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Research Article

Geometric Morphometric Analysis of Scapula at Cats and Dogs

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Abstract

The scapula in quadrupedal mammals is a flat bone that connects the thoracic limb with the trunk above the shoulder joint. For this purpose, scapulae of 34 dogs and 23 cats were modeled using computer tomography. 9 Landmarks and 50 semilandmarks were used. The cat and dog samples differ in scapula shape, as do male and female cats. Centroid size has no apparent covariance with shape, and we did not find any difference between the two sexes in dogs. The scapula of cats was wider. The scapula of dogs was narrower and longer. Margo cranialis was more oval in cats. Angulus cranialis border was not clear in cats. Angulus caudalis was sharper in dogs. Spina scapulae was closer to caudal in cats. In cats, the fossa supraspinata was wider than the fossa infraspinata. Also, the collum scapulae was narrower in cats. The scapula of male cats was wider than that of female cats. In shape, the fossa supraspinata was wider in male cats. In male cats, the spina scapulae were more caudal. Angulus caudalis was wider in female cats. The most significant gender differences in dogs were in tuberculum supraglenoidale and margo caudalis. Male dogs had larger tuberculum supraglenoidale in shape. Margo caudalis was more caudal in male dogs. Geometric morphometrics was found to be effective in distinguishing the scapula of cats and dogs. In addition, this method can be useful in sex estimation.

Keywords: Cat, Dog, Geometric morphometrics, Principal component analysis, Scapula, Sex differentiation, Taxonomy

Introduction

The scapula is a plane, triangular bone that connects the thoracic limb to the trunk. This bone is surrounded by the powerful muscles of the thoracic limb. Facies lateralis of scapula contains spina scapulae. The spina scapulae divide the facies lateralis into two faces, fossa infraspinata and fossa supraspinata. Unlike other species, spina scapulae in dogs is located in the middle of the facies lateralis. The spina scapulae terminate with the acromion near the angulus ventralis. However, spina scapulae terminate as processus hamatus in dogs, and additionally as processus suprahamatus in cats [1,2].

The margins of the scapula are called margo dorsalis, margo caudalis, and margo cranialis. Margo cranialis is flat in animals such as cattle and horses, but oval in cats and dogs. Margo caudalis is thicker than the other margins, because the musculus triceps brachii comes out of the margo caudalis and creates some bumps on the bone. It has been said that about these three margins of the scapula one can distinguish between species. However, the distinguishing information for the margins of the scapula for cats and dogs was not found in the reference information [1-3].

Geometric morphometry, unlike linear morphometry, detects shape differences between groups and reveals shape differences with statistical methods [4-7]. The term 'shape' here can be defined as the appearance obtained when we subtract the size of the example [8]. It was stated in the reference information that the shape showed greater variation among groups than in size [9]. With these features, geometric morphometry has been studied especially on bones in recent years, and the differences in structures between species and genders have been tried to be revealed.

Shape analysis in geometric morphometry is done by placing 'landmarks' on certain anatomical points. These points are homologous points applied in the same way to all samples used in the study [10]. These landmarks are classified into three types [11]. Type I are points that are clearly located, such as the junction of two structures. Type II can refer to the most extreme or most recessed parts of a structure. Type III landmarks, which can also be called semilandmarks, can be defined as landmarks determined on the basis of other landmarks and used along a curve or a boundary. These points are located on the x and y coordinates in the coordinate system in 2D analysis.

For taxonomy, osteological methods can be applied easily and the type and animal sex determination on the basis of bone can be easily done with reference supports [12-14]. These methods are important for the identification of bones examples as well as for archaeological bones [15,16]. The most commonly used element of skeleton in terms of taxonomy is the cranium [17-19]. There is a study showing that species and sex distinctions can be made for many skull-related specimens. However, in recent years, gender and species analysis has also been included in reference studies in bones except the skull [20-22]. Processus hamatus and processus suprahamatus are decisive for the distinction between cats and dogs. Moreover, the shape difference of the anatomical borders of the scapula will also provide important information for veterinary anatomy. In addition, some parts of the bone may be missing in areas such as archeology, causing difficulties in terms of taxonomy. In this respect, evaluating more than one point for bone separation can contribute to the reference information.

This study aimed to investigate the usability of geometric morphometric analysis in gender analysis.

Material and Methods

Ethical Statement

Before starting the study, an application was made to the Animal Experiments Local Ethics Committee Presidency of Istanbul University-Cerrahpasa Rectorate for the necessary permissions. Conditional approval received (Document Number: E-74555795-050.04-882352). Informed Consent Form was taken from the patient owners.

Animals

In this study, computed tomography images of the thoracic region of 34 cats and 23 dogs were used. The examined and imaged animals had no symptoms of any orthopedic disease. The cats and dogs used in the study, their average age and average weight are given in *Table 1* and *Table 2*.

Table 1. *The average age and weight of the cats used in the study*

Breed	Female	Male	Age (Year)	Body Weight (kg)
Mix breed	17	4	5.83	4.68
Ankara Cat	1	0	13	6
Van Cat	1	0	1	3.3
Scottish fold	0	3	1.17	3.53
Blue Point Siamese	1	0	1	3.6
British Shorthair	3	1	4.63	3.6
Persian	1	2	13	3.93

Table 2. *The average age and weight of the dogs used in the study*

Breed	Female	Male	Age (Year)	Body Weight (kg)
Mix breed	3	2	6	18.63
Beagle	1	0	13	15
English Bulldog	0	3	3.67	23.5
Chihuahua	1		10	5
Cocker Spaniel	1	1	13	16
German Shepherd	0	2	7	40
Siberian Husky	1	0	8.50	23
Labrador Retriever	0	1	9	36
Pekingese	0	1	11	6
Pomeranian	0	1	4.00	3.7
Pug	0	2	10.75	9.6
Rottweiler	1	1	10	33.5
Staffordshire Bull Terrier	0	1	1	20

Modeling and Acquisition of Images

Computed tomography scans were taken using Siemens (Somatom Scope vc30b) at the Animal Hospital, Faculty of Veterinary Medicine, Istanbul University-Cerrahpasa. Scanning parameters for all samples were 0.6 mm slice thickness, 110 kV, and 28 mA, total scanning time approximately - 14 sec. After the scanning process was completed, the images were transferred to the computer and the segmentation process was performed. 3D models of scapulae were made using the 3D Slicer (5.1.0 version) program. Soft tissues were removed from the image using the software.

Images were obtained from all samples from the same position and saved to the computer in ''pnp'' format. 57

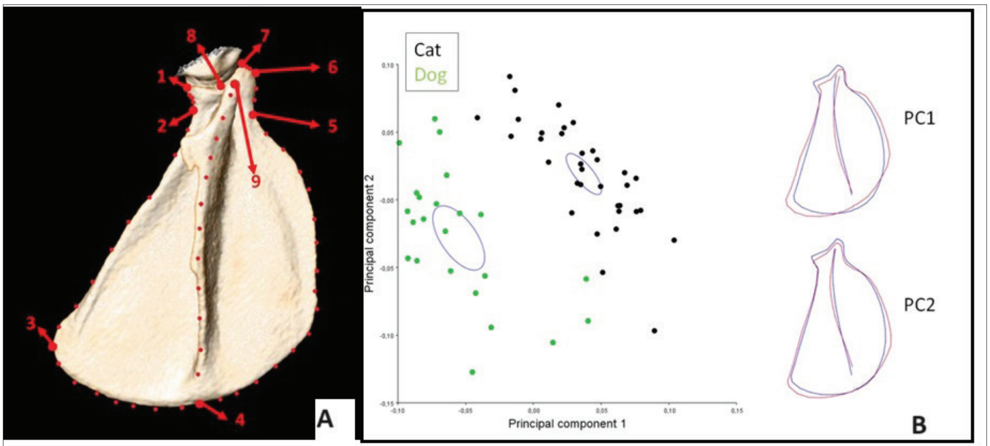

Fig 1. A: Landmarks (9) and Semilandmarks (50). *Red arrows:* landmarks, *small red points:* semilandmarks. **B:** Scatter plot of PC1 (30.1%) and PC2 (21.42%) of scapulae in lateral view for interspecies (left) (Confidence ellipses for means). Wire-frame warp plots of changes in the scapulas, as mapped by 9 land-marks, 50 semilandmarks (right). Blue outlines represent the mean shape configuration, while the red out-lines show the shape changes associated with the positive extremes of the PC axes

Table 3. *List of landmarks applied to the scapula in lateral view*

Landmark	Region
1	*Tuberculum infraglenoidale* 3 Semilandmarks
2	Caudal border of *collum scapulae* 8 Semilandmarks *(margo caudalis)*
3	*Angulus caudalis* 7 Semilandmarks
4	Middle point of *margo dorsalis* 15 Semilandmarks
5	*Incisura scapulae* 2 Semilandmarks
6	*Tuberculum supraglenoidale* 2 Semilandmarks
7	The most cranial point of *cavitas glenoidalis* 13 Semilandmarks
8	The middle point of the *cavitas glenoidalis*
9	The most ventral point of *spina scapulae*

images were converted to "tps" format using tpsUtil (version 1.74) [23]. 9 Landmarks (Type I) and 50 Semilandmarks (Type III) were used in the study *(Fig. 1-a)*. TpsDig2 (version 2.32) was used to insert the landmarks into the images [24]. The first, Landmarks were placed on all images *(Table 3)*. Then, 50 semi-landmarks were added. Finally, 'append tps curve to landmarks' was made using tpsUtil (version 1.74) again and semilandmarks were converted to Type I landmarks. Nomina Anatomica Veterinaria was used as a base for the anatomical terms of the landmarks used in the study [25].

Geometric Morphometrics

MorphoJ software was used for geometric morphometric analysis. Grouping operations were performed on the scapulae between species and sexes [26]. First, the differences in the shape of the scapula between cats and dogs were examined, followed by differences between sexes within species. Generalized Procrustes Analysis was performed before each group analysis. Then Principal Component Analysis (PCA) was applied. The shape and centroid size of these species and sexes were compared using Procrustes ANOVA. Discriminant function was used to reveal differences between cat and dog scapulae and between sexes.

Results

A total of 56 PCs were obtained to explain the morphological differentiation among cat and dog scapulae. PC1, which accounts for the most shape variation between species, explained 30.1% of the total variation *(Fig. 1-b)*. PC2 explained 21.42% of the total variation, while PC3 explained 12.64% of the total variation. The scatter plot of PC1 and PC2 of scapulae in lateral view is given in *Fig. 1-a*. Cats had higher PC1 values than dogs. The increase in PC1 value is correlated with a longer margo caudalis of the scapula. Also, when the PC1 value increased, spina scapulae were located more caudally. When the PC2 value increased, it was correlated with a longer caudal border of the scapula. PC1 would separate almost all cats and dogs. However, 3 dogs had a higher value for PC1 than the dog groups (Pekingese, male; mix, female and pug, male). One cat sample had a lower PC1 than the average cats (Mix, female).

Procrustes ANOVA results are given in *Table 4*. The cat and dog samples differ in scapula shape, as do male and female cats. Centroid size has no apparent covariance with shape, and we did not find any difference between the two sexes in dogs.

***Table 4.** Centroid size and the shape of scapula standard deviations of quails*

Individuals	Measurement	F	P-Value
Species	Centroid size	0.88	0.3522
	Shape	17.62	<.0001
Sex (cat)	Centroid size	0.48	0.4942
	Shape	2.43	<.0001
Sex (dog)	Centroid size	0.25	0.6256
	Shape	0.51	1

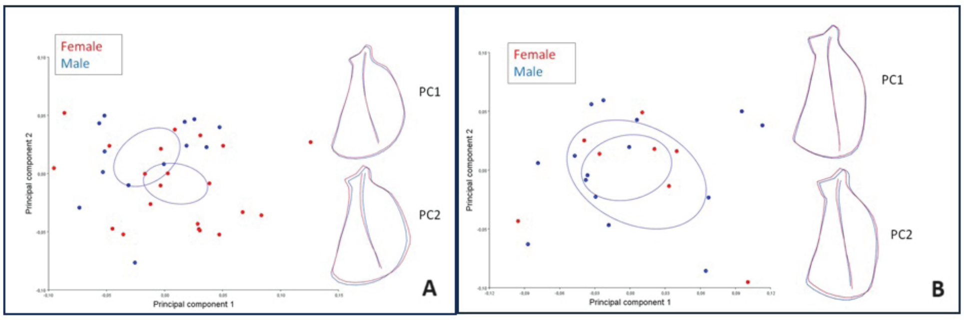

Fig 2. A: Scatter plot of PC1 (33.37%) and PC2 (16.77%) of scapulae in lateral view between sexes in cats (left) (Confidence ellipses for means). Wire-frame warp plots of changes in the scapulae, as mapped by 9 landmarks, 50 semilandmarks (right). Blue outlines represent the mean shape configuration, while the red outlines show the shape changes associated with the positive extremes of the PC axes. **B:** Scatter plot of PC1 (34.73%) and PC2 (19.23%) of scapulae in lateral view between sexes in dogs (left). (Confidence ellipses for means). Wire-frame warp plots of changes in the scapulae, as mapped by 9 landmarks, 50 semilandmarks (right). Blue outlines represent the mean shape configuration, while the red outlines show the shape changes associated with the positive extremes of the PC axes

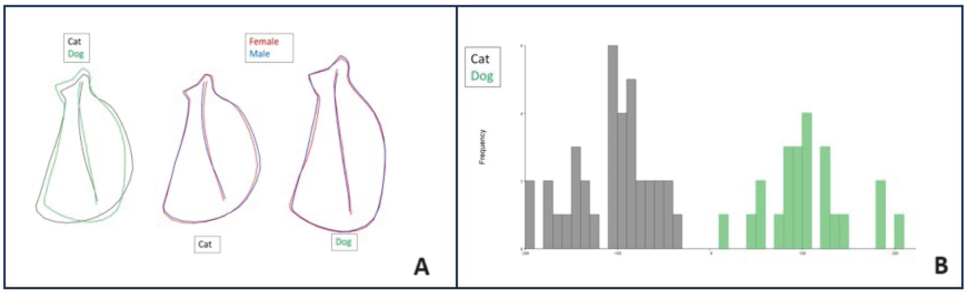

Fig 3. A: Wire-frame warp plots of changes in the scapulae. **B:** Distinctive scores and frequency for cats and dogs

A total of 32 PCs were obtained to explain the morphological differentiation between sexes in cats. PC1, which accounts for the most shape variation between sexes in cats, explained 33.37% of the total variation. PC2 explained 16.77% of the total variation, while PC3 explained 9.45% of the total variation. The scatter plot of PC1 and PC2 of scapulae is given in *Fig. 2-a*. The increase of PC1 is correlated with a wider tuberculum supraglenoidale. Females occupy the entire spectrum of PC1. Their values are not greater than those of males. However, males occupy mostly positive PC2 space, indicating a dorsoventrally elongated margo cranialis and a more posteriorly located spina scapulae in positive PC2 space.

A total of 21 PCs were obtained to explain the morphological differentiation between sexes in dogs. PC1, which accounts for the most shape variation between sexes in dogs, explained 34.73% of the total variation. PC2 explained 19.23% of the total variation, while PC3 had about 12.14% of the total variation. The scatter plot of PC1 and PC2 of scapulae in lateral view is shown in *Fig. 2-b*. No particular distribution was observed between males and females in the shape variation of the scapula.

The discriminant analysis result is given in shape difference *Fig. 3-a*. In shape, the scapula of cats was wider. The scapula of dogs was narrower and longer. Margo cranialis was more oval in cats. Angulus cranialis border was not

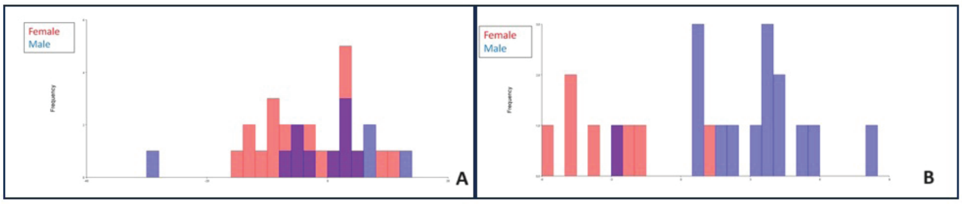

Fig 4. A: Distinctive scores and frequency for sexes for cats. **B:** Distinctive scores and frequency for sexes for dogs

clear in cats. Angulus caudalis was sharper in dogs. Spina scapulae was closer to caudal in cats. In cats, the fossa supraspinata was wider than the fossa infraspinata. Also, the collum scapulae was narrower in cats. According to the distinctive scores and frequency, it was seen that the cat and dog samples were completely separated *(Fig. 3-b)*.

The scapula of male cats was wider than that of female cats *(Fig. 3-a)*. In shape, the fossa supraspinata was wider in males. In males, the spina scapulae were more caudal. Angulus caudalis was wider in females. Distinctive scores and frequency for female and male cats are given in *Fig. 4-a*. There was no significant distribution in terms of gender of the samples used in the study.

The most significant gender differences in dogs were in tuberculum supraglenoidale and margo caudalis *(Fig. 3-a)*. Male dogs had larger tuberculum supraglenoidale in shape. Margo caudalis was more caudal in male dogs. Distinctive scores and frequency for female and male dogs are given in *Fig. 4-b*. There was no significant distribution in terms of gender of the samples used in the study like cats.

Discussion

The shape of the scapula in carnivores is strongly influenced by phylogeny, body size, and locomotion habits. [27]. Thus, when addressing the differences between cats and dogs, the type of locomotion in running dogs determines the long and slender scapula compared to the short and wide one in cats, adapted to climbing. The results of the study also supported this information. In shape, the scapula of cats was wider. The scapula of dogs was narrower and longer. The caudal border of the scapula in dogs is more developed than in cats, because of the active role of the m. serratus ventralis thoracis while running. The acromial part of the deltoid muscle is inserted on the processus hamatus. The morphometric analysis shows a more prominent processus hamatus in dogs than in cats. Given that the muscle acts in forelimb abduction, it may be assumed that this muscle is more active in dogs. Whilst sex distinction was statistically significant as a result of an analysis of the shape of the scapula in cats, no such differences were found in the dogs. This points to an aspect related to the greater allometric variability of shape in cats than in dogs [28].

In large ruminants, males have a wider basis of the scapula and higher spina scapulae, but the longitudinal dimension of the glenoid cavity is greater in females [29]. It is also reported [30], that basis scapulae are wider in male wild cats than in female individuals. It was also performed [31] a comparison between sexes in Van cats by using linear measurements of the scapula on the basis of three-dimensional reconstruction of computed tomography scans. The authors stated that the length of the spina scapulae, its height, the width of the fossa supraspinata, the width of the fossa infraspinata, the length of the tuberculum supraglenoidale, and the diameter of the cavitas glenoidalis were higher in male cats than in females. In this study, in which cats and dogs were used, geometric morphometry was used instead of linear measurements as in previous studies. By ignoring the size differences of the scapula, shape differences between species and genders were revealed. In the results of the shape analysis, it was seen that the scapula of male individuals for cats and dogs were wider than females.

In Van cats, both fossa supraspinata width and fossa infraspinata width were higher in males [31]. However, in the geometric analysis results, it was seen that the main difference here was in the fossa supraspinata. Because while margo caudalis was more caudal in shape, spina scapulae were in the same amount caudal in shape. While this ratio kept the width of the fossa infraspinale, wider fossa supraspinale was seen in males thanks to the margo cranialis, which is more cranial.

In a study it was observed [32], that processus hamatus exceeded the rim of the cavitas gelonidalis in wolves and foxes, but not in dogs. In Van cats, it was determined that processus hamatus slightly exceeded the border of cavitas glenoidalis as in wolves and foxes. In this study, we did not find that the processus hamatus extends beyond the outline of cavitas glenoidalis in dogs or cats.

In conclusion, although the cat and dog scapula were similar in shape, the difference between the two species could be revealed statistically with the geometric morphometrics. While there are not many size differences between scapulae of different breeds of cats, there are scapulae of different sizes among various breeds of dogs. The reason for this is that individual breeds vary considerably in body size. Regardless of this size variation, the shape of the scapula in dogs remains similar. While traditional morphometric methods cannot establish a standard of linear dimensions for this bone, a standard shape can be determined even for dogs via shape analysis. This method seems to be more effective in cats than in dogs that this was also thought due to the low number of dog samples or the fact that there were too many various breeds, belonging to different morphotypes.

Declarations

Availability of Data and Materials: The datasets analyzed during the current study are available from the corresponding author (D. Aydın Kaya) on reasonable request.

Funding Support: None.

Conflict of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

Ethical Statement: This study was approved by the Animal Experiments Local Ethics Committee of Istanbul University-Cerrahpasa (Approval Number: E-74555795-050.04-882352).

Author Contributions: Conceptualization, Z.S.A. and Z.N.A.; methodology, O.S. and N.M.; software, D.A.K and E.O.; validation, E.O., T.S. and M.C.S.; formal analysis, O.S and E.O.; resources, Z.N.A. and Z.S.A.; data curation, Z.N.A. and Z.A.; writing-original draft preparation, M.C.S. and T.S.; writing-review and editing, N.M., M.C.S. and T.S.; supervision, Z.N.A.; project administration, D.A.K; funding acquisition, Z.N.A. All authors have read and agreed to the published version of the manuscript.

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