The analysis of Norduz sheep mandible with DFA and PCA

Running title: Sex determination in mandible

Abstract

Norduz sheep are known as a variety of Akkaraman sheep bred in the Norduz region, which is located within the borders of the Gürpınar district of Van province . Norduz sheep are bred only in the Norduz region, adapt to the harsh climate of the Eastern Anatolia region, and show unique performance indicators that distinguish this breed from other breeds. In this study, the mandible of Norduz sheep was examined by geometric morphometric method and analyzed. Principal components in multidimensional data sets were determined by Principal Component Analysis. The differences between the samples were determined by Discriminant Function Analysis. For this purpose, study materials were obtained from slaughterhouses in Van. A total of 20 mandibles (10F/10M) were used since analyzes were made in terms of gender. The mandibles were first dissected from the skull. Later, the overlying muscles were dissected. After boiling, they were kept in hydrogen peroxide for 20-30 minutes and the mandibles were bleached. After drying, the mandibles were photographed from the same distance (20 cm). The left lateral sides of the mandibles were used for photographing. Statistical and formal analyzes of these variances were also performed. As a result of the analyzes made, 16 variances were obtained within the scope of principal component analyses. The first three of the variances obtained explained 58,647 of the shape differences. According to the discriminant function analysis, which gave the best results for gender discrimination, the results were very good and individuals were completely separated from each other. In line with these analyzes, information was obtained about the anatomical features and adaptations of the Norduz sheep mandible, and it became an exemplary study in this field.

Keywords: Mammalian morphology, discriminant function analysis, principal components analysis, geometric morphometry

1. Introductıon

Norduz sheep are known as a variety of Akkaraman sheep bred in the Norduz region, which is located within the borders of the Gürpınar district of Van province (1-3). Norduz sheep are bred only in the Norduz region, adapt to the harsh climate of the Eastern Anatolia region, and show unique performance indicators that distinguish this breed from other breeds (3). Various studies on Norduz sheep are available in the literature (3-5).

It is thought that studies conducted with classical morphometric methods (6-21) as well as geometric morphometric analysis studies will contribute to the literature in terms of both figural and gender analysis (22). In addition, geometric morphometry; It also allows the study of species patterns and evolutionary processes. This method determines the shape and position differences of the objects by using the coordinates of the points (23). In recent years, there have been many studies conducted on different species and different bones in order to determine the differences between the sexes of animals using the geometric morphometry method (24-31). In recent years, there are also geometric morphometric studies on three-dimensional bone materials (32).

In this study, it was aimed to reveal the shape differences between male and female individuals of the mandible in Norduz sheep by using analyzes based on geometric morphometry method.

2. Materials and Methods

Study materials were obtained from slaughterhouses in Van. A total of 20 mandibles (10F/10M) were used since analyzes were made in terms of gender. The mandibles were first dissected from the skull. Later, the overlying muscles were dissected. Boiling was applied to remove the muscles thoroughly. After boiling, they were kept in hydrogen peroxide for 20-30 minutes and

the mandibles were bleached. After drying, the mandibles were photographed from the same distance (20 cm). The left lateral sides of the mandibles were used for photographing. Photographs for punctuation were saved as tps file using tpsUtil (version 1.82). The tps file was imported into the tpsDig (version 2.31) program for marking. Marking was done with 10 selected points on each mandible using the TpsDig program. Each mandible photograph was marked from the same locations. The marked mandible data was converted to text file and imported into MorphoJ (version 1.07a) to perform geometric morphometric analysis. Principal Component Analysis (PCA) was performed and shape variations were obtained. Each component was ranked by percentage of variation. In addition, Discriminant Function Analysis (DFA) was performed and shape variations were obtained. The distinction between male and female groups was analyzed statistically and formally by Discriminant Function Analysis.

3.Results

In this study, 20 mandibles (10F/10M) of Norduz sheep were examined by using geometric morphometric analysis method, obtaining a total of 16 basic components with 10 punctuation. Among these principal components, the first principal component (TB1) alone constituted 27.49% of the total variation. The second principal component (TB2) alone constituted 17.25% of the total variation, and the third principal component (TB3) alone constituted 13.91% of the total variation. The data of principal component analysis are presented in Table 1.

Table 1: Principal components, eigenvalues and variances

TB No	Özdeğer	Varyans (%)	TB No	Özdeğer	Varyans (%)
TB1	0,00040929	27,487	TB9	0,00003808	2,557
TB2	0,00025680	17,246	TB10	0,00002842	1,909

TB3	0,00017209	13,914	TB11	0,00001513	1,016
TB4	0,00017209	11,557	TB12	0,00001255	0,843
TB5	0,00010624	7,135	TB13	0,00000714	0,480
TB6	0,00008472	5,689	TB14	0,00000273	0,184
TB7	0,00007692	5,166	TB15	0,00000201	0,135
TB8	0,00006869	4,613	TB16	0,00000103	0,069

Both the cumulative and individual variation distributions for 16 of the principal component analyzes are shown in (Figure 1). As can be seen from the graph, TB1, TB2 and TB3 cumulatively account for more than half of the total variation. It also has significant percentages of variation singularly. While performing principal component analysis on the findings, TB1, TB2 and TB3 were compared.

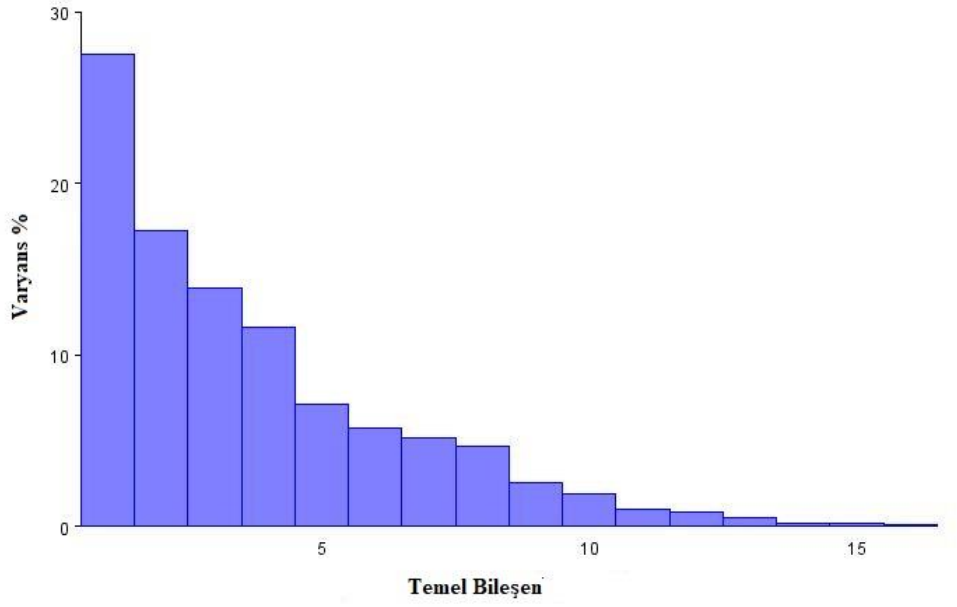
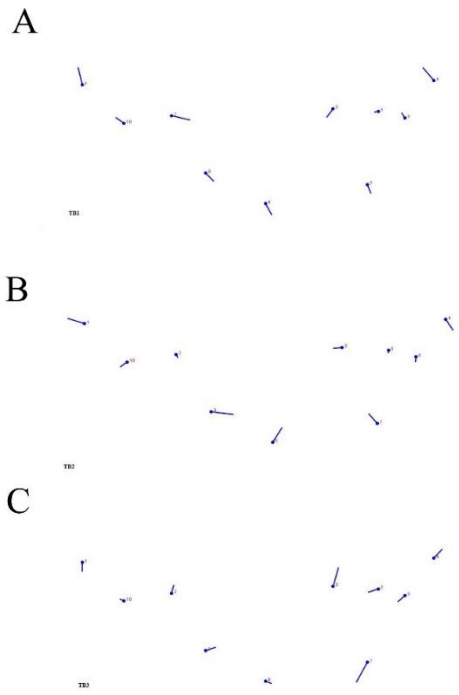


Figure 1: Variation scatter plot of principal component analysis

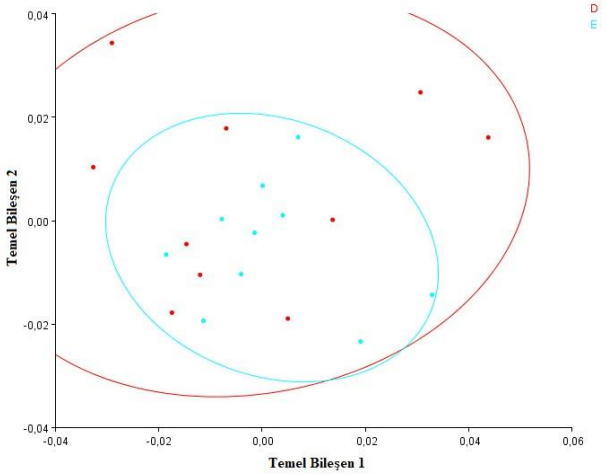
The shape variation obtained for the principal components 1, 2 and 3 is given in (Figure 2). The dots represent the mean shape. Extensions represent the positive limit for TB1, TB2 and TB3. Considering the remarkable deviations in TB1 and TB2, it is seen that the infradentale expands proximally. However, in TB3, it expanded distally. In TB1 and TB2, the anterior edge of the first molar tooth is enlarged distally and medially, and in TB3 proximally. In TB1 and TB3, the processus coronoideus expands proximally, while in TB2 it expands distally. In TB1 and TB3, the gonion caudale expands distally, while in TB2 it expands proximally. While the gonion expanded ventrally and distally in TB1 and TB3, it was analyzed that it expanded proximally in TB2. In TB1 and TB2, the end point of the second premolar tooth to the ventral edge enlarges distally and medially, while in TB3 it is observed to expand proximally and medially. In TB1 and TB3, the distance of the Foreman mentale to the ventral edge widened proximally, while in TB2 it expanded distally and laterally. In this context, as TB1 and TB3 values increased, it was observed that there was proximal and distal enlargement in the mandible. In TB2, a narrowing from distal to proximal was analyzed.

Figure 2: Shape variation of Principal Components 1,2 and 3. 1: Infradentale, 2: Anterior edge of the 1st molar tooth, 3: Anterior edge of the ramus mandible, 4: Processus coronoideus, 5: Incsura mandibulae, 6: Processus condylaris, 7: Gonion caudale, 8: Gonion ventrale, 9: End point of the 2nd premolar to the ventral edge, 10: Distal to the ventral edge of the foreman mentalkenera



The 16 principal components obtained in principal component analysis are shown in (Figure 3). The first three of these components (TB1, TB2 and TB3) explained 27,487, 17,246 and 13,914 of the total variance, respectively. In addition, although the total variance was high and the discriminant function analysis findings were significant, a complete gender separation was not observed in Principal Component Analysis.

Figure 3: Principal component 1-2 variation distributions and 95% confidence ellipses in Norduz sheep. Red dots: female, green dots: male



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١٠٣ Figure 4A,B: Norduz sheep Principal component 1-3 and 2-3 variation distributions and 95%

١٠٤ confidence ellipses. Red dots: female, green dots: male

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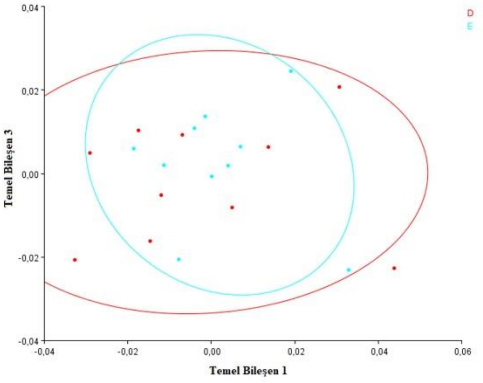
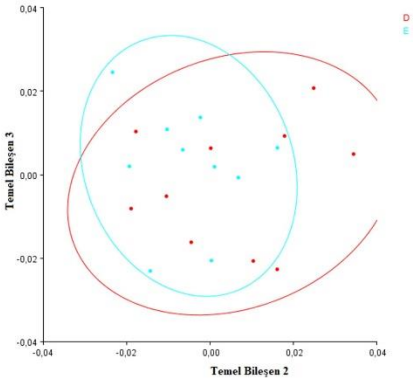


A



B

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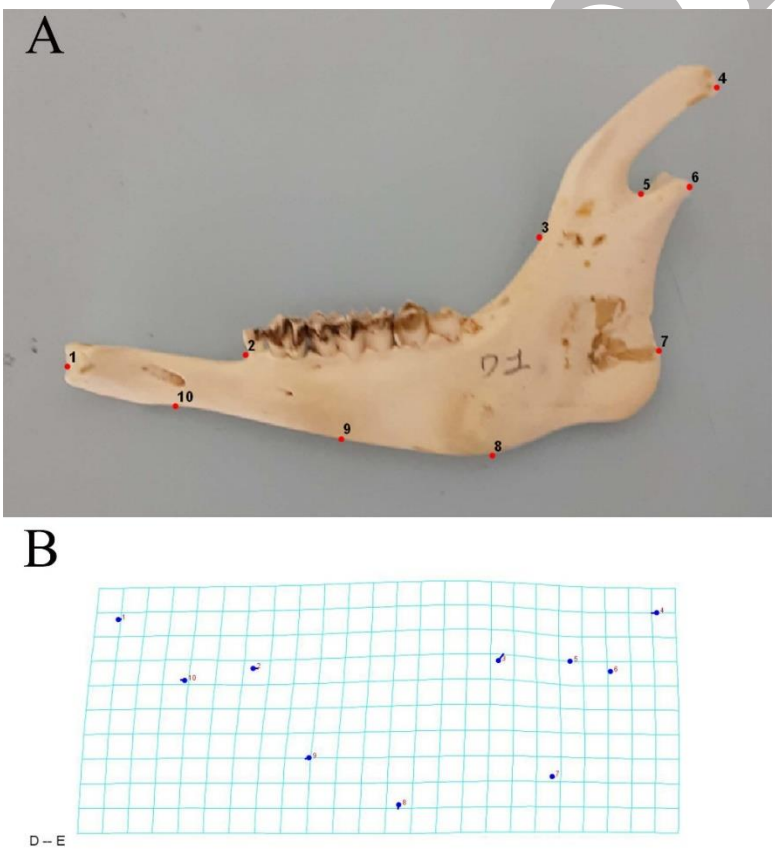
As the female-to-male variation increased, the anterior margin of the first molar tooth was enlarged medially in shape. The anterior margin of the ramus mandible is enlarged proximally. Processus coronoideus enlarged laterally. The gonion is enlarged ventrally distally. The distance of the foreman's mentale to the ventral edge has widened laterally. In this context, enlargement of the corpus mandible and narrowing of the ramus mandible were observed from female to male.

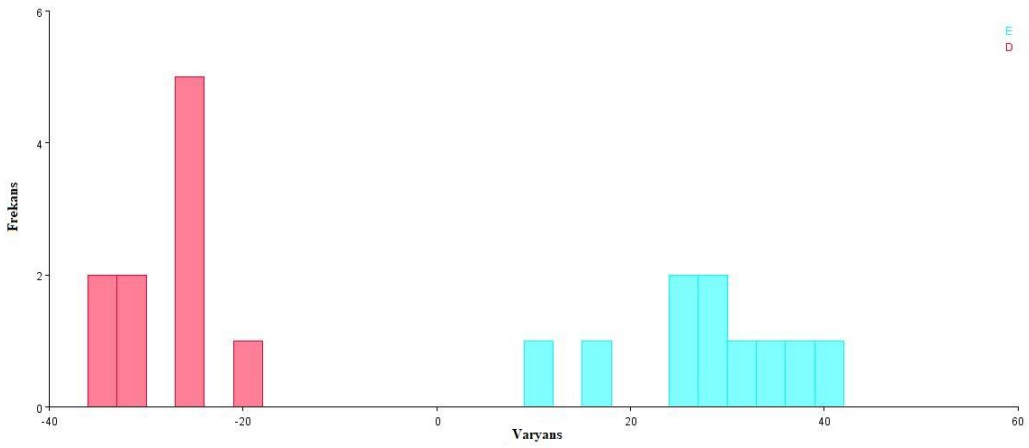
The gender distinction in Discriminant Function Analysis is presented both in shape variations and graphically in (Figures 5a,b and 6).

Figure 5A,B: 10 selected points on the mandible are shown in photograph A. B shows the differences between male and female with punctuation. The round dots represent the female, while the extensions from the dots represent the males.



Discriminant Function Analysis (DFA) was used to objectively evaluate gender differences. The variance and frequency distribution of individuals obtained by Discriminant Function Analysis for sex determination in the mandibles of Norduz sheep is given in (Figure 6). It is seen that the male and female groups are completely separated from each other. This distinction also indicates that the difference between the two groups is statistically significant. In the discriminant function analysis, the p value was below 0.05 (p=0.02).

Figure 6: Gender distribution graph in Discriminant Function Analysis. Red: female, green: male



4.Discussiion

In this study, 20 (10F/10M) mandibles of Norduz sheep were used. The mandibles of Norduz sheep were marked from 10 points and both Principal Component Analysis and Discriminant Function analyzes were performed with geometric morphometric analysis. Norduz sheep mandible was examined for sex determination. Analysis with geometric morphometry method was an important method that should be used to differentiate between sexes in Norduz sheep mandibles. In our study, although there was no separation between the sexes in Principal

Component Analysis, a complete separation between genders was observed in Discriminant Function Analysis. In Discriminant Function Analysis, while going from female to male, enlargement was observed in the corpus mandible, while narrowing was observed in the ramus mandible.

It has been previously stated that morphological data to be obtained from the skull and jawbone of living mammals can be used to reveal phylogenetic relationships thanks to the studies carried out with the geometric morphometric method (33). Researchers stated that principal component analysis performed under the scope of geometric morphometry explained 24.92% of the total shape difference of the first principal component, especially in the mandible in Awassi sheep (27). In the Norduz sheep mandible, it was determined that the first basic component explained 27.49% of the total shape difference.

In a study on the jawbone of Anatolian wild sheep, researchers (34) reported that no sex dimorphism was observed in the jawbone of Anatolian wild sheep. Similarly, there was no dimorphism in terms of principal component analysis in Norduz sheep, similar to the findings of researchers (Demircioğlu et al., 2023), who reported that no dimorphism was observed in the mandible in Avesi sheep. However, there was a complete separation between the sexes in terms of Discriminate Function Analysis.

Yalçın et al., (2010) working on Anatolian wild sheep suggested in their studies that the difference in mandibles at the level of LM9 parameters is quite significant and that this difference is in a relationship due to environmental conditions, feeding habits and adaptations in the domestication process. Similar to the parameter in the study, it is seen that the gonion ventral enlarges distally in TB1 and TB3 and proximal in TB2 in the parameter (gonion ventral) in Norduz sheep, which is similar to the parameter in the study. Significant differences in the LM9 parameter were also reported in Awassi sheep (Demircioğlu et al., 2023). In addition, it was stated that there were differences in LM2, LM8 and LM10 levels, but they were limited.

Duro et al., (2021), working on sexual dimorphism in turtles also benefited from the geometric morphometry method and revealed the differences. In addition to the skull studies in which dimorphism is clear in ruminants, various researchers have also conducted studies on the lower jawbone and brought the data to the literature (35).

Principal component variances, which express statistical and shape variations between groups, are related to the number of materials used, Koçak et al. (2023) (24) obtained 46 variances in their principal component analysis study, whereas in our study, 16 variances were obtained due to the difference in the number of animals. This method was used in our study, just like the researchers (21) who made gender discrimination with discriminant function analysis, and there was a complete separation between the genders.

Analyzes were made over the lengths determined by studies on skull (15), mandible (15,35) and metapodium (29,37) in sheep, and the unique anatomical differences of the species were evaluated with various methods in terms of species and sex, as in our study. With simpler analysis of variation and deviation, the effects of variables on gender were examined in Norduz sheep.

5. Conclusion

It is thought that this study will contribute to the morphometric analysis of the Norduz sheep mandible as well as the morphometric findings, and these analyzes will contribute significantly to the studies to be done in this area, the diagnosis and determination of the osteological materials obtained as a result of archaeological excavations, the creation of three-dimensional models and the use of these morphological analyzes in animal human models. In addition, principal component variation values between males and females were examined by Principal Component Analysis on the basis of race, and the shape changes between female and male were

interpreted in Principal Component Analysis. In addition, gender determination was also evaluated with Discriminant Function Analysis, which is the main element of the study.

Conflict of interest

The authors have declared no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgment

None.

Ethics

The study permit for animal experiments was approved by the local ethics committee Kafkas University with reference code 2023/070 (KAU/HADYEK).

Authors' Contribution

Study concept and design: S.D and S.K. Acquisition of data: S.D and S:K Analysis and interpretation of data: S.D. Drafting of the manuscript: S.D. Critical revision of the manuscript for important intellectual content: S.D. Statistical analysis: S.K.

References

1. Koyuncu, M. (2019). Sheep Breeding (in Turkish). Bursa, Turkey. Dora Publishing Distribution Ltd. Şti. 1st edn. 42.

2. Mis, L., Mert, H., Comba, A., Comba, B., Söğütlü, İD., Irak, K., Mert, N. (2018). Some mineral substance, oxidative stress and total antioxidant levels in Norduz and Morkaraman sheep. *Van Vet J*, 29 (3), 131-134.
3. Delibaş, V., Soygüder, Z., Çakmak, G., Gündüz, MS. (2023). Morphological Examination of Tongue Papillae in Norduz Sheep: A Scanning Electron Microscopic Study. *Van Veterinary Journa*l, 2023, 34 (1) , 75-80. DOI: 10.36483/vanvetj.1243598
4. Dalga, S., Aksu, Sİ., Aslan, K., Deprem, T., Uğran, R. (2021). Morphological, Morphometrical and Histological Structure of the Interdigital Gland in Norduz Sheep. *Kafkas Univ Vet Fak Derg*, 2021, 27 (6), 749-754.
5. Dalga, S., Aydın, U., Çal, T. (2022). Topographic, Morphological and Morphometric Investigation of Mandible in Norduz Sheep. *Türk Doğa ve Fen Dergisi*, 2022, 11 (3) , 129-133. DOI: 10.46810/tdfd.1100636
6. Ozcan, S., Aksoy, G., Kurtul, I., Aslan, K., Ozudogru, Z. (2010). A comparative morphometric study on the skull of the Tuj and Morkaraman sheep. *Kafkas Universitesi Veteriner Fakultesi Dergisi* 2010; (16):111–114. https:// doi. org/ 10. 9775/ KVFD. 2009. 518
7. Karimi, I., Onar, V., Pazvant, G., Hadipour, M., Mazaheri, Y. (2011). The cranial morphometric and morphologic characteristics of Mehraban sheep in Western Iran. *Glob Vet*, 2011, 6:111–117
8. Mohamed, R., Driscoll, M., Mootoo, N. (2016). Clinical anatomy of the skull of the Barbados Black Belly sheep in Trinidad. *Int J Curr Res Med Sci*, 2:8–19
9. Dalga, S., Aslan, K., Kırbaş, G. (2017). Hemşin Koyunu mandibulası üzerinde morfometrik bir çalışma. *Atatürk Üniv Vet Bil Derg*, 2017; 12(1): 22-7.

10. Dalga, S., Aslan, K., Akbulut, Y. (2018). A morphometric study on the skull of the adult Hemshin sheep. *Van Vet J*, 29:125–129

11. Dalga, S., & Aslan, K. (2020). Topographic and morphometric study on mental foramen in Hemshin sheep for local anesthesia. *MJAVL*, 2020, 10:93–97

12. Wehausen, JD., & Ramey, RR. (2000). Cranial morphometric and evolutionary relationships in the northern range of *Ovis canadensis*. J Mammal, 2000, 81:145–161. https:// doi. org/ 10. 1644/ 1545- 1542(2000) 081% 3c3C0 145: CMAERI% 3e3E2.0. CO;2

13. Dalga, S., & Aslan, K. (2021). Morphological and Osteometric Analysis of the Skull in Abaza goats (Capra aegagrus), *Dicle Üniv Vet Fak Derg*;14(1):35-38 DOI: 10.47027/duvetfd.883353

14. Dalga, S. (2020):Topographic and morphometric study of the mental foramina of Abaza goats with its clinical implication for regional anesthesia *Folia Morphol*, 2020; (79), No. 3, pp. 576–579 DOI: 10.5603/FM.a2019.0122

15. Jashari, T., Duro, S., Gündemir, O., Tomasz, S., Vlatko, I., Dashmir, M., Om Prakash, C. (2022). Morphology, morphometry and some aspects of clinical anatomy in the skull and mandible of Sharri sheep. *Biologia*, 2022, 77, 423–433, https://doi.org/10.1007/s11756-021-00955-y

16. Gündemir, O., Duro, S., Jashari, T., Kahvecioğlu, O., Demircioğlu, İ., Mehmeti, H. (2020). A study on morphology and morphometric parameters on skull of the Bardhoka autochthonous sheep breed in Kosovo. *Anat HistolEmbryol*. 2020;00:1–7, https://doi.org/10.1111/ahe.12538

17. Yılmaz, B., & Demircioglu, İ. (2020). İvesi Koyunlarda (*Ovis aries*) Kafatasının Morfometrik Analizi. Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi, 2020; 34:01–06

18. Özdoğru, Z., İlgün, R., Teke, BE. (2019). Konya Merinosunun Mandibula'sı Üzerine Morfometrik Bir Çalışma. *Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi*, 5(2): 392-395.

19. Babaei, M., Yavari, M., Zolhavarieh, S. M., Kalantari Hesari, A. A Case History of Gross and Radiological Observations of Agnathia: Otocephaly in a Mehraban Ewe-lamb. Iranian Journal of Veterinary Medicine, 2023; 17(4): 415-422. doi: 10.32598/ijvm.17.4.1005231

20. Marzban Abbasabadi, B., Ghojoghi, F., Rahmati, S., Hajian, O. Morphological Variations in the Skulls of Male and Female Persian Fallow Deer (Dama dama mesopotamica). Iranian Journal of Veterinary Medicine, 2021; 15(2): 221-232. doi: 10.22059/ijvm.2020.299875.1005075

21. Samuel, O., Korzerzer, B., Olopade, J. O., Onwuka, S. K. Acomparative osteometric evaluation of some cranial indices of clinical significance in goats (Capra hircus) from the middlebelt regions of Nigeria. Iranian Journal of Veterinary Medicine, 2013; 7(2): 111-116. doi: 10.22059/ijvm.2013.35315

22. Klingenberg, CP., & Marugan-Lobon, J. (2013). Evolutionary Covariation in Geometric Morphometric Data: Analyzing İntegration, Modularity, And Allometry İn A Phylogenetic Context. Syst. Biol, 62: 591–610, 2013.Ff

23. Szara, T., Duro, S., Gündemir, O., Demircioğlu, İ. (2022). Sex Determination in Japanese Quails (Coturnix japonica) Using Geometric Morphometrics of the Skull. Animals, 2022, 12: 302.

24. Koçak, S., Özaydın, İ., Gündemir, O. (2023). Sağlıklı ve Septik Artritli Buzağılarda Karpal Eklemin Radyometrik ve Geometrik Morfometrik Analizi. Kafkas Üniv, Sağlık Bilimleri Enstitüsü, Doktora Tezi, Kars, 2023, (in Turkish).

25. Duro, S., Gündemir, O., Sönmez, B., Jashari, T., Szara, T., Pazvant, G., Kambo, A. (2021). A Different Perspective on Sex Dimorphism in the Adult Hermann's Tortoise: Geometric Morphometry. *Zoological Studies*, 60 (9): 3-9.

26. Hadžiomerović, N., Gundemir, O., Tandir, F., Avdić, R., Katica, M. (2023). Geometric and Morphometric Analysis of the Auditory Ossicles in the Red Fox (*Vulpes vulpes*). *Animals* 2023, *13*, 1230. https://doi.org/10.3390/ani13071230

27. Demircioglu, I., Demiraslan, Y., Gurbuz, I., Dayan, MO. (2021). Geometric Morphometric Analysis of Skull and Mandible in Awassi Ewe and Ram. *Kafkas Üniv. Vet. Fakültesi Derg.* 2021, *27*, 43–49.

28. Gürbüz, I., Aytek, AI., Demiraslan, Y., Onar, V., Özgel, Ö. (2020). Geometric morphometric analysis of cranium of wolf (*Canis lupus*) and German shepherd dog (*Canis lupus* familiaris). *Kafkas Üniv. Vet. Fakültesi Derg.* 2020, *26*, 525–532.

29. Gündemir, O., Özkan, E., Dayan, MO., Aydogdu, S. (2020). Sexual analysis in turkey (*Meleagris gallopavo*) neurocranium using geometric morphometric methods. *Turk. J. Vet. Anim. Sci.* 2020, *44*, 681–687.

30. Parés-Casanova, PM., Tolić, A., Carnicero, R. (2020). Side differences in the skull of sheep: An assessment by geometric morphometrics. *Annu Res Rev Biol*, 34 (2): 1-7, 2020. DOI: 10.9734/arrb/2019/v34i230147

31. Parés Casanova, PM. (2015). Geometric morphometrics to the study of skull sexual dimorphism in a local domestic goat breed. J Fisheries Livest Prod, 3:141. DOI: 10.4172/2332-2608.1000141

32. Fernandez Blanco, MV., Cassini, GH., Bona, P. (2023). A three-dimensional geometric morphometric analysis of the morphological transformation

of *Caiman* lower jaw during post-hatching ontogeny. *PeerJ*, 11:e15548 https://doi.org/10.7717/peerj.15548

33. Marcus, LF., Hingst-Zaher, E., Zaher, H. (2000). Application of landmark morphometrics to skulls representing the orders of living mammals. Hystrix, 2000, 11, 27-47.
34. Yalçın, H., Kaya, MA., Arslan, A. (2010). Comparative geometrical morphometries on the mandibles of Anatolian wild sheep (Ovis gmelini anatolica) and Akkaraman sheep (Ovis aries). *Kafkas Univ Vet Fak Derg*, 16 (1): 55-61, 2010. DOI: 10.9775/kvfd.2009.385
35. Özkan, E., Jashari, T., Gündemir, O., İnce, NG. (2020). Morphometric Analysis of the Mandible of Bardhoka Autochthonous Sheep in Kosovo. *Anat Histol Embryol*, 2020, (1): 1-5.
36. Gündemir, O., Pazvant, G., Jashari, T., Dayan, MO. (2020). A morphometric study of metapodium in Bardhoka sheep. *Res Agric Vet Sci*, 2020, 4 (1): 30-38.
37. Kahraman, M., Değirmencioğlu, İ., Gülşah, G., Gündemir, O., Özkan, E., Das, A., Yılmaz, B. (2022). Determination of the correlations between the morphological characteristics and metapodial radiometric measurements of Awassi sheep. *Eurasian J Vet Sci*, 2022, 38 (2): 180-189.