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GEOMETRIC MORPHOMETRIC INVESTIGATION OF INCUS IN HORSE (EQUUS FERUS CABALLUS) AND DONKEY (EQUUS ASINUS)

İftar GÜRBÜZ[1]*, Yasin DEMİRASLAN[1]

[1]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Anatomy, 15100, Burdur, Türkiye

Abstract: In this study, it was aimed to determine the shape of the incus in horse and donkey by geometric morphometric method and to evaluate the shape differences between horse's and donkey's incus. The left incus bone of 5 adult horses and 5 donkeys were used in the study. Incus were photographed at same lateral direction. Thirteen homologous landmarks were marked from the photographs using TpsUtil (Version 1.79) and TpsDig2 (Version 2.31) software. As a result of the study, the first principal component explained 38,642% of the total shape variation. In the PC1 plot, samples were clearly clustered by group. Acording to canonical varians analysis, in the wire frame warp graphic, the corpus incudis edges (right, left, and bottom) were flatter in donkeys. Angle at the LM13 level was more pronounced on the crus breve. The apex of the crus longum (Landmark 4, 5, and 6) was wider in donkey. In the study, the morphological features of horse's and donkey's incus were determined by geometric morphometric method. This study is important in that it is the first geometric morphometric study on the incus that is one of the ossicula auditus in horse and donkey. We think that the study will contribute to the anatomy of the ossicula auditus in the equide family.

Keywords: Donkey, Geometric morphometry, Horse, Incus

***Corresponding author:** Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Anatomy, 15100, Burdur, Türkiye
E mail: iftargurbuz@mehmetakif.edu.tr (İ. GÜRBÜZ)
İftar GÜRBÜZ https://orcid.org/0000-0001-9460-0645
Yasin DEMİRASLAN https://orcid.org/0000-0003-3612-6142

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1. Introduction

The ossicula auditus are located in the pars petrosa of the os temporale, dorsal to the cavum tympani. These are located between the membrana tympanica and the fenestra vestibuli as the malleus, incus, and stapes, respectively (Pazvant and Gündemir, 2021). There is also os lenticulare between the incus and stapes in young animals. This ossicle fuses with the incus in later ages to form the processus (proc.) lenticulare. The ossicula auditus magnify the vibrations from the eardrum by 20 times and transmit them to the inner ear and cause the fluctuation in the endolymph (König and Liebich 2007). At the same time, the ossicula auditus can also reduce sound pressure by separating each other through certain muscles (musculus tensor tympani and musculus stapedius) (Reece, 2012).

In the studies carried out to date, there is information about the anatomy (Özgüden, 1962; Hebel and Stromberg, 1986; Masuda et al., 1986; Huang et al, 1996; Kristensen et al., 1996; Botti et al., 2006; Solntseva, 2013) and morphometry (Kürtül et al., 2003; Mohammadpour, 2011; Demiraslan et al., 2015; Gürbüz et al., 2016; Gürbüz et al., 2019; Dalga and Aslan, 2019; Gürbüz et al., 2020) of the ossicula auditus in different animal species However, no study was found in which the shape of the ossicula auditus was determined by the geometric morphometric method. For this reason, in this study, it was aimed to reveal the morphological anatomical values of the horse's and donkey's incus belonging to the Equidae family and to evaluate the shape differences between the horse's and donkey's incus.

2. Materials and Methods

2.1. Samples

In the study, the incus that one of the left ossicula auditus of 5 adult horses and 5 donkeys, were used.

2.2. Imaging and Digitization

Incus were photographed laterally with a stereo microscope (Leica S6D) focusing on the median line. The distance between the lens and the material was determined as 10 cm. The photos were saved on the computer with the Jpg extension. From the photographs, 13 homologous landmarks were marked using TpsUtil (Version 1.79) (Rohlf, 2019) and TpsDig2 (Version 2.31) (Rohlf, 2018) software (Figure 1 and 2). Thus, the x and y Cartesian coordinates of homologous anatomical points representing the general shape of the incus from the lateral direction were determined. Before statistical analysis, confirmation test was performed for landmarks in TpsSmall (Version 1.34) (Rohlf, 2017) program. In TPS small analysis, slope and corelation values of landmarks were found as 0.998850 and 1.000000, respectively.

These values show that the landmarks are placed correctly.

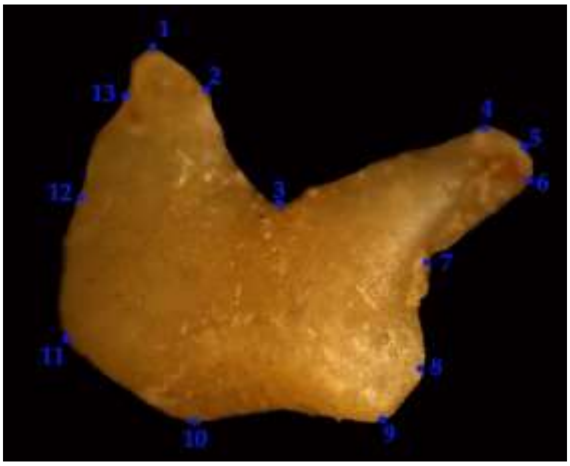

Figure 1. The Landmarks on Horse's incus.

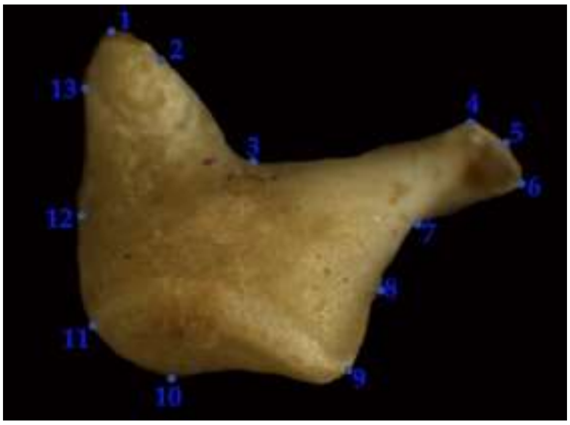

Figure 2. The landmarks on Donkey's incus.

For Figure 1 and 2; 1. The highest point of the crus breve, 2. The rightmost peak of the crus breve, 3. The angle of the crus breve with the crus longum, 4. The leftmost peak of the crus longum, 5. The midline of the crus longum of the peak point, 6. The righmost peak of the crus longum, 7. The angle of crus longum and corpus incudis, 8. The most protruding point of corpus incudis on the right edge, 9. Right corner point of corpus incudis, 10. The most protruding point of corpus incudis on ventral edge, 11. Left corner point of corpus incudis, 12. The most protruding point of corpus incudis on the left edge, 13. The rightmost peak of the crus breve.

2.3. Statistical Analysis

The differences in size, position and orientation of Incus' lateral photographs were superimposed by General Procrustes Analysis (superimposition) (Slice, 2007). PAST (Version 4.02) (Hammer et al., 2001) program was used for this analysis. With the same program, principal components analysis was performed on the new coordinates obtained as a result of the Procrustes analysis, and the components between the groups were calculated. In addition, 2-t test was applied to compare the landmark coordinate values (procrustes) according to the groups. The degree of closeness (Classical cluster) of individuals was analyzed in the PAST (version 4.02) program. Using the MorphoJ (Klingenberg, 2011) program, at which landmarks the shape differences were concentrated (PCA) and grouping characteristics (Canonical variance analysis-CVA) were analyzed.

3. Results

The results of principal component analysis performed with the landmark coordinates are shown in Table 1. Accordingly, the first principal component (PC.1) explained 38.642% of the total shape difference, and the first four principal components (PC1+PC2+PC3+PC4) explained 85.903%. Evident breakpoint among principal components was observed between PC1 and PC2. The distribution of samples according to PC1 was shown in the graph in Figure 3. Accordingly, the samples were clearly clustered according to the groups. It was observed that the donkey samples were collected on the right of the y axis, and 4 of the horse samples were collected on the left of the y axis.

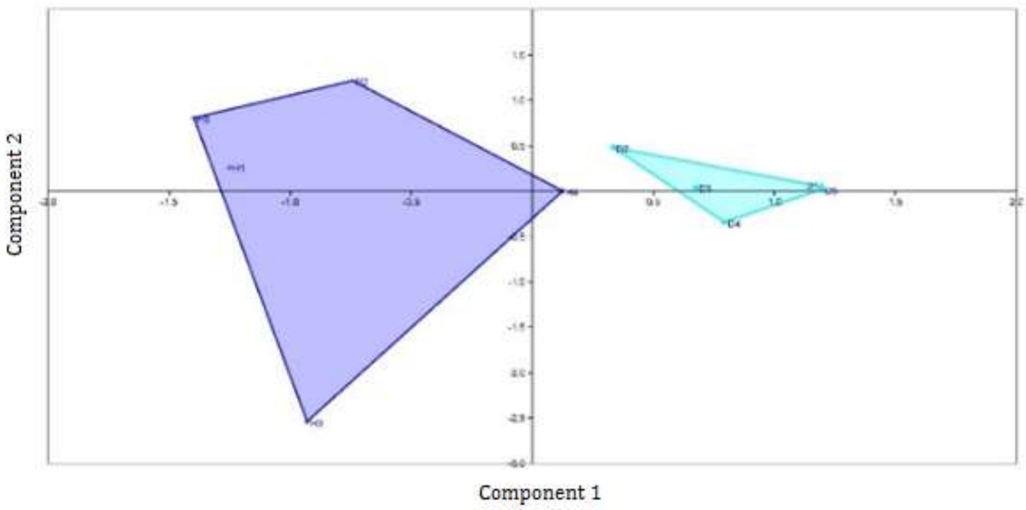

Figure 3. Distribution of samples on the graph over the first principal component (PC1), Light blue: Donkey's incus (D), Blue: Horse's incus (H).

In the study, the graph obtained as a result of the test performed to determine the proximity of the samples is given in Figure 4. Accordingly, the samples were largely grouped according to the race factor.

Table 1. Results of the principal component analysis, PC: principal component

PC	Eigenvalue	% variance
1	0.00407434	38.642
2	0.00244738	23.211
3	0.00151831	14.4
4	0.00101748	9.65
5	0.000636602	6.0377
6	0.000360864	3.4225
7	0.000296456	2.8116
8	0.00015774	1.496
9	3.46808E-05	0.32892

The graphs showing the shape differences at which landmarks (LM) according to PC1 were shown in Figure 5. Accordingly, shape differences for PC1 became evident in the landmarks except for LM1, LM2 and LM9.

Canonical variance analysis defined the between-group difference within a canonical variable (CV1). Shape variations with respect to CV1 were similar to anatomical points according to PC1. Mahalanobis and Procrustes distances values were determined as 3.2349 and 0.1098 (p: 0.0052), respectively. Shape differences and frequencies according to groups in incus' wire-frame warp graph were shown in Figure 6 and Figure 7. Accordingly, it was observed that the frequencies were homogeneously distributed between the groups.

According to canonical variance analysis, in the wire frame warp graphic, corpus incudis edges (right, left and bottom) were flatter in donkeys. Angle at the LM13 level was more pronounced on the crus breve. The apex of the crus longum (Landmark 4, 5, and 6) was wider in donkey.

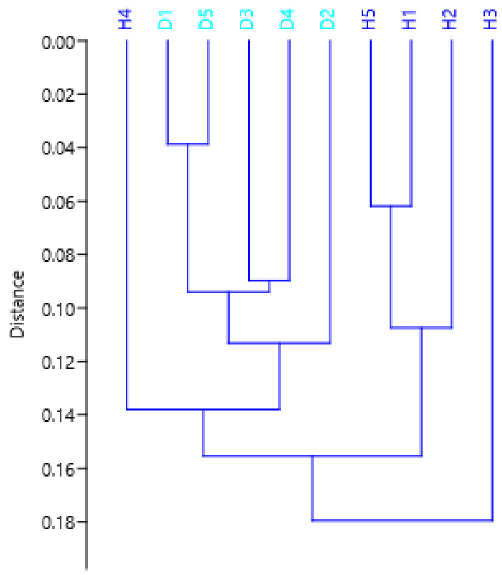

Figure 4. Graph of hierarchical proximity of individuals. Light blue: Donkey's incus (D), Blue: Horse's incus (H).

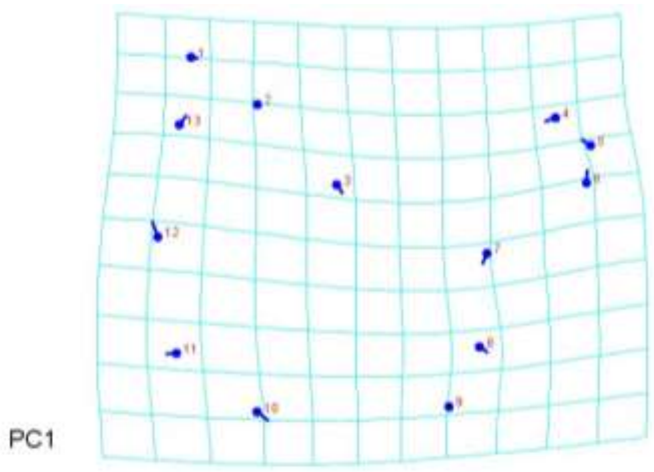

Figure 5. Landmark representation of shape differences of incus between donkey and horse for the first principal component (PC1). (Set scale factor: 0.05).

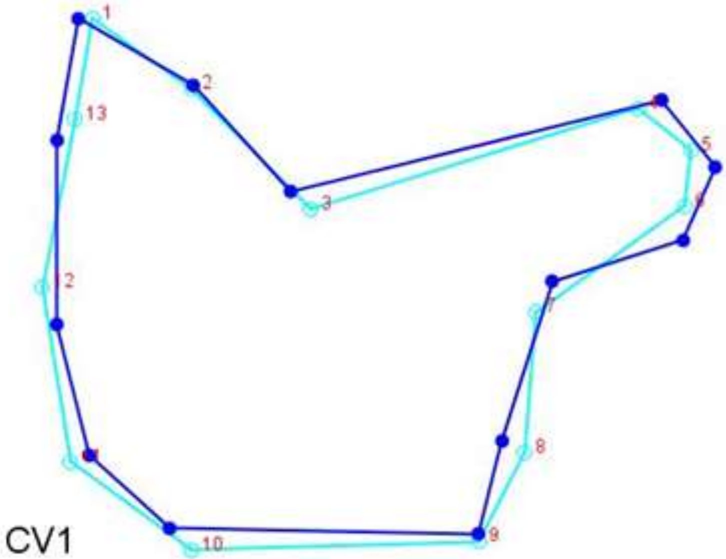

Figure 6. Canonical variance analaysis. Wire-frame warp plot (Set scale factor: 3.0). Dark blue: donkey, Light blue: Horse.

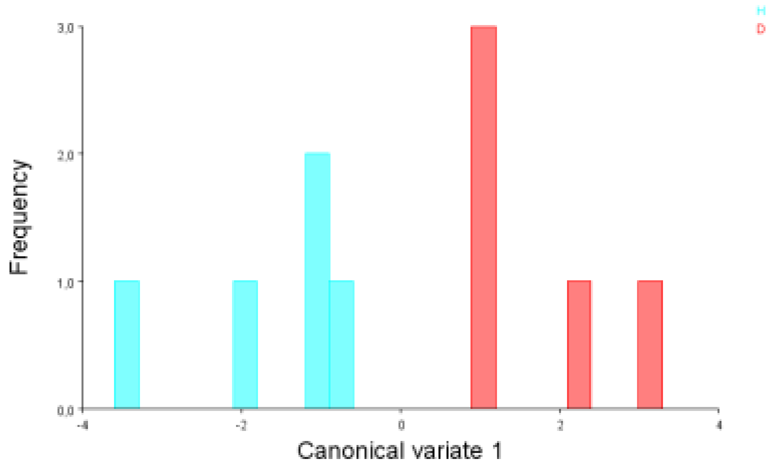

Figure 7. Grouping by Canonical Analysis of Variance. Light blue: Horse, Red: Donkey.

4. Discussion

The most important factor in the difference in bone shapes is genetic structure (Seeman, 2003). Anatomically, the bones of different members of a family subgroup are similar. However, the bone shape and size are different. Therefore, many studies have been carried out on the geometric morphometry of various bones (Gündemir et al., 2020; Gürbüz et al., 2020; Demircioğlu et al., 2021; Duro et al., 2021; Gündemir et al., 2021; Demiraslan et al., 2022; Gürbüz et al., 2022; Szara et al., 2022). In this study, it was aimed to reveal the anatomical features and differences of the incus between the donkey and horse belonging to the same family subgroup. In the study, the left incus of horse and donkey was examined by geometric morphometric method. The study contains restrictions in terms of the number of materials. However, it was determined that the incus shape of the horse and donkey were different from each other with the geometric morphometric method.

The incus hangs medial to the malleus and lateral to the stapes and connects these ossicles with synovial joints. Anatomically, the horse's and donkey's incus have a large body called the corpus incudis and two projections, the crus longum and the crus breve, which are separated from the body. The crus longum has a projection called the proccessus lenticulare, which articulates with the stapes (Demiraslan et al., 2015; Gürbüz et al., 2016). Incus length on the left side is 2.53 mm, corpus incudis width is 1.25 mm in donkeys (Demiraslan et al., 2015), and these lengths are 3.92 mm and 3.68 mm in horses (Gürbüz et al., 2016), respectively. According to the results of the previously reported morphometric study (Demiraslan et al., 2015; Gürbüz et al., 2016), the horse's incus was larger than the donkey's incus, that is, their sizes differ from each other. In the study, although the incus of the horse and donkey were similar anatomically, it was determined that there were some differences in shape by detailed geometric morphometric analysis.

Accordingly, the corpus incudis margins (right, left, and bottom) were flatter in donkeys. Angle at the LM13 level on the crus breve was more pronounced in horse. The apex of the crus longum (Landmark levels 4, 5 and 6) was wider in donkeys.

5. Conclusion

As a result, the shape differences of horse's and donkey's incus were determined by geometric morphometric method. Accordingly, in the PC1 graph, the principal component analysis, the samples were significantly clustered according to the race factor. The points of shape differences were determined by canonical variance analysis. It is important in that it is the first geometric morphometric study performed on the incus that is one of the ossicula auditus in horse and donkey. We think that this study will contribute to the morphology of the ossicula auditus.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	İ.G.	Y.D.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there is no live animal research. In this research, no ethical statement was needed since the experimental materials were obtained from previously dead animals (Animal experiment ethics committee regulation on working procedures and principles; Will data belonging to any living thing be used in the research? - No; Ethics committee permeation is not required).

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