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Identification of ambiguous fossil bone remains following $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signals on bone collagen

Aplicaciones inmediatas de las señales isotópicas ^{13}C y ^{15}N obtenidas en colágeno óseo de restos fósiles: identificación de ejemplares con caracteres ambiguos

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ABSTRACT

In this paper we deal with an useful application of isotopic studies on fossil bone remains. Following ^{13}C and ^{15}N signals recorded in bone collagen, it is possible to classify into the proper taxon some doubtful remains. Thus, three fossil ribs from Liñares site (Galicia, NW Iberian Peninsula) have been correctly classified as belonging to *Cervus elaphus* L., taking into account both some previous works on its isotopic signatures as well as morphological data.

Key words: *Ursus spelaeus*, *Cervus elaphus*, *Ursus arctos*, stable isotopes, ^{13}C , ^{15}N .

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INTRODUCTION

Carbon and nitrogen stable isotopes are extremely useful for different biological fields, for instance, in order to determine animal paleodiets and their relationship with the habitat and ecosystem changes (AMBROSE, 1991; AMBROSE & DE NIRO, 1989; BOCHERENS *et al.*, 1991; BOCHERENS *et al.*, 1994; BOCHERENS *et al.*, 1995; BOCHERENS *et al.*, 1996; BOCHERENS *et al.*, 1997a; BOCHERENS *et al.*, 1997b; BOCHERENS *et al.*, 1999; CERLING & HARRIS, 1999; CORMIE & SCHWARCZ, 1994; FERNÁNDEZ MOSQUERA, 1998; FERNÁNDEZ MOSQUERA *et al.*, 2000; HOBSON & MONTEVECCHI, 1991; KOCH *et al.*, 1997; VILA TABOADA *et al.*, 1999; WANG & CERLING, 1994; WANG *et al.*, 1994). Some striking works on this topic have been, for example, when using nitrogen and carbon collagen ^{15}N as a proxy for paleoprecipitation levels following analyses on fossil kangaroos ranging a specific period of time (GRÖCKE *et al.*, 1997). Or when revealing strong marine and El Niño effects on island food webs (STAPP *et al.*, 1999), and also when reflecting African winter quarters when examined across a migratory divide in different bird subspecies (CHAMBERLAIN *et al.*, 2000).

In this paper, carbon and nitrogen stable isotopes in bone collagen are proposed as paleontological tool in order to classify ambiguous fossil remains, when metric or anatomical comparisons are not significant enough, or when fragmented specimens have lost their key characters. This

issue will be focused on two Pleistocene mammals: *Cervus elaphus* L. and *Ursus spelaeus* Ros.-Hein., and the distinction between their anterior ribs.

The starting point of this suggestion comes from those plurispecific sites as Liñares (LÓPEZ GONZÁLEZ *et al.*, 1997) and the need of a new criterion in order to clarify difficult identifications: for instance, those generated by the genera *Bos* and *Bison* (LÓPEZ GONZÁLEZ *et al.*, 1999 *and included references*).

The rich site of Liñares (Lugo, Galicia, NW Spain) has provided with fossil bone remains from several species as large bovid (LÓPEZ GONZÁLEZ *et al.*, 1999), wild boar (*Sus scrofa*), horse (*Equus caballus*), though most of the remains belongs to red deer, 50.0 %, (*Cervus elaphus*) and cave bear, 30.9%, (*Ursus spelaeus*) (GRANDAL d'ANGLADE & LÓPEZ GONZÁLEZ, 1998). This site has been dated by ^{14}C AMS at 35.220 ± 1.440 years Before Present [yBP] (GRANDAL d'ANGLADE *et al.*, 1997) and $>38,000$ yBP (GRANDAL d'ANGLADE & LÓPEZ GONZÁLEZ, 1998).

It has been selected three ribs for this isotopic study, firstly identified as belonging to *Ursus spelaeus* regarding their anatomical description. They show strong and little arched body. Their articular head is at an almost right angle, level with the rib tuberosity. The problem lies in that these three ribs are the most anterior of the thoracic cage, just where it is most difficult to distinguish between *U. spelaeus* and *Cervus elaphus* ribs. Towards posterior positions, the ribs from each species are getting more and more different, espe-

cially at the body, the articular head and the articular surface. In any case, the

metrical analysis is useful to carry the distinction out.

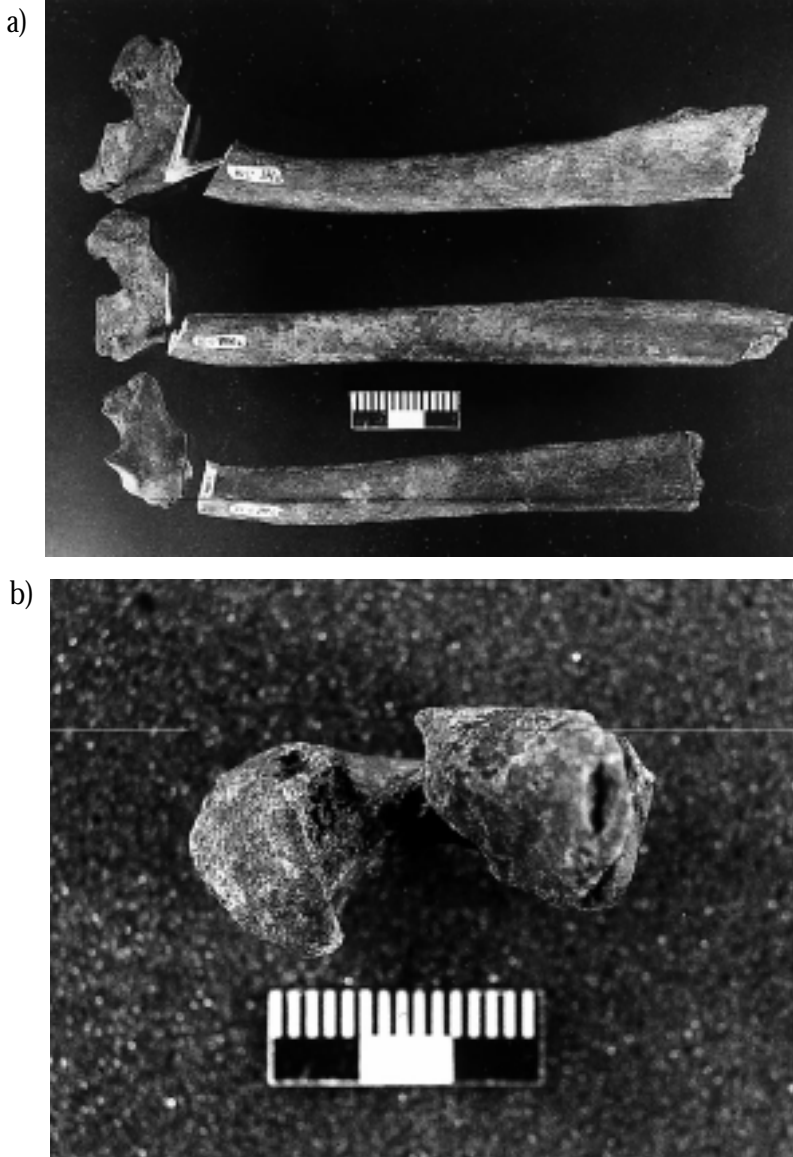


Figure 1. (a) The three analysed fossil ribs showing where they were cut in order to extract collagen. (b) Detail of one of the rib heads.

SAMPLES AND TECHNIQUES

The studied ribs were, named as LXL-400, LXL-372 and LXL-399, were provided by the *Laboratorio Xeolóxico de Laxe*. Their preservation condition was fairly good.

As %N is a first indicator about collagen preservation, 0.5 mg of bone powder (got after cleaning by sandblasting, alternative acetone/distilled water sonicating, sewing, crashing and finely ground) was analysed in a Carlo-Erba 1108 Elemental Analyser with analytical reproducibility better than 0.1 %. Once those bones not suitable enough for isotopic determination were evaluated (IACUMIN *et al.*, 1997), we followed a common collagen extraction method (BOCHERENS *et al.*, 1997b), based on alternative reactions and filtratings with HCl and NaOH, lyophilising and analysed by SIRMS (Stable Isotopic Ratios Mass Spectrometry). Carbon and nitrogen isotope measurements were performed on a Finnigan Mat Delta Plus spectrometer joint to an Elemental Analyser Carlo-Erba 1108 with analytic reproducibility better than 0.1‰ for carbon, and 0.2‰ for nitrogen. Results, ^{13}C and ^{15}N , are referred to

international standards: PDB (Pee Dee Bee limestone) and atmospheric N_2 , respectively. The atomic ratio of the heavy isotope to the lighter one ($R_x = {}^m\text{X}/{}^n\text{X}$, $m > n$) in the sample is compared to the one of a standard material. The difference between both, " ", can be calculated following this formula:

$$(\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$$

The computer program *Statgraphics® Plus 3.0 for Windows* has been used for statistical analysis.

RESULTS AND DISCUSSION

After considering which bones were confident enough for isotopic studies, following the atomic [2.9-3.6] C/N atomic ratio (DE NIRO, 1985), the initial number of 3 samples was reduced to 2. Data and information available about their origin are shown in table 1.

In order to classify fossil remains as belonging to a particular species taking as a basis isotopic values, some previous references are necessary. Literature provides with a lot of data from cave bears (BOCHERENS *et al.*, 1994; BOCHE-

Site	Bone number	Age class	Bone type	Absolute age (Ky) #	Altitude m a.s.l.*	Bone %N	Yield (mg%)	Atomic C/N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Lifaces	LXL400	Adult	Rib	35	1115	2,79	74,88	2,95	6,90	-19,39
Lifaces	LXL399	Adult	Rib	35	1115	2,64	57,38	3,08	6,12	-19,92

Table 1. Site information and isotopic data of bones whose isotopic information is proper for palaeoenvironmental inference. Absolute ages are shown as an average value, see exact radiocarbon datings in *Sample and Techniques*. [*] above sea level; [#] Kiloyears Before Present.

RENS *et al.*, 1997b; BOCHERENS *et al.*, 1999; FERNÁNDEZ MOSQUERA, 1998; LIDÉN & ANGERBJÖRN, 1999; NELSON *et al.*, 1998; VILA TABOADA *et al.*, 1999) ranging very different time-spans, individual age stage and type of site. The *Cervus elaphus* isotopic record is not so plentiful (BOCHERENS *et al.*, 1997a; IACUMIN *et al.*, 1997; NOENYGAARD, 1995; VILA TABOADA *et al.*, 1999), though rich enough to characterize our specimens.

Due to the high variability of the isotopic data for *Ursus spelaeus*—depending on the environment conditionings, diet and dormancy period (FERNÁNDEZ MOSQUERA *et al.*, 2000)—and also to the existence of isotopic data of both species at Liñares (VILA TABOADA *et al.*, 1999), we will only take these as reference for the statistical tests. Figure 2 shows a graphic approach to both species, even adding—in an indicative way—some data from fossil *Ursus arctos* (VILA TABOADA *et al.*, 1999) coming from a near site. Thus, it is shown that different species (*spelaeus-arc-tos*) from the same genus (*Ursus*) have no reason to show close values and that such values depend on the *ecological niche* and metabolism natural for each species.

Thus, the comparison of these isotopic values with those previously published for this site clearly shows (Kruskal-Wallis test, p -value < 0.005 , $n=28$) the similarity of the medians of the "ambiguous" bones

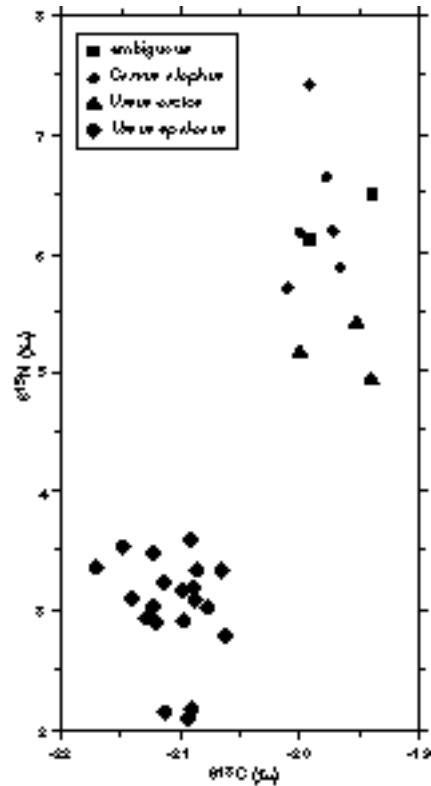


Figure 2. Isotopic results for Liñares *Ursus spelaeus*, Liñares *Cervus elaphus* and Tarelo *Ursus arctos*, as well as the LXL-399 and LXL-400 ribs.

(both variables: ^{13}C and ^{15}N) with the *Cervus elaphus* group. There is a significant difference with the *Ursus spelaeus* data. See figure 3.

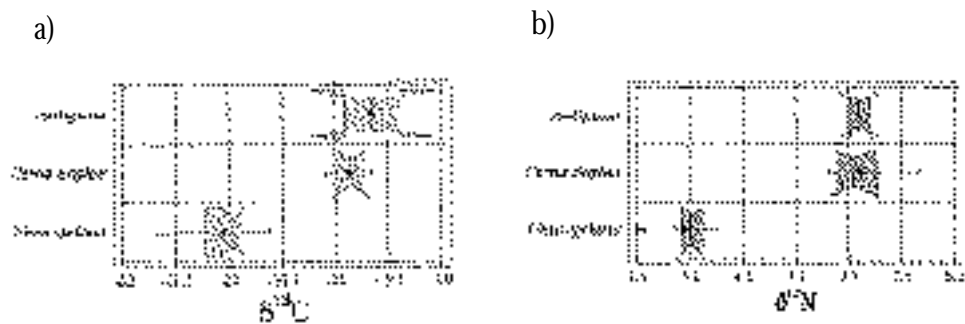


Figure 3. Box and Whisker plot for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ depending on the species. (a) $\delta^{13}\text{C}$ (b) $\delta^{15}\text{N}$. All data come from Liñares site. Notches mark the median, whereas dots show the sample mean. Bars length represents the standard deviation.

CONCLUSIONS

This paper shows a new application of stable isotopes as identification tool at species level, when morphometric determination is not possible. The increase of isotopic database in different species, proper age stage assignments of the specimens, as well as good datings for each sample will allow to rise the reliability of

the identifications and also the accuracy of the paleoenvironmental reconstructions.

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