Principal component and discriminant function analysis of cranium and mandible in domestic buffalo (*Bos bubalis*)

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ARTICLE INFO

Article History
Received : 06.09.2024
Accepted : 11.03.2025
DOI: 10.33988/auvfd.1544641

Keywords
Buffalo
Geometric morphometry
Mandible
Sex discrimination
Skull

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How to cite this article: Dalga S, Aslan K (2025): Principal component and discriminant function analysis of cranium and mandible in domestic buffalo (*Bos bubalis*). Ankara Univ Vet Fak Derg, 72 (3), 345-355. DOI: 10.33988/auvfd.1544641.

ABSTRACT

The purpose of this study was to use a geometric morphometric approach to ascertain the gender-related differences in the morphology of the domestic buffalo's skull and mandible. The skulls yielded a total of 20 main components. The first principal component (PC1) alone was responsible for 37.066% of the variation among these principal components. The first principal component (PC1) alone was responsible for 26.242% of the total variation among the lateral principal components. PC1 showed a medial extension of the posterior portion of the ectorbital, while PC2 showed a lateral extension. In PC2 and PC3, the right facial tuber displayed a cranial and linear expansion, respectively. In PC1, the left facial tuber was directed caudally, and in PC2, it was directed cranially. The anterior border of the first premolar had a caudo-ventral extension in PC1 and a cranio-dorsal extension in PC2 and PC3, according to lateral studies. In PC1 and PC2, the anterior side of the ectorbital displayed a caudo-dorsal extension, but in PC3, it displayed a dorsal extension. The anterior margin of the first premolar displayed a caudal extension in the extension evaluation of the three principal component analyses with the highest values in the mandibles, where the data are completely integrated with one another. PC1 showed a caudo-dorsal extension, PC2 showed a cranio-dorsal extension, and PC3 showed a dorsal extension of the landmark at the level of the incisura vasorum facialum. It is anticipated that this research will add to the body of knowledge about a particular breed, zoo archaeology, or be used as an animal model in relevant health professions.

Introduction

The buffalo, which has an important economic activity in the world as a dairy, meat, and draught animal, is raised in Southeast Asia, South America, North Africa, all Mediterranean countries except France, Balkan countries, some Central European countries, and Australia (39). The European bison (*Bison bonasus*), the largest mammal in Europe, is still a protected species, although its number has increased with breeding programs that put its species under protection after World War I (18). Water buffalo is a species that can cause confusion, especially in North America and Asia, about which animal is being referred to by the English term water buffalo. The word water buffalo in Turkish is thought to come from Manda, a geographical region in India. The domestication of the buffalo, whose domestication process dates back 5000 years (39). Buffalo breeding in the world has not lost its importance from past to present, and has even increased in both numbers and production over times (2), is now widely cultivated in 38 countries around the world (30). Buffaloes are a family of double-hoofed ruminant cattle. The first buffalo belongs to the Bubalus family. There are two types of Bubalus groups, and they are classified as Asian buffaloes (bubalina) and African buffaloes (synserina) (3).

Knowing the morphology of the skull is crucial to comprehending the systematics and phylogeny of the skull species under study (18). One sex-determining characteristic of male and female bovids is their horns, horn protrusions, and length, which have a robust structure (20, 21, 27, 40). The brain, hearing and balancing organs, respiratory and olfactory routes, and other vital tissues are all protected by the skull's mostly comprised bones (8, 26).

Markland (29) stated that in relation to nutritional factors, the facial tuber (tuber faciale) on the maxilla bone is related to the molar tooth articulation and the articulation role of the incisors in this region, as well as the changes in these regions and nutrition (29). The differences observed laterally in the points representing the rostral edge of the incisive bone and nasomaxillar fissura, which represent the mouth and nose regions, are thought to be related to diet and climate-altitude variations (28). Other points where the shape change is evident include the ventral margin of the jugular process, the midpoint of the margo supraorbitalis, and the external lacrimal fossa (5).

The majority of earlier research on large ruminants was based on traditional morphometric techniques, which included a variety of skull measurement instruments and reference data. Regarding species and sex relationships, these data are quite important (8). Skull morphology was described, and sex differences were attempted to be explained by researchers who performed a linear analysis and comparison of the skulls of domestic cattle and water buffalo (32). The study on the Indian Mithun's skull is the largest ruminant study currently accessible (7). In a similar vein, Ko-brýnczuk (27) used linear measures to analyze European bison and identify sex differences in skulls. Apart from cattle, numerous other animal species have been the subject of sex analysis research using the linear approach (16, 19, 22, 23).

The method of geometric morphometry, which easily reveals the shape differences between the skull and mandible, has become an important part of anthropology and archaeology studies in recently. It is possible both to evaluate discoveries and to re-evaluate old discoveries. Since the traditional morphometry method does not reveal all the information about the shape, this deficiency led to the birth of the geometric morphometry method. This new method reveals the whole geometric shape taken from the coordinates formed by anatomical points that are found in the same way in all samples. In this way, it reveals more information than the classical morphometry method (18, 36, 42). In recent years, there have been many studies on different species and different bones to determine the differences between the sexes of animals by the geometric morphometry method (1, 14). There have also been geometric morphometric studies on three-dimensional bone materials (5, 6, 15, 25, 33). There are also studies using the geometric morphometric method on materials obtained from archaeological excavations (35).

In this study, geometric morphometric approaches are used to assess the mandible and skull of farmed buffaloes based on the sex factor. We believe that the information gathered from the study will greatly advance real-world uses in buffalo management and breeding. In order to guide future research on buffaloes from microclimate regions and other parts of the world, as well as to support zooarchaeological studies, it is believed that the morphological characteristics of these unique animals, whose breeding dates back 5000 years, can be used for humanity in every aspect of life.

Materials and Methods

Sampling: Skulls obtained from local producers and slaughterhouses in Iğdır province were used in the study. 14 animals (7 female/7 male) of both sexes were used. All female and male subjects were 2 years old with an average body weight of 123-166 kg and 142-179 kg, respectively. Skulls weighed between 14 and 20 kg.

Preparation of Samples: They were separated from the skin and muscles. Then boiled and macerated. After bleaching and drying, mandibles were photographed from the left lateral direction and skulls from the dorsal and lateral directions. The recorded photographs were transferred to the computer in JPEG format and then converted to a Tps file with the TpsUtil (Version 1.79) program. Homologous landmarks were marked on the photographs with the TpsDig2 (Version 2.31) program (37). The Cartesian coordinates of the landmarks were determined in this way. A homologous landmark verification test was performed with the TpsSmall (Version 1.34) program (34, 36). Since there may be differences in size, direction, and position in the photographs obtained in the study, superimposition was applied (38).

Analysis: Principal Component Analysis (PCA) was performed on the new coordinates obtained as a result of superimposition. Thus, covariance analysis between factors was used to determine the degree of separation of specimens by sex (43). In addition, the MorphoJ program was used to determine the landmark level and direction of the shape difference (24).

Results

14 buffalo skulls, 7 female and 7 male, were analyzed laterally and dorsally for this study. Ten pointing procedures were used to investigate the dorsal and lateral directions in the geometric morphometry method. Twenty major components in all were found. The first principal component (PC1) alone was responsible for 37.066% of the variation among these principal components. Of the overall variation, 28.65% was explicated by the second main component (PC2) alone, and 12.65% was explained by the third principal component (PC3) alone. The first principal component (PC1) alone account for 26.242% of the total variation among the lateral principal components. Of the overall variation, 24.143% can be explicated by the

second principal component (PC2) alone, and 18.097% can be explained by the third main component (PC3) alone. The mandibles photographed from the left lateral direction yielded a total of 13 major components of variation. Of them, PC1 alone was responsible for 38.947% of the variation. The third principal component was responsible for 12.586%, whereas the second principal component (PC2) was in charge of 22.886%. More than half of the variation was explained by PC1, PC2, and PC3 in both materials, according to the principal component analysis. Significant percentages of variance were also found in each of the two materials after analysis. Table 1 contains the reference principal component analysis data for both materials. The plots show that PC1, PC2, and PC3 are accountable for over half of the variation. Significant percentages of variation are also present in each of the examined materials separately.

Figures 1, 2, and 3 display the shape variation for principal components 1, 2, and 3 derived from dorsal and lateral skulls and left lateral mandibles. Additionally, Figures 4, 5, and 6 display graphic representations of the variance distributions. The average form for each analysis is shown by dots. The extensions represent the positive bounds for PC1, PC2, and PC3. The posterior section of the occipital bone extends caudally in PC1 and PC2, particularly in PC2, as can be seen in the dorsal analysis when taking into account the significant deviations. In PC1 and PC2, the posterior region of the ectorbitale was found to exhibit a medial and lateral expansion, respectively. PC2 displays a cranial expansion of the right facial tuber, while PC3 has a linear extension. It was discovered that the left facial tuber had a cranial extension in PC2 and a caudal extension in PC1. It was observed that the septal process extended cranially in the direction of the skull's cranial aspect.

The anterior edge of the first premolar had a caudo-ventral expansion in PC1 and a cranio-dorsal extension in PC2 and PC3, according to the lateral studies. When seen from the lateral face, the root section of the cornual process (landmark number 5) had a ventral extension in PC3 and a linear cranial extension in PC1 and PC2. In PC1 and PC2, the anterior portion of the ectorbitale displayed a caudo-dorsal extension, but in PC3, it displayed a dorsal extension. In PC1 and PC2, the frontal tuber displayed a medially directed extension, whereas, in PC3, it displayed a caudo-ventrally directed extension. In PC1, the anterior border of the os incisivum seems to reach the skull's tip.

In the skulls of buffaloes obtained from the province with microclimate characteristics, stronger and larger anatomical formations are expected in male individuals in direct proportion to the purpose of use. Especially in females used for milk production, the horn size has strikingly attracted attention. It was observed that these formations remained weaker in male individuals where competition is common due to the environment and the labor force they use.

In both PC1 and PC3, the infradental gap appears to extend anteriorly from the cranial apex in the mandibles, where the data are completely integrated. According to the extension evaluation of the three principal component analyses with the highest values, the anterior border of the first premolar exhibits a caudal expansion. In PC1, the posterior border of the coronoid process extends ventrally, but in PC2 and PC3, it extends caudally. In every component analysis, the caudal gonion was revealed to have a cranio-dorsal extension. PC1 showed a caudo-dorsal extension, PC2 showed a cranio-dorsal extension, and PC3 showed a dorsal extension of the landmark at the level of Incisura vasorum facialum. The extension study of the projective landmark on the ventral margin of the mental foramen indicates that PC1 has a ventral extension, while PC2 and PC3 have a caudal extension. In PC2, the anterior border of the first premolar tooth also extends cranially.

Table 1. Component analysis obtained from the cranium and mandible

PC No	Eigenvalues (Dorsal)	Variance (%)	Eigenvalues (Lateral)	Variance (%)	Eigenvalues (Mandible)	Variance (%)
PC1	0.00112949	37.066	0.00034026	26.242	0.00138678	38.947
PC2	0.00087327	28.657	0.00031303	24.143	0.00081489	22.886
PC3	0.00038550	12.651	0.00023464	18.097	0.00044815	12.586
PC4	0.00033131	10.872	0.00015805	12.190	0.00040680	11.425
PC5	0.00011805	3.874	0.00008274	6.381	0.00022611	6.350
PC6	0.00009635	3.162	0.00005893	4.545	0.00010374	2.913
PC7	0.00007088	2.326	0.00004464	3.443	0.00008174	2.296
PC8	0.00002706	0.888	0.00003467	2.674	0.00003564	1.001
PC9	0.00000940	0.309	0.00002322	1.791	0.00002467	0.693
PC10	0.00000594	0.195	0.00000642	0.495	0.00001574	0.442

PC: Principal component

DOI: 10.33988/auvfd.1544641

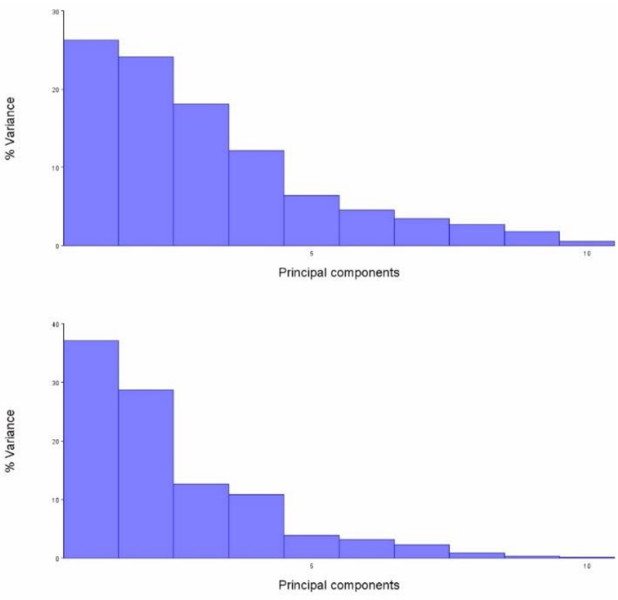

Figure 1. Variation distribution graph of principal component analysis (Dorsal (top), Lateral (bottom)).

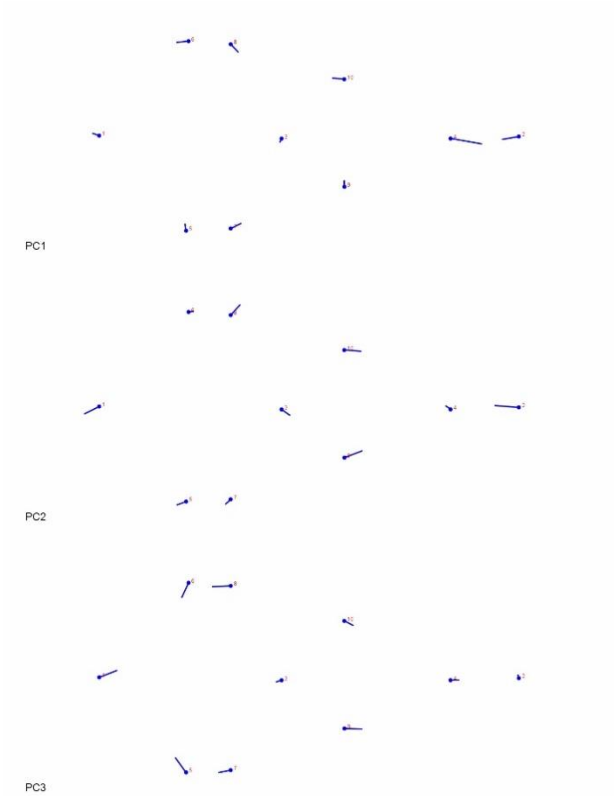

Figure 2. Shape variation of principal components 1, 2, and 3; 1: Posterior boundary of the occipital bone, 2: Tip of the incisive bone, 3: Fronto-nasal suture, 4: Tip of the nasal process, 5-6: Roots of the right and left cornual process, 7-8: Posterior edge of the ecto-orbital, 9-10: Right and left facial tuber (Dorsal Analysis). PC: Principal component

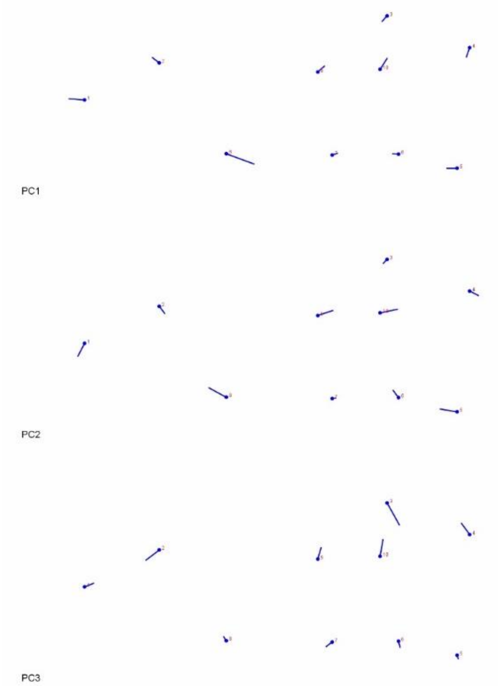

Figure 3. Shape variation of principal components 1, 2, and 3 in the lateral view; 1: Tip of the incisive bone, 2: Tip of the nasal process of the nasal boner 3: The frontal tuber of the frontal bone, 4-5: Roots of the condylar process, 6: Lateral edge of the muscular process, 7: Posterior edge of the last molar tooth, 8: Anterior edge of the first premolar tooth, 9: Anterior edge of the ectorbital, 10: Posterior edge of the ectorbital. PC: Principal component

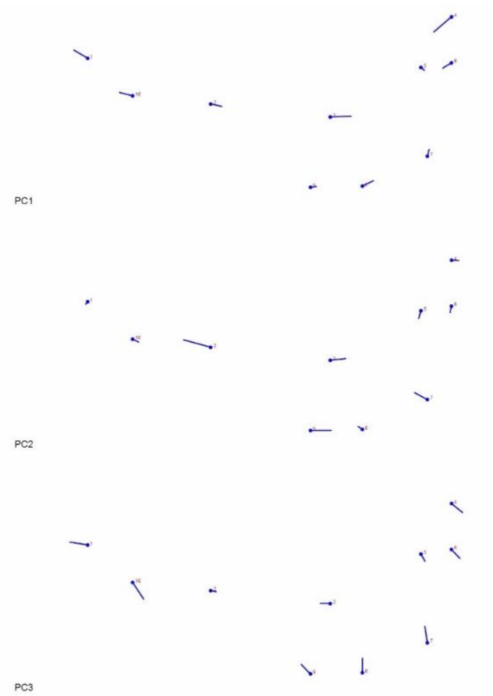

Figure 4. Shape variation of PC1, PC2, PC3; 1: Infradental space, 2: Anterior edge of the first premolar tooth, 3: Posterior edge of the last molar tooth, 4: Posterior edge of the coronoid process, 5: The ıncisura of mandible, 6: Posterior edge of the condylar process, 7: Caudal gonion, 8: Incisura vasorum facialum, 9: Ventral gonion, 10: Distance from the ventral edge of the mental foramen (Mandible). PC: Principal component

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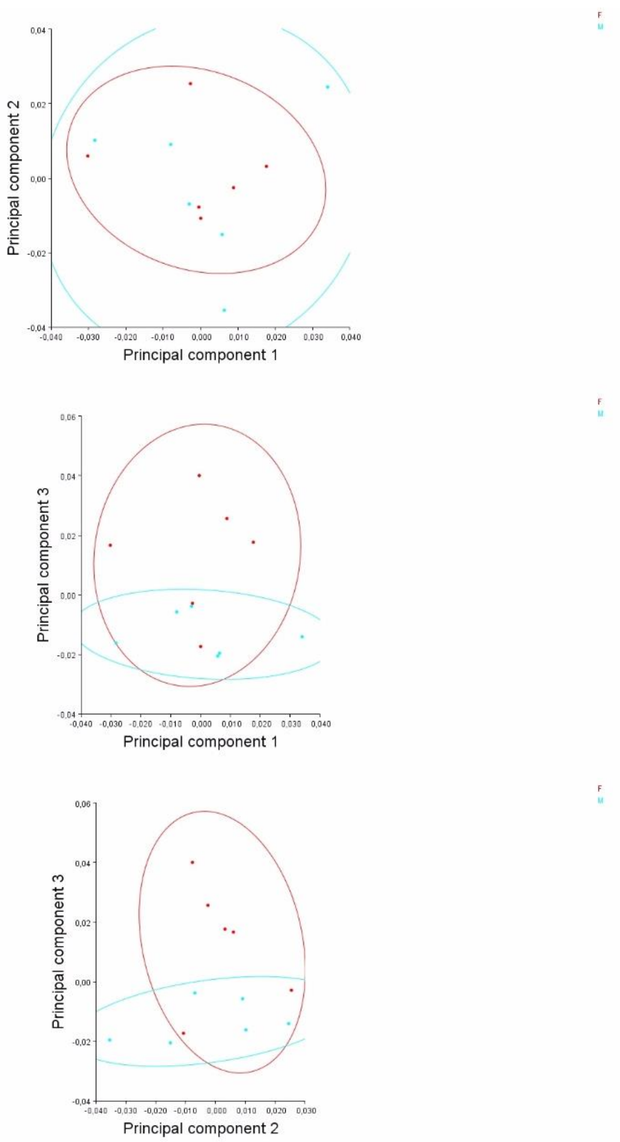

Figure 5. Variation distribution of principal components. Red points: Female, Green points: Male (Dorsal).

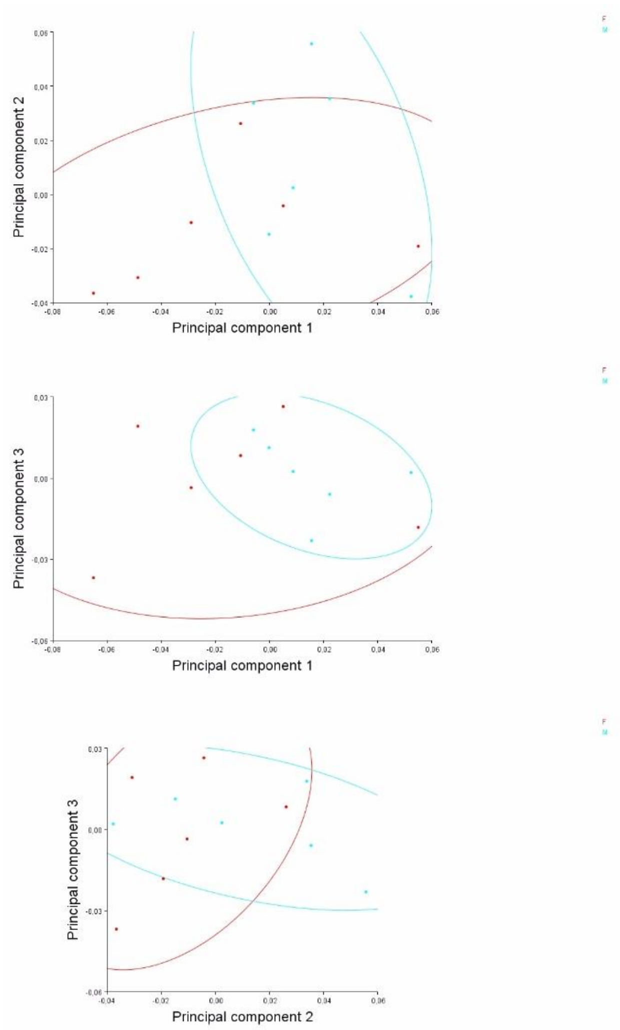

Figure 6. Variation distribution of principal components. Red points: Female, Green points: Male (Lateral).

Figures 7–11 display the primary components derived from the materials' principal component analysis. More than half of the variance was explained by the first three components (PC1, PC2, and PC3) for all materials. Despite having a large overall variance across all three tests, there was no statistically significant separation between the materials' shapes.

The study employed a discriminant function analysis (DFA) to evaluate sex differences objectively. The buffalo skull and mandible did not exhibit any statistically significant differences between the sexes, according to DFA. Nonetheless, the mandible and skull are both different shapes. Figure 12 shows the gender disparities in the discriminant function analysis graphically with shape modifications. The complete separation of both genders in terms of shape is also seen in the graph.

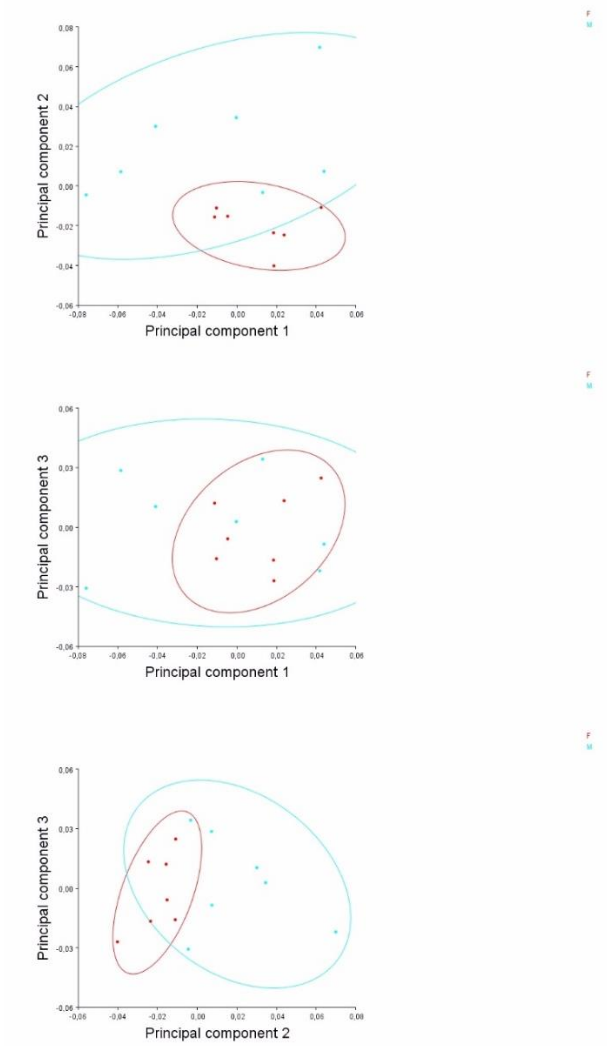

Figure 7. Variation distribution of principal components. Red points: Female, Green points: Male (Mandible).

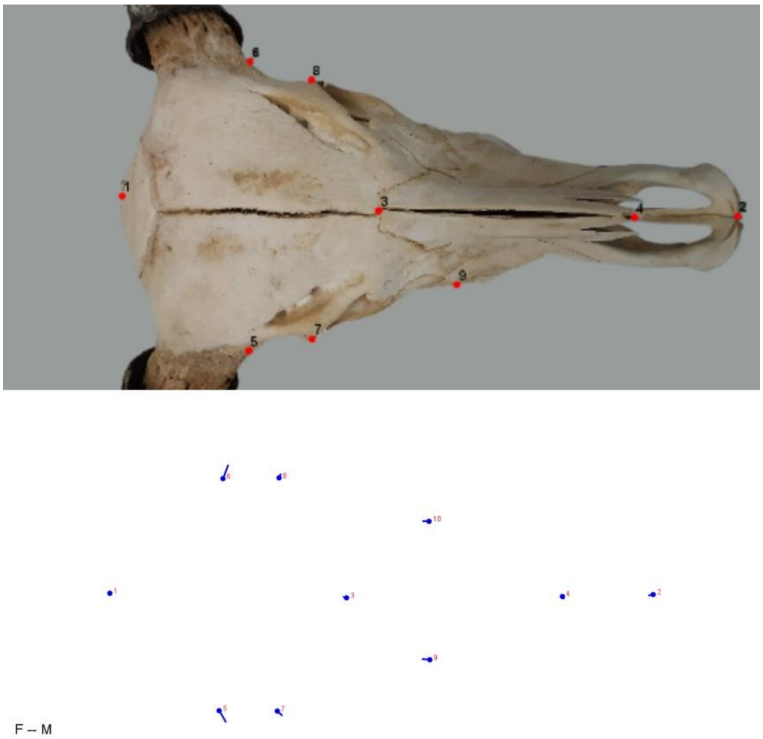

Figure 8. Principal component variation. 1: Posterior boundary of the occipital bone, 2: Apex of the incisive bone, 3: Fronto-nasal suture, 4: Apex of the nasal process, 5-6: Roots of the right and left cornual process, 7-8: Posterior edge of the ectorbitale, 9-10: Right and left sides of the facial tuber (Dorsal Analysis).

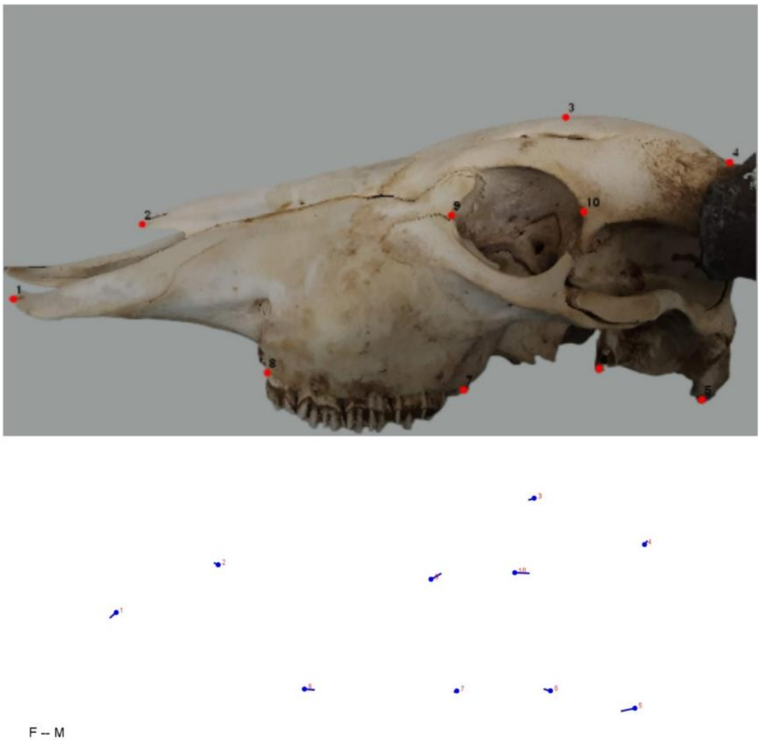

Figure 9. Principal component variation. 1: Apex of the incisive bone, 2: Apex of the nasal process of the nasal bone, 3: Frontal tuber of the frontal bone, 4-5: Root of the condylar process, 6: Lateral edge of the muscular process, 7: Posterior edge of the last molar tooth, 8: Anterior edge of the first premolar tooth, 9: Anterior edge of the ectorbitale, 10: Posterior edge of the ectorbitale.

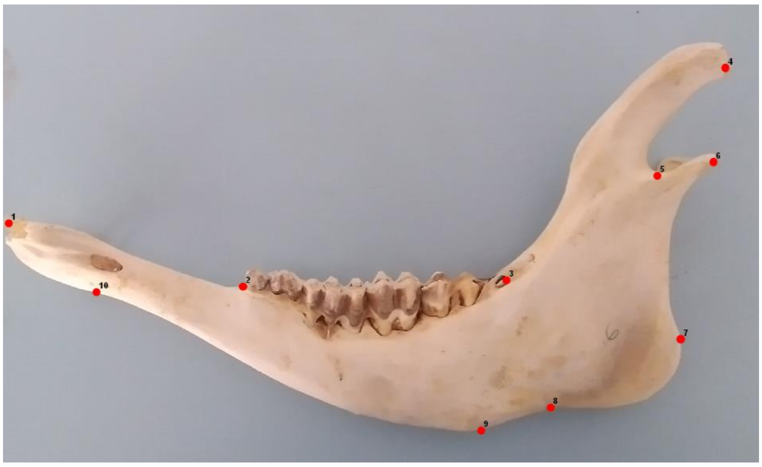

Figure 10. Principal component variation. 1: Infradental space, 2: Anterior edge of the first premolar tooth, 3: Posterior edge of the last molar tooth, 4: Posterior edge of the coronoid process, 5: Incisura mandibulae, 6: Posterior edge of the condylar process, 7: Caudal gonion, 8: Incisura vasorum facialum, 9: Ventral gonion, 10: Distance from the ventral edge of the mental foramen.

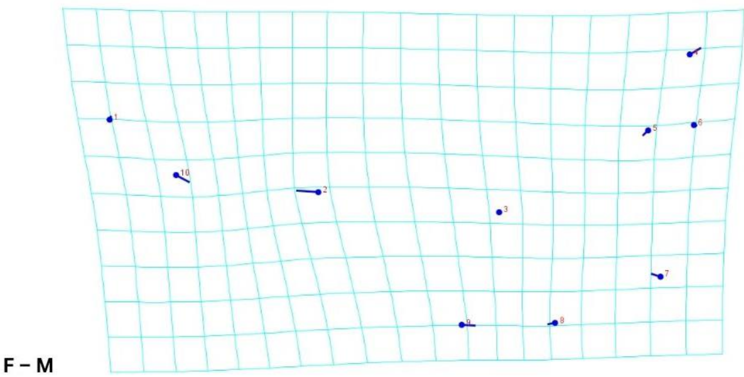

Figure 11. Discriminant fonction analyses of mandible; 1: Infradental space, 2: Anterior edge of the first premolar tooth, 3: Posterior edge of the last molar tooth, 4: Posterior edge of the coronoid process, 5: Incisura mandibulae, 6: Posterior edge of the condylar process, 7: Caudal gonion, 8: Incisura vasorum facilium, 9: Ventral gonion, 10: Distance from the ventral edge of the mental foramen. F: Female, M: Male

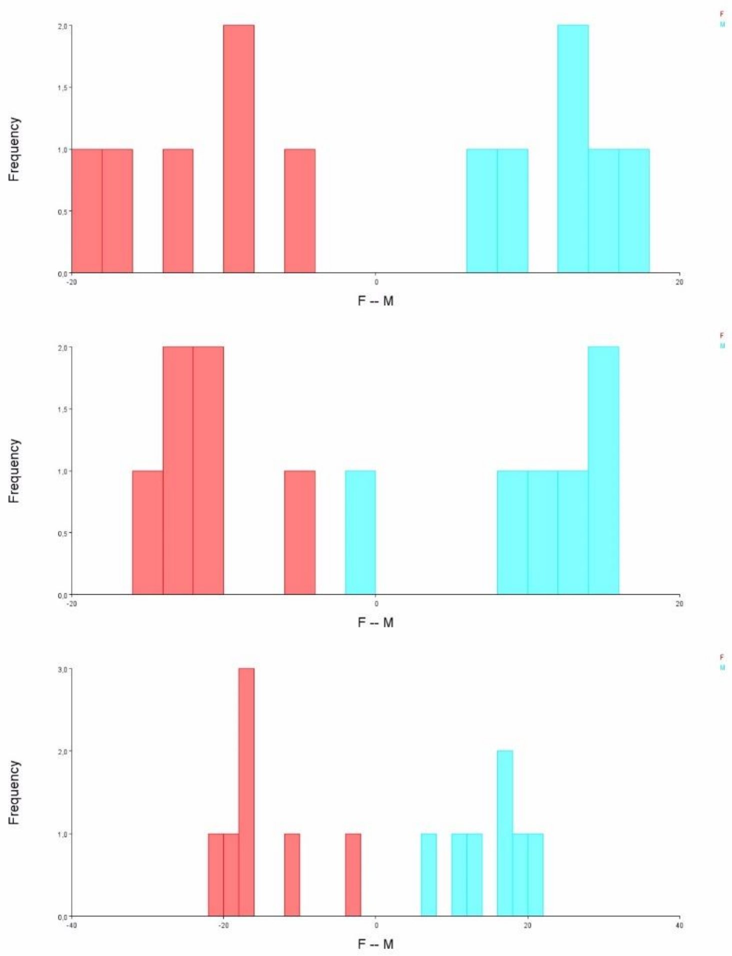

Figure 12. Sex distribution graph in Discriminant Function Analysis. Red: Female (F), Green: Male (M) (Top - Dorsal / Middle - Lateral / Bottom - Mandible).

Discussion and Conclusion

Male and female buffalo skulls from Eastern Anatolia were employed in this investigation. Dorsal and lateral markings were followed by a geometric morphometric examination of the materials. The geometric morphometric approach was used to conduct discriminant function analysis and principal component analysis. Among the variations derived from principal component analysis, the first three analyses were taken into account. The materials that did not exhibit a statistically complete separation varied in shape, even though the overall variance was considerable across all three tests. Similarly, sex differences were objectively evaluated using the discriminant function analysis (DFA). Gender differences in buffalo skulls and mandibles were not statistically significant (P>0.05), according to DFA. That being said, the mandibles and skulls differed in shape. There may not have been a statistically significant difference due to the small number of materials.

The skull and lower jaw bones of animals provide a great deal of morphological information for individuals and different races within the same family. Thanks to this morphological information, the effects of the environment in which living things are located on their morphological structures can be interpreted, and today's technologies allow the construction of human, animal-based surgical models to evaluate objects obtained from remains (25, 34, 42). Studies are conducted to comprehend how genetic variation, sexual selection, and environmental factors affect skull shape (16). According to previous reports, these morphological data can show relationships between living beings, especially when obtained using geometric methods (4, 10, 35, 43, 44). Although it has been widely used in recent years, classical morphological methods that have been used since ancient times are still used (31).

According to a study on male Holstein and Simmental cattle, PC1 accounted for 60.30% of the overall variation, whereas PC2 explained 12.67%. Additionally, it was highlighted that the Holstein breed's skull length was greater than that of the Simmental breed (8). According to a study by Gündemir and Szara (16) on the skulls of 57 European bison (*Bison bonasus*), males had larger heads and horns than females. Additionally, it was mentioned that the frontal, nuchal, and maxillary regions showed morphological modifications (18).

DOI: 10.33988/auvfd.1544641

It is easy to find studies on small ruminant skulls and mandibles that fall under the category of geometric morphometry in the literature. Apart from ruminant skull studies, where dimorphism is most evident, some researchers have also studied the mandible and reported their findings (15). Numerous research studies have been conducted on the metapodium (41), mandible (12, 15, 22), and skull (10, 11, 13) in sheep. The precise anatomical distinctions of the species were assessed using a variety of methodologies in terms of species and sex, just like in our investigation, and analyses were conducted over the determined durations in each of these studies. The geometric morphometry method has been used by researchers studying sexual dimorphism in several animals, including turtles (24), in addition to ruminants, to uncover the structural differences among related species. It must be because the materials belonging to small ruminants are easily obtained that they are frequently used by researchers in terms of understanding and interpretation. For this reason, the findings we obtained regarding our study materials, buffaloes, have always been compared with the existing literature on small ruminants.

When the mandibles of Honamlı and Hair goats were subjected to geometric morphometric analysis, the researchers found that there was a pronounced gender difference between the two species. They claimed that male goats were considerably more grouped than females in terms of race. In our investigation, there was a difference in shape even though the lower jaw was not fully sexually differentiated (11). Studies on Awassi sheep revealed that when utilizing the geometric morphometry approach for analysis, the initial PCA accounted for 24.92% of the total form difference (12). Furthermore, it was claimed that there was no discernible gender difference in the mandibles. In a similar vein, other researchers discovered that 30.409% of the Morkaraman sheep mandible's overall shape variance could be described by the first main component (9). However, it was shown that the first main component alone accounted for 38.947% of the overall shape variance in the studies taken from buffalo mandibles.

Using principal component analysis, researchers who attempted to assess mammalian morphology from a paleoecological standpoint reported that PC1 accounted for 45.59% of the variation in mandible morphophysiology (41). They added that dietary practices have an impact on the mandible's morphology. The researchers who studied Anatolian wild sheep (42) reported that there is a noticeable difference in the subjaws at the level of LM9 parameters, and this difference is related to environmental factors, feeding practices, and domestication adaptations. It was noted that the LM9 value in Awassi sheep varied significantly (12). Additionally, it was mentioned that although there were variations in LM2, LM8, and LM10 levels, they were minimal. The ventral gonion, identified as a marker, was observed to increase caudally at PC2 as a consequence of the form study of buffalo mandibles. The first two fundamental analyses in 2D image inspections were found to account for 45.59% and 14.70% of the shape variance, respectively, in the study looking at the association between cattle's mandible and nutrition (41). There was no gender dimorphism in the principal component analysis of buffalo mandibles, as reported in Anatolian wild sheep (42), Awassi sheep (12), and Morkaraman sheep (9), when we compared the study to other studies in the literature. However, changes in shape were noted when we examined the data using discriminant function analysis. Dorsal sexual dimorphism was not entirely isolated in our study. Once more, 37.066% of the form differences were explained by PCA-1 alone. By comparing two unique cattle breeds, the researchers found that there was a noticeable difference between the breeds based on principal component 2's measurements of the occipital bone's height and the frontal bone's width (8).

In a geometric morphometric analysis of skull bones from various breeds, the researchers found that PCA-1 accounted for 42.268% of the form variance in males and 50.628% in females. When they examined the dorsal side of the skulls of animals of various breeds and sexes, they discovered that sexual dimorphism was breed-specific. According to the same researchers, who dorsally analyzed the skulls of Honamlı, Kilis, Saanen, and Kıl goats, there was a notable clustering in female Honamlı goats and a limited amount of dorsal separation between the skulls. They reported that among males, there was a clear grouping among those from the Saanen and Honamlı families (43). According to a study on Balkan sheep, the first two primary components can account for 61.18% of the overall shape variation in the dorsal direction. This demonstrates the form differences between Bardhoka and Ivesi sheep (17). In both dorsal and ventral directions, the researchers examined the skulls of female Akkaraman and Anatolian wild sheep (42). Dorsal analyses revealed that both principal components accounted for 70.03% of the overall shape difference. The PCA-1 value for both breeds was determined to be 65.93% based on the ventral analysis. These studies all provide evidence for both intraspecific and interspecific variances. According to the same researchers, in PCA graphs created from the dorsal and basal sides, the skull bones of Anatolian wild sheep were clustered to the right of the “y” coordinate, whereas those of Akkaraman sheep were clustered to the left. The dorsal and left lateral sides of the Awassi sheep's skulls were examined by researchers, who found that PCA analysis explained 37.719% and 44.238% of the overall form variance for each side, respectively (13).

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In principal component analysis, PC1 is typically given more attention than the other three analyses. It was shown that PC2 accounted for 12.67% of the overall variation in Simmental and Holstein breeds for PC2 study. The second main component analyses revealed that Simmental cattle had a positive mean for metrics like the largest breadth of the skull, the length of the occipital bone, and the width of the frontal bone, but Holstein cattle had a negative mean for these same parameters. This difference was believed to be a reflection of the morphological traits unique to the species (8). This figure was reported to be 14.53% for the lateral area and 27.84% for the dorsal area in Awassi sheep in another study that focused on PC2 analysis (12). Although our study did not include any racial differences, PC2 accounted for 28.65% of the overall gender difference.

It is believed that the morphological analyses and morphometric findings of the native bison skull and mandible, as well as the identification and determination of osteological materials obtained from archaeological excavations, the development of three-dimensional models, and the application of these morphological analyses on animal and human models will greatly advance the research to be conducted in this field. We believe that the findings obtained from this study will also be valuable in terms of biogeographic, phylogenetic, and system studies in terms of their primary widespread impact. Principal Component Analysis was also used to evaluate the shape changes between males and females and to analyze the principal component variation values between males and females based on race. The primary component of the study, discriminant function analyses, was also used to evaluate sex determination. We think that there is no statistical difference due to the number of skulls used in the study. However, it was clearly demonstrated that the sexes are completely separate in terms of shape.

Acknowledgements

The abstract of this study was presented as an oral presentation at the 12th YGVA meeting held in Zagreb on July 17-19, 2024.

Financial Support

This research was supported by Kafkas University Scientific Research Grant No: 2022-TS-50.

Ethical Statement

This study was approved by the Kafkas University Animal Experiments Local Ethics Committee (Approval no: 2022/027).

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

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

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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