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RESEARCH ARTICLE

Allometry and Atlas Shape Analysis Between Tekir and Mix-breed Cats

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Abstract

The first cervical vertebra, the atlas, connects the skull to the body and plays a crucial role in supporting the skull and holds a distinct position within the atlanto-axial complex. In this study was aimed to examine the shape variation of the atlas in cats and to reveal shape changes related to variation in size via geometric morphometry. A total of 61 (31 mix-breed cats, 30 Tekir cats) cats were used. Shape differences were examined both for mix-breed cats and Tekir cats and between sexes. However, it was seen that the shape difference between species (P:0.1644) and between sexes (P:0.4801) was insignificant according to the discriminant function results. The average shape and shape variations of the atlas were obtained for all samples. A total of 52 principal components were obtained. PC1 explained 28.39% of the total variation. Tekir cats showed more shape variation than mix-breed cats for results of PC1 and PC2. Mix-breed cats had a larger *ala atlantis* than Tekir cats as shape. *Incisura alaris* were deeper in shape in mix-breed cats. *Foramen vertebrale laterale* of the male was more medial in shape than female. It was performed a multivariate regression of the procrustes coordinates as shape variables on the log-transformed centroid size values as a size variable to analyze the allometry of atlas for all samples. However, allometry results were statistically insignificant (P:0.3579). In this study, encompassing breed and sex comparisons in cats, will serve as a pioneering effort in future atlas research, offering substantial data concerning cat atlas bones.

Keywords: Geometric morphometry, shape variation, taxonomy, veterinary anatomy

INTRODUCTION

The atlas exhibits distinctive anatomical features when compared to the other cervical vertebrae [1] and plays a crucial role in supporting the skull and holds a distinct position within the atlanto-axial complex [2]. Due to its significant role in the region, this complex is one of the critical areas where surgical interventions are performed for congenital anomalies or trauma-related issues. Various surgical techniques are currently employed in human medicine to address instability in the atlanto-axial complex caused by a range of traumatic and nontraumatic conditions. However, during these procedures, there is a risk of causing harm to vital structures [1].

The importance of atlas morphometric data is valuable for enabling the safe execution of the mentioned procedures and the data is crucial for ensuring the secure implementation of the procedures in question [1,2]. There are numerous morphometric studies conducted on the human atlas bone, focusing on topics such as the general structure of the atlas and its anatomical features [2,3], asymmetry [4], as well as relationships among populations and sexes [5]. Some researchers have obtained materials using more modern methods, including 3D scanners, and have conducted analyses using the geometric morphometric approach, which is also becoming popular nowadays [6].

Studies on the morphology of the atlas bone in humans are generally conducted with the purpose of providing information for surgical interventions, and the same holds true for animals as well. In a study, African Giant Rat vertebrae were examined, and it was noted that the spinal canal is widest, particularly in the region where the atlas bone is located [7]. Another study on the axial skeletons of African lions, the axial bones of the animals were morphologically examined, and it was reported that the structures of their atlas bones resemble those of domestic cats [8]. There are also studies that compare atlases macro-anatomically with different species [9]. The mentioned studies provide valuable insights that can serve as fundamental knowledge for surgical endeavors, and there are also studies, such as the one by Parry et al.[10], which specifically focus on addressing these goals, like the presence of conditions like incomplete ossification in the atlas that could potentially pose significant neurological issues in dogs. Beside bone morphology research on sexual dimorphism using bone morphometrics in animals is a highly regarded and frequently conducted study. There is a study conducted on the differences between sexes using linear measurements taken from Gazelle pelvic computed tomography images [11]. In addition to classical linear measurements, sexual dimorphism studies have also been conducted in red foxes [12], sheep [13], Japanese quails [14], and cats [15,16] using geometric morphometric methods.

Bones find utility across a diverse spectrum of applications, spanning disciplines like anatomy [17], forensic science, anthropology, the study of evolution [16], zooarchaeology and anatomy education [11]. When considering the atlas bone, it is a scientific fact that there can be morphological differences among species, breeds, and sexes. In this study on the atlas bones of mix-breed cats and Tekir cats employed 3D tomography models and geometric morphometrics to reveal both interbreed and sex-based differences. The study aims to address this gap by examining differences between breeds and sexes in cat atlas bones, using modern techniques such as 3D tomography images and geometric morphometrics, in an effort to provide some data to alleviate this deficiency.

Material and Methods

Ethical Approval

Prior to conducting tomography scans, an "Informed Consent Form" was obtained from the animal owners, and the required ethics committee report for the study was obtained from the Istanbul University-Cerrahpasa Veterinary Faculty Ethics Committee (Report Number: 2022/38).

Animals

Sixty-one cats, which were over 1 year old, were used in the study. The average age of the cats was 3.79, and their average weight was 3.21 kg. Orthopedically healthy cats were used in the study, and their examination was performed before computerized tomography.

Modelling

Computed tomography scans were taken using "Siemens Somatom Scope vc30b". Scanning parameters for all samples were 0.6 mm slice thickness, 110 kV, and 28 mA, total scanning time app - 14 sec. After the scanning process was completed, the images were transferred to the computer and the segmentation process was performed. 3D models of atlas were made using the 3D Slicer (5.1.0 version) program. Soft tissues were removed from the image using the software. This way, atlas images of the samples were obtained from live animals, and then the dorsal side of the 3D specimens was photographed.

Landmarks

Images were obtained from all samples from the same angle and saved to the computer in ''pnp" format. 61 images were converted to ''tps" format using tpsUtil (version 1.74). Twenty-eight Landmarks were used in the study. TpsDig2 (version 2.32) was used to insert the landmarks into the images [18]. Nomina Anatomica Veterinaria was used as a base for the anatomical terms of the landmarks used in the study *(Fig. 1)*.

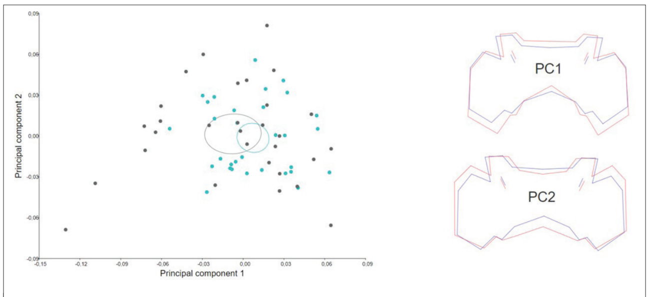

Fig 1. Scatter plot of PC1 (28.39%) and PC2 (16%) of atlas of cats in dorsal view (left) and confidence ellipses for means (Mix-breed cats: Green, Tekir cats: Grey). Wire-frame warp plots of changes in the atlas (right), as mapped by 28 landmarks. Blue outlines represent the mean shape configuration, while the red outlines show the shape changes associated with the positive extremes of the PC axes

Statically Analysis

"Morphoj" software was used for geometric morphometric analysis [19]. Principal component analysis (PCA) was performed to reveal the shape variation of the atlas. The discriminant function (DF) was applied to reveal the differences statistically for cat species and sexes. Incorrect grouping results were obtained by applying cross-validation. Average shapes between groups were examined. The intergroup procrustes distance and mahalanobis distance were obtained. It was performed a multivariate regression of the procrustes coordinates as shape variables (the regression score) on the log-transformed centroid size values (centroid size) as a size variable to analyze the allometry of atlas for all samples. Results are reported as a percentage value of the explained total shape variation from the size variation. Levels of statistical significance were computed by permutation tests, using 10,000 random permutations.

Results

Principal component (PC) analysis was carried out using a total of 61 cats. A total of 52 principal components were obtained. PC1 explained 28.39% of the total variation.

Table 1. *Principal components*		
Principal Component	**Eigenvalue**	**%Variance**
1	0.00167566	28.39
2	0.00094453	16.00
3	0.00044805	7.591
4	0.00035718	6.051
5	0.00035176	5.959
52	0.00000016	0.003

This value was 16% in PC2 and 7.59% in PC3. Principal components explaining the highest variation are given in *Table 1*.

Scatter plot of PC1 (28.39%) and PC2 (16%) of atlases of cats in dorsal view are given in *Fig. 1*. This scatter plot explained 44.39% of the total variation. Mix-breed cats mean PC1 value was higher than Tekir cats. The mean PC1 value of Tekir cats were negative. When considering the average values for Mix-breed cats and Tekir cats with respect to PC2, they are close to each other, although PC2 is found to be slightly higher. Tekir cats showed more shape variation than mix-breed cats for results of PC1 and PC2.

Fig. 1 shows wire-frame warp plots of changes for PC1 and PC2, with the average shapes in blue. The increased value for PC1 represented the larger *ala atlantis* posteriorly in shape. The borders of the *foramen vertebrale laterale* were more medial with increasing PC1 value. Also, the posterior border of the *arcus vertebralis* was narrower. *Incisura alaris* were also close to medial in shape. The increased value for PC2 represented the larger *ala atlantis* anteriorly in shape. The borders of the *foramen vertebrale laterale* were more lateral with increasing PC2 value. Also, the posterior border of the *arcus vertebralis* was wider. *Incisura alaris* was also close to medial for PC2.

The changes for PC1 and PC3 are shown in wire-frame warp plots (*Fig. 2*). The shape of *ala atlantis* was smaller and narrower in both anterior and posterior with the increasing value of PC3. The borders of the *foramen vertebrale laterale* were found to be more medial with increasing PC3 value, while the posterior border of the *arcus vertebralis* is closely matched the average width. The position of the *incisura alaris* was closer to the medial aspect compared to the mean shape.

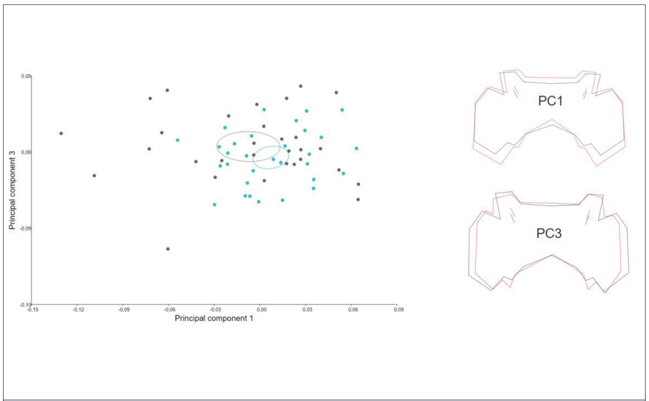

Fig 2. Scatter plot of PC1 (28.39%) and PC3 (7.59%) of atlas of cats in dorsal view (left) and confidence ellipses for means (Mix-breed cats: Green, Tekir cats: Grey). Wire-frame warp plots of changes in the atlas (right), as mapped by 28 landmarks. Blue outlines represent the mean shape configuration, while the red outlines show the shape changes associated with the positive extremes of the PC axes

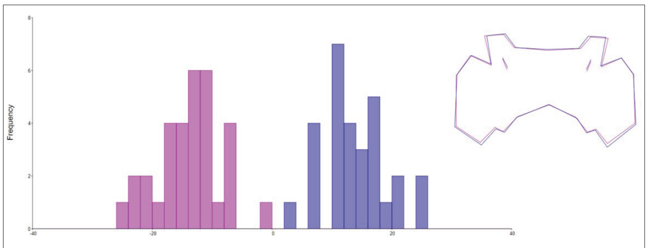

Fig 3. Discriminant function. Wire-frame warp plots of changes for sexes. Female: pink; Male: blue (Procrustes distance: 0.02585845; Mahalanobis distance: 5.2789; T-square: 423.9352; P:0.4801)

In *Fig. 3*, wire-frame warp plots of changes and average figures for both sexes are given. According to the discriminant function distribution, it was seen that the male and female were separated from each other for the samples used in the study. However, this distinction was statistically insignificant (P:0.4801). Even though the samples were completely separated according to the discriminant function, it was seen that not all of the samples were classified correctly according to the cross-validation results (*Table 2*). Male cats had a larger *ala atlantis* than females, with an average atlas shape. This enlargement was seen to occur at the caudal part of the

Table 2. *Discriminant function and cross-validation prediction accuracy*						
Groups		**Discriminant Function**		**Cross-validation**		**Rates (%)**
		Female	**Male**	**Female**	**Male**	**Prediction Rate (%)**
Sex	Female (n:32)	32	0	14	18	43.75
	Male (n:29)	0	29	17	12	41.38
Breed	Mix-breed (n:31)	31	0	21	10	67.74
	Tekir (n:30)	0	30	14	16	53.33

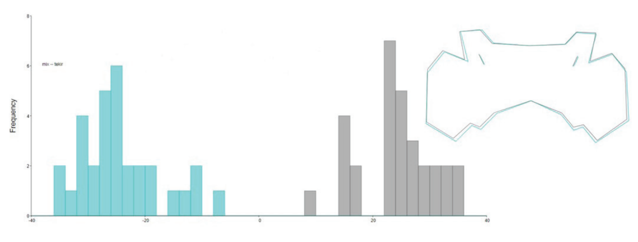

Fig 4. Discriminant function. Wire-frame warp plots of changes for mix-breed cats and Tekir cats. Mix-breed: Green, Tekir: Grey (Procrustes distance: 0.02255164; Mahalanobis distance: 6.9517; T-square: 736.7693; P:0.1644)

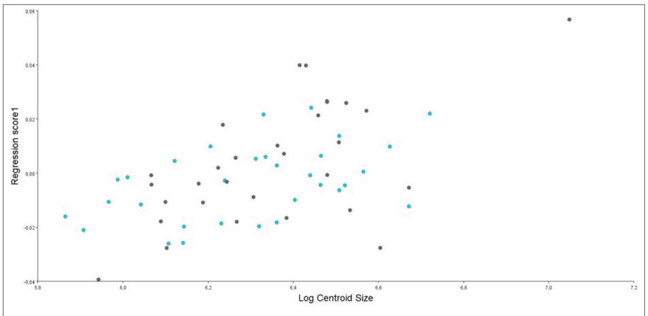

Fig 5. Regression trajectories for both sexes of mix-breed and Tekir cats by relating the centroid size Log-transformed values (Log Centroid Size) vs. the shape scores (the regression score obtained from the Procrustes coordinates). Regression of shape was not significant (n: 61; P:0.3579; 1.74 % of shape variation explained by size variation)

atlas. In addition, the *foramen vertebrale laterale* of the male was more medial in shape than female.

In *Fig. 4*, wire-frame warp plots of changes and average figures for mix-breed cats and Tekir cats are given. According to the discriminant function distribution, it was seen that the mix-breed cats and Tekir cats were separated from each other for the samples used in the study. However, this distinction was statistically insignificant (P:0.1644). Even though the samples were completely separated according to the discriminant function, it was seen that not all of the samples were classified correctly according to the cross-validation results in terms of breed *(Table 2)*. Mix-breed cats had a larger *ala atlantis* than Tekir cats, with an average atlas shape. This enlargement was seen to occur at the back of the atlas. Incisura alaris were deeper in shape in mix-breed cats.

Discriminant function and cross-validation results are given in *Table 2*. According to the discriminant function, samples were completely separated both between the sexes and between the species. Although, according to the cross-validation for both sex and breed discrimination, the results were not satisfactory. Regarding sex discrimination, 14 female cats were categorized correctly, while 18 were categorized as male within the female group. Within the male group, 12 were categorized as male, and 17 were categorized as female.

It was performed a multivariate regression of the procrustes coordinates as shape variables (the regression score) on the log-transformed centroid size values (centroid size) as a size variable to analyze the allometry of atlas for all samples. However, allometry results were statistically insignificant (P:0.3579) *(Fig. 5)*.

Discussion

Our study was conducted on a total of 61 cat atlases using geometric morphometric methods to reveal the shape differences between sexes and species on the atlas bone. When examining the shape differences among breeds in our study's DF results, it was observed that the *ala atlantis* in mix-breeds cats is wider towards the caudal direction compared to Tekir cats. This shape difference is also clearly evident in PC1 shape variation and slightly elevates the mix-breed cats PC average. It was observed that the *incisura alaris* is more medial in DF for mix-breed cats, and this reflects on PC1, PC2, and PC3 shape variations. Furthermore, the DF results also indicate that the posterior border of the *arcus vertebralis* is wider in mix-breed cats compared to Tekir cats. This variation is evident in PC2, which explains 16% of the shape variation.

Results of this study indicate that the atlas bone does not possess sufficient morphological differences statistically to distinguish between sexes. The lack of distinct sex differences in atlas for domestic cats is present in another article that have PCA clustering graph of overall cervical bone shapes showing there are not much discrimination between sexes [16]. Additionally, it has been stated that the average accuracy for sex prediction for the atlas is 63.10%. Although the bones show a complete separation by sex in the DF results in our study, when cross-validation is applied, it is observed that the rates decrease, and an average prediction accuracy of 42.57% is achieved. These results indicate that the shape changes in the atlas are not sufficient for sex predictions. In the same study [16], linear measurements were also taken, and the difference between sexes was found only in the measured caudal articular surface distance, which was statistically significant. Another measurement that is taken from the dorsal part of the bone (as our study contains dorsal view of the bone) was cranial articular surface distance and transverse process length (in cranio-caudal direction) and were statistically insignificant. A study conducted on the vertebral bones of domestic cats by Boonsri et al.[16] concludes that gender has a minimal impact on the cervical vertebrae, and this result is in line with the findings of our study. However, it is mentioned that there is a statistically significant difference between sexes for the caudal articular surface distance, and the shape differences in this study support the caudal expansion of the atlas in males compared to females.

Considering the crucial role of the neck as the cranium's support, the proportionate weight of the cranium in relation to the total body weight remains consistent between males and females in human [20]. In a study encompassing the analysis of human atlas bones, conducted through three-dimensional scanning and utilizing geometric morphometric methods on digitized images, it has been demonstrated that sex differences are statistically insignificant [6]. However, a morphological study conducted on Thai individuals' (human) atlas bones has indicated statistically significant sex differences [5]. In a study conducted by Parry et al.[10], which included various dog breeds and involved 120 dogs, the shape variations of the atlas were examined. Despite the differences among breeds, it was reported that no significant relationship could be found among the shape differences in the atlases. However, in the same study, it was mentioned that the length of the transverse process of the atlas increases with the weight of the dogs. In an anatomical study conducted on elephants [21], the cervical vertebrae of elephants were compared to those of horses and cattle in terms of their structures. It was observed that the cervical vertebrae of elephants are shorter in length compared to other species, and this has been associated with the need for a shorter neck to support their heavy heads. Additionally, it was noted that the width of the atlas bone in elephants is nearly as wide as the animal's neck. The lack of statistical significance in the shape variations in our PCA and DF analyses, despite the shape differences, has led us to consider that this may be due to the fact that the weights of the cats, despite their different breeds, are relatively close to each other. It is a reasonable hypothesis that the ambiguity in sex-related differences associated with head weight in humans and body weight in dogs and elephants could also apply to cats. In addition to head and body weights, among the many breeds of cats with various variations [22-24], it is believed that sex-related differences in atlas shape among cat breeds, similar to humans, may be associated with genetic backgrounds.

This study was based on the shape analyses performed on atlas images obtained from cats and aims to examine whether there are shape differences between sexes and breeds. Discriminant function analysis was conducted to analyze the differences between sexes, and it was observed that the shape differences between sexes resulted in a complete separation of the sex groups. However, during cross-validation analysis, individuals were poorly classified into their respective sex groups. As a result, the obtained P-value from the discriminant function analysis was found to be statistically insignificant (P:0.4801). Similar observations were made for breed differentiation, with a resulting P-value of 0.1644. The analyses revealed that there is no significance in terms of sex and breed differences among atlas bones. Considering the critical importance of the atlas bone's position, there is a need for more data on differences between cat breeds or sexes. In this context, our paper offers substantial data concerning cat atlas bones. We hope that our study will serve as a pioneering effort in future atlas research, encompassing breed and sex comparisons in cats.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author (Z. Mutlu) on reasonable request.

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There is no funding support.

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Authors Contributions

EÖ and ZM: Conceived and supervised this study; EÖ, BU, YA, and ZM: Collection of the data; EÖ and KA: Statistical analysis. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

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