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Unlocking phenotypic diversity of indigenous sheep ecotypes reared in contrasting agroecological zones using multivariate analysis of morphometric traits

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ABSTRACT

This study aimed to characterize the diversity of morphometric traits in indigenous sheep populations reared in the highland, lowland, and midland agroecological zones of the Sidama region. Morphometric measurements were collected from 621 sheep individuals sampled across four districts representing these zones. Multivariate analyses, including canonical discriminant analysis, cluster analysis, and stepwise discriminant analysis, were conducted to evaluate variation among the populations. The results revealed that most morphometric traits were significantly affected by the sex and age of the animals. Sheep from the lowland agroecological zone exhibited the highest values for body length and ear length among morphometric traits, while the lowest values were for head length. Stepwise discriminant analysis identified body length, rump length, and body weight as the three most important variables for distinguishing the sheep populations across the studied agroecological zones. The Mahalanobis distance between highland and lowland sheep populations was the largest, reflecting substantial phenotypic differentiation. Discriminant function analysis correctly classified 71.3% of highland, 67.3% of lowland, and 64.5% of midland sheep populations into their respective agroecological zones, with an overall accuracy rate of 67%. Canonical discriminant analysis further revealed considerable overlap between the midland sheep population and those from the highland and lowland agroecological zones, indicating significant admixture. In conclusion, this study highlights phenotypic variations among sheep populations reared in different agroecological zones of the Sidama region. The phenotypic diversity identified can therefore support the development of appropriate genetic improvement programs for indigenous sheep populations.

How to Cite

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INTRODUCTION

In Ethiopia, where agriculture accounts for 40% of gross domestic product (GDP) and employs 75% of the population, sheep play a critical role in the livelihoods of millions of smallholder farmers and pastoralists (Asefa et al., 2017; USAID, 2021). Besides providing income, mutton, and manure, sheep serve as an economic buffer in case of crop failure and fulfil a variety of other sociocultural functions (Edea et al., 2017; Yihun, 2023). They also play an important economic role and significantly contribute to Ethiopia's domestic and export markets (Derbie and Tilahun, 2023). The country has 38 million sheep (Ethiopian Statistical Services, 2022), with 9 genetically distinct breeds, and 14 traditionally classified sheep populations that are dispersed and adapted across diverse agroecological zones (Gizaw et al., 2007). This remarkable diversity is attributed to Ethiopia's historical role as a gateway for livestock migration from Asia to Africa (Abdurehman, 2019), as well as the influence of its varied ecology, diverse ethnic communities, and a wide range of production systems (Gizaw et al., 2007; Melesse et al., 2013; Deribe et al., 2021).

Despite their abundance, wide distribution, and diverse functions, sheep productivity in Ethiopia's smallholder production system remains relatively low (Abebe et al., 2020). This is mainly due to factors such as the low genetic potential of the animals, feed scarcity, prevalence of diseases and parasites, lack of marketing strategies, and lack of effective breeding strategies tailored to the production system (Gizaw et al., 2013; Taye et al., 2016). Additionally, the high genetic diversity of Ethiopian sheep poses challenges to their characterization and effective management (Hailu et al., 2020). To improve productivity and the sustainable use of local sheep, it is critical to implement improved management practices, including selective breeding (Gizaw et al., 2013; Fikadu and Shiferaw, 2022). A key step in developing sustainable breed improvement strategies and effective interventions compatible with the production system is identifying genetic diversity, understanding population structure, and genetic relationships among populations (Nigussie et al., 2019). Morphological characterization serves as a foundational approach to achieve this, as it helps assess the phenotypic variation arising from genotypic and environmental factors (Agaviezor et al., 2012; FAO, 2012; Melesse et al., 2013; Seid et al., 2016; Worku and Melesse, 2021; Melesse et al., 2022). This is valuable for identifying adaptable breeds, especially in the context of changing production environments driven by climate change (Hailu et al., 2020). A lack of detailed information on sheep breed characteristics may result in poor decisions and genetic degradation through crossbreeding, replacement, and dilution (Hussein et al., 2022).

Phenotypic comparison based on morphometric traits can provide a realistic picture of genetic variations among breeds, adaptation to local environments, and useful information on the animal's potential for selection, and it can serve as the basis for DNA analysis (Yunusa et al., 2013). However, previous studies on Ethiopian sheep populations have been limited in scope, focusing on a few well-known breeds or on-station-managed sheep flocks (Melesse et al., 2013; Taye et al., 2016; Hussein et al., 2022; Mustefa et al., 2022). The available literature generally categorizes sheep populations in the current study area as the Arsi-Bale traditional population and long fat-tailed (Gizaw, 2008). However, previous studies did not sample populations from areas adjacent to the current study sites. Most previous studies on the morphological characterization of sheep breeds have been limited to the use of univariate analysis methods, such as analysis of variance (ANOVA) (Tade et al., 2021; Worku and Melesse, 2021; Melesse et al., 2022), which evaluate traits individually and do not explain how the populations under study differ when all morphometric variables are analyzed together. When all morphometric traits are considered jointly, multivariate analysis of morphometric traits has demonstrated greater power in recovering the actual genetic variation and relationships within and between populations (Yakubu and Ibrahim, 2011). Such studies are essential for clarifying the presence of similarities or dissimilarities in their phenotypic expressions among the studied sheep populations. Additionally, multivariate analysis is effective in identifying morphological markers associated with desirable traits, such as meat quality, adaptation to harsh environments, and enhanced reproductive performance. These markers serve as valuable indicators for breed evaluation, selection, and identifying potentially suitable local sheep for commercial production. Understanding attributes such as body size and conformation also provides useful information for designing suitable breeding programs aimed at enhancing the resilience, productivity, and sustainability of the local sheep (Deribe et al., 2021). However, in the Sidama region, information on sheep characterization conducted so far has focused on a few selected areas (Melesse et al., 2013; Kenfo et al., 2017). Comprehensive data on genetic distances among indigenous sheep populations in the region, based on morphometric traits using multivariate analysis, remains scarce. Thus, this study was initiated to describe the morphometric diversity of indigenous sheep populations across contrasting agroecological zones of the Sidama regional state using multivariate discriminant analysis.

MATERIALS AND METHODS

Study area

This study was conducted in four districts, namely Hula, Chuko, Dale, and Boricha, which were selected from the Sidama Regional State of Ethiopia. The region is located between 5′45″ and 6′45″ N latitude and 38′00″ and 39′20″ E longitude. The Sidama region has varied agroclimatic conditions, from warm and dry in the lowlands to cold and humid conditions in the highland areas. Warm conditions cover 54% of the area, with elevations from 1500 m to 2500 m.a.s.l, known as the midland agroecological zone. The mean annual rainfall in this zone varies between 1200 mm and 1599 mm, with an average annual temperature of 15 °C to 19.9 °C. The hot climatic zone covers 30% of the total area, with elevations from 500 m to 1500 m.a.s.l, representing lowland agroecological zone. It has a mean annual rainfall of 400 mm - 799 mm, and a mean annual temperature of 20 °C to 24.9 °C. Cool climatic conditions exist in mountainous highlands, covering 16% of the total area, with elevations between 2500 m and 3500 m.a.s.l, representing the highland agroecological zone. This agroecological zone receives the highest amount of rainfall, ranging from 1600 mm to 1999 mm. It has a mean annual temperature of 15 °C to 19.9 °C. Livestock production is one of the major economic bases in the region (SZPEDD, 2022).

Sample size and sampling techniques

A purposive multi-stage sampling method was used to select the same representative sheep population from the three agroecological zones. In the first stage, the Hula district from the highland, Aleta Chuko and Dale districts from the midland, and the Boricha district from the lowland agroecological zones were purposively selected based on their sheep production potential. To ensure proportional representation of the agroecological zones, one district each from the highland and lowland zones, and two districts from the midland zone were included, as the midland zone predominates in the region. In the second stage, three kebeles (the smallest administrative units in a district) from each district were selected purposively, based on the size of sheep populations and accessibility. In the third stage, households that owned at least three sheep of both sexes and had experience in sheep rearing were identified. The number of sheep included in the study was determined based on the total sheep population from each district and by applying Yamane's sample size determination formula (Yamane, 1967). Accordingly, a total of 621 sheep individuals, comprising 156 from the highland, 155 from the lowland, and 310 from the midland agroecological zones, including 481 females and 140 males, were randomly selected for phenotypic measurement (Table 1).

Data collection

Live body weight (BW) was measured using a digital suspended weighing scale (50 kg capacity with 200 g precision) by placing each animal in a self-devised holding harness. Linear body measurements including body length (BL), height at withers (HW), chest girth (CG), paunch girth (PG), height at rump (RH), rump length (RL), rump width (RW), head length (HDL), head width (HDW), ear length (EL), ear width (EW), and fore cannon circumference (FCC) were recorded using tailor's measuring tape after restraining the animals in a natural position on a flat surface. All measurements were taken in the morning before the animals left for grazing. Pregnant, diseased, and castrated animals were not included in the linear body measurements. It is recommended that morphometric measurements of sheep be taken from adult animals with at least 3 pairs of permanent incisors (PPI) (FAO, 2012). However, it has always been challenging to find the required number of sheep above 3PPI. Additionally, many researchers in developing countries have used small ruminants starting from yearlings (1PPI) and above for morphological characterization studies (Taye et al., 2016; Melesse et al., 2022). Consequently, considering these factors, data on physical appearance and measurements of selected phenotypic traits were collected from both sexes of sheep aged 1PPI and above. Thus, sheep with 1 PPI, 2 PPI, 3 PPI, and 4 PPI were classified into the age groups of yearling, 2-year-old, 3-year-old, and 4-year-old and above, respectively. The owners' recall method, along with dentition classes, was used to estimate the age of the sheep (Elbeltagy et al., 2016).

Statistical analysis

All statistical analyses were performed using SAS (SAS, 2016, ver. 9.4). Data was first checked for normality using univariate analysis. The data on morphometric traits was analyzed using a mixed model by considering agroecological zone, age, sex and their three-way interactions as fixed effects and kebeles as a random effect. The following statistical model was applied:

$$Y_{ijkl} = \mu + S_i + A_j + L_k + (SL)_{ik} + (SA)_{ij} + (AL)_{jk} + kl + e_{ijkl}$$

where: Y_{ijk} = response of observed variables, μ = the overall mean, S_i = the fixed effect of the i^{th} sex (i = male and female), A_j = the fixed effect of the j^{th} age group (j = 1 PPI, 2 PPI, 3 PPI, 4 PPI), L_k = the effect of agroecological zones (k = highland, midland, and lowland), SL_{ik} = interaction effect of the i^{th} sex with the k^{th} agroecological zones, and SA_{ij} = the effect of the interaction of the i^{th} sex with the j^{th} age group, AL_{jk} the effect of the interaction of the j^{th} age group with the k^{th} agroecological zones, K_l = kebele (random) , e_{ijk} = residual error. For all post-hoc analyses, least square means were compared pairwise with an adjusted Tukey-Kramer test.

In addition to the aforementioned statistical analysis, various multivariate analysis procedures, including STEPDISC, CANDISC, DISCRIM, TEMPLATE, and SGRENDER, were applied to determine the divergence of the indigenous sheep populations. The STEPDISC procedure was used to select morphometric traits with the highest discriminatory power among populations. The relative importance of the morphometric variables in discriminating among the indigenous sheep populations was assessed using the level of significance ($p < 0.05$) and partial R^2 values. Canonical discriminant analysis, performed using the CANDISC procedure, enabled maximal differentiation between sheep populations, allowing the computation of the Mahalanobis distances between class means and eigenvalues to summarize variation among sheep populations. The TEMPLATE and SGRENDER procedures were further applied to visualize a scatter graph of the first two canonical variables for visual interpretation of the studied districts. Additionally, the nearest neighbor discriminant function analysis (DISCRIM) was conducted to verify the results from CANDISC and to determine the percentage of correctly classifying individual animals into their actual source, using a quadratic discriminant function for unequal covariance matrices within classes. Cluster analysis was conducted, and a dendrogram was performed based on average distance to illustrate the relationship between the sheep populations reared in the three agroecological zones.

Table 1. Sampled sheep populations from the selected districts using Yamane's formula (using 8% precision)

Agroecological zones	Districts	Total sheep population	Sampled sheep
Highland	Hula	38,199	156
Midland	Dale	27,912	155
	Aleta Chuko	19,572	155
Lowland	Boricha	21,261	155

RESULTS

Morphometric traits

Except for ER, sex had a significant ($p < 0.05$) effect on all studied morphometric traits (Table 2). Accordingly, rams consistently displayed higher values for BW and other linear body measurements than ewes across the three agroecological zones. All morphometric traits were significantly ($p < 0.01$) affected by the age of the animal and increased with advancing age. The increase in BW, CG, PG, and RH was significantly different across all age groups ($p < 0.01$). The BL, RL, RW, HDL, HDW, and EL values of 3PPI and 4PPI were similar but higher than those of 1PPI and 2PPI ($p < 0.05$). The effect of agroecological zones was significant ($p < 0.05$) only for BL and EL. Sheep with the highest BL were observed in the lowland agroecological zone and differed from those of midland and highland agroecological zones ($p < 0.05$). Sheep reared in the highland agroecological zone had the lowest EL as compared to those of midland and lowland agroecological zones ($p < 0.05$). The interaction of sex by agroecological zones had a significant impact ($p < 0.05$) on BW, BL, WH, CG, PG, RH, and RW. Accordingly, in sheep reared in the highland agroecological zone, rams were superior in all morphometric traits to females except for EL and EW ($p < 0.05$). Similarly, rams raised in the lowland agroecological zone had higher values ($p < 0.05$) than females for all morphometric traits except for RL, RW, EL, and EW.

Table 2. Least square mean values of body weight (kg) and linear body measurement traits (cm) of indigenous sheep in the Sidama region ($^{a-d}$ Means within a row with different superscripts are significantly different ($p < 0.05$); Agroec. zones = Agroecological zones, BW = Body weight, BL = Body length, WH = Height at withers, CG = Chest girth, PG = Paunch girth, RH = Rump height, RL = Rump length, RW = Rump width, HDL = Head length, HDW = Head width, EL = Ear length, EW = Ear width, FCC = Fore canon circumference, 1PPI = 1 Pair of permanent incisors; 2 PPI = 2 Pairs of permanent incisors; 4 PPI = 4 Pairs of permanent incisors)

Factors	Sex	BW	CG	WH	BL	PG	RH	RL	RW	HDL	HDW	EL	EW	FCC
Agroecological zones														
Highland	Male	26.8	73.7	62.3	63.1	85.3	63.9	15.1	15.0	17.2^{a}	13.4	10.8	5.74	7.10
	Female	25.3	72.2	61.5	62.6	83.8	63.6	15.0	15.0	16.5^{b}	13.0	10.7	5.65	6.94
Midland	Male	26.6^{a}	74.4^{a}	63.3^{a}	64.0^{a}	85.5^{a}	65.4^{a}	15.5^{a}	15.5^{a}	17.6^{a}	13.6^{a}	11.1	5.75	7.21^{a}
	Female	23.1^{b}	70.6^{b}	60.5^{b}	61.2^{b}	80.6^{b}	62.4^{b}	14.6^{b}	14.7^{b}	16.4^{b}	13.1^{b}	11.0	5.60	6.84^{b}
Lowland	Male	27.4^{a}	75.2^{a}	64.2^{a}	66.9^{a}	85.5^{a}	65.3^{a}	14.9	14.9	17.1^{a}	13.5^{a}	11.3	5.66	7.38^{a}
	Female	23.3^{b}	70.6^{b}	60.9^{b}	63.2^{b}	81.0^{b}	63.4^{b}	14.7	14.5	15.8^{b}	13.0^{b}	11.3	5.49	6.93^{b}
Highland		26.1	73.0	61.9	62.9^{b}	84.6	63.7	15.6	15.0	16.9^{a}	13.2	10.8^{b}	5.70	7.02
Lowland		25.3	72.9	62.5	65.1^{a}	83.3	64.3	15.4	14.7	16.5^{b}	13.3	11.3^{a}	5.58	7.16
Midland		24.9	72.5	61.9	62.6^{b}	83.1	63.8	16.4	15.1	17.0^{a}	13.3	11.1^{a}	5.68	7.02
SEM		0.476	0.869	0.372	0.451	1.083	0.473	0.934	0.272	0.391	0.158	0.116	0.065	0.084
Age														
1 PPI		21.5^{d}	68.1^{d}	59.8^{c}	60.1^{c}	77.8^{d}	61.9^{d}	14.6^{c}	13.7^{c}	15.9^{b}	12.8^{c}	10.7^{c}	5.41^{c}	6.95^{b}
2 PPI		24.5^{c}	71.7^{c}	61.9^{b}	62.9^{b}	82.3^{c}	63.6^{b}	15.5^{b}	14.7^{b}	16.8^{a}	13.2^{b}	11.1^{ab}	5.60^{b}	7.06^{b}
3 PPI		27.0^{b}	74.6^{b}	62.7^{ab}	64.6^{a}	85.9^{b}	64.3^{b}	16.2^{a}	15.4^{a}	17.1^{a}	13.5^{a}	11.1^{a}	5.70^{ab}	7.03^{ab}
4 PPI		28.6^{a}	76.6^{a}	64.0^{a}	66.4^{a}	88.5^{a}	65.9^{a}	17.0^{a}	15.9^{a}	17.3^{a}	13.6^{a}	11.4^{a}	5.89^{a}	7.23^{a}
SEM		0.381	0.610	0.336	0.401	0.781	0.381	0.575	0.199	0.249	0.111	0.102	0.059	0.064
***P*-values**														
Sex (S)		<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.009	<.001	<.001	0.569	0.014	<.001
Age (A)		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.003
Agroec. zone (AZ)		0.208	0.907	0.361	<.001	0.605	0.682	0.672	0.605	0.628	0.783	0.015	0.391	0.436
AZ*S		0.006	0.009	0.006	0.004	0.018	0.003	0.634	0.039	0.082	0.476	0.856	0.850	0.063
AZ*A		0.440	0.032	0.334	0.208	0.754	0.037	0.086	0.259	0.187	0.776	0.055	0.121	0.347
Sex*A		0.534	0.524	0.191	0.250	0.066	0.244	0.131	0.003	0.065	0.531	0.782	0.271	0.597

On the other hand, no significant differences were found between rams and ewes reared in the highland agroecological zone in any morphometric traits studied except for HDL. Ear length and EW did not differ between rams and ewes across agroecological zones. The analysis of the interaction between agroecological zones and sex further revealed that the BW of ewes reared in the highland agroecological zone was higher than that of those in the lowland ($p = 0.048$) and midland ($p = 0.007$) agroecological zones. Rams reared in the highland agroecological zone showed higher BL than those of the lowland agroecological zone ($p = 0.004$). Conversely, ewes and rams raised in the lowland agroecological zone had higher BL than those of the midland agroecological zone ($p = 0.001$ and $p = 0.012$, respectively). The EL of ewes raised in the highland agroecological zone was higher than that of the lowland agroecological zone ($p = 0.009$). The interaction of agroecological zones by age revealed significant differences only for CG and RH. Similarly, the interaction effect of sex by age was significant only for RW.

Stepwise discriminant analysis

The results of the stepwise discriminant analysis, based on the contribution to the differentiation of sheep populations in the study area, are presented in Table 3. Of sixteen morphometric traits subjected to the STEPDISC analysis, eight traits were identified with the best discriminating power. The BL, followed by the RL and BW, were the top three most important morphometric traits with the highest discriminatory power to separate the sheep populations. These were selected based on their higher R^2, F-values, and lowest ASCC values. Wilks' lambda test confirmed that all the selected variables had highly significant ($p < 0.001$) contributions to discriminate the total population into separate groups. When the eight most important morphometric traits were selected, Wilks' lambda dropped from 0.94 to 0.67, demonstrating a significant difference ($p < 0.001$) between the sheep populations reared in the three agroecological zones. For instance, BL had the highest value for Wilks' lambda, partial R^2, F-value, and the lowest value for ASCC, whereas PG had the lowest value for Wilks' lambda, partial R^2, and F-value, and the highest value for ASCC. The remaining six morphometric traits (WH, CG, RH, RW, and FCC) displayed poor discriminating power and were removed from the final model.

Canonical discriminant analysis

The Mahalanobis distance among the sheep populations based on morphometric traits sorted by means is shown in Table 4. All pairwise squared Mahalanobis distances obtained for the studied sheep populations were significant ($p < 0.001$). The largest Mahalanobis distance was observed between the lowland and highland sheep populations (2.10), followed by the midland and lowland sheep populations (1.62). The smallest Mahalanobis distance was observed between midland and highland (0.95).

Table 4. The Mahalanobis distance among three indigenous sheep populations in the study area

Agroecological zones	Highland	Midland	Lowland
Highland		0.95	2.10
Midland	<.001		1.62
Lowland	<.001	<.001	

The canonical discriminant analysis extracted two statistically significant canonical variates ($p < 0.001$) (Table 5). The first canonical variate (CAN1) explained the majority of the total variation, accounting for 65.2% with an eigenvalue of 0.46. The remaining 34.8% of the total variation was explained by the second canonical function (CAN2). Together, the two canonical variates contributed 100% to the cumulative variance. The first canonical correlation (46.2%) was the greatest multiple correlation with the classes, achieved by using the linear combination of quantitative variables. The different tests used in multivariate statistics, namely Wilks' lambda, Pillai's trace, Lawley-Hotelling trace, and Roy's greatest root test, showed significant differences ($p < 0.001$) between the three agroecological zones.

The plot of the two canonical variables illustrating the relationships of sheep populations in the three different agroecological zones is presented in Fig. 1. Highland sheep are clustered in the lower left-hand of the graph, while the lowland sheep populations occupy the lower right side, demonstrating a clear differentiation between the two populations.

Table 3. Summary of stepwise discriminant analysis for selection of morphometric traits with discriminant power (BL = Body length, RL = Rump Length, BW = Body weight, HDL = Head length, EL = Ear length, EW = Ear width, HDW = Head width, PG = Paunch girth, ASCC = Average squared canonical correlation)

Steps	Variables	Partial R^2	F - Value	Pr > F	Wilks' lambda	P < lambda	ASCC	*Pr* > ASCC
1	BL	0.060	19.8	<.0001	0.940	<.0001	0.030	<.0001
2	RL	0.058	19.2	<.0001	0.885	<.0001	0.058	<.0001
3	BW	0.054	17.5	<.0001	0.838	<.0001	0.084	<.0001
4	HDL	0.033	10.4	<.0001	0.769	<.0001	0.123	<.0001
5	EL	0.027	9.53	0.0002	0.720	<.0001	0.151	<.0001
6	EW	0.038	12.0	<.0001	0.693	<.0001	0.166	<.0001
7	HDW	0.018	5.59	0.0039	0.681	<.0001	0.173	<.0001
8	PG	0.012	3.80	0.0230	0.672	<.0001	0.179	<.0001

Table 5. Summary of canonical correlations, eigenvalues, and likelihood ratios of the studied sheep populations

Functions	Can. Corr.	Eigen Values			Likelihood ratio	F-value	P-value
		Eigenvalues	Proportion	Commutative			
CAN1	0.479	0.462	0.652	0.652	0.665	12.5	<0.001
CAN2	0.370	0.358	0.348	1.000	0.863	9.69	<0.001

CAN 1 = Canonical variable 1; CAN 2 = Canonical variable 2; Can. Corr. = Canonical Correlation

In contrast, the sheep populations in the midland agroecological zone are distributed across both canonical variables (CAN1 and CAN2), indicating relative homogeneity and overlapping among the sheep populations, which suggests the existence of admixture among them.

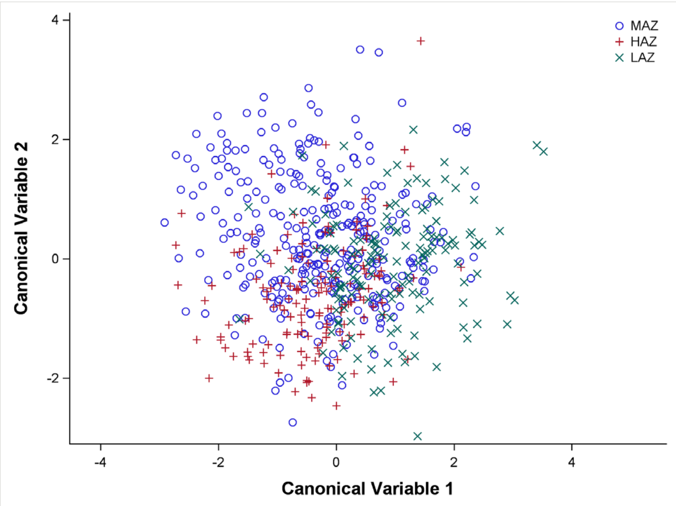

Figure 1. The canonical representation of sheep populations of the three agroecological zones based on morphometric traits (MAZ = Blue = Midland agroecological zone, HAZ = Red = Highland agroecological zone, LAZ = Green = Lowland agroecological zone)

Quadratic discriminant function

As indicated in Table 6, the correct assignment of sheep to their respective agroecological zones ranged from 71.3% for highland sheep to 64.5% for midland sheep, where 20.4 and 21.3 were misclassified as midland and highland, respectively. The highland agroecological zone had the highest correct assignment (71.3%), followed by lowland sheep, which scored 67.3% correct assignment of the sampled sheep within their original population. About 22% of the lowland sheep populations were misclassified as midland populations. Conversely, the midland sheep populations had the lowest percentage (64.5%) of individuals correctly classified into their source population. Based on the quadratic discriminant function analysis, 67% of the total sampled sheep populations were correctly classified into their source population.

Table 6. Number and percentage of individual sheep classified into their respective agroecological zones and cross-validation of classification (values in the brackets are the number of sheep)

Agroecological zones	Highland	Lowland	Midland	Total
Highland	71.3 (112)	8.28 (13)	20.4 (31)	156
Lowland	10.3 (16)	67.3 (105)	22.4 (34)	155
Midland	21.3 (65)	14.2 (44)	64.5 (201)	310
Error count rate	0.29	0.33	0.36	0.33
Cross-validation				
Highland	61.6 (96)	8.92 (14)	29.9 (46)	156
Lowland	10.3 (16)	56.4 (87)	33.3 (52)	155
Midland	23.2 (72)	16.5 (51)	60.3 (187)	310
Error count rate	0.39	0.44	0.40	0.41
Priors	0.25	0.25	0.50	

Cluster analysis

The dendrogram generated based on the genetic distance of sheep populations reared in the three agroecological zones was grouped into two main clusters as indicated in the dendrogram (Fig. 2).

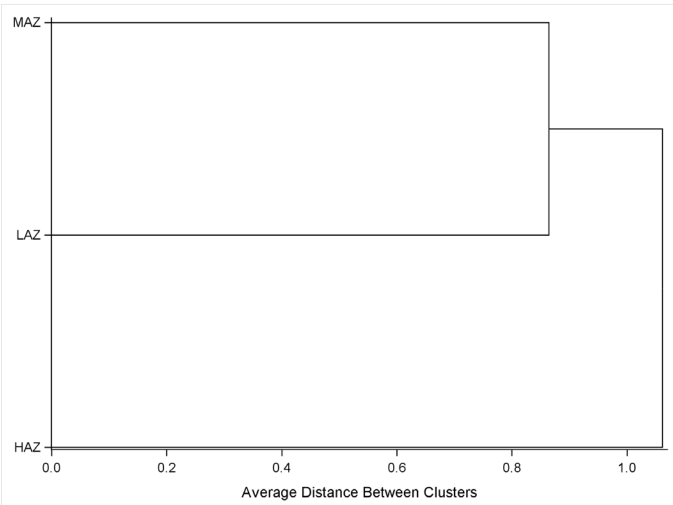

Figure 2. Dendrogram based on the average distance between the indigenous sheep populations reared in three contrasting agroecological zones using morphometric traits, MAZ = Midland agroecological zone, LAZ = Lowland agroecological zone, and HAZ = Highland agroecological zone

The first cluster contains midland and lowland sheep populations, while the second cluster consists of the highland sheep populations, which indicates that the highland sheep are relatively distinct from the lowland and midland sheep populations. The lowland and midland sheep populations are estimated to be more closely related to each other than to the highland sheep.

DISCUSSION

Information on BW and linear body measurements of the sheep population at a specific age is crucial for selecting genetically superior animals for production and reproduction purposes. In the current study, sex had a significant effect on almost all quantitative traits, which is consistent with the results obtained by Melesse et al. (2022). In contrast to the present findings, Alemayehu et al. (2022) reported the absence of a significant effect of sex on BW and other linear body measurements except for body condition scores. The higher values of BW and other linear body measurements in rams than in ewes may be due to sexual dimorphism, stemming from hormonal differences in growth rate between both sexes (Shibabaw et al., 2014). Ewes grow more slowly and reach maturity at a smaller size than rams due to the effect of estrogen, which restricts long bone growth (Sowande and Sobola, 2008). Similarly, the superiority of rams over ewes in most of the morphometric traits could be a result of the greater muscle mass and skeletal development associated with the secretion of testosterone hormone, which is secreted only in male animals (Hailu et al., 2020). In agreement with the present findings, the same authors reported non-significant differences in EL among rams reared in highland agroecological zones, suggesting that EL may be a stable trait not likely affected by husbandry practices.

All morphometric traits were significantly affected ($p < 0.01$) by age, which accords with the findings of Tade et al. (2021). All morphometric traits gradually increased with age, which is consistent with earlier findings reported by Gebreyowhens and Tesfay (2016). The increases in morphometric traits with advancing age may be related to enhanced muscle development, bone growth, and high-fat deposition. Similarly, Tade et al. (2021), Takele et al. (2021), Melaku et al. (2019), and Melesse et al. (2022) found that morphometric traits increased with the age of the animal in different Ethiopian sheep and goat breeds.

Agroecological zones had a significant effect only on BL and EL, which disagrees with other findings reported by various scholars. For instance, Hailemariam et al. (2018) reported that agroecological zones influenced most of the traits studied in sheep. Similarly, the study by Mebratie et al. (2022) in the Awi zone revealed a significant effect of agroecological zones on all quantitative measurements except for tail length. Additionally, Hailu et al. (2020) reported that agroecological zones significantly affected the linear body measurements of sheep in the Maychew districts of Ethiopia. These variations may be attributed to the fact that different sheep types have distinct physiological adaptation mechanisms to various environments, management styles, availability of feed and nutrition, and inherent genetic variations.

The higher values of morphometric traits observed in rams compared to ewes across all the studied agroecological zones may be attributed to sexual dimorphism, which is consistent with the results of Derbie and Tilahun (2023) for indigenous sheep in the Jimma zone. Furthermore, the aggressive behavior of rams during feeding and sucking, along with the anabolic effect of male sex hormones, may contribute to larger body sizes and heavier weights in rams compared to ewes (Gebreyowhens and Tesfay, 2016). In line with the findings of Hailu et al. (2020), rams in the lowland agroecological zone exhibited significantly higher values for BW and other morphometric traits. Beyond environmental adaptation, this disparity is likely influenced by traditional management practices in which superior males are retained to full maturity to serve as breeding stock or for specialized fattening, rather than being sold at an early market age for quick meat returns.

Some of the variables identified through stepwise discriminant analysis are similar to those reported in previous studies (Yakubu and Ibrahim, 2011; Zergaw et al., 2017; Hailu et al., 2020; Tade et al., 2021; Melesse et al., 2022). These results suggest that measurements using the top four variables could differentiate the three sheep populations more effectively than obtaining a large number of additional morphometric measurements.

The two statistically significant canonical variables observed in the current study are in line with the results of Abdallah and Omar (2017) for Awassi sheep. Contrary to this, Asamoah-Boaheng and Sam (2016) found that CAN1 was significant, while CAN2 was not significant and did not contribute significantly to the discrimination process as the first function. Wilks' lambda is the test statistic used in multivariate analysis of variance, which estimates the ratio between within-group variability and the total variability of the discriminant variables. It is an inverse measure of the importance of the discriminant functions (Tade et al., 2021; Melesse et al., 2022). Wilks' lambda was used to test the hypothesis that the means of agroecological zones are equal across the sheep populations studied, and the result was statistically significant ($p < 0.001$), confirming that the differences observed among the sheep populations from the three agroecological zones were statistically different from zero. Wilks' lambda values close to one indicate nearly all the variability in the discriminator variables is due to within-group differences (no significant differences between populations), whereas values close to zero indicate that within-group variation in discriminator variables is small compared to the total variability. In this case, the value of Wilks' lambda was 0.665, which indicates that the majority of the variability (66.5%) in discriminating variables originated from the difference within-group variability. The relative importance of the canonical function in partitioning the variance between populations depends on its eigenvalue; the higher the eigenvalue, the more variance it accounts for. Therefore, the first CAN1, with high eigenvalues, accounted for most of the variation.

Determining the morphometric distance helps to understand the genetic diversity of indigenous animal populations and allows for the design of suitable breeding programs that are useful for the conservation of animal genetic resources (Melesse et al., 2022). All the pairwise squared Mahalanobis distances obtained for the studied sheep populations were significant ($p < 0.001$), which aligns with the result of Alemayehu et al. (2022), indicating that the studied sheep populations exhibited measurable differences. The largest Mahalanobis distance was observed between lowland and highland agroecological zones, which may be attributed to geographic isolation and varying breeding objectives practiced, as well as differences in adaptation to environmental conditions (Ofori et al., 2021). The geographical isolation between the flocks disrupts the flow of genes across generations, which is typically facilitated by geographical proximity (Arandas et al., 2017). The longest distance between the sheep populations of highland and lowland agroecological zones may further be related to the variation among the studied sheep populations, which may maintain diversity, further genetic improvements, and conservation programs. Similarly, Hailu et al. (2020) also found the greatest distance between highland and lowland agroecological zone sheep populations in the Maichew district of northern Ethiopia. The observed short Mahalanobis distance between midland and highland sheep populations may suggest their similarity at a phenotypic level, likely due to extensive gene exchange between the two populations resulting from their geographic proximity. The dendrogram in Fig. 2 of the current study shows that the sheep population in the highland agroecological zone was greatly differentiated from those in the other agroecological zones. The close relationship between midland and lowland agroecological zones contrasts with the result of the Mahalanobis distance, which may be due to the double-sampled size of the midland sheep population. Additionally, the low Mahalanobis distance between the highland and midland populations may stem from continuous inbreeding, lack of selection, and a high rate of intermingling due to migration and a free-range management system (Melesse et al., 2022). All the pairwise squared Mahalanobis distances between the populations were significant ($p < 0.001$), which is in line with the result of Alemayehu et al. (2022), indicating that the studied sheep populations had measurable differences. Quadratic discriminant function allows for the classification of observations into pre-existing groups based on similarities and helps to describe the importance of variables within those groups. As indicated in Table 6, the correct assignment of sheep to their respective agroecological zones ranged from 71.3% for highland sheep to 64.5% for midland sheep, with an estimated total error count of 33%. Similarly, Hailu et al. (2020) found that 68.6, 69.6%, and 58.8% of the individual sheep were classified into their respective agroecological zones, with several individuals misclassified. The high Mahalanobis distance, coupled with the high correct assignment rate for the highland and lowland agroecological zones, indicates reduced gene flow between these populations. Conversely, midland sheep populations had the lowest percentage (64.5%) of individuals correctly classified into their original populations, suggesting a lack of distinctiveness within this group. A similar report by Guyo et al. (2023) reported the overall overlap of the midland agroecological zone with the other two agroecological zones. Additionally, it noted that the classification errors observed in all agroecological zones resulted from the interconnection between sheep flocks, driven by the exchange of males, as evidenced by the short distance between individuals. The overall rate of the error count estimate indicated a misclassification rate of 33%, suggesting heterogeneity among sheep populations for the variables included in the discriminant analysis (Worku and Melesse, 2021). This misclassification indicates that the sheep population in the midland may share a similar genetic basis with the other two sheep populations. This was supported by the canonical representation plot, which showed that the midland sheep population was distributed across the two canonical variables, indicating relative homogeneity of the midland with the other two agroecological zones. Worku and Melesse (2021) reported that about 36% of the original ATKJ sheep were correctly categorized into their original population, while the majority (approximately 74%) of the remaining were misclassified into other sheep populations, which is inconsistent with the current findings. Furthermore, there may be strong chances of gene flow among the studied sheep populations facilitated by the active marketing system in the region, which has existed for many generations.

CONCLUSION

The top three variables, including BL, HDL, and BW, selected through the stepwise discriminant analysis can differentiate the studied sheep populations. All squared Mahalanobis distances obtained among the agroecological zones were significant, indicating measurable differences among the sheep populations. The low level of similarity between highland and lowland sheep populations may be due to minimal interbreeding, resulting from large geographical isolation and variation in breeding objectives. The sheep population generally exhibits measurable morphometric divergence in the contrasting agroecological zones of the study area. Thus, the phenotypic diversity described would contribute to designing community-based breeding programs to facilitate genetic improvement and ensure the sustainable utilization of the indigenous sheep populations in the study region.

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AUTHORS CONTRIBUTION

Tariku Woldeyohannes, Aberra Melesse, and **Simret Betsha**: conceptualized the project. **Tariku Woldeyohannes**: conducted the fieldwork and collected the data. **Tariku Woldeyohannes** and **Aberra Melesse**: carried out the formal analysis. **Tariku Woldeyohannes**: prepared the original draft. **Aberra Melesse** and **Simret Betsha**: managed funding acquisition and supervision. **Simret Betsha**: administered the project. **Tariku Woldeyohannes, Aberra Melesse,** and **Simret Betsha**: contributed to editing, reviewing, and approving the final manuscript. All authors read and approved the final manuscript.

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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.

ETHICS APPROVAL

The ethical standards of the study were reviewed and approved by the Hawassa University Research and Ethics Review Committee (Reference No: RECO-16/23).

AVAILABILITY OF DATA AND MATERIALS

Data will be made available on reasonable request from the corresponding author.

AI USE STATEMENT

No AI-assisted technologies were employed in the preparation of this manuscript. All content was generated exclusively by the authors.

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