Isotopic evidence for omnivory among European cave bears: Late Pleistocene *Ursus spelaeus* from the Peştera cu Oase, Romania

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Previous bone collagen carbon and nitrogen isotopic studies of Late Pleistocene European cave bears (*Ursus spelaeus*) have shown that these bears frequently had low nitrogen isotope values, similar to those of herbivores and indicating either unusual physiology related to hibernation or a herbivorous diet. Isotopic analysis of animal bone from the Peştera cu Oase (Cave with Bones), Romania, shows that most of its cave bears had higher nitrogen isotope values than the associated herbivores and were, therefore, omnivorous. The Oase bears are securely identified as cave bears by both their morphology and DNA sequences. Although many cave bear populations may have behaved like herbivores, the Oase isotopic data demonstrate that cave bears were capable of altering their diets to become omnivores or even carnivores. These data therefore broaden the dietary profile of *U. spelaeus* and raise questions about the nature of the carnivore guild in Pleistocene Europe.

carbon isotopes | nitrogen isotopes | diets | ancient DNA

Late Pleistocene cave bear (*Ursus spelaeus*) diet and physiology is not well known, although based on skull, mandible, and tooth morphology, they have been inferred to have been largely herbivorous (1–4). Moreover, isotopic studies of central and western European cave bears have shown that, in most cases where their values are compared with herbivores and carnivores from the same site, they have $\delta^{15}N$ values that plot with, or lower than, herbivores (Table 1). These dietary inferences are in the context of bears that had a period of dormancy, similar to modern black bears (*Ursus americanus*) and brown bears (*Ursus arctos*), given the abundance of hibernation nests in European caves. This pattern has been interpreted in several ways.

Based on comparisons of cave bear $\delta^{15}N$ values to herbivore values from three Late Pleistocene sites in France and to modern bear isotope data, Bocherens *et al.* (7) concluded that the cave bears were herbivores. They ruled out the possibility that hibernation had an effect on $\delta^{15}N$ values of modern bears, and by inference, it should not have affected cave bear $\delta^{15}N$ values.

Fernández *et al.* (12) analyzed $\delta^{13}C$ and $\delta^{15}N$ data from central and western European cave bears and hypothesized that the $\delta^{15}N$ values could be linked to climate. They found a link between $\delta^{15}N$ values and temperature, with cave bears from colder environments having lower $\delta^{15}N$ values. Although there are connections between climate and plant $\delta^{15}N$ values (17) that show that colder environments often have lower $\delta^{15}N$ values, they argued instead that the lower $\delta^{15}N$ values observed in Alpine cave bears were linked to hibernation. They argued that, because dormancy may result in lower cave bear $\delta^{15}N$ values, following Nelson *et al.* (11), colder environments require periods of longer dormancy that results in lower $\delta^{15}N$ values.

An alternative possibility (18) is that they had a dietary preference for nitrogen-fixing plants, which have lower $\delta^{15}N$ values. Such a diet might result in the very low $\delta^{15}N$ values seen in some cave bear samples (Table 1). However, there are dietary constraints in terms of potential protein intake, feeding rates, and digestibility of plant matter for at least modern brown bears (19–21), suggesting that a dependence on such plants does not fully explain the cave bear $\delta^{15}N$ pattern.

A fourth possibility, proposed by Hilderbrand *et al.* (14, 22) on the basis of higher $\delta^{15}N$ values from a diverse sample of cave bears (Table 1), is that some cave bears were largely herbivorous, as are some modern bears, but that other cave bears were more carnivorous and hence exhibit $\delta^{15}N$ values similar to those of many modern omnivorous *U. arctos* and *U. americanus*. In this interpretation, the variation in trophic level indicated by $\delta^{15}N$ values would reflect ecological plasticity among *U. spelaeus*, in which they were more or less carnivorous depending on available food resources, body-size requirements, interspecific competition, seasonality, and temperature.

Regardless of the cause, 86.1% of the available individual European adult cave bear $\delta^{15}N$ values ($n = 101$) are $<6.0‰$, and hence within the basically vegetarian range [the number rises to 95.6%, $n = 91$ without Hilderbrand *et al.*'s (14) 10 specimens] (Table 1). In the context of this distribution of $\delta^{15}N$ values, routine isotopic assessment of a cave bear metapodial associated with an early modern human cranium in the Late Pleistocene Peştera cu Oase, Caraş-Severin, southwestern Romania (23–27) [supporting information (SI) *Text* and SI Figs. 4 and 5], provided an anomalously high $\delta^{15}N$ value of 7.8‰ (SI Table 2, Oase O34-232). To further evaluate this result and address the cause of the generally low cave bear $\delta^{15}N$ values, we measured the isotope values of cave bears and associated fauna from the Peştera cu Oase.

Affinities of the Peştera cu Oase Ursid Remains. Because the primary concern of isotope analysis of Late Pleistocene *U. spelaeus* remains relates to the persistently low $\delta^{15}N$ values obtained for them, in contrast to the variable but generally higher (more

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Table 1. Previously determined isotope values of European cave bears, with the mean ± SD for $N > 2$

Site	Region	$\delta^{13}C$	$\delta^{13}C$ range	$\delta^{15}N$	$\delta^{15}N$ range	N	Age, cal ka*	Ref
Liñares	NW Spain	−21.1 ± 0.3	−21.7 to −20.6	3.0 ± 0.4	2.1–3.6	20	40	5
Eirós	NW Spain	−21.1 ± 0.3	−21.3 to −20.4	5.1 ± 0.3	4.4–5.5	8	29	6
Aldène	S France	−20.8 ± 0.4	−21.3 to −20.5	3.1 ± 1.5	2.1–4.8	3	<30	7
Mialet	S France	−20.2 ± 0.4	−20.7 to −19.6	2.8 ± 1.6	0.8–4.4	5	—	7
Olaskoa	Pyrenees	−20.5	−20.5	2.7	2.7	1	12	7
Chauvet	S France	−20.5 ± 0.3	−21.2 to −20.1	3.8 ± 0.9	3.1–4.8	16	27.3–42.1	8
Scladina-1A	Belgium	−22.1 ± 0.2	−22.5 to −21.8	4.9 ± 1.2	3.0–6.1	7	44	9
Scladina-4	Belgium	−23.2 ± 0.3	−23.6 to −23.0	3.6 ± 0.6	3.0–4.5	4	120–130	10
Divje Babe	Slovenia	−20.5 ± 0.4	−19.9 to −21.0	1.9 ± 0.7	0.6–3.3	11	52	11
Ramesch	Austria	−22.5	−22.5	2.4	2.4	1	45–48	12
Herdengel	Austria	−22.0, −22.1	−22.1 to −22.0	2.3, 2.5	2.3–2.5	2	41.4–43.8	12
Schwabenreith	Austria	−22.9	−22.9	2.5	2.5	1	55–121	12
Lieglloch	Austria	−20.1, −20.2	−20.2 to −20.1	2.8, 4.1	2.8–4.1	2	30–40	12
Hartelsgraben	Austria	−20.8, −21.0	−21.0 to −20.8	0.8, 3.4	0.8–3.4	2	32–47	12
Conturines	Italy	−22.3 ± 0.9	−23.3 to −21.6	2.0 ± 0.5	1.6–2.5	3	45.3–51.3	12
Potočka Zijalka	Slovenia	−20.4	−20.4	4.2	4.2	1	35	12
Vindija	Croatia	−20.7, −21.1	−21.1 to −20.7	1.3, 1.5	1.3–1.5	2	>34	13
Muierii†	Romania	20.4 ± 0.2	−20.7 to −20.2	5.1 ± 1.6	3.4–7.3	4	44–53	
Cioclovina†	Romania	−20.3, −20.4	−20.3 to −20.4	3.1, 4.3	3.1–4.3	2	36, 45	
Various	Europe	−21.5 ± 0.5	−22.0 to −20.6	8.4 ± 0.5	7.7–9.1	10	—	14‡

All isotope data are from bone samples derived from mature individuals; data from teeth and immature individuals are not included here or in Fig. 3. The individual values are provided in SI Table 3.

*Radiocarbon ages were converted to calendar years by using CalPal (www.calpal.de).

†The Muierii and Cioclovina data are from OxA in the context of radiocarbon dating (15, 16).

‡The individual various European specimens are from Hilderbrand *et al.* (14) and were provided by Hilderbrand (personal commmunication).

omnivorous) values generally found for *U. arctos* (7, 28–30), it is essential to establish the taxonomic affinities of the bear bones from the Peştera cu Oase.

Morphological Affinities. A series of morphological contrasts between cave and brown bears are well established (31–34). These include, for *U. spelaeus*, broad molars with heavily wrinkled surfaces and additional cusps, loss of the first three premolars, pronounced doming of the forehead (especially in large males) (Fig. 1), large masseteric fossae and sagittal crests, large temporomandibular articulations, more robust long bones and metapodials, shorter extremities, pronounced tibial torsion, medial humeral curvature, and aspects of pedal facet morphology. All of these characteristics are found in all of the diagnostic bear remains from the Peştera cu Oase, and their morphology shows no distinctive differences from other cave bear samples that cannot be easily accommodated within models of normal, intraspecific temporal and spatial variation (25). There are no *U. arctos* in the sample.

Genetic Affinities. To provide additional confirmation of the morphological evidence, mitochondrial DNA (mtDNA) was extracted, amplified, and sequenced from 19 ursid samples (SI Table 2). All 19 individual sequences of the Peştera cu Oase ursids show clear affinity to central European cave bear sequences (35) rather than to brown bears. They do not form a monophyletic group within cave bear mtDNA variation, and the range of the Oase bear haplotypes is spread throughout most of the variability known for central European cave bear populations from southern Germany, Austria, Croatia, and Slovakia (35–37).

Stable Isotopes of the Peştera cu Oase *U. spelaeus* Remains. The Peştera cu Oase faunal isotope data are in Fig. 2 and SI Table 2. The herbivores (*Capra ibex* and *Cervus elaphus*) have values similar to those observed elsewhere for herbivores in Pleistocene Europe (38–40), with $\delta^{15}N$ values ranging between 2.2‰ and 5.4‰. The higher *Canis lupus* $\delta^{15}N$ values are similar to those for European carnivores (9, 10, 41, 42). Whereas the wolf and deer remains come from the same time period as the bears, the ibex remains are more recent. There is fluctuation in faunal $\delta^{15}N$ values over the past 50,000 years (38), and it is not necessarily valid to compare fauna from different time periods. The variation observed in European fauna occurs principally at ≈13 thousand years before the present (ka BP) and in the Holocene, where herbivores have reduced $\delta^{15}N$ values; this may have affected the ibex scapula but probably not the other samples.

The Oase cave bear isotope data (Fig. 2 and SI Table 2) are shifted negatively in $\delta^{13}C$ values (-21.5 ± 0.4‰, $n = 21$), but in this they match the values of most of the other European cave bear samples (Table 1 and SI Table 3). However, the Oase bear $\delta^{15}N$ values are principally between the relatively "carnivorous" values of 5.7–9.8‰, with one "herbivorous" value of 3.6‰ (7.8 ± 1.4‰, $n = 21$; 8.0 ± 1.1‰, $n = 20$ without the one low value). A Wilcoxon nonparametric comparison of the pooled herbivore versus cave bear $\delta^{15}N$ values for the Oase sample provides a $P < 0.001$ ($P = 0.001$ if the more recent ibex scapula is deleted from the sample); $P = 0.036$ between the Oase bears and more carnivorous wolf $\delta^{15}N$ values. These cave bear isotopic values cannot be the product of diagenesis, because the other fauna, including the contemporaneous canid and cervid remains, have expected $\delta^{15}N$ values, and all have expected C/N values.

These Oase bear data contrast strongly with all previously published cases of cave bear $\delta^{15}N$ values associated with herbivore data from the same site, in which the cave bears have $\delta^{15}N$ values that are similar to, or lower than, herbivores from the same site. Most of the unpublished specimens from the Romanian sites of Cioclovina and Muierii (15–16) follow the same pattern (Table 1 and SI Table 3), although two specimens (Muierii 05-6 and 05-11) have $\delta^{15}N$ values of 6.0‰ and 7.3‰, and three specimens from Scladina 1A [$\delta^{15}N$ of 5.7‰, 6.0‰, and 6.1‰ (9)] are close (Fig. 3). The museum-derived cave bear sample analyzed by Hilderbrand *et al.* (14) produced $\delta^{15}N$ values similar to the Oase ones, although that may be due in part to selection for large ("museum quality") specimens (22) because the degree of carnivory (and hence $\delta^{15}N$ values) tends to correlate with size, at least among North American brown bears (29).

EVOLUTION

Fig. 1. Left anterolateral views of Peştera cu Oase adult *U. spelaeus* crania. From top to bottom, N32.1 (female), O34.62b (female), N32.21 (male), and N37.49 (male). The protuberance on the N32.21 dorsal neurocranium is a stalagmite.

There is a modest inverse relationship between $\delta^{13}C$ and $\delta^{15}N$ values in the Oase cave bears ($r = -0.708$, $P < 0.001$; without the one low outlier, $r = -0.554$, $P = 0.011$). This pattern is occasionally observed in isotopic studies, and it is often interpreted as a mixing line between two food sources (43). In this case, it would indicate that the cave bears had two distinct dietary protein sources, one being plant foods and the other being animals with low $\delta^{13}C$ and high $\delta^{15}N$ values. None of the Oase herbivore species could be the main source of animal protein for the cave bears, because the $\delta^{13}C$ values are too different (there is almost no shift in $\delta^{13}C$ value between prey and consumer). Whatever the source of the animal protein, it is apparent from the Oase cave bear isotope data that *U. spelaeus*, as with *U. arctos*, was not strictly vegetarian.

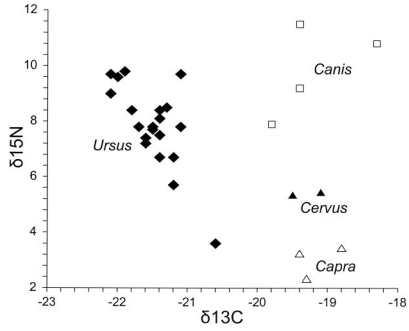

Fig. 2. Isotope data for cave bears and other fauna from the Peştera cu Oase, Romania. Filled diamonds, *U. spelaeus*; open squares, *C. lupus*; filled triangles, *C. elaphus*; open triangles, *C. ibex*. S-EVA values are used except for the specimens for which only OxA data are available.

Discussion

The Peştera cu Oase isotopic results indicate that cave bears do not always have low $\delta^{15}N$ values when compared with herbivores from the same site. Moreover, this pattern is evident, although less dominant, in samples from Muierii and Scladina 1A. Therefore, cave bears had the ability to consume animal protein and behaved like omnivores or even carnivores, even if in many cases they were herbivorous.

This inferred dietary flexibility for cave bears is evident in the stable isotope values within some samples, including Oase, Muierii, and Scladina 1A, as well as between samples. In this variation *U. spelaeus* closely parallels the range of isotopic values seen for modern European and North American brown bears (5, 7, 28–30) and the few data available for Late Pleistocene brown bears (10, 42).

As a consequence of these $\delta^{15}N$ values, the dietary ecology of modern, higher-latitude bears (excluding polar bears) is relevant for that of cave bears, especially the North American brown bears (*U. arctos*, including the Kodiak and grizzly bears) given their high-latitude range, body-size variation, occupation of regions with less human ecological impact than most of Eurasia, and extensive database. Brown bear diets range from almost completely vegetarian, including ones with substantial amounts of fruit/berries, to ones

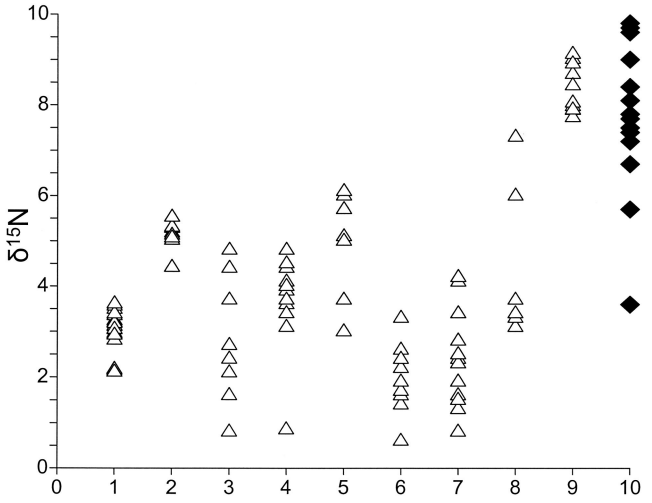

Fig. 3. Distributions of $\delta^{15}N$ values for *U. spelaeus* remains from European OIS 4–2 Late Pleistocene sites (Table 1) and the Peştera cu Oase (SI Table 2). Samples: Liñares (1), Eirós (2), southern France and Pyrenees (3), Chauvet (4), Scladina 1A (5), Divje Babe (6), eastern Alps (7), Muierii and Cioclovina (8), European (9), and Oase (10).

containing a substantial amount of fish and/or ungulate meat (19–21, 29, 30, 44, 45). All aspects of their omnivorous diets have limitations in availability, potential feeding rates, and nutritional value in any given environment; adequate weight gain for survival, reproduction, and hibernation therefore depends on a mix of as many food resources as are available (19, 21). Meat consumption, in particular, varies widely among and within brown bear populations, due, among nonmaritime bears, to the availability of ungulate fauna (29, 30, 44, 45). Large adult males also appear to be more carnivorous than females or subadult bears (28, 29). North American black bears (*U. americanus*) appear to have similar plant/meat dietary proportions as brown bears (29), except that the larger brown bears are frequently more carnivorous when the prime meat is maritime (e.g., salmon) (46). This ecological flexibility of modern brown bears therefore makes an appropriate model to understand the range of isotopic values now available for European cave bears, both within and between site-specific samples.

The Oase bear isotope data also indicate that there is no need to invoke special species-wide effects, such as a dormancy effect or a reliance on specific plant foods, to explain the frequently low δ^{15}N for European Late Pleistocene cave bears.

These isotopic data also imply that *U. spelaeus* participated, at least occasionally, in the European Late Pleistocene carnivore guild. This would have brought it into competition with the purer carnivores, especially cave lions and hyenas, as well as the more omnivorous ones such as brown bears and wolves. A very large bear obtaining much of its protein from vertebrate prey would have a serious effect on the dynamics of that guild. Moreover, contemporaneous European humans, both late archaic and early modern, consistently exhibit high δ^{15}N values (13, 47, 48), and an additional large-bodied carnivore would have further intensified any human–carnivore interactions within the ecosystem.

Conclusions

Isotopic analysis of the Late Pleistocene cave bears from the Peştera cu Oase, Romania demonstrates that at least this bear population had omnivorous dietary adaptations. This is in contrast to most of the other published cave bear isotope data, which have been taken to show that they were herbivorous. The Oase data confirm that cave bears, as with modern brown bears, were omnivorous, although they may have frequently had dietary regimes that were primarily herbivorous. In an ecological context containing an abundance of large social carnivores, such as Late Pleistocene Europe, bears would have needed the dietary flexibility that is emerging from these stable isotope data. The *U. spelaeus* stable isotope data from the Peştera cu Oase, in conjunction with the data for cave bears elsewhere in Europe, now permit us to view them in a broader dietary framework and thereby raise questions as to their roles within the ecosystems of Pleistocene Europe.

Materials and Methods

The Peştera cu Oase Ursid Sample. The overall assessment of the Peştera cu Oase ursid sample is based principally on those remains excavated from the Panta Strămoşilor, supplemented by surface materials in the other galleries of the Peştera cu Oase (see SI Text, SI Fig. 4 and 5). For isotopic and ancient DNA analysis, 22 ursid, and 10 other large mammals remains were selected from both the excavated and surface remains (see SI Table 2). Fifteen of the bear bones derive from excavations in the Panta Strămoşilor, whereas the others come from the surfaces of the adjacent galleries, some from hibernation nests. Other faunal species are rare within the Peştera cu Oase; sufficiently preserved bones for isotopic analysis include wolf ($n = 4$), ibex ($n = 4$), and red deer ($n = 2$). All of the specimens are bone and derive from fully mature individuals.

Given the mixed nature of the skeletal remains, there may be some duplication of individuals within species. The bear mtDNA haplotypes provide a minimum number of individuals of eight. The radiocarbon ages for the ibex provide a minimum of three individuals. The cervid hemimandibles, however, are right and left specimens, morphologically similar, may represent the same individual, and have similar isotopic values and radiocarbon ages; they are nonetheless plotted separately.

Morphological Assessment. The faunal remains from the Peştera cu Oase were determined to skeletal element and species based on morphological characteristics. Apart from a few fragments, morphological and morphometric species identification was readily apparent for the >5,000 bear remains excavated in the Panta Strămoşilor and collected from the cave surfaces.

Sample Collection. The analytical samples were derived from mature bone diaphyseal, rib, palatal, and basal mandibular cortical bone and removed by using a stainless-steel blade on a Dremel tool.

Ancient DNA. Ancient DNA was extracted from 170 to 750 mg of bone material as described in Hofreiter *et al.* (36) and Rohland and Hofreiter (49, 50). Two hundred eight-five base pairs (bp) of the mitochondrial control region were amplified by using regular PCR with primers and amplification conditions from Hofreiter *et al.* (35) or under multiplex PCR conditions as described by Rompler *et al.* (51) by using primers from Hofreiter *et al.* (35, 36). Amplification products were cloned by using the TOPO-TA cloning kit (Invitrogen) by following supplier recommendations. Four to eight clones of each amplification product were sequenced on an ABI 3730 by using the BigDye Terminator v1.1 Cycle Sequencing kit and M13 universal primers. Clone sequences were aligned by using BioEdit (52) and merged to reconstruct the complete 285-bp sequence for each individual. Every sequence position was determined from clone sequences derived from at least two primary amplification products to avoid errors caused by ancient DNA template damage (53, 54). In cases of consistent differences among clones derived from independent amplification products, a third primary amplification was used to clarify the correct state for each position.

Stable Isotopes. The key assumption behind the analysis of carbon and nitrogen stable isotopes is that bone collagen is formed throughout a mammal's life, and the carbon and nitrogen in collagen is ultimately derived from dietary protein (55–57). The isotope ratios of the two stable isotopes of carbon ($^{13}C/^{12}C$ or $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$) in dietary protein are commonly well preserved in European Late Pleistocene mammal bone collagen. Therefore, measurement of the $\delta^{13}C$ and $\delta^{15}N$ values of mammal bone collagen can be used to determine the main sources of dietary protein over the lifetime of that mammal. The bone collagen isotope values likely reflect dietary protein consumed over the past 10–20 years of life for an adult long-lived mammal (58). Although used to assess whether the dietary protein carbon is derived from marine versus terrestrial sources or from C_3 or C_4 plants, it is the trophic level of the protein consumed that is of interest here. Because the $\delta^{15}N$ of bone collagen is ≈2‰ to ≈4‰ higher than dietary protein (59, 60), one can use it to determine the main protein sources in the diets of cave bears, especially compared with other fauna from the same site, to see whether dietary protein derived mainly from plants, as indicated by other isotopic studies of cave bears, or from higher trophic level protein sources. Of particular relevance here, experimental work with living bears (14) has shown that bone collagen $\delta^{15}N$ values are an accurate reflection of diet.

A potential source of error for paleontological samples is postdepositional collagen bacterial and/or chemical diagenesis. Bone samples were therefore pretreated to remove exogenous carbon and isolate the original collagen. The resulting collagen extract is measured for a number of collagen quality indicators, including collagen yield, C/N ratio, percent C, and percent N in the collagen extract (61, 62).

We extracted collagen from 26 mammalian bones from the Oase site using standard extraction procedures [i.e., modified Longin (63) method (64) with the addition of an ultrafiltration step (65)]. Isotope measurements were made by using a ThermoFinnigan elemental analyzer coupled to a Delta Plus XP isotope ratio mass spectrometer at the Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany. Carbon isotope ratio measurements ($\delta^{13}C$) were made relative to the vPDB standard, and the nitrogen isotope ratio measurements ($\delta^{15}N$) were made relative to the AIR standard. All samples were measured in duplicate, and the errors (1σ) are better than ±0.2‰.

Additional isotopic data produced at the Oxford Radiocarbon Accelerator Unit (ORAU) during sample pretreatment for radiocarbon dating were included for 12 specimens. The ORAU extraction procedures are similar to those that we used, including ultrafiltration [Brown *et al.* (65)]. All of the data reported here have C/N ratios that are within the accepted range of 2.9–3.6 (61).

In addition, 15 of the same specimens were AMS radiocarbon dated, principally by ORAU (OxA); two specimens were dated by the University of Vienna laboratory (VERA), one by using ultrafiltration and the other of an intact molar, which experience has shown provides results similar to bone dates run with ultrafiltration.

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