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Three-dimensional (3D) geometric morphometrics of the carnivora axis: shape variation, allometry

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ABSTRACT

Objective: The *axis* is an important bone due to its adjacency to various structures and its contribution to the bony structure of the neck, as well as its responsibility for supporting the neck. Given the absence of similar studies in the literature, we sought to investigate the three-dimensional geometric morphometry of the *axis*. For this purpose, 17 *axis* bones from 8 different domestic species belonging to the carnivora family were analysed.

Materials and Methods: CT images of the bones were converted into three-dimensional (3D) bone surfaces. A total of 102 landmarks were applied to the images recorded with the generalized Procrustes method. Principal Component Analysis (PCA) was performed with SlicerMorph programme. As a result of this analysis, shape variations of the bones were analysed. In addition, Procrustes Distance and Centroid size values of the bones were measured with the same programme. These values were processed into SPSS programme and the effect of age and weight on size was investigated by regression analysis.

Results: The shape changes of the bones were analysed from five different perspectives: cranial, caudal, lateral, dorsal, and ventral. These analyses revealed shape variations, which were subsequently explained. Principal component analysis (PCA) showed that PC1 accounted for 21.04% of the total variation, PC2 explained 13.6%, and PC3 contributed 12%. Furthermore, regression analysis demonstrated that age and weight were statistically significant factors influencing centroid size.

Conclusion: Principal component analysis revealed significant shape variations in the axis bones of carnivora, particularly in key anatomical features such as the processus transversus and dens, with notable bilateral differences. The allometric analysis showed a significant relationship between centroid size, shape, age and weight, emphasizing the influence of allometry on axis morphology. These findings enhance our understanding of the morphological diversity and functional adaptations of the axis bone in carnivorous mammals.

Keywords: *Cat, Cervical, Computed tomography, Dog, Vertebra*

INTRODUCTION

The axis is the second of the vertebrae cervicales and is an important bone in terms of its location, vital adjacent formations such as cranial nerves, spinal cord and vertebral arteries, and its anatomical shape (Şengül and Kadıoğlu, 2006). Since the specialized shape of the axis is unique, it is considered special, much like the first vertebrae cervicale, the atlas. (Gosavi and Swamy, 2012). Vertebrae cervicales form the bony structure of the neck and also carry the weight of the neck (Dursun, 2007). As a whole, the foramen vertebralis of the columna vertebralis merge to form a canal called canalis vertebralis. This canal contains the medulla spinalis (Dursun, 2007).

It articulates with the atlas, the first vertebrae cervicales, through its protrusion dens, which is conical shaped in carnivora, located in front of the axis. In addition, at the front of the corpus, where the corpus and the dens meet, there is a facies articularis cranialis covered with cartilago articularis, which articulates with the fovea articularis caudalis of the atlas (Dursun, 2007). Dorsal to the axis is the processus spinossus. This is higher than the processus spinossus of other vertebrae cervicales (Demiraslan and Dayan, 2021). On the ventral side of the axis, there is a long crest called crista ventralis that extends up to the dens, which is characteristic of carnivora (Dursun, 2007; Demiraslan and Dayan, 2021).

Axis is the longest of the vertebrae cervicales for carnivora. Nutritional and behavioural changes of animals belonging to the carnivora family group can cause anatomical changes in their bones (Van Valkenburgh, 1987). For this reason, the mobility of the axis is higher in carnivora than in other mammals (Ewer, 1998). These changes in mobility can lead to anatomical alterations in the bones, as the stress on the bone generates varying degrees of strain (Bertram and Biewner, 1990).

Geometric morphometric methods have developed a lot over the years and still continue to develop. In the past, these methods were limited to various statistical analyses and linear measurements. Recently, new statistical analyses and different measurement methods have been developed (Adams et al., 2004). Landmark placement measurements are among these developments. Adams et al. (2004) evaluated the contribution of this emerging measurement method to geometric morphometric methods in their study. When measurements are made using landmarks, various coordinates are created on 2D and 3D images (Adams et al., 2004). By superimposing these coordinates, it is easier to perform shape analyses and statistical analyses (Gündemir et al., 2020; Rolfe et al., 2021; Demiraslan et al., 2024). Geometric morphometric analysis methods performed in 3D differ from linear measurement in terms of examining and investigating the bone as a whole (Rolfe et al., 2021).

In the literature, geometric morphometry studies with linear measurements on the axis were found (Gosavi and Swamy, 2012; Singla et al., 2015). Similarly, 3D geometric morphometric studies on vertebrae thoracicae were also found (Kikuchi and Ogihara, 2021). Nevertheless, no 3D geometry morphometry study was found among species belonging to the carnivora family group.

In this study, specimens belonging to carnivora were used and it was aimed to determine the shape changes on axis bones collected from these specimens. Since carnivora contains many species, morphological differences could be seen (Gündemir et. al., 2023; Manuta et. al., 2023). Thus, in the present study, specimens belonging to domestic species were selected among the felis and canis genus of this family group.

MATERIALS and METHODS

Samples

In this study, 17 axis bones belonging to the carnivora family were used. The selected specimens belonged to 8 different breeds. Descriptions of species, age, weight and sex were given in Table 1. The images of axis bones used in this study were obtained from Istanbul University-Cerrahpaşa Veterinary Faculty, Research and Application Animal Hospital. These were healthy bones without any pathological problems.

Ethical statement

The required ethics committee report for the study was obtained from the Istanbul University-Cerrahpaşa Veterinary Faculty Ethics Committee (Report Number: 2022/38).

Statistical analysis

Images were obtained with a Siemens SOMATOM brand 16-slice CT device. These images were converted into a three-dimensional (3D) bone surface. Afterwards, they were transferred to the computer in 'PLY' format. Landmark placement and geometric morphometric analyses were performed on these images using SlicerMorph program. A total of 102 landmarks were applied to the images. The landmarking process was visualized in SlicerMorph (Figure 1). Firstly, Generalized Procrustes Analysis was applied to the images transferred to the computer. Then Principal Component Analysis (PCA) was applied to explain the shape variations. PCA contains information about how variations within a dataset are separated from each other and is numbered proportionally to the number of data points (e.g., PCA1, PCA2, PCA3, etc.).

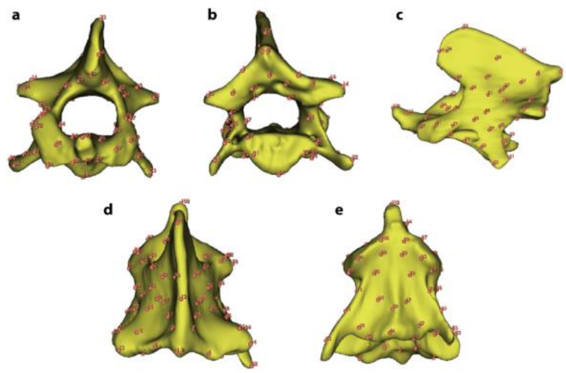

Figure 1. Landmarks on 3D models of *axis*.

The resulting PCAs explain certain percentages of the total variation based on the feature causing the variation. The sum of the variation percentages of these PCAs equals 100%, which corresponds to the total variation of the analyzed data (Aytek, 2016).

Table 1. Descriptions of species, age, weight and gender of specimens

Sample No	Species	Age	Weight (kg)	Gender
1	British	2 years	2	Male
2	British	5 months	1	Male
3	British	1.5 years	3.3	Female
4	British	3 years	4	Female
5	British	4.5 years	4	Male
6	British	4 months	1	Male
7	Chihuahua	9 months	1.8	Male
8	Chihuahua	3 years	3.3	Female
9	Cocker	8 years	13	Male
10	Cocker	8 years	18	Male
11	Golden	7 years	40	Male
12	Golden	8 years	33	Male
13	Labrador	6 years	28	Female
14	Pomerian	2 years	3.5	Female
15	Scottish	4 months	1.2	Male
16	Terrier	4 years	1	Female
17	Terrier	8 years	18	Male

In this study, a total of 10 principal components were obtained. Of these, the first three principal components with the highest explanation of total variation were visualized and explained in the results section. In addition, the values of the 10 principal components with the highest explanation are given in Figure 2 and Figure 3.

The centroid size and Procrustes distances obtained from the SlicerMorph programme were processed into SPSS in order to make size-related evaluations. In this program, multivariate regression analysis was performed between centroid size and Procrustes distances. In addition, the effects of age and weight changes on the size of the animals were investigated using the same program. For this purpose, first multivariate regression analysis was performed for age values and centroid size values. Then the same steps were applied for weight values.

RESULTS

Shape variations

Principal component analyses revealed 10 different principal components (Figure 2). Of these principal components, the first three principal components with the highest explanation rate were visualized and shape variations were explained (Figures 2). PC1 explained 21.04% of the total variation, PC2 explained 13.6% and PC3 explained 12% (Figure 2).

The shape variations between the maximum and minimum values for PC1 were quite evident in the processus transversus and processus articularis caudalis on the right side of the animal. For the maximum value, the processus articularis caudalis on the right side was shorter. For the minimum value, the processus articularis caudalis on the right side was longer. On the other hand, for the maximum value, the processus transversus was longer on the right side, while for the minimum value it was shorter and travelled more steeply caudally. At the cranial edge of the processus spinosus, different shape variations were formed for maximum and minimum values. In addition, the dorsal side of the processus spinosus formed different shape variations for maximum and minimum values. Processus spinosus exhibited a curved appearance with maximum and minimum values forming angles towards different sides. It was observed that the position between the maximum and minimum values in the dens was different from each other. Due to the shape variations between the maximum and minimum values of the bony roof forming the foramen vertebrale, the images in this region were different from each other. For PC1, the crista ventralis formed an oblique shape by making an angle at the minimum value, while it formed a straight line at the maximum value. In addition, when viewed from the lateral side, the axis bone in PC1 was dorsoventrally flattened compared to the maximum value of PC1.

Similar shape variations were observed in PC2 as in PC1. The processus articularis caudalis was shorter for the maximum value than for the minimum value, but unlike PC1, the processus articularis caudalis in PC2 was closer in distance for the maximum and minimum values. The cranial end of the processus spinosus showed a more flattened and curved shape variation at the maximum value. For the minimum value, it showed a longer and flatter shape variation compared to the maximum value. To explain this shape variation from the lateral side, the cranial end of the processus spinosus showed a more oval shape variation at the maximum value and a more angular shape variation at the minimum value. On the caudal side of the bone, the angle where the processus spinosus and processus articularis caudalis meet showed a flat junction in the maximum value, while a sharper angle was observed in the minimum value.

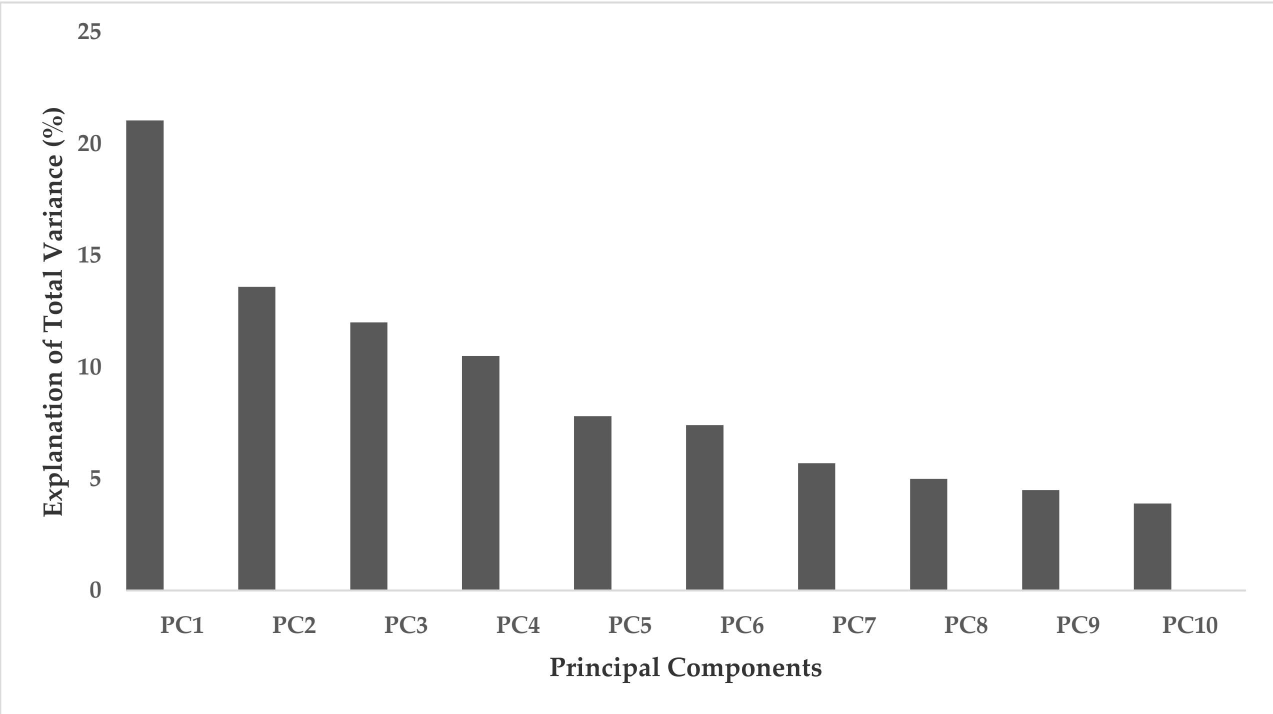

Figure 2. Percentage of Principal Components explaining total variation (%).

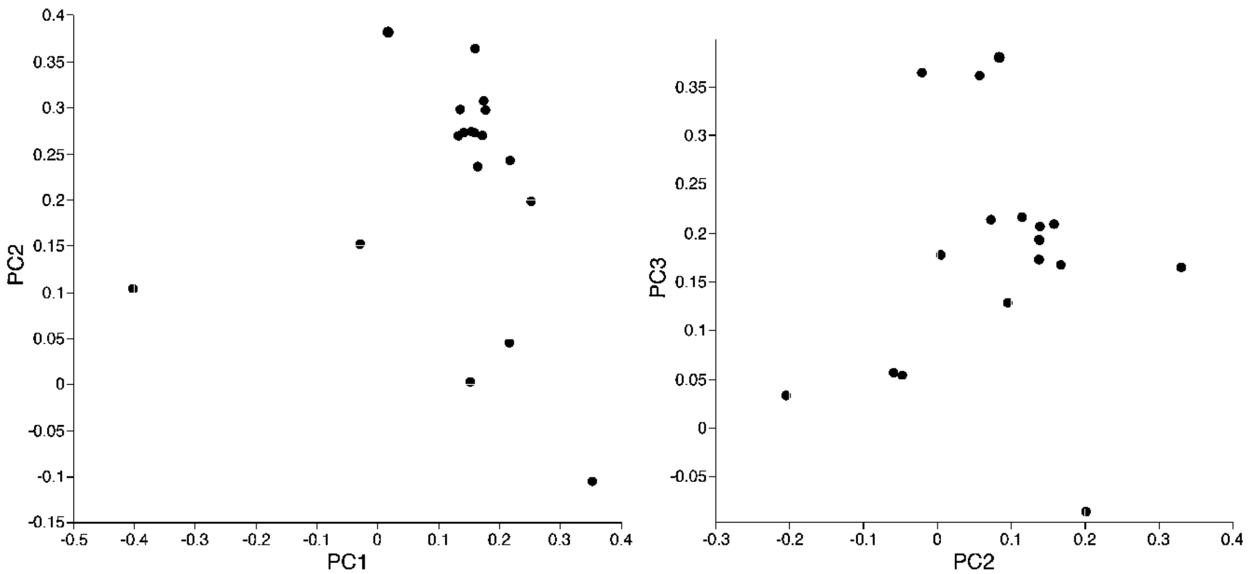

Figure 3. Scattter Plot based on the characteristics of the samples for PC1, PC2 and PC3 as a result of Principal Component Analysis.

Another change in PC2 was the processus transversus. This was more posterior and longer in the minimum value than in the maximum value. The dens was located in different positions at the minimum and maximum values of PC2. It was noticed in the ventral poses of PC2 that the dens was longer at the maximum value than at the minimum value. In PC2, the caudal end of the corpus vertebrae, the part where the fossa vertebrae is located, showed a flatter shape variation at the minimum value and a triangular shape variation at the maximum value. These shape variations were seen in both caudal and ventral poses. Additionally, the corpus vertebrae was shorter for the minimum value. It was observed that these differences in the corpus vertebrae also affected the crista ventralis. This triangular shape variation observed at the maximum value of the caudal end of the corpus vertebrae caused the crista ventralis to form an angular shape variation. At the minimum value, the crista ventralis was shorter due to the shorter corpus vertebrae. In addition, the crista ventralis showed a flatter shape variation due to the caudal end of the corpus vertebrae showing a minimum flat shape variation. The foramen vertebrale was larger at the maximum value than at the minimum value.

In PC3, the processus spinosus showed a shape variation in the form of an ellipse with a blunt cranial tip and a pointed caudal tip at the maximum value, while it showed a shape variation close to a rectangle at the minimum value. While the cranial tip of the processus spinosus showed an oblique shape variation for the maximum value, the shape variation occurred in an upright view for the minimum value. The caudal end of the processus spinosus showed a shape variation that extended to the caudal side for maximum value. However, on the contrary, for the minimum value, it formed a shape variation in the form of a triangle with its tip extending to the dorsal side. The processus transversus on the right side was longer and moved backwards for the maximum value. A similar shape variation was shaped on the left side for the minimum value. However, for the minimum value, the processus transversus on the left side extended obliquely forward. For the minimum value, the processus transversus on the right side showed a more horizontal shape variation. The left processus transversus, visualized at maximum value, was shorter than the right side. For PC3, the processus articularis caudalis showed a thicker and shorter shape variation than that seen in the other principal components for the maximum value and minimum value. The corpus vertebrae formed a longer and thinner shape variation at the maximum value than at the minimum value.

Allometry

The relationship between centroid size and Procrustes values was evaluated for allometric interpretation. A graphic plot of the results of the multivariate regression analysis is given in Figure 4. According to these results, allometry was statistically significant in carnivora axis bones ($p < 0.005$; Figure 4). Separately, another multivariate regression analysis was performed to investigate the effect of weight on size. The results of this analysis were statistically significant ($p < 0.005$).

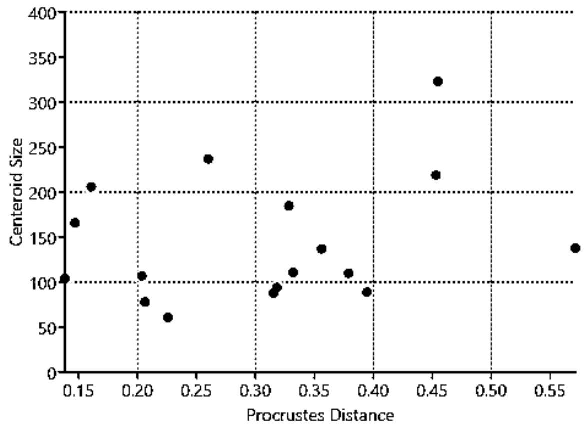

Figure 4. Scatter plot of the distribution of centroid size versus Procrustes distance of the samples, along with the relationship between the two variables.

DISCUSSION

The axis is a special bone in terms of shape and is also important due to the structures adjacent to the bone (Gosavi and Swamy, 2012). Therefore, knowing its anatomy and geometric morphometric studies are also important in terms of surgical interventions (Doherty and Heggeness, 1995). Additionally, as surgical techniques in this region improve, anatomical knowledge also needs to improve (Xu et al., 1995; Şengül and Kadıoğlu, 2006).

Although the samples belong to the same family group, it is common for them to show different shape variations in geometric morphometry studies performed on the same bone (Gündemir et al., 2023). In addition, vertebrae cervicales undertake the task of carrying the neck. Especially the axis bone participates with structures such as muscles and ligaments as a result of this task (Dursun, 2007). Some of these structures are m. longus colli, m.

spinalis, m. multifidus, m. intertransversus, lig. nuchae (Zileli, 2002; Dursun, 2007). Shape variations occurring in bones associated with muscles and ligaments are also observed in other studies in the literature (Reno, 2014). A change can be observed in the intensity and use of the muscles in the neck area (Mayoux-Benhamou et al., 1989; Fortin et al. 2018). As observed in our study, the shape differences between the right and left halves of the bone are thought to vary due to the similar reasons mentioned above.

The shape variations occurring between the maximum and minimum values for PC1 showed a serious change on the right and left sides of the bone. A similar shape variation was also observed in geometric morphometry studies in the literature where linear measurements were made. In the study conducted by Singla et al. (2015) on human axis bones, various measurements were made from the right and left sides with a line drawn from the median line of the bones. In these measurements, changes were observed between the right and left sides (Singla et al., 2015). Similarly, in Gosavi and Swamy's study in 2012, found that the difference in the width of the pedicle between the right and left sides was found to be statistically significant. Considering the structures on the right and left sides of the bones, previous studies have reported differences between these structures, a finding further supported by our study. Additionally, we believe that the difference between humans and animals in terms of normal posture also influences the use of neck muscles. However, it seems that, despite the differences in the muscle groups used, the distinction between the right and left sides in both humans and animals develops throughout life due to certain habits in neck movements and genetic factors.

As a result of the regression analysis, the relationship between size and age was examined and the results were statistically significant ($p<0.05$). A similar situation was observed in the study conducted by Johnson et al. (1988) on the vertebrae thoracicae of mice. In this study, the relationship between size and shape changes of vertebrae and age was examined by regression testing, and the results showed that age had an effect on size (Johnson et al., 1988). Since this study focuses on vertebral analysis in animals, it shows a similarity to our research, and the results have also been found to be similar, as expected. Accordingly, it can be said that the dimensional development and change of the vertebrae is related to age, as can be observed in many other bones (Mosekilde, 2000; Havill et al., 2007). In a study conducted on 7420 lumbar vertebrae in humans, it is reported that the body parts of the vertebrae increase in both length and width with age (Mavrych et al., 2014). As is evident in many studies, it is clear that the size of bones increases with age. However, although there are different opinions regarding the cause of this, it is suggested that the growth of adjacent formations over time may be a contributing factor. (Gosavi and Swamy, 2012). In our study, the significant allometric relationship between centroid size and Procrustes distance can be explained by the development of ligament and muscle tissues around the axis over time, and it is also our perspective that this may be a contributing factor. Additionally, the relationship between weight and size of the bone in our study shows similar results to a study conducted on humans, which found that size increases with weight (Mølgaard et al., 1998).

The principal component analysis revealed significant shape variations in the axis bones of carnivora, particularly in the first three principal components (PC1, PC2, and PC3), which accounted for a substantial portion of the total variation. These variations were primarily observed in key anatomical features such as the processus transversus, processus articularis caudalis, processus spinosus, dens, crista ventralis, and corpus vertebrae. Differences in the shapes of these structures were evident between maximum and minimum values across components. The findings highlight significant bilateral differences and provide evidence for shape variation across cranial, caudal, and ventral perspectives. Additionally, the observed variations in the bony roof of the foramen vertebrale and the crista ventralis suggest functional or developmental implications. Furthermore, the results of the allometric analysis demonstrated a statistically significant relationship between centroid size and shape, as well as between size and weight, emphasizing the influence of allometry on the morphological characteristics of carnivora axis bones. These findings contribute to a deeper understanding of the morphological diversity and functional adaptations of the axis bone in carnivorous mammals.

CONCLUSION

These findings contribute to a deeper understanding of the morphological diversity and functional adaptations of the axis bone in carnivorous mammals.

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