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The last of its kind? Radiocarbon, ancient DNA and stable isotope evidence from a late cave bear (*Ursus spelaeus* ROSENMÜLLER, 1794) from Rochedane (France)

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A B S T R A C T

We report here a new discovery of a cave bear left metatarsal 3 from Rochedane, an archaeological site near Montbeliard (French Jura) that yielded only Lateglacial and Holocene material, with no evidence of pre-LGM deposits, a context that made this bone a possible candidate for being a post-LGM cave bear in western Europe. To test this hypothesis, this bone was analyzed for mitochondrial DNA, which confirmed its attribution to cave bear of the *Ursus spelaeus* lineage, and a direct radiocarbon AMS dating on well preserved collagen (%C, %N and C/N well in the range of fresh collagen) yielded an age of 23,900 +110 −100 BP (28,730–28,500 cal BP, one sigma range). Its carbon and nitrogen isotopic values were similar to those of slightly older cave bears from the Swabian Jura, around 300 km to the East, suggesting that the ecological preferences of cave bears remained unchanged until the extirpation of this species in western Europe. Interestingly, the genetic type *U. spelaeus* was replaced by *Ursus ingressus* around 28,000 ^{14}C BP in the Swabian Jura. In contrast, the older type *U. spelaeus* apparently persisted in France ca. 3000 years longer. Traces left on the cave bear metapodium have been left by human activity on this bone, as it was the case for older cave bear bones from the Swabian Jura. This case study shows that cave bear remains found in post-LGM sites or layers may be candidates to be late survivors of this extinct species, but without direct radiocarbon AMS dated on well-preserved collagen (demonstrated by actual chemical composition results) and ancient DNA confirmation of the species attribution, such evidence can only be considered dubious.

1. Introduction

The Late Quaternary large mammal extinctions that took place between around 40,000 and 10,000 years ago in Europe are still a subject of intensive debate (e.g. Koch and Barnosky, 2006; Pushkina and Raia, 2008; Bridault and Chaix, 2009; Lorenzen et al., 2011; Prescott et al., 2012). Even fundamental aspects such as the timing of the extinctions, i.e. whether they took place before the Last Glacial Maximum (pre-LGM), at the end of the Pleistocene 12,000 years ago or later during the Holocene, are still under investigation. The ultimate causes that led to this large scale extinction, with humans and climate change being the main potential causes discussed, are not yet well understood. Both were probably playing a role, but to different extents for different species and in different areas (Prescott et al., 2012). In any case, a better understanding of the mechanisms that led to the extinction of several large mammal species in Europe in a context of climate instability and changing human impact on the ecosystems will have implications for anticipating possible future extinctions.

The cave bear (*Ursus spelaeus*) is a particularly interesting species to focus upon because it is probably the Late Quaternary

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mammal that yielded the largest amount of fossil material in Europe, with a large part associated with human deposits in archaeological sites. It is one of the large mammal species that disappeared during the last 30,000 years, but the exact time and cause of its extinction remain unclear (e.g. Stuart and Lister, 2007; Pacher and Stuart, 2009). Also, thanks to the very good general molecular preservation of cave bear bones in karst contexts, numerous studies dealing with stable isotopes of collagen and ancient DNA have yielded invaluable information about the palaeoecology and the phylogeography of this extinct species (e.g. Bocherens et al., 1994, 2006, 2011b; Hofreiter et al., 2002, 2007; Orlando et al., 2002; Stiller et al., 2010; Münzel et al., 2011). The combination of both approaches has also yielded interesting results about the population dynamics of this species in relationship with its ecological habits (e.g. Münzel et al., 2011; Bocherens et al., 2011b).

In the region including the French, Swiss, and German parts of the Jura Mountains, termed the northwestern alpine foreland in this paper, numerous cave bear bones have been recovered from archaeological layers attributed to the Magdalenian culture (Kurtén, 1976; Musil, 1981). Faunal remains from such contexts in this region are radiocarbon dated to between around 12,000 and 19,600 BP (13,800–23,900 years cal BP; Drucker et al., 2012). Magdalenian occupation of northwestern Europe started with the climatic warming following the Last Glacial Maximum (LGM) and probably ended with the climatic amelioration of the Greenland Interstadial GI-1e which coincided with a change in terrestrial ecosystems (Straus et al., 2012). However, evidence of cave bear survival after the LGM must be based on unambiguous taxonomic attribution, since the closely related brown bear (*Ursus arctos*) was present in this region at this time, and the chronological attribution must be established through direct radiocarbon dating of the same cave bear bones or teeth. Using mostly ancient DNA analysis and AMS radiocarbon dating of bone collagen, a number of alleged cave bear founds post-dating the LGM have been evaluated, and, although the taxonomic attribution was confirmed by palaeogenetic analysis, all of the studied samples yielded ^{14}C ages older than 24,000 BP (Table 1, Fig. 1). This apparent lack of evidence for cave bears younger than the LGM in the northwestern alpine foreland must be confirmed by further investigations of Magdalenian faunal assemblages in this region. In the present work, we report the result of such an investigation on one cave bear bone from a site in the French Jura, Rochedane, where so far the oldest archaeological layers contain evidence of the Magdalenian culture. We applied to this bone all the techniques that will allow us to fit this specimen into a chronological, phylogeographical and ecological context and yield further information that will help to understand the extinction process of cave bears in this region.

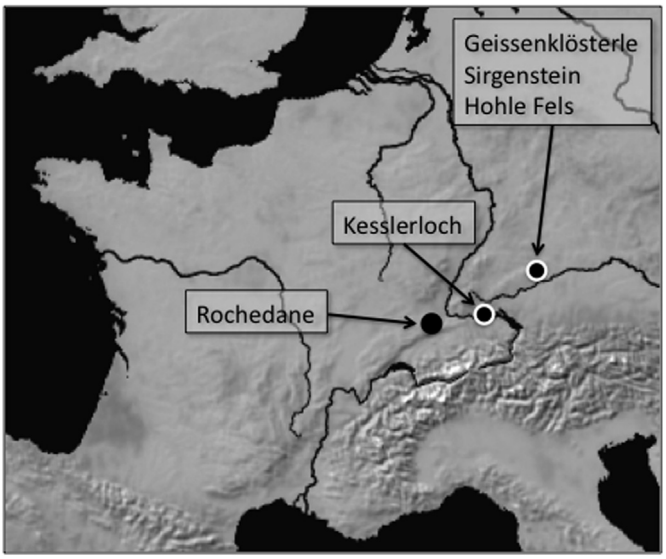

Fig. 1. Geographical location of sites with remains of cave bear supposedly associated with Magdalenian archaeological layers. The numbers correspond to sites from which cave bears for the osteometric comparisons come from: 1. Grotte de Vaucluse, 2. Grotte des Crosses, 3. Goyet, 4. Zoolithenhöhle-Spalte, 5. Hohle Fels, 6. Brillenhöhle VI, 7. Erpfingen, 8. Buse di Bernardo.

2. Material

The site of Rochedane (47°21′N, 6°46′E) is located approx. 25 km south of the town of Montbeliard on the left bank of the Doubs River. It is a rock-shelter dug into a calcareous cliff about 15 m high, at an altitude of 355 m above sea level in an area where the neighboring plateaus rise to 700 m above sea level. Excavation of Rochedane by André Thévenin and Jean Sainty (1968–1976) identified several archaeological layers ranging between ca. 12,500 and 8000 BP (e.g. Thévenin, 1982; Thévenin and Sainty, 1998). The deepest archaeological layer (D1) yielded ^{14}C dates ranging from 12,235 ± 85 to 12,420 ± 75 BP, but another layer called D2 without any archaeological artefact was also recognized below D1. A skeletal part attributed to cave bear, a third left metatarsal, was found outside a clear stratigraphical context, probably from D2 or lower levels (Fig. 2). An osteological examination suggested that this specimen had characteristics indicative of a cave bear *U. spelaeus* rather than of a brown bear *U. arctos*, present in the Holocene levels of the rock-shelter. As the archaeological excavation did not provide any evidence for pre-LGM deposits, this possible cave bear bone may be evidence of the post-LGM cave bear in Northeastern France. However, as many other similar occurrences proved to be older than expected, even in sites with no obvious evidence of deposits

Table 1
List of cave bear bones reported in post-LGM archaeological layers in the Northwestern alpine foreland. Italicized AMS 14C dating stands for discarded date due to poor collagen preservation.

Site	Country	Piece	Archaeological context	Species (method)	Direct AMS ^{14}C dating (BP)	Reference
Geißenklösterle	Germany	Maxilla (juvenile)	Magdalenian/Gravettian	*Ursus ingressus* (DNA)	*13,230 ± 130* (OxA-4854) 24,210 ± 100 (GrA-39687)	Münzel et al. (2007) Münzel et al. (2008)
Geißenklösterle	Germany	3rd upper incisor	Magdalenian/Gravettian	*Ursus ingressus* (DNA)	26,740 ± 120 (Beta-143244)	Münzel et al. (2008, 2011)
Geißenklösterle	Germany	3rd upper incisor	Magdalenian/Gravettian	*Ursus ingressus* (DNA)	27,240 ± 200 (Beta-161019)	Münzel et al. (2008, 2011)
Geißenklösterle	Germany	2nd lower incisor	Magdalenian/Gravettian	*Ursus ingressus* (DNA)	27,340 ± 180 (Beta-161018)	Münzel et al. (2008, 2011)
Geißenklösterle	Germany	2nd lower incisor	Magdalenian/Gravettian	*Ursus ingressus* (DNA)	27,870 ± 190 (Beta-161020)	Münzel et al. (2008, 2011)
Hohle Fels	Germany	3rd upper incisor	Magdalenian?	*Ursus ingressus* (DNA)	28,040 ± 200 (Beta-161021)	Münzel et al. (2008, 2011)
Sirgentein	Germany	1st lower molar	Magdalenian?	*Ursus ingressus* (DNA)	25,560 ± 130 (OxA-12013)	Münzel et al. (2008)
Sirgentein	Germany	1st lower molar	Magdalenian?	*Ursus ingressus* (DNA)	26,980 ± 130 (OxA-12014)	Münzel et al. (2008)
Sirgentein	Germany	1st lower molar	Magdalenian?	*Ursus ingressus* (DNA)	35,770 ± 330 (OxA-12015)	Münzel et al. (2008)
Kesslerloch	Switzerland	2nd upper molar	Magdalenian?	*Ursus* cf *spelaeus* (morpho.)	36,400 +420/−400 (KIA-33349)	Napierala (2008)

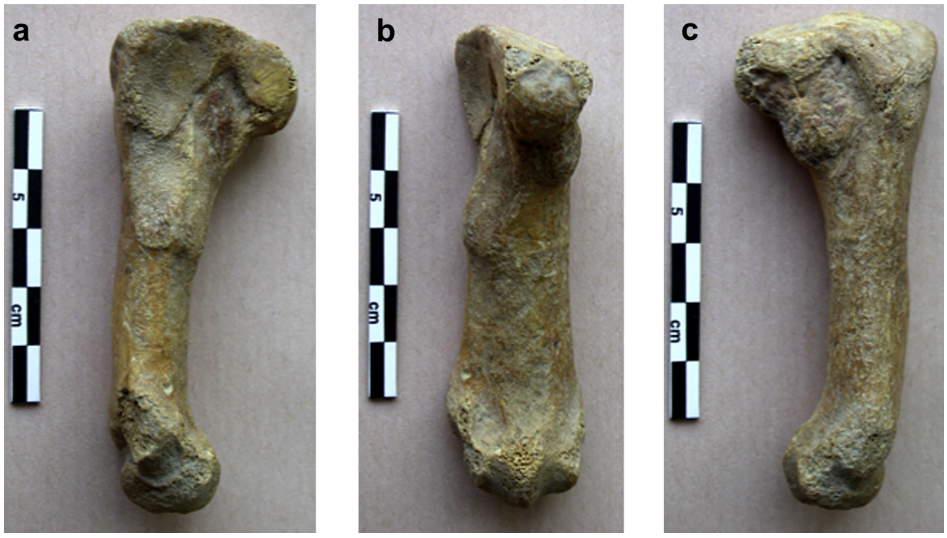

Fig. 2. Pictures of the studied bone: a and c: lateral views; b: ventral view.

older than the LGM, a complete analytical program was undertaken including taxonomical determination using osteometry and palaeogenetics, direct AMS radiocarbon dating, and stable isotopic tracking of palaeoecological parameters.

3. Methods

3.1. Osteometry

Cave bears are closely related to brown bears, both species showing large morphological variability due to different factors such as individual age, sexual dimorphism and geographical variation. Therefore, although many skeletal parts are similar in both bear species, it is possible to evaluate the range of variation for a given bone, in this case the third metatarsal, in order to identify the taxonomic status of the specimen.

To compare the size of the 3rd metatarsal from Rochedane, we used two combined measurements, the greatest length (GL) and the smallest breadth of diaphysis (SB), following Athen et al. (2005). As it is of general use, only fused specimens were taken into account, in order to eliminate variation due to lengthwise bone growth.

Cave bear assemblages from sites located in the circumscribed geographical areas mentioned above and included in a broad similar chronological range were selected for comparison (Fig. 1). The following caves are all radiocarbon dated to between ca. 40,000 and 25,000 ^{14}C BP: Goyet (Condroz, Belgium); Hohle Fels, Erpfingen and Brillenhöhle (Baden-Württemberg, Germany); Zoolithenhölhe-Spalte (Bavaria, Germany); Buse di Bernardo (Trentino Alto Adige, Italy). Grotte des Crosses (Doubs, France) is attributed to the Upper Pleistocene. Grotte de Vaucluse, nearby Rochedane, has yielded the richest corpus of third metatarsals ($N = 98$) (Schweizer, 1999, 2005), but it is undated. The gathered data are listed in Table 2.

Similar osteometric data are less numerous for brown bears and had to be collected from a larger sample of paleontological and archaeological sites located in various European areas (Table 3). Some data from reference collections (modern bears) were also added for comparison (Müller, 1971; Schweizer, 1999; Athen, 2007).

Bears, and particularly cave bears, display a strong sexual dimorphism (Grandal-d'Anglade, 2001). An unbalanced sex-ratio would then influence the average values of measurements in the distributions. Mixture analysis has proved to be a relevant method for sex determination when applied to cave bear assemblages (Quiles et al., 2005). "Mixture analysis is a maximum-likelihood method for estimating the parameters (mean, standard deviation and proportion) of two or more univariate normal distributions, based on a pooled univariate sample" (Hammer et al., 2001). It was applied here together with a normality statistical test (Anderson–Darling) using the software Past (Hammer et al., 2001), in order to first analyze if each distribution could be derived from a population with normal distribution (H0) or biased by an unbalanced sex ratio. The only sample for which normal distribution can be rejected is Grotte Vaucluse. It is significantly biased in favor of the highest values (71.5% and 77.5% for group#2, Table S3.), for males (Table S3).

3.2. Ancient DNA

A subsample of 185 mg was taken from the original metapodial specimen RCD10900cb that yielded the radiocarbon date. Ancient DNA was then extracted using a column based silica extraction protocol as described in Rohland et al. (2010). Extractions accompanied by negative controls were performed in a dedicated ancient DNA laboratory, physically separated from any post-PCR workspaces. We used 5 µl of ancient DNA extract and primers 2670a (5′-CTATTTAAACTATTCCCTGGTACATAC-3′) and 2719 (5′-ATGGGGG-CACGCCATTAATGC-3′) to generate a 165 bp PCR product (117 bp excluding primer sequences). Primers and amplification conditions were adopted from Hofreiter et al. (2004b). The amplification reactions were done in replicates and negative controls included at all times. PCR products were then cloned using the TOPO TA cloning kit (Life Technologies) and 6 clones were Sanger sequenced on an ABI 3730 capillary sequencing instrument. We then aligned all clone sequences of both PCR replicates and called a consensus sequence using the software BioEdit (Hall, 1999).

Table 2
Chemical and isotopic characteristics of the Rochedane cave bear collagen.

Species	Site	^{14}C age	Age cal BP (1s)	Date no.	Lab no.	Coll. yield (mg g^{-1})	%C	%N	C/N	Δ^{13}C (‰)	δ^{15}N (‰)
Ursus spelaeus	Rochedane	23,900 +110/−100	BP 28.730–28.500 cal	BP GrA-52632	RCD-10900cb	21	37.1	13.4	3.2	−21.0	4.6

Table 3
Radiocarbon dating of young cave bears in Europe.

Species	Site	Location	Piece	Age	Date no.	aDNA	Collagen yield (mg g^{-1})	%C	%N	C/N	Range calib 1s	Rang calib 2s	Reference
Ursus ingressus	Vindija Cave	Croatia	?	23,780 ± 120	Beta-156100	*U. ingressus*	nd	nd	nd	nd	28,322–28,733	28,059–29,044	Hofreiter et al. (2004a, 2004b)
Ursus spelaeus	Rochedane	French Jura	Metapodial	23,900 ± 110	GrA-52632	*U. spelaeus*	21	37.1	13.4	3.2	28,446–28,905	28,331–29,224	This study
Ursus spelaeus	Cova Eiros	NW Spain	Humerus	24,090 ± 440	Ua-4298	no (*U. spelaeus*)	nd	nd	nd	nd	28,502–29,392	28,458–29,476	Grandal-d'Anglade and Vidal Rominí (1997) and Stiller et al. (2010)
Ursus spelaeus or *U. ingressus*	La Bâme aux Pirôtas	Switzerland	Metapodial	24,170 ± 230	ETH-16879	no (*U. spelaeus* or *U. ingressus*)	nd	nd	nd	nd	28,695–29,290	28,458–29,476	Morel and Schifferdecker (1997)
Ursus spelaeus	Bärenloch	Switzerland	?	24,175 ± 365	Ua-24796	no (*U. spelaeus*)	108.8	37.8	14.6	3.0	28,614–29,378	28,094–29,671	Blant et al., 2010
Ursus ingressus	Sirgenstein	SW Germany	Lower M1	25,560 ± 130	OxA-12013	*U. ingressus*	81	39.9	13.7	3.4	30,266–30,545	29,757–30,819	Münzel et al. (2011)
Ursus ingressus	Geissenklösterle	SW Germany	Metacarpal I	26,230 ± 140	GrA-43386	*U. ingressus*	42.7	34.8	12.1	3.4	30,824–31,094	30,614–31,174	Münzel et al. (2011)

3.3. Collagen extraction and stable isotopes

A small bone fragment was carefully sawn on one side of the diaphysis of the metatarsus, ultrasonicated in acetone and water, rinsed with distilled water, dried and crushed to a powder of 0.7 mm grain size. The collagen was purified according to a well establish protocol (Bocherens et al., 1997). The elemental and isotopic measurements were performed at the Geochemical unit of the Geoscience Faculty at the University of Tübingen (Germany), using an elemental analyser NC 2500 connected to a Thermo Quest Delta + XL mass spectrometer. The isotopic ratios are expressed using the "δ" (delta) value as follows: $\delta^{13}C = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{reference} - 1] \times 1000‰$ and $\delta^{15}N = [(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{reference} - 1] \times 1000‰$, with the international reference being V-PDB for $\delta^{13}C$ values and atmospheric nitrogen (AIR) for $\delta^{15}N$ values. Measurements were normalized to $\delta^{13}C$ values of USGS24 ($\delta^{13}C = -16.00‰$) and to $\delta^{15}N$ values of IAEA 305A ($\delta^{15}N = 39.80‰$). The reproducibility was ±0.1‰ for $\delta^{13}C$ measurements and ±0.2‰ for $\delta^{15}N$ measurements, based on multiple analyses of purified collagen from modern bones.

The reliability of the isotopic signatures of the collagen extracts was addressed using their chemical composition (%C, %N, and C/N ratios). These values must be similar to those of collagen extracted from fresh bone to be considered reliable for isotopic measurements and radiocarbon dating. Several studies have shown that collagen with atomic C/N ratios lower than 2.9 or higher than 3.6 is altered or contaminated, and should be discarded, as well as extracts with %N < 5% (DeNiro, 1985; Ambrose, 1990).

3.4. Radiocarbon dating

Part of the collagen prepared for stable isotope analysis was used for radiocarbon dating. The collagen was combusted into CO_2 using an Elemental Analyzer, coupled to an Isotope Ratio Mass Spectrometer (Aerts et al., 2001). Part of the CO_2 was routed to a cryogenic trap to collect the samples for further processing for ^{14}C analysis. The CO_2 was transferred into graphite powder by the reaction $CO_2 + 2H_2 \rightarrow 2H_2O + C$ at a temperature of 600 °C and using Fe powder as catalyst. The graphite was then pressed into target holders for the ion source of the AMS. The AMS measured then the $^{14}C/^{12}C$ and $^{13}C/^{12}C$ ratios of the graphite (van der Plicht et al., 2000).

The ^{14}C age is reported as a conventional age, i.e. based on the Libby half-life value, oxalic acid as a reference and correction for isotopic fractionation using $^{13}C/^{12}C$ (van der Plicht and Hogg, 2006). The ^{14}C dates need to be calibrated into calendar ages. This is done by the recommended calibration curve Intcal09 (Reimer et al., 2009). The calibrated ages are reported in cal BP, which are calendar ages relative to 1950 AD.

4. Results

4.1. Osteometry

The greatest length (GL) of the Rochedane specimen is 80.3 mm while the shortest breadth (SB) is 17.4 mm. Compared to the reference dataset (Grotte Vaucluse excluded), the bivariate plot clearly indicates that the metatarsal from Rochedane falls within the variation of cave bears and outside the range of variation for brown bears (Fig. 3). These species are well differentiated by their metatarsal width, which is larger in cave bears. Compared to the Grotte Vaucluse cave bear sample, the Rochedane individual falls within the group of values for males as defined by the mixture analysis (Fig. 4).

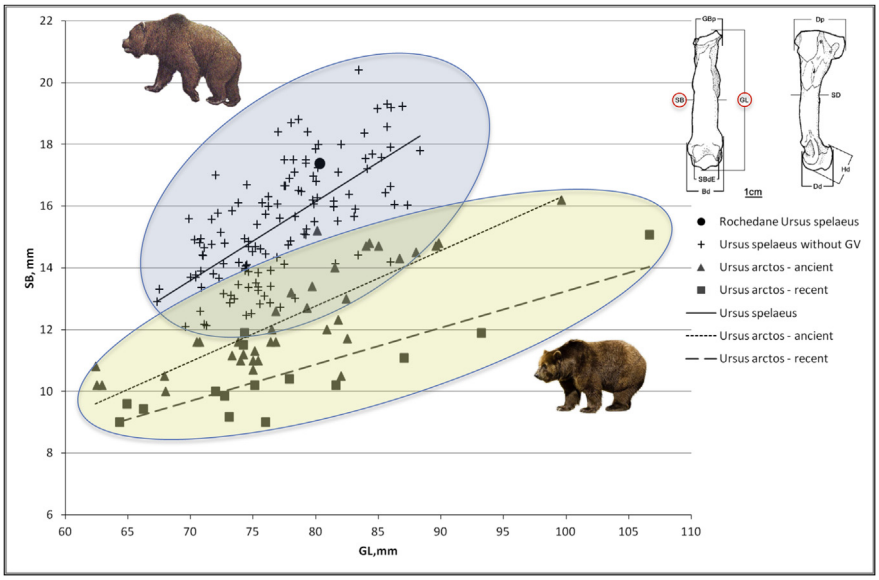

Fig. 3. Bivariate plot analysis – greatest length (GL, mm) and smallest breadth (SB, mm) of the third metatarsal – of cave bears from different sites (G. de Vaucluse excluded, Table. S1), brown bears from paleontological, archaeological sites ("ancient bears") and reference collections ("modern") listed in Table S2. The drawing showing how GL and SB were measured is from Athen (2007). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

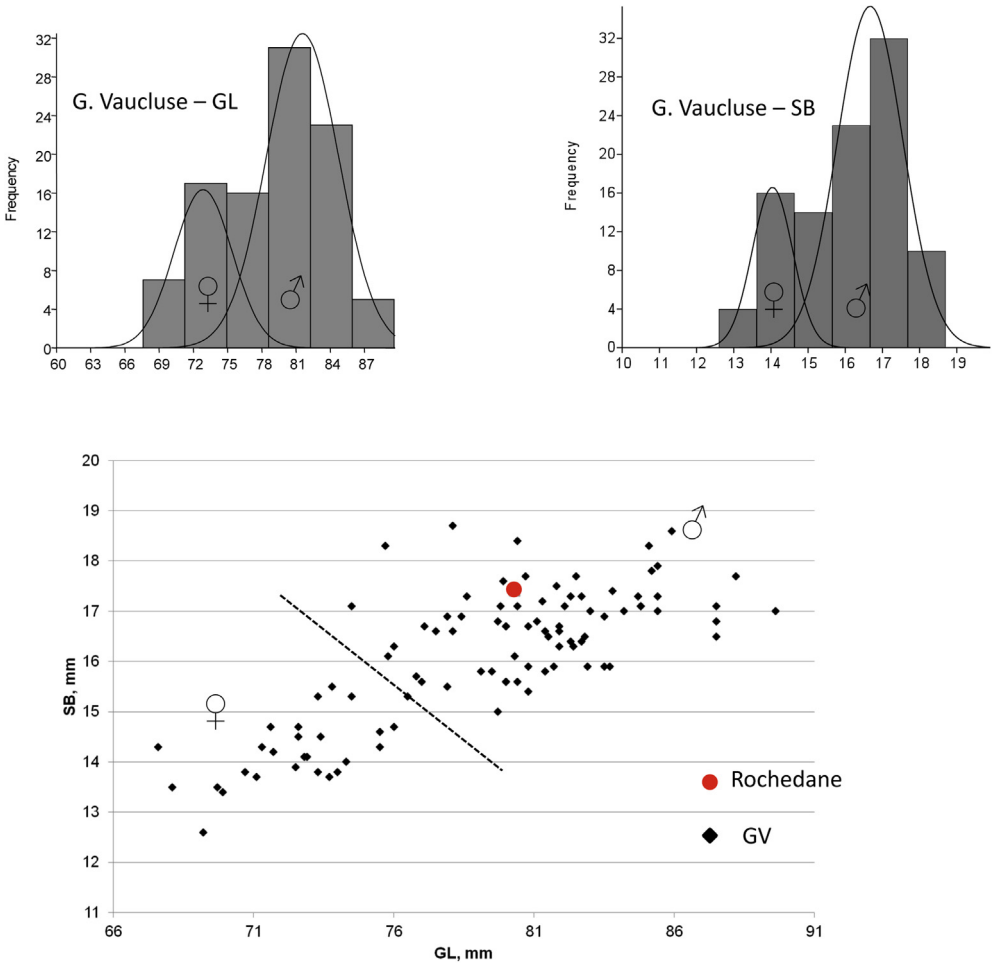

Fig. 4. Dimensions of the Rochedane third metatarsal compared to the specimens from Grotte de Vaucluse (Schweizer, 1999, 2005). Rochedane specimen fits in the values of males as defined by mixture analysis.

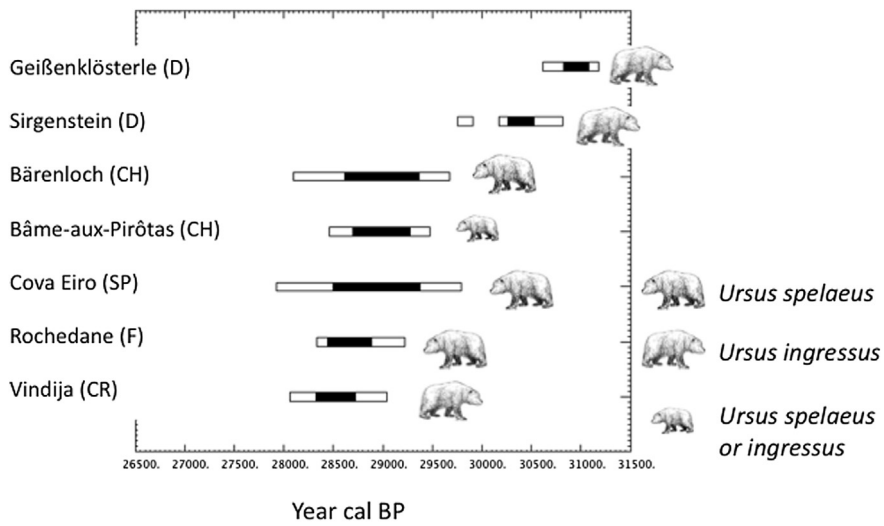

Fig. 5. Chronological position of Rochedane cave bears compared to other geologically young cave bears in Europe (range of calibrated dates using Calib 6.1 with INCAL09).

4.2. Ancient DNA

DNA preservation was sufficient to obtain a 117 bp sequence (165 bp including primers) of the mitochondrial control region with the following sequence: CATTATTTTACTCTATACCCTATTTATTTCA TATATACCATCTCATGTACTGTACCATCATAGTATGTCCTTAAATACTTTT TCCTTTATTTTTTTTTCCCCCCCTATGTATATCGT. A brief comparison to previously published sequence data using nucleotide blast (http://blast.ncbi.nlm.nih.gov/) clearly identified the specimen as cave bear rather than brown bear, confirming the taxonomic attribution based on the osteometric analysis. Specifically, the obtained mitochondrial DNA sequence belongs to the haplogroup *U. spelaeus* as opposed to the *Ursus ingressus* group. Both *U. ingressus* and *U. spelaeus* were present in the nearby Swabian Jura since 36,000 cal BP, but *U. spelaeus* disappeared from that area around 31,900 and 29,800 cal BP (Münzel et al., 2011), and only *U. ingressus* remained in the area until the final extinction of cave bears.

4.3. Stable isotopes and radiocarbon dating

The extracted collagen exhibits a chemical composition well within that of collagen extracted from fresh bone, with % C = 37.1%, %N = 13.4% and C/N = 3.2. It is therefore considered reliable in terms of carbon and nitrogen stable isotopic composition and radiocarbon dating (Table 2). The $\delta^{13}C$ value is −21.0‰ and the $\delta^{15}N$ value is 4.6‰. The radiocarbon age is measured as 23,900 +110/−100 BP. The calibrated age range is 28,730–28,500 cal BP (1 sigma range).

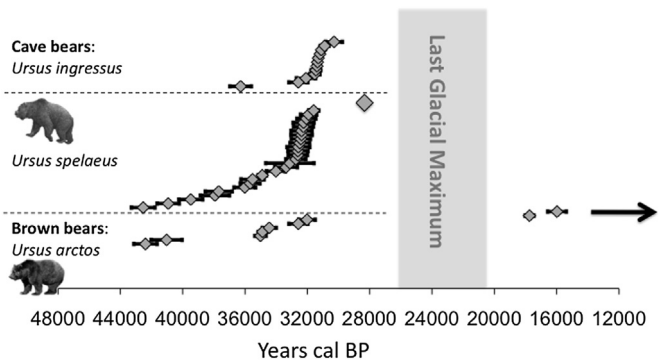

Fig. 6. Chronological position of Rochedane cave bears compared to other radiocarbon dated bears in the region (modified from Münzel et al., 2011). The radiocarbon dates have been calibrated according to Reimer et al. (2009).

5. Discussion

There is no doubt that the studied bone from Rochedane belongs to a cave bear and not to a brown bear. The results of the osteometric analysis unambiguously place the studied specimen within the range of the variation of the cave bears, most probably males, and rules out brown bears. The palaeogenetic analysis fully confirms this conclusion and furthermore indicates that this specimen belongs to the haplogroup *U. spelaeus* and not to *U. ingressus* (Münzel et al., 2011).

Radiocarbon dating shows that the age of the specimen is clearly older than the age range for the Magdalenian culture. Therefore, this cave bear is an additional case of a cave bear bone that does not belong to the cultural level it could (loosely) be associated with. In this case, there was no direct information that it came from a Magdalenian layer rather than from some other place not recognized in the site. However, the date is the youngest one so far recorded in the region, about 1000 years younger than the most recent radiocarbon date recorded in the Swabian Jura (around 29,700 cal BP; Münzel et al., 2011). Compared to the radiocarbon dates obtained from identified cave bear bones, the date of the Rochedane cave bear appears the second youngest one. The youngest one is from Vindija cave dated to 23,780 ± 120 BP (Hofreiter et al., 2004a). Three other directly dated cave bears, one from Spain and two from Switzerland, follow close behind (Table 3). After calibration and taking the standard deviations into account, all these cave bear bones yield age ranges that cannot be distinguished statistically (Fig. 5).

However, not all these radiocarbon dates are equally trustworthy. For instance, the radiocarbon dating of the Vindija cave bear is given without reliability criteria, and bones from this site have proven difficult to date due to poor collagen preservation (Higham et al., 2006). Therefore this date has to be taken with caution, and new dates on cave bear bones from this site should be undertaken with reliability criteria to confirm the occurrence of such young cave bears in Croatia. As well, the radiocarbon dates from Cova Eiros (Spain) and Bâme-aux-Pirôtas (Switzerland) were published without reliability criteria (Grandal-d'Anglade and Vidal Rominí, 1997; Morel and Schifferdecker, 1997). Only the

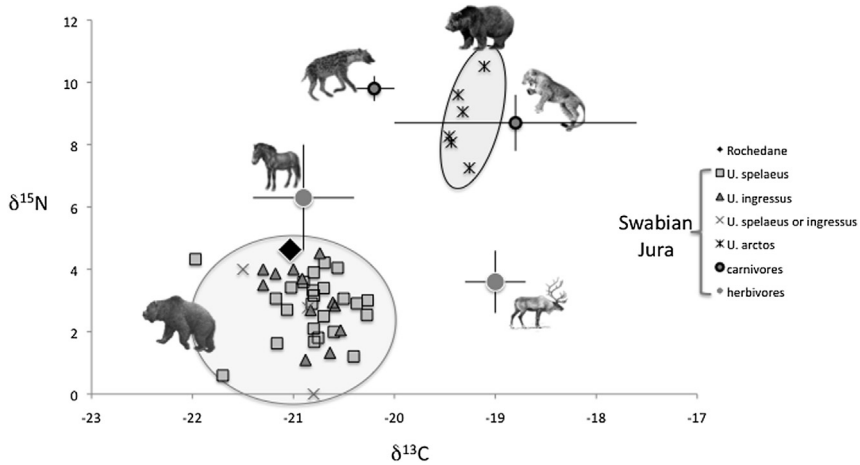

Fig. 7. $\delta^{13}C$ and $\delta^{15}N$ values of Rochedane cave bear compared to pre-LGM mammals from the Swabian Jura (data from Bocherens et al., 2011a, 2011b).

radiocarbon dated cave bear from Bärenloch fulfills the criteria for reliability (Table 3).

The genetic type of these young cave bears is not always known by direct palaeogenetic investigations, but some inferences can be made using morphological features or using genetic information on specimens from the same layer. For instance, the study of the teeth of the cave bears from Bärenloch suggests that they belong to the *U. spelaeus* lineage (Blant et al., 2010), and we can assume that the dated specimen also belongs to this lineage. In the case of the dated specimen from Cova Eiros, one cave bear specimen from the same stratigraphic unit was studied palaeogenetically. It belongs to the *U. spelaeus* lineage (Stiller et al., 2010), and thus we can reasonably assume that is also the case for the dated specimen. However, the genetic lineage of the specimen from Bâme-aux-Pirôtas has not been investigated. It is not known whether it belongs to *U. spelaeus* or *U. ingressus*. Further research should clarify this point. In any case, it would be ideal to have a direct palaeogenetic investigation on the same bone as the one dated, as changes of genetic types are known to occur in sites in relatively short periods (Hofreiter et al., 2007; Münzel et al., 2011). In conclusion, the Rochedane cave bear is so far the only one among the most recent representatives of this species for which direct AMS radiocarbon dating with reliability criteria as well as palaeogenetic investigations have been performed on the same bone.

In the regional context of cave bear population dynamics, which is well documented in Southwestern Germany (Hofreiter et al., 2007; Münzel et al., 2011), the Rochedane cave bear appears unusual since the lineage *U. spelaeus*, to which the Rochedane cave bear belongs, disappears two thousand years before the lineage *U. ingressus*. If we broaden the region to include the Swiss Jura, the cave bear from Bärenloch, which seems to belong to *U. spelaeus* and is contemporaneous with the cave bear from Rochedane, supports the scenario that 1) cave bear populations were isolated between the Swabian Jura on one side and the French and Swiss Jura on the

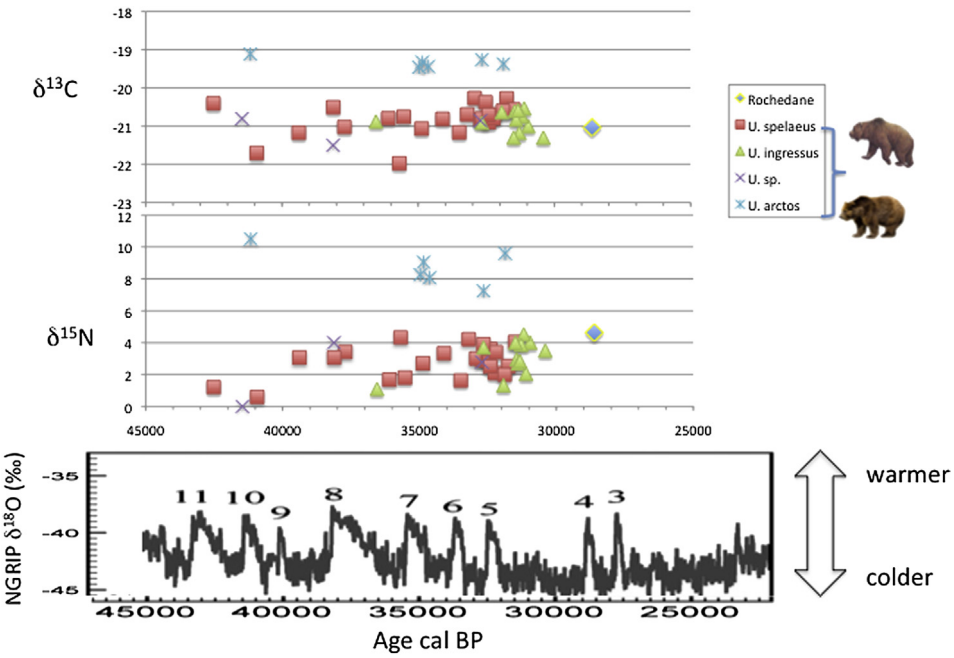

Fig. 8. Chronological trend in the $\delta^{13}C$ and $\delta^{15}N$ values of bears in cave bears and brown bears from French and Swabian Jura. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

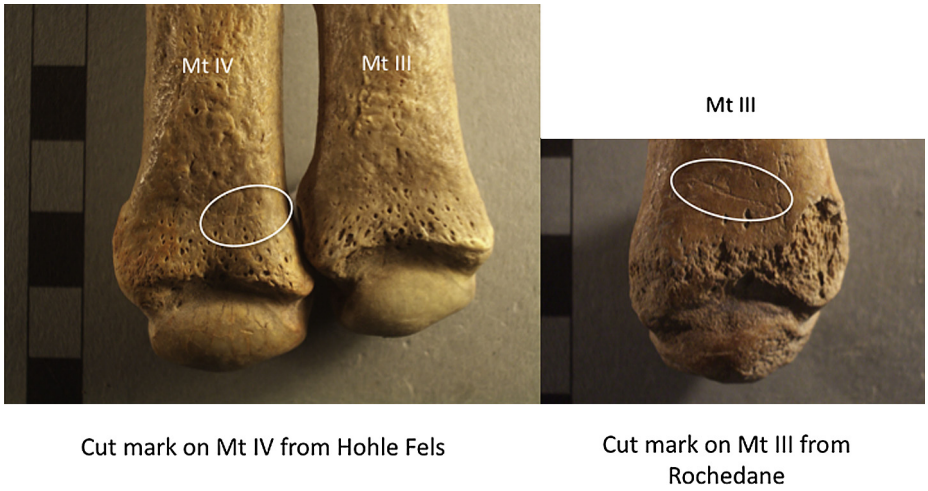

Fig. 9. Possible cut-marks on the Rochedane cave bear metatarsal compared to those observed on similar bones from Hohle Fels.

other side, and 2) the lineage *U. spelaeus* survived longer East and South of the Rhine river than *U. spelaeus* and thereafter *U. ingressus* on the other side of the Rhine river (Fig. 6). Such a pattern suggests that, during the last thousand years of cave bear occurrences in the northwestern alpine foreland, the different populations were fragmented, and that some local extirpation occurred, sometimes with re-immigration of cave bears in the same area, as shown by the replacement of *U. spelaeus* by *U. ingressus* in the Swabian Jura, or without re-immigration of this species, as it was probably the case in the French Jura after 28,500 cal BP.

Considering that the cave bear from Rochedane thus far is the youngest one known from this region, it is of interest to study the stable carbon and nitrogen isotope ratios. This enables us to investigate whether this late surviving individual exhibits any change in its ecological habits compared to older specimens of the same species in the region, namely cave bears from the Swabian Jura which have been studied extensively (Münzel et al., 2011). When plotted together with representatives of large mammal species in the Swabian Jura prior to the LGM, including cave bears as well as herbivores such as horse and reindeer, and predatory carnivores, such as hyenas and lions, the cave bear from Rochedane plots together with the vegetarian cave bears and very differently from the carnivorous brown bears (Fig. 7). This indicates that the cave bear from Rochedane did not differ in its dietary habits from the pattern of the species in western and central Europe, an essentially vegetarian diet (e.g. Bocherens et al., 1994, 1997, 2006, 2011a; Mosquera, 1998; Blant et al., 2010; Pacher et al., 2012).

However, the cave bear from Rochedane exhibits one of the most positive $\delta^{15}N$ values in comparison with the cave bears from the Swabian Jura, which could indicate an ecological shift. To test this hypothesis, we plotted all isotopic values for directly radiocarbon dated cave bears and brown bears from the Swabian Jura and Rochedane (Fig. 8). This graph does not show any clear trend in either $\delta^{13}C$ or $\delta^{15}N$ values during the last thousand years of occurrence of cave bears in the northwestern alpine foreland. The $\delta^{13}C$ values range from around −22 to −20‰, while the $\delta^{15}N$ values range from around 0 to 5‰, which is similar to the range exhibited by herbivorous species in the same region during the same period (Bocherens et al., 2011a). This suggests that cave bears did not change significantly their ecology during this period. The youngest cave bear from Rochedane presents $\delta^{13}C$ and $\delta^{15}N$ values similar to those measured in cave bears more than 10,000 years older in the same region, suggesting a relative ecological stability for this species in the region. It is not possible to correlate the fluctuations of $\delta^{13}C$ and $\delta^{15}N$ values with climatic oscillations due to the standard-deviation of calibrated radiocarbon dates, which are larger than the duration of interstadial episodes during this period. For the same reason, it is not possible to compare this last occurrence of cave bear in the French Jura with the global climatic context as recorded in Greenland ice, as it could fit either GS-4 or GI-4 using the terminology of Blockley et al. (2012). In any case, it seems that the last documented occurrence of cave bear in the region predates the extension of ice caps on the Jura Mountains by around 1000 years, since this initial glacial expansion is estimated to date to around 28,000 cal BP (Buoncristiani and Campy, 2004).

A final observation made on the metatarsal bone of the Rochedane cave bear suggests that humans may have had an impact in the final demise of the species in the region. A possible cut-mark was identified on the distal end of the metatarsal, in a very similar position as cut-marks observed on metatarsal bones from cave bears in Hohle Fels, where several evidence of human treatment of cave bear bones have been recognized (Münzel and Conard, 2004) (Fig. 9). Cut-marks in this position suggest skinning of the bear for fur exploitation (Münzel and Conard, 2004; Münzel et al., 2011). We cannot infer from this observation that humans were directly responsible for the death of this cave bear individual, but such a cut-mark indicates that cave bears were part of the prey spectrum of Upper Palaeolithic humans on both sides of the Rhine River, a situation that was probably not favorable to the survival of the species.

6. Conclusion

The cave bear from Rochedane demonstrates once more that the mere occurrence of cave bear remains in Magdalenian archaeological layers is not enough to demonstrate post-LGM survival of this species. Such claims need further confirmation 1) of the taxonomic attribution of the skeletal remains to cave bears by unambiguous morphological analysis and, as much as possible, by ancient DNA, which provides a secure specific attribution and a determination of the genetic type, and 2) by direct AMS dating to establish the chronological context.

The late cave bear in Rochedane belongs to haplogroup *U. spelaeus* that disappeared earlier in SW Germany (it is replaced by newer type *U. ingressus*). This indicates that cave bears occurred as fragmented populations across the Rhine with no genetic interchange between them. The cave bear from Rochedane is about 3000 years younger than the youngest *U. spelaeus* in SW Germany

and 2000 years younger than any cave bear in SW Germany. The youngest cave bear in French Jura occurred shortly before ice sheets extended at the beginning of the LGM in the Jura Mountains. However, its $\delta^{13}C$ and $\delta^{15}N$ values do not show any obvious ecological change since 40,000 years cal BP despite climatic fluctuations, suggesting a strong niche conservatism for this species, leading to a possible lack of ecological flexibility in the face of climate change. However, cave bears survived previous similar climatic changes during the Pleistocene. What was different around ca. 24,000 years ago that could have led to their demise? We found on the bone of the Rochedane cave bear some evidence of human impact. We can speculate that a combination of climatic deterioration and increasing human pressure could explain the extirpation of cave bears in Northwestern alpine foreland, on already fragmented populations. This scenario is unfortunately very similar to the situation of numerous mammal species nowadays.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.quaint.2013.05.021

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