RESEARCH ARTICLE

THREE-DIMENSIONAL MORPHOLOGICAL VARIATION AND ALLOMETRIC ANALYSIS IN DOG SCAPULA

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ABSTRACT

In canines, the scapula is a flat bone that serves to connect the thoracic limb with the trunk and the clavicle, which is located above the shoulder joint. In this study, computer tomography was employed to create models of the scapulae from 25 dogs. There are 12 breeds with different age, sex and weight. 562 semi landmarks were placed on the scapulae. Applying Procrustes analysis, variance in shapes of different breeds and ages of dogs were classified. Also, with the 3D Slicer Program extension called Dense correspondence analysis, we could show the shape variation with colorimetric measurement on a mean templated scapula. Lastly, using allometric analysis, this research also tried to point out if there is a shape change with the size change based on different breeds in dogs. In this study conducted on dogs, it was seen that PC1 explained the highest shape variation, explaining 25.4% of the total variation. It was seen that dogs were separated according to their size in PC1 values. While the PC1 value was high in small breed dogs, this value was generally negative in dogs with larger body size. While the scapula was wider in small breed dogs, the scapula in large breeds was thin and long in shape. The effect of size on shape was statistically significant. Geometric morphometrics is a valuable analytical approach for identifying the differences in animal species and their dimorphic features. This study has highlighted the effectiveness of geometric morphometrics in distinguishing shape variations and allometric differences among various breeds and age groups of dogs.

Keywords: Carnivore, shape analysis, veterinary anatomy

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INTRODUCTION

The dog exhibits an unparalleled range of skeletal diversity, unlike any other mammal. Dogs can span an extensive spectrum in terms of weight and height (Dyce et al., 1987; Hourdebaigt, 2003). Beyond variations in size, dogs can also display differences in the length and shape of their bones. This remarkable array of dog breeds, each with its unique shape, has been meticulously crafted through artificial selection processes. These practices have led to the establishment of distinctive physical traits within hundreds of unique breed populations. Scapula, also called the shoulder blade, is the largest flat bone located on lateral sides of the body, where the last cervical part of the columna vertebralis meets the costas (König, 2009). The scapula is a bone that connects the forelimb to the trunk via muscular connections. Positioned at the proximal end, the scapulae play a vital role in shaping the shoulder joint (Bahadır and Yıldız, 2012).

It features a prominent ridge or spine, *spina scapulae*, running down its lateral surface, which is called *facies lateralis*, effectively dividing this side into two distinct regions: *fossa supraspinata* and *fossa infraspinata* (König, 2009). Towards the end of this spine, there's a bony ledge known as the *acromion*. In dogs, acromion ends with *processus hamatus*. As the scapula extends towards its end, it tapers at the neck and forms a shallow articular socket called *cavitas glenoidalis* (Demiraslan and Dayan, 2021). This glenoid cavity combines with the head of the humerus to create the shoulder joint. The ridge on the craniodorsal side of the *cavitas glenoidalis* is referred as the *tuberculum supraglenoidale,* while the ridge on the caudoventral side is known as the *tuberculum infraglenoidale* (Bahadır and Yıldız, 2012). Notably, the *tuberculum infraglenoidale* is unique to dogs and cats. The medial surface of the scapula, *facies costalis,* is flat and relatively smooth (Demiraslan and Dayan, 2021).

Geometric morphometrics is a field that uses geometric principles and quantitative methods to analyze and compare the variations in size and shape of biological structures in animals (Adams et al., 2016) as well as in humans (Ajanović et al., 2023). It focuses on anatomical landmarks. Procrustes analysis is a foundational technique in geometric morphometrics (Slice et al., 2007). It aligns, scales, and superimposes shapes to eliminate differences in position, orientation, and size, allowing for the direct comparison of shapes (Mitteroecker and Gunz, 2009). Principal Component Analysis (PCA) is often used to analyze shape variations (Boz et al., 2023). It reduces complex shape data into a smaller set of variables (principal components) that capture the most significant variations in shape. In shape analysis, landmarks are specific locations on an animal's body or structure that hold anatomical or biological importance (Jashari et al., 2022). These landmarks are used to capture the shape information accurately. Landmarks can be 2D points (e.g., on photographs) or 3D points (in three-dimensional space) (Gurbuz et al., 2022; Hadžiomerović et al., 2023; Gundemir et al., 2023(a); Gundemir et al., 2023(b); Szara et al., 2023).

In osteology, taxonomy covers the systematic classification and grouping of skeletal remains according to their anatomical and morphological traits. This involves identifying and organizing bones from different species, breeds or individuals into distinct groups or categories. The goals of taxonomy in osteology include species identification, understanding individual variation, comparative anatomy, functional morphology, evolutionary studies, and forensic analysis. Being the largest flat bones in the body, scapulae still have limited reference stories of gender and breed analysis. Recent studies have given the scapulae more credit than before. Hence, investigating the allometry in the scapulae can help researchers understand how different bones grow and change in size relative to the overall size of a dog and also to estimate the age and sex of individuals based on the size and proportions of their skeletal parts. This information can also help to understand and classify the evolutionary relationships and species identification of skeletal remains.

MATERIALS AND METHODS

Specimens

For this research, computed tomography scans of the thoracic area from 24 dogs were employed. It's noteworthy that the subjects included in the imaging process did not exhibit any orthopedic issues. The dogs used in this study were given under in the **Table 1** with their gender, average age and weight.

Table 1 Dogs used in the study, their average age and average weight

Dogs	Female	Male	Age	Weight (kg)
GOLDEN RETRIEVER (n:5)	2	3	10.6	32.1
COCKER (n:4)	5	0	10.75	14.15
ROTTWEILER (n:4)	1	3	9	42.32
BULLDOG (n:2)	0	2	5	28.5
PEKINGESE (n:2)	1	1	10	5.75
BEAGLE (n:1)	1	0	13	15
PITBULL (n:1)	1	0	7	31
JACK RUSSELL (n:1)	0	1	5	11
DOGO ARGENTINO (n:1)	0	1	11	39
CAVALIER KING CHARLES (n:1)	1	0	8	10.8
CHIHUAHUA (n:1)	0	1	10	5
INGILIZ SETTER (n:1)	0	1	13	18

Computed tomography and modelling

For the acquisition of computed tomography scans, Siemens (Somatom Scope vc30b) was utilized at the Animal Hospital, Faculty of Veterinary Medicine, Istanbul University-Cerraphasa. Uniform scanning parameters, including a 0.6 mm slice thickness, 110 kV, and 28 mA, were applied to all samples, resulting in a total scanning time of approximately 14 seconds. Following the completion of the scanning process, the images were transferred to a computer, and subsequent segmentation was carried out. The 3D Slicer program (version 5.4.0) was used to create three-dimensional models of the scapulae, after removing the soft tissues from the bone.

In the Slicer program version 5.4.0, the PseudoLM Generator module in the GeoMorph extension was utilized to generate pseudo-landmarks. A source landmarks template was established using this plug in, employing a spacing tolerance of 3%. The 'Original Geometry' option was selected to derive a sampling pattern based on the model's geometry. The initial number of sampled points in the template was set to 15. Subsequently, a template mesh was generated using the 'Generate Template' function. A 'Project points to surface' operation was executed. Following this, an enforce spatial sampling rate was applied to eliminate samples with a point-to-point distance lower than the spatial sampling rate. As a result of these operations, a total of 562 pseudo-landmarks were obtained. The resulting draft of pseudo-landmarks was saved for application to other samples.

In the analysis, the ALPACA tool was used for the efficient transfer of landmarks from a draft pseudo-landmark to the target 3D model. A batch processing approach was implemented to apply the draft pseudo-landmark across 24 samples, using the "Single Template (ALPACA)" option. The

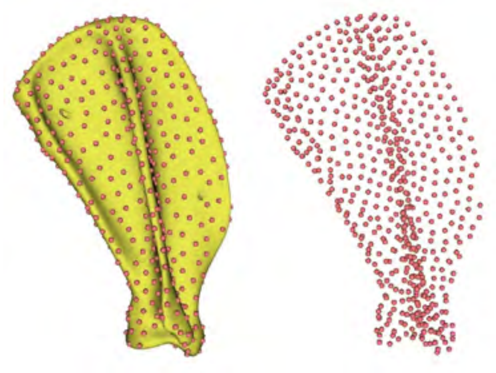

Figure 1 Semi-landmarks

identical mesh model served as both the source and the target, with the draft pseudo-landmark prepared for that specific sample, acting as the source landmark. The process concluded with the execution of Run-auto landmarking, resulting in the recording of 562 semi-landmark sets, each documented separately for all samples (Figure 1).

Shape analysis

Principal component analysis (PCA) was applied to reveal the shape variations of all scapulae. PCA changes were obtained in 3D from Slicer (version 5.4.0). Procrustes distances were obtained for all samples. R Studio (version 4.3.2) and Past (version 4.03) statistics program were used for statistical analysis.

3D Slicer Extension called "DeCa", A Dense Correspondence Analysis, was also utilized for colorimetric visualization on shape variation. (R) First, using the panel in DeCa, Rigid Alignment of Models, we aligned all the Scapula models of the specimens with the selected reference model. This step is mandatory because the results of aligned models and landmark files which we will use in the shape variation analysis will be saved in the data folder. Using the "Generate Mean" tab, after choosing a reference model, a mean template of the group is created with rigidly aligned models and landmarks. Additionally, the toolkit provides the visualization of heatmaps illustrating average and individual differences in shape.

Regression Analysis and allometry

After the centroid size and procrustes distance were obtained for each sample, multivariate regression was performed with these values. It was investigated whether allometry existed in the dog scapula and the statistical significance of the results.

RESULTS

PCA test was conducted to reveal the shape variations in the scapulae. As a result of PCA, a total of 24 PCs were obtained. PC1, which explains the highest variation in shape, accounts for 25.4%, while PC2 explains 14.7%. PC3 explains 9.7%. The scatter plot of PC1 and PC2 for the scapula is provided in Figure 2.

Golden Retrievers, Cockers and most of the big breed dogs are in the negative space of PC1. Pekingeses have the highest PC1 scores in all dog breeds, which makes them have a high rate in shape variation. Jack Russel, which is also a small breed of dog, also has positive PC1 score. As seen in the Table, Cockers have low rate in shape variation with negative PC1 and PC2. Rottweilers are also scattered in the negative PC1 space. Rottweilers are in the negative space of PC1, scattered around all the axes in the PC2 chart.

In breed base, smaller breeds seem to have higher PC1, when bigger breeds tend to have lower PC1 scores. PC2, on the other hand, isn't dependent on the breed size. We can see PC2 scores change in individual differences.

In the mean 3D mesh comparisons of maximum and minimum percentage of PC1, in maximum mean shape of the scapulae is wider and more sharp-edged (Figure 3). Increased PC1 explains anteriorly located margo cranialis though decreasing PC1 results in more oval margo dorsalis. Angle of the caudal border becomes more pronounced when the PC1 increases, in the mean template of the minimum percentage of PC1, angulus caudalis was not clear.

Spina scpaulae get elevated with the increase in PC1 mean, while the processus hamatus part

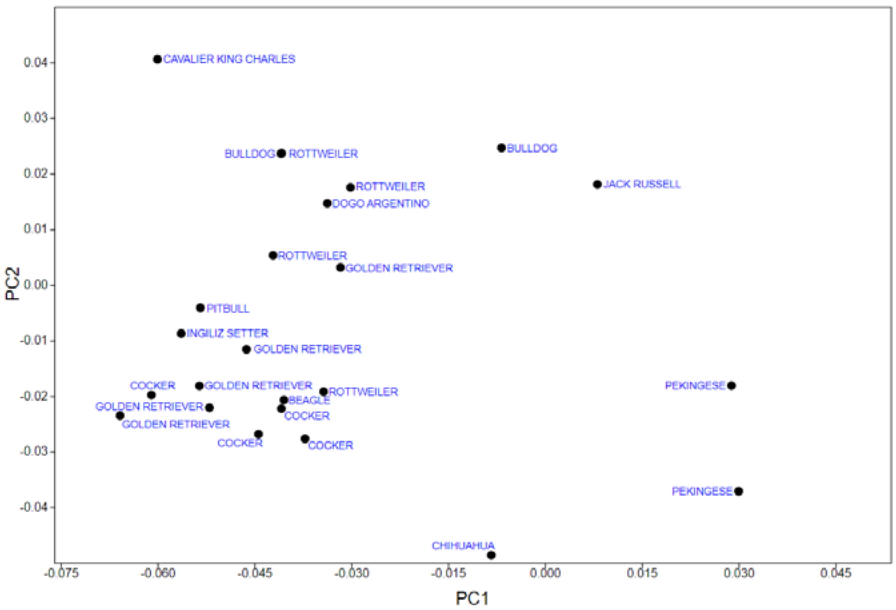

Figure 2 Principal component analysis scatter plot comparing scapula morphology

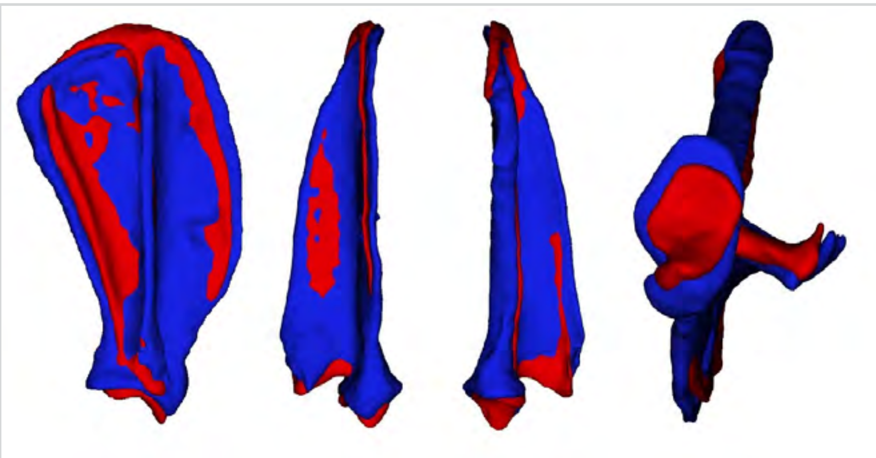

Figure 3 The negative and positive values of PC1 (red is negative limit, blue is positive limit)

in acromion gets more prominent/ more hook-looking as the rate of PC1 decreases. Cavitas glenoidalis in shape shows very distinct difference within maximum and minimum PC1 percentages. Decreased PC1 has smaller and squared formation, while an increase results in more elliptical and bigger cavitas glenoidalis. As seen in cavitas glenoidalis, maximum PC1 mean mesh has bigger, more evident and craniodorsally located tuberculum, but minimum has smaller, more hooked and ventrally located tuberculum.

In the mean mesh models of maximum and minimum percentages of PC2, both models have similar width and length (Figure 4). Margo dorsalis appears more rounded at increase. Angulus cranialis and specially angulus caudalis appear sharper and steeper in decrease of PC2. Acromion part of the maximum PC2 mesh is more elevated than minimum; meanwhile processus hamatus is located ventrally, is longer and more prominent in minimum PC2 mesh.

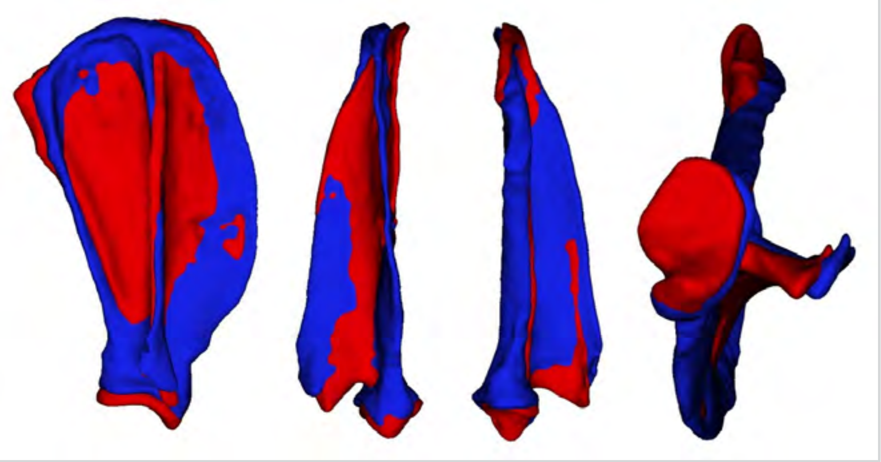

Figure 4 The negative and positive values of PC2 (red is negative limit, blue is positive limit)

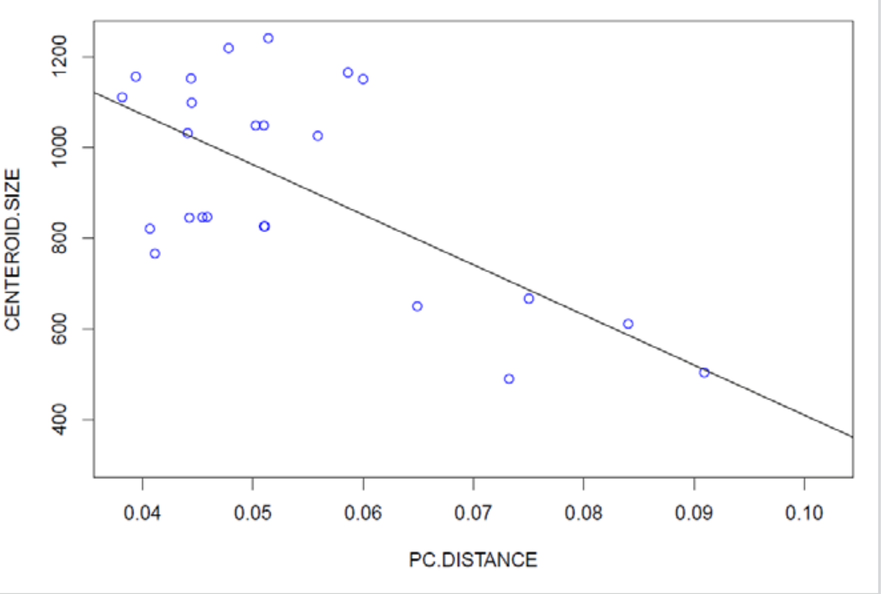

Figure 5 Allometry for scapula

In this chart (Figure 5), we analyzed the correlation of centroid size on procrustes distance, and it resulted in negative correlation. It is seen that specimens with low centroid size have higher procrustes distance scores mean, while most of our group consisting of big breed dogs with higher centroid sizes tend to have lower PC distance. As a result, the effect of size on shape was statistically significant.

Result of negative correlation can be explained with the number of big breed dogs compared to our total number of dogs. It is seen that they create the majority of the specimens. Because average procrustes distance is mostly based on larger dogs with high centroid size scores, small breed dogs such as Pekingese and Chihuahuas that we used in our analysis have high procrustes distance scores.

In DeCa analysis, colorimetric results indicate shape variations (Figure 6). Numbers are shown in milimetric scale between 16.7-1.4 mm. Ventral and dorsal part of the scapulae have the highest rate in shape variation. As seen in the PCA, tuberculum supraglenoidale and cavitas glenoidalis at the ventral section have a variation reaching up to 16.7 mm. Margo dorsalis part of the scapulae has a high rate of variation. As we reach the central part of the scapulae, the variation rate goes down to 1.4 mm between the breeds. Margo cranialis has a higher

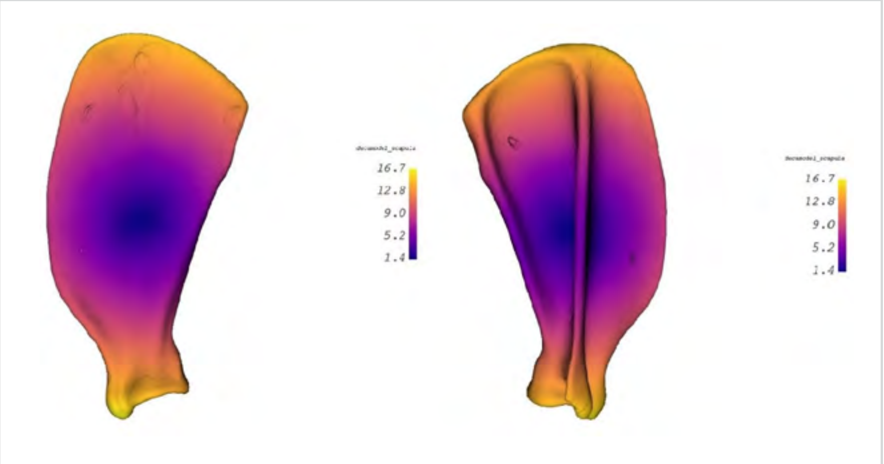

Figure 6 Results of DeCa

variation than margo caudalis, which is painted in deeper purple indicating smaller milimetric change in the shape of the bone.

DISCUSSION AND CONCLUSIONS

Geometric morphometrics is a valuable analytical approach for identifying the differences in animal species and their dimorphic features. This study has highlighted the effectiveness of geometric morphometrics in distinguishing shape variations and allometric differences among various breeds of dogs. In this study conducted on dogs, it was seen that PC1 explained the highest shape variation, explaining 25.4% of the total variation. It was seen that dogs were separated according to their size in PC1 values. While the PC1 value was high in small breed dogs, this value was generally negative in dogs with larger body size. While the scapula was wider in small breed dogs, the scapula in large breeds was thin and long in shape.

In a study conducted on a total of 36 cat scapulae, research focused on the utility of linear measurements taken over the bones for gender determination, and the relationship among the cat weights was observed (Oktay et al., 2023). According to the obtained results, it was observed that linear measurements, when evaluated both in terms of gender and the scapula length and width, showed variations in the calculated area measurements according to weight. In other words, changes in the bone area could be observed along with weight variations. The linear measurement changes in this article can be used to explain gender differences. Additionally, in various studies, it has been observed that these linear measurement methods can vary in explaining gender differences and also different breed types of animals. Therefore, a method other than the 3D landmarking system, as in our study, can define variations related to breed using linear measurements.

Oktay's (2023) study on cats investigated the effect of weight gain on scapula measurements. He said that in this study conducted on cats, the relationship between linear measurement results and weight was statistically significant ($p < 0.05$). In this study conducted on dogs, it was seen that size had an effect on shape. Size affected shape statistically. In these studies conducted on 2 different carnivore species, it was seen that the shape or measurements of the scapula were related to the size of the animal. In future studies, different hypotheses can be developed using different carnivore species, and research can be conducted on this subject.

In recent years, shape analysis studies have attracted attention in the field of veterinary anatomy. One of the most important operations in these analyses is landmark operations. These operations are performed manually through different software. However, with the developing technology in recent years, there are softwares that automatically perform landmark operations (Maga et al., 2017). In this study, landmarks were automatically placed on the scapula. Thanks to computer-aided landmark placement processes, large data was processed in a short time. Additionally, the accuracy of manual marking varies from person to person (Porto et al., 2021). It is thought that automatic landmark operations would have an important place in geometric morphometry.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – CNG, MK, NM; Design – B**Ç,** MTT; Supervision – EO, MK; Resources –CNG, NM, MTT; Materials – MTT; Data Collection and Processing – NM, EO; Interpretation – CNG, NM; Literature Search – MTT, EO; Writing Manuscript – NM, EO, MK; Critical Review – MK.

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TRODIMENZIONALNA MORFOLOŠKA VARIJACIJA I ALOMETRIJSKA ANALIZA SKAPULE KOD PASA

SAŽETAK

Skapula kod pasa je pljosnata kost koja služi za povezivanje torakalnih ekstremiteta sa trupom i klavikulom smještenom iznad ramenog zgloba. U našem istraživanju je korištena kompjuterizirana tomografija kako bi se kreirali modeli skapula 25 pasa. Uključeno je 12 pasmina različite starosti, spola i težine. Na skapule su postavljena 562 semi-orijentira. Primjenom Procrustes analize varijacije oblika, klasificirane su različite pasmine i starosne dobi. Korištenjem ekstenzije programa 3D Slicer Program zvane Denzna korespondentna analiza, bili smo u mogućnosti procijeniti varijacije oblika kolorimetrijskim mjerenjem skapule označene kao srednji uzorak. Na kraju smo koristeći alometrijsku analizu u ovom istraživanju pokušali dokazati da li promjenu veličine kod različitih pasmina prati i promjena oblika. U istraživanju provedenom na psima je vidljivo da je PC1 objasnio najveću varijaciju oblika i dao objašnjenje za 25.4% ukupnih varijacija. Vidljivo je da su psi kategorizirani prema veličinama u PC1 vrijednostima.

Dok je PC1 vrijednost bila visoka kod malih pasmina, kod velikih pasmina je generalno bila negativna. Dok je kod malih pasmina skapula bila šira, kod velikih pasmina je bila tanka i izdužena. Učinak veličine na oblik je bio statistički signifikantan. Geometrijska morfometrija predstavlja dragocjen analitički pristup za identifikaciju razlika kod životinjskih vrsta i njihovih dimorfnih karakteristika. Ovo istraživanje je naglasilo učinkovitost geometrijske morfometrije u razlikovanju varijacije oblika i alometrijskih analiza među različitim pasminama i starosnim skupinama pasa.

Ključne riječi: Analiza oblika, karnivor, veterinarska anatomija