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► To cite this version:

Marine Jeanjean, Krista Mcgrath, Silvia Valenzuela-lamas, Ariadna Nieto-Espinet, Renate Schafberg, et al.. ZooMS confirms geometric morphometrics species identification of ancient sheep and goat. Royal Society Open Science, 2023, 10 (9), pp.230672. 10.1098/rsos.230672 . hal-04250463

HAL Id: hal-04250463

<https://hal.science/hal-04250463>

Submitted on 19 Oct 2023

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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].

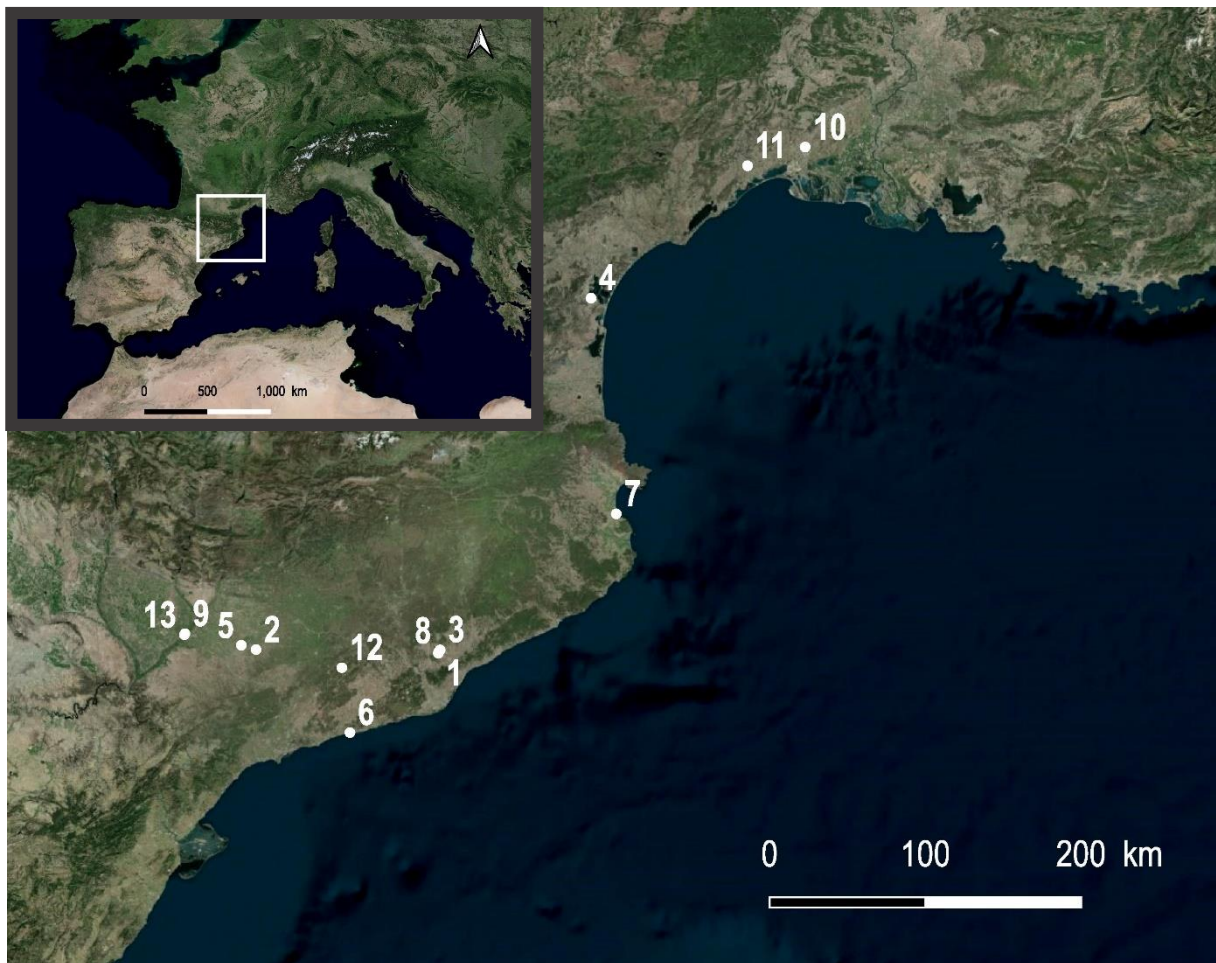
In addition, 109 individuals (SI-table 2) from 13 archaeological sites, selected for their chronological and spatial diversity, dated from the Neolithic to Modern period and located in the north-western part of the Mediterranean basin (present-day Catalonia region in Spain and Occitanie region in Southern 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]

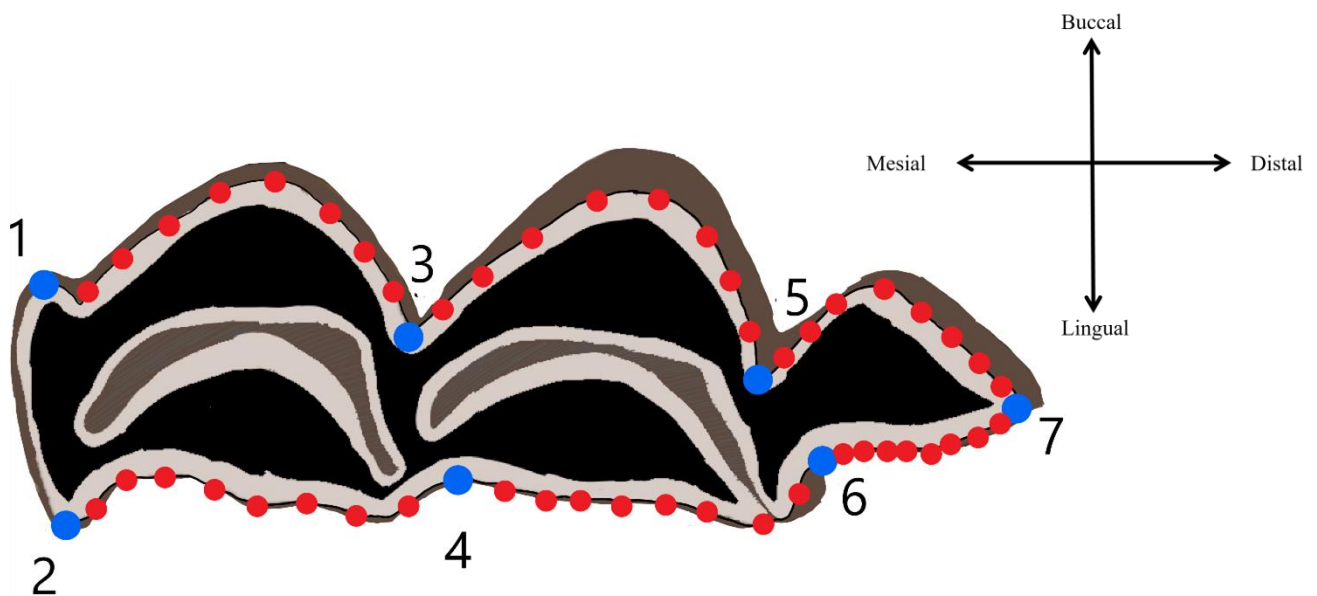
Figure 1 Location of the 13 archaeological sites of the sampled specimens. The numbers refer to the column ID of table 1.



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.

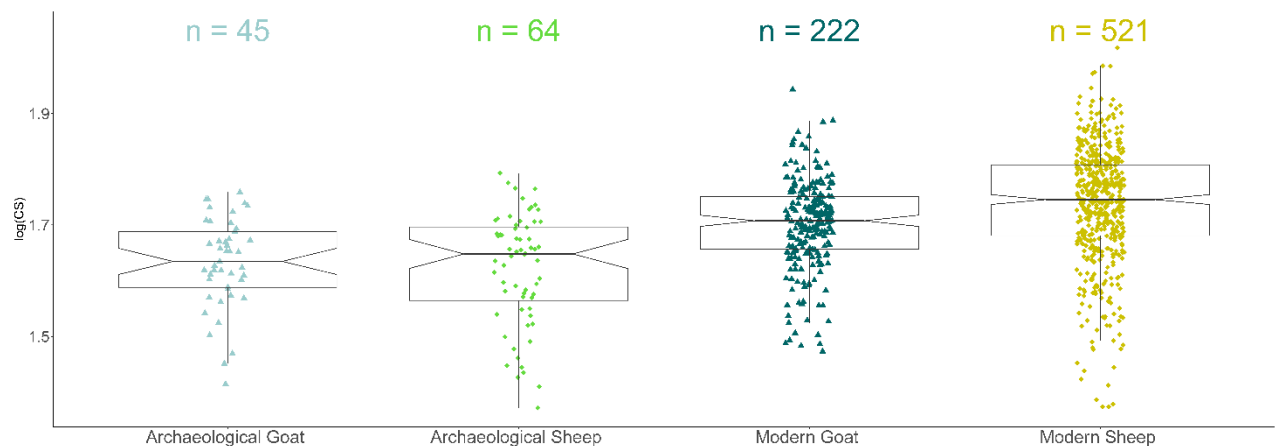


Table 2: Differences in size between modern sheep and goat, and the archaeological specimens identified by ZooMS. Pairwise Wilcoxon test were done between each group. The p-values in bold are significant (p-value < 0.05) after Bonferroni correction for multiple comparisons.

	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.

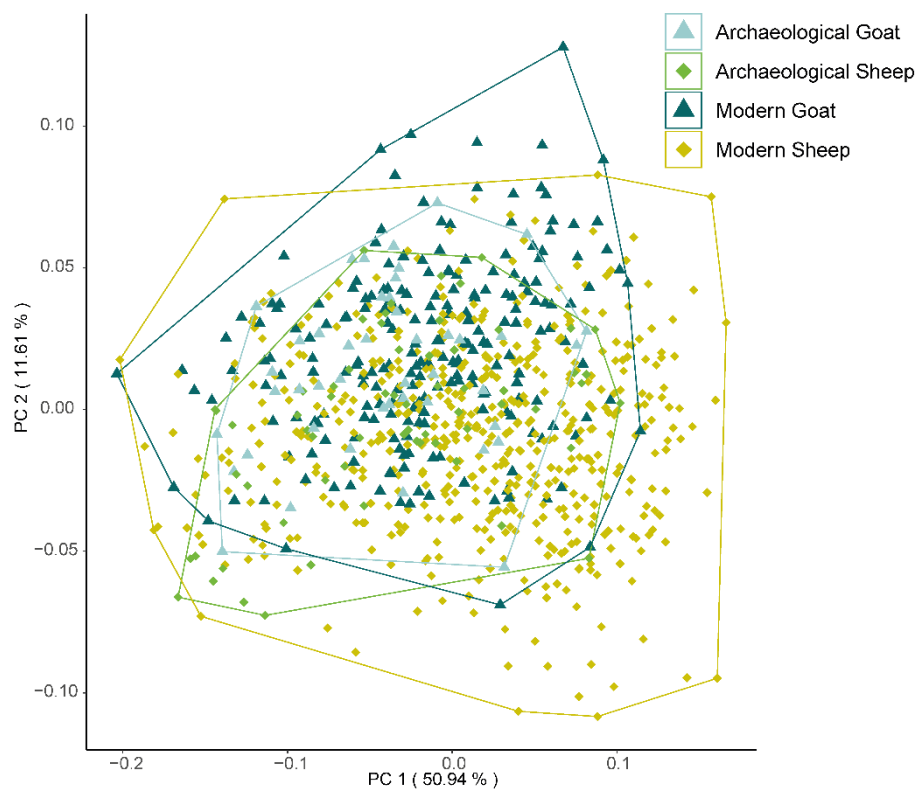
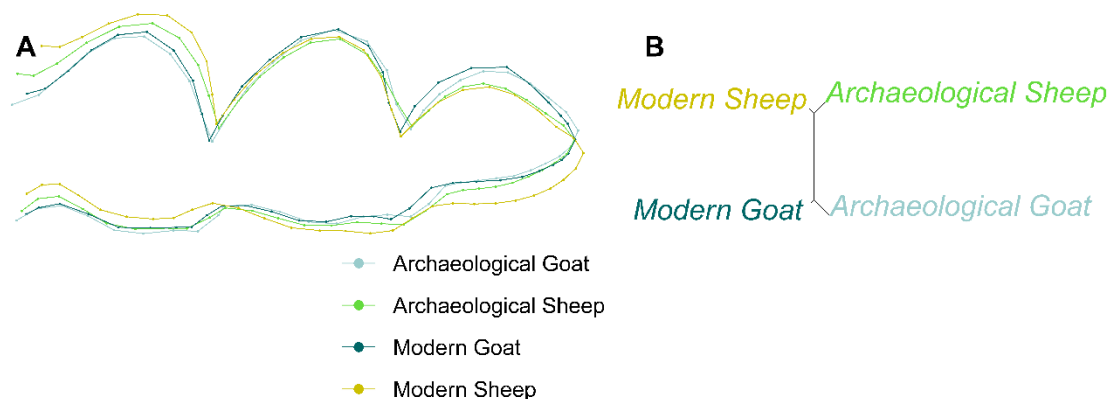


Figure 5: Visualisation of the mean shape (A) and dissimilarity network (B) between archaeological (light) and modern (dark) sheep (green) and goat (blue). Archaeological specimens are identified by ZooMS.



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 provided 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.

ACKNOWLEDGEMENTS

This project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (Grant Agreement No. 852573). We would like to thank the Laboratori de Proteòmica CSIC-UAB at the Universitat Autònoma de Barcelona for access to the Bruker Ultraflex III MALDI-TOF-MS, and the Centres Científics i Tecnològics of the Universitat de Barcelona for access to the AB SCIEX 4700 MALDI-TOF-MS. We acknowledge the Service Regional de l'Archéologie of the Occitanie Region for permission of studying and destructive sampling of the archaeological material, as well as the directors of the different excavations included in this study.

We also acknowledge Museum National d'Histoire Naturelle (Paris), Museum für Haustierkunde, Oniris Nantes and the Musée d'Histoire Naturelle de Nîmes for allowing to consult their collections.

CREDIT

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

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

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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