

## SHAPE ANALYSIS OF THE OLECRANON IN COWS, SHEEP AND HORSES

Nicoleta Manuta<sup>1</sup>, Ozan Gündemir<sup>2</sup>, Sokol Duro<sup>3\*</sup>

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### Abstract

With the geometric morphometrics method, bones and structures can be examined in terms of shape. In this study, the olecranon of sheep, cattle, and horses was examined by geometric morphometry method, and shape variations between species were revealed. 39 (15 cow, 9 sheep and 15 horse) olecranon was used for this study. Images of olecranon were acquired for geometric morphometrics. Five Landmarks and 37 semilandmarks were used. A total of 39 principal component analyses were obtained as a result of the shape analysis. PC1 is responsible mostly for the shape between species, declared 25.8 % of the total variation. PC2 declared 17.2 % of the total variation and PC3 declared 11.5 % of the total variation. Shape and centroid size were statistically different between groups ( $p < 0.0001$ ). The PC1 value of horses was generally lower than that of cows and sheep. For PC1, sheep had wider shape variation. Cows had the highest PC1 values. The CV1 value was in the lowest cow. The highest CV1 value was in the horses. The CV2 value was higher in sheep compared to other breeds. The olecranon of cows was larger in shape than other species. In horses, the olecranon was narrower. The anconeus process was narrower in shape in horses. Olecranon was longer in shape in sheep than in other species. The difference in shape was statistically significant between all three species for CVA. The biggest difference in shape was between horse and cow ( $p < 0.0001$ ). The highest Mahalanobis distance was between horse and cow (MD: 10.4659).

**Keywords:** Antebrachial bones, geometric morphometrics, veterinary anatomy, ulna.

<sup>1</sup> PhD student, Institute of Graduate Studies, Istanbul University- Cerrahpaşa, Istanbul 34500, Turkey, ORCID: 000-0002-2537-2973, nicoleta.manuta@mail.ru

<sup>2</sup> Associate Professor, Department of Anatomy, Faculty of Veterinary Medicine, Istanbul University- Cerrahpaşa, Istanbul 34500, Turkey, ORCID: 0000-0002-3637-8166, ozan.gundemir@iuc.edu.tr

<sup>3</sup> Associate Professor, Faculty of Veterinary Medicine, Agricultural University of Tirana, Tirana, Albania, ORCID: 0000-0002-6075-7342

\***Correspondence: Sokol Duro**

Faculty of Veterinary Medicine, Agricultural University of Tirana, Tirana, Albania

E-mail address: [durosokol@ubt.edu.al](mailto:durosokol@ubt.edu.al)

## 1. Introduction

Ossa antebrachium comprises of two long bones (the radius and ulna) which run parallel to each other, completely separate in carnivores but partly or completely fused in ruminants and equines respectively (Barone, 1986; König and Liebich 2020). The olecranon is the elongated and most developed part of the ulna, which projects dorsocaudally from the base of its coronoid process (Nickel, et al., 1986; Dursun, 2007). The olecranon tuberosity is the dorsal and thickest part of the olecranon. The olecranon tuberosity has a cranial and caudal protuberance where the triceps brachii muscle inserts (Aiello and Dean et al., 1990; Nickel, et al., 1986). The triceps brachii muscle is the extensor of the elbow joint and one of the largest muscles of the forearm which in ruminants has four heads (Amis et al., 1979; Nickel, et al., 1986). The ventrocranial part of the olecranon tuberosity consists of a prominence called the anconeus processes which arises from a separate ossification center (Dursun, 2007). Many authors have published the results of studies of olecranon bone with differences between many species (Grand et al., 1965; Barone, 1986).

Differences in scaling distinguishing between animals have often been observed (Bauchot et al., 1964). The variation reflects differences in adaptive strategy as well as changes due to functional requirements of size increase (Steudel, 1982). The difference between animals of the olecranon process apparently can be distinguished by the proximal part of it, relative length, and structure, by Dursun, (2017) equines olecranon tuberosity has only one prominence while ruminants have two projections which are projected cranial and caudal.

In recent years has been applied to examine osteological differences between species and sexes (Klingenberg et al., 2013). Therefore, many different alternative methods can be found in literature for using geometric morphometrics to landmarks based on protocols of data collection (Kranioti et al., 2009; Yilmaz and Demircioğlu, 2021). Through morphometric analyzes of carnivores compared to other species, Martin-Serra shows that phylogeny strongly influenced forelimb bone morphology, and biochemical requirements during locomotion correlate with variation in bone stability. In recent years, in traditional morphometric studies information on the distance between two points is provided, and geometric morphometry can detect the shape differences between reference points (Zelditch et al., 2012).

With the method of geometric morphometry, differences in the shape of structures in bones and various tissues and organs can be detected (Gündemir et al., 2022). For example, geometric morphometry was applied to parts of the tortoiseshell, plastron, and carapace, respectively, in order to determine their sex (Duro et al., 2021) or in quail's digits (Demircioğlu et al., 2022). At the same time, it has been shown that geometric analysis applied to the skull of different species was used for sex determination (Gündemir et al., 2020; Szara et al., 2022; Gürbüz et al., 2022; Jashari et al., 2022). Geometric analysis can be applied to two-dimensional images more in determining variations in shape than in sample size.

The olecranon is one of the important anatomical structures of the forelimb that also serves as a determinant landmark in living animals. The number of protrusions on the olecranon tuberosity can be used for species differences. However, morphological studies in different

species of the olecranon are limited. The aim of this study was to perform through geometric morphometric analysis the shape of olecranon tuberosity on cows, sheep, and horses.

## 2. Materials and Methods

### 2.1. Samples

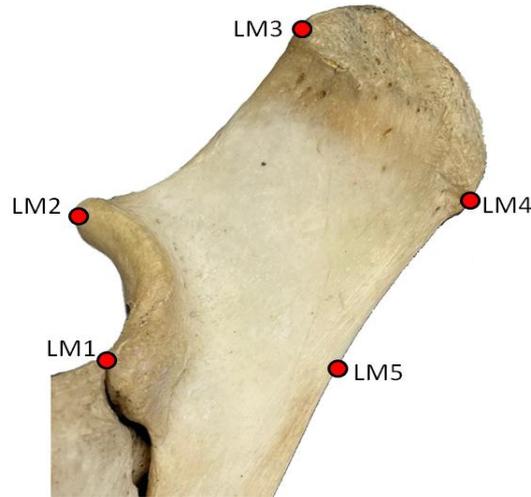
For this study, 39 (15 cow, 9 sheep, and 15 horse) olecranon from the right-side limbs were used. They were specifically pathogen free. Olecranon belonging to individuals over two years of age was used. Archival bones belonging to Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Anatomy were used in the study. No live animals were used in the study. Olecranon photos were taken by the same researcher. The photographs were taken from the medial angle, 20 cm from the same angle.

### 2.2. Modeling and Acquisition of Image

In this study, all sample images were taken from the same angle, same position (medial), and saved to the computer in “png” format. All images were converted to “tps” format (version 1.74) (Rohlf, 2004). Five Landmarks (LM) and 37 semilandmarks were used in this study (Table 1, Figure 1). The TpsDig2 (version 2.32) was used for the insertion of the Landmarks into the image (Rohlf, 2004). Firstly, all Landmarks were allocated on all images, and after 37 semilandmarks were added.

**Table 1.** List of landmarks and semilandmarks applied to olecranon in medial view

Landmarks	LM1	Medial coronoid processes
	LM2	Anconeus processes
	LM3	The cranial end of the olecranon tuberosity
	LM4	The caudal end of the olecranon tuberosity
	LM5	Caudal parallel point of coronoid processes
Semilandmarks	LM1 - LM2	Trochlear incisure
	LM2 - LM3	Cranial border of olecranon
	LM3 – LM4	Dorsal border of olecranon
	LM4 – LM5	Caudal border of olecranon



**Figure 1.** Landmarks applied to olecranon in medial view

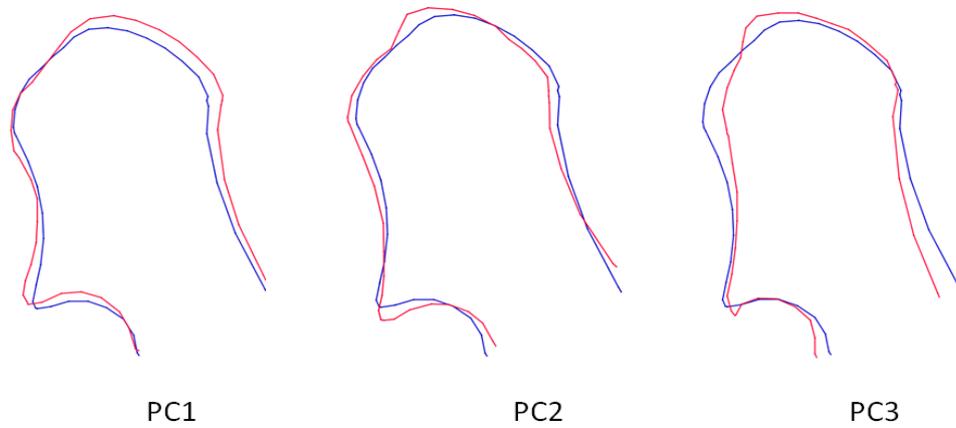
### 2.3. Geometric Morphometrics

For geometric morphometric analysis was used MorphoJ v1.06d software program (Klingenberg et al., 2011). Grouping operations were performed on the olecranon between species, then the differences in the shape and size of the olecranon between ruminants, and equines were examined. Geometric morphometrics analysis morphological features by collecting a series of coordinate data. Landmarks and semilandmarks, which are anatomically specific loci (Bookstein et al.,1991) were collected by using the TPS series program (TPSutil and TPSdig) of software (Rohlf, 2015). The tps file with the landmarks was added to the Morphoj program. The Procrustes fit was applied first, then Generalized Procrustes Analysis and the Principal Component Analysis (PCA) were performed for all samples. A PCA is an ordination method that is used for reducing the dimensionality of the data. The shape and size of these species were compared by using Procrustes ANOVA. Canonical analysis of variables (CVA) was used to reveal the olecranon variation between cows, sheep, and horses (Zelditch et al., 2012).

### 3. Results and Discussion

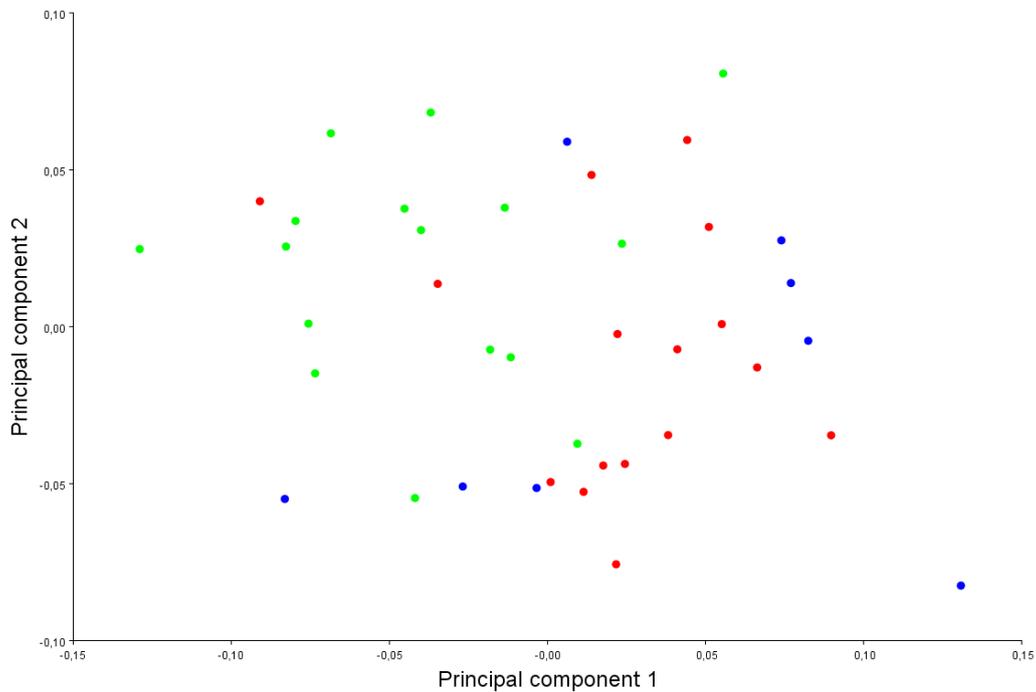
A total of 33 PCs were obtained to reveal the morphological differentiation among cows, sheep, and horse olecranon process. PC1, which is responsible mostly for the shape between species, declared 25.8 % of the total variation. PC2 declared 17.2 % of the total variation, and PC3 declared 11.5 % of the total variation. The scatter plot of PC1, PC2, and PC3 of the olecranon process is given in Figure 2. Along PC1, traits that accounted for variation were: an increase of the olecranon tuberosity and a width increase of the trochlear incisure. For PC2, traits accounted for variation were descending from the coronoid process to the anconeus process, a shortening of the region of the olecranon tuberosity. Along PC3, traits accounted for variation were similar to PC1, increasing maximum concavity point of the trochlear incisure, wide coronoid process, and increase the dorsal margin of the olecranon tuberosity. Cows and

sheep had higher PC1 values compared to horses. The increase of value in PC1 is correlated with wider olecranon tuberosity and the existence of two prominences of it.



**Figure 2.** Scatter plot of PC1 25.8 %, PC2 17.2 %, and PC3 11.5 % of the variation olecranon process in lateral view for interspecies. The wire-frame warp plots of changes in the olecranon, as mapped by 5 landmarks and 37 semi landmarks. Red lines describe the initial shape and blue lines describe the shape changes in the direction of PC1, PC2, and PC3 in their limits.

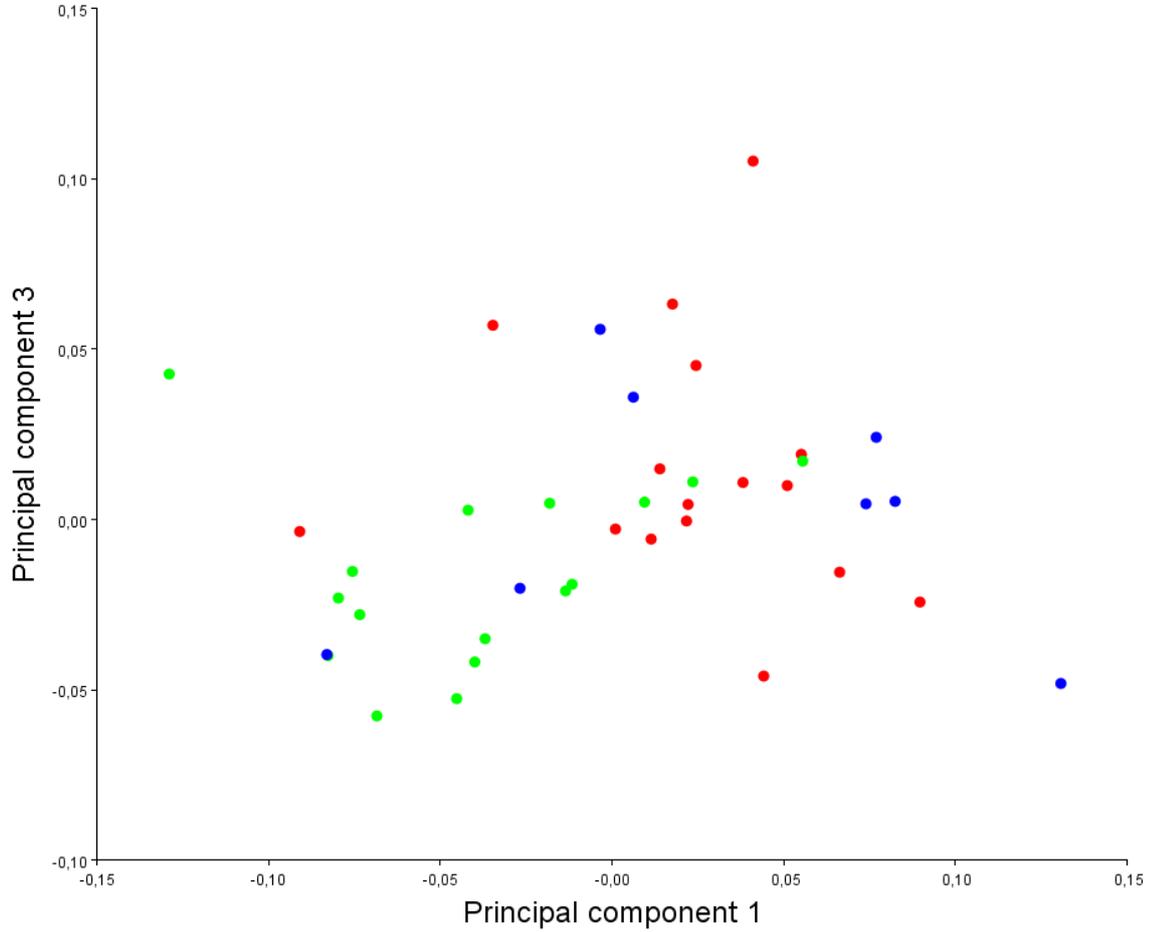
Shape variations for all samples according to PC1 and PC2 results are shown in Figure 3. The PC1 value of horses was generally lower than that of cows and sheep. For PC1, sheep had wider shape variation. Cows had the highest PC1 values. For PC2, interspecies shape variation was high for horses, cows, and sheep.



**Figure 3.** Scatterplot of the principal component analysis of cow (*Bos taurus*), sheep (*Ovis aries*), and horse (*Equus caballus*) based on the weight matrix of residuals form multivariate

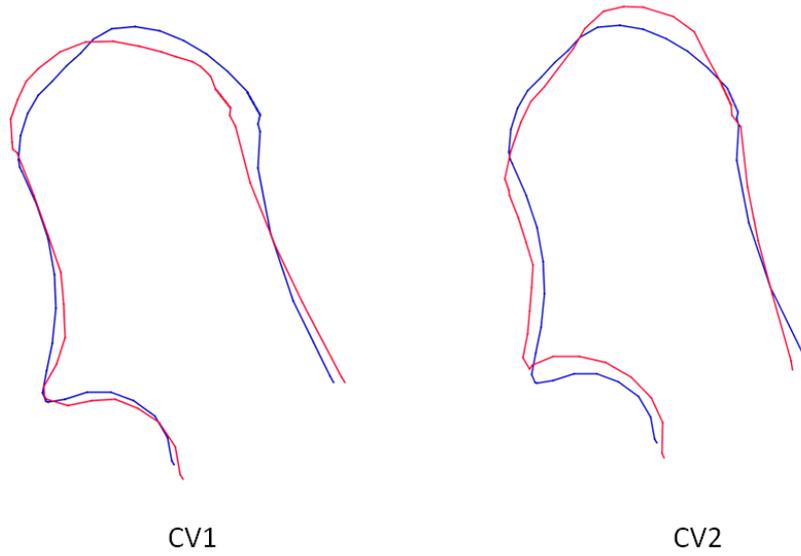
regression with the corresponding shape of olecranon of every axis. PC1: 25.8 % of the variance and PC2: 17.2 % of the variance. Red: cow, Green: horse, Blue: sheep.

Shape variations for PC1 and PC3 are shown in Figure 4. The variation in shape between species was high for PC3.



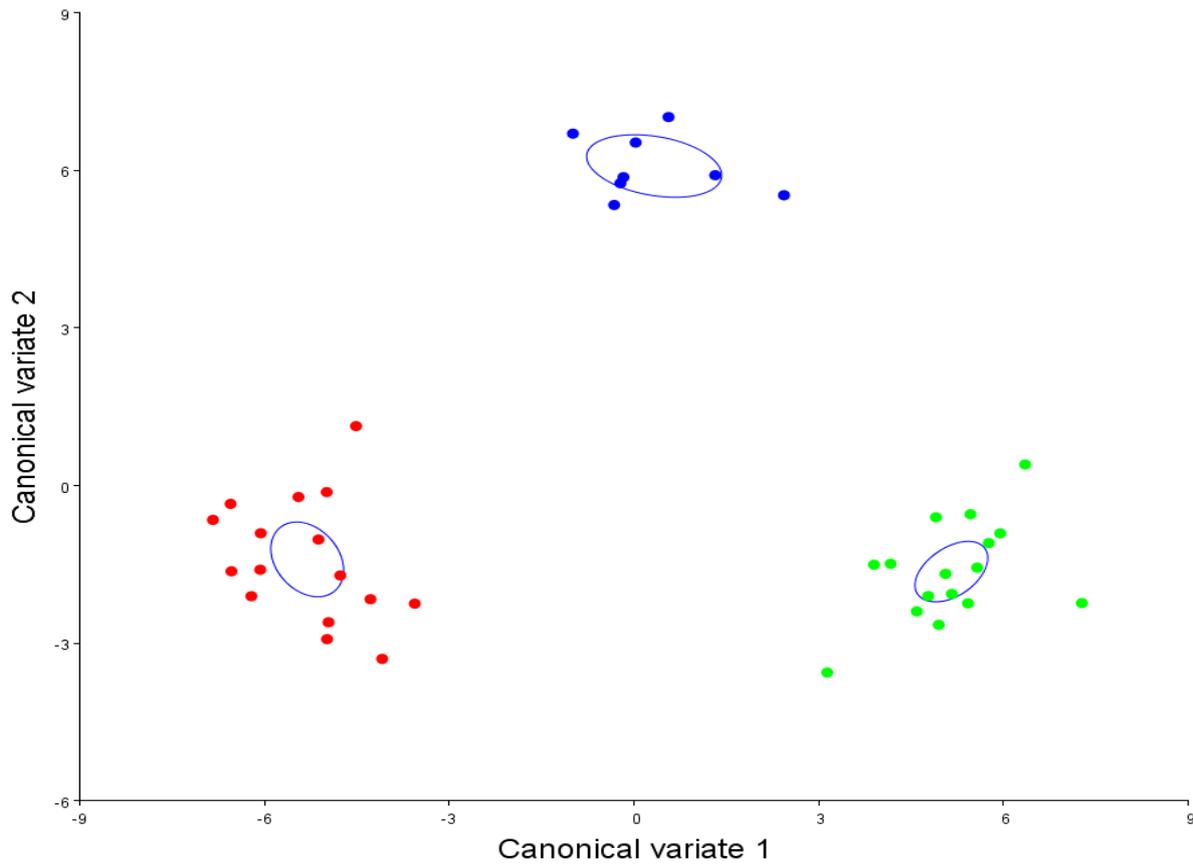
**Figure 4.** Scatter plot of PC1 (25.8 %) and PC3 (11.5 %) of quails for later view. Red: cow, Green: horse, Blue: sheep.

A high CV1 value represented narrower olecranon tuberosity. At high CV1, the anconeus process was narrower and the cranial end of the olecranon tuberosity was higher in shape. Also, in high CV2, the most important shape difference was in the caudal part of the olecranon tuberosity. Increased CV2 represented higher olecranon tuberosity. Also, at high CV2, the initial portion of the olecranon was narrower in shape (LM1-LM5).



**Figure 5.** Scatterplot of canonical variate analysis for olecranon (10000 permutation rounds). Red lines describe the initial shape and blue lines describe the shape changes in the direction of CV1 and CV2 in their limits.

Species distribution for CV1 and CV2 are shown in Figure 6. The CV1 value in cows was at the lowest. The highest CV1 value was in horses. The CV2 value was the same in horses and cows. However, the CV2 value was higher in sheep compared to other species. According to these values, the olecranon of cows was larger in shape than other species. In horses, the olecranon was narrower. The anconeus process in horses was narrower in shape. Olecranon was longer in shape in sheep than in other species.



**Figure 6.** Canonical variate analysis. Red: cows, Green: horses, Blue: sheep.

ANOVA results are shown in Table 2. The difference between species in terms of both centroid size and shape was statistically significant ( $p < 0.0001$ ).

**Table 2.** Procrustes ANOVA results for species with a difference between size (95.76) and shape (6.87).

Individuals		F	p-Value
Species	Centroid Size	95,76	<.0001
	Shape	6,87	<.0001

The Mahalanobis and Procrustes distance between species are given in Table 3. Also, p values from permutation tests (10000 permutation rounds) for the Mahalanobis distance and Procrustes distance among species are also given in Table 3. The distribution of PD-P between species was statistically significant. In canonical variate results, it was seen that the shape of the olecranon between samples differed in the medial view. The difference in shape was statistically significant between all three species. The biggest difference in shape was between horses and cows ( $p < 0.0001$ ). The highest Mahalanobis distance was between horses and cows (MD: 10.4659).

**Table 3.** Mahalanobis distance and Procrustes distances value and p-values.

	Cow				Horse			
	MD	MD-P	PD	PD-P	MD	MD-P	PD	PD-P
Horse	10,4659	<0.0001	0,1074	<0.0001				
Sheep	9,3682	<0.0001	0,0873	0.0001	9,1001	<0.0001	0,1055	0,0002

**MD:** Mahalanobis distance among the group. **MD-P:** p-values from permutation tests (10000 permutation rounds) for Mahalanobis distances among the group, **PD:** Procrustes distance among the group, **PD-P:** p-values from permutation tests (10000 permutation rounds) for Procrustes distance among the group.

The olecranon, part of the thoracic girdle bone, shows shape differences between cows, sheep, and equine. The large ruminants same to sheep, have a wider olecranon process with two prominences, described as well in various anatomy books (Getty et al., 1975; Barone, 1986; König and Liebich, 2020; Nickel et al., 1986). In this study, shape differences between olecranon species were also revealed. It has been shown that olecranon can be a discriminator between species in shape. The olecranon of cows was larger in shape than other species and maybe this is related to the insertions of four heads of the triceps brachii muscle (Barone, 1986; König and Liebich, 2020; Nickel et al., 1986). In horses, the olecranon and anconeus process was narrower in shape. Olecranon was longer in shape in sheep than in other species. The difference in shape was statistically significant between all three species for CVA.

Geometric morphometrics compared with linear-based analysis in terms, has the advantage of visualizing shape changes. Moreover, GM can be used to describe developmental pathways and symmetric patterns. Also, GM methods are able to observantly quantify the shape and size of bone rather than simply measurements of length and width, as linear methods do (Lemic et al., 2014). Another advantage of geometric morphometrics has the ability of easy use, low cost, and quick method. All these advantages make it attractive for sharing between researchers in the research centers. In this study, it can be recommended that shape is more allocated than size for distinguishing morphological similarities between species so, differentiation is more informative for specimens. Geometric morphometrics in this study was found to be purposeful in distinguishing between species of the olecranon of the ulna bone.

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