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ZooMS confirms geometric morphometrics species identification of ancient sheep and goat

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ABSTRACT

Geometric morphometrics can effectively distinguish isolated third lower molars of present-day sheep and goat, but its applicability to archaeological specimens has yet to be established. Using a modern reference collection of 743 sheep and goats and a 2D landmark based geometric morphometric (GMM) protocol, this study aimed to morphometrically identify 109 archaeological specimens, used as case studies, dating from the Late Neolithic to the modern period/era. These morphometric identifications were then compared to molecular identifications via collagen peptide mass fingerprinting, known as Zooarcheology by Mass Spectrometry (ZooMS). ZooMS confirmed the morphometric identifications for 104 specimens, with the five misidentified specimens all morphometrically identified as goat.

Modern sheep and goats have larger teeth and distinct shapes compared to their archaeological counterparts, suggesting strong differences between archaeological and modern specimens potentially linked with recent breed improvement or geographic origin of the specimens. In addition, for both species, some of the archaeological dental morphologies do not match with any of our modern references.

This study validates the applicability of geometric morphometrics for identifying isolated archaeological sheep and goat teeth. It represents a stepping stone for future, non-destructive, bioarchaeological studies of the two species.

Keywords: Caprines Identifications, GMM, *Ovis aries*, *Capra hircus*, Paleoproteins, Third lower molar

INTRODUCTION

Zooarchaeology by mass spectrometry (ZooMS) is increasingly used in archaeology for reliable taxonomic identification of organic materials [1] such as bones [2], leather [3] or parchment [4]. ZooMS, by using peptide mass fingerprinting of collagen, provides an inexpensive tool for archaeological studies and has been used to distinguish domestic caprine species and to detect their arrival in Africa [5–8], Asia [9,10], and Europe [11,12], and to study distinct farming practices [13–20].

Sheep (*Ovis aries*) and goat (*Capra hircus*) are two important species of agro-pastoral systems in the Mediterranean basin since the Neolithic and were often herded together [21–29]. However, they possess distinct ecological [30,31] and economical properties [32–36], making it of prime interest to establish secure identifications of ancient specimens before studying the two species separately. Criteria for distinguishing sheep and goat among archaeological assemblages exist, notably from post-cranial bones [37–39] and have greatly improved the ability to differentiate the two species. On teeth, discrete criteria have been proposed [31,40–45], but none has been universally used for identifying isolated lower third molars that are often found among archaeological assemblages, since, unlike bones, they are better preserved [46], but also often left unidentified because of the lack of secure identification criteria.

Geometric morphometrics (GMM) is more and more used in bioarchaeology [47]. This series of analytical tools are based on the multivariate statistical analysis of set of cartesian coordinate data allowing to explore in-depth morphometric variation [48]. Recently, such approaches have been used to quantify sheep and goat morphometric variation based on the size and shape of their lower third molar [49]. This study demonstrated the ability to identify adult modern specimens of the two species with a probability of 93.3%. In addition, the identification of Middle Ages specimens from the site of Missignac (Aimargues, southern France), used as a first case study, appeared congruent with the initial zooarchaeological faunal analyses and interpretations [50,51]. However, in order to assess whether the same geometric morphometrics (GMM) protocol can be used to identify ancient sheep and goat teeth originating from a broader spatio-temporal scale, it is necessary to validate the approach using securely identified archaeological specimens. Archaeological teeth can be found either enclosed in mandibular bone that possess distinct features between the two species [40,43], or isolated (i.e. without the bone), which make their identification particularly challenging even if some discrete criteria have been proposed for both mandible and teeth [31,40–45]. Being able to provide reliable identification is therefore of prime interest to bioarchaeological studies.

Here we aim to (1) identify archaeological specimens based on their third molar shape using geometric morphometrics (GMM), (2) compare those morphometric identifications with newly generated molecular Mass Spectrometry (ZooMS) identifications, and (3) compare the size and shape morphometric variation of modern sheep and goat with their securely identified archaeological counterparts.

MATERIAL AND METHODS

Third lower molars of 743 modern specimens, corresponding to 521 sheep and 222 goats of various breeds and geographical origins from Western Eurasia, including western Europe and north Africa, were used as a reference for the geometric morphometrics analysis (SI-table 1). Part of these specimens were already included in a previous study [49]. Only one tooth per specimen was considered. All studied specimens were adults, with fully erupted third lower molars. Tooth wear stages were used to estimate the age at death of the specimens following the classification established by Payne (1973, 1985) and they were ranging from stages 0 to 17G (*i.e.* from 1-2 years to more than 8 years). Sheep and goat display distinct sexual dimorphism and changes through age based on their third molar morphometry, but both factors were found to have little impact on the species differentiation [49].

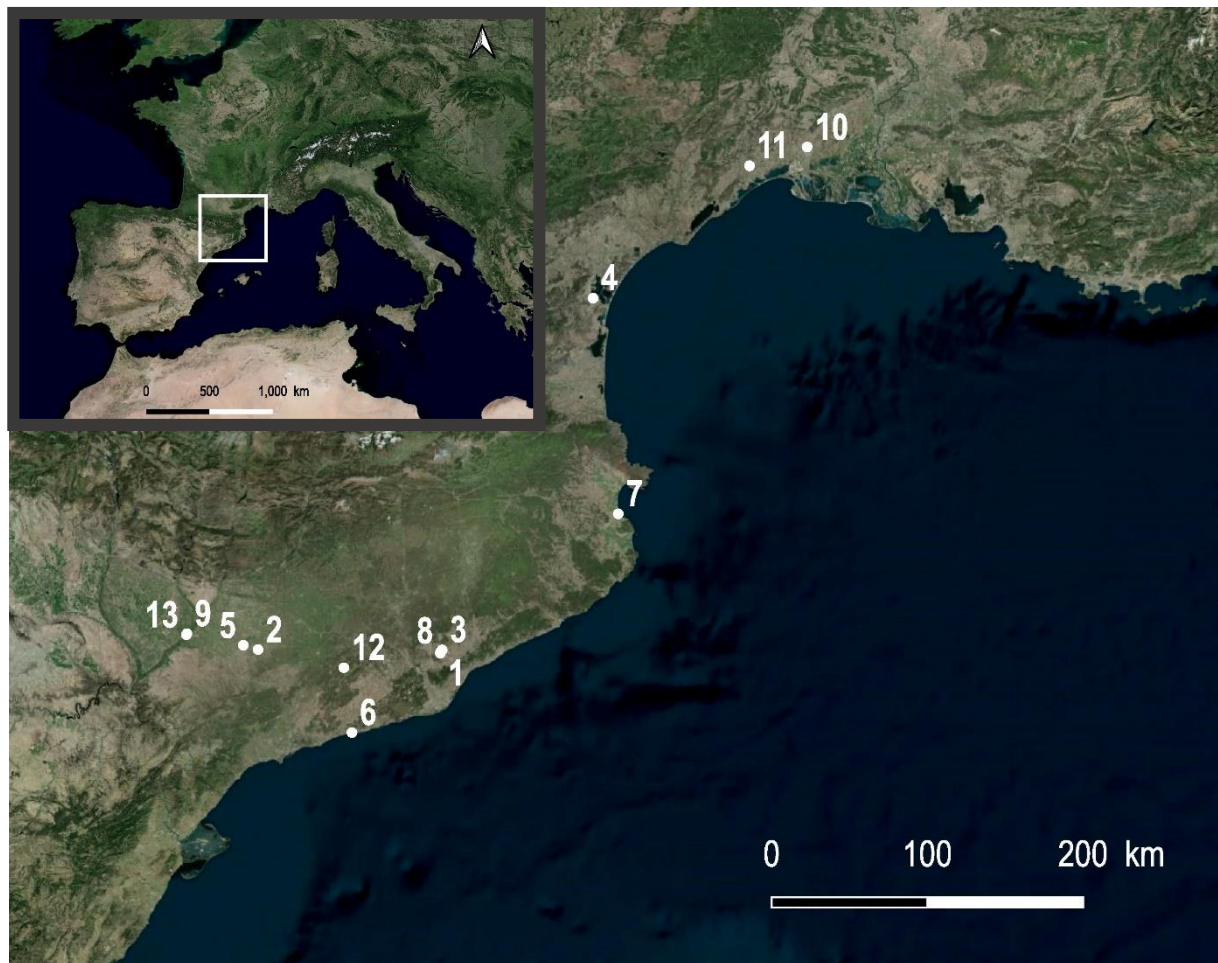
In addition, 109 individuals (SI-table 2) from 13 archaeological sites, selected for their chronological and spatial diversity, dated from the Neolithic to Modern period and located in the north-western part of the Mediterranean basin (present-day Catalonia region in Spain and Occitanie region in Southern France), were used to compare GMM and ZooMS identifications (figure 1, table 1). The selected archaeological specimens consisted of third lower molars with at least a small portion of adherent mandibular bone. For each specimen, the size and shape of the third lower molar was quantified through geometric morphometrics and used for morphometric identification, and a fragment of mandibular bone (normally between 10 to 50 mg) was sampled for molecular ZooMS identification.

Table 1 Description of the 13 archaeological sites sampled, indicating geographic origin, chrono-cultural period of occupation, and number of specimens studied by geometric morphometrics and ZooMS.

ID	Country	City	Name of the Site	Chrono-cultural Occupation	Number of specimens	References
1	Spain	Sant Quirze del Vallès	Bòbila Madurell-Mas Duran	Neolithic	1	[53]
2	Spain	Maldà	Cantorella	Neolithic	1	[54]
2	Spain	Maldà	Cantorella	Bronze Age	1	[54]
3	Spain	Sabadell	Bòbila Madurell - Can Gambús 1	Bronze Age	2	[55]
1	Spain	Sant Quirze del Vallès	Bòbila Madurell-Mas Duran	Iron Age	2	[53]
4	France	Sigean	Pech Maho	Iron Age	15	[56]
5	Spain	Arbeca	Vilars	Iron Age	12	[57]
6	Spain	Calafell	Alorda Park	Iron Age	1	[58]
7	Spain	L'Escala	Empúries	Iron Age	1	[59]
8	Spain	Sabadell	Can Feu	Iron Age	1	[60]
4	France	Sigean	Pech Maho	Iron Age/ Antiquity transition	1	[56]

3	Spain	Sabadell	Bòbila Madurell - Can Gambús 1	Antiquity	21	[55]
9	Spain	Lleida	L'Antic Portal de Magdalena	Antiquity	1	[61]
10	France	Aimargues	Missignac	Middle Age	18	[62,63]
11	France	Montpellier	CNR	Middle Age	20	[64]
12	Spain	La Llacuna	Vilademàger	Middle Age	1	[65]
13	Spain	Lleida	La Cuirassa	Middle Age	4	[66,67]
13	Spain	Lleida	La Cuirassa	Modern period	1	[66,67]
5	Spain	Arbeca	Vilars	Out stratigraphy of	4	[57]

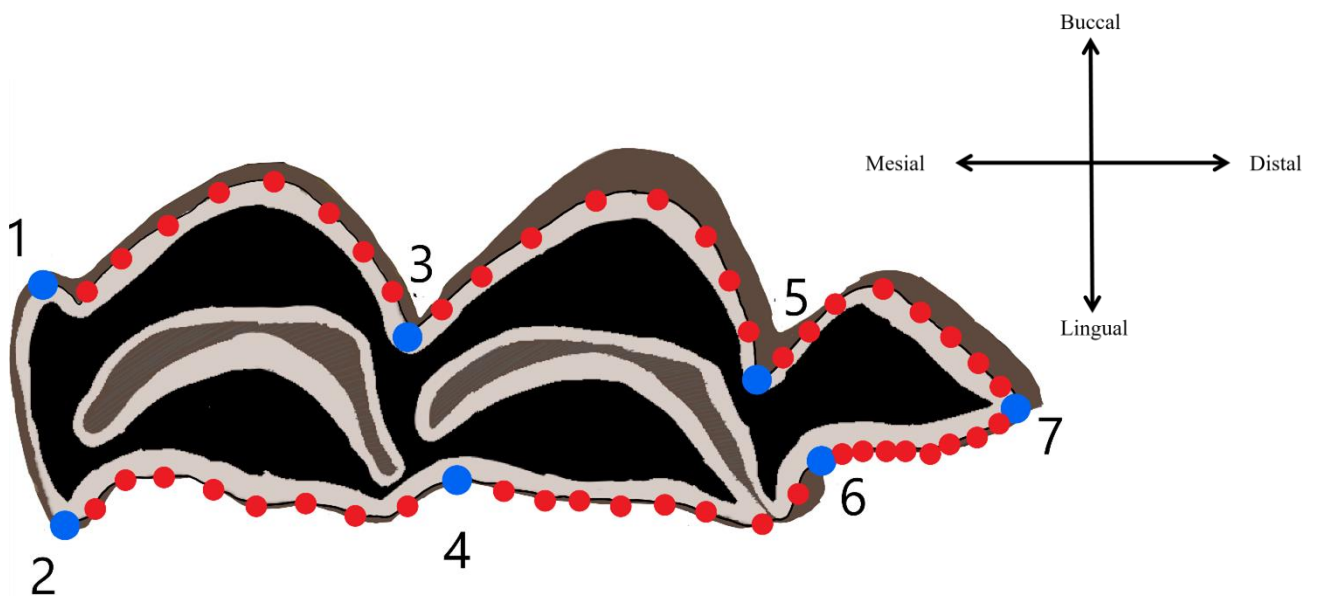
Figure 1 Location of the 13 archaeological sites of the sampled specimens. The numbers refer to the column ID of table 1.



Geometric morphometrics

The occlusal view of the third lower molars was photographed using a Nikon d90 LSR camera paired with a 60 mm macro lens (AF-S Micro NIKKOR) attached to a photographic arm (manfrotto 244RC) following a previously published protocol [49]. Right molars were preferentially photographed, and when necessary, photographs of left molars were mirrored prior to GMM data acquisition. Third molar size and shape were quantified using two dimensional landmarks and sliding landmarks based geometric morphometrics approaches following the protocol developed in our previous study [49] (figure 2). The coordinates of seven landmarks (in blue) and of 48 sliding semi-landmarks distributed along six curves (eight equidistant points in each) were acquired using TPSdig2 [68] (for a formal description of the landmark positions see SI table 2 of [49]) (figure 2). This protocol does not include points along the mesial part of the teeth that is often affected by lateral wear (Jeanjean et al., 2022). All data are available in SI table 3.

Figure. 2 Geometric morphometrics protocol: Position of the seven landmarks (in blue) and 48 sliding semi-landmarks (in red) whose coordinates were acquired on a right third lower molar of a sheep. Landmark positions are described in SI table 2 of [49].



Morphometrics identification

All modern and archaeological specimens were superimposed together using a Generalized Procrustes Analysis (GPA) during which the sliding semi-landmarks were allowed to slide by minimizing the sum of the Procrustes distances between each individual and the mean conformation [69–71]. The Procrustes residuals were first analysed using a Principal Component Analysis (PCA) [72–74] (SI figure 1). A predictive linear discriminant analysis (pLDA), computed on the 20 first PCA scores that maximise the differences between groups [75], was then used to predict to which species the archaeological specimens belonged. Because unbalanced sample size can have a profound effect on discriminant analyses [76], and the fact that sheep greatly outnumber goat in the reference dataset, we used a resampling procedure to down sample the number of sheep to the smaller number of goats, as recommended by previous analysis [77]. The morphometrics identification was therefore based on 100 predictive discriminant analyses (pLDA) performed on two equal size samples (here 222 in each group) whose results were

summarized by the percentage of times a specimen was identified to its correct group [78]. All discriminant analyses were retained and no threshold was fixed for posterior probabilities (i.e. all identifications were based on the 100 pLDA). Only LDA with a cross validation percentage (CVP) above the 3rd quartile of all CVP were retained in order to select the most discriminant analyses. Morphometric identifications were only based on molar shape data, and not size, since it has been previously identified as the most discriminative criteria between sheep and goat [49].

ZooMS

Between 10 and 50 mg of bone was weighed out for each specimen. 250 μ L of 0.6 M hydrochloric acid (HCl) was added to each sample then stored at 4°C until demineralised. Once demineralised, the acid was discarded and three washes of 200 μ L each of ammonium bicarbonate (AmBic, NH_4HCO_3 , pH8) were performed to remove any remaining acid. 100 μ L of AmBic was added and the samples were gelatinised at 65°C for 1 hour. Samples were centrifuged for 1 minute and 50 μ L of the gelatinised supernatant was transferred to a new tube and 0.4 μ g/ μ L of trypsin was added. The trypsinated samples were digested at 37°C overnight, after which the samples were acidified with 1 μ L of 5% trifluoroacetic acid to stop the trypsin. C18 zip tips (PierceTM, Thermo ScientificTM) were used to purify the samples which were eluted in a 50 μ L volume. 1 μ L of sample topped with 1 μ L of matrix (α -cyano-4-hydroxycinnamic acid) was spotted in triplicate along with calibration standards on either a Bruker MTP 384 ground steel MALDI plate and analysed on a Bruker Ultraflex III MALDI-ToF-MS, or onto a SCIEX Opti ToF stainless steel MALDI plate and analysed with an AB SCIEX 4700 MALDI-ToF-MS in the Department of Prehistory & Institute of Environmental Science and Technology (ICTA) at Universitat Autònoma de Barcelona (Barcelona, Spain). Triplicate spectra for each sample were averaged and analysed using mMass [79] and compared to a database of known reference species [2,80–82].

Comparison of ancient and modern sheep and goat

The overall percentage of correct identification was computed comparing the geometric morphometrics and ZooMS identifications. Securely ZooMS-identified archaeological sheep and goat were then compared with their modern counterparts using Wilcox-tests for size and Procrustes Anova (procD.lm function from geomorph package [83]) for shape. Size and shape variation were visualised using boxplot and PCA, respectively. Homogeneity of size and shape difference between species and between archaeological vs. modern specimens were tested using 2-way Anova and 2-way Procrustes Anova, respectively.

Morphometrics proximities between modern sheep and goat and the archaeological specimens identified via ZooMS were visualized by a neighbour-joining network computed on Mahalanobis distances. Graphics were created in R using the ‘ggplot2’ package [84]

Change in molar shape and size variance between archaeological and modern specimens of sheep and goat was assessed, respectively, using the disparity test of the geomorph R package [85,86] and Fligner test (stats package,[87]).

All the analysis were performed in R version 6.1.524 [87] through Rstudio version 4.2.2[88].

RESULTS

Identification for archaeological specimens

Based on their third molar shape, 59 archaeological specimens were identified as sheep (54.1%) and 50 as goat (45.9%) (figure 3). The 109 identified were based on 31 LDA with cross-validation percentages above 94.22% [90% Confidence Interval, CI: 93.01-95.50].

For the same 109 specimens, ZooMS identified 64 (58.7%) specimens as sheep and 45 (41.3%) as goat (all spectra supporting the results are available in the Dryad Digital Repository <https://doi.org/10.5061/dryad.fj6q5741m> [89]).

The two approaches provided the same species identification for 104 (95.4%) of the archaeological specimens corresponding to 59 sheep and 45 goats. The five (4.6%) remaining specimens are from the sites of Can Gambús (Catalonia, Late Antiquity) (n=3) and Missignac (France, Late Antiquity and Middle Age) (n=2). These misidentified specimens are all morphometrically identified as goat but molecularly identified as sheep. All of these misidentified specimens fell into the most common age category of 4-8 years old (*i.e.* 11G) (SI Table 2). Looking more closely at these misidentifications, it appears that three out of the five specimens were identified 100% of the time to goat via GMM (*i.e.* all LDA give 'goat' as identification, no matter the probability of each of the 100 LDAs), while the two last ones were attributed respectively to goat only in 74.2% and 81.8% of the cases (See SI figure 1 for probability of each LDA).

Morphometrics comparison with modern specimens

The ancient samples studied were selected to represent a diversity of geographic origin (Catalonia and the South of France) and chrono-cultural periods (Late Neolithic to modern period) but certainly do not cover the entire past sheep and goat diversity in the North Western Mediterranean basin. Sample sizes per region and chrono-cultural period were too small to envision detailed morphometrics comparison and all archaeological specimens were analysed jointly (See SI-figure-2 for a visualization of size differences between sites and chrono-cultural periods). Overall, the archaeological sheep and goat possessed smaller third lower molars than their modern relatives (figure 3, table 2). Modern sheep also appear larger than goat, contrary to the pattern observed for archaeological specimens (figure 3, table 2).

Figure 3 Third lower molar size variation: Boxplot showing variation in log centroid size between modern (dark) and archaeological (light) sheep (green) and goat (blue) identified by ZooMS. For differences between sites and chronologies, see SI figure 2.

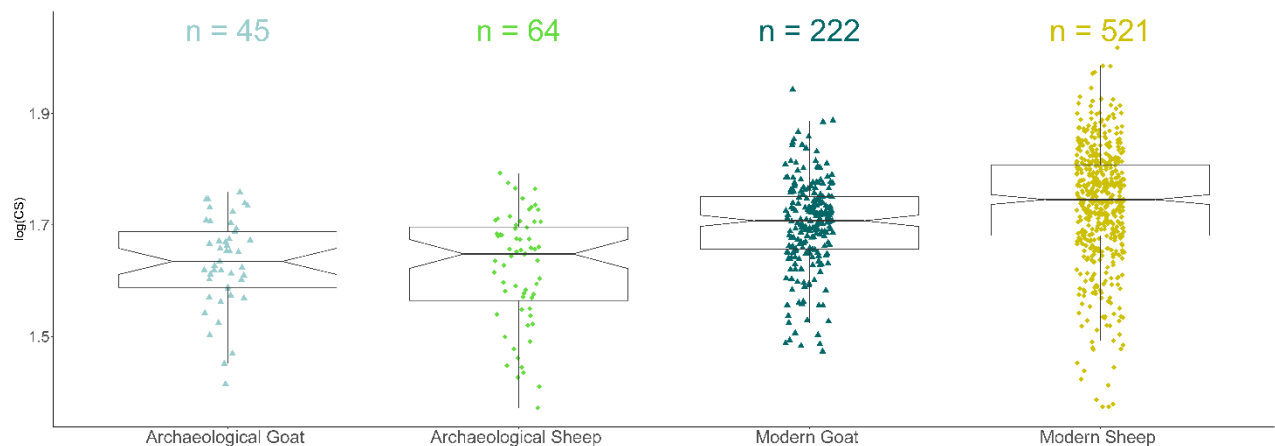


Table 2: Differences in size between modern sheep and goat, and the archaeological specimens identified by ZooMS. Pairwise Wilcoxon test were done between each group. The p-values in bold are significant (p-value < 0.05) after Bonferroni correction for multiple comparisons.

	Archaeological Goat	Archaeological Sheep	Modern Goat
Archaeological Sheep	1		
Modern Goat	3.9×10^{-6}	1.4×10^{-7}	
Modern Sheep	9.6×10^{-11}	2.9×10^{-14}	9.1×10^{-8}

A PCA of all archaeological and modern sheep and goat shows that the modern specimens studied show new morphologies that didn't exist in the past (figure 4).

Figure 4: Two first axes of the PCA showing variation between modern (dark) and archaeological (light) sheep (green) and goat (blue) identified by ZooMS. PCA for each species are available in SI figure 3.

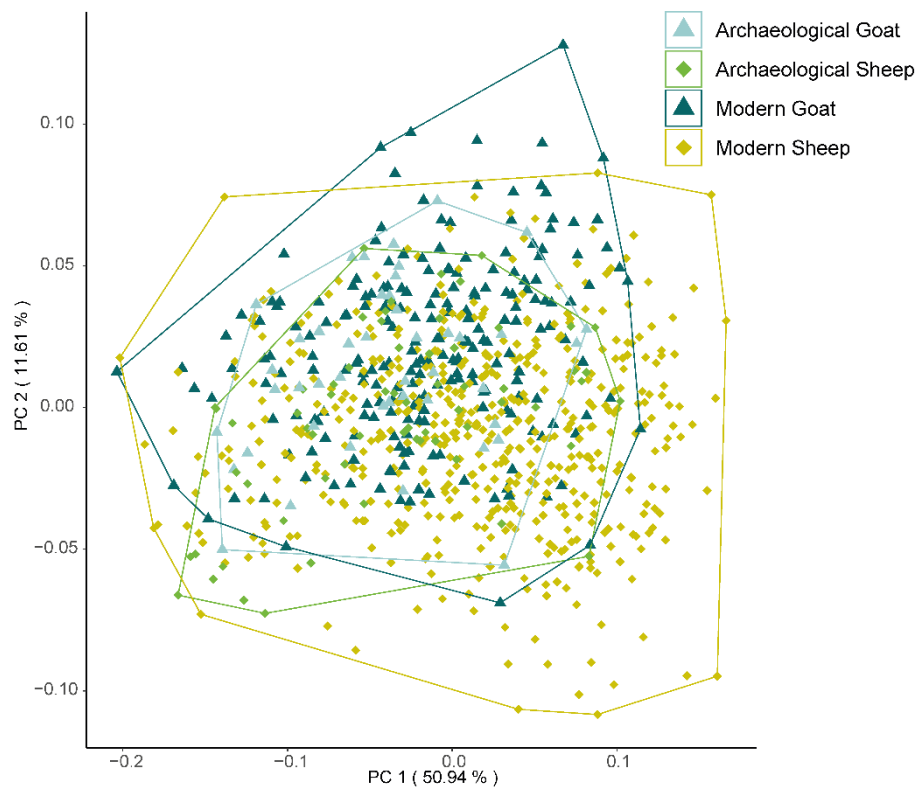
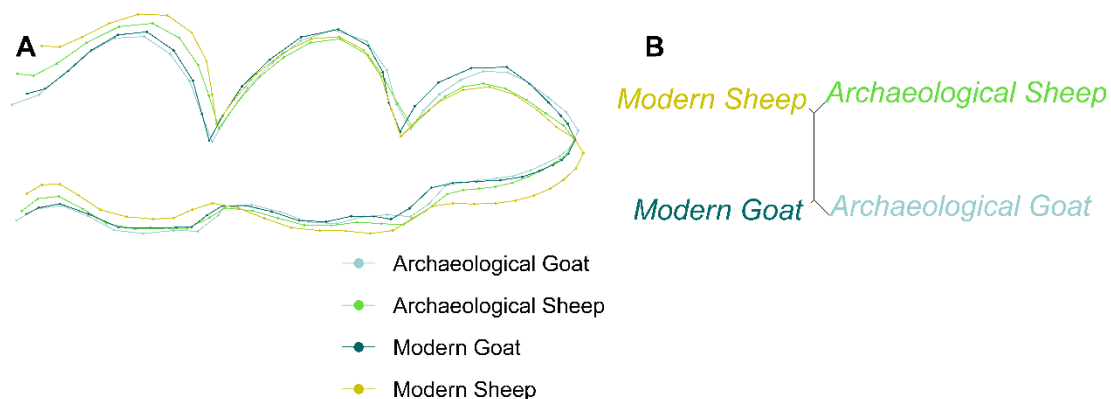


Figure 5: Visualisation of the mean shape (A) and dissimilarity network (B) between archaeological (light) and modern (dark) sheep (green) and goat (blue). Archaeological specimens are identified by ZooMS.



Shape differences between sheep and goat are present for both modern and archaeological specimens (respectively p-value=0.013 and p-value=0.001). Mean shape visualisation and dissimilarity network on shape confirm that there is a higher proximity within species than within periods, that is to say the archaeological goat (identified through ZooMS) reassemble more to modern goat than to archaeological sheep (and conversely) (figure 5), even if there are shape differences between archaeological and modern sheep (p-value= 0.001) and goat (p-value=0.004).

In addition, differences between sheep and goat are homogeneous between modern and archaeological specimens for both shape (interaction term of a 2-way Procrustes ANOVA, p-value= 0.463) and size (p-value= 0.138)

The only difference in terms of size or shape variance is seen between modern sheep and goat, with modern sheep showing more variation than modern goat (table 3).

Table 3 Comparison of size and shape variance between modern and archaeological sheep and goat. Size variance was tested with Fligner test, with a Khi^2 test statistic, and shape variance with a disparity approach (pairwise test on Procrustes variance, distance between each Procrustes variance).

	Size		Shape	
	Khi^2	p-value	distance	p-value
Archaeological Sheep/Archaeological Goat	2.34	0.12	$1,71 \times 10^{-3}$	0.26
Modern Sheep/Modern Goat	15.41	8.63×10^{-5}	$2,07 \times 10^{-3}$	0.002
Archaeological Sheep/Modern Sheep	3.64×10^{-4}	0.98	$1,69 \times 10^{-3}$	0.11
Archaeological Goat/Modern Goat	0.11	0.73	$1,34 \times 10^{-3}$	0.31

DISCUSSION

We demonstrate, for the first time, that geometric morphometrics can be an efficient alternative to molecular identification with 95.4% of the morphometric identifications being congruent with those provide by the molecular ZooMS identification.

Identification of paleontological or archaeological remains is often done by actualism, defined as ‘the methodology of inferring the nature of past events by analogy with processes observable and in action in the present’ [90]. In order to fully explain the morphological diversity in the past, a better understanding of current diversity and its incorporation in the analyses is needed [91,92]. The hypothesis based on these identifications assumed that modern specimens are relevant for studying remains from the past, with negligible influence of extrinsic (e.g. temporal or geographical) variation. Modern specimens are often the only available reference of securely identified species, however, it has been demonstrated that modern references can lead to misidentifications. For example, modern dog breeds are not a good proxy to identify archaeological specimens [93]. This is also the case for other domestic species for which it is well known that direct selection over the last two centuries was intensified, followed by standardization of morphology and performance with the emergence of the breed concept [94–97]. This also applies to sheep and goat, whose breeds became genetically more and more uniformized [95], even if our morphometric results do not detect increase in diversity between our ancient and modern datasets. However, we detected morphologies that exist today that were not present in the studied archaeological dataset. This result will have to be explored further using more archaeological specimens.

We found that a large majority (95.4%) of the specimens were correctly identified based on their third molar shape, suggesting that the morphometric integrity, and thus the distinction of the species, are maintained through time, at least for the third lower molar. This means that, based on their third molar shape, the differences between sheep and goat remain the same through time. On the other hand, modern specimens of both species possess larger teeth than their archaeological counterparts with a strong overlap between sheep and goat, confirming the impossibility of using size alone for species identification. This size difference between archaeological and modern specimens, although little studied, has also been suggested for cattle [98] and pigs [99].

The five misaligned specimens, those identified as sheep by ZooMS and goat by geometric morphometrics suggest that some ancient sheep dental morphologies were more similar to the here-captured present-day goat dental morphologies. It seems important to note that the percentage of correct identification of 95.4% observed here is similar to the 93.3 % percentage of correct cross-validation between modern sheep and goat obtained in a previous study [49]. At least four hypotheses could be envisaged to explain the misidentified specimens.

First, even if our modern reference collection includes 521 sheep and 222 goats belonging to a range of breeds originating from Europe and North Africa, the entire existing modern diversity is certainly not represented. Furthermore, this reference collection was purposely built to study the north western Mediterranean region, and so may not be directly suitable for other geographic areas. Similarly, two waves of exotic foods are known during Iron Age [100], the Roman and medieval periods [101,102] that may have influenced the diversity we observe in our archaeological dataset. Consequently, we cannot exclude that these five individuals may reflect animal translocation across regions, from areas where sheep dental morphology was similar to that of present-day goats.

Second, these animals could correspond to taxa not included in our analyses – e.g. the mouflon (*Ovis orientalis musimon*, a feral form of sheep), the alpine (*Capra ibex*) or Iberian ibex (*Capra pyrenaica*) or the Barbary sheep (*Ammotragus lervia*)– whose dental morphology and geographical distributions in the past are largely unknown, and whose distinction based on collagen peptide sequences is limited. Due to the highly conserved nature of the amino acid sequence of collagen, ZooMS cannot be used to

distinguish between domestic and wild specimens of the same species, and sometimes cannot distinguish between closely related taxa, as their collagen sequences are identical or near identical [80]. For example, domestic sheep, mouflon and Barbary sheep cannot be distinguished from another using ZooMS as their collagen peptide markers are the same. It is possible that these five samples belong to taxa that are closely related to domestic sheep however have dentition more similar to that of domesticated goats. This hypothesis could be tested in the future through e.g. palaeogenomics.

Third, age and sex are known to have a significant impact on sheep and goat third molar morphology, but that both factors have limited impact on the ability to discriminate the two species [49]. Indeed, even if age (estimated through wear stages) affects the third lower molar shape of both species, a closer proximity between species than between age categories has been found [49]. In addition, it was demonstrated that the impact of age on the between species differentiation was mostly due to the oldest specimens (more than 8 years) [49]. Because the mis-identified specimens had an estimated age of 4-8 years, it seems unlikely that age caused the mismatch between the morphometric and molecular identifications. Sexual dimorphism in teeth size and shape has been identified in both species; but as for age, a greater proximity has been found between species than sex, and the sexual dimorphism has no impact on modern caprine identification [49].

Finally, even if very rare, sheep-goat hybrids exist (e.g. [103,104]). Such hybrids would likely be difficult, if not impossible, to identify using either ZooMS or GMM, as presumably the results for both analyses would simply indicate either sheep or goat, rather than a mix.

Nevertheless, with a ~95% success rate, this newly generated data allows to build a secure reference collection of ancient morphologies for the two species from the Neolithic to Modern periods in the North Western Mediterranean basin. This will enable non-molecular, and thus non-destructive, methods of analysis of dentition for larger zooarchaeological assemblages to improve our understanding of the husbandry history of these two species in space and time. Because this study aims only at validating the methodological approach, the archaeological interpretations require further consideration. While the number of specimens studied here did not allow detailed analyses, adding more specimens will allow further exploration of the environmental and socio-cultural factors that shaped the past agrobiodiversity and its evolution. While it is known that modern [31] and ancient goat and sheep have different diet [105], the impact of diet in tooth morphology in those species remain to be explored, but in any case did not have a major impact on the taxonomic identification.

The observed size and shape differences between archaeological and modern specimens of both sheep and goats are in agreement with previous zooarchaeological results. Here we observed that archaeological specimens have on average smaller molars than their modern counterparts. Sheep and goat are known to have increased in size during Modern period [106–108]. Caprines were particularly improved upon over the last centuries [108–110], which could explain the differences noticed between archaeological and modern groups. However, despite the presence of both primitive and standardised breeds in our referential, we did not find that modern specimens were more or less diverse than the archaeological groups. The only observed variance difference is between modern sheep and goat, where modern sheep are, for both size and shape, more variable than modern goat. This result could be due to the greater number of sheep compared to goat in the modern referential. Moreover, today officially recognised sheep breeds are also more numerous than goat, with for example around 50 different French sheep breeds compared to only 15 breeds of goat [97,111]. Moreover goats were mainly selected for the purpose of milk, while sheep have been selected for both meat, milk and wool [97,111], which could explain this greater morphological diversity, and the outnumber of sheep breeds compare to goat.

Archaeological sheep and goat dental remains can prove difficult to identify to the species level [38,40,42,43,112,113]. This study is a new contribution toward analyses dedicated to understanding the

evolution of both sheep and goat in archaeological records using non-destructive morphometric identification of dental specimens. Being able to identify isolated third molars, that often remain unidentified in archaeozoology, allows new perspectives for further research. It is now possible to use the powerful and non-invasive quantitative tools of geometric morphometrics, via archaeophenomics [47], to explore in more depth the separate evolution of sheep and goat through time, and follow their socio-economic and cultural roles in past animal husbandry. Such studies require secure identification and quantification which, particularly for large scale assemblages, is best accomplished through integrating molecular and non-molecular methodologies. Integrative approaches that combine GMM and biomolecular methods, such as ZooMS, are in high demand, with such integration leading to clearer perceptions of domestic species evolution through time and space.

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CREDIT

Marine Jeanjean : Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing

Krista McGrath : Methodology, Resources, Writing – review & editing

Silvia Valenzuela-Lamas : Resources, Writing – review & editing

Ariadna Nieto-Espinet : Resources, Writing – review & editing

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Claude Guintard : Resources, Writing – review & editing

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Rania Ridouh: Resources

Cyprien Mureau: Visualization, Writing – review & editing

Allowen Evin: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

REFERENCES

1. Richter KK, Codlin MC, Seabrook M, Warinner C. 2022 A primer for ZooMS applications in archaeology. *Proc. Natl. Acad. Sci. U. S. A.* **119**. (doi:10.1073/pnas.2109323119)
2. Buckley M, Collins M, Thomas-Oates J, Wilson JC. 2009 Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **23**, 3843–3854. (doi:10.1002/rcm)
3. Ebsen JA, Haase K, Larsen R, Sommer DVP, Brandt LØ. 2019 Identifying archaeological leather – discussing the potential of grain pattern analysis and zooarchaeology by mass spectrometry (ZooMS) through a case study involving medieval shoe parts from Denmark. *J. Cult. Herit.* **39**, 21–31. (doi:10.1016/j.culher.2019.04.008)
4. Fiddyment S *et al.* 2015 Animal origin of 13th-century uterine vellum revealed using noninvasive peptide fingerprinting. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 15066–15071. (doi:10.1073/pnas.1512264112)
5. Coutu AN, Taurozzi AJ, Mackie M, Jensen TZT, Collins MJ, Sealy J. 2021 Palaeoproteomics confirm earliest domesticated sheep in southern Africa ca. 2000 BP. *Sci. Rep.* **11**, 1–11. (doi:10.1038/s41598-021-85756-8)
6. Culley C *et al.* 2021 Collagen fingerprinting traces the introduction of caprines to island Eastern Africa. *R. Soc. Open Sci.* **8**. (doi:10.1098/rsos.202341)
7. Janzen A *et al.* 2021 Distinguishing African bovids using Zooarchaeology by Mass Spectrometry (ZooMS): New peptide markers and insights into Iron Age economies in Zambia. *PLoS One* **16**, 1–36. (doi:10.1371/journal.pone.0251061)
8. Culley C, Janzen A, Brown S, Prendergast ME, Shipton C, Ndiema E, Petraglia MD, Boivin N, Crowther A. 2021 Iron Age hunting and herding in coastal eastern Africa: ZooMS identification of domesticates and wild bovids at Panga ya Saidi, Kenya. *J. Archaeol. Sci.* **130**. (doi:10.1016/j.jas.2021.105368)
9. Taylor W *et al.* 2018 Early pastoral economies along the Ancient Silk Road: biomolecular evidence from the Alay Valley, Kyrgyzstan. *PLoS One* **13**, 1–19.
10. Taylor WTT *et al.* 2021 Evidence for early dispersal of domestic sheep into Central Asia. *Nat. Hum. Behav.* **5**, 1169–1179. (doi:10.1038/s41562-021-01083-y)
11. Martins H, Xavier Oms F, Pereira L, Pike AWG, Rowsell K, Zilhão J. 2015 Radiocarbon dating the beginning of the Neolithic in Iberia: New results, new problems. *J. Mediterr. Archaeol.* **28**, 105–131. (doi:10.1558/jmea.v28i1.27503)
12. Sjögren KG, Buckley M, Vretemark M, Axelsson T. 2023 Evaluating caprine remains of the Swedish Funnel Beaker culture through ZooMS. *Archaeol. Anthropol. Sci.* **15**. (doi:10.1007/s12520-023-01743-2)
13. Buckley M, Kansa SW. 2011 Collagen fingerprinting of archaeological bone and teeth remains from Domuztepe, South Eastern Turkey. *Archaeol. Anthropol. Sci.* **3**, 271–280. (doi:10.1007/s12520-011-0066-z)
14. Price MD, Buckley M, Kersel MM, Rowan YM. 2013 Animal Management Strategies during the Chalcolithic in the Lower Galilee: New Data from Marj Rabba (Israel). *Paléorient* **39**, 183–200. (doi:10.3406/paleo.2013.5527)

15. Vaiglova P, Bogaard A, Collins M, Cavanagh W, Mee C, Renard J, Lamb A, Gardeisen A, Fraser R. 2014 An integrated stable isotope study of plants and animals from Kouphovouno, southern Greece: A new look at Neolithic farming. *J. Archaeol. Sci.* **42**, 201–215. (doi:10.1016/j.jas.2013.10.023)
16. Pilaar Birch SE, Scheu A, Buckley M, Çakırlar C. 2019 Combined osteomorphological, isotopic, aDNA, and ZooMS analyses of sheep and goat remains from Neolithic Ulucak, Turkey. *Archaeol. Anthropol. Sci.* **11**, 1669–1681. (doi:10.1007/s12520-018-0624-8)
17. Prendergast ME, Janzen A, Buckley M, Grillo KM. 2019 Sorting the sheep from the goats in the Pastoral Neolithic: morphological and biomolecular approaches at Luxmanda, Tanzania. *Archaeol. Anthropol. Sci.* **11**, 3047–3062. (doi:10.1007/s12520-018-0737-0)
18. Gron KJ, Rowley-Conwy P, Jensen TZT, Taurozzi AJ, Marciniak A. 2020 Separating caprine (*Capra/Ovis*) distal tibiae: A case study from the Polish Neolithic. *Int. J. Osteoarchaeol.* **30**, 170–179. (doi:10.1002/oa.2844)
19. Ananyevskaya E, Buckley M, Pal Chowdhury M, Tabaldiev K, Motuzaitė Matuzevičiūtė G. 2021 Specialized wool production economy of prehistoric farmstead of Chap I in the highlands of Central Tian Shan (Kyrgyzstan). *Int. J. Osteoarchaeol.* **31**, 18–28. (doi:10.1002/oa.2921)
20. Bläuer A, Solala H, Kinnunen J, Rannamäe E. 2022 Multidisciplinary History of Goats in Finland: A Comparative Approach. *Heritage* **5**, 1947–1959. (doi:10.3390/heritage5030101)
21. Boyazoglu J. 2002 Livestock research and environmental sustainability with special reference to the Mediterranean basin. In *Small Ruminant Research*, (doi:10.1016/S0921-4488(02)00100-1)
22. Galaty J., Johnson D. 1990 *Pastoral Systems*. New York: Guilford Press.
23. Enne G, Zucca C, Montoldi A, Noe L. 2004 The role of grazing in agropastoral systems in the Mediterranean region and their environmental sustainability. *Adv. Geoecology* **37**, 29–46.
24. Durmus M, Agossou DJ, Koluman N. 2019 Sustainability of Small Ruminant Production in Mediterranean Region. *J. Environ. Sci. Eng. B* **8**, 241–248. (doi:10.17265/2162-5263/2019.06.005)
25. Marandet MC. 2021 *L'homme et l'animal dans les sociétés méditerranéennes*. Presses universitaires de Perpignan.
26. Nieto-Espinete A, Huet T, Trentacoste A, Guimarães S, Orengo H, Valenzuela-Lamas S. 2021 Resilience and livestock adaptations to demographic growth and technological change: A diachronic perspective from the Late Bronze Age to Late Antiquity in NE Iberia. *PLoS One* **16**, 1–47. (doi:10.1371/journal.pone.0246201)
27. Valenzuela-Lamas S, Albarella U. 2017 Animal husbandry across the Western Roman Empire: Changes and continuities. *Eur. J. Archaeol.* **20**, 402–415. (doi:10.1017/ea.2017.22)
28. Halstead P. 1996 Pastoralism or household herding? problems of scale and specialization in early Greek animal husbandry. *World Archaeol.* **28**, 20–42. (doi:10.1080/00438243.1996.9980329)
29. Trentacoste A, Nieto-Espinete A, Guimarães Chiarelli S, Valenzuela-Lamas S. 2022 Systems change: Investigating climatic and environmental impacts on livestock production in lowland Italy between the Bronze Age and Late Antiquity (c. 1700 BC – AD 700). *Quat. Int.* **662**, 26–36. (doi:10.1016/j.quaint.2022.11.005)
30. Shackleton DM, Shank CC. 1984 A Review of the Social Behavior of Feral and Wild Sheep and Goats. *J. Anim. Sci.* **58**, 500–509.
31. Balasse M, Ambrose SH. 2005 Distinguishing sheep and goats using dental morphology and stable carbon isotopes in C4 grassland environments. *J. Archaeol. Sci.* **32**, 691–702. (doi:10.1016/j.jas.2004.11.013)

32. De Serre O. 1600 Le théâtre d'agriculture et ménage des champs. Arles: Actes Sud.
33. Bourrier T. 1897 *Les industries des abattoirs*. Paris: Librairie J.-B. Baillière et Fils.
34. Helmer D, Vigne J-D. 2004 La gestion des cheptels de caprinés au Néolithique dans le Midi de la France. In *Approches fonctionnelles en Préhistoire* (eds P Bodu, C Constantin), pp. 397–407. Paris: Société Préhistorique Française Édition.
35. Helmer D, Gourichon L, Sidi Maamar H, Vigne J. 2005 L'élevage des caprinés néolithiques dans le sud-est de la France : saisonnalité des abattages, relations entre grottes-bergeries et sites de plein air. *Anthropozoologica* **40**, 167–189.
36. Blaise E. 2009 Economie animale et gestion des troupeaux au Néolithique final en Provence. Université de Provence - Aix-Marseille I.
37. Boessneck J. 1971 Osteological Differences between Sheep (*Ovis aries* Linné) and Goat (*Capra hircus* Linné). In *Science in Archaeology: a survey of progress and research* (eds D Brotherweel, E Higgs), pp. 331–358. London: Thames and Hudson.
38. Zeder M, Lapham HA. 2010 Assessing the reliability of criteria used to identify postcranial bones in sheep, *Ovis*, and goats, *Capra*. *J. Archaeol. Sci.* **37**, 2887–2905. (doi:10.1016/j.jas.2010.06.032)
39. Prummel W, Frisch HJ. 1986 A guide for the distinction of species, sex and body side in bones of sheep and goat. *J. Archaeol. Sci.* **13**, 567–577. (doi:10.1016/0305-4403(86)90041-5)
40. Halstead P, Collins P, Isaakidou V. 2002 Sorting the sheep from the goats: Morphological distinctions between the mandibles and mandibular teeth of adult *Ovis* and *Capra*. *J. Archaeol. Sci.* **29**, 545–553. (doi:10.1006/jasc.2001.0777)
41. Helmer D. 2000 Discrimination des genres *Ovis* et *Capra* a l'aide des premolaires inferieures 3 et 4 et interpretation des ages d'abattage: l'exemple de dikili tash (Grece). *Anthropozoologica* **31**, 29–38.
42. Payne S. 1985 Morphological distinctions between the mandibular teeth of young sheep, *Ovis*, and goats, *Capra*. *J. Archaeol. Sci.* **12**, 139–147. (doi:10.1016/0305-4403(85)90058-5)
43. Zeder M, Pilaar SE. 2010 Assessing the reliability of criteria used to identify mandibles and mandibular teeth in sheep, *Ovis*, and goats, *Capra*. *J. Archaeol. Sci.* **37**, 225–242. (doi:10.1016/j.jas.2009.10.002)
44. Gillis R, Chaix L, Vigne JD. 2011 An assessment of morphological criteria for discriminating sheep and goat mandibles on a large prehistoric archaeological assemblage (Kerma, Sudan). *J. Archaeol. Sci.* **38**, 2324–2339. (doi:10.1016/j.jas.2011.04.012)
45. Grine FE, Fosse G, Krause DW, Jungers WL. 1986 Analysis of enamel ultrastructure in archaeology: The identification of *Ovis aries* and *Capra hircus* dental remains. *J. Archaeol. Sci.* **13**, 579–595. (doi:10.1016/0305-4403(86)90042-7)
46. Lyman RL. 1984 Bone density and differential survivorship of fossil classes. *J. Anthropol. Archaeol.* **3**, 259–299. (doi:10.1016/0278-4165(84)90004-7)
47. Evin A, Bouby L, Bonhomme V, Jeanty A, Jeanjean M, Terral J-F. 2022 Archaeophenomics of ancient domestic plants and animals using geometric morphometrics : a review. *Peer Community J.* **2**. (doi:10.24072/pcjournal.126)
48. Rohlf FJ, Marcus LF. 1993 A Revolution in Morphometrics. *Trends Ecol. Evol.* **8**, 129–132. (doi:10.2307/20047487)

49. Jeanjean M *et al.* 2022 Sorting the flock: Quantitative identification of sheep and goat from isolated third lower molars and mandibles through geometric morphometrics. *J. Archaeol. Sci.* **141**, 105580. (doi:10.1016/j.jas.2022.105580)
50. Maufras O, Hernandez J, Rochette M, Thomas B. 2015 Genèse, évolution et désertion de Missignac (Aimargues, Gard), villa des Ve-XIIIe siècles. *Archéologie du Midi Médiéval*
51. Bardot-Cambot A, Fabre M, Forest V. 2017 Étude archéozoologique : Ostéologie, conchyliologie. In *Lattes, Castelle, Pahon, Pinède. Se déplacer, cultiver et mourir dans la campagne lattoise de l'Antiquité à nos jours* (ed M Compan), pp. 256–274. Montpellier: Rapport final d'opération – Fouille préventive, Service Régional de l'Archéologie Occitanie.
52. Payne S. 1973 Kill-off Patterns in Sheep and Goats : The Mandibles from Aşvan Kale. *Anatol. Stud.* **23**, 281–303.
53. Bordas A, Pou R, Parpal A, Martín A. 1994 Excavacions arqueològiques 1991-1992 a la Bòbila Madurell - Mas Duran (Sant Quirze del Vallès, Vallès Occidental). *Trib. d'Arqueologia* **1992–1993**, 31–47.
54. Abad ÒE, Garra AM, Bieto ET, Aixalà AV, Oller NA. 2012 Cantorella (Maldà, Urgell), un nou assentament a l'aire lliure del neolític final - calcolític i del bronze ple a la vall del Corb. *Trib. d'arqueologia* , 129–172.
55. Roig J, Coll JM, Gibaja JF, Chambon P, Villar V, Ruiz J, Terradas X, Subirà ME. 2010 La necrópolis de Can Gambús-1 (Sabadell, Barcelona) nuevos conocimientos sobre las prácticas funerarias durante el Neolítico medio en el noreste de la Península Ibérica. *Trab. Prehist.* **67**, 59–84. (doi:10.3989/tp.2010.10031)
56. Gailledrat E *et al.* 2012 Rapport de fouille Pech Maho (Sigean, Aude).
57. Alonso N, Junyent E, Lafuente Á, López JB, Tartera E. 2000 La fortaleza de Arbeca. El proyecto Vilars 2000. Investigación, recuperación y socialización del conocimiento y del patrimonio. *Trab. Prehist.* **57**, 161–173. (doi:10.3989/tp.2000.v57.i2.255)
58. Pou J, Sanmartí J, Santacana J. 1995 La reconstrucció del poblat ibèric d'Alorda Park o de les Toixoneres (Calafell, Baix Penedès). *Trib. d'Arqueologia* **1993–1994**, 51–62.
59. Aquilué X, Castanyer P, Santos M, Tremoleda J. 2009 Resultats de les darreres intervencions arqueològiques a la Neàpolis de la ciutat grega d'Empòrion (Empúries, l'Escala , Alt Empordà). *Trib. d'Arqueologia* , 121–147.
60. Martín A, Miret J, Blanch RM, Aliaga S, Enrich R, Colomer S, Albizuri S, Bosch J. 1988 Campaña d'excavacions arqueològiques 1987-88 al jaciment de la Bòbila Madurell-Can Feu (Sant Quirze del Vallès, Vallès occidental). *Arraona Rev. d'història* **3**, 9–23.
61. Julià M, Lázaro P, Lorencio C, Lorient A, Oliver A, Plens M, Puig F. 1989 Excavacions d'urgència a l'antic Portal de Magdalena. Lleida (Segrià). *Excavacions Arqueol. d'urgència a les Comarq. Lleida* , 203–226.
62. Maufras O, Mercier C. 2002 Habitat et terroir du IVE au XIIe s. à Saint- Gilles-le-Vieux (Aimargues, Gard). In *Archéologie du TGV Méditerranée. Fiche de Synthèse, tome 3. Antiquité, Moyen âge, Epoque moderne*, pp. 945–972. Lattes: Association pour la Recherche Archéologique en Languedoc Oriental.
63. Maufras O, Hernandez J, Rochette M, Thomas B. 2018 Aimargues - Madame - Saint-Gilles le Vieux: Missignac, villa médiévale et ses abords (Ve - XIIIe s.).
64. Henry E. 2016 Montpellier, CNR rue de la Vieille Coraterie, Rapport final d'opération.,

65. Romeu PG, Masachs JM. 1968 Hallazgos prehistóricos en los alrededores de La Lacuna. Castillo de Vilademager. *Empúries Rev. món clàssic i Antig. tardana*, 207–213.
66. Morán Álvarez M, Payà Mercè FX. 2021 Fonts documentals i arqueològiques per al coneixement de la Cuirassa jueva de Lleida. *Urtx. Rev. d'Humanitats l'Urgell* **35**, 133–177.
67. Nieto-Espinet A, Valenzuela-Lamas S. 2019 Muerte o conversión. Del bienestar a la hambruna en el barrio judío de la Cuirassa de Lleida (siglos xii-xiv). In *Comunicació presentada en Workshop. Minorías y transiciones en la edad media a través de la zooarqueología (s. x-xv). 28 i 29 de novembre del 2019. Universitat de Granada, Universidad de Granada*.
68. Rohlf FJ. 2006 Tps Dig v2.32, Ecology and Evolution.
69. Perez SI, Bernal V, Gonzalez PN. 2006 Differences between sliding semi-landmark methods in geometric morphometrics, with an application to human craniofacial and dental variation. *J. Anat.* **208**, 769–784. (doi:10.1111/j.1469-7580.2006.00576.x)
70. Sampson P, Bookstein F, Sheenan F, Bolson E. 1996 Eigenshape analysis of left ventricular outlines from contrast ventriculograms. In *Advances in morphometrics* (eds L Marcus, M Corti, L Loy, G Naylor, D Slice), pp. 211–233. New-York: NATO ASI Series A: Life Sciences, Plenum.
71. Sheets HD, Kim K, Mitchell CE, Sheets DH, Kim K, Mitchell CE. 2004 A combined landmark and outline-based approach to ontogenetic shape change in the Ordovician trilobite *Triarthrus becki*. In *Morphometrics* (ed AM. Elewa), pp. 67–81. New-York: Springer. (doi:10.1007/978-3-662-08865-4)
72. Gower J. C. 1975 Generalized procrustes analysis. *Psychometrika* **40**, 33–51. (doi:10.1007/BF02291478)
73. Rohlf FJ, Slice D. 1990 Extensions of the procrustes method for the optimal superimposition of landmarks. *Syst. Zool.* **39**, 40–59. (doi:10.2307/2992207)
74. Goodall CR. 1995 Procrustes methods in the statistical analysis of shape revisited. In *Current Issues in Statistical Shape Analysis* (eds K V Mardia, CA Gill), pp. 18–33. Leeds: University of Leeds Press.
75. Baylac M, Friess M. 2005 Fourier descriptors, Procrustes superimposition, and data dimensionality: an example of cranial shape analysis in modern human populations. In *Modern morphometrics in physical anthropology* (ed DE Slice), pp. 145–165. Boston: Springer.
76. Kovarovic K, Aiello L, Cardini A, Lockwood CA. 2011 Discriminant function analyses in archaeology: Are classification rates too good to be true? *J. Archaeol. Sci.* **38**, 3006–3018. (doi:10.1016/j.jas.2011.06.028)
77. Evin A, Cucchi T, Cardini A, Strand Vidarsdottir U, Larson G, Dobney K, Strand U, Larson G, Dobney K. 2013 The long and winding road: identifying pig domestication through molar size and shape. *J. Archaeol. Sci.* **40**, 735–743. (doi:10.1016/j.jas.2012.08.005)
78. Evin A *et al.* 2015 Unravelling the complexity of domestication: A case study using morphometrics and ancient DNA analyses of archaeological pigs from Romania. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 1–8. (doi:10.1098/rstb.2013.0616)
79. Strohmalm M, Kavan D, Novák P, Volný M, Havlíček V. 2010 MMass 3: A cross-platform software environment for precise analysis of mass spectrometric data. *Anal. Chem.* **82**, 4648–4651. (doi:10.1021/ac100818g)
80. Buckley M, Whitcher Kansa S, Howard S, Campbell S, Thomas-Oates J, Collins M. 2010 Distinguishing between archaeological sheep and goat bones using a single collagen peptide. *J. Archaeol. Sci.* **37**, 13–20. (doi:10.1016/j.jas.2009.08.020)

81. Kirby DP, Buckley M, Promise E, Trauger SA, Holdcraft TR. 2013 Identification of collagen-based materials in cultural heritage. *Analyst* **138**, 4849–4858. (doi:10.1039/c3an00925d)
82. Welker F, Soressi M, Rendu W, Hublin JJ, Collins M. 2015 Using ZooMS to identify fragmentary bone from the Late Middle/Early Upper Palaeolithic sequence of Les Cottés, France. *J. Archaeol. Sci.* **54**, 279–286. (doi:10.1016/j.jas.2014.12.010)
83. Adams DC, Collyer M, Kaliontzopoulou A. 2020 Geomorph: Software for geometric morphometric analyses.
84. Wickham H. 2016 ggplot2: Elegant Graphics for Data Analysis.
85. Collyer M, Adams DC. 2018 RRPP: An R package for fitting linear models to high-dimensional data using residual randomization.
86. Collyer M, Adams DC. 2019 RRPP: Linear Model Evaluation with Randomized Residuals in a Permutation Procedure. R package version 0.4.0.
87. R Core Team. 2022 R: a language and environment for statistical computing.
88. Posit team. 2023 RStudio: Integrated Development Environment for R.
89. Jeanjean M *et al.* 2023 ZooMS Spectra from: ZooMS confirms geometric morphometrics species identification of ancient sheep and goat. *Dryad, Dataset* (doi:https://doi.org/10.5061/dryad.fj6q5741m)
90. Rudwick MJS. 1971 Uniformity and progression: reflections on the structure of geological theory in the age of Lyell. In *Perspectives in the History of Science and Technology* (ed D Roller), pp. 209–227. Norman: University of Oklahoma Press.
91. Kitts DB. 1977 *The Structure of Geology*. Dallas: Southern Methodist University Press.
92. Pobiner BL, Braun DR. 2005 Applying Actualism: Considerations for Future. *J. Taphon.* **3**, 57–66.
93. Brassard C *et al.* 2022 Unexpected morphological diversity in ancient dogs compared to modern relatives. *Proc. R. Soc. B Biol. Sci.* **289**. (doi:10.1098/rspb.2022.0147)
94. Taberlet P, Valentini A, Rezaei HR, Naderi S, Pompanon F, Negrini R, Ajmone-Marsan P. 2008 Are cattle, sheep, and goats endangered species? *Mol. Ecol.* **17**, 275–284. (doi:10.1111/j.1365-294X.2007.03475.x)
95. Taberlet P, Coissac E, Pansu J, Pompanon F. 2011 Conservation genetics of cattle, sheep, and goats. *Comptes Rendus - Biol.* **334**, 247–254. (doi:10.1016/j.crv.2010.12.007)
96. Denis B. 2010 Races Bovins: Histoire, Aptitudes, Situation actuelle. Chaumont: Castor & Pollux.
97. Denis B, Vaissaire J-P. 2021 *Les Races d'animaux domestiques en France. Etude générale et inventaire*. Paris: Chartes Delachaux et Niestlé.
98. Albarella U. 1997 Shape variation of cattle metapodials: age, sex or breed? Some examples from Medieval and Postmedieval sites. *Anthropozoologica* **25–26**, 37–48.
99. White S. 2011 From globalized pig breeds to capitalist pigs: A study in animal cultures and evolutionary history. *Environ. Hist. Durh. N. C.* **16**, 94–120. (doi:10.1093/envhis/emq143)
100. Gaastra JS. 2014 Shipping sheep or creating cattle: Domestic size changes with Greek colonisation in Magna Graecia. *J. Archaeol. Sci.* **52**, 483–496. (doi:10.1016/j.jas.2014.08.030)

101. Livarda A. 2011 Spicing up life in northwestern Europe: Exotic food plant imports in the Roman and medieval world. *Veg. Hist. Archaeobot.* **20**, 143–164. (doi:10.1007/s00334-010-0273-z)
102. Aubaile F. 2012 Pathways of diffusion of some plants and animals between Asia and the Mediterranean region. *Rev. d'ethnoécologie*, 0–32. (doi:10.4000/ethnoecologie.714)
103. Bunch TD, Foote WC, Spillett JJ. 1976 Sheep-goat hybrid karyotypes. *Theriogenology* **6**, 379–385.
104. Mine OM, Kedikilwe K, Ndebele RT, Nsoso SJ. 2000 Sheep-goat hybrid born under natural conditions. *Small Rumin. Res.* **37**, 141–145. (doi:10.1016/S0921-4488(99)00146-7)
105. Jiménez-Manchón S. 2020 Pratiques d'élevage entre l'Empordà et le Languedoc à l'âge du Fer: Archéozoologie, alimentation animale et saisonnalité. Université Paul Valéry - Montpellier III.
106. Robin O, Clavel B. 2018 The diversity evolution of sheep morphology in French zooarchaeological remains from the 9th to the 19th century: Analysis of pastoral strategy. *J. Archaeol. Sci.* **99**, 55–65. (doi:10.1016/j.jas.2018.08.017)
107. Clavel B. 2001 L'animal dans l'alimentation médiévale et moderne en France du Nord (XIIIe - XVIIe siècles). *Rev. archéologique Picardie. Numéro spécial* **19**, 9–204. (doi:10.3406/pica.2001.3065)
108. Grau-Sologestoa I. 2015 Livestock management in Spain from Roman to post-medieval times: A biometrical analysis of cattle, sheep/goat and pig. *J. Archaeol. Sci.* **54**, 123–134. (doi:10.1016/j.jas.2014.11.038)
109. Audoin-Rouzeau F. 1991 La taille du mouton en Europe de l'Antiquité aux Temps Modernes. *Fiches d'ostéologie Anim. pour l'archéologie. CRA-CNRS* **3**, 36.
110. Davis SJM, Gonçalves MJ, Gabriel S. 2008 Animal remains from a Moslem period (12 th/13 th century AD) lixeira (garbage dump) in Silves, Algarve, Portugal. *Rev. Port. Arqueol.* **11**, 183–258.
111. Babo D. 2000 *Races ovines et caprines françaises*. 1ère. Paris: Editions France Agricole.
112. Davis SJM. 2017 Towards a metrical distinction between sheep and goat astragali. In *Economic Zooarchaeology* (eds P Rowley-Conwy, D Serjeantson, P Halstead), pp. 50–82. Oxford: Oxbow Books. (doi:10.2307/j.ctt1s4751q.14)
113. Salvagno L, Albarella U. 2017 A morphometric system to distinguish sheep and goat postcranial bones. *PLoS One* **12**, 1–37. (doi:10.1371/journal.pone.0178543)

SI SUPPLEMENTARY DATA

SI Table 1 : List of modern specimens used as geometric morphometrics modern reference.

SI Table 2 : List of archaeological specimens sampled for ZooMS analyses.

SI Table 3 : Geometric morphometrics data set with scaled coordinated of modern and archaeological specimens.

SI Figure 1: Identification probability of each LDAs for each archaeological specimen, sorted by archaeological sites numbers. The upper part of the graph, in green, corresponds to the probability of each LDAs for specimens identified as sheep by the geometric morphometrics, while the lower part, in blue, corresponds to the goats identified by the geometric morphometrics. The colour of each boxplot correspond to the ZooMS identification. Specimens with a red star are misidentified by geometric morphometrics. Specimens with a black star are those which are both identify as goat or sheep depending on the LDAs. The most numerous identification was kept.

SI Figure 2: Boxplot showing differences of log centroid size for goat and sheep between archaeological sites and chrono-cultural occupation. Refers table 1 for archaeological sites numbers. N refers to Neolithic ; Br to Bronze Age ; Ir to Iron Age ; Ant to Antiquity ; MA to Middle Ages ; MOD to Modern period ; ACT to modern referential and Out Str to Out of Stratigraphy.

SI Figure 3: Two first axes of the PCA showing variation between modern (dark) and archaeological (light) independently for sheep and goat.

SI File 1: ZooMS MALDI-TOF-MS spectra for each archaeological sample. The first column corresponds to mass to charge (m/z) ratio and the second to absorbance intensity. Each sample was analysed in triplicate, resulting in three files per sample corresponding to spectrum a, b and c, which where then averaged. See SI Table 2 for sample names