Analysis of tail morphology and osteology in Ethiopian indigenous sheep

Agraw Amane[1,2,3*], Gurja Belay[2], Adebabay Kebede[1], Tadelle Dessie[3], Setegn Worku[3], Olivier Hanotte[3,4,5*], Emmanuelle Vila[6*]

[1]Amhara Regional Agricultural Research Institute, Bahir Dar, Ethiopia

[2]Microbial, Cellular and Molecular Biology, Addis Ababa University, Addis Ababa, Ethiopia

[3]LiveGene, International Livestock Research Institute (ILRI), P.O. 5689, Addis Ababa, Ethiopia

[4]School of Life Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK

[5]Centre for Tropical Livestock Genetics and Health, The Roslin Institute, Edinburgh EH25 9RG, UK

[6]CNRS/Univ. Lyon 2, UMR 5133 Archéorient, Maison de l'Orient et de la Méditerranée, Lyon, France

*Corresponding author.

E-mail addresses: agraw29@yahoo.com (A. Amane), o.hanotte@cgiar.org (O. Hanotte), emmanuelle.vila@mom.fr (E. Vila).

Orcid ID: https://orcid.org/0000-0001-7693-0173

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 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 groups. 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, which further differentiates its tail morphotypes. Significant differences ($P < 0.05$) were also observed in individual caudal vertebra length and breadth, tail width and tail circumference, type 2, 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, as well as an osteological baseline for archaeozoological work aiming to understand the history of sheep husbandry and breed development in past societies.

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

1. Introduction

Sheep were 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 allowing us to distinguish sheep breeds, tail type and length are one of 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, extreme 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 periods of drought and famine (Moradi et al., 2012). Sheep breeds from across the world are classified today on the basis of their tail phenotypes as thin-tailed, short fat-tailed, long fat-tailed and fat-rumped sheep breeds (Epstein, 1971; Gizaw et al., 2007; Muigai and Hanotte, 2013; Ahbara et al., 2019; Whannou et al., 2021).

The founding populations of current African sheep breeds were thought to have been introduced from Asia in three waves of migration of corresponding to the modern thin-tailed, fat-tailed and fat-rumped sheep (Epstein, 1971), 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 (Epstein, 1954; Epstein, 1971; Ryder, 1984). Indigenous African sheep genetic resources are geographically distributed largely across three non-overlapping geographic ranges. Thin-tailed sheep are currently found in West Africa and East Africa up to Sudan (IEMVT-FRA, 1950), with distributions are bordering Ethiopia. Fat-tailed sheep are found in North, East and Southern Africa and fat-rumped sheep are found across the Horn of African (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 iconography, 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; 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 at the Horn of the African continent (Devendra and McLeroy 1982). The country comprises one of the most diversified sheep populations in Africa (Devendra and McLeroy 1982). Ethiopian indigenous sheep are adapted to diverse agro-ecological 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 reported between 3 to more then 24 caudal vertebrae according to breeds (Cornevin and Lesbre, 1897). 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) among others) 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 term of meat production. On the contrary, the tail region is only of interest for 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 and adaptation studies, as well as baseline osteological information for archaeological studies and sheep breed origins. Moreover, estimation of the relationship between tail osteological and morphological traits in sheep may help to provide a means for predicting traits which are not normally and easily measured from the live sheep, e.g., osteological traits are recorded from slaughtered sheep but 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 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, as well as 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 (TT: Gumz, n = 10), long fat-tail (LFT: Bonga, n = 10), short fat-tail (SFT: Menz and Washera, n = 10) and fat-rump (FR: Afar and Blackhead Somali, n = 10). The sheep breeds, adapted to diverse agro-ecological 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	Group	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.

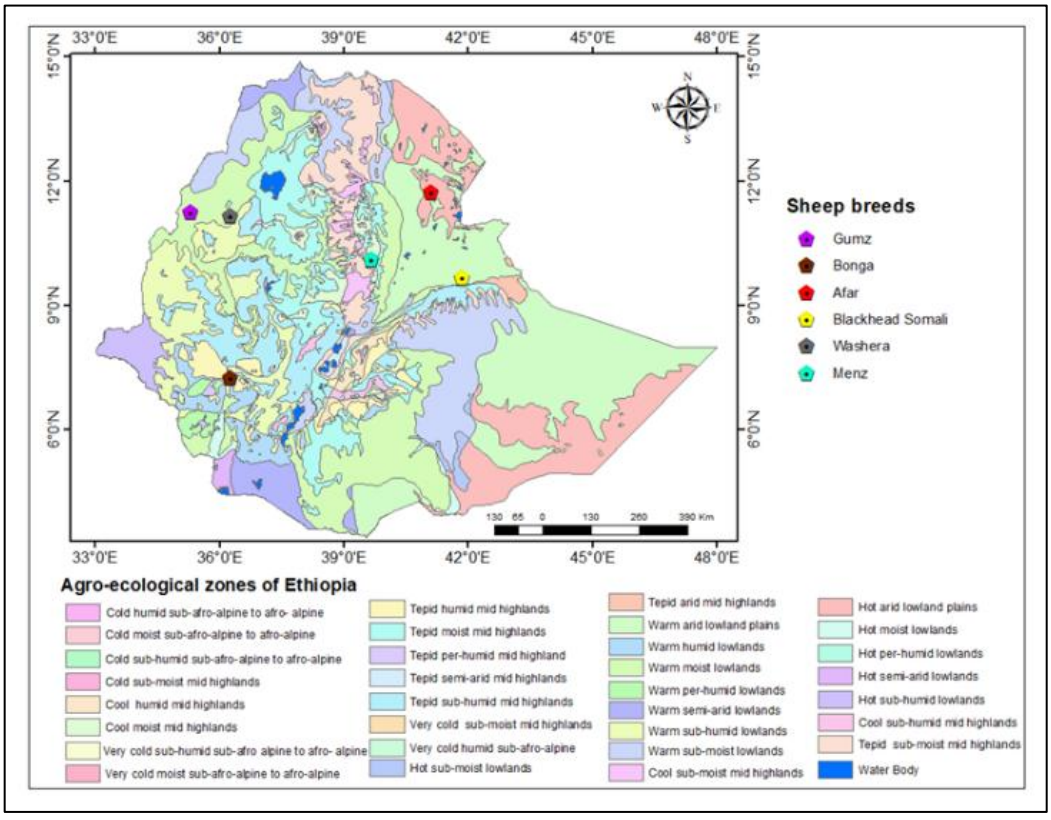


Fig. 1. Geographical location of the studied sheep breeds in relation to agro-ecological zones of Ethiopia.



Fig. 2. Iconographic examples of the six sheep breeds examined with their skin peeled out tail, (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 represents the long fat-tail morphotype and D sheep represents 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 the tail of a sheep. See Fig. 4 for a description of a 'typical vertebra', as relevant to this study.



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

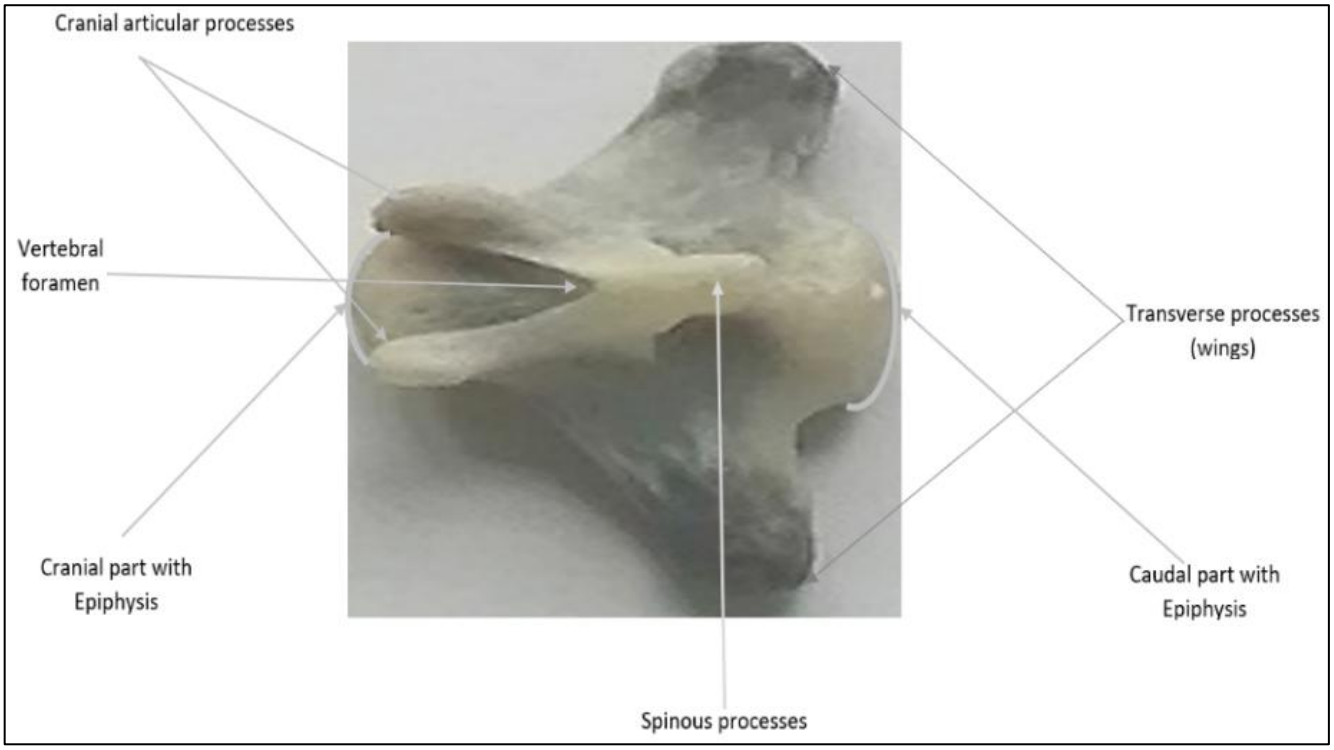


Fig. 4. Anatomical parts of a caudal vertebra.

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 own protocol to qualitatively describe each caudal vertebra by setting various criteria (Table 3).

Measurements for quantitative traits were taken from 40 live sheep and a total of 705 caudal vertebrae. The quantitative traits in live sheep measured in centimetres using a flexible tape and measuring stick were tail length (TL), tail width/breadth (TW), and tail circumference (TC). The quantitative traits for each caudal vertebra were measured in millimetres using a calliper (Fig. 5A, B). They included the greatest length (GL) and greatest breadth (GB) and the smallest breadth (SBV) of each caudal vertebra. Detailed descriptions of quantitative morphological and osteological tail traits are presented in Table 2.

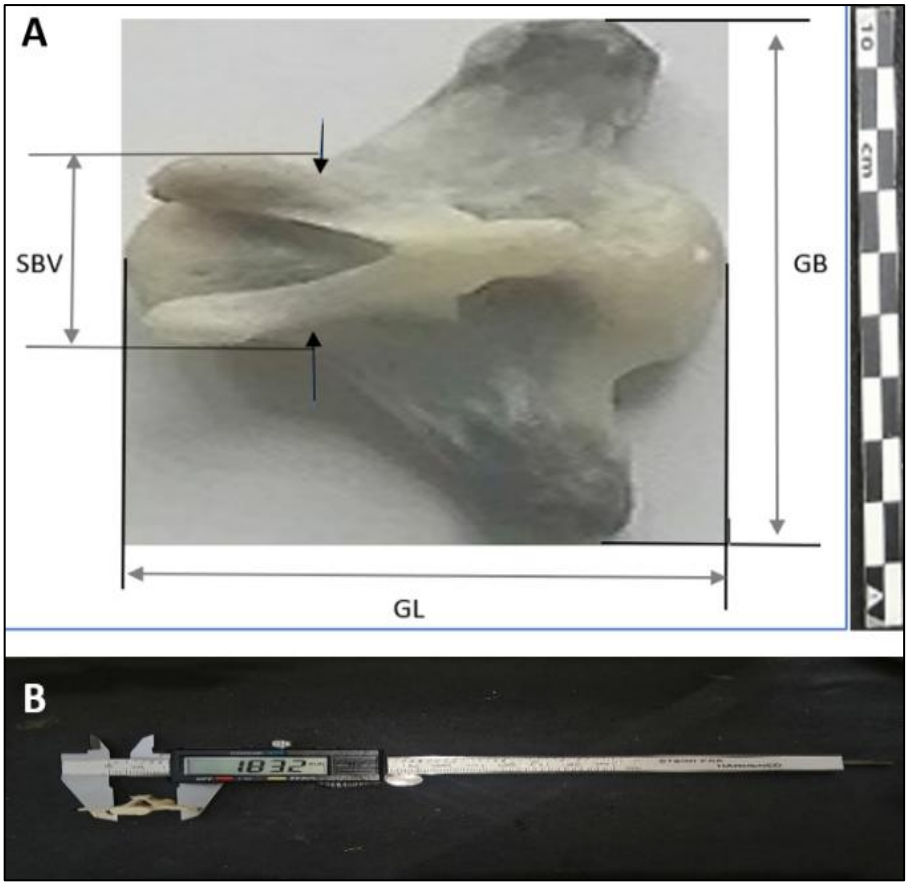


Fig. 5. Quantitative osteological tail traits, (A) measurements of each caudal vertebra (mm) and (B) calliper. GL = Greatest Length, GB = Greatest Breadth over the wings, SBV = Smallest Breadth of the vertebra.

Qualitative osteological traits observed and recorded for each caudal vertebra (including type, shape and symmetry categories) are presented in Fig. 6-8 and Table 3. Various qualitative characteristics of each caudal vertebra were recorded after careful observationfrom different angles (cranial *versus* caudal; top *versus* bottom view) by two observers (EV and AA). The greatest length and the smallest breadth measurements were taken from all caudal vertebra types (T1: Type 1, T2: Type 2, T3: Type 3 and T4: Type 4), but the greatest breadth measurements were only taken from the first three types (T1, T2, and T3), since transverse processes (wings) are absent on T4 types. Data on the shape and symmetry of the caudal vertebrae were only taken from type 1 and type 2 caudal vertebrae as two types have a well-developed transverse process. Detailed descriptions of qualitative osteological tail traits are presented in Table 3.

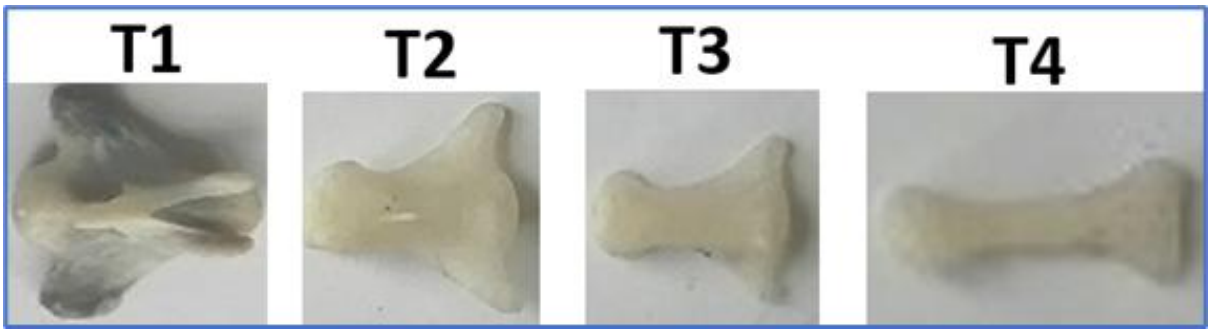


Fig. 6. Categories of caudal vertebra type. The abbreviations are defined in Table 3.

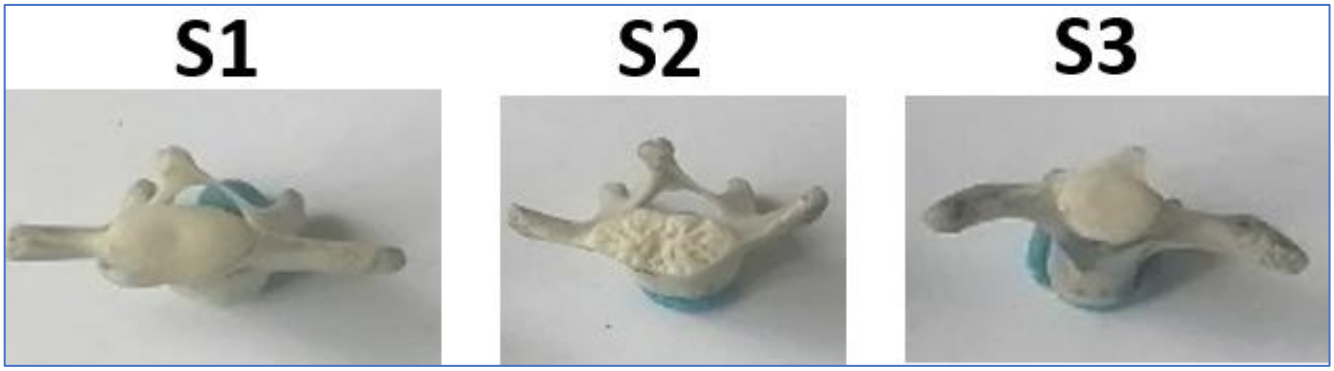


Fig. 7. Categories of caudal vertebra shape. The abbreviations are defined in Table 3.



Fig. 8. Categories of caudal vertebra symmetry. The abbreviations are defined in Table 3.

The age of the studied sheep was identified based on dentition (tooth eruption and dental wear) (Silver, 19 69 ; Schmid, 1972; Payne, 1973 ; Habermehl, 1975). According to age estimation and the fusion status of the cranial and caudal annular ring (epiphysis) on the body of caudal vertebrae, deux groups were made: subadults and adults. Subadult sheep are younger than two years old, with unfused cranial and/or caudal annular epiphyses on caudal vertebrae. Adult sheep are older than two years old, with fused cranial and/or caudal annular epiphyses to the body of caudal vertebrae.

241 **Table 2**

242 Descriptions of the quantitative morphological and osteological tail traits.

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

243

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 (T1)	Caudal vertebrae with a vertebral foramen, a well-developed spinous process and a well- developed cranial articular and transverse processes
Type 2 (T2)	Caudal vertebrae with no vertebral foramen, with vestigial spinous and cranial articular processes and moderately-developed transverse processes
Type 3 (T3)	Caudal vertebrae with no vertebral foramen and no spinous process, with vestigial cranial articular and transverse processes
Type 4 (T4)	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 (S1)	Caudal vertebrae with horizontal transverse processes (straight position)
Concave (S2)	Caudal vertebrae with dorsally angled transverse processes (upward position)
Convex (S3)	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 (S)	Caudal vertebrae with symmetrical transverse processes
Asymmetry (A)	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 analysis, principal component analysis and canonical correlation analysis) were performed using different packages and functions in the software R v4.0.1 (R Core Team, 2020)

2.4.1. Means of tail morphological and osteological traits

We checked the normality of the data and the homogeneity of variance using Q-Q plot and the 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 (SFT: short fat-tail, LFT: long fat-tail, FR: fat-rump, and TT: thin-tail). As the ANOVA test was significant, a Tukey multiple pairwise-comparisons test (TMPCT) 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 group (i = SFT, FR, LFT, TT)

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 6 and Fig. 11. Regression models were developed for significantly and positively correlated osteological and morphological tail characters (Table 6, Fig. 11). Moreover, as shown by the analysis of variance results, these characters clearly allow for separation of the studied Ethiopian sheep into three main tail morphotypes (short-tail, medium-tail, 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, from the external tail measurement, tail length, were developed (Fig. 12A 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. 13A and B, respectively).

2.2.3. Multivariate analyses

Multivariate statistical analysis (MVA) techniques such as linear discriminant analysis (LDA), principal component analysis (PCA), and canonical correlation analysis (CCA) were employed to investigate the morphological structure of the studied sheep tail groups or sheep breeds. The analyses were performed separately for sheep tail groups and breeds based on both morphological and osteological tail traits. A separate analysis was performed for sheep tail groups 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 (LDA) generates useful linear discriminant functions (LDs) for discriminating sheep tail groups or breeds. The canonical correlation (CC), in canonical correlation analysis, measures the correlation between each LD and sheep tail groups or breeds. The CC value obtained indicates the ability of the LD to separate sheep tail groups or breeds. The closer the CC value is to 1, the higther the discriminating ability. One way of displaying LDA results is to create a stacked histogram of the values of linear discriminant functions for the samples from different sheep tail groups or breeds. It is also important to investigate how each LD separates the studied sheep tail groups or breeds. Principal component analysis (PCA), another multivariate analysis technique, also generates useful principal components (PCs) for discriminating the sheep tail groups or breeds.

The percentage of separation (proportion of trace) achieved by each linear discriminant function (LD) was calculated from the loadings (value of each LD). The Eigen value, percentage of separation and canonical correlation of LDs performed for sheep tail groups or breeds tell us

how important the linear discriminant function is to discriminate the studied sheep tail groups 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 4. 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 (LFT) compared to the three other tail morphotypes, as well as for the thin-tail (TT) 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 4). Measurements for caudal vertebra breadth (CVB) were significantly different ($P < 0.05$) for long-tailed (LFT, TT) and short-tailed (SFT, FR) 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 width/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 width: 19.95 ± 2.22, tail circumference: 36.73 ± 7.75 cm) and the lowest values in the long-tailed sheep (tail width: 14.3 ± 2.17, tail circumference: 23.45 ± 6.49 cm). Individual caudal vertebra length is significantly ($P < 0.05$) different for the short fat-tail (SFT) compared to the three other tail morphologies, 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 (LFT, TT) (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 (CVT) were significantly different ($P < 0.05$) for the fat-rumpedd (FR) 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, morphological and osteological tail traits of the four sheep tail morphotypes are shown in Fig. 9A, B, and Fig. 10A, B.

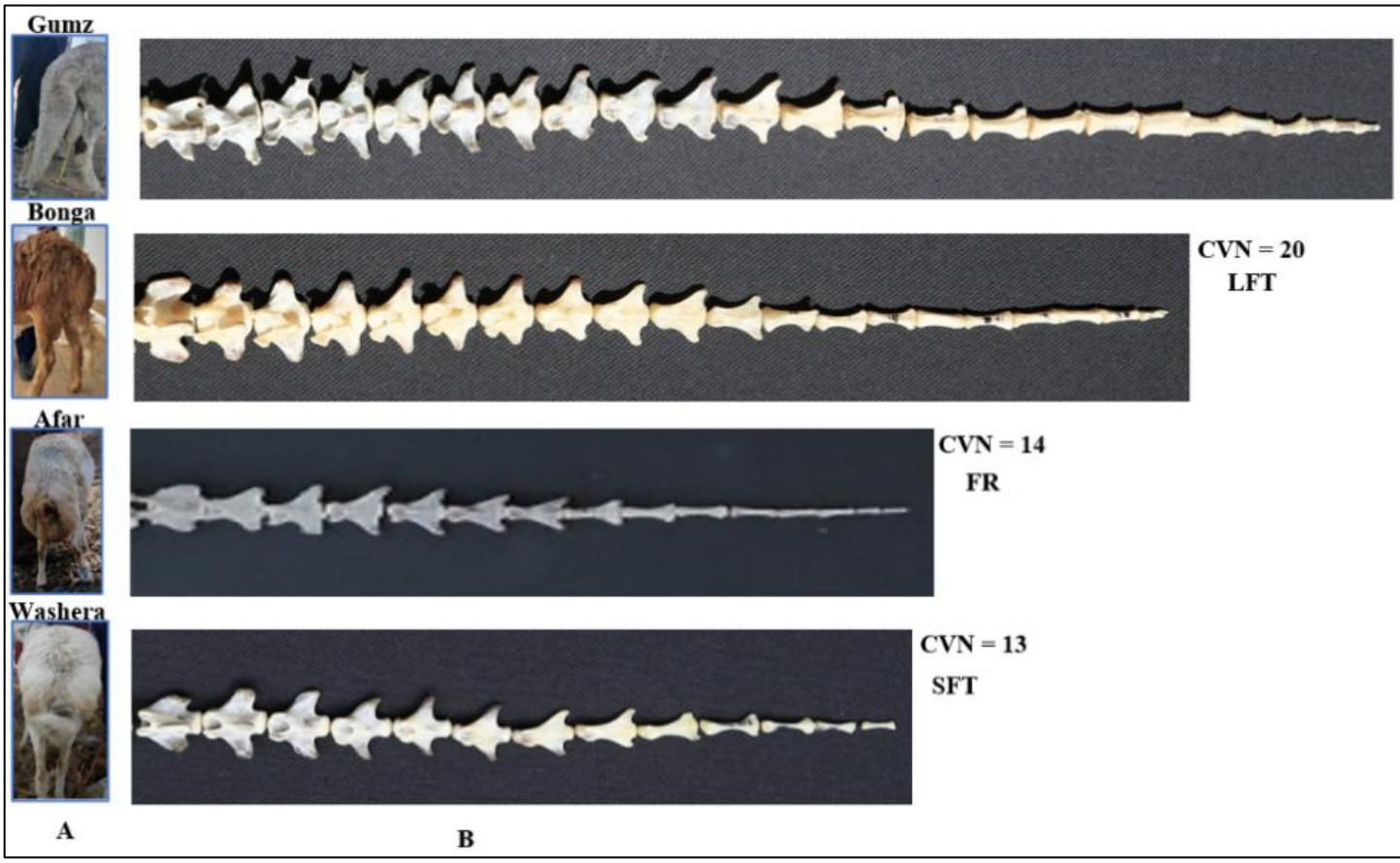


Fig. 9. Sheep tail morphotypes with visual tail phenotype and caudal vertebrea number, (A) visual tail lengths, (B) number of caudal vertebrae (CVN). SFT = short fat-tail (Washera) , FR = fat-rump (Afar), LFT = long fat-tail (Bonga) and TT = thin-tail (Gumz).

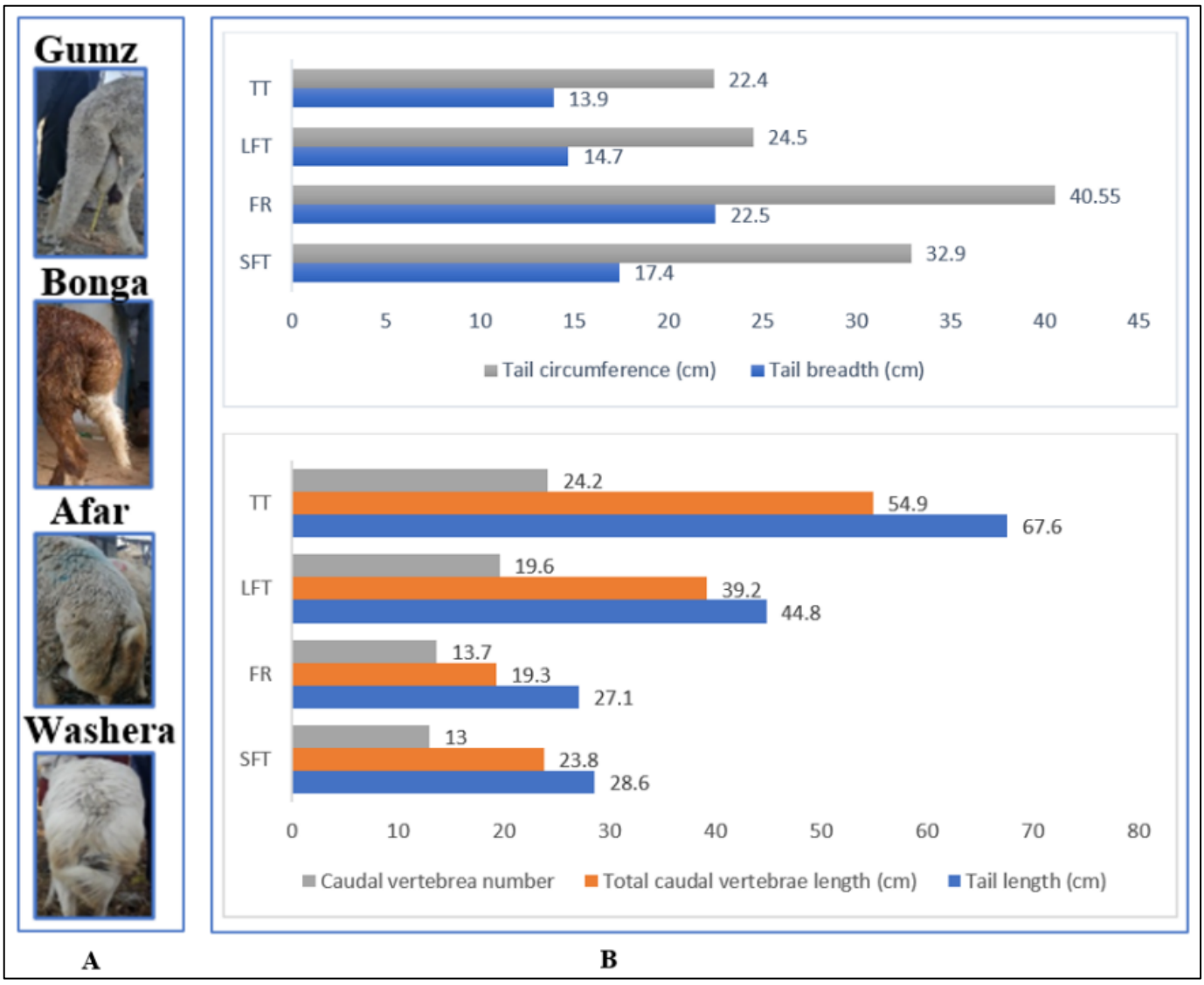


Fig. 10. Morphological and osteological tail traits of the four sheep tail morphotypes, (A) visual tail shape and size, (B) mean values of tail circumference (cm), tail breadth (cm), caudal vertebrae number (count), total caudal vertebrae length (cm) and tail length (cm). SFT = short fat-tail, FR = fat-rump, LFT = long fat-tail and TT = thin-tail.

Table 4

Mean values of quantitative morphological and osteological tail traits of the four sheep tail morphotypes (10 sheep/tail group) 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*	28.6 ± 3.81[a]	27.1 ± 7.53[a]	44.8 ± 5.85[b]	67.6 ± 4.62[c]
Tail width*	17.4 ± 2.12[a]	22.5 ± 2.32[a]	14.7 ± 2.16[b]	13.9 ± 2.18[b]
Tail circumference*	32.9 ± 7.92[a]	40.55 ± 7.57[a]	24.50 ± 9.44[b]	22.40 ± 3.53[b]
Caudal vertebrae number	13.0 ± 1.25[a]	13.7 ± 1.02[a]	19.6 ± 1.26[b]	24.2 ± 1.48[c]
ICVL**	18.2 ± 1.18[a]	13.9 ± 1.51[b]	20.0 ± 1.25[c]	22.7 ± 2.38[c]
TCVL*	23.8 ± 3.12[a]	19.3 ± 2.70[a]	39.2 ± 3.50[b]	54.9 ± 6.38[c]
Caudal vertebrae breadth**	21.2 ± 2.24[a]	19.4 ± 2.96[a]	22.5 ± 1.99[b]	23.2 ± 3.20[b]
Caudal vertebrae thickness**	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). ICVL: Individual caudal vertebra length. TCVL: Total caudal vertebrae length. *Measurements in cm. **Measurements in mm.*

The mean number of qualitative osteological tail traits (type, shape and symmetry categories) of the four sheep tail morphotypes are presented in Table 5. Type 1 (T1) caudal vertebrae are significantly ($P < 0.05$) different for the short fat-tail (SFT) compared to the three other tail morphotypes, as well as for the fat-rump compared to the three other sheep tail morphotypes. The highest mean number of T1 caudal vertebrae was observed in the long-tailed sheep (LFT, TT) (8.15 ± 1.23), and the lowest mean number in the fat-rumped (3.9 ±1.29) and the short fat-tailed (6.1 ± 1.61) sheep. Type 4 (T4) caudal vertebrae are significantly different ($P < 0.05$) for the long fat-tail and fat-rump compared to the two other sheep tail morphotypes as well as for the short fat-tail compared to the three other sheep tail morphotypes ($P < 0.05$), with the highest and the lowest mean numbers observed in the thin-tailed (8.9 ± 2.02) and the short fat-tailed (2.2 ± 0.63) sheep, respectively. The mean number of Type 2 (T2), flat-shaped (S1), and concave-shaped (S2) caudal vertebrae was significantly different ($P < 0.05$) for long-tailed (LFT, TT) and short-tailed (SFT, FR) 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 (Table 5). Moreover, the mean number of asymmetric (A) caudal vertebrae is significantly ($P < 0.05$) different for the fat-rump (FR) 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 (S) 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 5).

Table 5

Mean number of qualitative osteological tail traits (type, shape and symmetry) of the four sheep tail morphotypes (10 sheep/ tail group). The units are counted for all types.

Traits	Sheep tail morphologies or groups			
	short fat-tail	fat-rump	long fat-tail	thin-tail
Type category				
Type 1 (T1)	6.1 ± 1.61^{a}	3.9 ±1.29^{b}	7.4 ± 1.17^{c}	8.9 ± 1.28^{c}
Type 2 (T2)	2.6 ± 0.70^{a}	2.1 ± 1.20^{a}	3.9 ± 0.74^{b}	3.0 ± 1.25^{b}
Type 3 (T3)	2.1 ± 0.74^{a}	2.1 ± 0.57^{a}	3.1 ± 0.74^{a}	3.4 ± 1.17^{a}
Type 4 (T4)	2.2 ± 0.63^{ba}	5.6 ± 1.07^{ab}	5.2 ± 1.40^{ab}	8.9 ± 2.02^{c}
Shape category				
Flat (S1)	4.3 ± 1.95^{a}	3.4 ± 1.07^{a}	6.3 ± 2.36^{b}	6.7 ± 2.21^{b}
Concave (S2)	1.6 ± 0.84^{a}	0.8 ± 1.03^{a}	2.2 ± 1.40^{b}	2.9 ± 1.10^{b}
Convex (S3)	2.8 ± 1.48^{a}	1.8 ± 0.92^{a}	2.8 ± 2.66^{a}	2.3 ± 1.76^{a}
Symmetry category				
Asymmetry (A)	7.8 ± 1.61^{a}	2.3 ± 2.06^{b}	8.7 ± 2.26^{a}	7.4 ± 3.06^{a}
Symmetry (S)	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 6, Fig. 11). The Pearson correlation coefficient was represented by $r_{p.}$ A $r_p > 0.7$ was defined as a high correlation (Dormann et al. 2013). Among the external morphological tail traits, tail width/breadth and tail circumference show a high correlation ($r_p = 0.88$, $P < 0.001$), whereas tail

length against tail circumference show a moderate correlation (r_p = -0.57, $P < 0.001$). However, tail length showed a high 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, $P < 0.001$), type 1 caudal vertebrae (r_p = 0.73, $P < 0.001$) and a moderate correlation with type 4 caudal vertebrae (r_p = 0.61, $P < 0.001$). Among the osteological tail traits, high 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. A moderate correlation was observed between type 2 and symmetry caudal vertebrae (r_p = 0.53, $P < 0.001$), flat-shaped and type 1 caudal vertebrae (r_p = 0.60, $P < 0.001$), symmetry and type 4 caudal vertebrae (r_p = 0.52, $P < 0.001$), and among concave-shaped, asymmetric and type 1 caudal vertebrae (r_p = 0.50 to 0.62, $P < 0.001$) (Table 6, Fig.11).

Table 6

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

Traits	TL	TW	TC	CVN	ICVL	TCVL	CVB	CVT
TW	-0.38*							
TC	-0.57***	0.88***						
CVN	0.95***	-0.44**	-0.62***					
ICVL	0.78***	-0.46**	-0.61***	0.73***				
TCVL	0.96***	-0.45**	-0.63***	0.96***	0.89***			
CVB	0.46**	-0.15	-0.24	0.49**	0.69***	0.61***		
CVT	0.38*	-0.32*	-0.34*	0.36*	0.68***	0.47**	0.63***	
S1	0.50***	-0.42**	-0.43**	0.56***	0.41**	0.51***	0.21	0.40*
S2	0.51***	-0.37*	-0.46**	0.55***	0.64***	0.62***	0.63***	0.47**
S3	0.12	-0.07	0.12	0.05	0.16	0.09	0.07	0.22
S	0.34*	0.05	0.07	0.36*	0.01	0.24	-0.03	-0.15
A	0.38*	-0.52***	-0.59***	0.39*	0.62***	0.49**	0.45**	0.67***
T1	0.73***	-0.64***	-0.66***	0.74***	0.79***	0.79***	0.55***	0.65***
T2	0.35*	-0.13	-0.29	0.38*	0.32*	0.35*	0.19	0.40**

T3	0.54***	-0.46**	-0.55***	0.55***	0.40*	0.51***	-0.08	0.10
T4	0.61***	-0.03	-0.21	0.62***	0.38*	0.65***	0.36*	-0.12
Traits	S1	S2	S3	S	A	T1	T2	T3
S2	0.10							
S3	-0.49**	0.03						
S	0.23	-0.02	-0.21					
A	0.33*	0.50**	0.32*	-0.62***				
T1	0.60***	0.62***	0.09	0.14	0.61***			
T2	0.19	0.19	0.41**	-0.17	0.53***	0.12		
T3	0.32*	0.12	0.06	0.17	0.18	0.27	0.37*	
T4	0.29	0.32*	-0.19	0.52***	-0.12	0.33*	-0.00*	0.22

**** Correlation is significant at the 0.001 level; ** Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level. $r_p > 0.70$ was defined as high correlation (Dormann et al. 2013). TL: Tail length; TW: Tail width; TC: Tail circumference; CVN: Caudal vertebrae number; ICVL: Individual caudal vertebra length; CVL: Caudal vertebrae length; CVB: Caudal vertebrae breadth; CVT: Caudal vertebrae thickness; S1: Flat-shaped caudal vertebrae; S2: Concave-shaped caudal vertebrae; S3: Convex-shaped caudal vertebrae; S: Symmetric caudal vertebrae; A: Asymmetric caudal vertebrae; T1: Type 1 caudal vertebrae; T2: Type 2caudal vertebrae; T3: Type 3 caudal vertebrae; T4: Type 4 caudal vertebrae.*

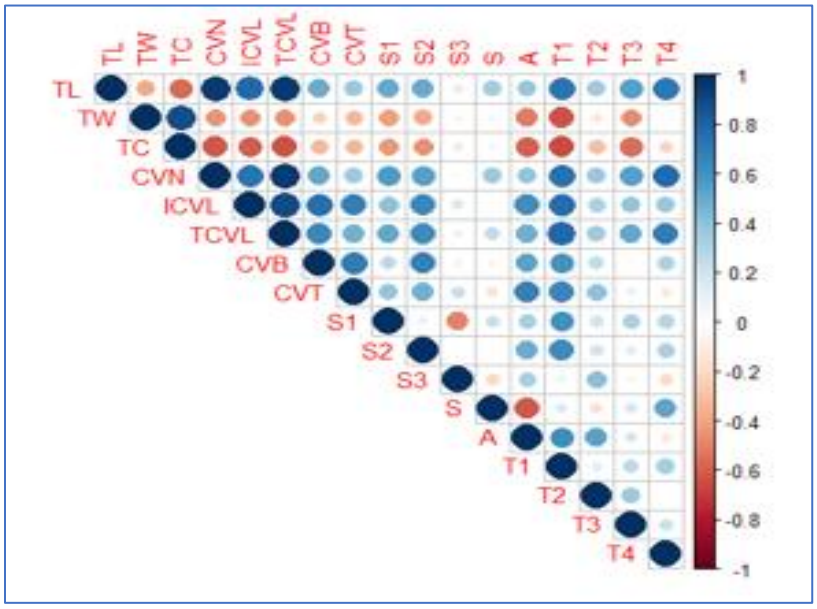


Fig. 11. Pearson correlation analysis between pairs of all morphological and osteological tail traits. All abbreviations are defined in Tables 2 and 3. $r_p > 0.70$ was defined as a high correlation (Dormann et al. 2013).

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 6, Fig. 11). Based on this, regression models for total caudal vertebrae length and for caudal vertebrae number from the tail length of the sheep were developed as indicated in Fig. 12A and 12B, respectively. We also developed regression models for total caudal vertebrae length and for tail length from individual caudal vertebra length of the sheep (Fig. 13A and 13B, respectively).

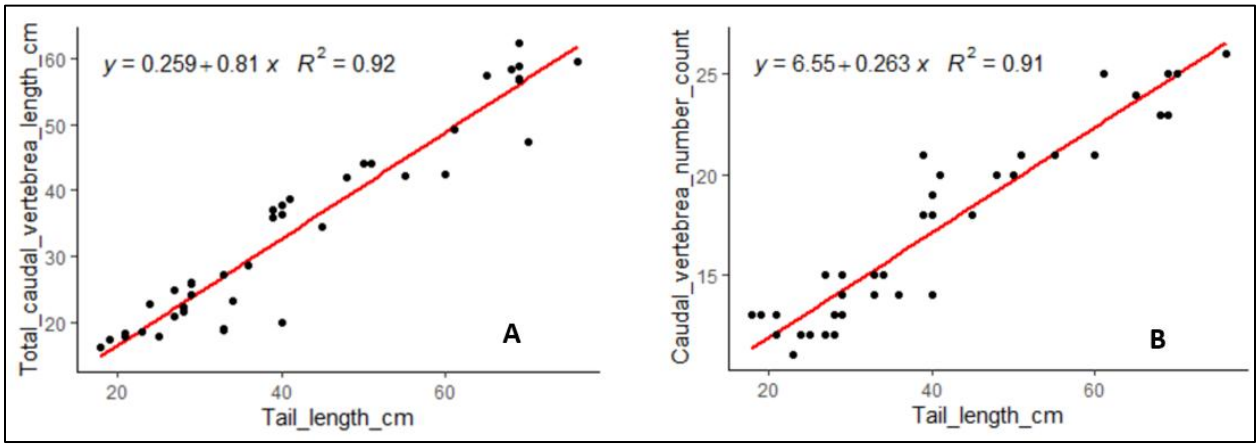


Fig. 12. Linear regression models, (A) estimation of total caudal vertebrae length using external tail measurement, tail length, (B) estimation of total caudal vertebrae number using tail length.

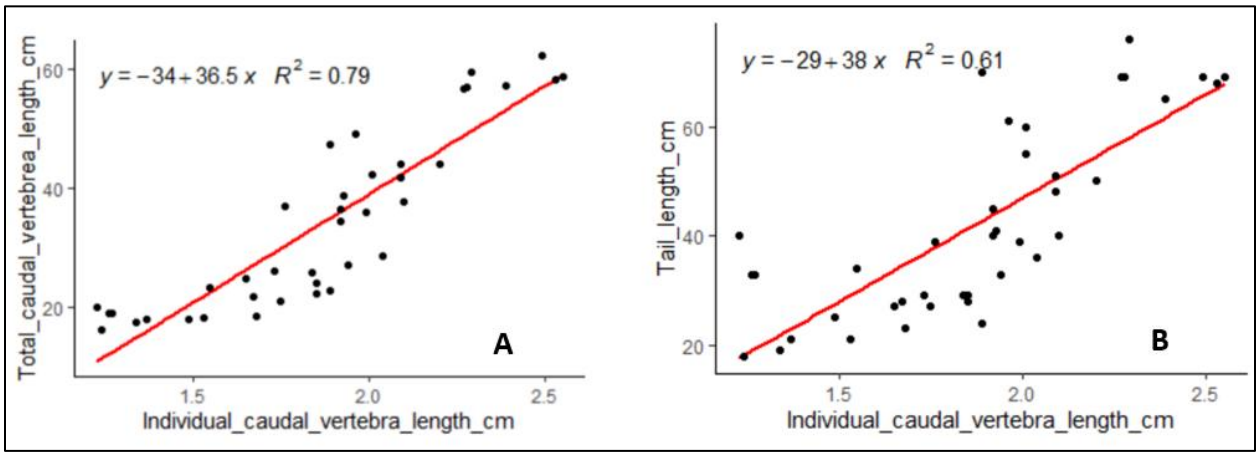


Fig. 13. Linear regression model, (A) estimation of total caudal vertebrae length using individual vertebra length, (B) estimation of tail length using individual caudal vertebra length.

3.3. Linear discriminant analysis

The scatterplot (linear discriminant analysis: LDA plot) for sheep tail morphotypes based on morphological and osteological tail traits (Fig. 14A) indicates that the first two linear discriminant functions (LDs: LD1 and LD2) account for 86% and 12% of the total variation, respectively, and clearly differentiates the sheep tail morphotypes according to tail morphology and osteology. LD1 separates the fat-rumped (FR) and short fat-tailed (SFT) sheep from long fat-tailed (LFT) and thin-tailed (TT) sheep. LD2 separates the FR and most of the samples of TT sheep from the SFT and LFT sheep. The finding was well supported by a separate scatter plot (LDA plot) performed for sheep tail groups based on osteological tail traits only (osteological traits LDA plot: Fig. 14B). Similarly, LD1 separates the FR and SFT sheep from LFT and TT sheep, whereas LD2 separates the FR and most of the samples of TT sheep from the SFT and LFT sheep. Furthermore the LDA plot result obtained for sheep tail morphotypes was supported by a stacked histogram of the values of linear discriminant function for the samples from different sheep tail morphotypes (Supplementary Fig. 1A1, A2).

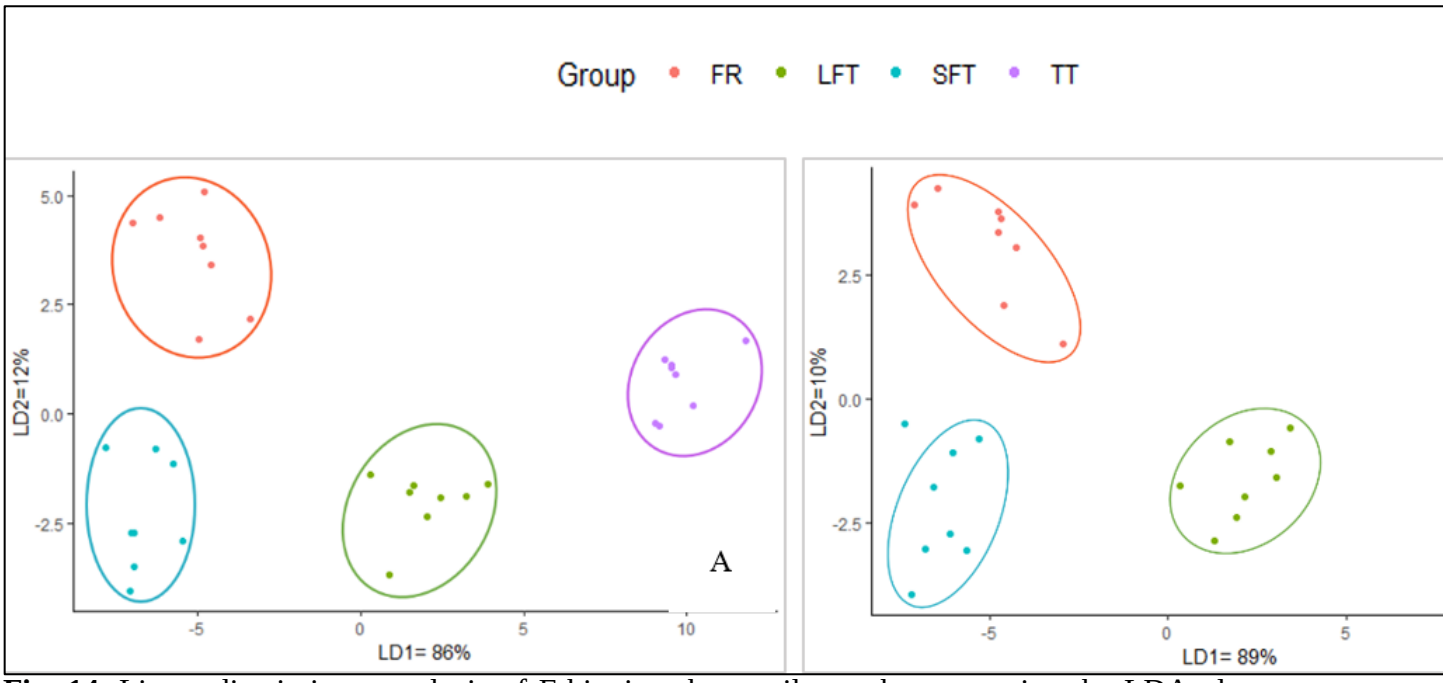


Fig. 14. Linear discriminant analysis of Ethiopian sheep tail morphotypes using the LDA plot

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 (LDA plot) performed for sheep breeds based on both morphological and osteological tail traits (Fig. 15A) indicates that the first two LDs account for 72% and 22% of the total variation, respectively, and categorizes the six sheep breeds into four sheep tail groups according to tail morphology and osteology: the two fat-rumped (FR) sheep breeds (Afar and BHS) form one cluster tail morphotype (FR tail morphotype). Similarly, the two short fat-tailed (SFT) sheep breeds (Washera and Menz) form another cluster tail morphotype (SFT tail morphotype). LD1 clearly separates the FR and SFT sheep breeds from long fat-tailed (LFT) (Bonga) and thin-tailed (TT) (Gumz) sheep breeds, whereas LD2 separates the FR and TT sheep breeds from the SFT and LFT sheep breeds. The finding was well supported by a separate LDA 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 LDA plot: Fig. 15B). LD1 clearly separates the FR and SFT breeds from LFT (Bonga) and TT (Gumz) breeds, whereas LD2 separates the FR and TT breeds from the SFT and LFT sheep breeds. A separate LDA plot result obtained for sheep breeds was further supported by a stacked histogram of the LD values for the samples from different sheep breeds (Supplementary Fig. 2B1, B2).

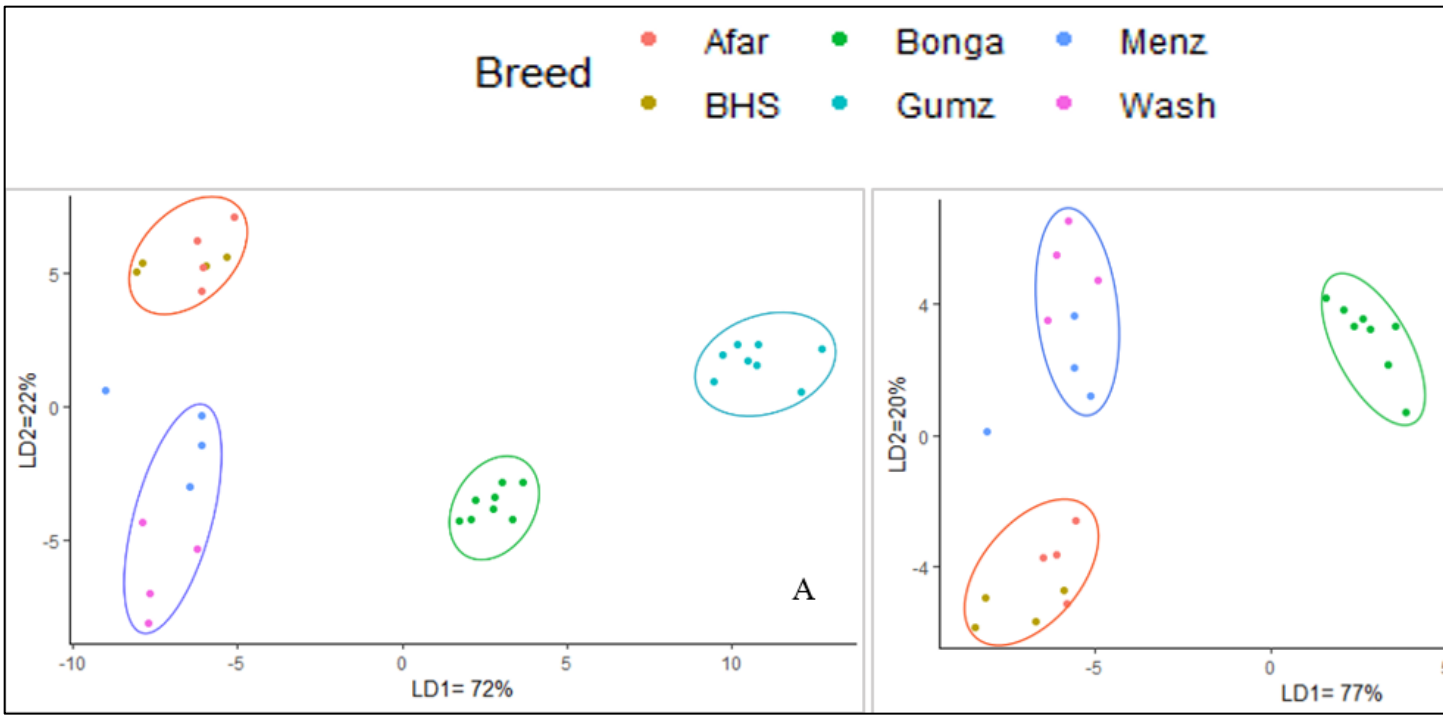


Fig. 15. Linear discriminant analysis of Ethiopian sheep breeds using the LDA plot based on, (A) morphological and osteological tail traits and (B) osteological tail traits only. BHS: Blackhead Somali; Wash: Washera.

The linear discriminant analysis results for sheep tail morphotypes and breeds were further supported by the eigen value and percentage of separation (proportion of trace) achieved by LD1 and LD2, and the canonical correlation analysis (CCA) result (Table 7). The eigen value and percentage of separation result indicated that the first two linear discriminant functions (LDs) accounted for 98% (LD1: 86%, LD2: 12%) of the total variation in differentiating the four sheep tail morphotypes, as well as 94% (LD1: 782%, LD2: 22%) in differentiating the six sheep breeds (Table 7). The canonical correlation analysis (CCA) result indicates the presence of high values of canonical correlation (CC) between each linear discriminant function (LD) and sheep tail morphotypes (LD1 = 99%, LD2 = 93%), as well as between each LD and the six sheep breeds (LD1 = 99%, LD2 = 98%), as indicated in Table 7.

Table 7

Eigen values, proportion of variability (%) and canonical correlation (%) explained by the first two discriminant functions (LD1 and LD2).

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 (PCA) based on morphological and osteological tail traits (Fig. 16) indicated that the first two principal components (PCs) accounted for 62.7% (PC1: 46.5%, PC2: 16.2%) of the total variation and clearly differentiated the four sheep tail morphotypes. PC1 separates the two tail morphotypes very well: the short-tailed (SFT: short fat-tail and FR: fat-rump) from the long-tailed (LFT: long fat-tail and TT: thin-tail) sheep tail morphotypes. PC2 separates FR and most of the TT tail morphotypes samples from SFT and some of the LFT tail morphotype samples. The result obtained (all traits PCA) was backed up by a separate PCA performed for sheep tail morphotypes based on osteological tail straits only (Osteological PCA: Fig. 16B). Similarly, PC1 separates the two tail morphotypes very well: the short-tailed from the long-tailed sheep. PC2 separates FR and most of the TT tail morphotypes samples from SFT and some of the LFT tail morphotype samples.

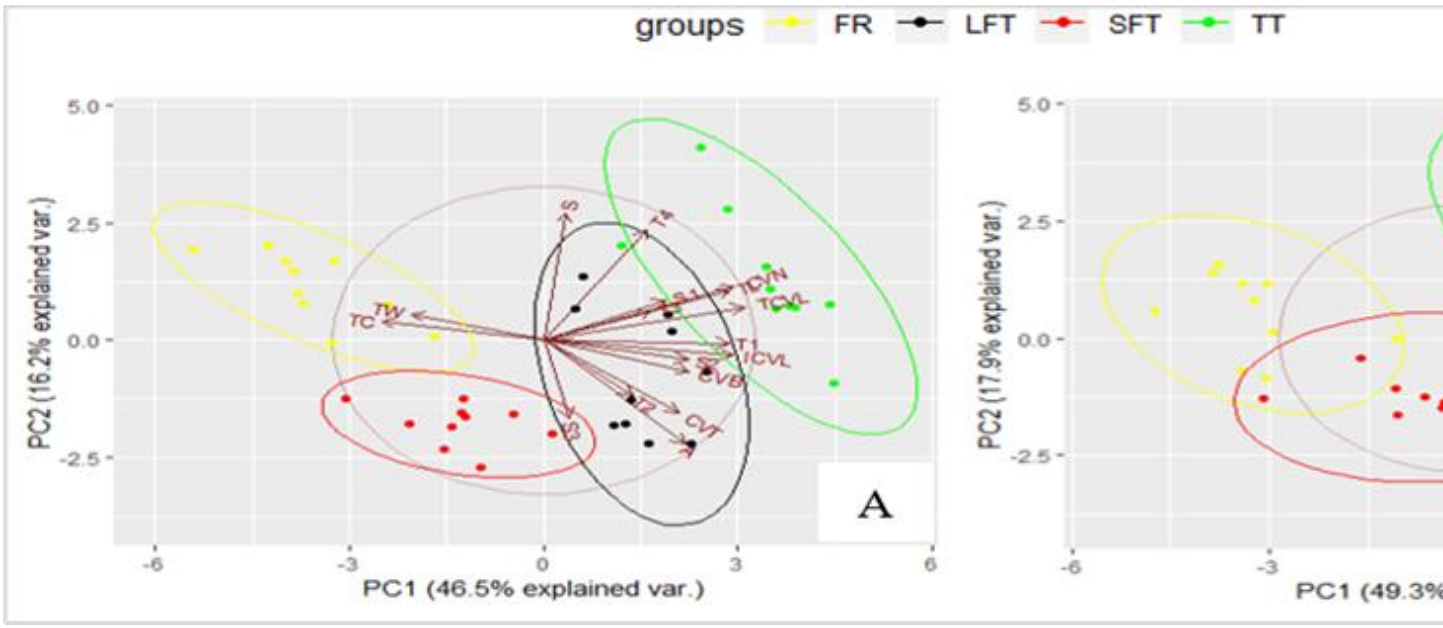


Fig. 16. Principal component analysis of Ethiopian sheep tail morphotype based on: (A) morphological and osteological tail traits and (B) osteological tail traits only. SFT = short fat-tail; FR = fat-rump; LFT = long fat-tail; TT =thin-tail.

A separate PCA based on morphological and osteological tail traits (Fig. 17A) indicated that the first two principal components (PCs) accounted for 62.7% (PC1: 47.9%, PC2: 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 (FR) sheep breeds (Afar and BHS) forms one cluster tail morphotype (FR tail morphotype). Similarly, the result indicated that close clustering of the two short fat-tailed (SFT) sheep breeds (Washera and Menz) forms another one cluster tail morphotype (SFT tail morphotype). PC1 clearly separates the FR and SFT sheep breeds from LFT (Bonga) and TT (Gumz) sheep breeds, whereas PC2 separates the FR and most of the TT sheep breed samples from SFT and some of the LFT sheep breed samples. The result obtained (all traits PCA) was supported by a separate PCA for sheep breeds based on osteological tail traits only (osteological traits PCA: Fig. 17B). Similarly, PC1 clearly separates FR and SFT sheep breeds from LFT and TT sheep breeds, whereas PC2 separates the FR and most of the TT sheep breed samples from SFT and some of the LFT sheep breed samples.

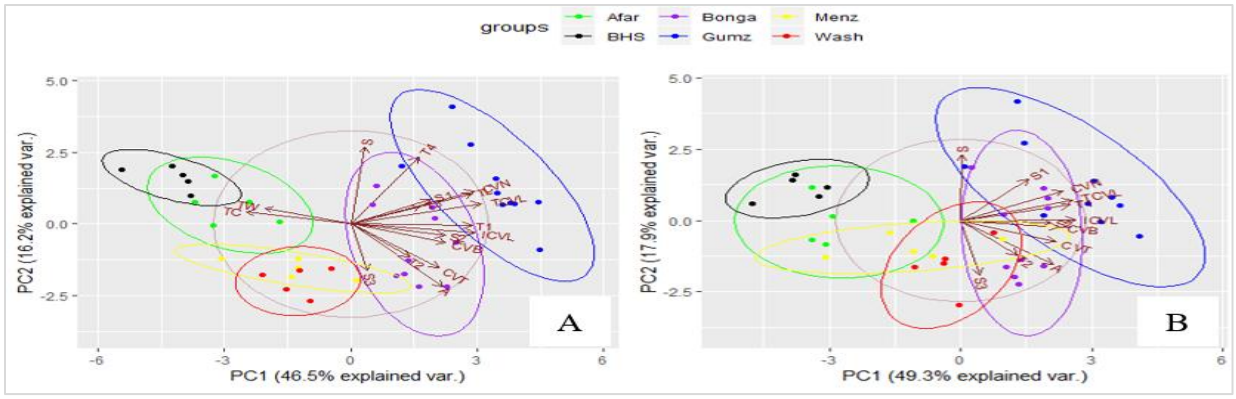


Fig. 17. Principal component analyses of Ethiopian sheep breeds based on: (A) morphological and osteological tail traits and (B) osteological traits only. BHS: Blackhead Somali; Wash: Washera.

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 types found in Ethiopia at morphological and osteological levels; to provide baseline osteological information on sheep caudal vertebra morphologies as reference material for zooarchaeological studies of sheep husbandry; to provide accurate phenotypic descriptions for genome mapping studies aiming to elucidate the genetic control of sheep tail morphologies.

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

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

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

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

609 The correlation analysis indicated that the osteological tail (individual caudal vertebra length,
610 total caudal vertebrae length, and number of caudal vertebrae) and external measurement (tail
611 length) characters were significantly ($P < 0.05$) and positively correlated with each other. Based
612 on this result, regression models were developed for these traits (Fig. 12A, B and Fig. 13A, B).
613 The higher association of total caudal vertebrae length and caudal vertebrae number with tail
614 length over the other osteological measurement, individual caudal vertebra length, (Table 6, Fig.

11) indicates that tail length alone can estimate both osteological tail traits (Fig. 12A, B) without killing the animal, simply by measuring tail length on live animals. ~~Moreover, the higher association of total caudal vertebrae length with individual caudal vertebra length over the other two osteological tail traits (total caudal vertebrae length and caudal vertebrae number) and tail length indicates that individual caudal vertebra length can estimate the total caudal vertebrae length of the animal and hence estimate the tail length of that animal (Fig. 13A, B). Therefore, based on a specific vertebra length measurement of the animal, osteologists can identify which tail morphotype vertebrae come from by knowing or predicting the total caudal vertebrae length, caudal vertebrae count and tail length of that animal, as the analysis of variance indicated that these traits support separation of the studied Ethiopian sheep into three main tail morphotypes (short-tail, medium-tail, long-tail).~~

The linear discriminant analysis indicated that the first two linear discriminant functions (LD1 and LD2) 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 (Fig. 14,15), which further indicates that LD1 achieves a good separation of the four sheep tail groups as well as the six sheep breeds, but that LD2 only slightly improves the separation. Therefore, to achieve a better separation of the sheep tail groups and breeds, it is necessary to use both the first and second discriminant functions (LD1 and LD2) to differentiate the four sheep tail groups, as well as the six sheep breeds, as they accounted for 98% and 94% of the total variation in the dataset, respectively (Fig. 14, 15). Moreover, the observed high canonical correlation (CC) between the first two linear discriminant functions and sheep tail morphotypes (LD1 = 99%, LD2 = 93%), and sheep breeds (LD1 = 99%, LD2 = 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 7). The principal component analysis (PCA) showed that most of the variation between samples in the whole dataset can be captured using the first two principal components (PC1 and PC2), as they accounted for over 62% of the total variation (Fig. 16, 17).

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 types can provide informative elements for archaeozoological research on the development and diffusion of sheep breeds. The remains of complete sheep tails are very rare 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 type of tail, fat or thin, and a better understanding of the routes of introduction of 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 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 breadth), morphological characteristics (type 2, flat and concave-shaped caudal vertebrae), and tail measurements (tail width and tail circumference) clearly separate the short-tailed sheep from the long 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 groups: 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. Both the linear discriminant (LDA) and principal component (PCA) analyses revealed four distinct sheep tail morphotypes, categorizing the six breeds of sheep examined according to their tail morphology and osteology. Moreover, the LDA and canonical correlation analysis revealed that the first two discriminant functions are efficient and are more effective in discriminating between the four sheep tail morphotypes as well as 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. 12A, B). Moreover, by measuring sheep caudal vertebrae,

osteologists can estimate other osteological traits and the tail length of that sheep (Fig. 13A, 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 the investigation of the evolution of sheep tails since domestication and for an enhanced understanding of the history of the introduction of 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

We would like to acknowledge the staff of Debre Berhan Agricultural Research Center, Bahir Dar Regional Animal Health Laboratory, Bonga Agricultural Research Center, and Guba & Almeha office of Agriculture for their kind support in sheep skeleton preparation in their respective institutions and farmers` training centres (FTC). We thank the Authority for Research and Conservation of the Cultural Heritage (ARCCH) and the Centre Français des Etudes Ethiopiennes (CFEE) for providing facilities for skeleton preparation. The authors would also like to acknowledge Mr Michael Temesgen and Anteneh Hailemariam for their kind support and cooperation in providing driving services and their involvement in reference bone sample preparation. Field data collection (sheep purchase and skeleton collection) was co-financed by the CGIAR Livestock CRP and the EvoSheep ANR project (France). In particular, this research was conducted as part of the CGIAR Research Program on Livestock, and supported by contributors to the CGIAR Trust Fund. It was also conducted as part of the

EvoSheep project on the origin and development of sheep breeds funded by the French National Research Agency (ANR-17-CE27-0004). We would also like to thank Amhara Regional Agricultural Research Institute (ARARI) and Addis Ababa University, department of cellular, microbial & molecular biology, for their financial support.

References

Ahbara, A., Bahbahani, H., Almathen, F., Al Abri, M., Agoub, M. O., Abeba, A., Kebede, A., Musa, H. H., Mastrangelo, S., Pilla, F., Ciani, E., Hanotte, O., Mwacharo, J. M., 2019. Genome-wide variation, candidate regions and genes associated with fat deposition and tail morphology in Ethiopian indigenous sheep. Front. Genet. 9, 699. doi: 10.3389/fgene.2018.00699.

Amane, A., Belay, G., Nasser, Y., Kyalo, M., Dessie, T., Kebede, A., Getachew, T., Entfellner, J.B.D., Edea, Z., Hanotte, O. and Tarekegn, G.M., 2020. Genome-wide insights of Ethiopian indigenous sheep populations reveal the population structure related to tail morphology and phylogeography. Genes & Genomics. 42, 1169–1178. https://doi.org/10.1007/s13258-020-00984-y.

Atti, N., Bouquier, F., Khaldi, K., 2004. Performance of the fat tailed Barbarine sheep in its environment: adaptive capacity to alternation of underfeeding and re-feeding periods. A review. Anim. Res. 53, 165–176.

Chaix, L., Grant, A., 1987. A study of a prehistoric population of sheep (Ovis aries L.) from Kerma (Sudan). Archaeozoologia. 1, 77-92.

Chilliard, Y., Ferlay, A., Faulconnier, Y., Bonnet, M., Rouel, J., Bocquier, F., 2000. Adipose tissue metabolism and its role in adaptations to undernutrition in ruminants. Proceedings of the Nutrition Society. 59(1), 127-134. doi:10.1017/S002966510000015X.

761 Cornevin, M. and Lesbre, F.-X., 1897. Mémoire sur les variations numériques de la colonne
762 vertébrale et des côtes chez les mammifères domestiques. In : Bulletin de la Société
763 d'anthropologie de Lyon, tome. 16/2, 343-383. https://doi.org/10.3406/linly.1897.16390.
764 Devendra, C. and McLeroy, G., 1982. Goat and Sheep Production in the Tropics. Intermediate
765 Tropical Agriculture Series. Longman Group UK Ltd Essex. 271 pp.
766 Dormann, C. F., Elith, J., Bacher, Sv., Buchmann, C., Carl, G., Carr é, G., Marquéz, J.R.G.,
767 Gruber, B., Lafourcade, B., Leitão, P.J., Münkemüller, T., McClean, C., Osborne, P.E.,
768 Reineking, B., Schröder, B., Skidmore, A.K, Zurell, D., Lautenbach, Sv., 2013.
769 "Collinearity: a review of methods to deal with it and a simulation study evaluating their
770 performance." Ecography. 36 (1), 27-46.
771 Economides, K. D., Zeltser, L., Capecchi, M. R., 2003. Hoxb13 mutations cause overgrowth of
772 caudal spinal cord and tail vertebrae. Dev Biol. 256, 317-330. doi:10.1016/S0012-
773 1606(02)00137-9.
774 Edea, Z., Dadi, H., Dessie, T., Kim, K. S. 2019. Genomic signatures of high-altitude adaptation
775 in Ethiopian sheep populations. *Genes & genomics*, 41:973-981.
776 Epstein, H., 1954. The fat-tailed sheep of East Africa. The East African Agricultural Journal. 20,
777 109-117.
778 Epstein, H., 1971. The Origin of Domestic Animals of Africa. Vol.2. Africana Publication
779 Corporation. New Yor. pp. 719.
780 Ermias, E., Yami, A., Rege, J., 2002. Fat deposition in tropical sheep as adaptive attribute to
781 periodic feed fluctuation. J. Anim. Breed. Genet. 119, 235–246.
782 FAO., 1986. Animal Genetic Resources Data Banks. 2. Descriptor Lists for Cattle, Buffalo, Pigs,
783 Sheep and Goats. Animal Production and Health Paper. 59/2. FAO, Rome, Italy. pp. 96-129.
784 Fariello, M. I., Servin, B., Tosser-Klopp, G., Rupp, R., Moreno, C., San Cristobal, M., Boitard,
785 S., 2014. Selection signatures in worldwide sheep populations. PLoS ONE 9, e103813. doi:
786 10.1371/journal.pone.0103813.
787 Gebremichael, S. (2008). "Sheep resources of Ethiopia: genetic diversity and breeding strategy,"
788 Wageningen University, The Netherlands.
789 Gizaw, S., Van Arendonk, J. A., Komen, H., Windig, J., Hanotte, O., 2007. Population structure,
790 genetic variation and morphological diversity in indigenous sheep of Ethiopia. Animal
791 Genetics. 38, 621-628.

Gizaw, S., 2009. Sheep Breeds of Ethiopia: a guide for identification and utilization. Technical Bulletin.

Hopkins Sr, C. E., Hamm Jr, T. E., Leppart, G. L. 1970. *ATLAS OF GOAT ANATOMY. PART 1. OSTEOLOGY*. EDGEWOOD ARSENAL ABERDEEN PROVING GROUND MD.

IEMVT-FRA., 1950. Les races ovines et caprines de l'Afrique occidentale française (conférence de Luknow, 13-22 février 1950). Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux. 4(4), 193–201.

Kang, D., Zhou, G., Zhou, S., Zeng, J., Wang, X., Jiang, Y., Yang, Y., Chen, Y., 2017. Comparative transcriptome analysis reveals potentially novel roles of Homeobox genes in adipose deposition in fat-tailed sheep. Sci. Rep. 7, 14491. doi: 10.1038/s41598-017-14967-9.

Korecha, D., and Sorteberg. A., 2013. Validation of operational seasonal rainfall forecast in Ethiopia. Water Resource. Res. 49, 7681–7697. doi:10.1002/2013WR013760.

Levene, H. 1960. In Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling, I. Olkin et al. eds., Stanford University Press, pp. 278-292.

Lv, F.-H., Peng, W.-F., Yang, J., Zhao, Y.-X., Li, W.-R., Liu, M.-J., Ma, Y.-H., Zhao, Q.-J., Yang, G.-L., Wang, F., Li J.-Q., Liu, Y.-G., Shen, Z.-Q., Zhao, Sh.-G., Hehua, EE., Gorkhali, N.A., Vahidi, F. S. M., Muladno, M., Naqvi, A.N., Tabel, J., Iso-Touru, T., Bruford, M.W., Kantanen, J., Han, J.-L., Li, M.-H., 2015. Mitogenomic meta-analysis identifies two phases of migration in the history of eastern Eurasian sheep. Mol. Biol. Evol. 32, 2515-2533. doi: 10.1093/molbev/msv139.

May, N. D. 1964. The anatomy of the sheep. *Journal of the South African Veterinary Association*, *35*(4).

Miller, M. E., Christensen, G. C., Evans, H. E. 1965. Anatomy of the Dog. *Academic Medicine*, *40*(4), 400.

Moradi, M. H., Nejati-Javaremi, A., Moradi-Shahrbabak, M., Dodds, K. G., McEwan, J. C., 2012. Genomic scan of selective sweeps in thin and fat tail sheep breeds for identifying of candidate regions associated with fat deposition. BMC Genet. 13:10. doi: 10.1186/1471-2156-13-10.

Muigai, A. W., & Hanotte, O. 2013. The origin of African sheep: archaeological and genetic perspectives. *African Archaeological Review,* 30(1): 39-50.

Pourlis, A. F., 2011. A review of morphological characteristics relating to the production and reproduction of fat-tailed sheep breeds. Trop. Anim. Health Prod. 43, 1267-1287. doi: 10.1007/s11250-011-9853-x.

R Core Team., 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rege, J. E. O., Yapi-Gnaore, C. V., Tawah, C. L., 1996. The indigenous domestic ruminant genetic resources of Africa. In H. H. Meissner (Ed.), Food security in Africa. Challenges, opportunities and targets for animal production. Proceedings of a conference (pp. 57–77). Germiston: South African Society of Animal Science.

Ruiz-Larrañaga, O., Langa, J., Rendo, F., Manzano, C., Iriondo, M., Estonba, A., 2018. Genomic selection signatures in sheep from the Western Pyrenees. Genet Sel Evol. 50: 9. doi: 10.1186/s12711-018-0378-x.

Ryder, M.L., 1984. Sheep In: Mason, I.L., Ed., Evolution of Domesticated Animals. Longman, New York. 63- 84.

Schmid, E., 1972. Atlas of animal bones. Elsevier Publishing Company, New York and Amsterdam, 1972. vii and 159 pp. Bibliography. Index. $29.00. Quaternary Research, 2(2), 258-259.

Trut, L., Oskina, I., Kharlamova, A., 2009. Animal evolution during domestication: the domesticated fox as a model. BioEssays. 3, 349–360.

Vatankah, M. and Talebi, M. A., 2008. Genetic parameters of body weight and fat-tail measurements in lambs. Small Ruminant Research. 75, 1–6.

Vigne, J.-D., 2011. The origins of animal domestication and husbandry: a major change in the history of humanity and the biosphere. C. R. Biol. 334, 171–181.

Vila, E., Abrahami, P., Albesso, M., Amane, A., Bader, C., Berthon, R., Vuillien, M., 2021. EVOSHEEP: the makeup of sheep breeds in the ancient Near East. *Antiquity*, *95*(379).

Von den Driesch, A., 1976. A guide to the measurements of animal bones from archaeological sites (Peabody Museum Bulletin 1). Cambridge (MA): Peabody Museum.

Whannou, H. R. V., Afatondji, C. U., Ahozonlin, M. C., Spanoghe, M., Lanterbecq, D., Demblon, D., Dossa, L. H., 2021. Morphological variability within the indigenous sheep population of Benin. *PloS one*, *16*(10), e0258761.

Wilke, H. J., Kettler, A., Wenger, K. H., Claes, L. E., 1997. Anatomy of the sheep spine and its comparison to the human spine. *The Anatomical Record: An Official Publication of the American Association of Anatomists*, *247*(4), 542-555.

Wilson, R.T., 1991. Small ruminant production and the small ruminant genetic resource in tropical Africa, Food & Agriculture Org. Rome, Italy. Springer. ISBN 0387981403.

Zamiri, M. J. and Izadifard, J., 1997. Relationship of fat-tail weight with fat-tail measurements and carcass characteristics of Mehraban and Ghezel rams. Small Ruminant Research. 26, 261–266.

Zeng, J., Zhou, S., Yang, Y., Du, J., Knag, D., Wang, X., Chen, Y., 2020. Effect of dietary nutrition on tail fat deposition and evaluation of tail related genes in fat-tailed sheep. Electron J. Biotechnol. 46. https://doi.org/10.1016/j.ejbt.2020.05.004.

Zhang, H., Zhang, X., Wang, Z., Dong, X., Tan, C., Zou, H., Peng, Q., Xue, B., Wang, L., Dong, G., 2015. Effects of dietary energy level on lipid metabolism related gene expression in subcutaneous adipose tissue of yellow breed x Simmental cattle. Anim. Sci J. 86(4), 392–400. https://doi.org/10.1111/asj.12316 PMid: 25440457