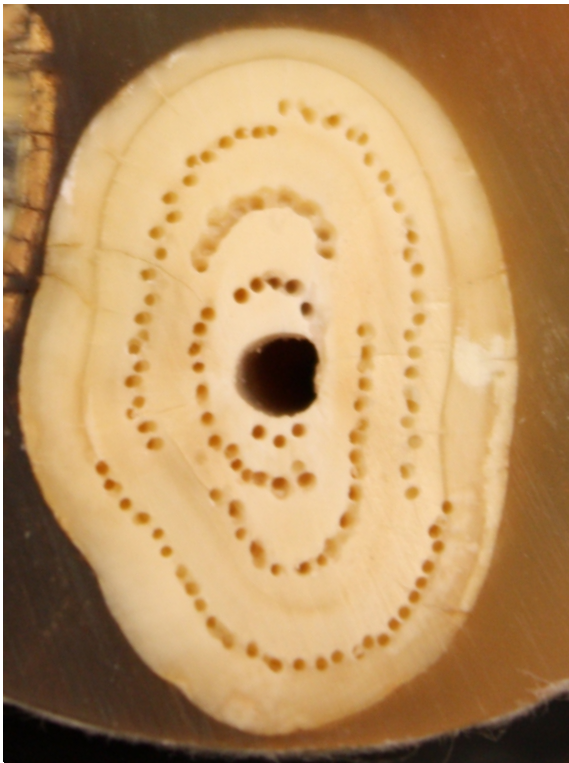


Fig. 3

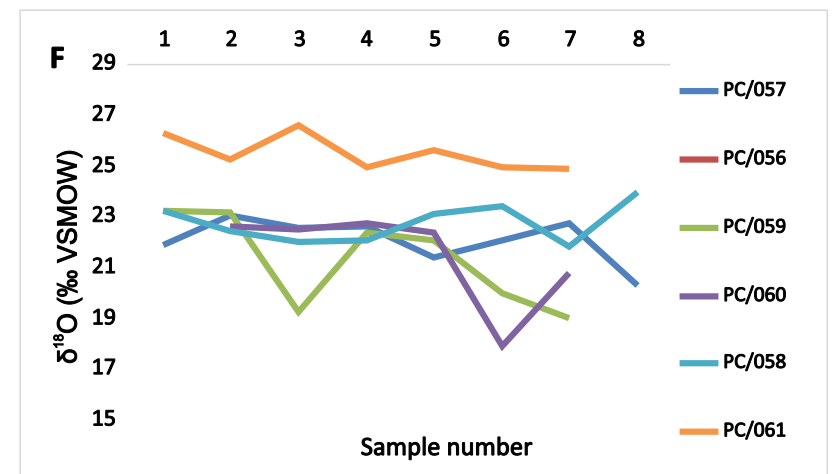
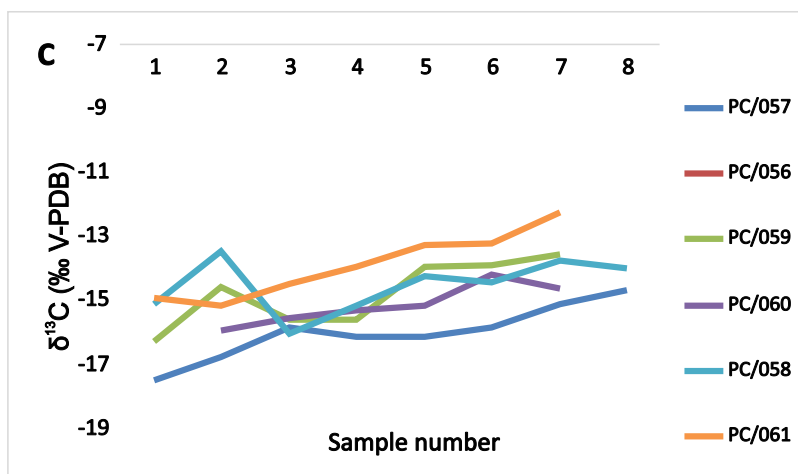
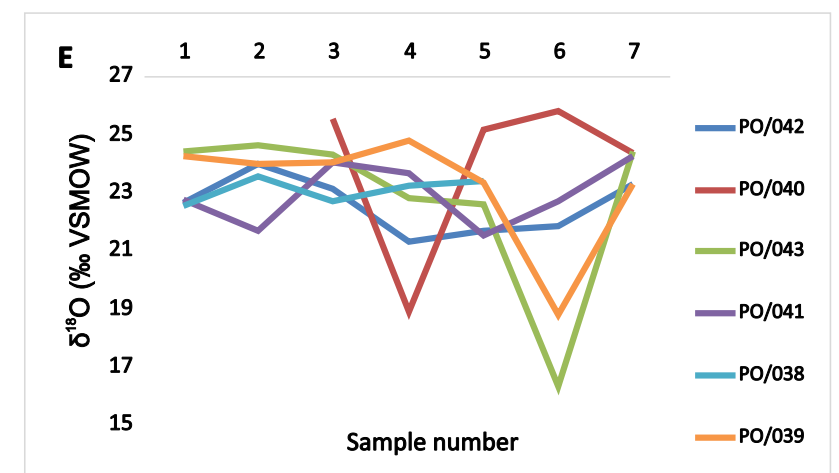
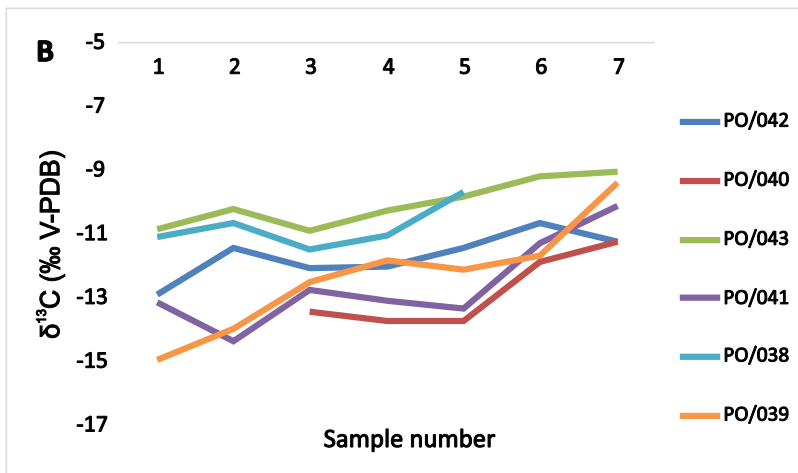
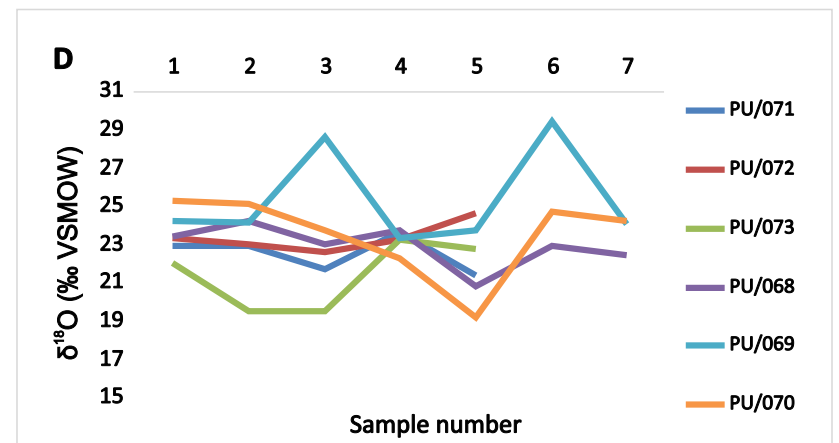
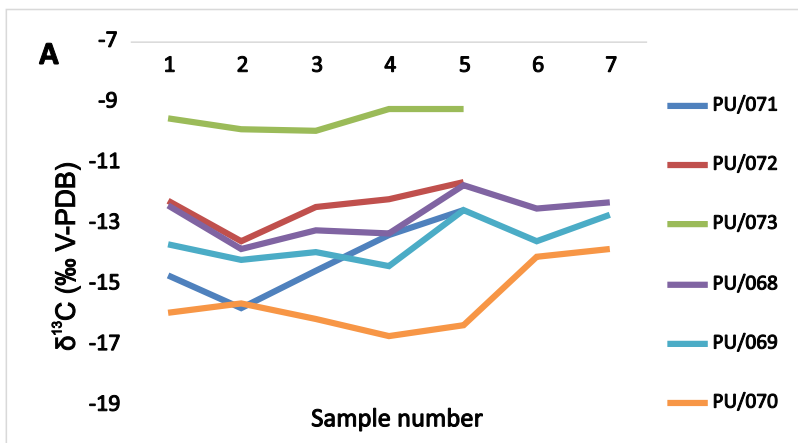


Fig. 4

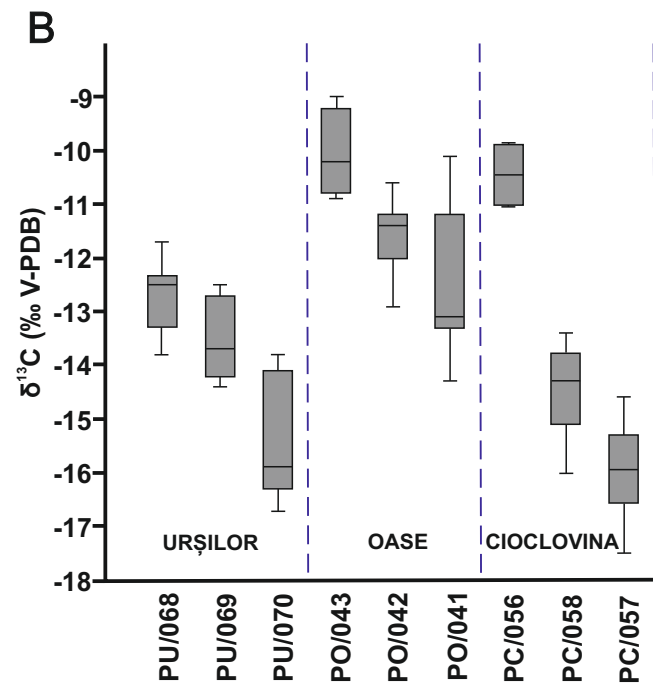
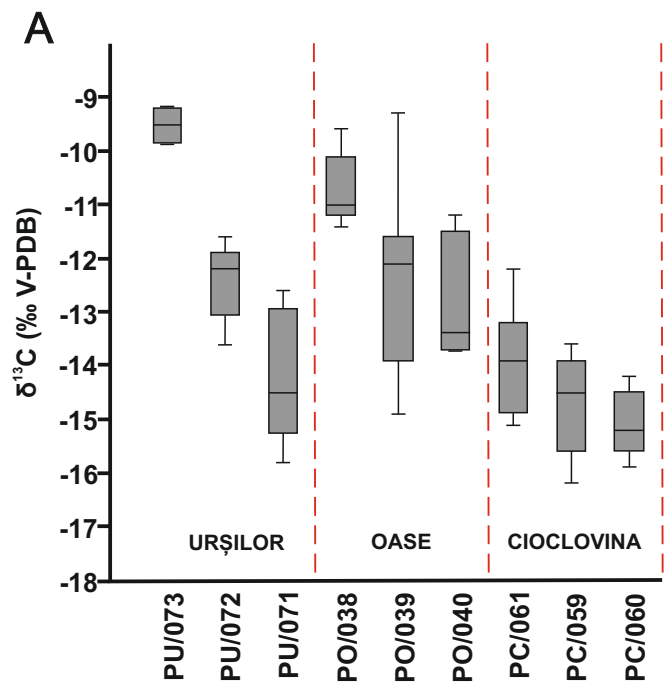


Fig. 5

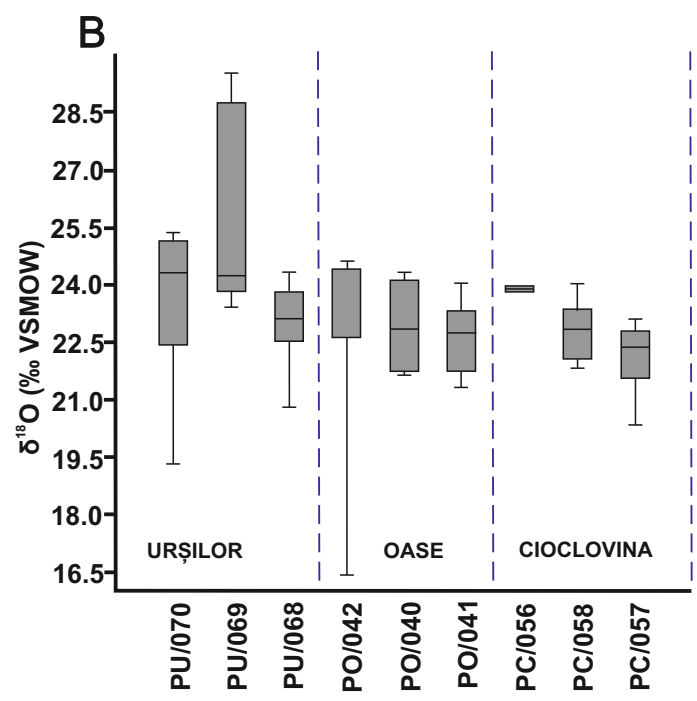
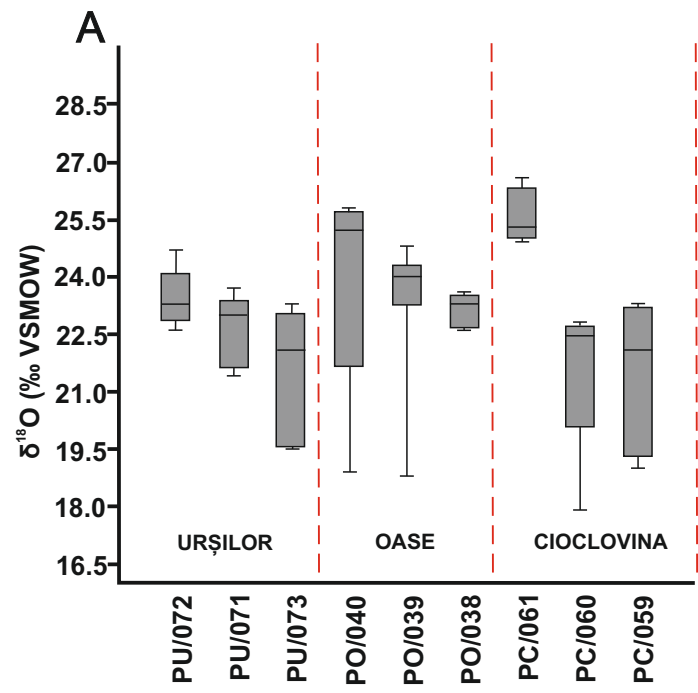


Fig. 6

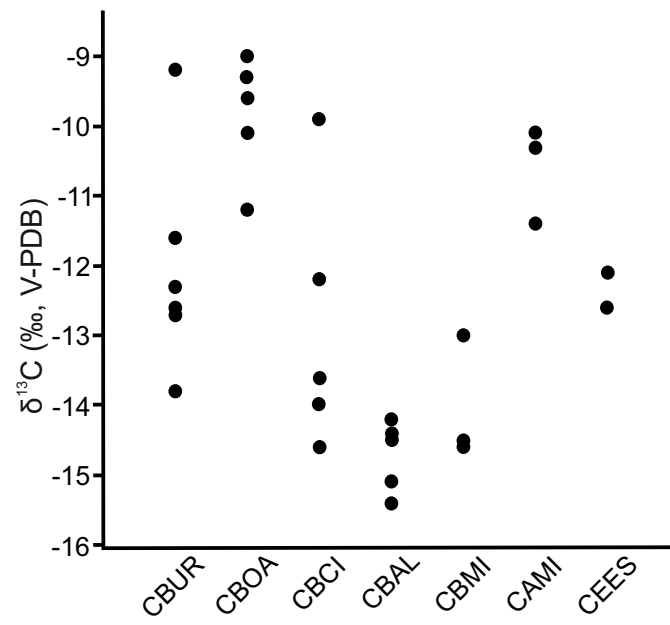


Fig 7

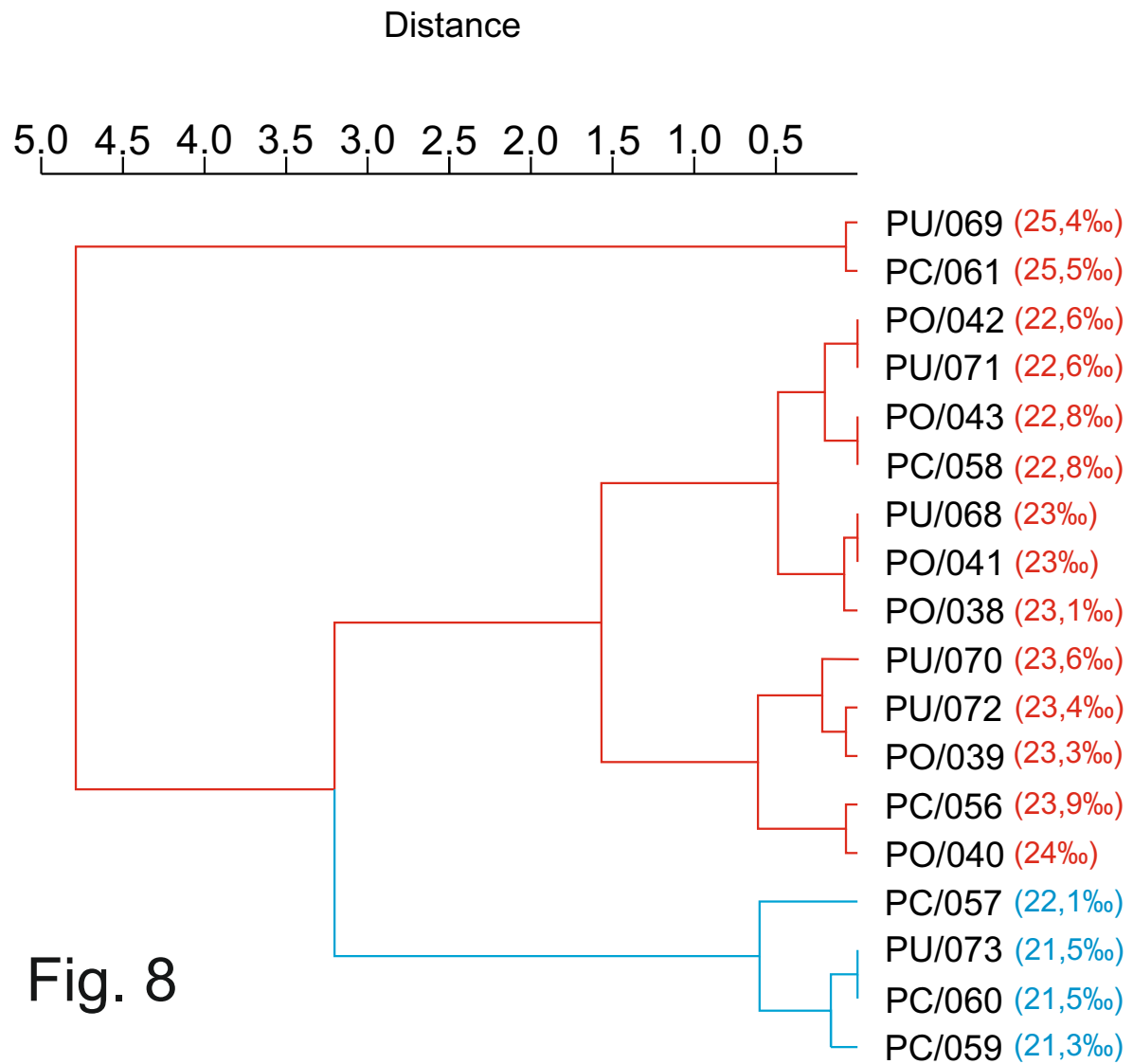


Fig. 8

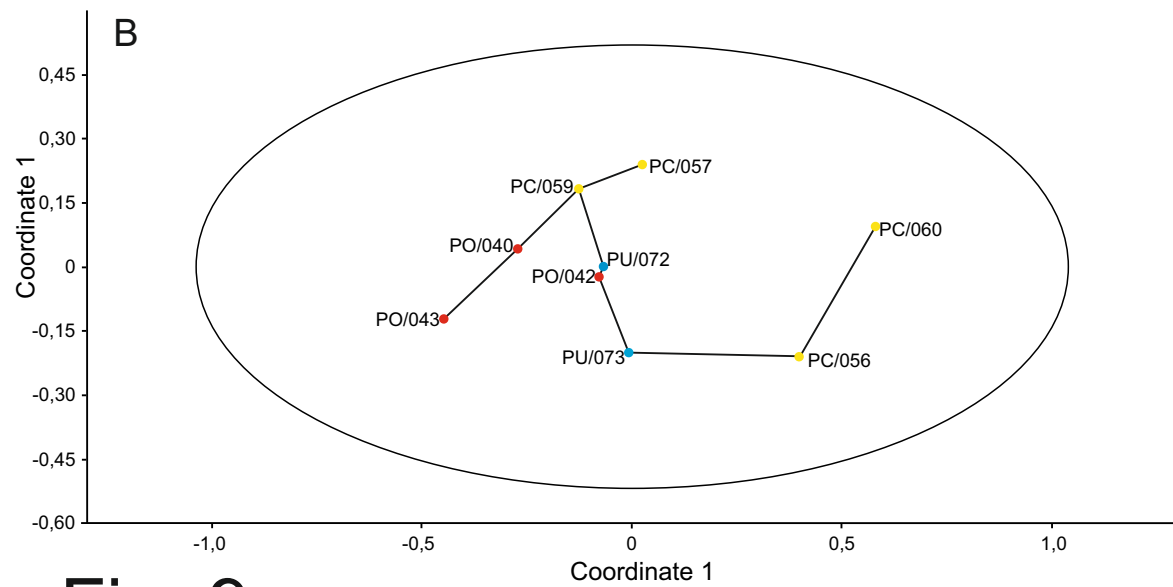
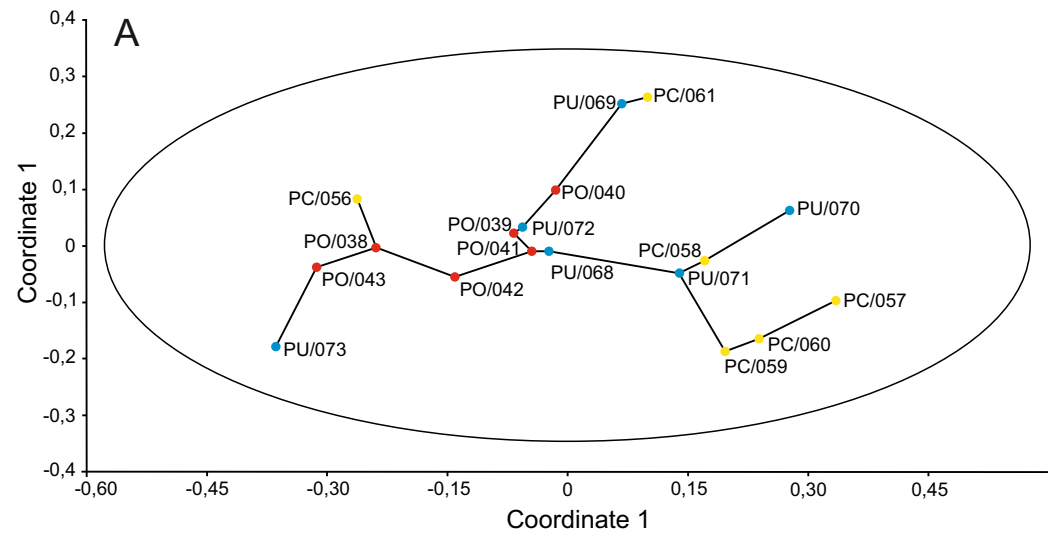


Fig. 9

Sample	Site	Species	Sex	Age	$\delta^{13}\text{C}$ (‰V-PDB)	$\delta^{18}\text{O}$ (‰V-PDB)	%CO3	$\delta^{18}\text{O}$ (‰SMOW)	Radiocarbon age (calibrated; 95.4% probability)
PU/068 1	Urñilor	<i>U. spelaeus</i>	Male	Adult	-12,4	-7,1	6,0	23,5	N/A
PU/068 2	Urñilor	<i>U. spelaeus</i>	–	–	-13,8	-6,4	3,5	24,3	N/A
PU/068 3	Urñilor	<i>U. spelaeus</i>	–	–	-13,2	-7,5	4,5	23,1	N/A
PU/068 4	Urñilor	<i>U. spelaeus</i>	–	–	-13,3	-6,8	5,9	23,8	N/A
PU/068 5	Urñilor	<i>U. spelaeus</i>	–	–	-11,7	-9,7	5,3	20,8	N/A
PU/068 6	Urñilor	<i>U. spelaeus</i>	–	–	-12,5	-7,6	8,2	23,0	N/A
PU/068 7	Urñilor	<i>U. spelaeus</i>	–	–	-12,3	-8,1	3,2	22,5	N/A
PU/069 1	Urñilor	<i>U. spelaeus</i>	Male	Adult	-13,7	-6,4	5,0	24,3	N/A
PU/069 2	Urñilor	<i>U. spelaeus</i>	–	–	-14,2	-6,5	5,2	24,2	N/A
PU/069 3	Urñilor	<i>U. spelaeus</i>	–	–	-13,9	-2,1	3,1	28,7	N/A
PU/069 4	Urñilor	<i>U. spelaeus</i>	–	–	-14,4	-7,3	5,0	23,4	N/A
PU/069 5	Urñilor	<i>U. spelaeus</i>	–	–	-12,5	-6,8	5,9	23,8	N/A
PU/069 6	Urñilor	<i>U. spelaeus</i>	–	–	-13,6	-1,4	4,4	29,5	N/A
PU/069 7	Urñilor	<i>U. spelaeus</i>	–	–	-12,7	-6,5	4,2	24,1	N/A
PU/070 1	Urñilor	<i>U. spelaeus</i>	Male	Adult	-15,9	-5,4	N/A	25,3	N/A
PU/070 2	Urñilor	<i>U. spelaeus</i>	–	–	-15,6	-5,6	N/A	25,1	N/A
PU/070 3	Urñilor	<i>U. spelaeus</i>	–	–	-16,1	-6,8	N/A	23,8	N/A
PU/070 4	Urñilor	<i>U. spelaeus</i>	–	–	-16,7	-8,2	N/A	22,4	N/A
PU/070 5	Urñilor	<i>U. spelaeus</i>	–	–	-16,3	-11,2	N/A	19,3	N/A
PU/070 6	Urñilor	<i>U. spelaeus</i>	–	–	-14,1	-5,9	N/A	24,7	N/A
PU/070 7	Urñilor	<i>U. spelaeus</i>	–	–	-13,8	-6,4	N/A	24,3	N/A
PU/071 1	Urñilor	<i>U. spelaeus</i>	Female	Adult	-14,7	-7,6	5,1	23,0	42,707 - 37,913 cal yr BP
PU/071 2	Urñilor	<i>U. spelaeus</i>	–	–	-15,8	-7,6	2,9	23,0	N/A
PU/071 3	Urñilor	<i>U. spelaeus</i>	–	–	-14,5	-8,8	4,4	21,8	N/A
PU/071 4	Urñilor	<i>U. spelaeus</i>	–	–	-13,3	-6,9	4,1	23,7	N/A
PU/071 5	Urñilor	<i>U. spelaeus</i>	–	–	-12,6	-9,1	3,6	21,4	N/A
PU/072 1	Urñilor	<i>U. spelaeus</i>	Female	Adult	-12,2	-7,3	6,6	23,4	42,144 - 38,286 cal yr BP
PU/072 2	Urñilor	<i>U. spelaeus</i>	–	–	-13,6	-7,6	7,6	23,1	–
PU/072 3	Urñilor	<i>U. spelaeus</i>	–	–	-12,5	-8,0	6,9	22,6	–
PU/072 4	Urñilor	<i>U. spelaeus</i>	–	–	-12,2	-7,3	5,6	23,3	–
PU/072 5	Urñilor	<i>U. spelaeus</i>	–	–	-11,6	-6,0	5,2	24,7	–
PU/073 1	Urñilor	<i>U. spelaeus</i>	Female	Adult	-9,5	-8,5	7,1	22,1	41,490 - 37,276 cal yr BP
PU/073 2	Urñilor	<i>U. spelaeus</i>	–	–	-9,8	-10,9	6,0	19,6	–

PU/073 3	Urñilor	<i>U. spelaeus</i>	–	–	-9,9	-11,0	5,7	19,5	–
PU/073 4	Urñilor	<i>U. spelaeus</i>	–	–	-9,2	-7,4	6,2	23,3	–
PU/073 5	Urñilor	<i>U. spelaeus</i>	–	–	-9,2	-7,8	5,0	22,8	–
PO/038 1	Oase	<i>U. spelaeus</i>	Female	Adult	-11,0	-8,0	4,8	22,6	N/A
PO/038 2	Oase	<i>U. spelaeus</i>	–	–	-10,6	-7,1	3,9	23,6	N/A
PO/038 3	Oase	<i>U. spelaeus</i>	–	–	-11,4	-7,9	4,9	22,7	N/A
PO/038 4	Oase	<i>U. spelaeus</i>	–	–	-11,0	-7,3	4,0	23,3	N/A
PO/038 5	Oase	<i>U. spelaeus</i>	–	–	-9,6	-7,2	3,9	23,4	N/A
PO/039 1	Oase	<i>U. spelaeus</i>	Female	Adult	-14,9	-6,4	2,2	24,3	N/A
PO/039 2	Oase	<i>U. spelaeus</i>	–	–	-13,9	-6,6	3,9	24,0	N/A
PO/039 3	Oase	<i>U. spelaeus</i>	–	–	-12,5	-6,6	2,5	24,1	N/A
PO/039 4	Oase	<i>U. spelaeus</i>	–	–	-11,8	-5,9	2,5	24,8	N/A
PO/039 5	Oase	<i>U. spelaeus</i>	–	–	-12,1	-7,3	2,6	23,4	N/A
PO/039 6	Oase	<i>U. spelaeus</i>	–	–	-11,6	-11,7	3,4	18,8	N/A
PO/039 7	Oase	<i>U. spelaeus</i>	–	–	-9,3	-7,3	1,9	23,3	N/A
PO/040 3	Oase	<i>U. spelaeus</i>	Female	Adult	-13,4	-5,14	N/A	25,6	45,911 - 40,842 cal yr BP
PO/040 4	Oase	<i>U. spelaeus</i>	–	–	-13,7	-11,6	N/A	18,9	–
PO/040 5	Oase	<i>U. spelaeus</i>	–	–	-13,7	-5,5	N/A	25,2	–
PO/040 6	Oase	<i>U. spelaeus</i>	–	–	-11,8	-4,9	N/A	25,8	–
PO/040 7	Oase	<i>U. spelaeus</i>	–	–	-11,2	-6,3	N/A	24,4	–
PO/041 1	Oase	<i>U. spelaeus</i>	Male	Adult	-13,1	-7,8	6,3	22,8	N/A
PO/041 2	Oase	<i>U. spelaeus</i>	–	–	-14,3	-8,9	6,2	21,7	N/A
PO/041 3	Oase	<i>U. spelaeus</i>	–	–	-12,7	-6,6	6,1	24,1	N/A
PO/041 4	Oase	<i>U. spelaeus</i>	–	–	-13,1	-7,0	6,3	23,7	N/A
PO/041 5	Oase	<i>U. spelaeus</i>	–	–	-13,3	-9,0	4,7	21,6	N/A
PO/041 6	Oase	<i>U. spelaeus</i>	–	–	-11,2	-7,9	5,5	22,7	N/A
PO/041 7	Oase	<i>U. spelaeus</i>	–	–	-10,1	-6,4	5,5	24,3	N/A
PO/042 1	Oase	<i>U. spelaeus</i>	Male	Adult	-12,9	-8,0	6,4	22,7	42,230 - 38,560 cal yr BP
PO/042 2	Oase	<i>U. spelaeus</i>	–	–	-11,4	-6,7	5,4	24,0	–
PO/042 3	Oase	<i>U. spelaeus</i>	–	–	-12,0	-7,5	5,1	23,2	–
PO/042 4	Oase	<i>U. spelaeus</i>	–	–	-12,0	-9,3	5,7	21,3	–
PO/042 5	Oase	<i>U. spelaeus</i>	–	–	-11,4	-8,9	4,5	21,7	–
PO/042 6	Oase	<i>U. spelaeus</i>	–	–	-10,6	-8,7	4,9	21,9	–
PO/042 7	Oase	<i>U. spelaeus</i>	–	–	-11,2	-7,3	5,2	23,3	–
PO/043 1	Oase	<i>U. spelaeus</i>	Male	Adult	-10,8	-6,2	N/A	24,4	49,347 - 42403 cal yr BP

PO/043 2	Oase	<i>U. spelaeus</i>	–	–	-10,2	-6	N/A	24,6	–
PO/043 3	Oase	<i>U. spelaeus</i>	–	–	-10,9	-6,3	N/A	24,4	–
PO/043 4	Oase	<i>U. spelaeus</i>	–	–	-10,2	-7,8	N/A	22,8	–
PO/043 5	Oase	<i>U. spelaeus</i>	–	–	-9,8	-8	N/A	22,6	–
PO/043 6	Oase	<i>U. spelaeus</i>	–	–	-9,2	-14,1	5,2	16,4	–
PO/043 7	Oase	<i>U. spelaeus</i>	–	–	-9	-6,3	N/A	24,4	–
PC/056 1	Cioclovina	<i>U. spelaeus</i>	Male	Adult	-11	-6,8	N/A	23,8	34,166 - 32,924 cal yr BP
PC/056 6	Cioclovina	<i>U. spelaeus</i>	–	–	-9,9	-6,7	N/A	23,9	–
PC/057 1	Cioclovina	<i>U. spelaeus</i>	Male	Adult	-17,5	-8,7	3,0	21,9	40,888 - 38,215 cal yr BP
PC/057 3	Cioclovina	<i>U. spelaeus</i>	–	–	-16,7	-7,5	2,4	23,1	–
PC/057 4	Cioclovina	<i>U. spelaeus</i>	–	–	-15,8	-8,0	2,5	22,6	–
PC/057 5	Cioclovina	<i>U. spelaeus</i>	–	–	-16,1	-8,0	2,7	22,7	–
PC/057 6	Cioclovina	<i>U. spelaeus</i>	–	–	-16,1	-9,2	3,4	21,4	–
PC/057-7	Cioclovina	<i>U. spelaeus</i>	–	–	-15,8	-8,5	3,9	22,1	–
PC/057 8	Cioclovina	<i>U. spelaeus</i>	–	–	-15,1	-7,8	4,0	22,8	–
PC/057 9	Cioclovina	<i>U. spelaeus</i>	–	–	-14,6	-10,2	1,6	20,3	–
PC/058 1	Cioclovina	<i>U. spelaeus</i>	Male	Adult	-15,1	-7,4	2,5	23,2	N/A
PC/058 2	Cioclovina	<i>U. spelaeus</i>	–	–	-13,4	-8,1	3,7	22,5	N/A
PC/058 3	Cioclovina	<i>U. spelaeus</i>	–	–	-16,0	-8,6	3,3	22,0	N/A
PC/058 4	Cioclovina	<i>U. spelaeus</i>	–	–	-15,1	-8,5	3,7	22,1	N/A
PC/058 5	Cioclovina	<i>U. spelaeus</i>	–	–	-14,2	-7,5	2,4	23,1	N/A
PC/058 6	Cioclovina	<i>U. spelaeus</i>	–	–	-14,4	-7,2	2,5	23,4	N/A
PC/058 7	Cioclovina	<i>U. spelaeus</i>	–	–	-13,7	-8,7	3,1	21,8	N/A
PC/058 8	Cioclovina	<i>U. spelaeus</i>	–	–	-14	-6,6	N/A	24,0	N/A
PC/059 1	Cioclovina	<i>U. spelaeus</i>	Female	Adult	-16,2	-7,4	4,6	23,3	43,046 - 40,446 yr cal BP
PC/059 2	Cioclovina	<i>U. spelaeus</i>	–	–	-14,5	-7,5	N/A	23,2	–
PC/059 3	Cioclovina	<i>U. spelaeus</i>	–	–	-15,5	-11,2	4,4	19,3	–
PC/059 4	Cioclovina	<i>U. spelaeus</i>	–	–	-15,6	-8,2	4,1	22,4	–
PC/059 5	Cioclovina	<i>U. spelaeus</i>	–	–	-13,9	-8,5	4,6	22,1	–
PC/059 6	Cioclovina	<i>U. spelaeus</i>	–	–	-13,9	-10,5	1,9	20,0	–
PC/059 7	Cioclovina	<i>U. spelaeus</i>	–	–	-13,6	-11,5	2,3	19,0	–
PC/060 2	Cioclovina	<i>U. spelaeus</i>	Female	Adult	-15,9	-7,9	3,2	22,7	31,860 - 30,974 cal yr BP
PC/060 3	Cioclovina	<i>U. spelaeus</i>	–	–	-15,5	-8,1	2,0	22,5	–
PC/060 4	Cioclovina	<i>U. spelaeus</i>	–	–	-15,3	-7,8	3,6	22,8	–
PC/060 5	Cioclovina	<i>U. spelaeus</i>	–	–	-15,1	-8,2	3,2	22,4	–

PC/060 6	Cioclovina	<i>U. spelaeus</i>	–	–	-14,2	-12,6	2,2	17,9	–
PC/060 7	Cioclovina	<i>U. spelaeus</i>	–	–	-14,6	-9,7	1,9	20,8	–
PC/061 1	Cioclovina	<i>U. spelaeus</i>	Female	Adult	-14,9	-4,4	–	26,3	N/A
PC/061 2	Cioclovina	<i>U. spelaeus</i>	–	–	-15,1	-5,4	–	25,3	N/A
PC/061 3	Cioclovina	<i>U. spelaeus</i>	–	–	-14,5	-4,1	–	26,6	N/A
PC/061 4	Cioclovina	<i>U. spelaeus</i>	–	–	-13,9	-5,7	–	25,0	N/A
PC/061 5	Cioclovina	<i>U. spelaeus</i>	–	–	-13,2	-5	–	25,7	N/A
PC/061 6	Cioclovina	<i>U. spelaeus</i>	–	–	-13,2	-5,7	–	25,0	N/A
PC/061 7	Cioclovina	<i>U. spelaeus</i>	–	–	-12,2	-5,8	–	24,9	N/A

Table S1. Romanian/Carpathian MIS 3 cave bear carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) stable isotope samples and results.