Sorting the flock: Quantitative identification of sheep and goat from isolated third lower molars and mandibles through geometric morphometrics

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ARTICLE INFO

ABSTRACT

Sheep and goat are often herded together and show morphological similarities in their skeleton. Being able to identify archaeological remains of these two taxa to species level is particularly important for understanding and characterising past herding practices. Discrete criteria are now available to identify a large number of their bones and teeth, and quantitative approaches have been developed for post-cranial elements but not for mandible and isolated teeth. In this paper we explore the discriminating potential of geometric morphometrics to identify modern sheep and goat third lower molar and mandible and its application on archaeological specimens. The size and shape of the mandible and the third lower molar of 143 modern specimens (101 sheep and 42 goats) were quantified using 2D-landmark and sliding semi-landmarks geometric morphometric approaches. The results show that sheep and goat differ in terms of the size, shape, and form (*i.e.* size and shape together) in both studied elements. Classification accuracy of the two species reaches 93.3% (CI: 90.0–95.7%) for third lower molar shape, 62.7% (CI 57.1–68.6%) for third lower molar size, 95.2% (CI: 92.0–97.4%) for mandible shape and 84.0% (CI 81.6–86.8%) for mandible size. Form does not provide better classification than shape alone. Sex and age appear to have little impact on the ability to differentiate between sheep and goat, despite the two species displaying distinct sexual dimorphism and changes through age. The same methodology was then applied on 32 Middle Ages third lower molars from Missignac-Saint Gilles le Vieux, Aimargues, France. The identifications obtained through geometric morphometrics were only partially congruent with the identifications based on visual observations calling for caution in the interpretation and further investigations. Further research should include molecular identification of the archaeological specimens to assess whether the geometric morphometric identification can be made with confidence for all periods and all geographic areas. Nevertheless, the results obtained with the newly developed geometric morphometric protocols represent an important contribution toward a better understanding of past livestock husbandry practices.

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1. Introduction

Sheep and goat were among the first animals to be domesticated (Naderi, 2007; Vigne et al., 2011) about 11,000–10,500 years ago in south-eastern Anatolia, and spread across Europe from approximately 8000 years ago as part of the Neolithic revolution, expanding beyond the range of their wild ancestors, *Ovis orientalis* and *Capra aegagrus* (Clutton-Brock, 1989; Peters et al., 2002; Vigne et al., 2015). Since then, sheep and goat became ubiquitous; regardless of the type of archaeological site and the geographic area considered, they are often among the most represented species as they formed the basis of many agropastoral societies and still are two inseparable emblematic species of the Mediterranean basin landscape (Altuna and Mariezkurrena, 2009; Álvarez-Fernández et al., 2014; Cubas et al., 2016; Peters et al., 1999; Vigne, 1988, 2011).

Sheep and goats have been selected and bred for multiple purposes, including secondary products such as milk, wool/hair and manure, which can be collected during the life of the animal, and final products such as skin, meat, horn and bones (*e.g.* Gillis et al., 2019). Because of their morphometric similarity and the fact that they readily form mixed herds, sheep and goat are often studied has a single entity (*e.g.* French, 1970; Grau-Sologestoa, 2015; Helmer, 2000; Payne, 1973; Salvagno, 2020). However, the two species have different feeding behaviour, with sheep generally preferring to graze on grass and soft plants while goats browse on leaves and bushes (Balasse and Ambrose, 2005). As a general trend, goat is preferred for clothing or wineskin and milk (Bourrier, 1897; De Serre, 1600), while sheep is preferred for meat and wool (Blaise, 2009; Helmer et al., 2005; Helmer and Vigne, 2004).

Differentiating and identifying sheep and goat is a very well-known longstanding challenge in zooarchaeology (*e.g.* Cornevin and Lesbre, 1891) and many studies have proposed discrete morphological criteria that has improved greatly our ability to differentiate between the two of them (*e.g.* Balasse and Ambrose, 2005; Boessneck et al., 1964; Fernandez, 2001; Halstead et al., 2002; Helmer, 2000; Payne, 1985; Prummel and Frisch, 1986; Zeder and Pilaar, 2010). More recently, criteria using linear measurements, such as those traditionally collected in archaeozoology (*e.g.* Von Den Driesch, 1976), have been developed for the petrous bone (Mallet et al., 2019; Mallet and Guadelli, 2013) and most postcranial bones (Gron et al., 2020; Salvagno and Albarella, 2017; Zedda et al., 2017).

In parallel, with the increased application of geometric morphometrics approaches (GMM, *i.e.* a set of statistical methods and visualizations based on the analysis of landmarks, outline or surface coordinates. See Rohlf and Marcus, 1993; Rohlf and Slice, 1990) to bioarchaeology, a number of studies have been published focusing on the bioarchaeological history of several domestic ungulate species. These include the taxonomic identification of archaeological wild and domestic populations as well as the study of the spatio-temporal variation of ancient and modern domestic populations of pig (*e.g.* Cucchi et al., 2011, 2009; Duval, 2015; Evin et al., 2015; Harbers et al., 2020b, 2020a; Krause-Kyora et al., 2013; Neaux et al., 2020b, 2020a; Ottoni et al., 2013; Owen et al., 2014), cattle (*e.g.* Csippán, 2016; Cucchi et al., 2019), and horse (*e.g.* Chuang and Bonhomme, 2019; Cucchi et al., 2017; Hanot et al., 2017; Seetah et al., 2014). However, only few studies have employed geometric morphometrics on caprine bones. For sheep and goat, this includes studies of the talus with the aim of distinguishing archaeological sheep and goat (Haruda, 2017), differentiate ancient wild sheep from modern and archaeological domestic sheep (Pöllath et al., 2018, 2019), and investigate the variation between archaeological sheep morphotypes (Colominas et al., 2019; Haruda et al., 2019; Vuillien, 2020).

Mandibular teeth are often targeted by GMM studies because, due to their internal structure, they survive very well deposition and are often found numerous in archaeological assemblages (Binford and Betram, 1977). In addition, they carry a taxonomic signal. The third lower molar in particular, as it is the last tooth in the jaw and is less constrained in its posterior part compared to the other teeth, has been considered a phenotypic marker of adaptation to natural or anthropic environment (Butler, 1939; Cucchi et al., 2019; Dahlberg, 1945). Nevertheless, the very few GMM studies exist on sheep and goat focus only on caprine mandibles are all exclusively based on modern specimens (Demiraslan et al., 2020; Demircioğlu et al., 2021; Parés-Casanova, 2013; Yalçin et al., 2010).These studies studied mandibular growth (Parés-Casanova, 2013), sexual dimorphisms (Demiraslan et al., 2020; Demircioğlu et al., 2021), and differences between wild and domestic sheep (Yalçin et al., 2010).

Very little attention has also been given to the measurement of teeth for species identification purposes despite the fact that they are among the most commonly found anatomical elements in archaeozoological assemblages (*e.g.* Buckley et al., 2010; Gerbault et al., 2016; Halstead et al., 2002; Payne, 1973). This is probably due to the fact that, until recently, sheep and goat teeth were considered to be too affected by occlusal wear to be used for bioarchaeological studies using a geometric morphometrics approach. Such an approach however, could not only help in the species identification of loose teeth, a practice which is notoriously difficult (especially to an untrained eye), but it also has the potential to greatly contribute to the quantitative exploration of the intra-specific diversity of sheep and goat in the past.

In that context, the major aims of this study are to: (1) establish protocols to quantify size and shape of modern sheep and goat third lower molar and mandible through the use of geometric morphometrics; (2) assess, for these two elements, the classification accuracy of size, shape, form and allometries (size and shape relationship) to identify sheep and goat; (3) explore the effect of age and sexual dimorphism on tooth and mandible morphometric variation, and their impact on identification accuracy; (4) identify to species level sheep/goat medieval teeth to test whether the new proposed methodology works on archaeological material.

2. Materials

2.1. Modern reference collection

A total of 102 mandibles and 133 third lower molars belonging to 143 specimens of modern breeds are included in this study. Sheep (*Ovis aries*) are represented by 101 specimens from 6 breeds (*Préalpes, Lacaune, Merinos, Negretti, Blanche du massif Central* and *Rouge du Roussillon*). Goats (*Capra hircus*) are represented by 42 specimens from 5 breeds (*Rove, Cabra Catalana, Angora, Corse* and *Cachemire*) (SI Table 1). All studied specimens were older than 1 year. Since tooth wear is directly linked to age, and wear can potentially have a significant impact on the tooth size and shape, the age of the specimens was established following Payne's method (1973). Specimens were primarily divided into the following categories: 1–2 years, 2–4 years, 4–6 years, 6–8 years and 8–10 years (Table 1) to which was added the category 4-8 years that includes specimens that could not be attributed more precisely. Both

Table 1
Age at death of the modern specimens for each species (third lower molar and mandible). Sex information, when available, is provided in brackets as follows (female/male). Mandibles with no useful teeth for age estimation are classified as 'undetermined age' (Undet. age).

	Sheep		Goat	
	Mandible	Third lower molar	Mandible	Third lower molar
1–2 years	6 (4/1)	5 (5/0)	2 (0/1)	0
2–4 years	9 (8/2)	12 (9/1)	5 (0/1)	4 (0/1)
4–6 years	30 (22/3)	34 (25/3)	14 (9/5)	19 (8/11)
6–8 years	6 (6/0)	9 (8/0)	7 (2/1)	5 (2/1)
4-8 years	2 (1/1)	20 (1/1)	0	0
8–10 years	13 (10/2)	17 (10/2)	5 (4/3)	8 (5/3)
Undet. age	0	0	3 (0/3)	0
Total	66 (51/9)	97 (58/7)	36 (15/14)	36 (15/16)

sexes were present in the sample however, the majority of individuals of known sex were female (Table 1).

2.2. Archaeological specimens

Thirty-two archaeological third lower molars from the site of Missignac-Saint Gilles le Vieux (Aimargues, Gard, France) were analysed, they dated between the end of the fifth century and the beginning of the thirteenth century AD and were either isolated teeth or teeth embedded in a mandible (SI Table 4).

The site our archaeological sample comes from is located in the South-East of France about 15 km from the Mediterranean Sea (Maufras et al., in press; Maufras and Mercier, 2002; Mercier and Barberan, 1996; Mion et al., 2019) and it was occupied from the second century BC to the thirteenth century AD. Preventive archaeology excavation carried out between 2012 and 2013 concerned the heart of a medieval village and its 4 ha peripheral storage district, with 3950 silos (Maufras et al., 2018; Maufras and Mercier, 2002). While the first occupation found consists of a villa dated to antiquity, later periods attest to an increase in density of population starting from the fifth century AD and continuing in the eighth and ninth century AD. The site is then abandoned during the twelfth century AD, even though burials continued in the following century (Maufras et al., 2018).

3. Methods

3.1. Data acquisition

Landmark coordinates were acquired from 2D-images. Third lower molars and mandibles were 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). One ramus of each mandible was positioned with its labial side facing upward paying particular attention to the flat position of the angular process. Planarity was assessed with a spirit level. Third lower molars were photographed in their occlusal view and were positioned perpendicular to the lens with the tooth root equally visible on both lateral sides. A millimetre scale was included in all pictures. Pictures were acquired both by MJ and AE with negligible inter-operator differences (lower than 11.6% following Claude, 2008). Mandibles were measured with 9 landmarks (see SI Table 2 for a formal description) and 70 sliding semi-landmarks (Fig. 1). The sliding semi-landmarks were distributed along four curves as follows: 13 points between landmarks 1 and 2; 18 points between landmarks 3 and 5; 11 points between landmarks 5 and 7; and 28 points between landmarks 7 and 8. Third lower molars were measured with 6 landmarks and a total of 48 sliding semi-landmarks distributed along 6 curves (8 equidistant points in each) along the outer outline of the tooth

(Fig. 1). Point coordinates were acquired using TpsDig (v2.32) (Rohlf, 2006) by a single operator (MJ).

Coordinates were superimposed using a Generalized Procrustes Analysis (GPA) (Goodall, 1995; Gower, 1975; Rohlf and Slice, 1990). During this procedure, sliding semi-landmarks were allowed to slide by minimizing the sum of the Procrustes distances between each individual and the mean conformation (Perez et al., 2006; Sampson et al., 1996; Sheets et al., 2004). Prior to further analyses, a Principal Component Analysis (PCA) on Procrustes residuals (coordinates after superimposition) was used to visualize and quantify the shape heterogeneity of individuals (Bookstein, 1991; Cooke and Terhune, 2015; Dryden and Mardia, 1998; Zelditch et al., 2012). Size was quantified as the log-transformed centroid size in all analyses.

All analyses were performed first by using only the homologous landmarks and then, by using the complete protocol including the full set of coordinates, hence combining the landmarks and sliding semi-landmarks. This allows us to compare performances of a simple protocol with that of a more complex one, which is more time-consuming and potentially more affected by wear. All raw coordinates are available in supplementary material (SI 1 for the mandibles, SI 2 for the lower third molars).

3.2. Repeatability test

In order to quantify error measurement, third lower molars and mandibles from five individuals of the French breed "Blanche du Massif Central (BMC)" were photographed 3 times, and coordinates were acquired 3 times on each picture by a single person (MJ). In addition, since two different people acquired the pictures of the remaining specimens in this study (MJ and AE), the differences between operators were also assessed. The measurement repeatability was quantified using Procrustes ANOVAs (Claude, 2008; Evin et al., 2020).

3.3. Inter species comparison

Differences in the mandible and the third lower molar between sheep and goat were first visualized using boxplot for size and principal component analysis (PCA) for shape. Linear discriminant analysis (LDA), paired with a leave-one-out correct cross-validation (CVP), were then performed in order to obtain classification accuracy. LDA was performed separately for size and for shape. LDA on shape data were computed on the first PCA axes maximizing the between species discrimination while taking into account unbalanced group sample sizes (using the "mevolCVP" R function (Evin et al., 2013)) Visualisation of shape changes along the discriminant axis were performed following Claude (2008). Allometry was explored using Procrustes MANCOVAs within species while the homogeneity of allometric trends among the
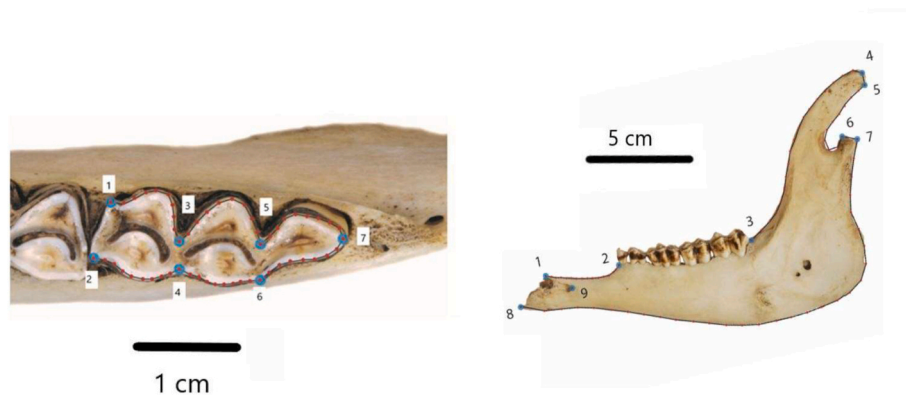

Fig. 1. Geometric morphometric protocols: Position of the landmarks (in blue) and sliding semi-landmarks (in red) measured on the mandible (right, specimen ISEM_926Ng) and third lower molar (left, specimen ISEM_926Nj) of a sheep. Landmark positions are described in SI Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

two species was explored using a 2-way Procrustes MANCOVA (using shape as the dependent variable, the log centroid size as a covariate and the species as a factor). Allometry free shape was then calculated using a multivariate regression.

3.4. Effect of age and sexual dimorphism

Differences in size and shape between age categories and between sexes were tested using Procrustes ANOVAs. First, tests were performed for each species separately using one-way ANOVAs for overall and pairwise comparisons. The homogeneity of size and shape variation through age and between sexes was explored using two-way Procrustes ANOVAs, utilising respectively size or shape data as the dependent variable, age or sex as first factor and, species as second factor. Differences between age categories were explored for categories ranging from 1-2 years to 8–10 years for the mandibles and from 2-4 years to 8–10 years for the third lower molar (Table 1). Similarities between sheep and goat for the various age categories were visualized by a neighbour-joining network computed on Mahalanobis distances.

3.5. Identification of archaeological specimens

Archaeological specimens were superimposed along the modern specimens, and a linear predictive discriminant analysis was performed on shape data. The identification of the archaeological specimens was based on a resampled and balanced sample size (Evin et al., 2015) ('pldam' function)); this in order to avoid the effect of uneven sample size due to the higher number of sheep present in the modern dataset. In addition, four experienced zooarchaeologists provided a first taxonomic identification (*i.e.* sheep, goat, or unknown) mainly based on the discrete morphological criteria proposed by Halstead et al. (2002). This was based solely on the pictures of the occlusal and buccal views of the teeth and not on the specimens themselves. We then compared the GMM identifications with those based on visual observation.

When multiple comparisons were made, the *p*-values of the tests were adjusted according to the Benjamini Hochberg method in order to avoid false recovery rate (Benjamini and Hochberg, 1995). All the analyses were carried out using the R language (R Development Core Team, 2012), the packages 'MASS' (Venables and Ripley, 2002), 'Morpho' (Schlager, 2017) and 'Geomorph' (Adams et al., 2020) and the function provided in Claude (2008).

4. Results

4.1. Repeatability and measurement error

Repeatability tests confirm the variation between repeated measurements to be negligible compared to the between specimens variation. The error due to both landmarking and photographing was similar for both the third lower molar and the mandible: for the third lower molar the error was 3.6% when the landmarks alone were measured and 5.1% when the complete protocol was used, while for the mandible the error was respectively 3.3% and 5.7%. The inter-operator error ranged from 4.6% to 11.6% and was considered negligeable.

4.2. Sheep and goat differences

In all comparisons, goats have smaller measurements than sheep (Table 2, Fig. 2). Based on size, the cross-validation percentage of the discriminant analysis is higher for the mandible than for the third lower molar (Table 2). This percentage decreases slightly when only the landmarks are used (as opposed to the use of the complete protocol, though the confidence intervals overlap). Based on the size, sheep and goat can be identified with an accuracy, at its best, of 62.7% (CI: 57.1–68.6%) for the third lower molar and 84.0% (CI: 81.6–86.8) for the mandible.

Table 2
Differences in size, shape, form, allometry, and allometry free shape between sheep and goat for the third lower molar and the mandible. Procrustes ANOVA results and correct cross-validation percentages (CVP) of the discriminant analysis are provided. For allometry, only the interaction term of the 2-way ANOVA, comparing the allometric trend between the two species, is provided. The p-values in bold are significant (p < 0.05) after adjustment for multiple comparisons.

		landmarks		complete	
		Test	CVP	Test	CVP
Mandible	Size	**F = 7.3456, p = 0.001**	**83.9 [81.6–86.8]**	**F = 5.5993, p = 0.001**	**84 [81.6–86.8]**
	Shape	**F = 12.138, p = 0.001**	**84.1 [80.3–88.6]**	**F = 8.8521, p = 0.001**	**95.2 [92–97.4]**
	Form	**F = 12.248, p = 0.001**	**84.5 [80.3–88.2]**	**F = 9.3447, p = 0.001**	**95.3 [92.1–97.4]**
	Allometric trend	**F = 1.9823, p = 0.038**		F = 1.7404, P = 0.067	
	Allometry free shape	**F = 4.4066, p = 0.001**	**71.7 [67–76.4]**	**F = 3.6787, p = 0.001**	**75.8 [71–80.3]**
Third lower molar	Size	**F = 63.756, p = 0.001**	**59 [54.3–64.3]**	**F = 63.072, p = 0.001**	**62.7 [57.1–68.6]**
	Shape	**F = 12.457, p = 0.001**	**89.2 [85.6–92.9]**	**F = 9.1407, p = 0.001**	**93.3 [90–95.7]**
	Form	**F = 11.855, p = 0.001**	**88.6 [84.3–92.9]**	**F = 8.6862, p = 0.001**	**93.1 [90–97.1]**
	Allometric trend	F = 1.5911, p = 0.141		F = 0.9588, p = 0.4	
	Allometry free shape	**F = 11.591, p = 0.001**	**88.2 [84.3–91.4]**	**F = 9.1747, p = 0.001**	**91.3 [87.1–95.7]**

Principal component analysis on shape revealed a strong overlap between sheep and goat (SI 3) on the first two axes. However, the two species differ in both their mandibular and third lower molar shape regardless of the protocol used (Table 2). The identification accuracy of shape data is higher than those obtained for size. An exception to this pattern is the mandible when quantified only with landmarks as it shows the same cross validation percentages for size and shape (Table 2). For shape, the use of the complete protocol provides higher percentages of correct classification when compared to the results obtained when only landmarks were used, with the cross-validation percentage reaching 93.3% (CI: 90.0–95.7%) for the third lower molar and 95.2% (CI: 92.0–97.4%) for the mandible (Table 2). Form analysis provides cross-validation percentages very similar to those obtained for shape (Table 2). Sheep and goats show similar allometric trends for all comparisons except for the third lower molar when measured only with landmarks (Table 2). For both protocols, the relationship between size and shape appeared relatively weak: for the mandible, when measured with landmarks, the adjusted R^2 value is 0.3% while it increases to 6.0% when measured with the complete protocol. When the third lower molar is considered, the R^2 value is 17.0%, when only landmarks are included, and of 5.0% when the complete protocol is used. Allometry-free shape
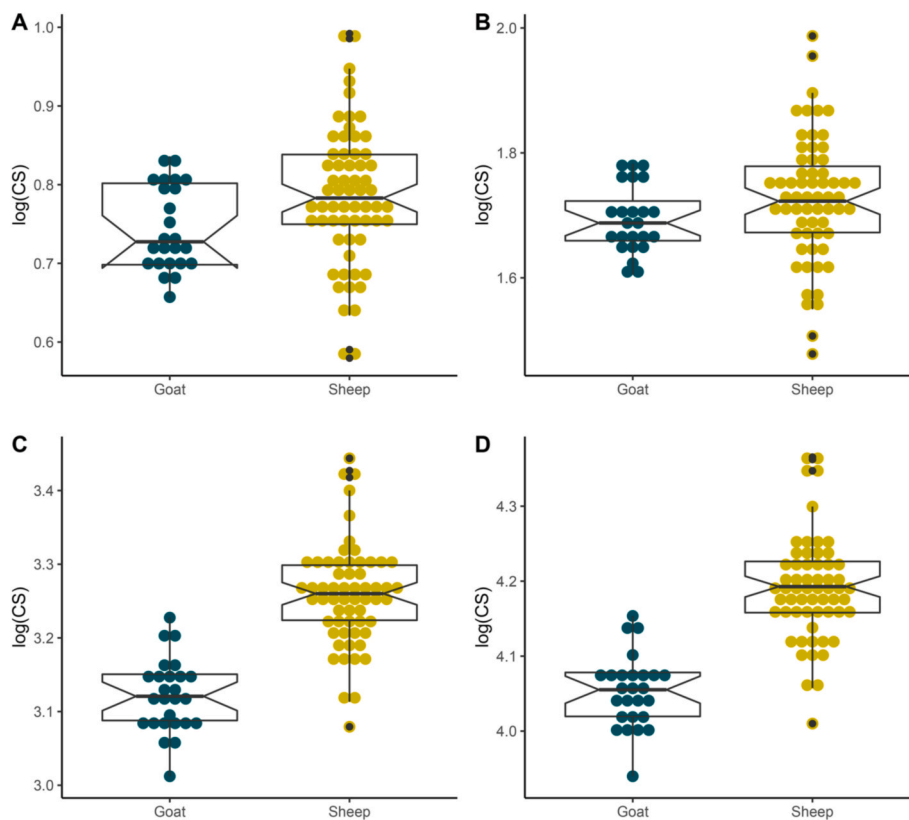
Fig. 2. Boxplots showing the differences in size for the third lower molar (A-landmarks only, B- complete protocol) and the mandible (C and D respectively) between sheep and goat.

provided lower correct cross-validation percentages compared to those obtained for shape (including allometry) for the mandibles but this is not the case for the third lower molar which shows similar cross validation percentages in both allometry-free shape and shape including allometry (Table 2).

Along the between species discriminant axis (Fig. 3), goats have, compared to sheep, a proportionally thinner third lower molar, with a proportionally reduced mesial edge and a more elongated distal lobe (Fig. 3A and 3B). The goat mandibular ramus is proportionally thinner compared to the one of sheep, this is especially visible in the mandibular condyle and notch. The angle formed by the ramus and the corpus of the mandible is also proportionally slightly acuter for goats than sheep (Fig. 3C and 3D).

4.3. Effect of age and sex

When all age categories were compared, the analysis revealed that age has little influence on the mandible and the third lower molar size on both protocols; the only exception is the third lower molar when measured by using the complete protocol (Table 3, Fig. 4). Pairwise comparisons of age categories did not reveal significant differences in size (SI Table 3).

Age affects the third lower molar shape of both species, but only sheep mandibular shape (Table 3). Pairwise comparisons of age categories detect differences in shape that always affect the age category 8–10 years (SI Table 3). Dissimilarity networks between species and age categories confirmed a closer proximity between species than age categories (Fig. 5). The two species share homogenous changes through age except for their third lower molar shape when measured with the complete protocol (Table 3). If the 8–10 years age category is removed from the analysis, the interaction term between shape, age and species becomes non-significant (landmarks: F = 1.23, p = 0.261, complete protocol: F = 1.32, p = 0.183).

Sheep and goat show sexual dimorphism in the size of their lower third molar, with the males having larger teeth than their female counterparts (Table 4, Fig. 6). Males and females sheep also differ in their molar and mandible shape, but this sexual dimorphism impact the between species differences only for the mandible when measured only with landmarks (Table 4).

4.4. Aimargues-missignac: archaeological application

The geometric morphometric identification of the 32 archaeological third lower molars revealed a mixed assemblage dominated by sheep (62.5%) and followed by goat (37.5%). Two specimens were left un-identified since they were identified with high probability (81.5%–100%) as belonging to either sheep or goat depending of the protocol used (SI Table 4). These geometric morphometric identifications match only partially (between 37.5% and 62.5%) the macroscopic identifica-tions (SI Table 4) that also varied depending on the archaeozoologist who carried them out.

The shape of the archaeological specimens overlap with those of both modern sheep and goat based on the two first axes of the PCAs (Fig. 7A, 7B) that represents respectively 61.9% (landmarks data) and 59.4% (complete protocol) of the total variance in the sample. The archaeo-logical specimens were, on average, smaller than the modern sheep and goat (all p = 0.001, Fig. 7). In addition, both the archaeological speci-mens identified as sheep (Landmarks: F = 67.59, p = 0.001 and com-plete protocol: F = 59.21, p = 0.001) and goat (Landmarks: F = 19.93, p = 0.001, complete protocol: F = 15.83, p = 0.002) were smaller than their modern counterparts. We detected no size differences between the sheep and goat archaeological specimens (all p < 0.001).
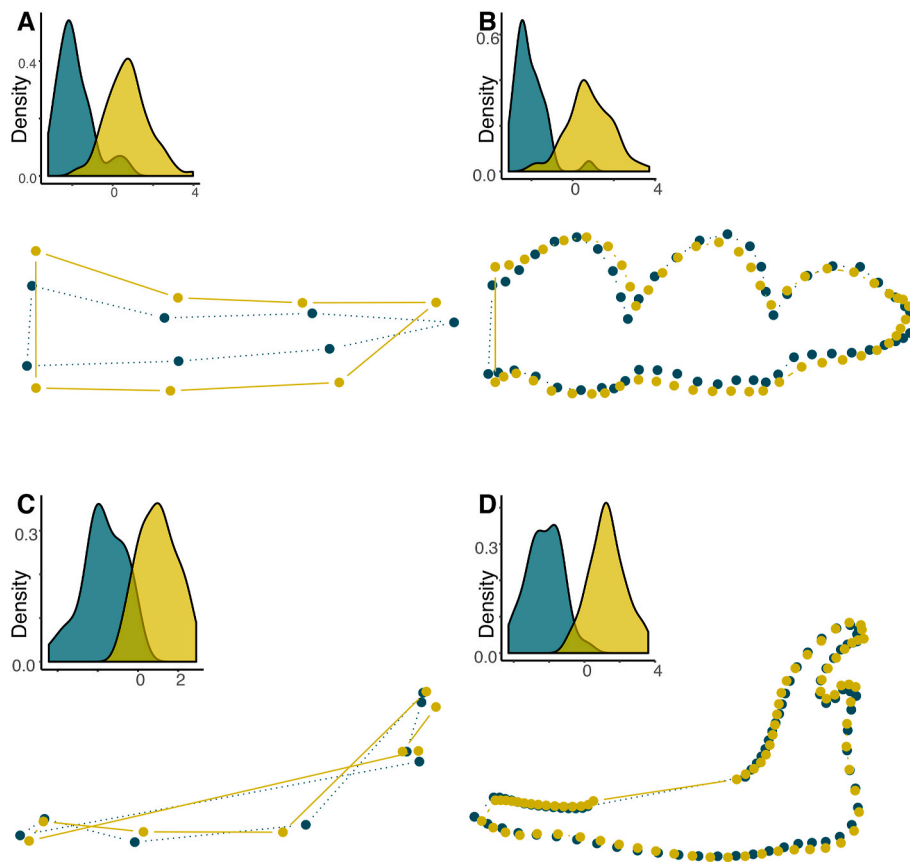
Fig. 3. *Shape differences between sheep (light green) and goat (dark blue). The distribution of the specimens along the discriminant axis and the visualisation of the shape differences between the two species are represented for both the lower third lower molar (A-landmarks only, B- complete protocol) and the mandible (C-landmarks only, D-complete protocol). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)*

Table 3
Influence of age on tooth and mandible size and shape. Results of one-way Procrustes ANOVAs for sheep and goat separately, and of two-way ANOVAs for testing the interaction between age and species (only the interaction term is provided). Results are provided for the landmarks only and complete protocol. The p-values in bold are significant (p < 0.05) after adjustment for multiple comparisons.

		Mandible				Third lower molar			
		Landmarks		Complete		Landmarks		Complete	
		F	p	F	p	F	p	F	p
Sheep	Shape	**1.9321**	**0.006**	**2.1427**	**0.003**	**9.1306**	**0.001**	**12.25**	**0.001**
	Size	0.9491	0.418	0.8754	0.468	1.1792	0.339	**5.3384**	**0.003**
Goat	Shape	1.7216	0.034	1.5906	0.026	**3.0503**	**0.004**	**3.707**	**0.001**
	Size	3.1687	0.038	3.0543	0.042	2.4466	0.081	0.6088	0.577
Interaction	Size	1.1442	0.298	1.3072	0.234	1.4376	0.225	1.1445	0.321
	Shape	1.1183	0.198	1.0870	0.243	1.7083	0.028	**2.1501**	**0.003**

5. Discussion

5.1. Repeatability of the protocols

Our two protocols for measuring third lower molar and mandibles were found to be repeatable, with the variation between replicates taken by the same operator being smaller than the variation between the specimens used in the repeatability test. In our study we found that the cumulative error in picturing and landmarking is, at its highest, around 11%. This is relatively similar to other protocols such as the one used by Evin et al. (2020) to measure pig third lower molars were inter-operator error averaged around 13% when both landmarks and sliding semi-landmarks were used. In addition, our study showed that the amount of error increases only marginally when pictures are acquired by multiple operators.

5.2. Sheep/goat differences

Our study revealed clear differences between modern sheep and goat based on the size and shape of their third lower molar and mandible. Specimens can be correctly identified with up to 93.3% probability for the third lower molar, and 95.2% for the mandible. Modern sheep have larger measurements than goat, which is congruent with previous studies on postcranial elements (Fernandez, 2001; Haruda, 2017). As far as size is concerned, the correct cross-validation percentages range from as low as 59.0% for the third lower molar (landmarks) to up to 84.0% for the mandible (complete protocol). Sheep and goat bones are also known to vary in size diachronically (*e.g.* Davis, 2008; Espinet et al., 2021; Grau-Sologestoa, 2015). From the results provided by the analysis of 143 modern specimens from 13 different breeds, we conclude that the lower third molar size has a low discriminatory power and, as such, it has a
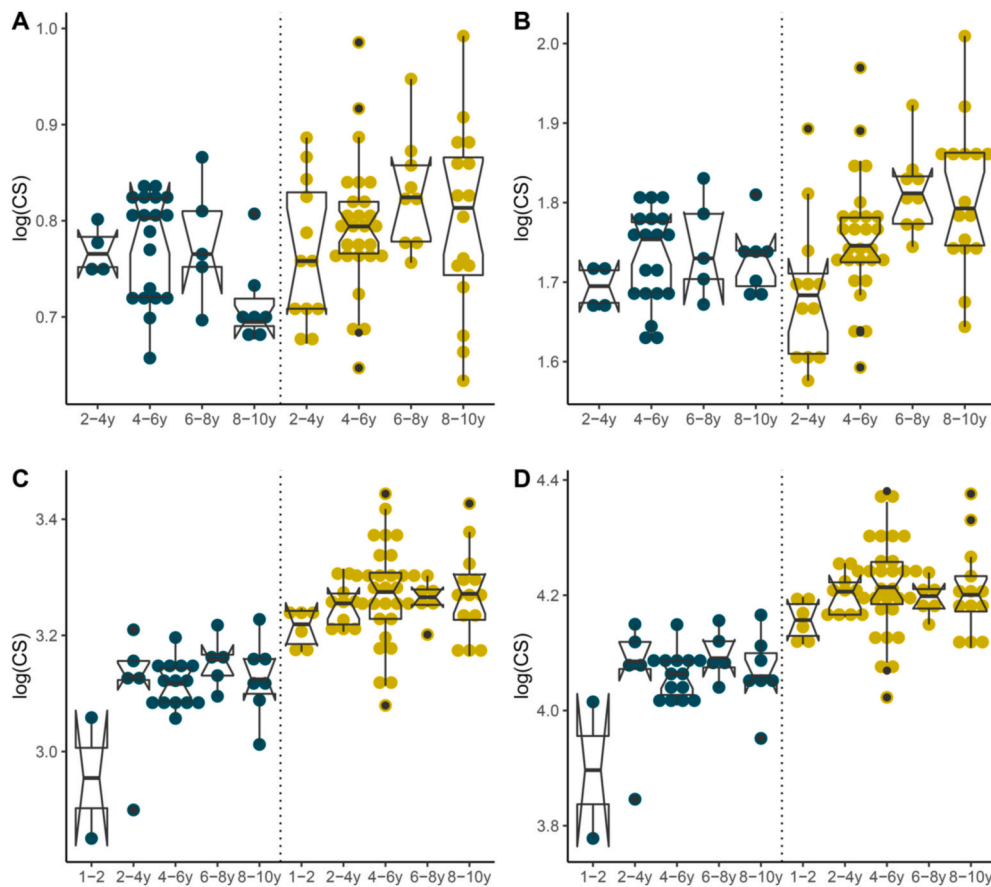
Fig. 4. Boxplots showing size (log (CS), logarithm of the centroid size) variation through age in the mandible (C-landmarks only, D-complete protocol) and the third lower molar (A-landmarks only, B- complete protocol) of sheep and goat. Goat is in dark blue (on the left) and sheep in light green (on the right). The two species are separated by a dotted line. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

very limited use for the identification of sheep and goat archaeological specimens. The size of the mandible, on the other hand, has provided more promising results however, it must be bore in mind that this element is less likely to be found complete in high numbers in archaeozoological assemblages than isolated teeth, though with some exceptions.

Importantly, sheep and goats also differ in the shape of their third lower molar and mandible. Specimens can be correctly identified to species level with probability ranging from 84.1% to 95.2% for the mandible, and from 89.2% to 93.3% for the third lower molar (depending on the protocol used). Correct cross-validation percentages of the discrete morphological criteria proposed by Halstead et al. (2002) range from 63.8% to 85.4% for the criteria on the third lower molar, while the two mandibular criteria provided a percentage of 84.2 and 88.3 of correct identification (% derived from Halstead et al. (2002) Table 2). A similar approach was used by Zeder and Pilaar (2010) and provided correct cross-validations ranging from 42.9% to 89.5% using individual criteria (% derived from Zeder and Pilaar (2010) Table 2; values were adjusted to take into account the non-identified specimens). Identifications carried out by using a combination of individual diagnostic criteria, practice routinely adopted in archaeozoology, allowed from 82.8% to 100% correct identifications for the third lower molar and from 71.3% to 78.1% correct identifications for the mandible (% derived respectively from Zeder and Pilaar (2010) Table 4 and Gillis et al. (2011), table 10). Although not based on the same sample and statistical approach (*e.g.* our approach takes into account unbalanced sample sizes), our geometric morphometric protocols performed at least as well as the discrete criteria used in isolation.

The shape differences we observed using the geometric morphometric protocols reflect the variations described by the discrete morphological criteria. Among the criteria proposed by Halstead et al. (2002), three clearly mirror our observations. According to Halstead et al. (2002:547) "the buccal edge of the centro-buccal cusp of third lower molar often points strongly in a posterior direction in goat, while it is relatively symmetrical in sheep" (criterion M3.2) and this is reflected in the fact that the distal part of the centro-buccal cusp is proportionally thicker in goats than in sheep. This criterion correctly identified the specimens with a probability of 76.7% (% derived from Halstead et al. (2002) Table 2). The second criterion on the third lower molar is that "the distal margin of the distal cusp of third lower molar often has a buccally defined "flute'' in sheep, rarely so in goat" (M3.5 of Halstead et al. (2002:549)). This criterion provided 84.1% of correct identification (% derived from Halstead et al. (2002) Table 2) and it mirrors the fact that sheep have a proportionally more pointed distal part of the distal cusp compared to goats. The proportionally reduced mesial edge in goat compared to sheep could also reflect the Halstead et al. (2002:549) M3.6 criterion described as "The flange on the mesial face of third lower molar tends to be broad in sheep and narrow in goat", (70.9% of corrected identification, derived from Table 2), noticed also by Balasse and Ambrose (2005). Conversely to Halstead et al. obersvations (2002), when the shape of the mesial part of the buccal edge of the mesio-buccal cusp (M3.1), and the shape of mesial and central part (M3.3) were considered, we did not notice shape differences between sheep and goat.

Size and shape appeared only weakly correlated, with the allometric trends being mainly homogeneous between sheep and goats. While the third lower molar form and allometry-free shape performed equally as well as shape in separating sheep and goat, mandibular allometry-free
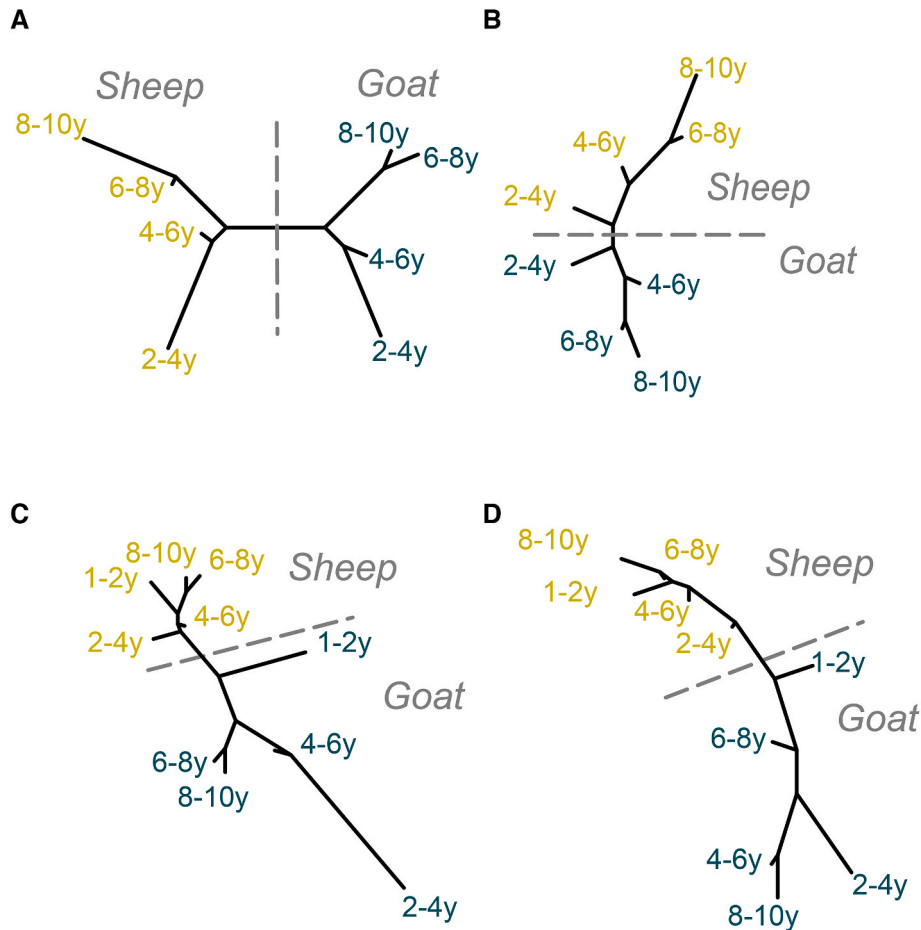
Fig. 5. Third lower molar and mandibular shape variation through age: Dissimilarity networks between age classes for the lower third lower molar (A-landmarks only, B- complete protocol) and mandible (C-landmarks only, D-complete protocol). Goat is in dark blue and sheep in light green. The two species are separated by a dotted line.

Table 4
Sexual dimorphism in tooth and mandible size and shape. Results of one-way Procrustes ANOVAs for sheep and goat separately, and of two-way ANOVAs used for testing the homogeneity of sexual dimorphism (only the interaction term is provided). Results are provided for the landmarks only and complete protocol. The p-values in bold are significant (p < 0.05) after adjustment for multiple comparisons.

		Mandible				Third lower molar			
		Landmarks		Complete		Landmarks		Complete	
		F	p	F	p	F	p	F	p
Sheep	Shape	**5.2088**	**0.001**	**3.5185**	**0.002**	*3.4913*	*0.023*	*2.9595*	*0.04*
	Size	0.0182	0.896	0.023	0.889	**10.666**	**0.004**	**13.359**	**0.002**
Goat	Shape	0.935	0.468	0.8592	0.563	0.706	0.599	0.815	0.524
	Size	0.7409	0.38	1.2001	0.269	*4.7747*	*0.032*	*6.5906*	*0.017*
Interaction	Size	0.3414	0.571	0.1145	0.739	2.0450	0.147	3.3232	0.062
	Shape	**3.0138**	**0.003**	1.7987	0.052	1.2837	0.225	1.0832	0.316

shape was less efficient to do so than shape and form. Allometries are, therefore, at least partially involved in the mandibular shape differences noticed between the two species.

Two different protocols were used on both the third lower molar and the mandible, one based only on landmark data, the second also including sliding semi-landmarks. In both cases landmark data provided lower correct cross-validation percentages and a simplified description of the shape differences compared to the results obtained when the complete protocol was used. However, to its advantage, the landmark protocol is quicker and simpler to apply and correctly identified the two species with a high probability for both for the third lower molar (84.1%) and the mandible (89.2%).

5.3. Age

Because of the well-known influence of wear on ungulate hypsodont teeth, in the past, it has been assumed that a geometric morphometric approach on teeth could not work. However, a recent geometric morphometric study of the upper second permanent premolar and third lower molar of domestic horses has revealed that age has no effect on the size and shape of their occlusal folding pattern (Seetah et al., 2014). Conversely, the study by (Cucchi et al., 2019) on bovid molars revealed that age-related variations in size and shape do exist, but are homogeneous between species. Therefore, hypsodont teeth appear perfectly suitable for geometric morphometric studies and such an approach will most likely be extensively applied in the near future.
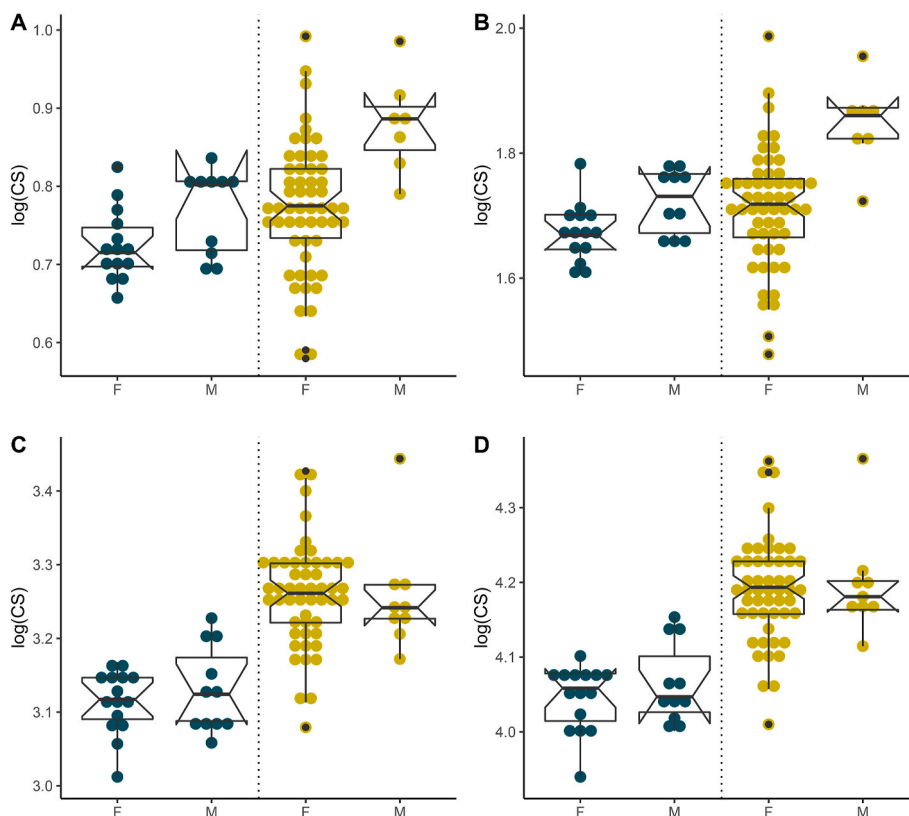
Fig. 6. Boxplots showing sexual dimorphism in the size of the mandible (C-landmarks only, D-complete protocol) and the lower third molar (A-landmarks only, B-complete protocol). Goat is in dark blue, and sheep in light green. The two species are separated by a dotted line. F: female, M: male. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
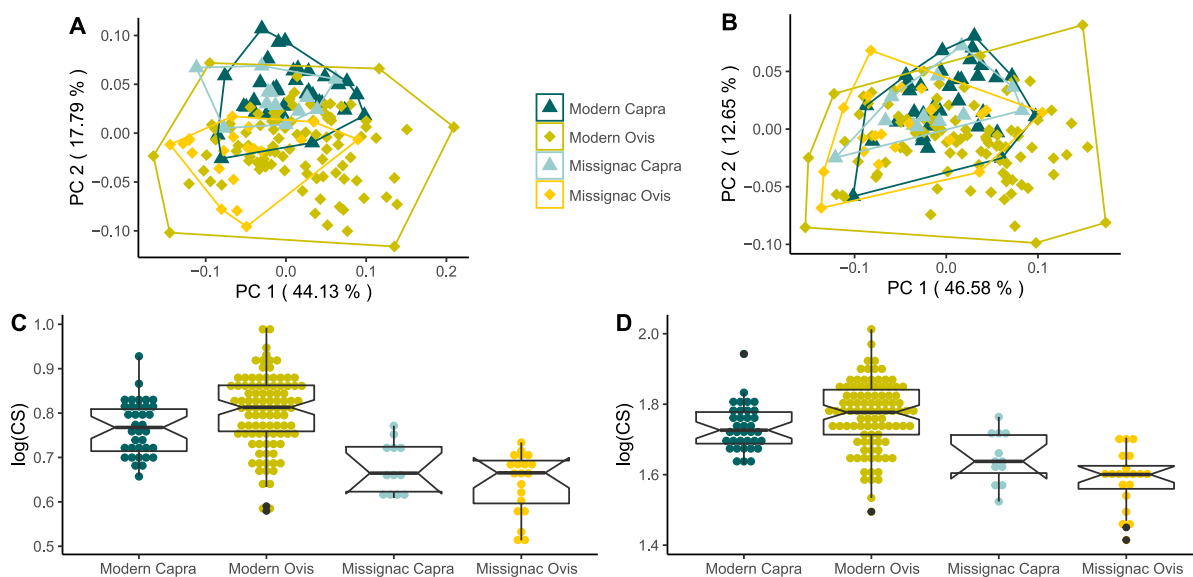

Fig. 7. Top: shape variation between the modern and the archaeological specimens identified as sheep and goat. Two first axes of PCAs based on the landmarks only (A) and the complete protocol (B). Bottom: boxplots showing size variation of the third lower molar (C-landmarks, D-complete protocol).

In this study, age appeared to have a limited impact on the size and shape of both the third lower molar and the mandible in comparison to the differences between the two species. Most of the differences noticed affected older specimens, *i.e.* 8–10 years old animals; such old animals are usually not the most abundant in the archaeological record (*e.g.* Blaise, 2005; Payne, 1973) and, even in modern husbandry practices animals are not keep alive for so long (Blaise, 2006). In particular, only the third lower molar shape showed some age-related differences between sheep and goats in the above mentioned age category. However, there is less variation between age groups than between species, revealing that age has little impact on distinction between taxa. Available discrete morphological criteria for the distinction of sheep and goat

teeth are highly dependent on age (Zeder and Pilaar, 2010) but can be applied to younger specimens (0–1.5 years of age) that those composing our sample (2–4 years old for the teeth and 1–2 years old for the mandible). The age-related mandibular variations we observed appeared less pronounced than those observed in the study conducted by Parés-Casanova (2013) where shape differences between age categories, especially visible on the molar row, were due to allometry and linked to morpho-functional changes.

5.4. Sexual dimorphism

Our study confirmed that generally, despite some overlap between sexes, in both species, males have larger third lower molars than females. In addition, our results suggest that the molar size of male goats overlap with that of female sheep. Although further analyses and additional samples are needed to confirm the observed trend, our study seem to suggest that size differences between sexes may be higher for sheep than for goat.

According to previous literature, the ratio of body to tooth size is greater for females than males (Carranza and Pérez-Barbería, 2007; Fortelius, 1985); this is the case for ungulates but also other mammals (Cochard, 1987; Lucas, 2004; Lucas et al., 1986). Sheep and goat are known to be sexually dimorphic species (Pérez-Barbería and Gordon, 1999), even if they show less sexual dimorphism in size than their wild relatives, with an almost similar size reduction in both species (Polák and Frynta, 2009). The same study also revealed that sheep and goat breeds follow the Rensch's rule, according to which larger species exhibit higher sexual size dimorphism (Rensch, 1950, 1959). Several studies have demonstrated that this rule has exceptions depending on the species and the element considered. For example, while goat shows sexual size dimorphism in the skull (Parés-Casanova, 2015), sheep do not (Parés-Casanova, 2014). The opposite pattern is true for shape with male and female sheep showing the most differences in the tibia, metapodials, femur, pelvis, radius and humerus (with females being less variable than males) (Popkin et al., 2012), while goats showed none. Clearly, not all skeletal elements display to the same extent sexual dimorphism.

In addition, it cannot be excluded that, the absence of significant sexual dimorphism in goats is the result of a smaller sample size for this species compared to sheep. Moreover, we did not explore the effect of castration known to have an impact on animal size (Davis, 2000; Popkin et al., 2012), as this information was not always recorded in the collections used for this study.

5.5. Archaeological specimens

During the Middle Age, inhabitants from Aimargues Missignac ate mainly cultivated and wild fruits, with a meat diet based mostly on the main domestic animals. Caprine remains were abundant at this site, with sheep and goat forming respectively 85% and 15% of the total of the identified specimens (Bardot-Cambot et al., 2018), but with high variation between skeletal elements (*e.g.* 72% of mandibles and 100% of metacarpals were identified as sheep) (Bardot-Cambot et al., 2018; Mureau, 2020). The geometric morphometric identifications revealed a mixed assemblage dominated by sheep (62.5% sheep and 37.5% goat), but with a larger proportion of goat than originally identified. However, the initial archaeozoological study focussed only on a selection of the total assemblage (Bardot-Cambot et al., 2018). When we restricted our geometric morphometric analyses to the specimens coming from the same contexts as analysed in (Bardot-Cambot et al., 2018), the proportion of identified sheep amounted to 76%, a percentage that is in line with the previous study (72%). It should also be mentioned that our study did not include the very young individuals which were part of the previous archaeozoological analysis (Bardot-Cambot et al., 2018) and that might explain the small discrepancy between the two studies. Regardless, the sheep-goat ratio that our study has revealed fits very

well into the regional pattern: during the medieval period in Languedoc, sheep are usually more numerous than goat (Forest, 1997) though goat can be locally particularly abundant (Forest et al., 2004; Rodet-Belarbi et al., 2002).

Finally, it should also be mentioned that, because of the nature of the discrete morphological criteria used to identify sheep and goat third lower molars, identifications may vary from one experienced archaeozoologist to another. Those macroscopic identifications also differ from the ones obtained through geometric morphometrics. As a consequence, future research may require the additional use of molecular identification methods based on aDNA or palaeoproteomics (*e.g.* ZooMS) to confirm identification for indeterminate specimens and assess the extent to which such identification can be made for all time period and areas of the world.

6. Conclusions and future perspectives

Geometric morphometrics provide a new and efficient way of identifying third lower molars and mandibles of sheep and goat. Although based on 143 specimens of 13 modern European local breeds, which do not likely reflect the full diversity of ancient sheep and goat globally, this study revealed clear differences between modern sheep and goat based on the size and shape of their third lower molar and mandible. In particular, this work opens new perspectives when it comes to the identification of isolated teeth which are abundant but largely ignored in archaeozoological studies.

Third lower molar size appeared to have little value for the identification of archaeological specimens, contrary to mandible size that appeared more promising. Shape, on the other hand, was the marker of choice for identifying archaeological specimens. Geometric morphometric based identifications have the advantage of being simultaneously less dependent of the operator, based on quantitative data and provide high degree of confidence in the identifications. Once all caprine specimens are identified to the different genus and species, it will be possible to study in detail the spatio-temporal variation in taxa proportions, to explore further the relationships between human populations, domestic animal species, and past husbandry practices. Further studies are needed to fully explore the discrepancy between the geometric morphometric based identifications and those based on discrete morphological criteria. The next step forward will be to confirm archaeological identifications through ancient DNA or palaeoproteomic analysis so that it will be possible to assess to what extent the different methodologies can be used with confidence. Once confirmed to be accurate when applied on archaeological assemblages, this 2D GMM protocol will represent an easy to set up, non-destructive, repeatable, objective and quantitative identification protocol that will complement discrete morphological criteria.

Declaration of competing interest

7. Acknowledgement

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jas.2022.105580.

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