MJAVL
Manas Journal of Agriculture Veterinary and Life Sciences
ISSN 1694-7932 | e-ISSN 1694-7932
Volume 14 (Issue 1) (2024) Pages 11-20

Research Article

Examination of Mandible in Morkaraman Sheep Using Geometric Morphometry Method

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ABSTRACT

The aim of this study is to examine and analyze the mandible of Morkaraman sheep without any skeletal disorders using the geometric morphometric method. For this purpose, a total of 14 mandibles from male and female individuals were used in the study. The main components in multidimensional data sets were determined with Principal Component Analysis, used within the scope of the geometric morphometry method, and the differences between the samples were determined with Discriminant Function Analysis. Statistical and formal analyzes of these variances were also performed. A total of 12 principal components were obtained with 12 punctuations selected on a total of 14 mandibular (7 female, 7 male) images. Among these principal components, the first principal component (PC1) alone accounted for 30.409% of the total variation. The second principal component (PC2) alone accounted for 22.265% of the total variation, and the third principal component (PC3) alone accounted for 14.893% of the total variation. The first three of the variances obtained explained 67,567 of the shape differences. Discriminant Function Analysis (DFA) was used to objectively evaluate gender differences. In the discriminant function analysis, the p value was above 0.05 (p = 0.7). Although there was a complete separation between genders formally and statistically, no significant p value was obtained. In line with these analyses, information was obtained about the anatomical features and adaptations of the Morkaraman sheep mandible, which is one of the important economic resources of our country and is bred in a wide area, and it has become an exemplary study in this field.

ARTICLE INFO

Received: 05.02.2024

Accepted: 18.03.2024

Keywords: *Geometrik morphometry, PC Analysis, Mandible, Discriminant Fonction Analysis*

Cite this article as: Dalga, S., & Koçak, S. (2024). Examination of Mandible in Morkaraman Sheep Using Geometric Morphometry Method. Manas Journal of Agriculture Veterinary and Life Sciences, 14(1), 11-20. https://doi.org/10.53518/mjavl.1431805

INTRODUCTION

In the Eastern Anatolia Region, which has the majority of our sheep existence; Morkaraman breed is widely bred in Erzurum, and Karakaş sheep, a variety of Akkaraman breed, is widely bred in Bitlis and Van regions (Karaca et al., 1996). Again, Tuj is grown in Kars and Hamdani is grown in the southern parts of the region (Geliyi and İlaslan, 1978).

Morkaraman breed constitutes 21.5% of our existing sheep. This breed is grown in a wide region from the east of Sivas and Malatya provinces to Kars and Van. However, it is mostly grown in the provinces of Erzurum, Van, Ağrı, Kars and Muş. Sheep wealth in the Eastern Anatolia region constitutes 30.6% of Türkiye's sheep wealth. Morkaraman breed sheep constitute 61.1% of the sheep population in the Eastern Anatolia region. As a result of the research, the ram breeding of the Morkaraman sheep breed is generally done in September and October; Its birth takes place in February and March. Its morphological characteristics are: withers height 70 cm, back height 68 cm, rump height 69 cm, body length 65 cm, chest width 18 cm, front rump width 18.5 cm, middle rump width 21 cm, front shin circumference 7.4 cm, rear shin circumference. It was reported as 9 cm, leg height as 38.7 cm, head length and width as 22.4 cm, and 12.5 cm, and ear length as 14.7 cm, (Kayalık and Bingöl, 2015).

The geometric morphometric method, which measures the position differences of objects and the amount of shape changes, determines the shape differences according to the sign coordinates (Kimmerle et al. 2008). Markings are made on the figures with homologous landmarks. Semilandmarks allow the measurement of two- or three-dimensional homologous curves and surfaces and their analysis together with traditional marks (Gunz and Mitteroecker 2013).

The lower jaw is considered one of the strongest skull bones for sex determination and has therefore been one of the most studied bones. It accurately reflects gender characteristics (Okkesim and Erhamza 2020).

Studies conducted on many different species and races using traditional morphometric methods are widely available in the literature (Dalga et al. 2021; Dalga et al. 2022; Özcan et al. 2010; Karimi et al. 2011; Mohamed et al. 2016; Dalga et al. 2017; Dalga et al.2018; Dalga and Aslan 2020; Wehausen and Ramey 2000; Dalga and Aslan 2021; Dalga 2020; Jashari et al. 2022; Gundemir et al. 2020; Yılmaz and Demircioğlu 2020; Özüdoğru et al. 2019). In addition to examining evolutionary processes, there are many studies that analyze two- and three-dimensional images using the geometric morphometry method (Klingenberg and Marugan 2013; Szara et al. 2022; Koçak et al. 2023; Pares 2015; Fernandez et al. 2023).

This study was conducted to reveal the shape differences of the mandible between male and female individuals in Morkaraman sheep, using analyzes based on the geometric morphometry method.

MATERIAL AND METHODS

In the study, the mandible of the Morkaraman sheep, which is located in the Eastern Anatolia region and bred in Kars, Ardahan and Iğdır provinces, was used. A total of 14 mandibles (7F/7M) were obtained from slaughterhouses in the relevant provinces. The number was found sufficient for parametric analyzes in terms of gender. The study permit for animal experiments was approved by the local ethics committee unit with reference code 2023/071. Mandibles were first separated from the skull. Then, the muscles on it were dissected. Boiling was applied to thoroughly remove the muscles. After the boiling process, the mandibles were kept in hydrogen peroxide for 20-30 minutes and the mandibles were whitened. After the drying process, the mandibles were photographed from the same distance (15 cm,). The left lateral sides of the mandibles were used for photography. For punctuation, photographs were saved as tps files using tpsUtil (version 1.82). The tps file was imported into the tpsDig (version 2.31) program for marking. Marking was made with 12 selected points on each mandible using the TpsDig program. Each mandible photograph was marked at the same locations. The marked mandible data were converted to text file and imported into the MorphoJ (version 1.07a) program to perform geometric morphometric analysis. Principal Component Analysis (PCA) was performed and shape variations were obtained. Each component was ranked by percentage of variation. Additionally, Discriminant Function Analysis (DFA) was

performed and shape variations were obtained. The distinction between male and female groups was examined statistically and formally with Discriminant Function Analysis.

RESULTS

A total of 12 principal components were obtained with 12 punctuations selected on 14 mandible (7 female, 7 male) images of Morkaraman sheep (Figure 1). Among these principal components, the first principal component (PC1) alone accounts for 30.409% of the total variation. The second principal component (PC2) alone accounts for 22.265% of the total variation, and the third principal component (PC3) alone accounts for 14.893% of the total variation. Data from PCA are presented in Table 1.

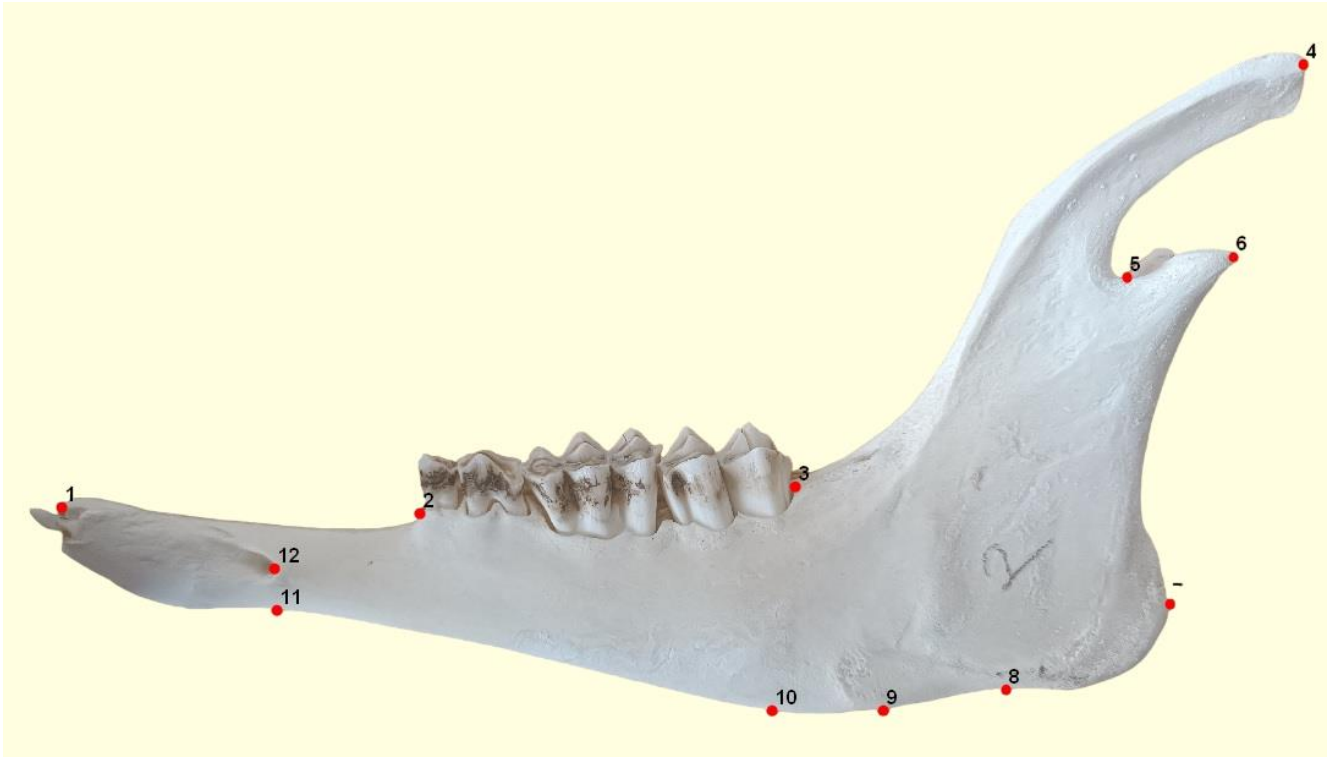

Figure 1: *The Landmarks point of on Mandible; 1: İnfradentale, 2: First premolar teet oral border, 3: Last molar teet aboral border, 4: Processus coronoideus, 5: İncisura mandibulae, 6: Processus condylaris, 7: Caudal end point, 8: İncisura vasorum facialum, 9: Ventral point, 10: the length of from molar teeth to ventral border, 11: the ventral point of under diastema, 12: Foramen mentale*

Table 1: *Eigenvalues and variances obtained as a result of principal component analysis*

PC No	Eigenvalues	Varyans(%)	PC No	Eigenvalues	Varyans (%)
PC1	0,00054645	30,409	**PC7**	0,00005990	3,334
PC2	0,00040009	22,265	**PC8**	0,00005671	3,156
PC3	0,00026763	14,893	**PC9**	0,00003081	1,715
PC4	0,00022065	12,279	**PC10**	0,00001543	0,859
PC5	0,00010948	6,093	**PC11**	0,00000567	0,316
PC6	0,00008056	4,483	**PC12**	0,00000360	0,200

Figure 2 shows both cumulative and individual variation distribution plots for 12 of the principal component analyses. As can be seen from the graph, PC1, PC2 and PC3 cumulatively account for more than half of the total variation. Additionally, these three variations individually have significant variation

percentages. With these findings, PC1, PC2 and PC3 were compared while performing principal component analysis.

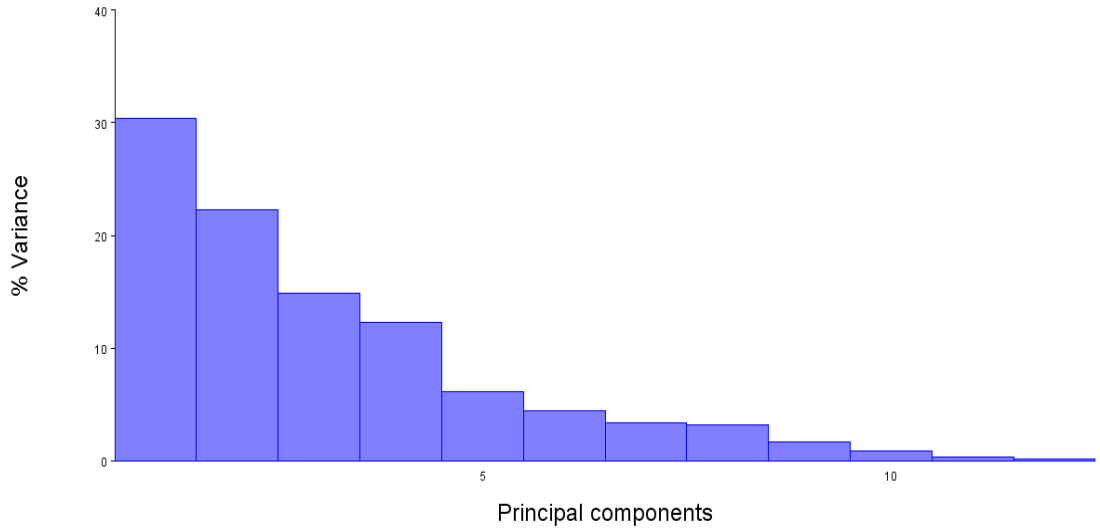

Figure 2: *Variation distribution plot of principal component analysis*

The shape variation for PC1, PC2 and PC3 is shown in Figure 3. Dots represent the average shape. The extensions represent the positive limit for PC1, PC2 and PC3. For PC1, it narrows infradentally, distally and medially, while for PC2 and PC3, it widens proximally. For PC1, the first premolar teet oral border expanded proximally, while for PC2 and PC3 it expanded distally. For PC1, PC2 and PC3, the last molar teet aboral border has expanded distally. For PC1, the processus coronodieus expanded proximally, while in PC2 and PC3 it expanded distally. For PC1, the incisura mandibulae expanded proximally, while in PC2 and PC3 it expanded distally. For PC1, the processus condylaris expanded proximally, while in PC2 and PC3 it expanded distally. While the caudal end point expanded distally for PC1, it expanded proximally for PC2 and PC3. For PC1 and PC3, the incisura vasorum facialum expanded distally, while for PC2 it expanded proximally. For PC1 and PC3, the ventral end point expanded distally, while for PC2 it expanded proximally. For PC1, the distance of the molar teeth to the ventral edge expanded proximally, while in PC2 and PC3 it expanded distally. For PC1, the distance of the premolar to the ventral edge narrows medially, while for PC2 it widens distally. For PC3, it expanded proximally. For PC1, the mental foramen expanded proximally, while for PC2 it expanded distally, and for PC3 it expanded proximally. As a result of the statistical and formal analysis of the Morkaraman sheep mandible, it was obtained that the formal variation in the distal region was higher than in other regions.

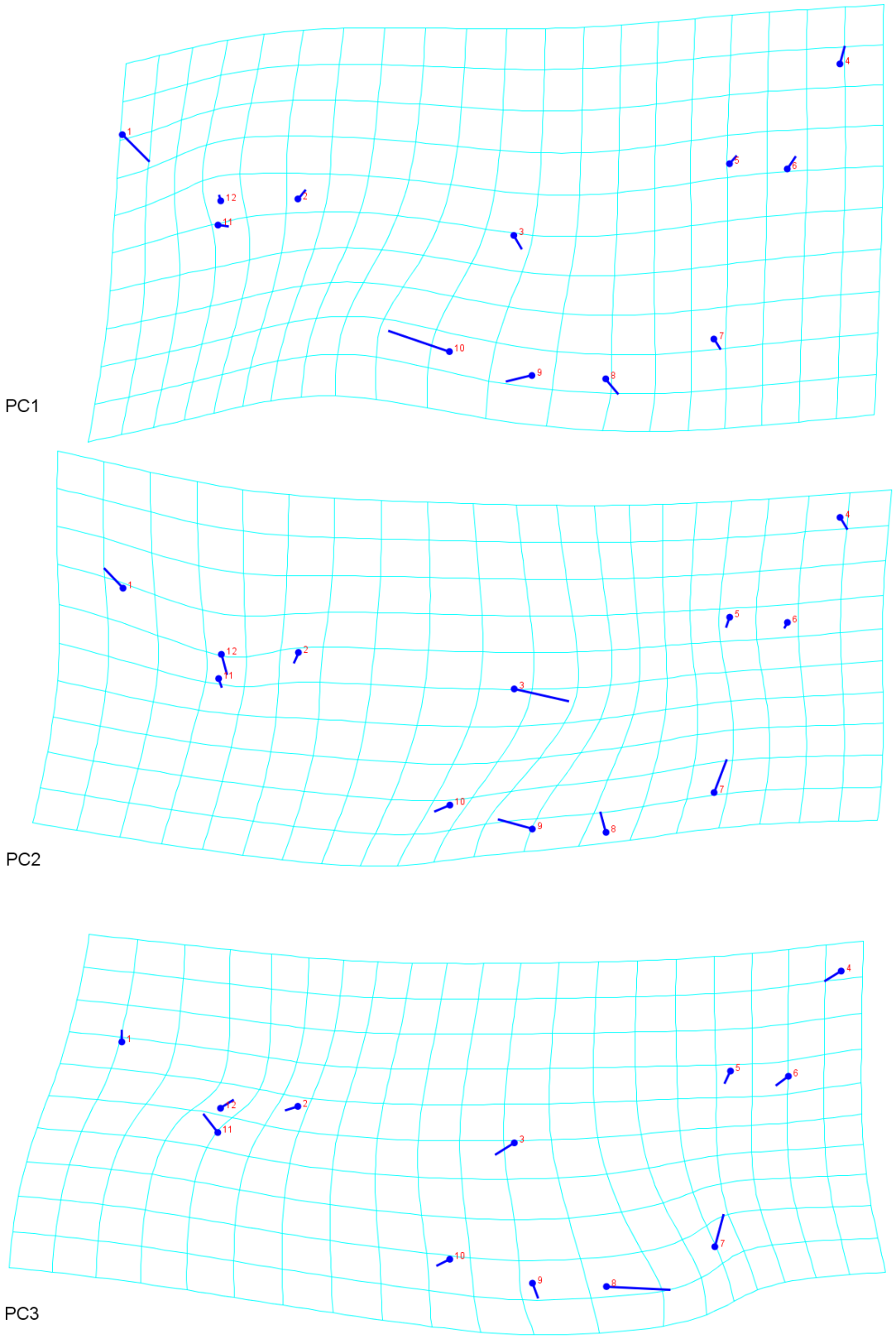

Figure 3: *1: İnfradentale, 2: First premolar teet oral border, 3: Last molar teet aboral border, 4: Processus coronoideus, 5: İncisura mandibulae, 6: Processus condylaris, 7: Caudal end point, 8: İncisura vaorum facialum, 9: Ventral point, 10: the length of from molar teeth to ventral border, 11: the ventral point of under diastema, 12: Mental foramen*

A total of 12 principal components were obtained in the Principal component analysis in figures 4 and 5. The first three principal components (PC1, PC2 and PC3) explained 30.409, 22.265 and 14.893% of the total variance, respectively.

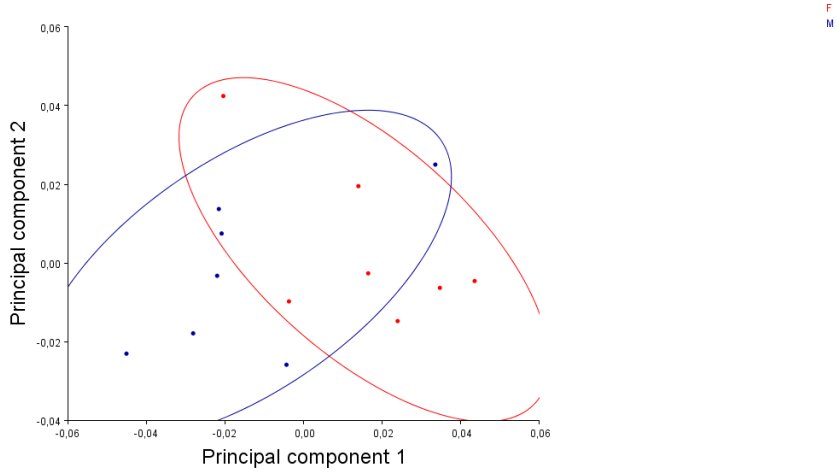

***Figure 4**: Morkaraman sheep Principal component 1-2 variation distributions and 95% confidence ellipses. Red dots are female, green dots are male.*

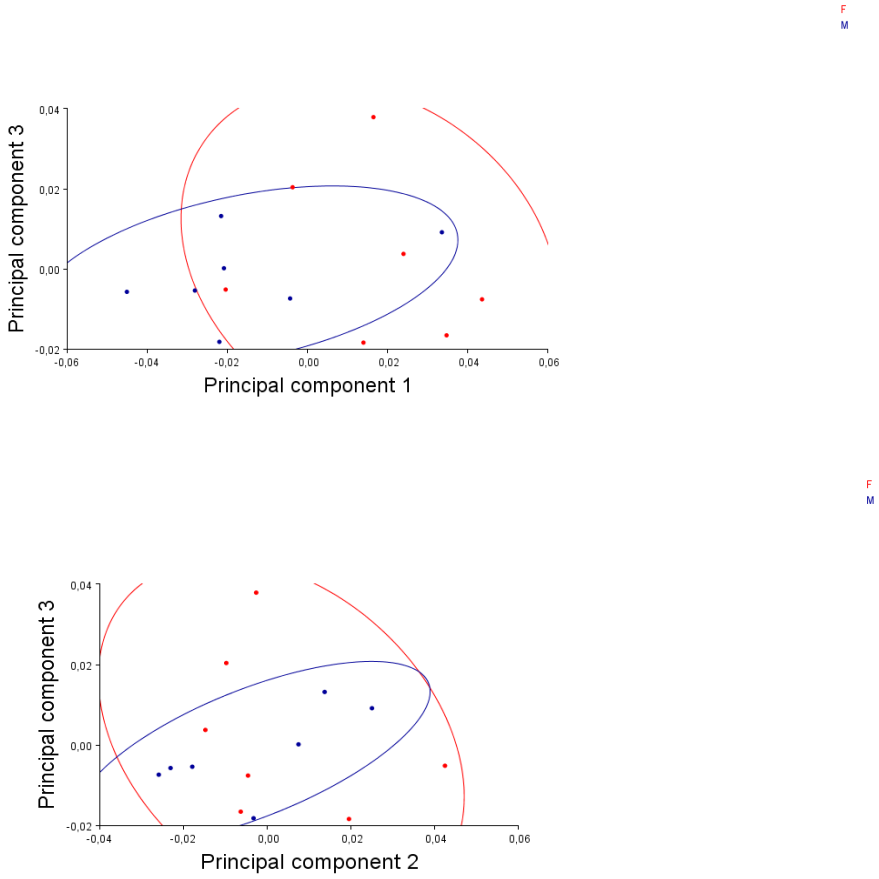

Figure 5: *Morkaraman sheep Principal component 1-3 and 2-3 variation distributions and 95% confidence ellipses. Red dots are female, green dots are male.*

Discriminant Function Analysis (DFA) was used to objectively evaluate gender differences. In the discriminant function analysis, the p value was above 0.05 (p = 0.7). Although there was a complete separation between genders formally and statistically, no significant p value was found.

In Figure 6, there are points that show significant changes in shape as the variation from female to male increases. The first premolar teet oral border has expanded proximally. The last molar teet aboral border has expanded ventrally. Processus coronoideus has expanded distally. The caudal end point narrows proximally. Incicura vasorum facialum is narrowed proximally. The distance of the molar teeth to the ventral edge has increased distally. Foramen mentale has expanded ventrally.

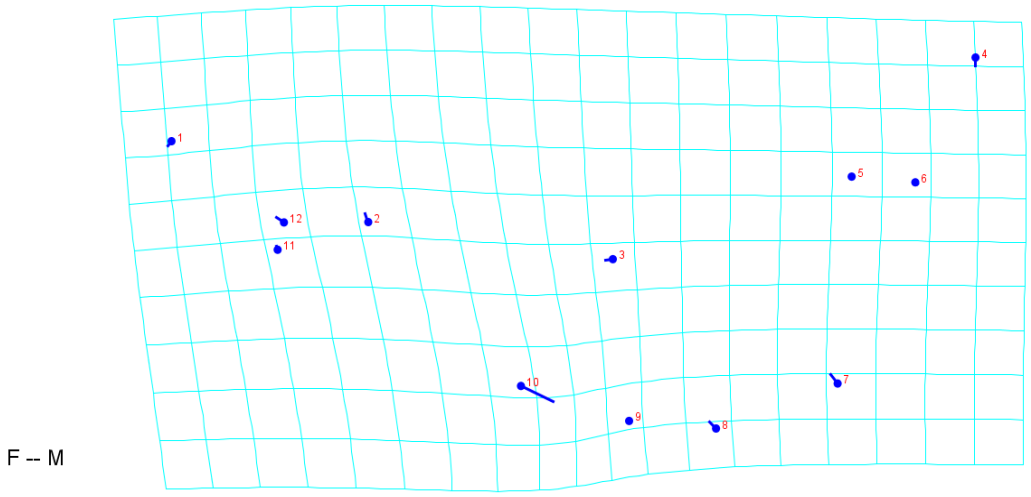

Figure 6: *Differences between males and females are shown with punctuation. While the round dots represent females, the extensions coming from the dots represent males.*

Discriminant Function Analysis (DFA) was performed on the mandibles of Morkaraman sheep for gender determination. The variance and frequency distribution of individuals is given in Figure 7. It can be seen that the male and female groups are completely separated from each other.

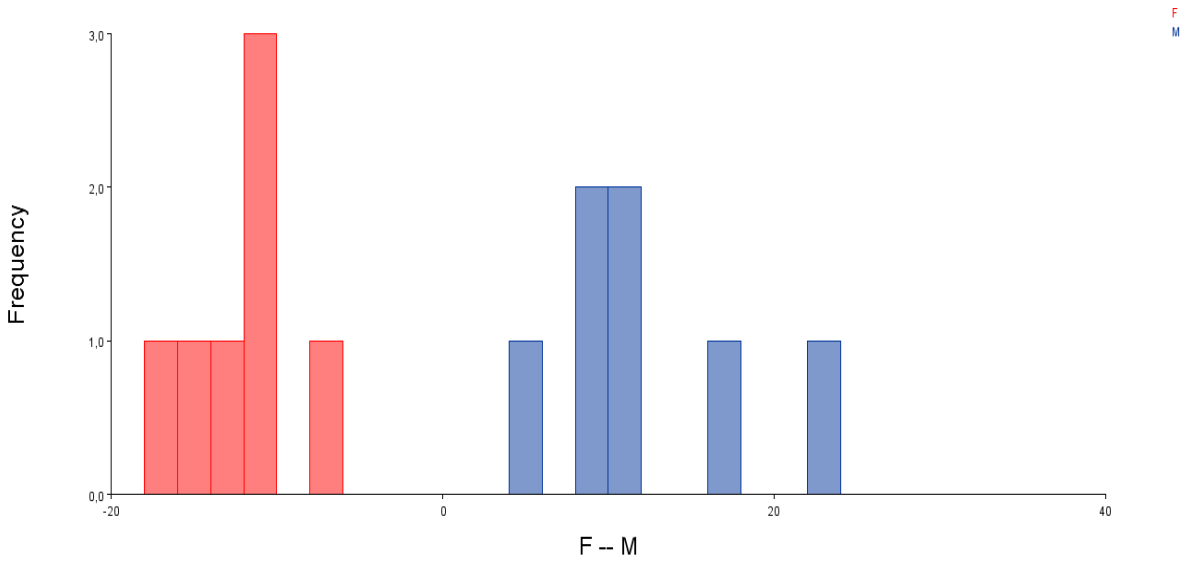

Figure 7: *Gender distribution graph in Discriminant Function Analysis. Red ones are female, green ones are male.*

DISCUSSION

In this study, 14 (7F/7M) mandibles from Morkaraman sheep were used. Morkaraman sheep mandibles were marked from 12 points and both Principal Component Analysis and Discriminant Function analyzes were performed with geometric morphometric analysis. Morkaraman sheep mandible was examined for gender determination. Geometric morphometry was an important method that should be used to distinguish gender between Morkaraman sheep mandibles. In our study, although there was no separation between the genders in the Principal Component Analysis, a complete formal separation was observed between the genders in the Discriminate Function Analysis.

Geometric morphometric method-based analyzes performed on the skulls and mandibles of mammals are used to explain the phylogenetic relationships between living things (Marcus et al. 2000). In addition to skull studies in ruminants, where dimorphism is clearly evident, various researchers have also conducted studies on the lower jaw bone and contributed the data to the literature (Özkan et al. 2020).

Analyzes were made on the lengths determined by studies on the skull (Jashari et al. 2022), mandible (Jashari et al. 2022; Özkan et al. 2020) and metapodium (Gündemir et al. 2020; Kahraman et al. 2022) in sheep, and our As in our study, the unique anatomical differences of the species were evaluated with various methods in terms of species and gender. With simpler variation and deviation analyses, the effects of variables on gender were examined in morkaraman sheep.

There are many studies in the literature in which the obtained principal component variances, especially the first three, are evaluated and discussed as a result of the analysis. In such a study conducted on the mandible of Awassi sheep, researchers stated that especially the first principal component explained 24.92% of the total shape difference (Demircioğlu et al. 2021). Examining the variations in different sheep breeds using the geometric morphometry method, Boz et al. (2023) obtained a total of 14 components. In the components they obtained, they stated that PC1 explained 29.668% of the total variation and PC2 explained 22.169%, and both PC values explained more than half of the total variation. They also reported 13.643% in PC3. In Morkaraman sheep (PC1), it alone explained 30.409% of the total variation. PC2 and PC3 explained 22.265% and 14.893% respectively. Demiraslan et al. (2024), who examined the mandible of Hamdani and Awasi sheep using the geometric morphometric method, stated that, according to their findings, PC1 explained 36.52% of the total difference, and the sum of the other two PC analyzes and PC1 explained 63.822% of the shape difference. In Morkaraman sheep, the sum of PC1 and PC2 alone explained more than half of the shape variation.

Demiraslan et al. (2021), conducted geometric morphometric analysis on the mandibles of Honamlı and Kıl goats. They reported in their study that hair goats showed a very distinct gender difference compared to honamlı goats. They also stated that in terms of race, male goats are clearly clustered compared to females. Demircioğlu et al. (2021) also stated that there was no significant difference between genders between mandibles.

Researchers who studied Anatolian wild sheep (Yalçın and Kaya, 2009) and Awassi sheep (Demircioğlu et al. 2021) reported that no dimorphism was observed in terms of gender. In our study, when we compared the study with other studies in the literature in terms of gender dimorphism, similar findings were obtained. However, when we looked at it in terms of discriminant function analysis, formal differences were observed.

Principal component variances, which express statistical and shape variations between groups, are related to the number of materials used, Koçak et al. (2023) obtained 46 variances in their principal component analysis study, whereas in our study, 12 variances were obtained due to the difference in the number of animals. Like the researchers who discriminate between genders with discriminant function analysis (Koçak et al. 2023), this method was used in our study and there was a complete separation between genders.

Researchers who studied the Anatolian wild sheep (Yalçın and Kaya 2009), in which the shape variations in the gonion ventrale are quite prominent, suggested that this difference is related to environmental conditions and nutrition, and also due to adaptations during the domestication process. Among the first three analyzes obtained

in Morkaraman sheep, it is seen that the gonion ventrale expands distally in PC1 and PC3, and proximally in PC2. It has been reported that there are significant differences in this parameter in Awassi sheep (24). It was also stated that there were differences in LM2, LM8 and LM10 levels, but they were limited.

CONCLUSION

This study aims to analyze the morphometric findings of the Morkaraman sheep mandible as well as its shape, and it is thought that these analyzes will contribute significantly to the studies to be carried out in this field, the identification and determination of osteological materials obtained as a result of archaeological excavations, the creation of three-dimensional models and the use of these shape analyzes in animal human models. In addition, principal component variation values between males and females were examined on a race basis with Principal Component Analysis, and shape changes between males and females were interpreted in Principal Component Analysis. In addition, gender determination was evaluated with Discriminant Function Analysis, which is the main element of the study.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

AUTHORS CONTRIBUTION

SD and SK designed the study. SD, and SK performed the geometrical and morphometric analysis. SD and SK carried out the statistical analysis. SD and SK the imaging all section. The manuscript was written by SD and SK. All authors approved the final version.

ETHICAL STATEMENT

During the writing process of the study titled "**Examination of Mandible in Morkaraman Sheep Using Geometric Morphometry method**", scientific rules, ethical and citation rules were followed; No falsification has been made on the collected data and this study has not been sent to any other academic media for evaluation. The study protocol was approved by the Ethics Committee Kafkas University (20.06.2023, KAU/HADYEK-2023/07).

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