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RESEARCH ARTICLE

Geometric Morphometric Analysis of Skull and Mandible in Awassi Ewe and Ram

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Abstract

The aim of the study was to analyze Awassi ewe and ram skull and mandible by geometric morphometric methods. For this purpose, different numbers of skulls and mandibles of adult Awassi sheep were used based on sexual dimorphism. The skull was analyzed using the photos taken from 17 samples from dorsal side and 16 samples from the left lateral side, and the mandible was analyzed using the photos taken from 20 samples from the left lateral side. In the comparison of Awassi sheep skull from the dorsal and lateral sides between the sexes, the first principal component accounted for 37.719% and 44.238% of the total shape difference, respectively. In mandible, the first principal component accounted for 24.92% of the total shape difference. The skull had an apparent dimorphism in both sides between the sexes but the same effect was not observed in mandible. As a result, it is considered that the results obtained would contribute to the future studies related to ruminant cranium to be performed using geometric morphometric method.

Keywords: *Geometric morphometry, Awassi sheep, Principal components analysis, Relative warp analysis*

İvesi Koyunu ve Koçunda Kafatası ve Mandibulanin Geometrik Morfometrik Analizi

Öz

Çalışmada İvesi koç ve koyun kafatası ve mandibula'sının geometrik morfometrik yöntemlerle analizi amaçlandı. Bu amaçla cinsiyet farkı gözetilerek farklı sayıda ergin İvesi koyunu kafatası ve mandibula'ları kullanıldı. Kafatası dorsal yönden 17, sol lateral yönden 16, mandibula ise sol lateral yönden 20 örnekten alınan fotoğraflardan analiz edildi. İvesi koyunu kafatasında dorsal ve lateral yönden cinsiyetler arası yapılan karşılaştırmada birinci temel bileşen toplam şekil farklılığının sırasıyla %37.719 ve %44.238'ini açıkladı. Mandibula'da ise birinci temel bileşen toplam şekil farklılığının %24.92'sini açıkladı. Kafatası her iki yönde cinsiyetler arası belirgin bir dimorfizm gösterirken, aynı etki mandibula'da gözlenmedi. Sonuç olarak elde edilen bulguların ileride planlanacak ruminantia cranium'u ile ilgili geometrik morfometrik yöntemle yapılacak çalışmalara katkı sağlayacağı düşünülmektedir.

Anahtar sözcükler: *Geometrik morfometri, İvesi koyunu, Temel bileşenler analizi, Relative warp analizi*

INTRODUCTION

Geometric morphometric method determines shape differences by landmark (LM) coordinates and measures the amount of shape changes using the location differences of objects [1]. Superimposition (General Procrustes Analysis) is one of the most important points of this method. By applying this method, variations of the objects related to their shape such as their location, direction, and scale are removed [2]. Therefore, the coordinates are aligned and the size and direction of the movement between different populations or samples are mapped [3].

Awassi Sheep's name is originated from the El Awas tribe between Tigris river and Euphrates river and it is a combined, fat-tailed sheep species and named after different names based on regions. This sheep has completely adapted to the harsh climate conditions of South-West Asia [4]. Awassi is the most common sheep species which is not Europe-origin [5].

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In recent years, geometric morphometry method analysis of cranium has started to be used on different species in the field of veterinary anatomy [6-11]. Cranium is one of the main parts of skeleton demonstrating the taxonomic relationship in animals [12]. In the literature review, the cranium in Awassi sheep is analyzed by geometric morphometric method according to gender, and no other result has been found. In this study, it was aimed to determine whether gender makes a difference on the cranium in terms of shape or not by geometric morphometric method in Awassi sheep.

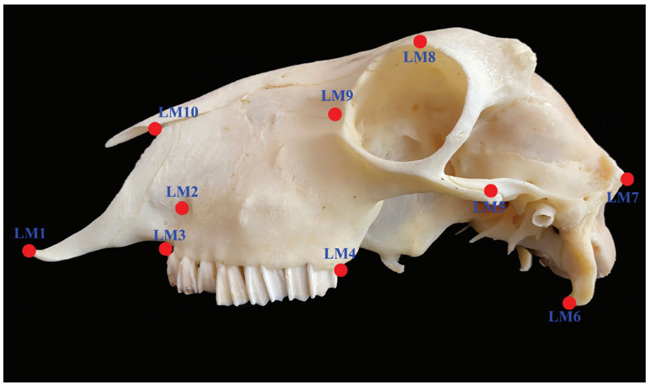


Fig 2. View of lateral landmarks on skull. LM1: Anterior edge of os incicivum, LM2: Foramen infraorbitalis, LM3: Anterio-dorsal edge of PM1, LM4: Caudal edge of M3, LM5: Middle point of arcus zygomaticus, LM6: Ventral edge of processus jugularis, LM7: External occipital protuberance, LM8: Middle point of margo supraorbitalis, LM9: Fossa lacrimalis externa, LM10: Fissura nasomaxillaris

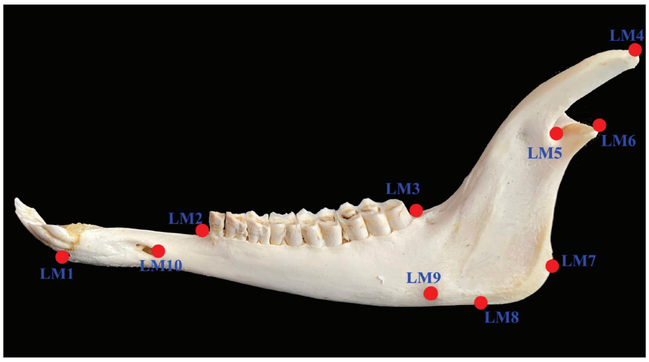


Fig 3. View of landmarks on mandible. LM1. Aboral anterioventral end point of alveoli dentales of L1, LM2. Anterioventral edge of P2, LM3. Posterioventral edge of M3, LM4. End-middle point of Processus coronoideus, LM5. Medioventral point of Incisura mandible, LM6. Posterior end point of Condylus mandible, 7. Posterioventral corner of angulus mandible, LM8. Incisura vasorum facialium. LM9. Anterior junction point of the dorsal and ventral axes of fossa masseterica, LM10. Posterior margin of Foramen mentale

Material and Methods

The permissions were obtained from Harran University Animal Experiments Local Ethics Committee (Decision no: 2020/003-01-12).

In the study, different numbers of adult Awassi ewe and ram skull and mandible were used by considering sexual dimorphism. The materials were boiled and macerated. While the skull was analyzed using the photos taken from 17 (10 ewe, 7 ram) samples from dorsal side and 16 (9 ewe, 7 ram) samples from the left lateral side, the mandible was analyzed using the photos taken from 20 (10 ewe, 10 ram) samples from the left lateral side. The photos were taken from a 30-cm distance by focusing on frontonasal suture from the dorsal side, the ventral edge of orbita from the lateral side for skull and between the second and third premolar teeth for mandible. The photos were saved in a computer in JPEG format. Firstly, the photos were converted into a tps file using TpsUtil software (Version 1.79) [13]. 10 homolog LMs [11,14] *(Fig. 1, Fig. 2,* and *Fig. 3)* were marked from all directions on the photos by TpsDig2 software (Version 2.31) [11,14,15]. The confirmation test of the homolog LMs, the cartesian coordinates of which were determined, was performed by TpsSmall (Version 1.34) [16] software. As a result of this analysis, it was determined that the slope and correlation values were 0.999412 and 1.0 and 0.999741 and 1.0 from the dorsal and left lateral sides, respectively. These values were 0.999853 and 1.0 in mandible. All these values demonstrated that LMs were accurate.

As there are differences between the mandible in terms of size, position and direction etc., General Procrustes Analysis (superimposition) were performed [17]. PAST (Version 4.02) [18] software was used for this analysis. Principal components analysis (PCA) was performed through the same software on the new coordinates obtained as a result of Procrustes analysis. Thus, the degree of diverging of the samples based on sex was determined using covariance analysis [2]. In addition, MorphoJ software was used to demonstrate shape differences at which LM levels and directions. In this software, the average shapes were determined with their differences based on sex using discriminant function analysis.

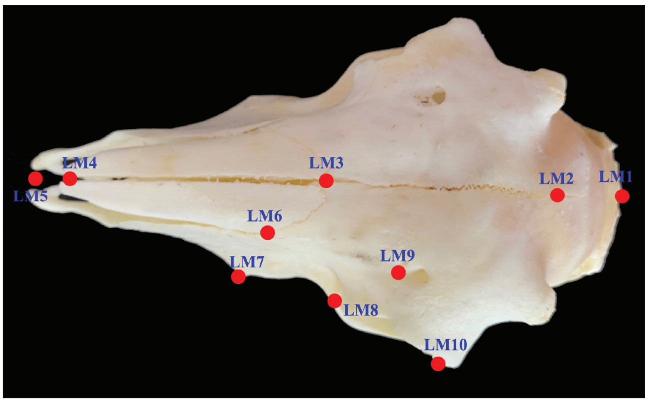


Fig 1. View of dorsal landmarks on skull. LM1: External occipital protuberance, LM2: Junction of sutura coronalis and sutura interfrontalis, LM3: Junction of sutura interfrontalis, sutura internasalis and frontonasal suture, LM4: Anterior edge of sutura internasalis, LM5: Anterior edge of fissura interincisiva, LM6: Fissura nasomaxillaris, LM7: Tuber faciale, LM8: Medial angle of orbita, LM9: Foramen supraorbitale, LM10: Posterio-ventral corner of margo supraorbitalis

In the study, relative warp analysis (RWA) was performed by TpsRelw (Version 1.70) [19] software and the consensus graphics of the groups were formed. Also, distribution of the groups on graphic was also tested by this analysis. The statistical analysis of LM coordinate values based on the groups was performed by 2-t test in PAST (Version 4.02) software.

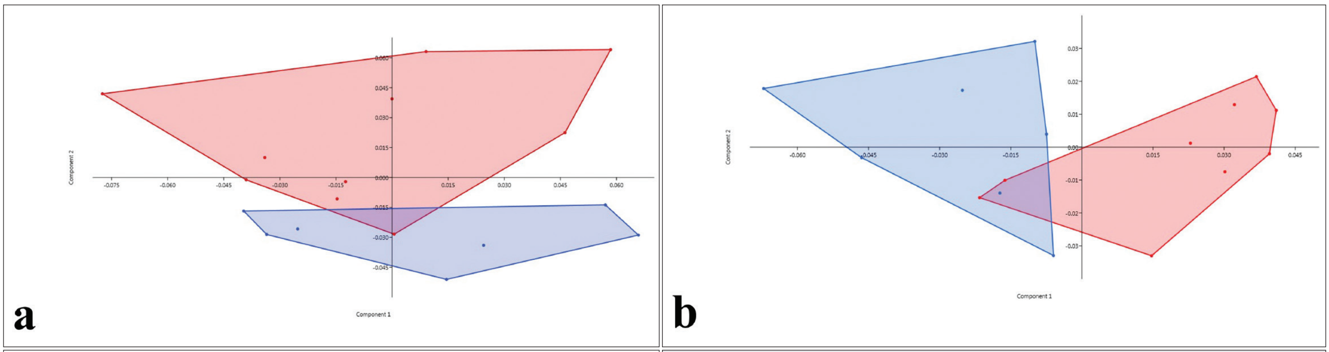
Results

Skull Analysis

Table 1 shows the results related to the skull principal components analysis in the study. Accordingly, in the

Table 1.*Values obtained as a result of the principal component analysis*

PC	Skull Dorsal		Skull Lateral		Mandible	
	Eigenvalue	% Variance	Eigenvalue	% Variance	Eigenvalue	% Variance
1	0.00165069	37.719	0.00105427	44.238	0.000412877	24.92
2	0.00121854	27.844	0.000346344	14.533	0.000306917	18.525
3	0.000456679	10.435	0.000243111	10.201	0.000230935	13.939
4	0.000340601	7.7829	0.000198678	8.3366	0.000208795	12.602
5	0.000233552	5.3368	0.000156905	6.5838	0.000134089	8.0934
6	0.000130795	2.9887	0.000151965	6.3765	8.93702E-05	5.3942
7	0.000105017	2.3997	7.0557E-05	2.9606	7.58173E-05	4.5762
8	9.10019E-05	2.0794	4.94674E-05	2.0757	6.44643E-05	3.8909
9	5.55349E-05	1.269	4.20146E-05	1.7629	4.64634E-05	2.8044
10	3.87545E-05	0.88556	2.14639E-05	0.90063	3.78018E-05	2.2816
11	3.1854E-05	0.72788	2.09338E-05	0.87839	2.06527E-05	1.2466
12	1.02154E-05	0.23343	1.39763E-05	0.58645	1.40721E-05	0.84936
13	8.47604E-06	0.19368	6.2137E-06	0.26073	7.50213E-06	0.45281
14	4.16989E-06	0.095284	4.54143E-06	0.19056	3.92625E-06	0.23698
15	3.69238E-07	0.0084373	2.76267E-06	0.11592	2.1843E	0.13184
16	2.8442E-09	6.4992E-05			8.97427E	0.054167
17					1.46284E	0.00088294
18					2.39165E	1.4436E-11
19					1.20709E	7.2857E-13



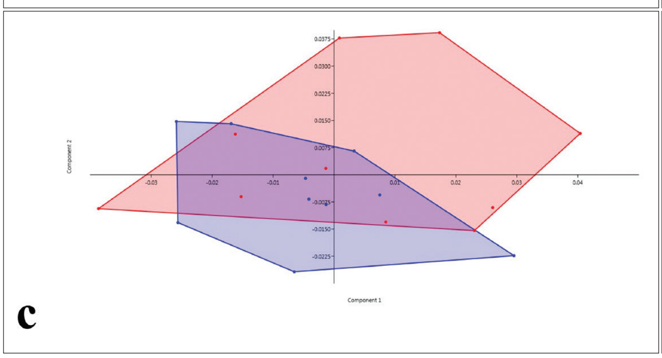


Fig 4. Graphical demonstration of the results obtained based on the first principal component. **a.** Skull dorsal, **b.** Skull lateral, **c.** Mandible

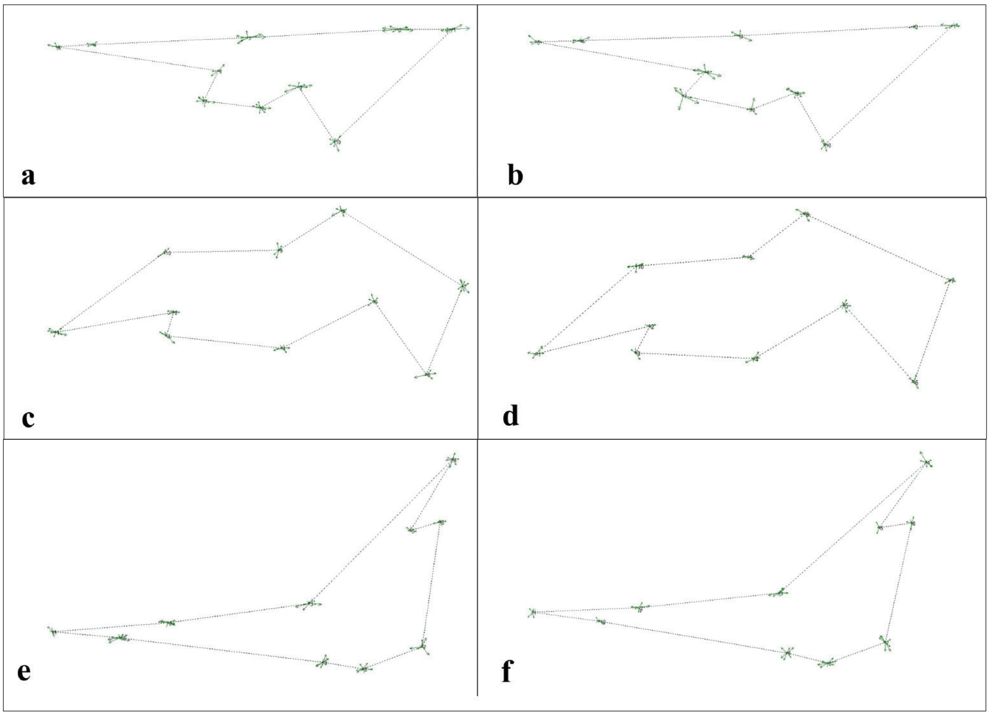


Fig 5. Consensus graphics based on groups, **a.** Skull ewe dorsal, **b.** Skull ram dorsal, **c.** Skull ewe lateral, **d.** Skull ram lateral, **e.** Ewe mandible, **f.** Ram mandible

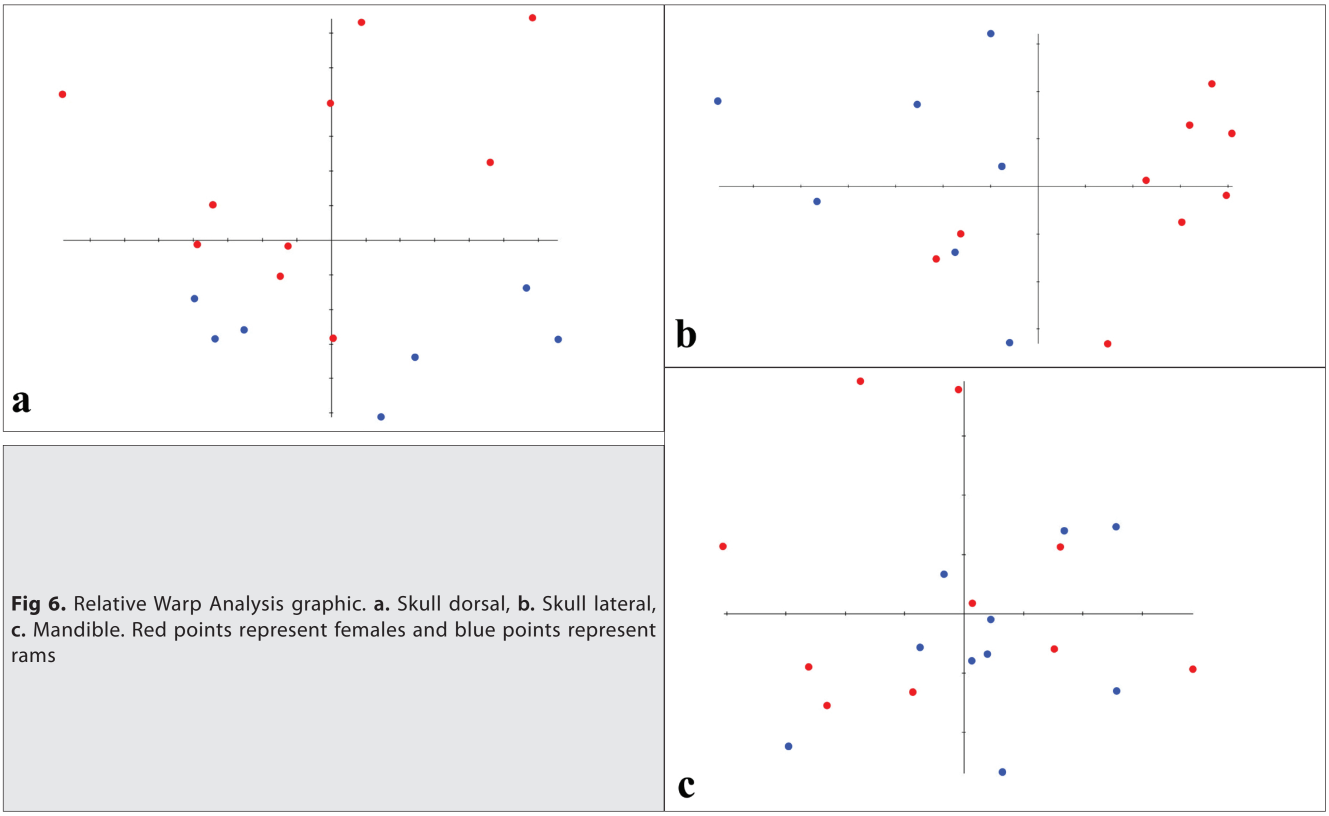


Fig 6. Relative Warp Analysis graphic. **a.** Skull dorsal, **b.** Skull lateral, **c.** Mandible. Red points represent females and blue points represent rams

comparison of Awassi ewe and ram skull from the dorsal and lateral sides between the sexes, the first principal component accounted for 37.719% and 44.238% of the total shape difference, respectively. The graphics in *Fig. 4-a,b* shows the sexual dimorphism in terms of the first principal component. Accordingly, it was observed that the crania of the ewe and ram individuals formed groups apparently in both dorsal and lateral side.

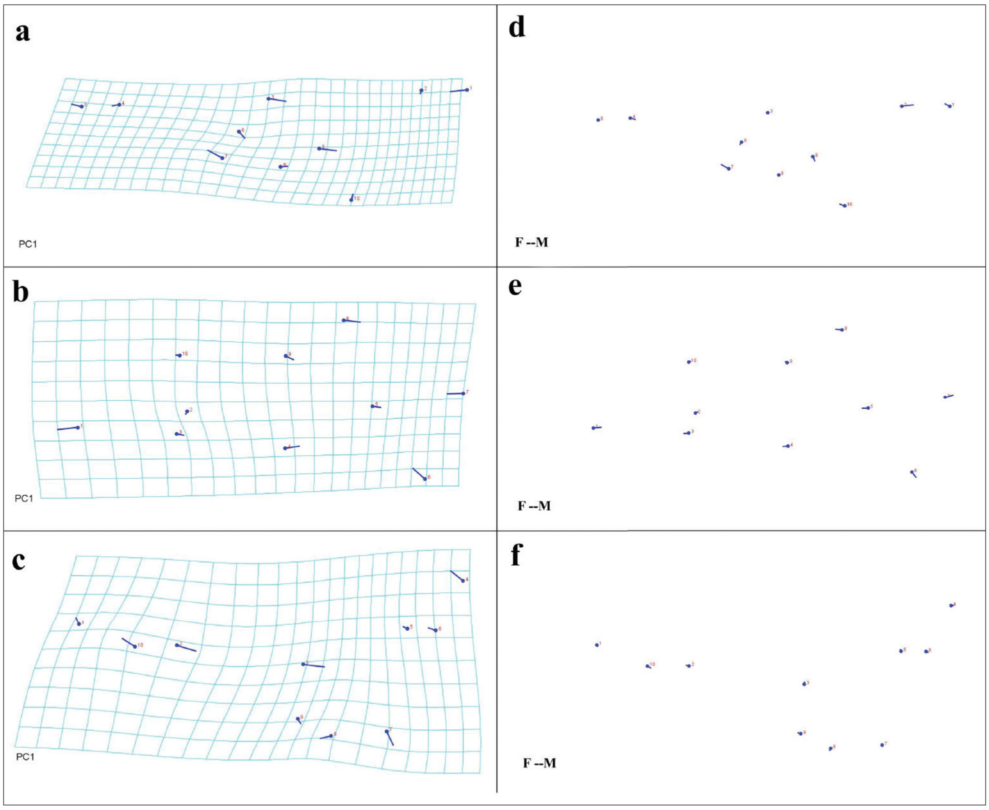


Fig 7. The lollipop demonstration of the shape differences occurring on the landmarks based on the first principal component and the discriminant function analysis. **a, d.** Skull dorsal, **b, e.** Skull lateral, **c, f.** Mandible. Point represents ewes

Fig. 5-a,b,c,d show the consensus graphics obtained as a result of relative warp analysis along with change vectors in the study. Accordingly, vector intensity was determined at LM2, 3 and 9 levels from the dorsal side and at LM1, 6 and 7 levels from the lateral side in the Awassi ewe. Vector intensity was determined in the Awassi ram at LM6 and 7 levels from the dorsal side and at LM1 and 8 levels from the lateral side. Also, *Fig. 6-a,b* shows the graphics obtained as a result of the between-groups relative warp analysis. As a result of this analysis, it was observed that the ram differentiates from ewe under the x axis from the dorsal side and on the left part of y axis from the lateral side (Dorsal RWA1: 37.75%, RWA2: 27.85%, RWA3: 10.44%, Lateral RWA1: 44.26%, RWA2: 14.53%, RWA3: 10.20%).

Fig. 7-a,b,d,e show in terms of PCA1 and sexes at which LM levels shape differences occurred in the study. From the dorsal side, a shape difference was determined from anteriodorsal side at LM1 and LM7 and from caudoventral side at LM4 and 9 in the ram compared to the ewe. From the lateral side, a shape difference was determined from caudal side at LM1 and LM7, from caudoventral side at LM6 and from anterior side at LM4 and 8 in the ram compared to the ewe. In the comparison of LMs performed in terms of coordinate value, a statistically significant difference was determined at LM2, 4, 9 and 10 dorsally and at LM1, 5, 7 and 8 laterally between the sexes ($P<0.05$).

Mandible Analysis

Table 1 shows the results of the principal components analysis related to mandible in the study. Accordingly, in the comparison between the sexes in mandible of Awassi sheep, the first principal component accounted for 24.92% of the total shape difference. The graphics in *Fig. 4-c* shows the sexual dimorphism in terms of the first principal component. Accordingly, it was observed that the mandibles of ewe and ram individuals were not separated with apparent borders.

Fig. 5-e,f show the mandible consensus graphics obtained as a result of relative warp analysis along with change vectors in the study. Accordingly, the vector intensity in the ewe and ram was at similar LMs. Also, *Fig. 6-c* shows the graphics obtained as a result of the between-groups relative warp analysis. The result of this analysis indicated that the discrimination was not apparent between the ewe and ram individuals as in the principal components analysis (RWA1: 24.92%, RWA2: 18.53%), RWA3: 13.94%).

Fig. 7-c shows at which LM levels shape differences occurred in mandible in the study. A shape difference was determined from caudal side at LM2 and 3, from caudoventral at LM10 and from the anteriodorsal side at LM9 in the male sheep compared to the ewe. In the comparison of LMs in terms of coordinate value, no statistical difference was observed between sexes ($P>0.05$).

Discussion

In the study, the shape difference of skull and mandible in Awassi sheep breed with a common breeding potential was analyzed using geometric morphometric methods based on sex factor. There are classic morphometric studies in the literature conducted on sheep skull and mandible related to sexual dimorphism [20,21]. However there is only one study examining sheep skull using geometric morphometric analysis [9].

Jaslow [22] stated that horn affected the cranial morphology and craniometry significantly in wild sheep [23]. Also, in the present study, LMs were selected cautiously from both lateral and dorsal sides for geometric morphometric method in order to minimize the effect of horn.

Sexual dimorphism is one of the most interesting phenotypic variation sources in animals and plants. Sexual differences in morphological characteristics in animal taxonomy are a general phenomenon. The most apparent one of this phenomenon is the body size difference based on sex [23]. There are important results of size difference based on sex for ecology, behavior, generation mobility and evolution [24]. Although it is stated in the literature [25] that sexual dimorphism is significant in sheep, it is important to analyze the points apart from horn and the shape of mandible based on sheep breeds, especially in skull. Abbasabadi et al.[21] stated that there was no sexual dimorphism in skull of Zell sheep using classic morphometric method, Pares-Casanova et al.[26] stated that there was no sexual dimorphism in skull of Gwembe-Dwarf goat using classic morphometric method and Pares-Casanova [9] expressed that there was no sexual dimorphism in skull of Fardasca sheep using geometric morphometric method. Pares-Casanova et al.[27] stated in their study conducted by geometric morphometric method that the skull of White Rasquera goats included sexual dimorphism. Therefore, in the present study, it was observed that skull of Awassi sheep had an apparent differentiation from the lateral side compared to the dorsal side. In their study, Yalçın et al.[11] reported that gender dimorphism was not observed in mandible of Anatolia Wild sheep. Likewise, in the present study, the grouping based on gender in the mandible was not apparent.

Pares-Casanova [5] used the crania of 16 ewe and 2 ram individuals of native sheep breed in his study. A limited number of male individual materials was remarkable. In the present study, 17 samples (10 ewe, 7 ram) were used from the dorsal side and 16 samples (9 ewe, 7 ram) were used from the lateral side. Also, the geometric morphometric analysis of mandible was performed.

Pares-Casanova [9] reported in his study that the first three principal components (PC) accounted for 63.68% (PC1: 30.43%, PC2: 18.77%, PC3: 14.47%) of the total shape variation in Fardasca sheep skull. In the present study, it was determined that the first three principal components explained 68.97% (PC1: 44.14%, PC2: 14.53%, PC3: 10.2%) of the total shape variation from the lateral side in skull of Awassi sheep.

The data obtained from the archaeological bone residues is important as it allows the estimation of the morphological characteristics of animals, determination of fauna or other socio-economic comparisons [27-30]. The morphological data to be obtained in the skull and mandible of the living mammals through geometric morphometric method may be used to reveal the phylogenetic relations [31]. For this reason, the information obtained in the present study is important as they provide basic shape information for the small ruminant skull or mandible remains to be found in the archaeological excavations especially in Mesopotamia region including Gobeklitepe.

In their study, Yalçın and Kaya [14] compared Akkaraman and Anatolian Wild Sheep crania by taking 13 LM from the dorsal side using a geometric morphometric method. It was stated in this study that the first and second principal components accounted for 58.55% and 11.75% of the total shape difference, respectively. In the present study, the first and second principal components accounted for 37.72% and 27.84% of the total shape difference from dorsal side between sexes in Awassi sheep, respectively. These rates showed that skull of Awassi sheep had an apparent difference from the dorsal side based on sex.

Yalçın et al.[11] reported in their study that the difference in mandibles at LM9 level is quite apparent and this may be associated with the differences such as environmental conditions and feeding habits as well as adaptation to the domestication process. In the present study, although it was observed that the most apparent differences in mandible were at levels of LM2, 8, 9 and 10, the differences were quite limited.

Consequently, Awassi sheep skull and mandible were analyzed in this study using geometric morphometric methods in terms of presence of sexual dimorphism. In the study, it was an important finding that Awassi sheep crania formed groups considerably as ram and ewe from both the dorsal and lateral sides in principal components and relative warp analyses. However, the mandibles did not exhibit an apparent difference in the same analyses based on sex. It is considered that the data found in this study would contribute to the possible ruminant skull and mandible studies by using geometric morphometric method. Also, we think that these data would be useful in the distinction of the cranium remains uncovered in zooarcheological excavations and, especially assessed as ovicapri (sheep-goat).

Author Contributions

Demircioğlu İ and Demiraslan Y designed and directed the study. Dayan MO, Demiraslan Y and Gürbüz İ conducted geometric morphometric application. Demircioğlu İ and Demiraslan Y provided Awassi sheep skulls. Demircioğlu İ, Demiraslan Y and Gürbüz İ and Dayan MO cowrote the overall paper.

Conflicts of Interest

The authors declare no conflict of interest.

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