

Osteological identification criteria for *Capra pyrenaica* and *Capra hircus* validated with DNA

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ABSTRACT

The taxonomic identification of *Capra hircus* and *Capra pyrenaica* from bone remains is essential in order to be able to infer a prehistoric society's subsistence strategy. Although osteometry is the criterion that is typically used to distinguish both of these species, Sarrión (1988) also defined morphological characteristics for distinguishing them. The objective of this work is to validate these morphological criteria with the genetic identification of ovicaprine bone remains from Palaeolithic and Neolithic levels of the Chaves site (Sierra de Guara, Huesca). The preliminary results indicate that the astragalus and calcaneus are more adequate for identifying *Capra pyrenaica* and *Capra hircus* and the discrimination criteria of mandibles and metacarpals are called into question.

Keywords: *Capra pyrenaica*, *Capra hircus*, DNA, Neolithic, Palaeolithic, Chaves site.

RESUMEN

La identificación taxonómica de *Capra hircus* y *Capra pyrenaica* a partir de sus restos óseos es esencial para inferir la estrategia de subsistencia de una sociedad prehistórica. Aunque el criterio de diferenciación entre ambas especies más utilizado es la osteometría, también se han definido caracteres morfológicos óseos que las diferencian (Sarrión, 1988). El objetivo de este trabajo es validar estos criterios morfológicos a partir de la identificación genética del material óseo de ovicápridos (ovejas y cabras: domésticas y salvajes) de los niveles paleolíticos y neolíticos del yacimiento de Chaves (sierra de Guara, Huesca). Los resultados preliminares indican que los astrágalos y calcáneos son los elementos anatómicos más adecuados para su identificación y se discuten los criterios de discriminación en las hemimandíbulas y metacarpos.

Palabras clave: *Capra pyrenaica*, *Capra hircus*, ADN, Neolítico, Paleolítico, yacimiento de Chaves.

1. INTRODUCTION

The discrimination of Iberian ibex (*Capra pyrenaica*) and domestic goat (*Capra hircus*) (Artiodactila; Mammalia) (Wilson & Reeder, 2005) is essential for inferring the subsistence strategy or economy of a population: hunter, livestock breeder or mixed economy. Therefore, an adequate taxonomic classification of goats at an archaeopalaeontological site is necessary in order to gain an understanding of prehistoric societies' way of life.

The differentiation between Iberian ibex (*Capra pyrenaica*) and domestic goat (*Capra hircus*) at a peninsular site is based on their difference in size: the Iberian ibex is bigger than the domestic goat. Nevertheless, the osteometric overlap among the big females and the small males must be considered (Altuna, 1978). On few occasions, the morphology of the bone remains, defined by Sarrión (1988), is used by zooarchaeologists for their differentiation.

Sarrión (1988) observed morphological differences in anatomical elements, such as mandibles, astragali, calcanei, metapodials (basically metacarpals) and phalanges. This study focuses on all of these anatomical elements.

The first objective is to carry out a detailed study of the diagnostic characteristics, which morphologically distinguish the bones of Iberian ibex and domestic goats from the Iberian Peninsula. The next objective is to validate the currently proposed morphological criteria, through genetic samples extracted from the bone remains of goats from Palaeolithic and Neolithic levels of the Chaves site. A complete morphological review, which distinguishes between wild and domestic goats, has been carried out. Some characteristics have been validated and some of them have been called into question.

2. MATERIAL AND METHODS

2.1. Material

The material is composed of 23 bone remains (five mandibles, 11 astragali, two calcanei, three metapodials and two phalanges are included) of Iberian ibex and domestic goats from Palaeolithic and Neolithic levels of the Chaves site, according to zooarchaeological (osteometric and morphological methodology) criteria. Genetic information was recovered from 18 of the 23 bone remains using ancient DNA techniques. The results indicated that 18 bone remains belong to the genus *Capra* (*Capra hircus* or *Capra pyrenaica*). These 18 bone remains are the elements that have been used for the morphological analysis, because both genetics and zooarchaeological criteria that indicated that they belong to wild or domestic goats.

The Chaves cave is located in the Sierra de Guara (Huesca, Aragón). It comprises Upper Palaeolithic and Neolithic levels, clearly separated by a speleothem (Utrilla, 1992). The high frequency of wild goats, which were identified within Magdalenian (level IIa: 12,020 BP and level IIb: 12,800 BP), is noteworthy, and the site served as an area for hunting bucardos (Castaños, 1993). Bucardo is the Spanish common name of *Capra pyrenaica pyrenaica*: subspecies of Iberian wild goat from the Pyrenees which was recently extinct. In Neolithic levels, the Iberian goat constituted the most abundant wild ungulate followed by the deer (Castaños, 2004). The ovicaprines (*Ovis aries* and *Capra hircus*) are the best represented group from the Neolithic with 6,163 identified bone remains and a ratio (*Ovis aries*/*Capra hircus*) of 1.34 in level Ia and 1.76 in level Ib (Castaños, 2004).

2.2. Zooarchaeological method

In order to conduct palaeontological discrimination (*Capra hircus* versus *Capra pyrenaica*), metric data, considering the range of values between wild and domestic form, according to Altuna (1978) and morphological criteria following Sarrión (1988) were used.

In Neolithic levels, the presence of sheep (*Ovis aries*), in addition to goats (both Iberian ibex and domestic goat), has been considered. The osteometric (Payne, 1969) and morphological criteria for postcranial (Boessneck *et al.*, 1964; Boessneck, 1969; Kratochvil, 1969; Prummel & Frisch, 1986) and cranial elements (Payne, 1985; Helmer, 2000; Halstead *et al.*, 2002) have been applied for their discrimination. A critical review is found in Zeder & Pilaar (2010) and Zeder & Lapham (2010).

2.3. Genetic method

All the DNA extractions were performed at the ancient DNA laboratory (Centro Mixto UCM-ISCI, Madrid and EBC, Uppsala). Between 150 and 200 mg of bone/tooth powder were used to conduct DNA extractions following a silica extraction method (Yang *et al.*, 1998). For every 10 samples, 2 water negative controls were used. Each extract was eluted in a final volume of 60 µl Elution Buffer).

In order to identify the samples, a short fragment of the mitochondrial DNA was amplified. The primers Mbos L1269 and Mbos H1346 (Rollo *et al.*, 2002) were used to amplify a 117 bp fragment of the mitochondrial gene 12S).

The PCR amplification was conducted in 25 µl using 1 X Smart-Taq Hot 10X PCR Buffer (Naxo), 1mM Smart-Taq Hot 25mM MgCl₂ solution (Naxo), 0.1 mM dNTPs (Naxo), 0.2 µM of each tagged primer (Biomers), one unit of Smart Taq Hot Polymerase 10U/µl (Naxo) and 2 µl of

DNA extract. One PCR water control was prepared for every 10 samples. Thermal cycling conditions were 95°C for 15 min and 52 cycles of 94°C for 30 sec, 51-54°C for 30 sec, 72°C for 30 sec, and 72°C for 10 min. Amplicons were purified using ExoStar™ and sequenced at Centro Nacional de Investigaciones Oncológicas (the National Cancer Research Centre) or at Macrogen.

The results obtained were compared using BLAST with the available sequences in the GenBank for the different species of mammals.

3. RESULTS

DNA recovery and genetic identification were possible for 18 of the 23 samples: two of them were identified as *Capra hircus*, 16 as *Capra pyrenaica*.

The five mandibles were identified as belonging to *Capra pyrenaica* using osteometric (Table 1), as well as morphological and genetic criteria (CH7, CH41, CH25, CH27, and CH40) (Table 6).

The zooarchaeological identification (Table 2) of the metacarpal CH1 (Neolithic) suggested that it belonged to *Capra pyrenaica*; nevertheless, the genetic analysis indicated that this element belonged to *Capra hircus* (Table 6).

Nine out of the 11 astragali that were studied gave positive results from a DNA analysis. Eight of these nine were identified as Iberian ibex or domestic goat by zooarchaeological and genetic criteria: seven were identified as *Capra pyrenaica* (Tables 3, 6) (CH76, CH77, CH80, CH82, CH83, CH10, CH20) and one as *Capra hircus* (CH98) (Table 6). The astragalus CH93 was identified as *Ovis aries* by osteometric and morphological criteria; nevertheless, the genetic criteria indicate that it belonged to *Capra pyrenaica* (Tables 3, 6).

The two analysed calcanei (CH18 from the Palaeolithic level and CH8 from the Neolithic) were identified as *Capra pyrenaica*, based on their large size (Table 4) and morphology. The genetic analysis indicated the same taxonomical identification (Table 6).

The second phalanx (CH6) was identified as *Capra pyrenaica* by zooarchaeological (Table 5 for osteometry) and genetic analysis (Table 6).

Table 1. Metric data of goat mandibles from Chaves. LM₁-M₃: occlusal length M₁-M₃; LP₂-P₄: occlusal length P₂-P₄; LM₁: length M₁; LM₃: length M₃ (all values in mm). Values [min-max, mean] of LP₂-P₄ for mandibles of Iberian ibex (Altuna, 1978): [21-30, 25.45].

		LM ₁ -M ₃	LP ₂ -P ₄	LM ₃	BM ₃	Osteometric identification	
Mandibles	CH7	CH.90.8D.112.242	54.7	23.56	28.5	9.35	<i>Capra pyrenaica</i>
	CH25	CH.4G.292.54	50.7	22.48	22.94	10.43	<i>Capra pyrenaica</i>
	CH27	CH.5G.281.35	74.03	22.5	39.72	12.3	<i>Capra pyrenaica</i>
	CH40	CH.2G.290.88	72.31	X	31.43	10.1	<i>Capra pyrenaica</i>
	CH41	CH.2E.282.308 (9)	51	X	20.5	5.3	<i>Capra pyrenaica</i>

Table 2. Metric data of goat metacarpal from Chaves. Bd: distal width; WCM: medio-lateral width of the condyle; DEM: antero-posterior width of its external trochlea (all values in mm). Values [min-max, mean] of Bd for metacarpal of Iberian ibex (Altuna, 1978): [31.5-41.5, 36.63] and for domestic goat: [23.5-28.4, 25.9] (Iborra, 2004).

		Bd	WCM	DEM	Osteometric identification	
Metacarpal	CH1	CH.7C.1b.36	37.65	16.44	13.33	<i>Capra pyrenaica</i>
	CH17	CH.2E.284.21	35.52	16.44	14.22	<i>Capra pyrenaica</i>
	CH29	CH.E.2.285.33	36.63	17.49	15.91	<i>Capra pyrenaica</i>

Therefore, 18 anatomical cranial and postcranial elements of *Capra pyrenaica* and *Capra hircus* have been analysed (* in Table 6): nine astragali, one metacarpal, two calcanei, five mandibles and one second phalanx, which they were identified as either Iberian ibex or as domestic goats, by both zooarchaeological and genetic analysis.

Next, the morphological description of some samples is indicated.

On the left astragalus CH10 (Fig. 1). From plantar aspect, the junction between the proximo-plantar and lateral trochlea is rounded. The surface from medial to lateral side (c-d), if the astragalus were cut transversally, it

Table 3. Metric data of goat astragalus from Chaves. GLl: greatest lateral length; GLm: greatest medial length; BL: lateral width; Bm: medial width; Bd: distal width (all values in mm). Values [min-max] for GLl for astragalus [31.5-40.5, 36.68] and Bd [20-28, 23.88] of Iberian ibex (Altuna, 1978).

		GLl	GLm	BL	Bm	Bd	Osteometric Identification	
Astragalus	CH10	CH.D.1b.103	40.73	36.54	22.3	22.57	24.36	<i>Capra pyrenaica</i>
	CH20	CH.E2.280.11	39.34	36.72	21.9	22.43	26.38	<i>Capra pyrenaica</i>
	CH76	CH.89A.1b.301	34.5	32.6	18.93	18.5	21.92	<i>Capra pyrenaica</i>
	CH77	CH.92.14C.1a.199	38.08	36.62	20.97	21.75	23.76	<i>Capra pyrenaica</i>
	CH78	No label	38.7	34.88	20.97	20.88	25.29	<i>Capra pyrenaica</i>
	CH80	No label	34.38	32.06	X	X	X	<i>Capra pyrenaica</i>
	CH81	No label	34.95	32.43	18.59	18.72	21.85	<i>Capra pyrenaica</i>
	CH82	No label	33.74	31.6	18.58	18.01	21.92	<i>Capra pyrenaica</i>
	CH83	No label	34.03	32.61	18.47	18.85	22.84	<i>Capra pyrenaica</i>
	CH93 ^N	No label	26.54	25.75	15.31	14.74	16.72	<i>Ovis aries</i>
CH98 ^N	CH.92.12E.1a.71	28	25.8	16.6	17.01	17.5	<i>Capra hircus</i>	

Table 4. Metric data of goat calcaneus from Chaves. GL: greatest length; Bd: greatest width (all values in mm). Uf: unfused. Values [min-max, mean] of GL for calcaneus of Iberian ibex (Altuna, 1978): [65-86, 75.52] and for domestic goat (Iborra, 2004): [43.6-52, 47.8].

		GL	Bd	Osteometric identification	
Calcaneus	CH8	CH.6D.220.102	uf 68.8	24.5	<i>Capra pyrenaica</i>
	CH18	CH.E4.280.270	70.97	24.33	<i>Capra pyrenaica</i>

Table 5. Metric data of goat second phalanx from Chaves (all values in mm).

		GL	Bp	Bd	SD	Osteometric identification	
II phalanx	CH6	CH.92.10D.1b.92	28.4	16.2	12.36	12.32	<i>Capra pyrenaica</i>
	CH15	CH.E4.288.299	31.42	16.77	13.5	12.43	<i>Capra pyrenaica</i>

would be slightly concave (a). Nevertheless, this surface is flat in domestic goats. The cavity (b) located among plantar and proximal trochlea (i.e., the contact point between it and the coracoid process of the calcaneus) is deep and sharpened. From a lateral aspect, the distal articular surface (e) is elongated and forms a kidney shape.

All morphological criteria (according to Sarrión, 1988) and genetic analysis indicate that this astragalus belongs to *Capra pyrenaica*.

These diagnostic criteria for *Capra pyrenaica* have been observed on six additional astragali (CH20, CH76, CH77, CH80, CH82, CH83), which are consistent with the taxonomic identification achieved through DNA analysis. The zooarchaeological and genetic criteria indicate that one of the astragalus (CH98) belongs to *Capra hircus*. Just one of the astragalus was identified as *Ovis aries* with zooarchaeological criteria, and the genetic results indicate that it belongs to *Capra pyrenaica* (CH93 in Table 6). Thus, seven of nine astragali (77.7 %) were

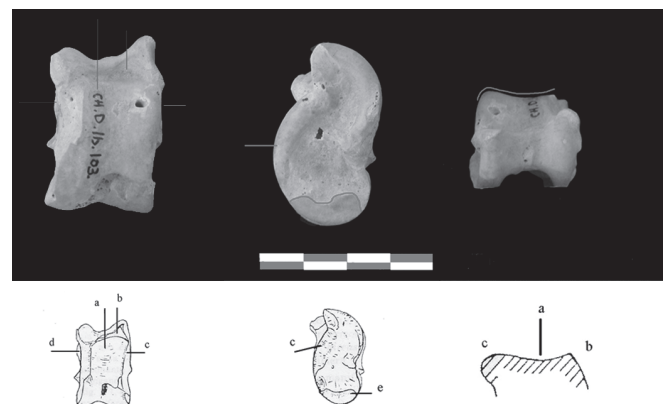


Figure 1. Picture of left astragalus (CH10) of *Capra pyrenaica* from Neolithic levels at the Chaves site. From left to right: plantar, lateral and proximal views. Scale: 4 cm. Below: diagnostic criteria to distinguish astragalus of *Capra pyrenaica* and *Capra hircus* (taken from Sarrión, 1988). a, b, c, d, e: see description in the text.

Table 6. Anatomical elements used for the study. AST: astragalus; MC: metacarpal; II FALN: II phalanx; MAN: mandible; CALC: calcaneus. *O.a.*: *Ovis aries*; *C.h.*: *Capra hircus*; *C.py*: *Capra pyrenaica*.

	Anatomical element	Zooarchaeological criteria	DNA criteria
CH93 ^N	AST	<i>O.a.</i>	<i>C.py</i> *
CH98 ^N	AST	<i>C.h.</i>	<i>C.h.</i> *
CH76 ^N	AST	<i>C.py</i>	<i>C.py</i> *
CH77 ^N	AST	<i>C.py</i>	<i>C.py</i> *
CH78	AST	<i>C.py</i>	X
CH80 ^N	AST	<i>C.py</i>	<i>C.py</i> *
CH81	AST	<i>C.py</i>	X
CH82 ^N	AST	<i>C.py</i>	<i>C.py</i> *
CH83 ^N	AST	<i>C.py</i>	<i>C.py</i> *
CH1 ^N	MC	<i>C.py</i>	<i>C.h.</i> *
CH6 ^N	II FALN	<i>C.py</i>	<i>C.py</i> *
CH7 ^N	MAND	<i>C.py</i>	<i>C.py</i> *
CH8 ^N	CALC	<i>C.py</i>	<i>C.py</i> *
CH10 ^N	AST	<i>C.py</i>	<i>C.py</i> *
CH15 ^P	II FALN	<i>C.py</i>	X
CH17 ^P	MC	<i>C.py</i>	<i>C.py</i> *
CH18 ^P	CALC	<i>C.py</i>	<i>C.py</i> *
CH20 ^P	AST	<i>C.py</i>	<i>C.py</i> *
CH25 ^P	MAND	<i>C.py</i>	<i>C.py</i> *
CH27 ^P	MAND	<i>C.py</i>	<i>C.py</i> *
CH29 ^P	MC	<i>C.py</i>	X
CH40 ^P	MAND	<i>C.py</i>	<i>C.py</i> *
CH41 ^P	MAND	<i>C.py</i>	<i>C.py</i> *

identified as *Capra pyrenaica* through the use of diagnostic morphological and genetic criteria. Nevertheless, just one of the nine samples (11.11 %) that belongs to *Capra hircus* as identified using morphological criteria (Zeder & Lapham, 2010), was identified as *Capra hircus* through genetic criteria. One of the nine astragali was identified as *Ovis aries* by morphological criteria and as *Capra pyrenaica* with DNA identification.

In the case of the calcaneus CH8 (Fig. 2), the junction between the coracoid process and the articular facet for the os malleolare is rounded (a) (the negative of the astragalus morphology) and the side of the coracoid is curved (c). On the cavity's articular surface for the trochlea of the astragalus, the surface is joined in proximal parts (b). Both morphological and genetic criteria indicate the calcaneus CH8 belongs to *Capra pyrenaica*.

The calcaneus CH18 presents diagnostic morphological characteristics of *Capra pyrenaica* so that, through the use of palaeontological criteria like genetics, it concurs with the taxonomic identification.

The mandible CH7 (Fig. 3) includes the almost complete cheektooth row (P₃-M₃), missing P₂. The lack of

a second premolar is a characteristic that is typical of the *Capra pyrenaica* mandible, according to Sarrión (1988). This missing second premolar is due to lack of entity in the roots, which causes its loss from the alveoli (Sarrión, 1988). The index that indicates this characteristic has been calculated:

$$\text{Index (CH7)} = (\text{LP}_2 * 100) / \text{LP}_3 = (3.5 * 100) / 5.5 = 63.63$$

where LP: alveolar length of premolar; P₂: second premolar; P₃: third premolar.

This result is closer to the average value from the *Capra hircus* index than to the *Capra pyrenaica* index average value, according to Sarrión (1988).

The mandibles CH40 and CH41 have no P₂, and the alveoli are reabsorbed, so the metric data of the alveoli length have not been taken and the index could not be calculated. The genetic analysis indicates that these mandibles belong to *Capra pyrenaica*.

The P₂ is missing in mandible CH25, which is a typical characteristic of *Capra pyrenaica*, and the index = (2.8*100)/4.5 = 62.22 indicates a value closer to *Capra*



Figure 2. Left: calcaneus (CH8) of *Capra pyrenaica* from the Neolithic levels of Chaves, in dorsal view. Scale: 4 cm. Right: diagnostic morphological criteria of *Capra pyrenaica*, according to Sarrión (1988). a, b, c: see description in the text.



Figure 3. Picture of right mandible (CH7) of *Capra pyrenaica* from Neolithic levels of the Chaves site. Scale: 3 cm. LP2: length of second premolar; LP3: length of third premolar.

hircus than the index of *Capra pyrenaica*. The genetic analysis indicates that this mandible belongs to *Capra pyrenaica*.

The P₂ is missing in the mandible CH27 and the index = (3.9*100)/6 = 65 indicates that the value is closer to *Capra hircus* than the index of *Capra pyrenaica*. The genetic analysis indicates that this mandible belongs to *Capra pyrenaica*.

The size of the mandibles and the chronology of the level (Palaeolithic) indicate that the five mandibles

belong to *C. pyrenaica*. The missing P₂ is observed in all mandibles. The index could be calculated in three of the five mandibles. The three mandibles have an index closer to *C. hircus* than *C. pyrenaica*. The DNA analysis conducted on the mandibles indicates that they belonged to *Capra pyrenaica*.

The morphological criteria of the metacarpal CH1 (Fig. 4) have been described in order to check the taxonomic identification. In dorsal aspect, the distal third of the diaphysis is flat (b) without epiphysis protuberances and links to distal articular surface without (or with slight) cavities above (a). The cavities, located above the distal articular surfaces, are slightly marked, opposite of the *Capra hircus* metacarpal, which has very marked cavities. In lateral or medial aspect, if a line was extended from the anterior surface of the diaphysis to the distal articular surface, the line should cut the distal articular surface (c). All morphological characteristics indicate that this metacarpal belongs to *Capra pyrenaica* according to Sarrión (1988). However, the DNA analysis showed haplotypes of *Capra hircus*.

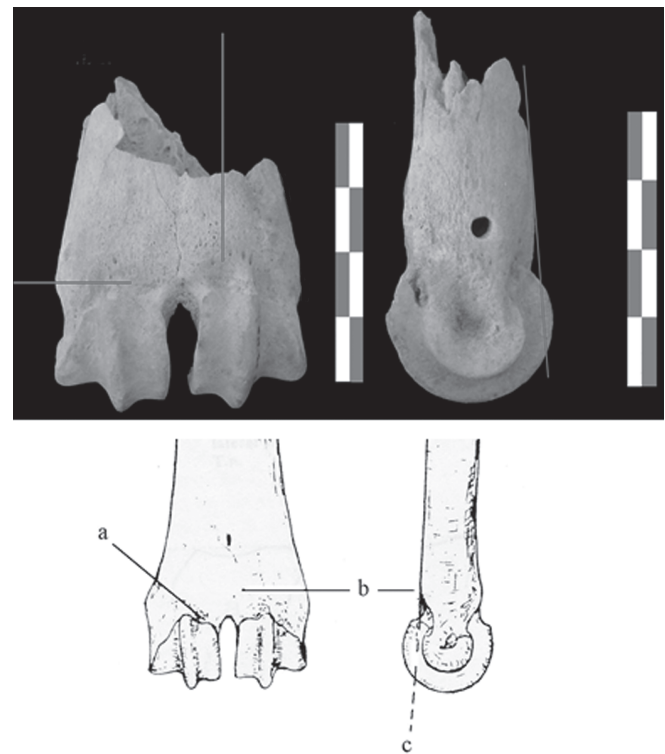


Figure 4. Metacarpal (CH1) of *Capra pyrenaica* from the Neolithic levels of the Chaves site. From left to right: dorsal view and lateral view. Scale: 4 cm. a, b: See description in the text.

The morphological and genetic criteria are in agreement with regard to the second phalanx (CH6) in that it belongs to *Capra pyrenaica*.

4. DISCUSSION

The morphological and genetic criteria gave the same taxonomical identification of goat (wild or domestic) in 91.67 % (11 of 12 anatomical elements) of the analysed samples of astragalus, calcaneus and second phalanx. Therefore, these anatomical elements are considered appropriate for their identification.

A missing P_2 is observed in 100 % of the analysed mandibles and thus, we consider it to be a valid criterion for taxonomic identification of *Capra pyrenaica* (Table 7). Nevertheless, the calculation of the dental index does not match the expected rate in any of the analysed mandibles. Therefore, this criterion is not valid for distinguishing *C. hircus* and *C. pyrenaica*.

Table 7. Summary of the zooarchaeological and DNA results for the identification of goats using the mandibles from the Chaves site.

Mandibles	Missing P_2	Index	Size	Chronology	DNA analysis
CH7	Yes	63.63 <i>C.hi</i>	<i>C.py</i>	Palaeolithic (<i>C.py</i>)	<i>C.py</i>
CH25	Yes	62.22 <i>C.hi</i>	<i>C.py</i>	Palaeolithic (<i>C.py</i>)	<i>C.py</i>
CH27	Yes	65 <i>C.hi</i>	<i>C.py</i>	Palaeolithic (<i>C.py</i>)	<i>C.py</i>
CH40	Yes	X	<i>C.py</i>	Palaeolithic (<i>C.py</i>)	<i>C.py</i>
CH41	Yes	X	<i>C.py</i>	Palaeolithic (<i>C.py</i>)	<i>C.py</i>

In the case of the metacarpal, the criteria according to Sarrión (1988) for identifying *C. pyrenaica* does not concur with genetic results, so these must be used with caution.

5. CONCLUSIONS

The morphological criteria of the anatomical elements described by Sarrión (1988) for distinguishing between *Capra pyrenaica* and *Capra hircus* have been assessed using DNA analysis. The zooarchaeological analysis is consistent with the genetic analysis on astragalus, calcaneus and second phalanges from Palaeolithic and Neolithic levels at the Chaves site. Some morphological criteria of mandibles and metacarpals are not consistent with the genetic analysis for their identification, so these criteria must be used with caution.

Despite the fact that this analysis is preliminary and more samples are necessary in order to assess the

morphological criteria, the criteria considered a valid link to osteometry are sufficient to identify *Capra pyrenaica* without DNA analysis.

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