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Agraw Amane, Gurja Belay, Adebabay Kebede, Tadelle Dessie, Setegn Worku, et al.. Analysis of tail morphology and osteology in Ethiopian indigenous sheep. *Journal of Archaeological Science: Reports*, 2023, pp.103776. 10.1016/j.jasrep.2022.103776 . halshs-04004487

HAL Id: halshs-04004487

<https://shs.hal.science/halshs-04004487>

Submitted on 24 Feb 2023

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1 **Analysis of tail morphology and osteology in Ethiopian indigenous sheep**

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26 **ABSTRACT**

27 Sheep adaptive diversity, including tail morphology, has been shaped by various factors
28 including natural and/or artificial selection for different traits. The Horn of Africa has historically
29 been a major livestock entry point on the African continent from the Near Eastern centers of
30 initial domestication. Ethiopia, in particular, possesses a marked sheep diversity, including the
31 presence of breeds with four distinct tail morphotypes (short fat-tail, long fat-tail, fat-rump, and
32 thin-tail) that do not co-exist elsewhere. The origin and development of the fat tail are still poorly
33 known and the osteological and metrical differences between the fat tail morphotypes have never
34 been studied. Here, we characterized the phenotypic diversity of Ethiopian sheep tails from
35 morphological and osteological perspectives. Three tail measurements and 14 osteological traits
36 were recorded in six breeds (Menz, Washera, Afar, Blackhead Somali, Bonga, and Gumz),
37 representative of the four sheep tail morphotypes. Both linear discriminant and principal
38 component analyses categorize the six sheep breeds into four distinct tail groups. Analysis of
39 variance of the morphological and osteological traits shows significant differences ($P < 0.05$)
40 between the four tail morphotypes. The highest mean values of tail length, total caudal vertebrae
41 length and the number of caudal vertebrae were recorded in the thin-tailed sheep, followed by the
42 long fat-tailed sheep whereas the lowest average values were recorded in the fat-rumped and
43 short fat-tailed sheep. These traits are significantly and positively correlated with each other.
44 Based on regression model analysis, it is possible to use tail length alone as a predictive tool to
45 estimate the sheep tail osteology without killing the animal. Moreover, based on measurements
46 of sheep caudal vertebrae, the osteologist can estimate other osteological traits and the tail length
47 of that sheep, which further differentiates its tail morphotypes. Significant differences ($P < 0.05$)
48 were also observed in individual caudal vertebra length and breadth, tail width and tail
49 circumference, type 2, flat and concave-shaped caudal vertebrae between the short-tailed and
50 long-tailed sheep. Our results provide important phenotypic baselines for genome diversity and
51 adaptation studies, as well as an osteological baseline for archaeozoological work aiming to
52 understand the history of sheep husbandry and breed development in past societies.

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

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58 **1. Introduction**

59 Sheep were domesticated in the Fertile Crescent approximately 11,000 years ago (Vigne et
60 al., 2011). Since then, sheep have been selected for production by human societies for their meat,
61 milk, and wool (Ruiz-Larrañaga et al., 2018), behavioural traits (e.g., tameness), and
62 environmental adaptation (Trut et al., 2009). Among the key physical characteristics that
63 allowing us to distinguish sheep breeds, tail type and length are one of the most discriminating
64 features (Gebremichael, 2008; Gizaw, 2009). In particular, the morphology and fat content of the
65 sheep tail are considered to be a reservoir of energy for the animal to cope with harsh
66 environmental challenges such as drought, extreme cold winters, and food shortages (Chilliard et
67 al., 2000; Pourlis, 2011; Moradi et al., 2012; Lv et al., 2015). The fat in the sheep tail also
68 represents an essential source of dietary fat for human societies lacking other fat producing
69 animals, particularly during periods of drought and famine (Moradi et al., 2012). Sheep breeds
70 from across the world are classified today on the basis of their tail phenotypes as thin-tailed,
71 short fat-tailed, long fat-tailed and fat-rumped sheep breeds (Epstein, 1971; Gizaw et al., 2007;
72 Muigai and Hanotte, 2013; Ahbara et al., 2019; Whannou et al., 2021).

73 The founding populations of current African sheep breeds were thought to have been
74 introduced from Asia in three waves of migration of corresponding to the modern thin-tailed, fat-
75 tailed and fat-rumped sheep (Epstein, 1971), with two types of fat-tailed sheep - long and short
76 ones. It is thought that the first sheep to enter the African continent were thin-tailed hairy sheep
77 types. Fat-tailed sheep were introduced into Africa during the second wave of migration
78 followed by the fat-rumped sheep (Epstein, 1954; Epstein, 1971; Ryder, 1984). Indigenous
79 African sheep genetic resources are geographically distributed largely across three non-
80 overlapping geographic ranges. Thin-tailed sheep are currently found in West Africa and East
81 Africa up to Sudan (IEMVT-FRA, 1950), with distributions are bordering Ethiopia. Fat-tailed
82 sheep are found in North, East and Southern Africa and fat-rumped sheep are found across the
83 Horn of African (Ethiopia, Djibouti, Somali, Kenya, and Sudan) (Wilson, 1991; Rege et al.,

84 1996). Archaeological and molecular genetic information indicate separate introductions and
85 dispersion histories for the African thin-tailed and fat-tailed sheep (Muigai and Hanotte, 2013).
86 Thin-tailed sheep entered the African continent through Egypt and the Sinai Peninsula, and fat-
87 tailed sheep entered through the Horn of Africa and Northeast Africa. This scenario is supported
88 by ancient iconography, such as illustrations of sheep tail morphology (Gootwine, 2018; Vila et
89 al., 2021) and the current geographic distribution of sheep tail morphotypes (Gizaw, 2009;
90 Muigai and Hanotte, 2013; Amane et al., 2020; Whannou et al., 2021).

91 Ethiopia is one of the major gateways for domestic sheep migration from Asia to Africa
92 owing to its ancient civilization and geographic position at the Horn of the African continent
93 (Devendra and McLeroy 1982). The country comprises one of the most diversified sheep
94 populations in Africa (Devendra and McLeroy 1982). Ethiopian indigenous sheep are adapted to
95 diverse agro-ecological environments with the presence of the four tail-type groups (short fat-
96 tail, long fat-tail, fat-rump, and thin-tail) (Gizaw, 2009). This tail group classification is to a large
97 extent arbitrary, as it is based on the external visual examination of sheep tails (tail type and
98 length) and does not necessarily provide accurate information on tail morphology. Furthermore,
99 the approach does not consider other important tail measurement traits, such as tail width and
100 circumference, which are used as a measure of tail weight in breeding programs (Vatankah and
101 Talebi, 2008). Moreover, it does not take any osteological characters into consideration (caudal
102 vertebrae), which can provide precise and detailed osteological information on sheep caudal
103 vertebrae. Only a few studies mention variability in the number of caudal vertebrae in domestic
104 sheep breeds compared to its wild ancestor, the mouflon (*Ovis orientalis* or *Ovis gmelini*). The
105 mouflon has a short tail with 12-13 caudal vertebrae whereas this number can rise to 35 in
106 domestic sheep (Zeuner 1963) Cornevin and Lesbre reported between 3 to more than 24 caudal
107 vertebrae according to breeds (Cornevin and Lesbre, 1897). The number of caudal vertebrae is
108 between 8-10 in short-tailed and 16-18 in long-tailed European sheep (Dýrmundsson and
109 Niznikowski, 2010). Several studies have been carried out on the skeletal anatomy (osteology) of
110 sheep (Wilke et al., 1997, Boessneck et al. 1964), Boessneck 1969, Prummel and Frisch 1986,
111 Clutton-Brock and Pemberton, 2004, Salvagno and Albarella 2017, Haruda et al. 2019) among
112 others) but there is no readily available information on their tail osteology (caudal vertebrae),
113 unlike for other spinal regions (atlas, cervical, thoracic, and lumbar vertebrae) (May, 1964;
114 Wilke et al., 1997, Donaldson et al. 2013). Studies of vertebral variation in the thoracolumbar

115 (thoracic and lumbar) region are of particular interest to livestock breeders in term of meat
116 production. On the contrary, the tail region is only of interest for fat-tailed and fat-rumped sheep
117 breeders. On the other hand, detailed information on sheep tail morphology and osteology may
118 provide accurate phenotypes for genome mapping and adaptation studies, as well as baseline
119 osteological information for archaeological studies and sheep breed origins. Moreover,
120 estimation of the relationship between tail osteological and morphological traits in sheep may
121 help to provide a means for predicting traits which are not normally and easily measured from
122 the live sheep, e.g., osteological traits are recorded from slaughtered sheep but there should be a
123 method for estimating them without killing the animal. On the other hand, there should also be a
124 method enabling osteologists to differentiate one tail morphotype from the other based on caudal
125 vertebra measurements. Therefore, we must develop regression models to predict one trait from
126 the other.

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

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

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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

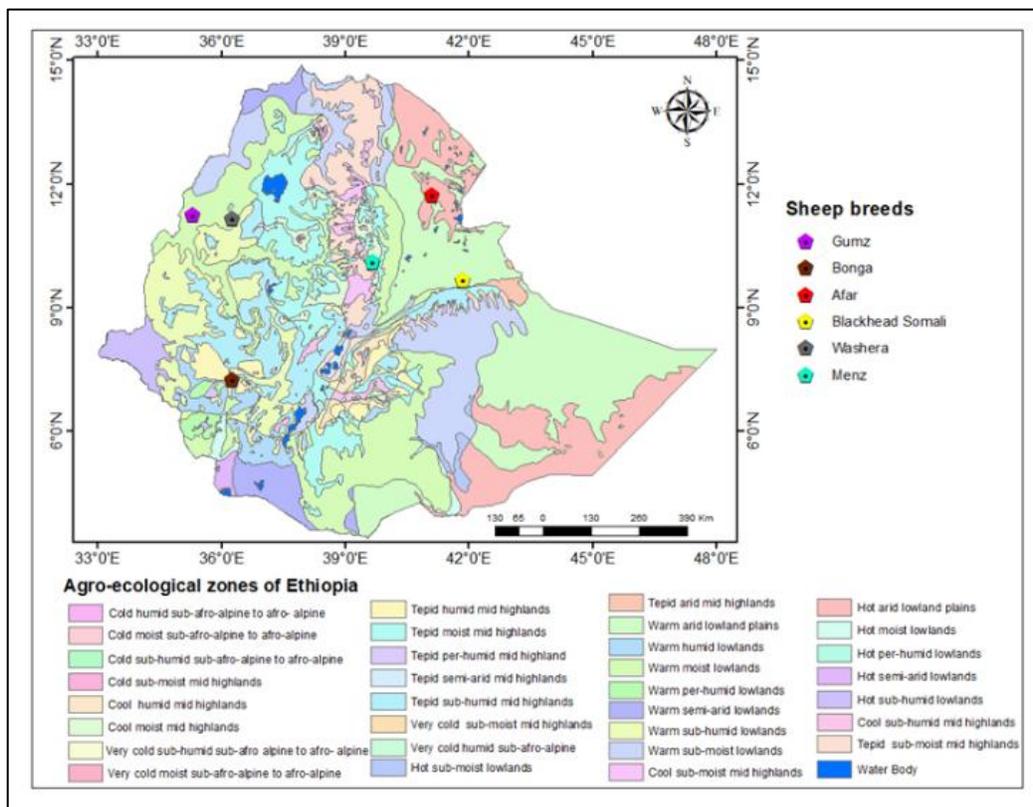
Sampling information for the studied sheep breeds.

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				

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

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



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 176 **Fig. 2.** Iconographic examples of the six sheep breeds examined with their skin peeled out tail,
 177 (A1) Menz sheep, (A2) Washera sheep, (B1) Afar sheep, (B2) Blackhead Somali sheep, (C)
 178 Bonga sheep, (D) Gumz sheep. A1 and A2 sheep represent the short fat-tail morphotype; B1 and
 179 B2 sheep represent the fat-rump morphotype; C sheep represents the long fat-tail morphotype
 180 and D sheep represents the thin-tail morphotype.

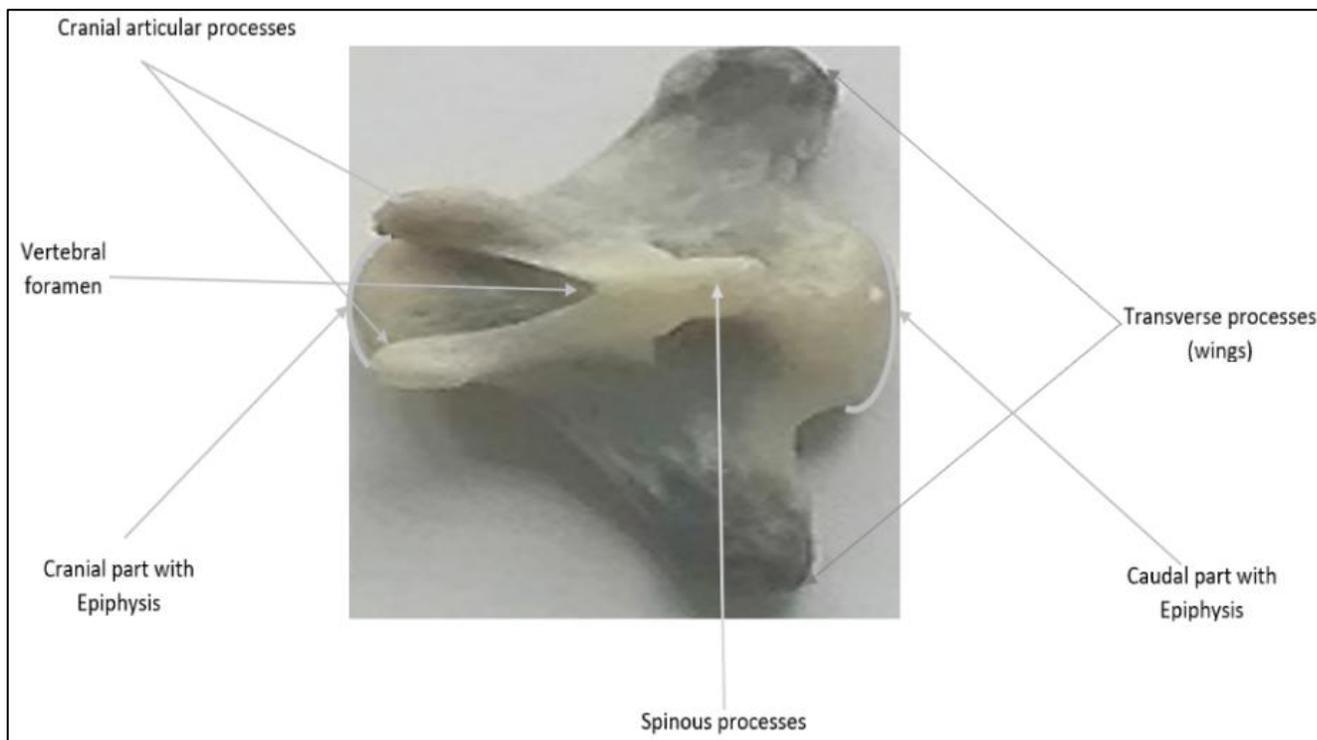
181 *2.2. Preparation of reference skeleton and caudal vertebrae*

182 Reference bone samples (skeleton) in general, and caudal vertebrae in particular, were
 183 obtained through a series of processing steps, including sheep slaughtering, evisceration;
 184 removal of the skin, meat, and muscle tissue using a knife; cooking, cleaning, and washing bones
 185 using washing powders; sun-drying the cleaned bones, and finally packing the dried bones in
 186 plastic boxes (Fig. 3). The bones were initially cooked for two hours, followed by two to three
 187 changes of water, depending on the level of fat deposition on the meat and bones of the animals,
 188 and further cooking. The tails (sacrum and caudal vertebrae) were prepared with particular care,
 189 separately from the rest of the skeleton, considering that caudal vertebrae, especially at the tip of

190 the tail, are extremely small. Caudal vertebrae refer to the variable number of bones (vertebrae)
191 in the tail of a sheep. See Fig. 4 for a description of a ‘typical vertebra’, as relevant to this study.



192
193 **Fig. 3.** Reference bone sample preparation, (a) washing and cleaning, (b) sun-drying and (c)
194 packing of bones.



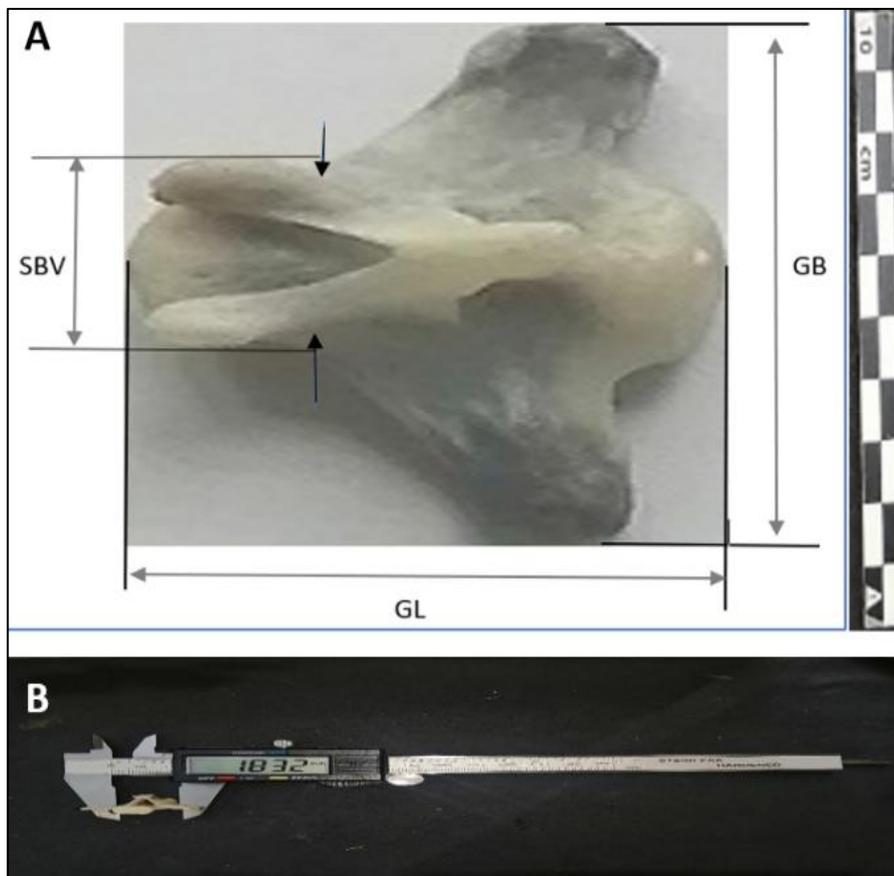
195
196 **Fig. 4.** Anatomical parts of a caudal vertebra.

197 2.3. Morphological and osteological data collection

198 To characterize the tail of the studied sheep, morphological and metric data were collected
199 from the tail and caudal vertebrae. The FAO (1986) sheep breed descriptor lists, and the Von den
200 Driesch (1976) animal bone measurement manual were used to quantitatively characterize the

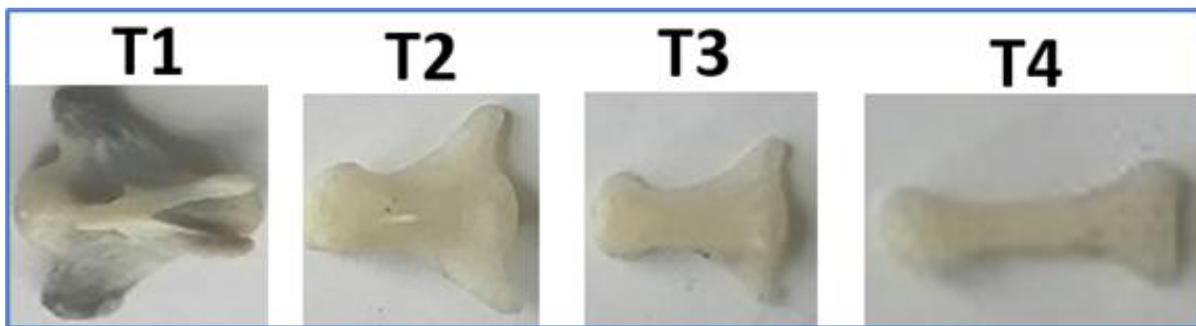
201 tail and caudal vertebrae of each sheep (Table 2). We developed our own protocol to
202 qualitatively describe each caudal vertebra by setting various criteria (Table 3).

203 Measurements for quantitative traits were taken from 40 live sheep and a total of 705 caudal
204 vertebrae. The quantitative traits in live sheep measured in centimetres using a flexible tape and
205 measuring stick were tail length (TL), tail width/breadth (TW), and tail circumference (TC). The
206 quantitative traits for each caudal vertebra were measured in millimetres using a calliper (Fig.
207 5A, B). They included the greatest length (GL) and greatest breadth (GB) and the smallest
208 breadth (SBV) of each caudal vertebra. Detailed descriptions of quantitative morphological and
209 osteological tail traits are presented in Table 2.



210
211 **Fig. 5.** Quantitative osteological tail traits, (A) measurements of each caudal vertebra (mm) and
212 (B) calliper. GL = Greatest Length, GB = Greatest Breadth over the wings, SBV = Smallest
213 Breadth of the vertebra.

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



224

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



226

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



228

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

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

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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

244 **Table 3**

245 Descriptions of the qualitative osteological tail traits.

Qualitative traits	Description
<i>1. Type category</i>	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)
<i>2. Shape category</i>	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)
<i>3. Symmetry category</i>	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

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247 2.4. *Statistical analyses*

248 Various analyses, including analysis of variance, correlation analysis, regression model
249 development, and multivariate analyses (linear discriminant analysis, principal component
250 analysis and canonical correlation analysis) were performed using different packages and
251 functions in the software R v4.0.1 (R Core Team, 2020)

252 2.4.1. *Means of tail morphological and osteological traits*

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

$$260 Y_{ij} = u + G_i + e_{ij}$$

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

262 u = overall mean

263 G_i = Fixed effect of sheep group (i = SFT, FR, LFT, TT)

264 e_{ij} = effect of random error

265 2.4.2. *Correlation analysis and regression model development*

266 Pearson correlation analysis was used to determine and statistically test the nature of the
267 association of pairs of morphological and osteological tail traits in the whole dataset (17 traits:
268 three tail measurements and 14 osteological tail traits), as presented in Table 6 and Fig. 11.
269 Regression models were developed for significantly and positively correlated osteological and
270 morphological tail characters (Table 6, Fig. 11). Moreover, as shown by the analysis of variance
271 results, these characters clearly allow for separation of the studied Ethiopian sheep into three
272 main tail morphotypes (short-tail, medium-tail, long-tail). These characters are the three

273 osteological tail traits (total caudal vertebrae length, caudal vertebrae number, individual
274 vertebra length) and one tail measurement character (tail length). Regression models for the two
275 osteological tail characters, total caudal vertebrae length and caudal vertebrae number, from the
276 external tail measurement, tail length, were developed (Fig. 12A and B, respectively). Moreover,
277 based on the individual caudal vertebra measurement of the animal, we developed regression
278 models for the other two osteological tail traits, caudal vertebrae length and caudal vertebrae
279 number, and for the external tail measurement, tail length (Fig. 13A and B, respectively).

280

281 *2.2.3. Multivariate analyses*

282 Multivariate statistical analysis (MVA) techniques such as linear discriminant analysis
283 (LDA), principal component analysis (PCA), and canonical correlation analysis (CCA) were
284 employed to investigate the morphological structure of the studied sheep tail groups or sheep
285 breeds. The analyses were performed separately for sheep tail groups and breeds based on both
286 morphological and osteological tail traits. A separate analysis was performed for sheep tail
287 groups and breeds based only on osteological tail traits to assess possible differences in
288 osteological traits linked to the tail of the studied sheep. Linear discriminant analysis (LDA)
289 generates useful linear discriminant functions (LDs) for discriminating sheep tail groups or
290 breeds. The canonical correlation (CC), in canonical correlation analysis, measures the
291 correlation between each LD and sheep tail groups or breeds. The CC value obtained indicates
292 the ability of the LD to separate sheep tail groups or breeds. The closer the CC value is to 1, the
293 higher the discriminating ability. One way of displaying LDA results is to create a stacked
294 histogram of the values of linear discriminant functions for the samples from different sheep tail
295 groups or breeds. It is also important to investigate how each LD separates the studied sheep tail
296 groups or breeds. Principal component analysis (PCA), another multivariate analysis technique,
297 also generates useful principal components (PCs) for discriminating the sheep tail groups or
298 breeds.

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

302 how important the linear discriminant function is to discriminate the studied sheep tail groups or
303 breeds.

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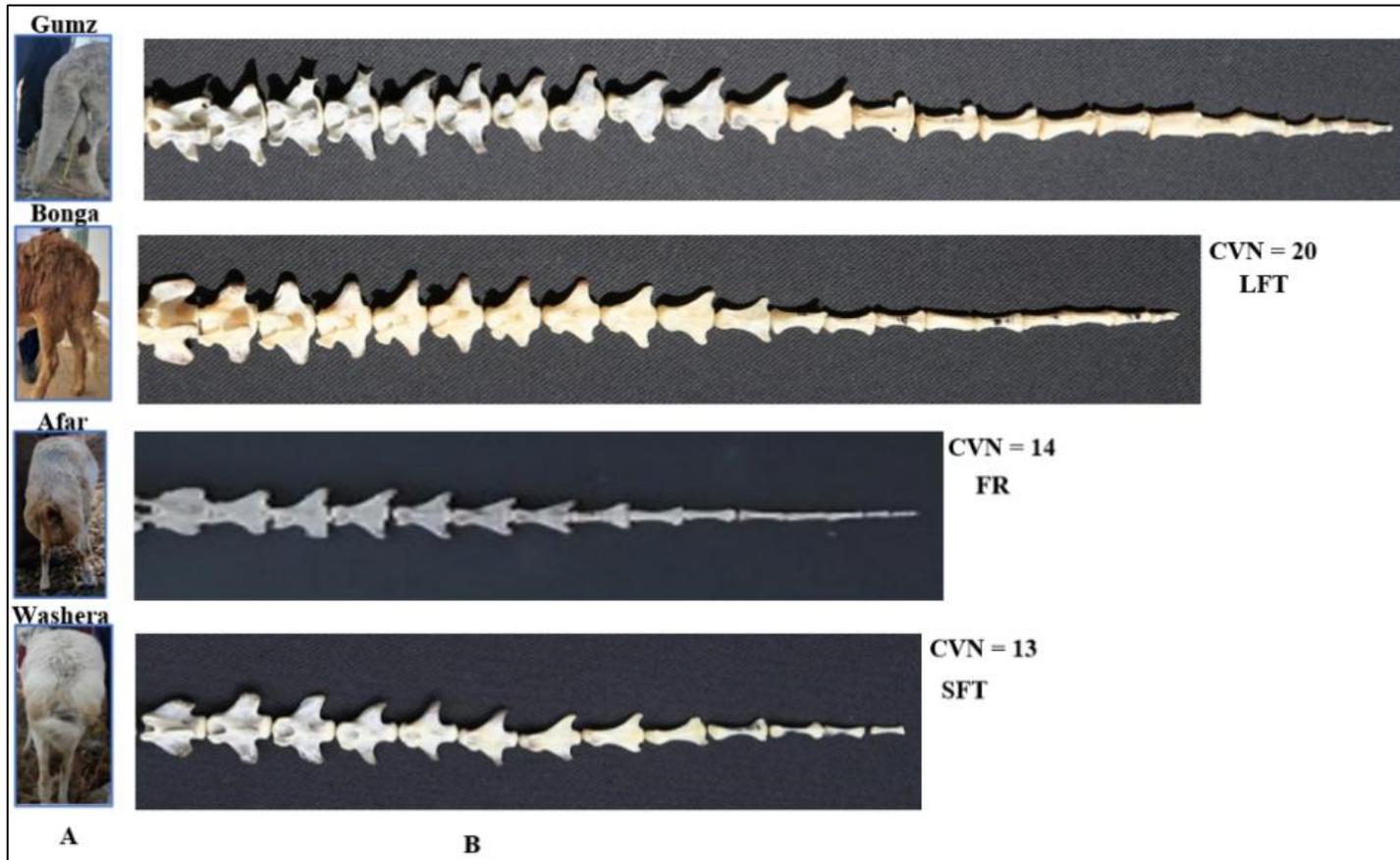
324

325 3. Results

326 3.1. Mean values of tail morphological and osteological traits.

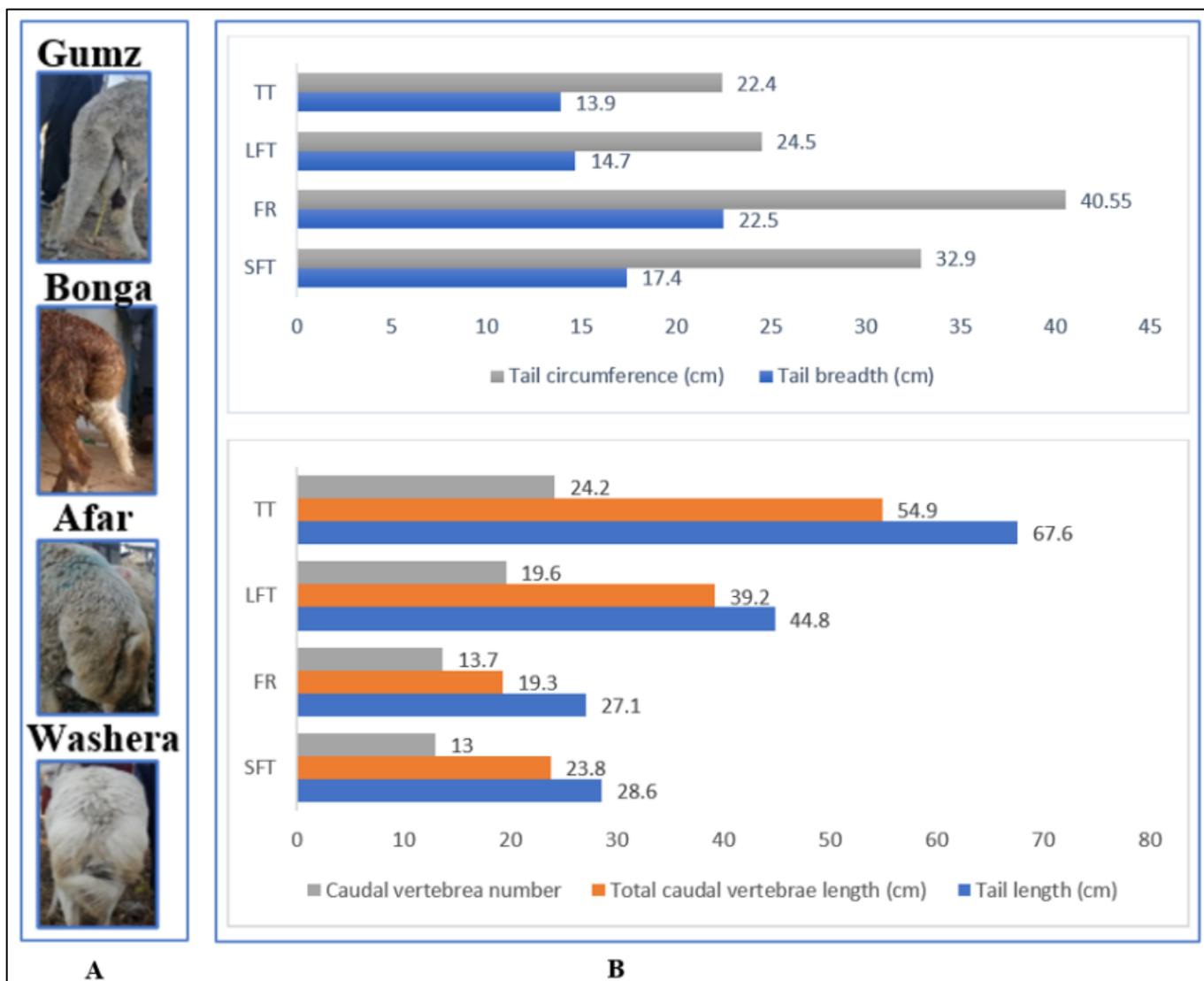
327 The mean values of quantitative morphological and osteological tail traits of the four
328 sheep tail morphotypes are presented in Table 4. The age of the animals (adult and subadults) did
329 not have a significant ($P > 0.05$) effect on the measurements and counts of the different caudal
330 vertebra types for the four sheep tail morphotypes (Supplementary Table 1). Accordingly, all the
331 comparisons were made between animals regardless of their age. Total caudal vertebrae length,
332 caudal vertebrae number, and tail length were significantly different ($P < 0.05$) for the long fat-
333 tail (LFT) compared to the three other tail morphotypes, as well as for the thin-tail (TT)
334 compared to the other three other tail morphotypes, with the highest mean values recorded for
335 the thin-tailed sheep (total caudal vertebrae length: 54.9 ± 6.38 cm, caudal vertebrae number:
336 24.2 ± 1.48 , range 21-26, tail length: 67.6 ± 4.62 cm), followed by the long fat-tailed sheep (total
337 caudal vertebrae length: 39.2 ± 3.50 cm, caudal vertebrae number: 19.6 ± 1.26 , range 18-21, tail
338 length: 44.8 ± 5.85 cm). The lowest mean values were observed in the fat-rumped (total caudal
339 vertebrae length: 19.3 ± 2.70 cm, caudal vertebrae number: 13.7 ± 1.02 , range 11-15, tail length:
340 27.1 ± 7.53 cm) and the short fat-tailed (total caudal vertebrae length: 23.8 ± 3.12 cm, caudal
341 vertebrae number: 13.0 ± 1.25 , range 12-15, tail length: 28.6 ± 3.81 cm) sheep (Table 4).
342 Measurements for caudal vertebra breadth (CVB) were significantly different ($P < 0.05$) for
343 long-tailed (LFT, TT) and short-tailed (SFT, FR) sheep, with the highest mean value observed in
344 the long-tailed sheep (22.85 ± 2.60 mm) and the lowest value in the short-tailed sheep ($20.3 \pm$
345 2.6 mm). Tail width/breadth, and tail circumference were significantly different ($P < 0.05$) for
346 the long-tailed and short-tailed sheep with the highest mean values observed in the short-tailed
347 sheep (tail width: 19.95 ± 2.22 , tail circumference: 36.73 ± 7.75 cm) and the lowest values in the
348 long-tailed sheep (tail width: 14.3 ± 2.17 , tail circumference: 23.45 ± 6.49 cm). Individual caudal
349 vertebra length is significantly ($P < 0.05$) different for the short fat-tail (SFT) compared to the
350 three other tail morphologies, as well as for the fat-rump compared to the three other sheep tail
351 morphotypes, with the highest mean value in the long-tailed sheep (LFT, TT) (21.35 ± 1.82 mm),
352 and the lowest mean value in the fat-rumped sheep (13.9 ± 1.51 mm), followed by the short fat-
353 tailed sheep (18.2 ± 1.18 mm). Measurements for caudal vertebra thickness (CVT) were
354 significantly different ($P < 0.05$) for the fat-rumped (FR) sheep and the three other sheep tail

355 morphotypes, with the lowest mean value observed in the fat-rumped sheep (4.9 ± 0.84 vs $8.07 \pm$
356 1.05 mm). Visual tail lengths, tail shape, size, morphological and osteological tail traits of the
357 four sheep tail morphotypes are shown in Fig. 9A, B, and Fig. 10A, B.



358
359 **Fig. 9.** Sheep tail morphotypes with visual tail phenotype and caudal vertebrae number, (A)
360 visual tail lengths, (B) number of caudal vertebrae (CVN). SFT = short fat-tail (Washera) , FR =
361 fat-rump (Afar), LFT = long fat-tail (Bonga) and TT = thin-tail (Gumz).

362



363

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

368 **Table 4**

369 Mean values of quantitative morphological and osteological tail traits of the four sheep tail
 370 morphotypes (10 sheep/tail group) from different caudal vertebrae categories (type, shape and
 371 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

372 ^{a,b,c}Means within a row with different superscript letters are significantly different ($P < 0.05$).
373 ^{a,b,c}Means within a row with the same superscript letters are not significantly different ($P >$
374 0.05). ICVL: Individual caudal vertebra length. TCVL: Total caudal vertebrae length.
375 *Measurements in cm. **Measurements in mm.

376

377 The mean number of qualitative osteological tail traits (type, shape and symmetry
378 categories) of the four sheep tail morphotypes are presented in Table 5. Type 1 (T1) caudal
379 vertebrae are significantly ($P < 0.05$) different for the short fat-tail (SFT) compared to the three
380 other tail morphotypes, as well as for the fat-rump compared to the three other sheep tail
381 morphotypes. The highest mean number of T1 caudal vertebrae was observed in the long-tailed
382 sheep (LFT, TT) (8.15 ± 1.23), and the lowest mean number in the fat-rumped (3.9 ± 1.29) and
383 the short fat-tailed (6.1 ± 1.61) sheep. Type 4 (T4) caudal vertebrae are significantly different (P
384 < 0.05) for the long fat-tail and fat-rump compared to the two other sheep tail morphotypes as
385 well as for the short fat-tail compared to the three other sheep tail morphotypes ($P < 0.05$), with
386 the highest and the lowest mean numbers observed in the thin-tailed (8.9 ± 2.02) and the short
387 fat-tailed (2.2 ± 0.63) sheep, respectively. The mean number of Type 2 (T2), flat-shaped (S1),
388 and concave-shaped (S2) caudal vertebrae was significantly different ($P < 0.05$) for long-tailed
389 (LFT, TT) and short-tailed (SFT, FR) sheep, with the highest and the lowest mean numbers
390 observed in the long-tailed (type 2: 3.45 ± 1.0 , flat-shaped: 6.5 ± 2.29 , concave-shaped: $2.55 \pm$
391 1.25) and short-tailed (type 2: 2.35 ± 0.95 , flat-shaped: 3.85 ± 1.51 , concave-shaped: 1.2 ± 0.94)
392 sheep, respectively (Table 5). Moreover, the mean number of asymmetric (A) caudal vertebrae is
393 significantly ($P < 0.05$) different for the fat-rump (FR) compared to the three other tail
394 morphotypes. The lowest mean number was observed in the fat-rumped sheep (2.3 ± 2.06 vs 7.97

395 ± 2.31), whereas the mean number of symmetric (S) caudal vertebrae is significantly ($P < 0.05$)
 396 different for the short fat-tailed sheep compared to the three other tail morphotypes, with the
 397 lowest mean number observed in the short fat-tailed sheep (0.9 ± 0.99 vs 3.57 ± 1.98) (Table 5).

398

399 **Table 5**

400 Mean number of qualitative osteological tail traits (type, shape and symmetry) of the four sheep
 401 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

402 ^{a,b,c}Means within a row with different superscript letters are significantly different ($P < 0.05$).

403 ^{a,b,c}Means within a row with the same superscript letters are not significantly different ($P >$
 404 0.05).

405 **3.2. Correlation analysis and regression model development**

406 The correlation analysis was performed on pairs of all traits for the whole dataset (Table 6,
 407 Fig. 11). The Pearson correlation coefficient was represented by r_p . A $r_p > 0.7$ was defined as a
 408 high correlation (Dormann et al. 2013). Among the external morphological tail traits, tail
 409 width/breadth and tail circumference show a high correlation ($r_p = 0.88$, $P < 0.001$), whereas tail

410 length against tail circumference show a moderate correlation ($r_p = -0.57, P < 0.001$). However,
 411 tail length showed a high correlation with osteological tail traits such as total caudal vertebrae
 412 length ($r_p = 0.96, P < 0.001$), caudal vertebrae number ($r_p = 0.95, P < 0.001$), individual caudal
 413 vertebrae length ($r_p = 0.78, P < 0.001$), type 1 caudal vertebrae ($r_p = 0.73, P < 0.001$) and a
 414 moderate correlation with type 4 caudal vertebrae ($r_p = 0.61, P < 0.001$). Among the osteological
 415 tail traits, high correlations ($r_p = 0.73$ to $0.96, P < 0.001$) were observed among total caudal
 416 vertebrae length, caudal vertebrae number, individual caudal vertebrae length, and type 1 caudal
 417 vertebrae. A moderate correlation was observed between type 2 and symmetry caudal vertebrae
 418 ($r_p = 0.53, P < 0.001$), flat-shaped and type 1 caudal vertebrae ($r_p = 0.60, P < 0.001$), symmetry
 419 and type 4 caudal vertebrae ($r_p = 0.52, P < 0.001$), and among concave-shaped, asymmetric and
 420 type 1 caudal vertebrae ($r_p = 0.50$ to $0.62, P < 0.001$) (Table 6, Fig.11).

421

422 **Table 6**

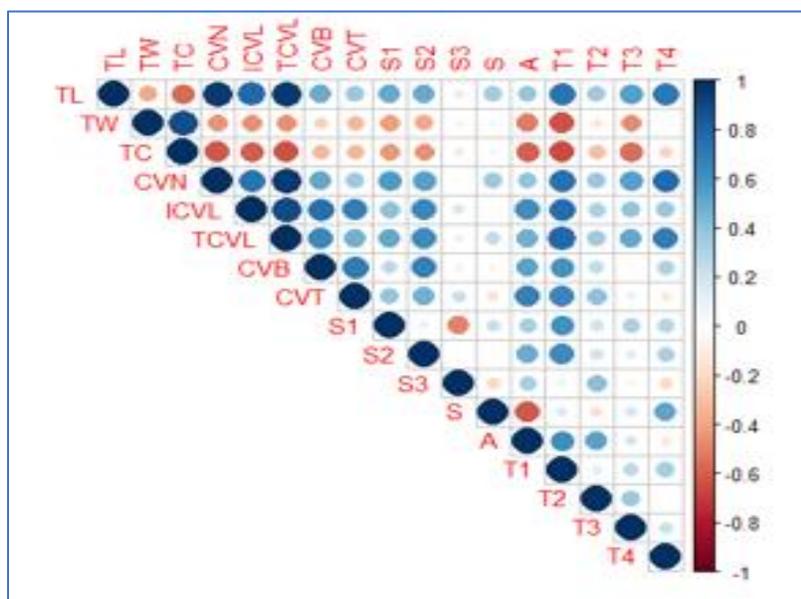
423 Correlation among pairs of morphological and osteological tail traits in the studied sheep.

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

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

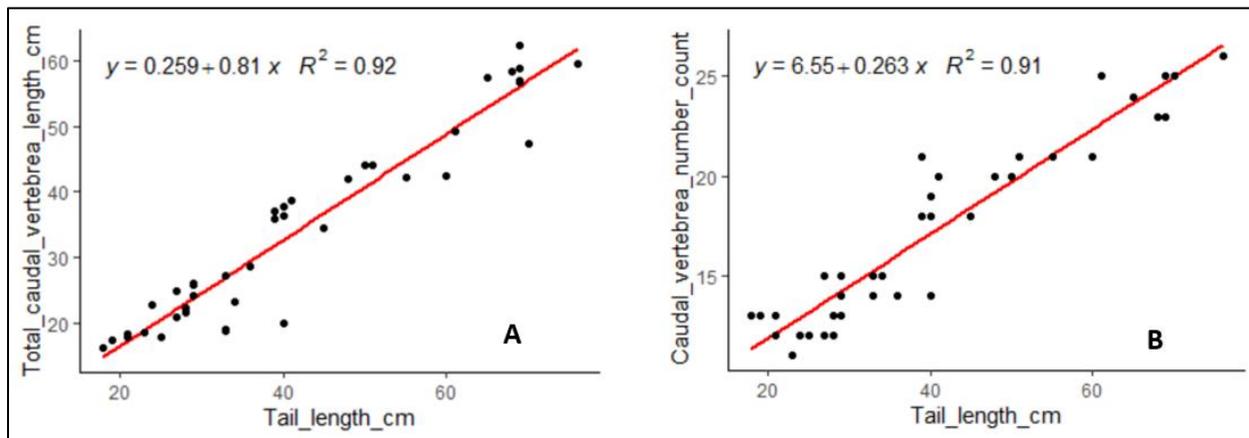
432



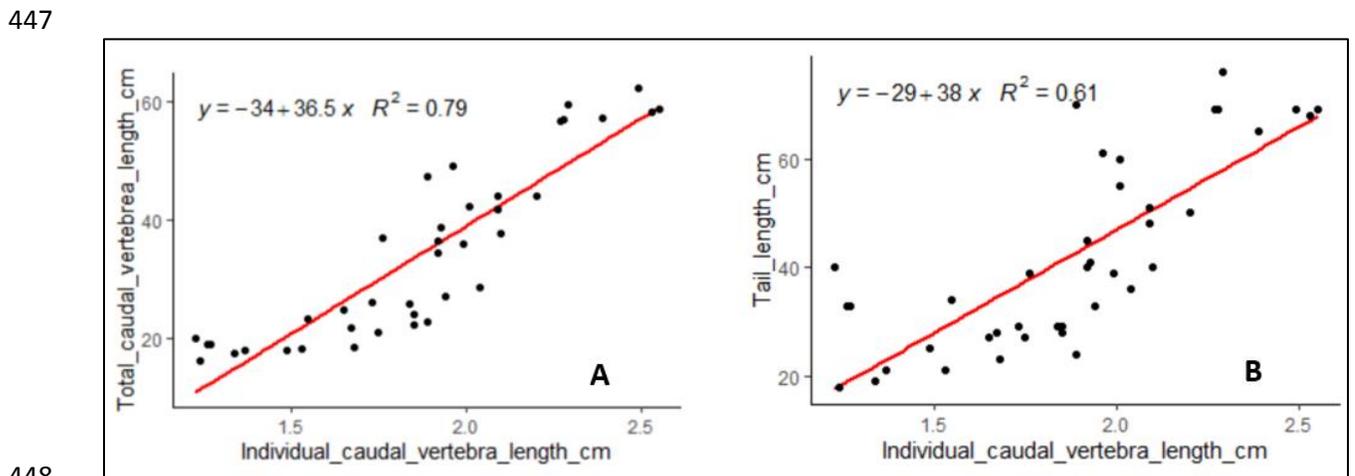
433

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

437 For regression model development, we considered three osteological (total caudal vertebrae
438 length, caudal vertebrae number, and individual caudal vertebra length) and one external tail
439 measurement (tail length) characters that significantly and positively correlate (Table 6, Fig. 11).
440 Based on this, regression models for total caudal vertebrae length and for caudal vertebrae
441 number from the tail length of the sheep were developed as indicated in Fig. 12A and 12B,
442 respectively. We also developed regression models for total caudal vertebrae length and for tail
443 length from individual caudal vertebra length of the sheep (Fig. 13A and 13B, respectively).



444
445 **Fig. 12.** Linear regression models, (A) estimation of total caudal vertebrae length using external
446 tail measurement, tail length, (B) estimation of total caudal vertebrae number using tail length.

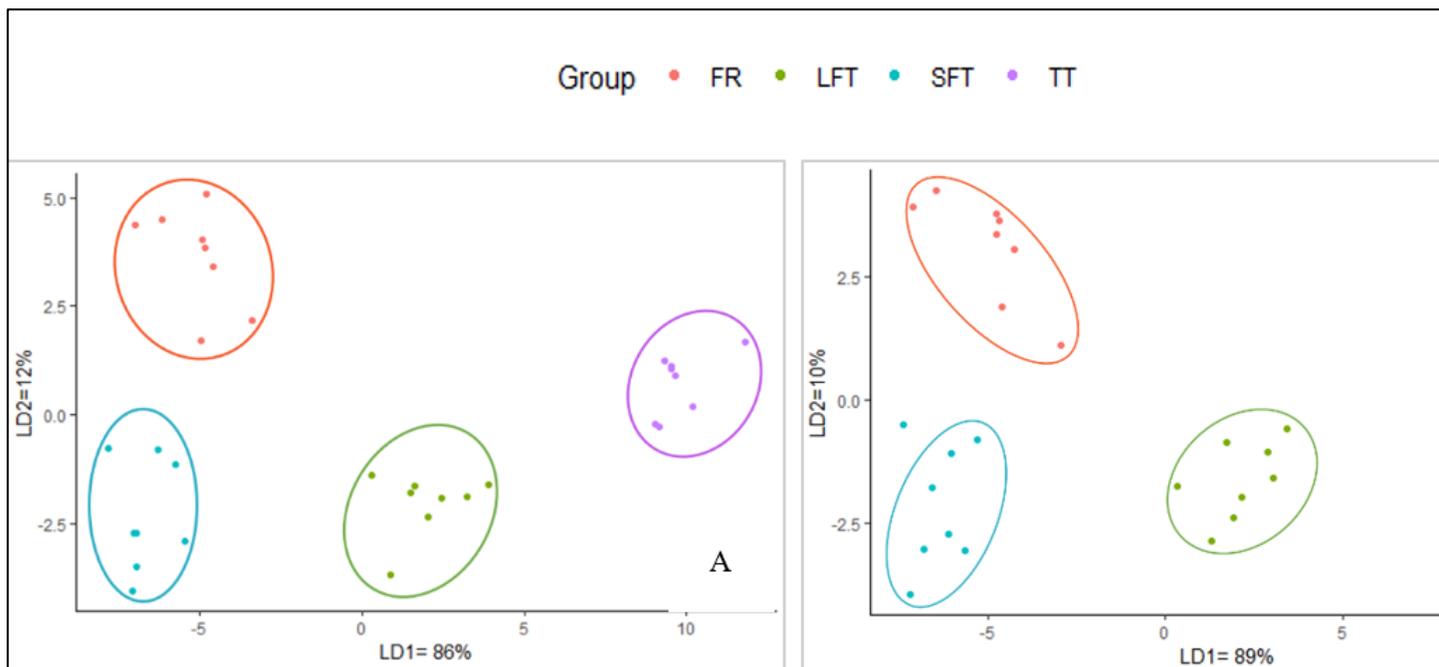


448

449 **Fig. 13.** Linear regression model, (A) estimation of total caudal vertebrae length using individual
450 vertebra length, (B) estimation of tail length using individual caudal vertebra length.
451

452 3.3. Linear discriminant analysis

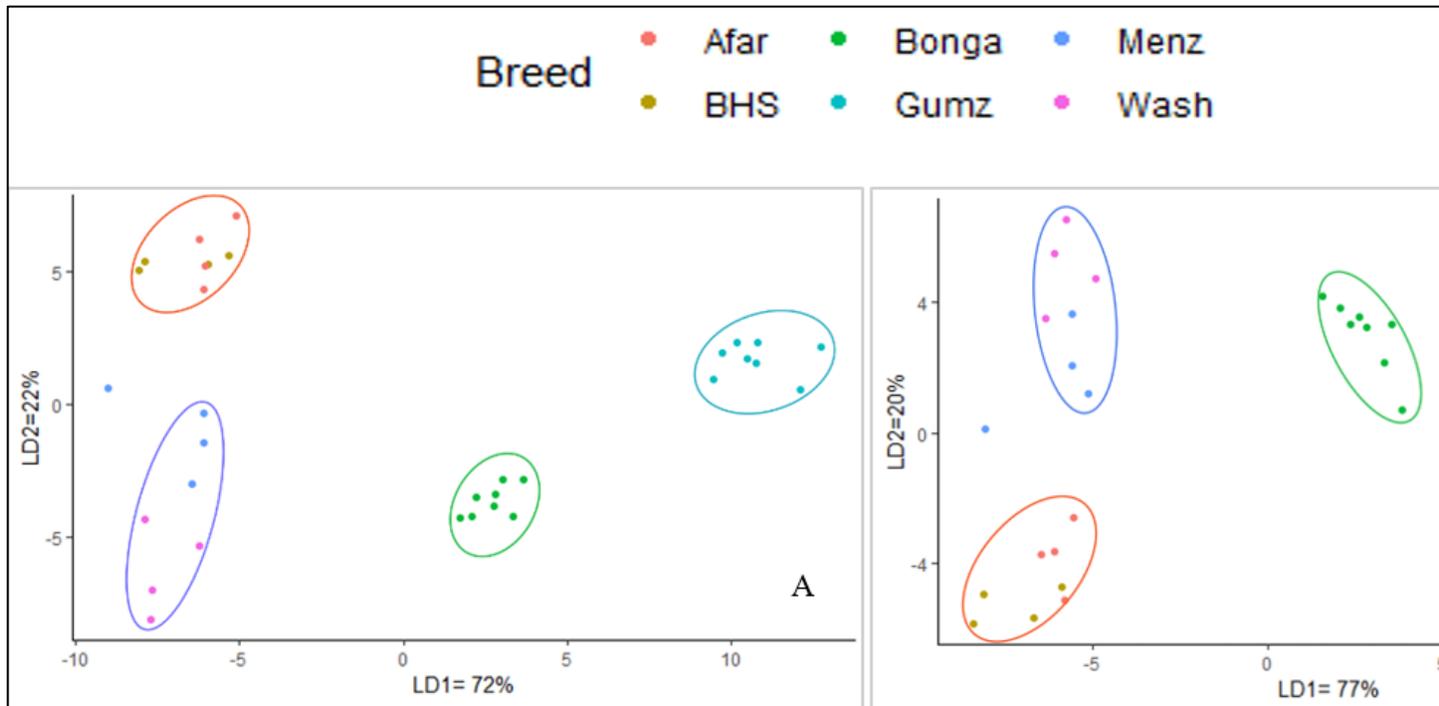
453 The scatterplot (linear discriminant analysis: LDA plot) for sheep tail morphotypes based on
454 morphological and osteological tail traits (Fig. 14A) indicates that the first two linear
455 discriminant functions (LDs: LD1 and LD2) account for 86% and 12% of the total variation,
456 respectively,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



466 **Fig. 14.** Linear discriminant analysis of Ethiopian sheep tail morphotypes using the LDA plot
467

468 based on: (A) morphological and osteological tail traits and (B) osteological tail traits only. FR:
469 fat-rump; LFT: long-fat tail; SFT: short fat-tail; TT: thin-tail.

470 Linear discriminant analysis (LDA plot) performed for sheep breeds based on both
471 morphological and osteological tail traits (Fig. 15A) indicates that the first two LDs account for
472 72% and 22% of the total variation, respectively, and categorizes the six sheep breeds into four
473 sheep tail groups according to tail morphology and osteology: the two fat-rumped (FR) sheep
474 breeds (Afar and BHS) form one cluster tail morphotype (FR tail morphotype). Similarly, the
475 two short fat-tailed (SFT) sheep breeds (Washera and Menz) form another cluster tail
476 morphotype (SFT tail morphotype). LD1 clearly separates the FR and SFT sheep breeds from
477 long fat-tailed (LFT) (Bonga) and thin-tailed (TT) (Gumz) sheep breeds, whereas LD2 separates
478 the FR and TT sheep breeds from the SFT and LFT sheep breeds. The finding was well
479 supported by a separate LDA plot performed for sheep breeds based on osteological tail traits
480 only, which indicates that the first two linear discriminant functions account for 77% and 20% of
481 the total variation, respectively (osteological traits LDA plot: Fig. 15B). LD1 clearly separates
482 the FR and SFT breeds from LFT (Bonga) and TT (Gumz) breeds, whereas LD2 separates the
483 FR and TT breeds from the SFT and LFT sheep breeds. A separate LDA plot result obtained for
484 sheep breeds was further supported by a stacked histogram of the LD values for the samples
485 from different sheep breeds (Supplementary Fig. 2B1, B2).



486

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

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

500

501 **Table 7**

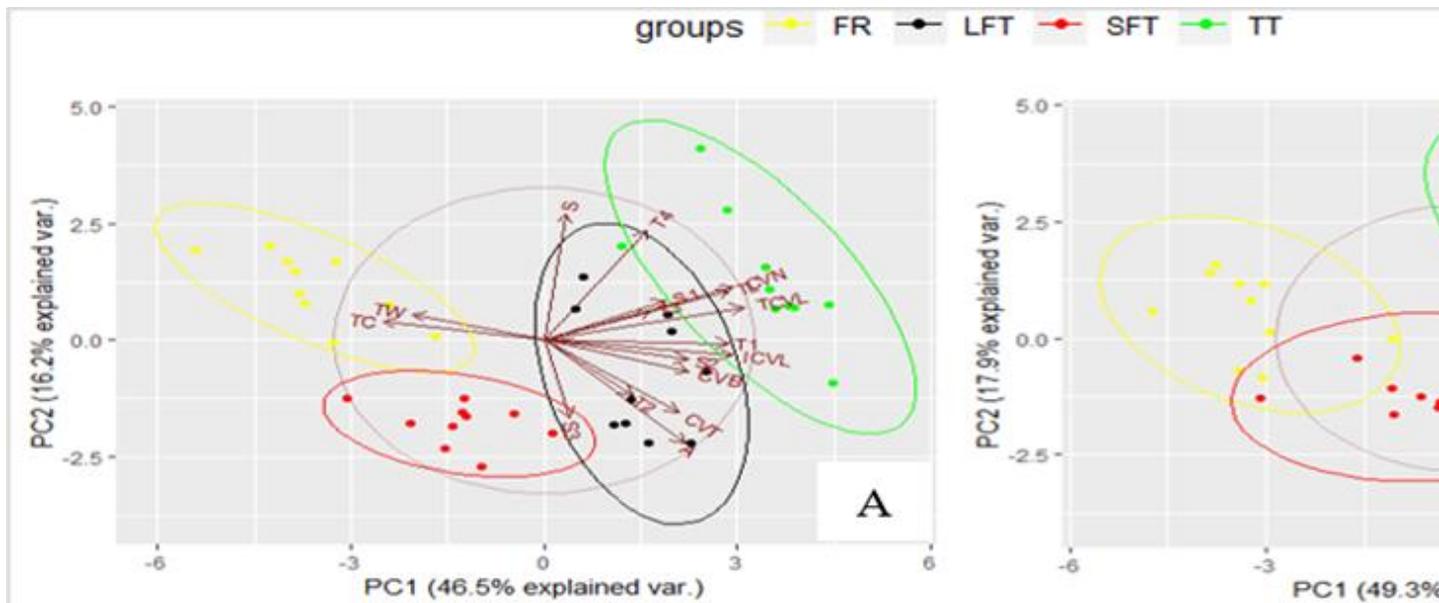
502 Eigen values, proportion of variability (%) and canonical correlation (%) explained by the first
503 two discriminant functions (LD1 and LD2).

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

504

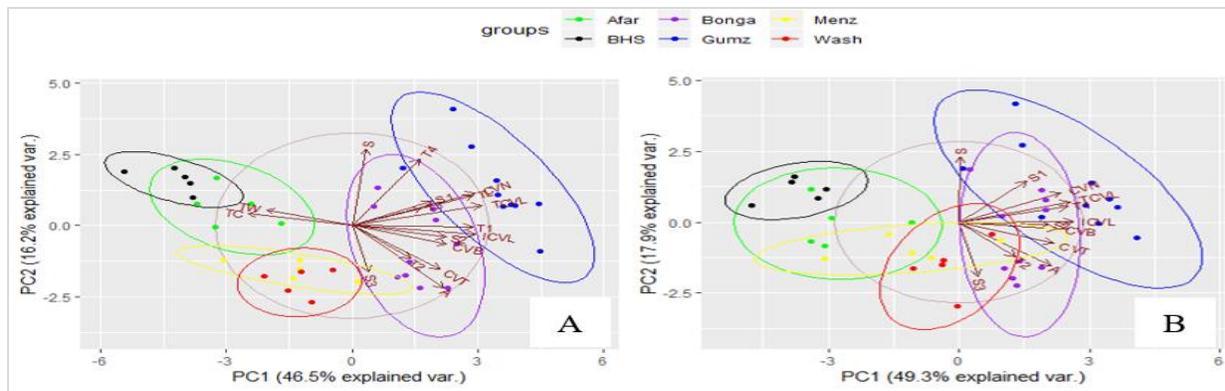
505 3.4. Principal component analysis

506 The principal component analysis (PCA) based on morphological and osteological tail traits
507 (Fig. 16) indicated that the first two principal components (PCs) accounted for 62.7% (PC1:
508 46.5%, PC2: 16.2%) of the total variation and clearly differentiated the four sheep tail
509 morphotypes. PC1 separates the two tail morphotypes very well: the short-tailed (SFT: short fat-
510 tail and FR: fat-rump) from the long-tailed (LFT: long fat-tail and TT: thin-tail) sheep tail
511 morphotypes. PC2 separates FR and most of the TT tail morphotypes samples from SFT and
512 some of the LFT tail morphotype samples. The result obtained (all traits PCA) was backed up by
513 a separate PCA performed for sheep tail morphotypes based on osteological tail traits only
514 (Osteological PCA: Fig. 16B). Similarly, PC1 separates the two tail morphotypes very well: the
515 short-tailed from the long-tailed sheep. PC2 separates FR and most of the TT tail morphotypes
516 samples from SFT and some of the LFT tail morphotype samples.



517
 518 **Fig. 16.** Principal component analysis of Ethiopian sheep tail morphotype based on: (A)
 519 morphological and osteological tail traits and (B) osteological tail traits only. SFT = short fat-
 520 tail; FR = fat-rump; LFT = long fat-tail; TT =thin-tail.

521 A separate PCA based on morphological and osteological tail traits (Fig. 17A) indicated
 522 that the first two principal components (PCs) accounted for 62.7% (PC1: 47.9%, PC2: 17.05%)
 523 of the total variation and categorizes the six sheep breeds into four tail morphotypes. The result
 524 revealed that close clustering of the two fat-rumped (FR) sheep breeds (Afar and BHS) forms
 525 one cluster tail morphotype (FR tail morphotype). Similarly, the result indicated that close
 526 clustering of the two short fat-tailed (SFT) sheep breeds (Washera and Menz) forms another one
 527 cluster tail morphotype (SFT tail morphotype). PC1 clearly separates the FR and SFT sheep
 528 breeds from LFT (Bonga) and TT (Gumz) sheep breeds, whereas PC2 separates the FR and most
 529 of the TT sheep breed samples from SFT and some of the LFT sheep breed samples. The result
 530 obtained (all traits PCA) was supported by a separate PCA for sheep breeds based on
 531 osteological tail traits only (osteological traits PCA: Fig. 17B). Similarly, PC1 clearly separates
 532 FR and SFT sheep breeds from LFT and TT sheep breeds, whereas PC2 separates the FR and
 533 most of the TT sheep breed samples from SFT and some of the LFT sheep breed samples.



534

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

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546 **4. Discussion**

547 In this study, we used morphological and osteological tail data to characterize and
 548 differentiate the tail morphotypes of Ethiopian sheep. Our objectives were to document in detail
 549 the characteristics of the different sheep tail types found in Ethiopia at morphological and
 550 osteological levels; to provide baseline osteological information on sheep caudal vertebra
 551 morphologies as reference material for zooarchaeological studies of sheep husbandry; to provide
 552 accurate phenotypic descriptions for genome mapping studies aiming to elucidate the genetic
 553 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.

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

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628 The linear discriminant analysis indicated that the first two linear discriminant functions
629 (LD1 and LD2) accounted for 72-86% and 12-22% of the total variation in differentiating the
630 four sheep tail morphotypes as well as the six sheep breeds, respectively (Fig. 14,15), which
631 further indicates that LD1 achieves a good separation of the four sheep tail groups as well as the
632 six sheep breeds, but that LD2 only slightly improves the separation. Therefore, to achieve a
633 better separation of the sheep tail groups and breeds, it is necessary to use both the first and
634 second discriminant functions (LD1 and LD2) to differentiate the four sheep tail groups, as well
635 as the six sheep breeds, as they accounted for 98% and 94% of the total variation in the dataset,
636 respectively (Fig. 14, 15). Moreover, the observed high canonical correlation (CC) between the
637 first two linear discriminant functions and sheep tail morphotypes (LD1 = 99%, LD2 = 93%),
638 and sheep breeds (LD1 = 99%, LD2 = 98%) indicates that the two linear discriminant functions
639 are more effective in discriminating the studied sheep according to tail morphology and
640 osteology, as their canonical correlation is close to 1 (Table 7). The principal component analysis
641 (PCA) showed that most of the variation between samples in the whole dataset can be captured
642 using the first two principal components (PC1 and PC2), as they accounted for over 62% of the
643 total variation (Fig. 16, 17).

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

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

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679 **5. Conclusions**

680 The significant ($P < 0.05$) differences observed in tail measurements, osteological
681 measurements, and caudal vertebra characteristics among the four sheep tail morphotypes
682 indicate differences in tail length, tail shape, tail size, and morphometry of each caudal vertebra
683 of the studied sheep. Osteological tail traits (total caudal vertebrae length and caudal vertebrae
684 number), and tail measurements (tail length) clearly allow for separation of the studied Ethiopian
685 sheep into three main tail morphotypes (short-tail, medium-tail, long-tail). We also observe that
686 osteological measurements (individual caudal vertebra breadth), morphological characteristics
687 (type 2, flat and concave-shaped caudal vertebrae), and tail measurements (tail width and tail
688 circumference) clearly separate the short-tailed sheep from the long ones. Moreover, caudal
689 vertebra characteristics (particularly their symmetry or asymmetry), and osteological
690 measurements (individual caudal vertebra length and thickness) of the fat-rumped and the short
691 fat-tailed sheep are clearly distinct from the other tail morphotypes. Based on the combination of
692 the measured, observed, and recorded osteological and morphological tail traits, Ethiopian sheep
693 could be categorized into three major tail groups: Short-tailed sheep with a large fat-tail or a
694 massive fat-rump, medium-tailed sheep with a small fat-tail, and long-tailed sheep with a thin
695 fat-tail. Both the linear discriminant (LDA) and principal component (PCA) analyses revealed
696 four distinct sheep tail morphotypes, categorizing the six breeds of sheep examined according to
697 their tail morphology and osteology. Moreover, the LDA and canonical correlation analysis
698 revealed that the first two discriminant functions are efficient and are more effective in
699 discriminating between the four sheep tail morphotypes as well as the sheep breeds. It is possible
700 to use the external tail measurement, tail length, alone to estimate the sheep tail osteology
701 without killing the animal (Fig. 12A, B). Moreover, by measuring sheep caudal vertebrae,

702 osteologists can estimate other osteological traits and the tail length of that sheep (Fig. 13A, B),
703 which further enables them to differentiate its tail morphotype. The previous classification of
704 Ethiopian sheep based on external visual examination of their tails (tail length and type) (Gizaw,
705 2009), should be supported by detailed osteological and morphological analyses of their tail
706 which might provide accurate information about tail morphology, as well as a precise and
707 detailed osteological information about sheep caudal vertebrae. This study combined for the first
708 time systematic external tail morphology with a detailed osteological analysis of sheep tails. The
709 results are of great interest for archaeozoological studies and provide a baseline for the
710 investigation of the evolution of sheep tails since domestication and for an enhanced
711 understanding of the history of the introduction of sheep breeds on the African continent. The
712 results are also of great interest for genome mapping studies which aim to elucidate the genetic
713 control of sheep tail morphotypes.

714

715 **Declaration of competing interest**

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

718

719 **Acknowledgements**

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

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

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742 **References**

743 Ahbara, A., Bahbahani, H., Almathen, F., Al Abri, M., Agoub, M. O., Abeba, A., Kebede, A.,
744 Musa, H. H., Mastrangelo, S., Pilla, F., Ciani, E., Hanotte, O., Mwacharo, J. M., 2019.
745 Genome-wide variation, candidate regions and genes associated with fat deposition and tail
746 morphology in Ethiopian indigenous sheep. *Front. Genet.* 9, 699. doi:
747 10.3389/fgene.2018.00699.

748 Amane, A., Belay, G., Nasser, Y., Kyalo, M., Dessie, T., Kebede, A., Getachew, T., Entfellner,
749 J.B.D., Edea, Z., Hanotte, O. and Tarekegn, G.M., 2020. Genome-wide insights of Ethiopian
750 indigenous sheep populations reveal the population structure related to tail morphology and
751 phylogeography. *Genes & Genomics.* 42, 1169–1178. [https://doi.org/10.1007/s13258-020-](https://doi.org/10.1007/s13258-020-00984-y)
752 [00984-y](https://doi.org/10.1007/s13258-020-00984-y).

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

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

758 Chilliard, Y., Ferlay, A., Faulconnier, Y., Bonnet, M., Rouel, J., Bocquier, F., 2000. Adipose
759 tissue metabolism and its role in adaptations to undernutrition in ruminants. *Proceedings of*
760 *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.

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

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

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

799 Kang, D., Zhou, G., Zhou, S., Zeng, J., Wang, X., Jiang, Y., Yang, Y., Chen, Y., 2017.
800 Comparative transcriptome analysis reveals potentially novel roles of Homeobox genes in
801 adipose deposition in fat-tailed sheep. Sci. Rep. 7, 14491. doi: 10.1038/s41598-017-14967-
802 9.

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

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

807 Lv, F.-H., Peng, W.-F., Yang, J., Zhao, Y.-X., Li, W.-R., Liu, M.-J., Ma, Y.-H., Zhao, Q.-J.,
808 Yang, G.-L., Wang, F., Li J.-Q., Liu, Y.-G., Shen, Z.-Q., Zhao, Sh.-G., Hehua, EE.,
809 Gorkhali, N.A., Vahidi, F. S. M., Muladno, M., Naqvi, A.N., Tabel, J., Iso-Touru, T.,
810 Bruford, M.W., Kantanen, J., Han, J.-L., Li, M.-H., 2015. Mitogenomic meta-analysis
811 identifies two phases of migration in the history of eastern Eurasian sheep. Mol. Biol. Evol.
812 32, 2515-2533. doi: 10.1093/molbev/msv139.

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

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

817 Moradi, M. H., Nejati-Javaremi, A., Moradi-Shahrbabak, M., Dodds, K. G., McEwan, J. C.,
818 2012. Genomic scan of selective sweeps in thin and fat tail sheep breeds for identifying of
819 candidate regions associated with fat deposition. BMC Genet. 13:10. doi: 10.1186/1471-
820 2156-13-10.

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

823 Pourlis, A. F., 2011. A review of morphological characteristics relating to the production and
824 reproduction of fat-tailed sheep breeds. *Trop. Anim. Health Prod.* 43, 1267-1287. doi:
825 10.1007/s11250-011-9853-x.

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

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

832 Ruiz-Larrañaga, O., Langa, J., Rendo, F., Manzano, C., Iriondo, M., Estonba, A., 2018. Genomic
833 selection signatures in sheep from the Western Pyrenees. *Genet Sel Evol.* 50: 9. doi:
834 10.1186/s12711-018-0378-x.

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

837 Schmid, E., 1972. *Atlas of animal bones.* Elsevier Publishing Company, New York and
838 Amsterdam, 1972. vii and 159 pp. Bibliography. Index. \$29.00. *Quaternary Research*, 2(2),
839 258-259.

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

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

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

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

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

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

- 853 Wilke, H. J., Kettler, A., Wenger, K. H., Claes, L. E., 1997. Anatomy of the sheep spine and its
854 comparison to the human spine. *The Anatomical Record: An Official Publication of the*
855 *American Association of Anatomists*, 247(4), 542-555.
- 856 Wilson, R.T., 1991. Small ruminant production and the small ruminant genetic resource in
857 tropical Africa, Food & Agriculture Org. Rome, Italy. Springer. ISBN 0387981403.
- 858 Zamiri, M. J. and Izadifard, J., 1997. Relationship of fat-tail weight with fat-tail measurements
859 and carcass characteristics of Mehraban and Ghezel rams. *Small Ruminant Research*. 26,
860 261–266.
- 861 Zeng, J., Zhou, S., Yang, Y., Du, J., Knag, D., Wang, X., Chen, Y., 2020. Effect of dietary
862 nutrition on tail fat deposition and evaluation of tail related genes in fat-tailed sheep.
863 *Electron J. Biotechnol.* 46. <https://doi.org/10.1016/j.ejbt.2020.05.004>.
- 864 Zhang, H., Zhang, X., Wang, Z., Dong, X., Tan, C., Zou, H., Peng, Q., Xue, B., Wang, L., Dong,
865 G., 2015. Effects of dietary energy level on lipid metabolism related gene expression in
866 subcutaneous adipose tissue of yellow breed x Simmental cattle. *Anim. Sci J.* 86(4), 392–
867 400. <https://doi.org/10.1111/asj.12316> PMID: 25440457