Analysis of tail morphology and osteology in Ethiopian indigenous sheep

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ABSTRACT

Sheep adaptive diversity, including tail morphology, has been shaped by various factors, including natural and/or artificial selection for different traits. The Horn of Africa has historically been a major livestock entry point on the African continent from the Near Eastern centers of initial domestication. Ethiopia, in particular, possesses a marked sheep diversity, including the presence of breeds with four distinct tail morphotypes (short fat-tail, long fat-tail, fat-rump, and thin-tail) that do not co-exist elsewhere. The origin and development of the fat-tail, as well as the fat-rump, are still poorly known, and the osteological and metrical differences between the fat-tail morphotypes have never been studied. Here, we characterized the phenotypic diversity of Ethiopian sheep tails from morphological and osteological perspectives. Three tail measurements and 14 osteological traits were recorded in six breeds (Menz, Washera, Afar, Blackhead Somali, Bonga, and Gumz), representative of the four sheep tail morphotypes. Both linear discriminant and principal component analyses categorize the six sheep breeds into four distinct tail morphotypes. Analysis of variance of the morphological and osteological traits shows significant differences ($P < 0.05$) between the four tail morphotypes. The highest mean values of tail length, total caudal vertebrae length and the number of caudal vertebrae were recorded in the thin-tailed sheep, followed by the long fat-tailed sheep, whereas the lowest average values were recorded in the fat-rumped and short fat-tailed sheep. These traits are significantly and positively correlated with each other. Based on regression model analysis, it is possible to use tail length alone as a predictive tool to estimate the sheep tail osteology without killing the animal. Moreover, based on measurements of sheep caudal vertebrae, the osteologist can estimate other osteological traits and the tail length of that sheep, further differentiating its tail morphotypes. Significant differences ($P < 0.05$) were also observed in individual caudal vertebra length and breadth, tail breadth and tail circumference, and flat and concave-shaped caudal vertebrae between the short-tailed and long-tailed sheep. Our results provide important phenotypic baselines for genome diversity and adaptation studies and an osteological baseline for archeozoological work aiming to understand the history of sheep farming and breed development in past societies.

Keywords: Ethiopia, sheep breed, tail, morphometry, osteometry.

1. Introduction

Sheep domesticated in the Fertile Crescent approximately 11,000 years ago (Vigne et al., 2011). Since then, sheep have been selected for production by human societies for their meat, milk, and wool (Ruiz-Larrañaga et al., 2018), behavioural traits (e.g., tameness), and environmental adaptation (Trut et al., 2009). Among the key physical characteristics that distinguish sheep breeds, tail type and length are among the most discriminating features (Gebremichael, 2008; Gizaw, 2009). In particular, the morphology and fat content of the sheep tail are considered to be a reservoir of energy for the animal to cope with harsh environmental challenges such as drought, extremely cold winters, and food shortages (Chilliard et al., 2000; Pourlis, 2011; Moradi et al., 2012; Lv et al., 2015). The fat in the sheep tail also represents an essential source of dietary fat for human societies lacking other fat-producing animals, particularly during drought and famine (Moradi et al., 2012). Sheep breeds from across the world are classified today based on their tail phenotypes as thin-tailed, short fat-tailed, long fat-tailed and fat-rumped sheep breeds (Gizaw et al., 2007; Gifford-Gonzalez and Hanotte, 2011; Muigai and Hanotte, 2013; Ahbara et al., 2019; Whannou et al., 2021).

The founder populations of current African sheep breeds were thought to have been introduced from Asia in three waves of migration corresponding to the modern thin-tailed, fat-tailed and fat-rumped sheep (Gifford-Gonzalez and Hanotte, 2011), with two types of fat-tailed sheep - long and short ones. It is thought that the first sheep to enter the African continent were thin-tailed hairy sheep types. Fat-tailed sheep were introduced into Africa during the second wave of migration, followed by the fat-rumped sheep (Ryder, 1984; Gifford-Gonzalez and Hanotte, 2011). Indigenous African sheep genetic resources are distributed geographically, mainly across three non-overlapping geographic ranges. Thin-tailed sheep are currently found in West Africa and East Africa up to Sudan (IEMVT-FRA, 1950), bordering Ethiopia. Fat-tailed sheep are found in North, East and Southern Africa, and fat-rumped sheep are found across the Horn of Africa (Ethiopia, Djibouti, Somali, Kenya, and Sudan) (Wilson, 1991; Rege et al., 1996). Archaeological and molecular genetic information indicate separate introductions and dispersion histories for the African thin-tailed and fat-tailed sheep (Muigai and Hanotte, 2013). Thin-tailed sheep entered the African continent through Egypt and the Sinai Peninsula, and fat-tailed sheep entered through the Horn of Africa and Northeast Africa. This scenario is supported by ancient iconographies/images, such as illustrations of sheep tail morphology (Gootwine, 2018; Vila et al., 2021) and the current geographic distribution of sheep tail morphotypes (Gizaw, 2009; Muigai and Hanotte, 2013; Amane et al., 2020 and 2022; Whannou et al., 2021).

Ethiopia is one of the major gateways for domestic sheep migration from Asia to Africa, owing to its ancient civilization and geographic position in the Horn of the African continent (Devendra and McLeroy 1982). The country comprises one of Africa's most diversified sheep populations (Devendra and McLeroy 1982). Ethiopian indigenous sheep are adapted to diverse agroecological environments with the presence of the four tail-type groups (short fat-tail, long fat-tail, fat-rump, and thin-tail) (Gizaw, 2009). This tail group classification is, to a large extent, arbitrary, as it is based on the external visual examination of sheep tails (tail type and length) and does not necessarily provide accurate information on tail morphology. Furthermore, the approach does not consider other important tail measurement traits, such as tail width and circumference, which are used as a measure of tail weight in breeding programs (Vatankah and Talebi, 2008). Moreover, it does not take any osteological characters into consideration (caudal vertebrae), which can provide precise and detailed osteological information on sheep caudal vertebrae. Only a few studies mention variability in the number of caudal vertebrae in domestic sheep breeds compared to its wild ancestor, the mouflon (*Ovis orientalis* or *Ovis gmelini).* The mouflon has a short tail with 12-13 caudal vertebrae, whereas this number can rise to 35 in domestic sheep (Zeuner, 1963). Cornevin and Lesbre (1897) reported between 3 to more than 24 caudal vertebrae according to breeds. The number of caudal vertebrae is between 8-10 in short-tailed and 16-18 in long-tailed European sheep (Dýrmundsson and Niżnikowski, 2010). Several studies have been carried out on the skeletal anatomy (osteology) of sheep (Wilke et al., 1997; Boessneck et al., 1964); Boessneck,1969; Prummel and Frisch, 1986; Clutton-Brock and Pemberton, 2004; Salvagno and Albarella 2017; Haruda et al., 2019) but there is no readily available information on their tail osteology (caudal vertebrae), unlike for other spinal regions (atlas, cervical, thoracic, and lumbar vertebrae) (May 1964; Wilke et al., 1997; Donaldson et al., 2013). Studies of vertebral variation in the thoracolumbar (thoracic and lumbar) region are of particular interest to livestock breeders in terms of meat production. On the contrary, the tail region is only interested in fat-tailed and fat-rumped sheep breeders. On the other hand, detailed information on sheep tail morphology and osteology may provide accurate phenotypes for genome mapping, adaptation studies, and baseline osteological information for archaeological studies and sheep breed origins. Moreover, estimating the relationship between tail osteological and morphological traits in sheep may help to predict traits that are not commonly and easily measured from live sheep, e.g., osteological traits are recorded from slaughtered sheep. Still, there should be a method for estimating them without killing the animal. On the other hand, there should also be a method enabling osteologists to differentiate one tail morphotype from the other based on caudal vertebra measurements. Therefore, we must develop regression models that allow us to predict one trait from the other.

Genetic studies have been carried out to identify candidate regions and genes associated with tail morphotypes of Ethiopian indigenous sheep (Ahbara et al., 2019). This finding should be confirmed by further investigation of the morphometry of tail morphology variation, which might provide accurate phenotypes based on external tail morphological traits and baseline osteological information on sheep caudal vertebrae (Amane et al., 2020).

Accordingly, this study aims to address the following two main objectives: (i) to characterize the tail of Ethiopian sheep using morphological and osteological (caudal vertebrae) traits, and (ii) to provide baseline osteological information on sheep caudal vertebrae as baseline information for zooarchaeological studies.

2. Material and methods

2.1. Sheep populations/breeds and sampling

A total of 40 reference skeletons, including tail bones (caudal vertebrae), were prepared from six adult and subadult Ethiopian sheep breeds, representing four tail morphotypes (Gizaw et al., 2007; Table 1; Fig. 2). The four tail morphotypes are thin-tail (Gumz, n = 10), long fat-tail (Bonga, n = 10), short fat-tail (Menz and Washera, n = 10) and fat-rump (Afar and Blackhead Somali, n = 10). The sheep breeds, adapted to diverse agroecological environments, were bought from different households in the geographic areas where they are predominantly bred (Fig. 1). Sampling information for the studied sheep breeds is presented in Table 1.

Table 1

Sampling information for the studied sheep breeds.

Breed	Tail morphotype	N	Location	Sampling date	Latitude (*N*)	Longitude (*E*)	Altitude (m.a.s)
Menz	SFT	5	Molale	20-21.2.2019	10° 7’ 0"	39° 40’ 0"	3068
Washera	SFT	5	Banja	26-28.2.2019	11° 10' 0"	36° 15' 0"	2500
BHS	FR	5	Shinile	1-3.2.2020	9° 41’ 0"	41° 51’ 0"	986
Afar	FR	5	Dubti	1-3.2.2020	11° 44’ 10"	41° 5’ 7"	570
Bonga	LFT	10	Bonga	9-13.07.2019	7° 16' 0"	36° 15' 0"	1788
Gumz	TT	10	Guba	25-30.12.2019	11° 16' 0"	35° 17' 0"	620
Total	4	40	6				

N: number of animals sampled from each breed; BHS: Blackhead Somali; SFT: Short fat-tail; FR: Fat-rump; LFT: Long fat-tail; TT: Thin-tail.

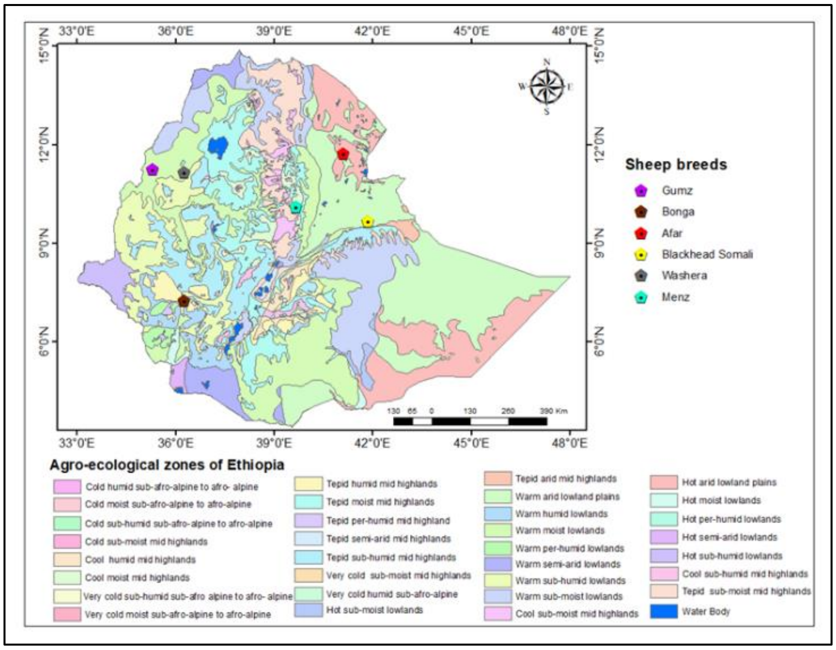

Fig. 1. The geographical location of the studied sheep breeds in relation to agroecological zones of Ethiopia.

Fig. 2. Images of the six sheep breeds examined with their tail skinned, (A1) Menz sheep, (A2) Washera sheep, (B1) Afar sheep, (B2) Blackhead Somali sheep, (C) Bonga sheep, (D) Gumz sheep. A1 and A2 sheep represent the short fat-tail morphotype; B1 and B2 sheep represent the fat-rump morphotype; C sheep represent the long fat-tail morphotype, and D sheep represent the thin-tail morphotype.

2.2. Preparation of reference skeleton and caudal vertebrae

Reference bone samples (skeleton) in general, and caudal vertebrae in particular, were obtained through a series of processing steps, including sheep slaughtering, evisceration; removal of the skin, meat, and muscle tissue using a knife; cooking, cleaning, and washing bones using washing powders; sun-drying the cleaned bones, and finally packing the dried bones in plastic boxes (Fig. 3). The bones were initially cooked for two hours, followed by two to three changes of water, depending on the level of fat deposition on the meat and bones of the animals, and further cooking. The tails (sacrum and caudal vertebrae) were prepared with particular care, separately from the rest of the skeleton, considering that caudal vertebrae, especially at the tip of the tail, are extremely small. Caudal vertebrae refer to the variable number of bones (vertebrae) in a sheep's tail. See Fig. 4 for a description of a 'typical vertebra' relevant to this study.

Fig. 3. Reference bone sample preparation, (a) washing and cleaning, (b) sun-drying and (c) packing of bones.

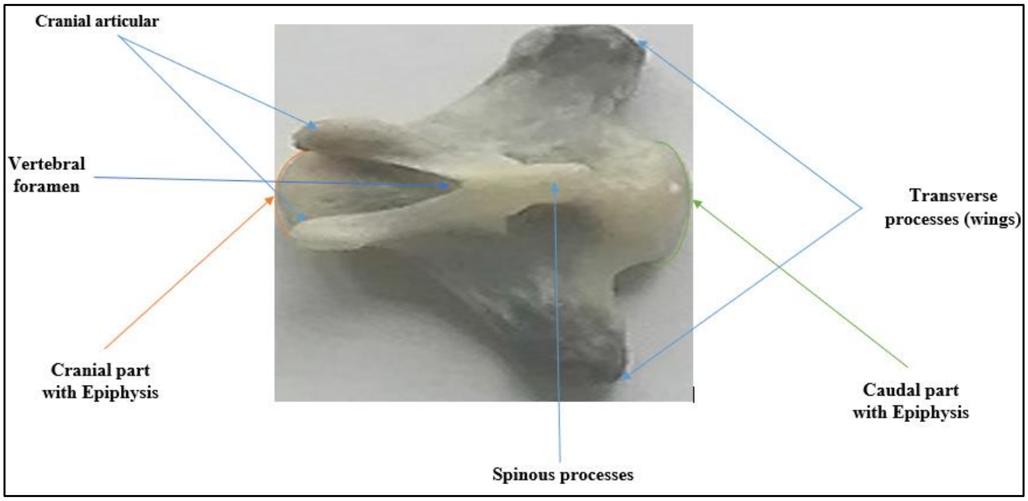

Fig. 4. Anatomical parts of a caudal vertebra.

2.3. Morphological and osteological data collection

To characterize the tail of the studied sheep, morphological and metric data were collected from the tail and caudal vertebrae. The FAO (1986) sheep breed descriptor lists and the Von den Driesch (1976) animal bone measurement manual were used to quantitatively characterize the tail and caudal vertebrae of each sheep (Table 2). We developed our protocol to qualitatively describe each caudal vertebra by setting various criteria (Table 3).

Measurements for quantitative traits were taken from the 40 live sheep selected for the osteological collection and 705 caudal vertebrae. The quantitative traits measured from the live sheep included tail length, tail breadth, and tail circumference. They were measured in centimeters using a flexible tape and measuring stick. On the other hand, the quantitative traits measured from each caudal vertebra included the greatest length, the greatest breadth, and the smallest breadth, and they were measured in millimeters (mm) using a calliper (Fig. 5). Detailed descriptions of quantitative morphological and osteological tail traits are presented in Table 2.

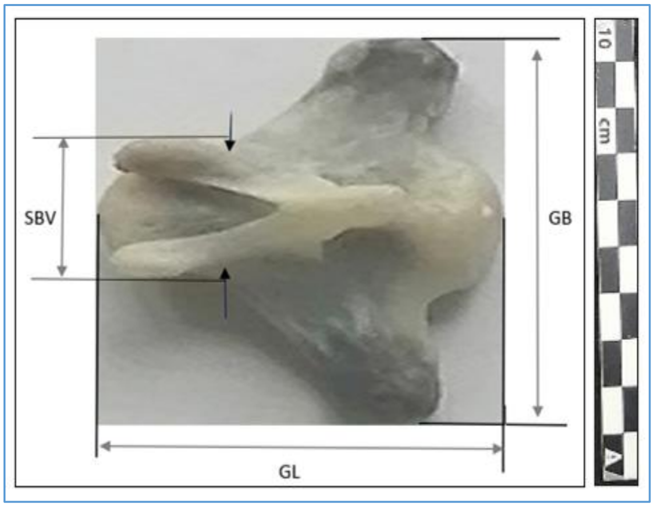

Fig. 5. Quantitative osteological tail traits measured from each caudal vertebra (mm). GL = Greatest Length, GB = Greatest Breadth over the wings, SBV = Smallest Breadth of the vertebra.

Qualitative osteological traits observed and recorded for each caudal vertebra (including type, shape, and symmetry categories) are presented in Figs. 6-8 and Table 3. Various qualitative characteristics of each caudal vertebra were recorded after careful observation from different angles (cranial *versus* caudal; top *versus* bottom view) by two observers (Emmanuelle Vila and Agraw Amane). Four caudal vertebra types were distinguished (Type 1, Type 2, Type 3 and Type

4) (Fig. 6). We took the greatest length and the smallest breadth measurements from all caudal vertebra types (Type 1, Type 2, Type 3 and Type 4), but the greatest breadth measurements were only taken from the first three types (Type 1, Type 2, and Type 3) since transverse processes (wings) are absent on type 4 caudal vertebra types. Data on the shape and symmetry of the caudal vertebrae were only taken from type 1 and type 2 caudal vertebrae, as the two types have a well-developed transverse process. Detailed descriptions of qualitative osteological tail traits are presented in Table 3.

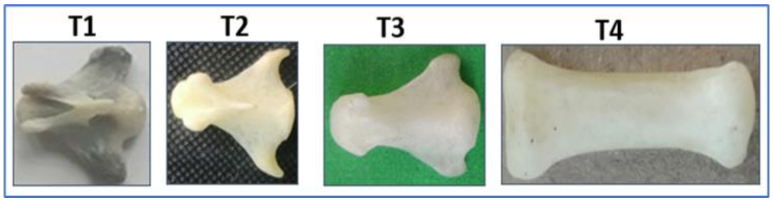

Fig. 6. Categories of caudal vertebra type. T1 = Type 1, T2 = Type 2, T3 = Type 3, T4 = Type 4.

Fig. 7. Categories of caudal vertebra shape. S1 = flat-shaped, S2 = Concave-shaped, S3 = Convex-shaped.

Fig. 8. Categories of caudal vertebra symmetry. S = Symmetry, A = Asymmetry.

Based on the fusion status of epiphyses to the body of each caudal vertebra (Schmid, 1972) and dentition (tooth eruption and dental wear: Payne 1973; Payne 1987), the studied sheep were grouped into two age groups: subadults and adults. Epiphyses are rounded structures that fuse cranially and/or caudally to the body of each caudal vertebra as the bone grows (Fig. 4). Subadult sheep are those sheep less than or equal to two years old, and they have unfused cranial and/or caudal epiphyses caudal vertebrae. Adult sheep are those older than two years old and have caudal vertebrae with epiphyses that fused cranially and/or caudally to their body (Schmid, 1972).

Table 2

Descriptions of the quantitative morphological and osteological tail traits.

Quantitative traits	Description
Tail length	Distance from the base to the tip of the tail on the outer side of the tail in cm
Tail breadth	Distance between both sides of the tail measured at the widest part in cm
Tail circumference	Circumference of the tail of the animal at the widest part in cm
Individual caudal vertebra length	Greatest length of a vertebra from the ventral side measured in a cranio-caudal direction in mm (cf. Driesch 1976)
Caudal vertebra breadth	Greatest breadth of a vertebra that measured across the transverse processes (wings) in mm (cf. Driesch 1976)
Caudal vertebra thickness	Smallest breadth of a vertebra measured in a medio-lateral direction in mm (cf. Driesch 1976)
Total caudal vertebrae length	Individual caudal vertebra length value of all the caudal vertebrae of a sheep
Caudal vertebrae number	Caudal vertebrae count of a sheep

Table 3

Descriptions of the qualitative osteological tail traits.

Qualitative traits	Description
1. Type category	Characteristics of the caudal vertebrae grouped into four different types
Type 1	Caudal vertebrae with a vertebral foramen, a well-developed spinous process, and a well- developed cranial articular and transverse processes
Type 2	Caudal vertebrae with no vertebral foramen, with vestigial spinous and cranial articular processes and moderately developed transverse processes
Type 3	Caudal vertebrae with no vertebral foramen and no spinous process, with vestigial cranial articular and transverse processes
Type 4	Caudal vertebrae with a simple cylindrical shape (no vertebral foramen and no spinous process; cranial articular and transverse processes cannot be clearly distinguished)
2. Shape category	Characteristics of the caudal vertebrae grouped into three different shapes (Type 1 and Type 2)
Flat	Caudal vertebrae with horizontal transverse processes (straight position)
Concave	Caudal vertebrae with dorsally angled transverse processes (upward position)
Convex	Caudal vertebrae with ventrally angled transverse processes (downward position)
3. Symmetry category	Characteristics of the caudal vertebrae grouped into two categories of symmetry (Type 1 and Type 2)
Symmetry	Caudal vertebrae with symmetrical transverse processes
Asymmetry	Caudal vertebrae with asymmetrical transverse processes

2.4. Statistical analyses

Various analyses, including analysis of variance, correlation analysis, regression model development, and multivariate analyses (linear discriminant, canonical correlation, and principal component analyses), were performed using different packages and functions in the software R v4.0.1 (R Core Team, 2020). The various r packages and their r library locations (web addresses) used to perform different statistical measures and procedures are presented in Table 4.

Table 4

The various r packages and their r library locations (web addresses).

<table>
<tr><td colspan="2">Analysis of variance</td></tr>
<tr><td>Package</td><td>Their r library locations (web addresses)</td></tr>
<tr><td>car</td><td>https://cran.rstudio.com/src/contrib/4.0.1/car_3.1-1.tar.gz</td></tr>
<tr><td>dplyr</td><td>https://dplyr.tidyverse.org, https://github.com/tidyverse/dplyr</td></tr>
<tr><td>stats</td><td>https://CRAN.R-project.org/package=STAT</td></tr>
<tr><td>agricolae</td><td>https://cran.rstudio.com/bin/windows/contrib/4.0.1/agricolae_1.3-5.zip</td></tr>
<tr><td colspan="2">Correlation analysis and regression model development</td></tr>
<tr><td>GGally</td><td>https://cran.rstudio.com/bin/windows/contrib/4.0.1/GGally_2.1.2.zip</td></tr>
<tr><td>MVN</td><td>https://cran.rstudio.com/bin/windows/contrib/4.0.1/MVN_5.9.zip</td></tr>
<tr><td>psych</td><td>https://personality-project.org/r/psych-manual.pdf</td></tr>
<tr><td>corrplot</td><td>https://cran.rstudio.com/bin/windows/contrib/4.0.1/corrplot_0.92.zip</td></tr>
<tr><td>tidyverse</td><td>https://cran.rstudio.com/bin/windows/contrib/4.0.1/tidyverse_1.3.2.zip</td></tr>
<tr><td>ggpmisc</td><td>https://cran.rstudio.com/src/contrib/ggpmisc_0.5.1.tar.gz</td></tr>
<tr><td colspan="2">Linear discriminant and canonical correlation analyses</td></tr>
<tr><td>tidyverse</td><td>https://cran.rstudio.com/bin/windows/contrib/4.0.1/tidyverse_1.3.2.zip</td></tr>
<tr><td>caret</td><td>https://cran.rstudio.com/bin/windows/contrib/4.0.1/caret_6.0-93.zip</td></tr>
<tr><td>dplyr</td><td>https://dplyr.tidyverse.org , https://github.com/tidyverse/dplyr</td></tr>
<tr><td>digest</td><td>https://cran.rstudio.com/bin/windows/contrib/4.0.1/digest_0.6.29.zip</td></tr>
<tr><td>MASS</td><td>https://cran.rstudio.com/bin/windows/contrib/4.0.1/MASS_7.3-58.1.zip</td></tr>
<tr><td>ggplot2</td><td>https://cran.rstudio.com/bin/windows/contrib/4.0.1/ggplot2_3.3.6.zip</td></tr>
<tr><td>ggbiplot</td><td>http://github.com/vqv/ggbiplot</td></tr>
</table>

Principal component analysis	
stats	https://CRAN.R-project.org/package=STAT
devtools	https://cran.rstudio.com/src/contrib/4.0.1/devtools_2.4.5.tar.gz
factoextra	https://cran.rstudio.com/bin/windows/contrib/4.0.1/factoextra_1.0.7.zip
ggplot2	https://cran.rstudio.com/bin/windows/contrib/4.0.1/ggplot2_3.3.6.zip

2.4.1. *Means of tail morphological and osteological traits*

We checked the normality of the data and the homogeneity of variance using the Q-Q plot and Levene's tests (Levene, 1960), respectively. A one-way ANOVA test was used to compare means of morphological and osteological tail traits for the four sheep tail morphotypes (short fat-tail, long fat-tail, fat-rump, and thin-tail). As the ANOVA test was significant, a Tukey multiple pairwise comparisons test was used to distinguish which pairs of means were significant. The following general linear model was used for the analysis of morphological and osteological tail traits:

$$Y_{ij} = u + G_i + e_{ij}$$

Where: Y_{ij}= observation on tail morphological and osteological traits

u = overall mean

G_i = Fixed effect of sheep tail morphotypes (*i* = short fat-tail, long fat-tail, fat-rump, thin-tail)

e_{ij}= effect of random error

2.4.2. *Correlation analysis and regression model development*

Pearson correlation analysis was used to determine and statistically test the nature of the association of pairs of morphological and osteological tail traits in the whole dataset (17 traits: three tail measurements and 14 osteological tail traits), as presented in Table 7. Regression models were developed for significantly and positively correlated osteological and morphological tail characters (Table 7). Moreover, as shown by the analysis of variance results, these characters allow for separation of the studied Ethiopian sheep into three main tail morphotypes (short-tail, medium-tail, and long-tail). These characters are the three osteological tail traits (total caudal vertebrae length, caudal vertebrae number, individual vertebra length) and one tail measurement character (tail length). Regression models for the two osteological tail characters, total caudal vertebrae length and caudal vertebrae number were developed from the external tail measurement, tail length (Fig. 11A and B, respectively). Moreover, based on the individual caudal vertebra measurement of the animal, we developed regression models for the other two osteological tail traits, caudal vertebrae length and caudal vertebrae number, and for the external tail measurement, tail length (Fig. 12A and B, respectively).

2.4.3. Multivariate analyses

Multivariate statistical analysis techniques such as linear discriminant analysis, principal component analysis, and canonical correlation analysis were employed to investigate the morphological structure of the studied sheep tail morphotypes or breeds. The analyses were performed separately for sheep tail morphotypes and breeds based on morphological and osteological traits. We performed a separate analysis for sheep tail morphotypes and breeds based only on osteological tail traits to assess possible differences in osteological traits linked to the tail of the studied sheep. Linear discriminant analysis generates useful linear discriminant functions for discriminating sheep tail morphotypes or breeds. In canonical correlation analysis, the canonical correlation measures the correlation between each linear discriminant function and sheep tail morphotypes or sheep breeds. The canonical correlation value obtained indicates the ability of the linear discriminant function to separate sheep tail morphotypes or breeds. The closer the canonical correlation value is to 1, the higher the discriminating ability. One way of displaying linear discriminant analysis results is to create a stacked histogram of the values of linear discriminant functions for the samples from different sheep tail morphotypes or breeds. It is also important to investigate how each linear discriminant function separates the studied sheep tail morphotypes or breeds. Principal component analysis, another multivariate analysis technique, generates useful principal components for discriminating the sheep tail morphotypes or breeds.

We calculated the percentage of separation (proportion of trace) achieved by each linear discriminant function from the loadings (value of each linear discriminant function). The Eigenvalue, percentage of separation and canonical correlation of linear discriminant functions performed for sheep tail morphotypes or breeds tell us how important the linear discriminant function is to discriminate the studied sheep tail morphotypes or breeds.

3. Results

3.1. Mean values of tail morphological and osteological traits.

The mean values of quantitative morphological and osteological tail traits of the four sheep tail morphotypes are presented in Table 5. The age of the animals (adult and subadults) did not have a significant ($P > 0.05$) effect on the measurements and counts of the different caudal vertebra types for the four sheep tail morphotypes (Supplementary Table 1). Accordingly, all the comparisons were made between animals regardless of their age. Total caudal vertebrae length, caudal vertebrae number, and tail length were significantly different ($P < 0.05$) for the long fat-tail compared to the three other tail morphotypes, as well as for the thin-tail compared to the other three other tail morphotypes, with the highest mean values recorded for the thin-tailed sheep (total caudal vertebrae length: 54.9 ± 6.38 cm, caudal vertebrae number: 24.2 ± 1.48, range 21-26, tail length: 67.6 ± 4.62 cm), followed by the long fat-tailed sheep (total caudal vertebrae length: 39.2 ± 3.50 cm, caudal vertebrae number: 19.6 ± 1.26, range 18-21, tail length: 44.8 ± 5.85 cm). The lowest mean values were observed in the fat-rumped (total caudal vertebrae length: 19.3 ± 2.70 cm, caudal vertebrae number: 13.7 ± 1.02, range 11-15, tail length: 27.1 ± 7.53 cm) and the short fat-tailed (total caudal vertebrae length: 23.8 ± 3.12 cm, caudal vertebrae number: 13.0 ± 1.25, range 12-15, tail length: 28.6 ± 3.81 cm) sheep (Table 5). Measurements for caudal vertebra breadth were significantly different ($P < 0.05$) for long-tailed (long fat-tail, thin-tail) and short-tailed (short fat-tail, fat-rump) sheep, with the highest mean value observed in the long-tailed sheep (22.85 ± 2.60 mm) and the lowest value in the short-tailed sheep (20.3 ± 2.6 mm). Tail breadth and tail circumference were significantly different ($P < 0.05$) for the long-tailed and short-tailed sheep, with the highest mean values observed in the short-tailed sheep (tail breadth: 19.95 ± 2.22 cm, tail circumference: 36.73 ± 7.75 cm) and the lowest values in the long-tailed sheep (tail breadth: 14.3 ± 2.17 cm, tail circumference: 23.45 ± 6.49 cm). Individual caudal vertebra length is significantly ($P < 0.05$) different for the short fat-tail compared to the three other tail morphotypes, as well as for the fat-rump compared to the three other sheep tail morphotypes, with the highest mean value in the long-tailed sheep (long fat-tail, thin-tail) (21.35 ± 1.82 mm), and the lowest mean value in the fat-rumped sheep (13.9 ± 1.51 mm), followed by the short fat-tailed sheep (18.2 ± 1.18 mm). Measurements for caudal vertebra thickness were significantly different ($P < 0.05$) for the fat-rumped sheep and the three other sheep tail morphotypes, with the lowest mean value observed in the fat-rumped sheep (4.9 ± 0.84 *vs* 8.07 ± 1.05 mm). Visual tail lengths, tail shape, size, and morphological and osteological tail traits of the four sheep tail morphotypes are shown in Fig. 9A, B, and Fig. 10A, B.

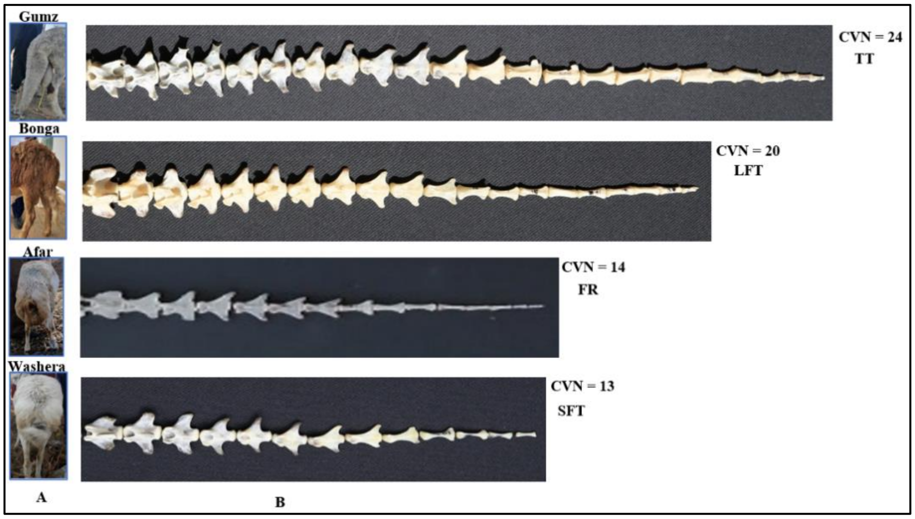

Fig. 9. Sheep tail morphotypes with visual tail phenotype and caudal vertebrae number, (A) visual tail lengths, and (B) CVN = number of caudal vertebrae. SFT = short fat-tail (Washera) , FR = fat-rump (Afar), LFT = long fat-tail (Bonga) and TT = thin-tail (Gumz).

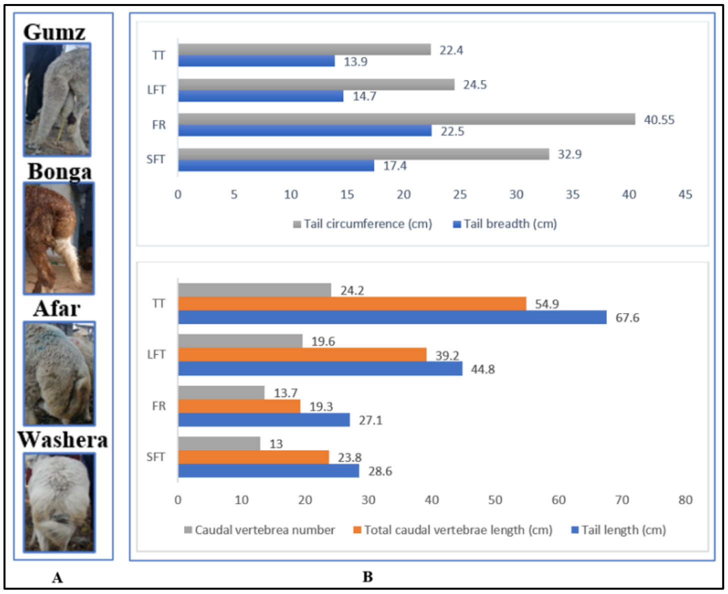

Fig. 10. Morphological and osteological tail traits of the four sheep tail morphotypes, (A) visual tail shape and size, (B) mean values of the tail circumference (cm), tail breadth (cm), caudal

vertebrae number (count), total caudal vertebrae length (cm) and tail length (cm). SFT = short fat-tail, FR = fat-rump, LFT = long fat-tail and TT = thin-tail.

Table 5

Mean values of quantitative morphological and osteological tail traits of the four sheep tail morphotypes (10 sheep/tail morphotype) from different caudal vertebrae categories (type, shape and symmetry).

Traits	Sheep tail morphologies or groups			
	short fat-tail	fat-rump	long fat-tail	thin-tail
Tail length (cm)	28.6 ± 3.81^{a}	27.1 ± 7.53^{a}	44.8 ± 5.85^{b}	67.6 ± 4.62^{c}
Tail breadth (cm)	17.4 ± 2.12^{a}	22.5 ± 2.32^{a}	14.7 ± 2.16^{b}	13.9 ± 2.18^{b}
Tail circumference (cm)	32.9 ± 7.92^{a}	40.55 ± 7.57^{a}	24.50 ± 9.44^{b}	22.40 ± 3.53^{b}
Caudal vertebrae number (count)	13.0 ± 1.25^{a}	13.7 ± 1.02^{a}	19.6 ± 1.26^{b}	24.2 ± 1.48^{c}
Individual caudal vertebra length (mm)	18.2 ± 1.18^{a}	13.9 ± 1.51^{b}	20.0 ± 1.25^{c}	22.7 ± 2.38^{c}
Total caudal vertebrae length (cm)	23.8 ± 3.12^{a}	19.3 ± 2.70^{a}	39.2 ± 3.50^{b}	54.9 ± 6.38^{c}
Caudal vertebrae breadth (mm)	21.2 ± 2.24^{a}	19.4 ± 2.96^{a}	22.5 ± 1.99^{b}	23.2 ± 3.20^{b}
Caudal vertebrae smallest breadth (cm)	8.1 ± 1.60^{a}	4.9 ± 0.84^{b}	8.4 ± 0.98^{a}	7.7 ± 0.56^{a}

^{a,b,c}*Means within a row with different superscript letters are significantly different ($P < 0.05$).*
^{a,b,c}*Means within a row with the same superscript letters are not significantly different ($P > 0.05$).*

The mean qualitative osteological tail traits (type, shape, and symmetry categories) of the four sheep tail morphotypes are presented in Table 6. The mean number of Type 2, flat-shaped, and concave-shaped caudal vertebrae was significantly different ($P < 0.05$) for long-tailed (long fat-tail, thin-tail) and short-tailed (short fat-tail, fat-rump) sheep, with the highest and the lowest mean numbers observed in the long-tailed (type 2: 3.45 ± 1.0, flat-shaped: 6.5 ± 2.29, concave-shaped: 2.55 ± 1.25) and short-tailed (type 2: 2.35 ± 0.95, flat-shaped: 3.85 ± 1.51, concave-shaped: 1.2 ± 0.94) sheep, respectively. Moreover, the mean number of asymmetric caudal vertebrae is significantly ($P < 0.05$) different for the fat-rump compared to the three other tail morphotypes. The lowest mean number was observed in the fat-rumped sheep (2.3 ± 2.06 *vs* 7.97 ± 2.31), whereas the mean number of symmetric caudal vertebrae is significantly ($P < 0.05$) different for the short fat-tailed sheep compared to the three other tail morphotypes, with the lowest mean number observed in the short fat-tailed sheep (0.9 ± 0.99 *vs* 3.57 ± 1.98) (Table 6).

Table 6

Mean qualitative osteological tail traits (type, shape and symmetry) of the four sheep tail morphotypes (10 sheep/ tail morphotype). The unit is count for all caudal vertebra types.

Traits	Sheep tail morphotypes			
	short fat-tail	fat-rump	long fat-tail	thin-tail
Type category				
Type 1	6.1 ± 1.61^{a}	3.9 ±1.29^{b}	7.4 ± 1.17^{c}	8.9 ± 1.28^{c}
Type 2	2.6 ± 0.70^{a}	2.1 ± 1.20^{a}	3.9 ± 0.74^{b}	3.0 ± 1.25^{b}
Type 3	2.1 ± 0.74^{a}	2.1 ± 0.57^{a}	3.1 ± 0.74^{a}	3.4 ± 1.17^{a}
Type 4	2.2 ± 0.63^{ba}	5.6 ± 1.07^{ab}	5.2 ± 1.40^{ab}	8.9 ± 2.02^{c}
Shape category				
Flat	4.3 ± 1.95^{a}	3.4 ± 1.07^{a}	6.3 ± 2.36^{b}	6.7 ± 2.21^{b}
Concave	1.6 ± 0.84^{a}	0.8 ± 1.03^{a}	2.2 ± 1.40^{b}	2.9 ± 1.10^{b}
Convex	2.8 ± 1.48^{a}	1.8 ± 0.92^{a}	2.8 ± 2.66^{a}	2.3 ± 1.76^{a}
Symmetry category				
Asymmetry	7.8 ± 1.61^{a}	2.3 ± 2.06^{b}	8.7 ± 2.26^{a}	7.4 ± 3.06^{a}
Symmetry	0.9 ± 0.99^{a}	3.7 ± 1.49^{b}	2.5 ± 2.12^{b}	4.5 ± 2.32^{b}

^{a,b,c}*Means within a row with different superscript letters are significantly different ($P < 0.05$).*
^{a,b,c}*Means within a row with the same superscript letters are not significantly different ($P > 0.05$).*

3.2. Correlation analysis and regression model development

The correlation analysis was performed on pairs of all traits for the whole dataset (Table 7). The Pearson correlation coefficient was represented by $r_{p.}$ A $r_p > 0.7$ was defined as a strong correlation (Dormann et al., 2013). Among the external morphological tail traits, tail breadth and tail circumference show a strong correlation ($r_p = 0.88$, $P < 0.001$). The other external morphological tail trait, tail length, showed a strong correlation with osteological tail traits such as total caudal vertebrae length ($r_p = 0.96$, $P < 0.001$), caudal vertebrae number ($r_p = 0.95$, $P < 0.001$), individual caudal vertebrae length ($r_p = 0.78$, $P < 0.001$) and type 1 caudal vertebrae ($r_p = 0.73$, $P < 0.001$). Among the osteological tail traits, strong correlations ($r_p = 0.73$ to 0.96, $P < 0.001$) were observed among total caudal vertebrae length, caudal vertebrae number, individual caudal vertebrae length, and type 1 caudal vertebrae (Table 7).

Table 7

Correlation among pairs of morphological and osteological tail traits in the studied sheep.

Traits	Tail length	Tail breadth	Tail circumference	Caudal vertebrae number	Individual caudal vertebra length	Total caudal vertebrae length	Caudal vertebrae breadth	Caudal vertebrae thickness
Tail breadth	*-0.38*							
Tail circumference	**-0.57**	**0.88**						
Caudal vertebrae number	**0.95**	**-0.44**	**-0.62**					
Individual caudal vertebra length	**0.78**	**-0.46**	**-0.61**	**0.73**				
Total caudal vertebrae length	**0.96**	**-0.45**	**-0.63**	**0.96**	**0.89**			

Caudal vertebrae breadth	**0.46**	-0.15	-0.24	**0.49**	**0.69**	**0.61**		
Caudal vertebrae thickness	*0.38*	*-0.32*	*-0.34*	*0.36*	**0.68**	**0.47**	**0.63**	
Flat-shaped caudal vertebrae	**0.50**	**-0.42**	**-0.43**	**0.56**	**0.41**	**0.51**	0.21	*0.40*
Concave-shaped caudal vertebrae	**0.51**	*-0.37*	**-0.46**	**0.55**	**0.64**	**0.62**	**0.63**	**0.47**
Convex-shaped caudal vertebrae	0.12	-0.07	0.12	0.05	0.16	0.09	0.07	0.22
Symmetric caudal vertebrae	*0.34*	0.05	0.07	*0.36*	0.01	0.24	-0.03	-0.15
Asymmetric caudal vertebrae	*0.38*	**-0.52**	**-0.59**	*0.39*	**0.62**	**0.49**	**0.45**	**0.67**

Type 1 caudal vertebrae	**0.73**	**-0.64**	**-0.66**	**0.74**	**0.79**	**0.79**	**0.55**	**0.65**
Type 2 caudal vertebrae	*0.35*	-0.13	-0.29	*0.38*	*0.32*	*0.35*	0.19	**0.40**
Type 3 caudal vertebrae	**0.54**	**-0.46**	**-0.55**	**0.55**	*0.40*	**0.51**	-0.08	0.10
Type 4 caudal vertebrae	**0.61**	-0.03	-0.21	**0.62**	*0.38*	**0.65**	*0.36*	-0.12

Traits	Flat-shaped caudal vertebrae	Concave-shaped caudal vertebrae	Convex-shaped caudal vertebrae	Symmetric caudal vertebrae	Asymmetric caudal vertebrae	Type 1 caudal vertebrae	Type 2 caudal vertebrae	Type 3 caudal vertebrae
Concave-shaped caudal vertebrae	0.10							
Convex-shaped caudal vertebrae	**-0.49**	0.03						
Symmetric caudal vertebrae	0.23	-0.02	-0.21					

Asymmetric caudal vertebrae	*0.33*	**0.50**	*0.32*	**-0.62**				
Type 1 caudal vertebrae	**0.60**	**0.62**	0.09	0.14	**0.61**			
Type 2 caudal vertebrae	0.19	0.19	**0.41**	-0.17	**0.53**	0.12		
Type 3 caudal vertebrae	*0.32*	0.12	0.06	0.17	0.18	0.27	*0.37*	
Type 4 caudal vertebrae	0.29	*0.32*	-0.19	**0.52**	-0.12	*0.33*	*-0.00*	0.22

Correlation which is significant at the 0.001 level is indicated by bold type and underlining; Correlation which is significant at the 0.01 level is indicated by bold type; Correlation which is significant at the 0.05 level is indicated by italics. $r_p > 0.70$ *was defined as strong correlation (Dormann et al. 2013).*

For regression model development, we considered three osteological (total caudal vertebrae length, caudal vertebrae number, and individual caudal vertebra length) and one external tail measurement (tail length) characters that significantly and positively correlate (Table 7). Based on this, regression models that allow estimating total caudal vertebrae length and caudal vertebrae number of a sheep from its tail length were developed (Fig. 11A and 11B, respectively). We also developed regression models that allow estimating total caudal vertebrae length and tail length from individual caudal vertebra lengths of the sheep (Fig. 12A and 12B, respectively).

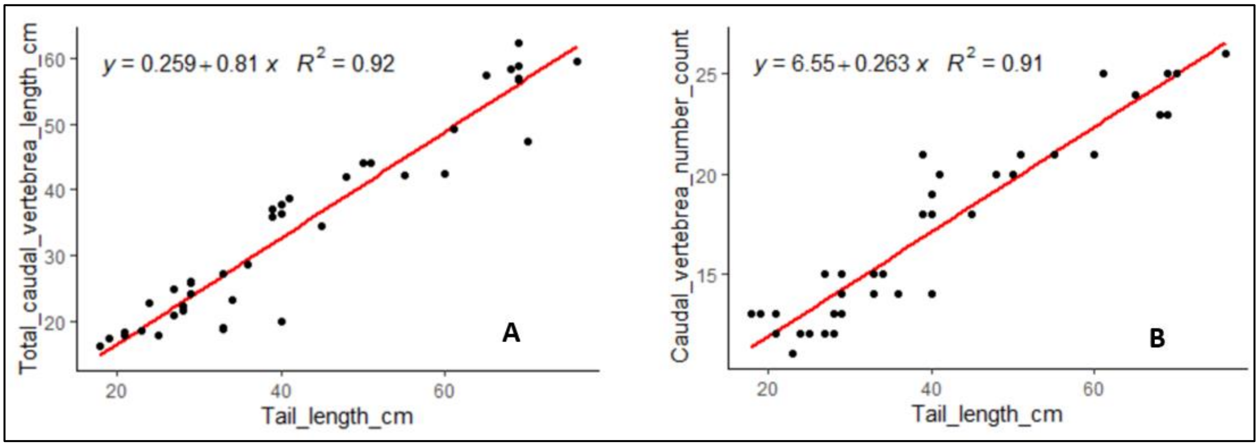

Fig. 11. Linear regression models, (A) estimation of total caudal vertebrae length using external tail measurement, tail length, (B) estimation of total caudal vertebrae number using tail length.

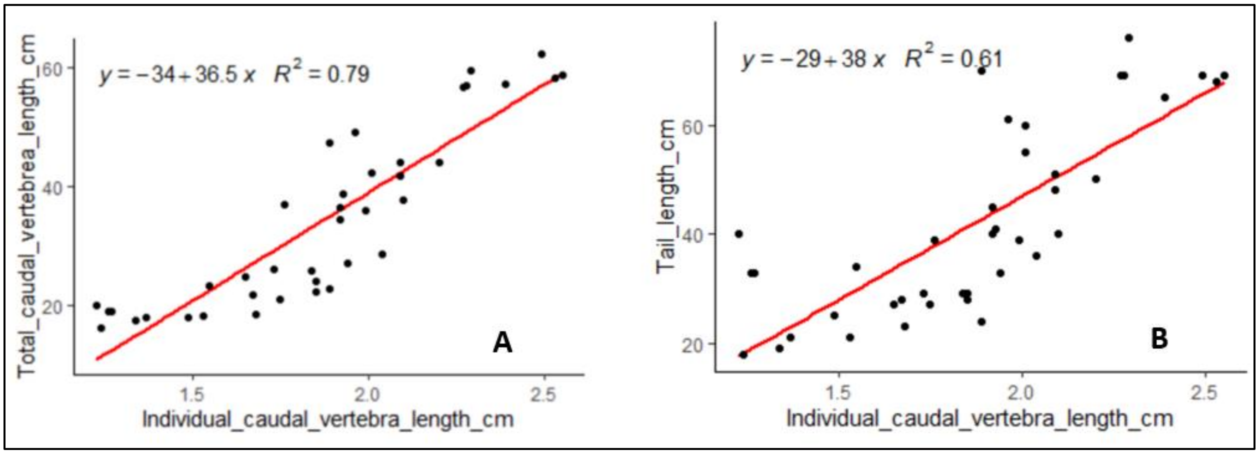

Fig. 12. Linear regression models, (A) estimation of total caudal vertebrae length using individual vertebra length, (B) estimation of tail length using individual caudal vertebra length.

3.3. *Linear discriminant analysis*

The scatterplot (linear discriminant analysis plot) for sheep tail morphotypes based on morphological and osteological tail traits (Fig. 13A) indicates that the first two linear discriminant functions account for 86% and 12% of the total variation, respectively, and clearly differentiates the sheep tail morphotypes according to tail morphology and osteology. The first linear discriminant function separates the fat-rumped and short fat-tailed sheep from long fat-tailed and thin-tailed sheep. The second linear discriminant function separates the fat-rumped and most of the samples of thin-tailed sheep from the short fat-tailed and long fat-tailed sheep. The finding was well supported by a separate scatter plot performed for sheep tail morphotypes based on osteological tail traits only (osteological traits linear discriminant analysis plot: Fig. 13B). Similarly, the first linear discriminant function separates the fat-rumped and short fat-tailed sheep from long fat-tailed and thin-tailed sheep, whereas the second linear discriminant function separates the fat-rumped and most of the samples of thin-tailed sheep from the short fat-tailed and long fat-tailed sheep. Furthermore, the linear discriminant analysis plot result obtained for sheep tail morphotypes was supported by a stacked histogram of the values of a linear discriminant function for the samples from different sheep tail morphotypes (Supplementary Fig. 1A1, A2).

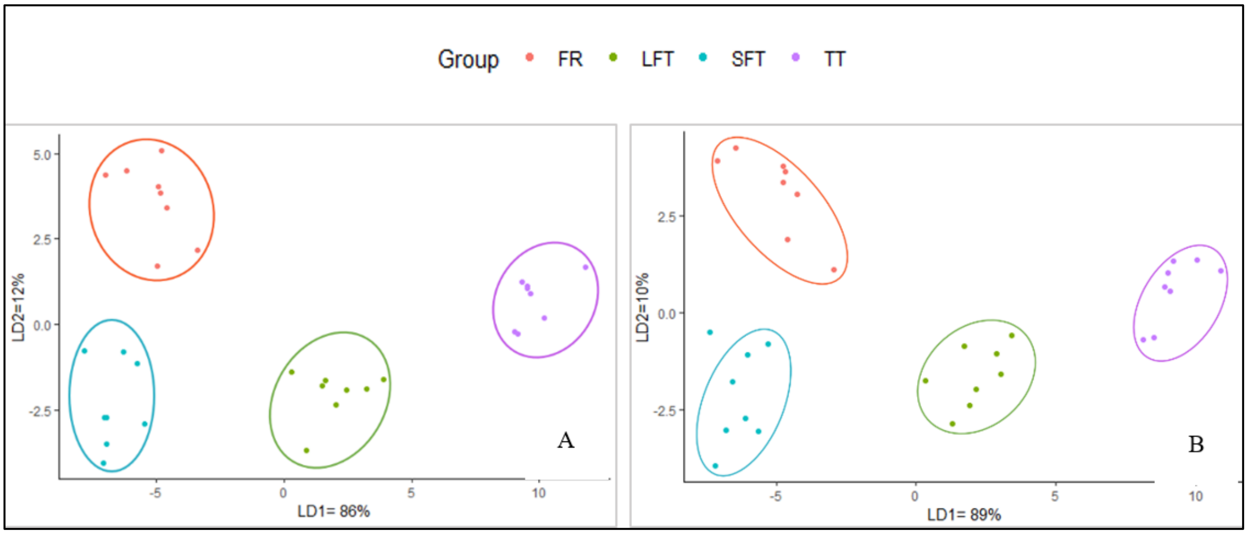

Fig. 13. Linear discriminant analysis of Ethiopian sheep tail morphotypes using the linear discriminant analysis plot based on: (A) morphological and osteological tail traits and (B) osteological tail traits only. FR: fat-rump; LFT: long-fat tail; SFT: short fat-tail; TT: thin-tail.

Linear discriminant analysis performed for sheep breeds based on both morphological and osteological tail traits (Fig. 14A) indicates that the first two linear discriminant functions account for 72% and 22% of the total variation, respectively, and categorizes the six sheep breeds into four sheep tail morphotypes according to their tail morphology and osteology: the two fat-rumped sheep breeds (Afar and Blackhead Somali) form one cluster tail morphotype (fat-rump tail morphotype). Similarly, the two short fat-tailed sheep breeds (Washera and Menz) form another cluster tail morphotype (short fat-tail tail morphotype). The first linear discriminant function separates the fat-rumped and short fat-tailed sheep breeds from long fat-tailed (Bonga) and thin-tailed (Gumz) sheep breeds, whereas the second linear discriminant function separates the fat-rumped and thin-tailed sheep breeds from the short fat-tailed and long fat-tailed sheep breeds. The finding was well supported by a separate linear discriminant analysis plot performed for sheep breeds based on osteological tail traits only, which indicates that the first two linear discriminant functions account for 77% and 20% of the total variation, respectively (osteological traits linear discriminant analysis plot: Fig. 14B). The first linear discriminant function clearly separates the fat-rumped and short fat-tailed breeds from long fat-tailed (Bonga) and thin-tailed (Gumz) breeds, whereas the second linear discriminant function separates the fat-rumped and thin-tailed breeds from the short fat-tailed and long fat-tailed sheep breeds. A separate linear discriminant analysis plot result obtained for sheep breeds was further supported by a stacked histogram of the linear discriminant function values for the samples from different sheep breeds (Supplementary Fig. 2B1, B2).

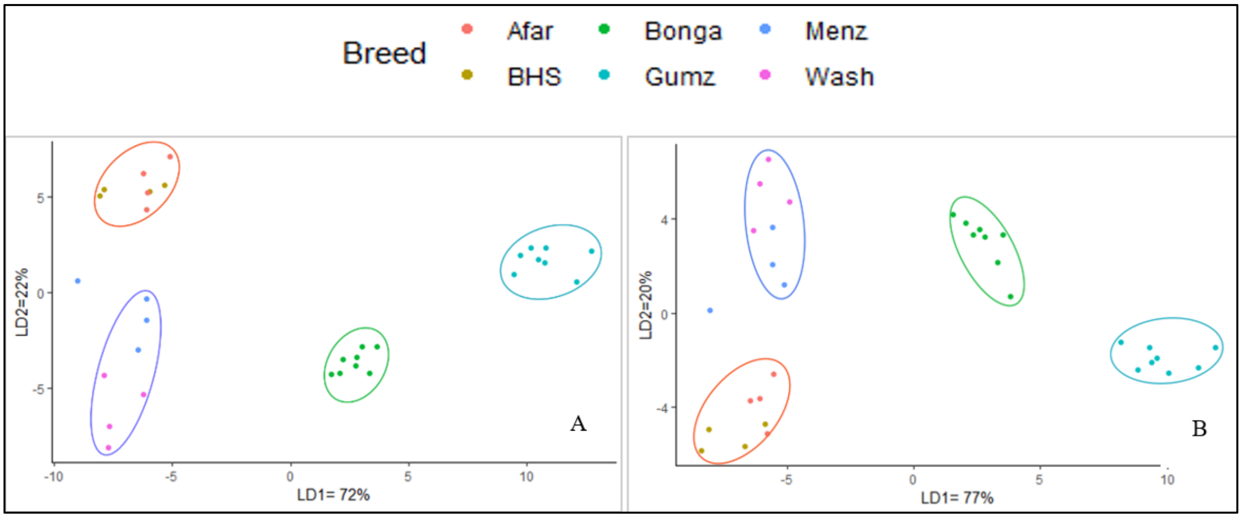

Fig. 14. Linear discriminant analysis of Ethiopian sheep breeds using the linear discriminant analysis plot based on (A) morphological and osteological tail traits and (B) osteological tail traits only. BHS: Blackhead Somali; Wash: Washera.

The linear discriminant analysis results for sheep tail morphotypes and breeds were further supported by the eigenvalue and percentage of separation (proportion of trace) achieved by the first and the second linear discriminant functions and the canonical correlation analysis result (Table 8). The eigen value and percentage of separation result indicated that the first two linear discriminant functions accounted for 98% (first linear discriminant function = 86%, second linear discriminant function = 12%) of the total variation in differentiating the four sheep tail morphotypes, as well as 94% (first linear discriminant function = 782%, second linear discriminant function = 22%) in differentiating the six sheep breeds (Table 8). The canonical correlation analysis result indicates the presence of high values of canonical correlation between each linear discriminant function and sheep tail morphotypes (first linear discriminant function = 99%, second linear discriminant function = 93%), as well as between each linear discriminant function and the six sheep breeds (first linear discriminant function = 99%, second linear discriminant function = 98%), as indicated in Table 8.

Table 8

Eigen values, proportion of variability (%) and canonical correlation (%) explained by the first two discriminant functions.

Discriminant function	Eigen values		Proportion of trace		Canonical correlation	
	Group	Breed	Group	Breed	Group	Breed
First discriminant function	48.65	66.01	86	72	99	99
Second discriminant function	6.66	20.40	12	22	93	98

3.4. Principal component analysis

The principal component analysis based on morphological and osteological tail traits (Fig. 15) indicated that the first two principal components accounted for 62.7% (first principal component = 46.5%, second principal component = 16.2%) of the total variation and clearly differentiated the four sheep tail morphotypes. The first principal component separates the two tail morphotypes very well: the short-tailed (short fat-tail and fat-rump) from the long-tailed (long fat-tail and thin-tail) sheep tail morphotypes. The second principal component separates fat-rump and most of the thin-tail tail morphotype samples from short fat-tail and some long fat-tail tail morphotype samples. The result obtained (all traits principal component analysis) was backed up by a separate principal component analysis performed for sheep tail morphotypes based on osteological tail traits only (Osteological principal component analysis: Fig. 15B). Similarly, the first principal component separates the two tail morphotypes very well: the short-tailed from the long-tailed sheep. The second principal component separates fat-rump and most of the thin-tail tail morphotype samples from short fat-tail and some long fat-tail tail morphotype samples.

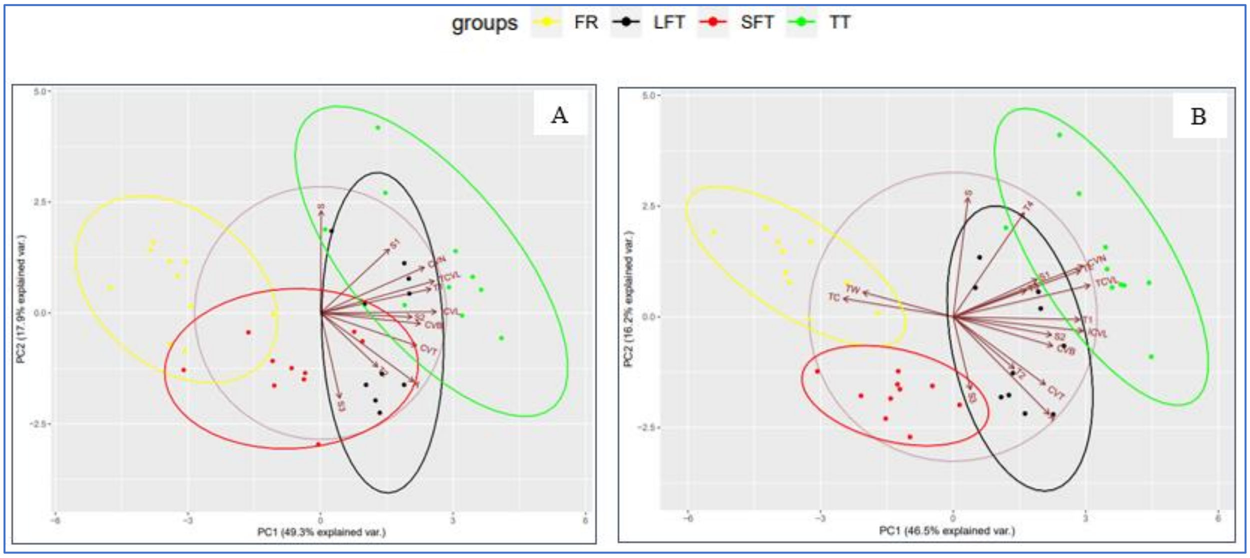

Fig. 15. Principal component analysis of Ethiopian sheep tail morphotypes based on: (A) morphological and osteological tail traits and (B) osteological tail traits only. SFT = short fat-tail; FR = fat-rump; LFT = long fat-tail; TT =thin-tail.

A separate principal component analysis based on morphological and osteological tail traits (Fig. 16A) indicated that the first two principal components accounted for 62.7% (first principal component = 47.9%, second principal component = 17.05%) of the total variation and categorizes the six sheep breeds into four tail morphotypes. The result revealed that close clustering of the two fat-rumped sheep breeds (Afar and Blackhead Somali) forms one cluster tail morphotype (fat-rump tail morphotype). Similarly, the result indicated that close clustering of the two short fat-tailed sheep breeds (Washera and Menz) forms another cluster tail morphotype (short fat-tail tail morphotype). The first principal component clearly separates the fat-rumped and short fat-tailed sheep breeds from long fat-tailed (Bonga) and thin-tailed (Gumz) sheep breeds, whereas the second principal component separates the fat-rumped and most of the thin-tailed sheep breed samples from short fat-tailed and some of the long fat-tailed sheep breed samples. The result obtained (all traits principal component analysis) was supported by a separate principal component analysis for sheep breeds based on osteological tail traits only (osteological traits principal component analysis: Fig. 16B). Similarly, the first principal component clearly separates the fat-rumped and short fat-tailed sheep breeds from long fat-tailed and thin-tailed sheep breeds, whereas the second principal component separates the fat-rumped and most of the thin-tailed sheep breed samples from short fat-tailed and some of the long fat-tailed sheep breed samples.

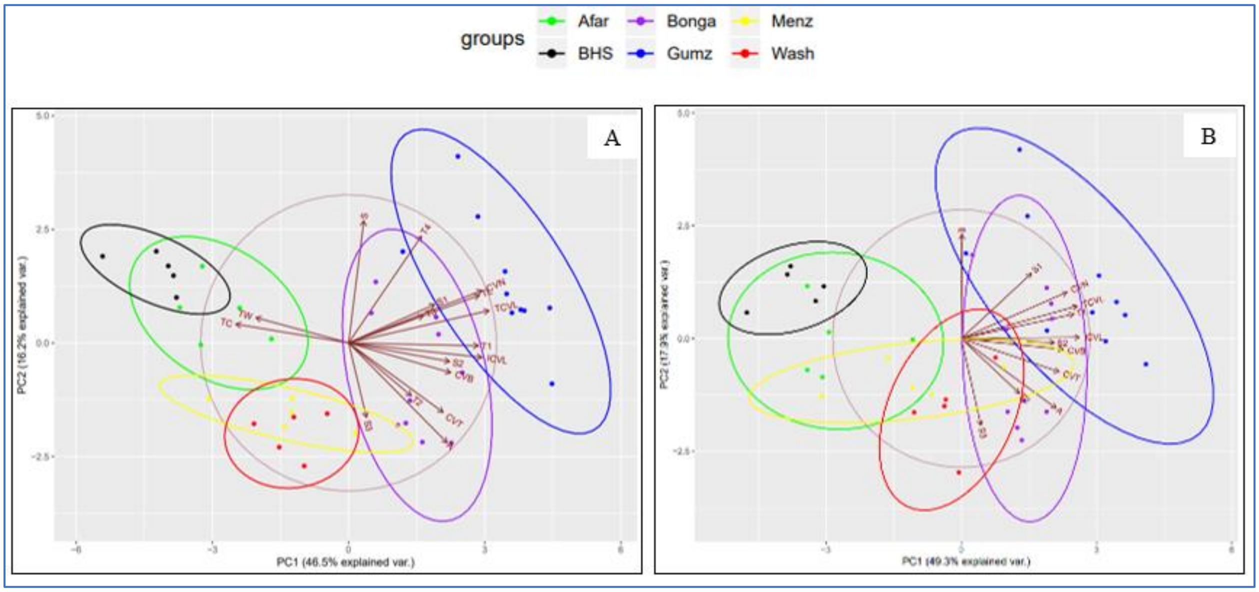

Fig. 16. Principal component analyses of Ethiopian sheep breeds are based on: (A) morphological and osteological tail traits and (B) osteological traits only. BHS: Blackhead Somali; Wash: Washera.

4. Discussion

In this study, we used morphological and osteological tail data to characterize and differentiate the tail morphotypes of Ethiopian sheep. Our objectives were to document in detail the characteristics of the different sheep tail morphotypes found in Ethiopia from morphological and osteological perspectives: to provide baseline osteological information on sheep caudal vertebra morphologies as reference material for zooarchaeological studies of sheep farming; to provide accurate phenotypic descriptions for genome mapping studies aiming to elucidate the genetic control of sheep tail morphologies.

Significant ($P < 0.05$) differences were observed in tail length (external tail character), total caudal vertebrae length and number of caudal vertebrae (osteological tail characters) for the four sheep tail morphotypes with the highest average values in the thin-tailed sheep followed by the long fat-tailed sheep, and the lowest average values in the short-tailed sheep (short fat-tail, fat-rump). This supports the separation of Ethiopian sheep into three broad sheep tail morphotypes (short-tailed, medium-tailed, and long-tailed sheep).

We also observed significant ($P < 0.05$) differences in tail breadth and tail circumference, the external tail characters, between the short-tailed (short fat-tail, fat-rump) and the long-tailed (long fat-tail, thin-tail) sheep with the highest and the lowest average values in the short-tailed and long-tailed sheep, respectively. This supports categorizing the studied sheep into two broad sheep tail morphotypes (short-tailed and long-tailed). Short-tailed sheep, including the fat-rumped sheep, are characterized by a short or large fat-tail and/or a massive fat-rump, whereas long-tailed sheep are described as sheep with a medium to long fat-tail and/or a much less massive thin-tail (Fig. 10). Thus, the two external tail morphology characters, tail width and tail circumference could be used as a measure of tail weight in breeding programs as they provide enough information on the shape and size of the sheep tail (Vatankah and Talebi, 2008). However, the amount of fat in the sheep tail varies between breeds and according to the time of the year of sampling, which might be related to the food availability (Zamiri and Izadifard, 1997; Zhang et al., 2015; Gootwine, 2018). This variation could also be due to variations in the genetic basis of tail shape, size, fat allocation, fat deposit, and fat development in the tail of sheep (Kang et al., 2017; Ahbara et al., 2019). Zeng et al. (2020) reported that sheep nutrition, which depends on seasonal food availability, is linked to levels of tail-fat deposition in the tail and, thus, to the expression of tail-related (lipolytic and lipogenic) genes. The six sheep breeds were not killed at the same time of the same year, which may have influenced the results presented here (Table 1) as the sampling season is associated with variable food availability in terms of quality and quantity (Korecha and Sorteberg, 2013). Fat-tailed sheep breeds, including fat-rumped sheep, are widely recognized as more tolerant to severe and prolonged undernutrition owing to the supplementary tail or rump fat deposit that serves as a steady but slow-releasing source of fatty acids for the metabolism (Atti et al., 2004). At an osteological level, we also observe significant ($P < 0.05$) differences in caudal vertebra breadth between the short-tailed and long-tailed sheep, with the highest mean value in the long-tailed sheep, allowing separation of the two tail morphotypes (short-tail and long-tail).

Moreover, the significant ($P < 0.05$) differences observed in individual caudal vertebra length and individual caudal vertebra thickness between the fat-rump and the other three sheep tail morphotypes, as well as between the short fat-tail and the other three sheep tail morphotypes, indicate that individual caudal vertebra measurements may provide a departure point for distinguishing the fat-rumped sheep from the three other sheep morphotypes, as well as the short fat-tailed sheep from the three other sheep morphotypes (Table 5). It should be noted that variation in individual caudal vertebra length between and within sheep tail morphotypes may be associated with differences in the fusion status of the annular epiphysis of each caudal vertebra (Fig. 4). Sheep with caudal vertebrae with unfused cranial and/or caudal epiphyses have lower individual caudal vertebra length than sheep with fused epiphyses. Moreover, the lowest mean value of individual caudal vertebra thickness observed in the fat-rumped sheep compared to the three other sheep tail morphotypes may suggest the possible influence of fat location in sheep tails on the thickness of each caudal vertebra. This is due to the deposition of the fat reserve in the fat-tailed sheep in the tail, whereas the fat in the fat-rumped sheep is located in the rump (Ermias et al., 2002).

Interestingly, our detailed osteological examination of caudal vertebrae reveals the presence of different numbers of asymmetric and symmetric caudal vertebrae for the four sheep tail morphotypes. In particular, the mean number of asymmetric caudal vertebrae clearly separates the fat-rumped sheep from the three other sheep tail morphotypes. Similarly, the mean number of symmetric caudal vertebrae separates the short fat-tailed sheep from the three other sheep tail morphotypes. It may indicate that fat accumulation in fat-tailed or fat-rumped sheep during their lifespan may interfere with the development of the caudal vertebra, providing a possible osteological marker for sheep tail morphotypes.

The correlation analysis indicated that the osteological tail (individual caudal vertebra length, total caudal vertebrae length, and number of caudal vertebrae) and external measurement (tail length) characters were significantly ($P < 0.05$) and positively correlated with each other. This result allows for the development of regression models for these traits (Fig. 11A, B and Fig. 12A, B). The higher association of total caudal vertebrae length and caudal vertebrae number with tail length over the other osteological measurement, individual caudal vertebra length (Table 7), indicates that tail length alone can estimate both osteological tail traits (Fig. 11A, B) without killing the animal, simply by measuring tail length on live animals. Moreover, based on measurements of sheep caudal vertebrae, the osteologist can estimate other osteological traits and the tail length of that sheep (Fig. 12A, B), which further differentiates its tail morphotypes.

The linear discriminant analysis indicated that the first two linear discriminant functions accounted for 72-86% and 12-22% of the total variation in differentiating the four sheep tail morphotypes as well as the six sheep breeds, respectively (Figs. 13,14), which further indicates that the first linear discriminant function achieves a good separation of the four sheep tail morphotypes as well as the six sheep breeds, but the second linear discriminant function only slightly improves the separation. Therefore, to achieve a better separation of the sheep tail morphotypes and breeds, it is necessary to use both the first and second discriminant functions to differentiate the four sheep tail morphotypes, as well as the six sheep breeds, as they accounted for 98% and 94% of the total variation in the dataset, respectively (Figs. 13, 14). Moreover, the observed high canonical correlation between the first two linear discriminant functions and sheep tail morphotypes (first linear discriminant function = 99%, second linear discriminant function = 93%) and sheep breeds (first linear discriminant function = 99%, second linear discriminant function = 98%) indicates that the two linear discriminant functions are more effective in discriminating the studied sheep according to tail morphology and osteology, as their canonical correlation is close to 1 (Table 8). The principal component analysis showed that most of the variation between samples in the whole dataset could be captured using the first two principal components, as they accounted for over 62% of the total variation (Figs. 15, 16).

Several studies have investigated the genetic control of tail morphology in sheep. For example, in agreement with Economides et al. (2003), who reported that mutations in HOXB13 result in overgrowth of the caudal spinal cord and tail vertebrae number in mice, several studies have now similarly identified candidate positive signature signals in genome regions in sheep overlapping with members of the HOX gene family (Fariello et al., 2014; Kang et al., 2017; Ahbara et al., 2019). However, until now, no study has been able to provide a direct link between vertebra measurement, numbers and/or types with specific genome haplotype(s). We collected blood samples of all the sheep studied here, which may allow us to address these issues in the future.

The morphological and osteometric differences highlighted here on caudal vertebrae according to tail morphotypes can provide informative elements for archaeozoological research on the development and diffusion of sheep breeds. The remains of complete sheep tails are scarce and are only found in specific archaeological contexts where the animal was deposited in its entirety, in pits or tombs, as is the case at the prehistoric site of Kerma (dated from 2400 to 1400 BC) in Sudan (Chaix and Grant, 1987; Chaix and Callou 2011). Counting the tail vertebrae of 30 sheep deposited in graves at Kerma shows that these were short-tailed sheep, with an average of 15 caudal vertebrae. This information complements other osteological data obtained at Kerma on other skeletal parts and suggests that the phenotypes of second-millennium sheep in Sudan were similar to those of Egyptian sheep depicted in funerary and religious contexts in the third millennium BC (Chaix and Grant, 1987; Boessneck et al., 1989). Applying our analysis method to such archaeological finds could allow for more precise identification of the tail type, fat or thin, and a better understanding of the routes of introducing the different sheep breeds in Africa. Morphometric analysis of individual vertebrae and the identification of tail type could also be applied to sets of caudal vertebrae from the same individual, even if the tail is incomplete.

5. Conclusions

The significant ($P < 0.05$) differences observed in tail measurements, osteological measurements, and caudal vertebra characteristics among the four sheep tail morphotypes indicate differences in tail length, tail shape, tail size, and morphometry of each caudal vertebra of the studied sheep. Osteological tail traits (total caudal vertebrae length and caudal vertebrae number) and tail measurements (tail length) clearly allow for the separation of the studied Ethiopian sheep into three main tail morphotypes (short-tail, medium-tail, long-tail). We also observe that osteological measurements (individual caudal vertebra length and breadth), caudal vertebra morphological characteristics (type 2, flat and concave-shaped caudal vertebrae) and tail measurements (tail breadth and tail circumference) clearly separate the short-tailed sheep from the long-tailed ones. Moreover, caudal vertebra characteristics (particularly their symmetry or asymmetry) and osteological measurements (individual caudal vertebra length and thickness) of the fat-rumped and the short fat-tailed sheep are clearly distinct from the other tail morphotypes. Based on the combination of the measured, observed, and recorded osteological and morphological tail traits, Ethiopian sheep could be categorized into three major tail morphotypes: Short-tailed sheep with a large fat-tail or a massive fat-rump, medium-tailed sheep with a small fat-tail, and long-tailed sheep with a thin fat-tail. The linear discriminant and principal component analyses revealed four distinct sheep tail morphotypes, categorizing the six breeds of sheep examined according to tail morphology and osteology. Moreover, the linear discriminant and canonical correlation analyses revealed that the first two discriminant functions are more efficient in discriminating between the four sheep tail morphotypes and the sheep breeds. It is possible to use the external tail measurement, tail length, alone to estimate the sheep tail osteology without killing the animal (Fig. 11A, B). Moreover, by measuring sheep caudal vertebrae, osteologists can estimate other osteological traits and the tail length of that sheep (Fig. 12A, B), which further enables them to differentiate its tail morphotype. The previous classification of Ethiopian sheep, based on external visual examination of their tails (tail length and type) (Gizaw, 2009), should be supported by detailed osteological and morphological analyses of their tail, which might provide accurate information about tail morphology, as well as a precise and detailed osteological information about sheep caudal vertebrae. This study combined, for the first time, systematic external tail morphology with a detailed osteological analysis of sheep tails. The results are of great interest for archaeozoological studies and provide a baseline for investigating the evolution of sheep tails since domestication and an enhanced understanding of the history of introducing sheep breeds on the African continent. The results are also of great interest for genome mapping studies which aim to elucidate the genetic control of sheep tail morphotypes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge the following institutions and personnel for facilitating and funding the research. The staff of Debre Berhan Agricultural Research Center, the Bahir Dar Regional Animal Health Laboratory, Bonga Agricultural Research Center, and the Guba & Almeha office of Agriculture for their kind support in sheep skeleton or reference bone sample preparation in their respective institutions and farmers` training centres (FTC). The French Centre for Ethiopian Studies (CFEE) and the Authority for Research and Conservation of Cultural Heritage (ARCCH) for their warm welcome and for facilitating skeleton preparation in Addis Ababa. Mr Michael Temesgen and Anteneh Hailemariam for their kind support and cooperation in providing driving services and their involvement in reference bone sample preparation. The International Livestock Research Institutes, livestock genomics program, supported by the CGIAR Research Program on Livestock (CRP livestock project) sponsored by the CGIAR funding contributors to the Trust Fund (http://www.cgiar.org/about-us/our-funders/), partly by Bill and Melinda get Foundation and UK aid from UK Foreign, Commonwealth and Development Office (Grant Agreement OPP1127286) under the auspices of the Centre for Tropical Livestock Genetics and Health (CTLGH), established jointly by the University of Edinburgh, SR UC (Scotland's Rural College). This research was also conducted as part of the EvoSheep project on sheep breeds' origin and development, funded by the French National Research Agency (ANR-17-CE27-0004).

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