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Geometric morphometric investigation of ossicula auditus in two breed sheep

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Abstract

The ossicular chain consists of three joined ossicles: the first and largest bone is the auditory bone, the malleus, consisting of a stalk, head, and neck (collum mallei) in direct contact with the tympanic membrane; the second bone, the incus, is divided into a body, long crus, lenticular extension (processus lenticulare), and short crus. Geometric morphometry is used in a number of fields (e.g. paleontology, anthropology, biology, zooarchaeology, medicine, engineering, etc.) where the measurement of morphological variation is important. The aim of this study was to reveal the differences and similarities in shape by geometric morphometric analysis of the ossicula auditus of akkaraman and morkaraman sheep. For this purpose, ossicula of male and female Akkaraman and Morkaraman breeds were removed, imaged under steromicroscope and geometric morphometric analyses were performed. In the study, 16, 12 and 8 principal components were calculated for sheep malleus, incus and stapes. The separation of individuals according to the breed factor was more pronounced than the sex factor. Accordingly, shape differences became evident in the medial surface of the corpus mallei and the processus muscularis in the malleus, in the peripheral corners in the incus, and in the caput and crus rostrale in the stapes. In terms of breed, shape variation between groups was only partially seen in LM9 in malleus, LM6 and 7 in incus. As a result of the discriminant function analysis, the weakest and strongest grouping was seen in the stapes in terms of gender factor.

Introduction

The ossicles of the ear mediate the transmission of vibrations from the tympanic cavity to the inner ear (Miller 1964; Kurtul et al. 2003). The ossicular chain consists of three joined ossicles: the first and largest bone is the auditory bone, the malleus, consisting of a stalk (manubrium mallei), head (caput mallei), and neck (collum mallei) in direct contact with the tympanic membrane; the second bone, the incus, is divided into a body (corpus incudis), long crus (crus longum), lenticular extension (processus lenticulare), and short crus (crus breve). The third and smallest auditory bone, the stapes, consists of the head (caput stapedis), rostral and caudal crus (crus rostrale, crus caudale), and the base (basis stapedis), directly connected to the vestibular fenestra (Demiraslan et al. 2015; Gürbüz et al. 2015; Martonos et al 2021). In young animals, there is an os lenticulare between the incus and stapes. This ossicle forms the processus (proc.) lenticulare later in life. The ossicula auditus transmits vibrations from the ear bones to the inner ear, magnifying them 20 times and causing fluctuations in the endolymph (Konig and Liebich 2007).

In general, morphometrics seeks to evaluate shape variation and how it varies with other variables (Bookstein 1991). The approach described as traditional morphometrics is typically based on univariate and multivariate analysis of variables such as distance measures, angles and ratios (Rohlf and Marcus 1993). Although effective in many aspects and remaining prevalent across various domains, these methods are insufficient for fully delineating the complete morphology of an organism or structure (Klingenberg 2008; Weber and Bookstein 2011). The absence of geometric preservation of the object or region under examination resulted in the subsequent emergence of novel morphometric techniques known as "geometric morphometrics," which seek to maintain the geometric shape of samples for analysis (Bookstein et al. 1991; Polly 2008; Klingenberg 2015).

Geometric morphometry is used in a number of fields (e.g. paleontology, anthropology, biology, zooarchaeology, medicine, engineering, etc.) where the measurement of morphological variation is important. These studies have been used in different research areas, such as assessing variation within taxa (Cardini and O'Higgins 2004; Baab 2011), investigating the relationship between shape and phylogeny (Lockwood et al. 2004), and identifying ancestral morphotypes (Wiley et al. 2005).

Ruminates are one of the most diverse classes of mammals, with more than 200 species adapted to a wide range of environmental conditions. Their social behavior ranges from solitary to herding. This diversity has made them the species of choice for the study of bone structure (Nowak 1999; Costeur et al. 2017; Demiraslan et al. 2024). Geometric

morphometry is preferred for both intraspecific and interspecific variation studies (Fruciano 2016; Tamagnini et al. 2017; Gürbüz et al. 2020; Demircioğlu et al. 2021). The aim of this study was to reveal the differences and similarities in shape by geometric morphometric analysis of the ossicula auditus of akkaraman and morkaraman sheep, which are two closely related breeds living in the same region.

Materials and Methods

Samples

In this study, auditory ossicles of Akkaraman and Morkaraman sheep breeds were used to investigate whether breed and gender factors have an effect on shape. The numerical information (n: number of specimens) about the specimens collected for this study is given in the Table 1. The samples obtained from both breeds were numerically similar in terms of gender.

Table 1
Number of materials by race and gender

	Breed		**Gender**	
	Akkaraman	**Morkaraman**	**Male**	**Female**
Malleus	20	18	19	19
Incus	21	23	23	21
Stapes	19	21	20	20

Table 1. Number of materials by breed and sex

Imaging and Digitization

The right ossicula auditus were dissected from the skull and photographed at the same magnification using a stereomicroscope. The obtained photographs were saved to the computer with Jpg extension. A “tps.” file was created from the photographs using TpsUtil (Version 1.79) (Rohlf 2019) and 10 homologous landmarks from malleus, 8 from incus and 6 from stapes were marked using TpsDig2 (Version 2.64) (Rohlf 2018). Descriptions of these landmarks are given in the Table 2. With the landmark marking, the x and y Cartesian coordinates of the points representing the general shape of the material were determined in the analytical plane. Before statistical analysis, a verification test was performed for the landmarks in TpsSmall (Version 1.34) (Rohlf 2017). The results of this test revealed the accuracy of the marked landmarks.

Table 2
Landmark descriptions of the ossicula auditus

LM number	Malleus	Incus	Stapes
1	The tip of Manubrium mallei	Leftmost summit of the crus longum	Highest point of connection of the crus caudale with the caput stapedis
2	Distal segment of the processus lateralis	Rightmost summit of the crus longum	The highest point of connection of the caput stapedis and crus rostrale
3	Proximal segment of the processus lateralis	Angle of the crus breve with the crus longum	Connection point of the crus rostrale and basis stapedis
4	Lateral beginning of the collum mallei	The highest point of the crus breve	Connection point of the crus caudale and basis stapedis
5	Lateral beginning of the caput mallei	Right corner point of the corpus incudis	Uppermost point of the foramen intercrurale
6	Lateral segment of th caput mallei	Midpoint of the incudomalleolaris joint	The lowest point of the foramen intercrurale
7	Madial segment of th caput mallei	Left corner point of the corpus incudis	
8	Processus anterior	The beginning of the crus longum	
9	Processus muscularis		
10	Medial beginning of the manubrium mallei		
LM: Landmark			

Table 2: Landmark descriptions of the ossicula auditus

Statistical analysis

General Procrustes Analysis (superimposition) was performed on all bones separately since there were differences in size, position and orientation in the photographs (Slice 2007). Principal component analysis was performed on the new coordinates obtained from Procrustes analysis. In addition, areas where shape differences were concentrated, allometric effect and grouping characteristics (Discriminant Function Analysis-DFA) were determined. MorphoJ (Klingenberg, 2011) was used for all these analyses.

Results

As a result of the regression analysis performed to determine whether size (centroid size) has an effect on shape (PCs), it was determined that 3.6401% ($p = 0.1970$), 4.6035% ($p = 0.0443$) and 5.5559% ($p = 0.0515$) of the shape could be predicted in terms of size in malleus, incus and stapes, respectively (95% confidence interval). To eliminate the statistically significant allometric effect in the incus, the residuals were subtracted from the procrustes coordinates and the analyses continued.

In the study, 16, 12 and 8 principal components were calculated for sheep malleus, incus and stapes, respectively. The results of this analysis are shown in the Table 3. For malleus, the first principal component explained 35.353% of the total shape variation and the first three principal components (PC1 + PC2+PC3) explained 63.858%. For Incus, the first principal component explained 27.276% of the total shape variation and the first three principal components (PC1 +

PC2+PC3) explained 59.479%. In the stapes, the first principal component explained 35.864% of the total shape variation and the first three principal components (PC1 + PC2+PC3) explained 74.706%. The distribution of samples according to PC1 and PC2 is shown in Figures. 1 and 2. The separation of individuals according to the breed factor was more pronounced than the sex factor. Therefore, the contribution of the breed factor to the variation in the shape of the ossicula auditus in sheep was greater than that of sex.

Table 3
Results of the principal component analysis, PC: principal component

PC	Malleus		Incus		Stapes	
	Eigenvalue	% Variance	Eigenvalue	% Variance	Eigenvalue	% Variance
1	0,001683	35,353	0,002297	27,276	0,004639	35,864
2	0,000760	15,963	0,001530	18,174	0,003329	25,737
3	0,000597	12,542	0,001181	14,029	0,001695	13,105
4	0,000333	7	0,000880	10,446	0,001377	10,642
5	0,000308	6,463	0,000746	8,864	0,000782	6,044
6	0,000219	4,59	0,000439	5,210	0,000475	3,669
7	0,000181	3,807	0,000398	4,722	0,000382	2,955
8	0,000152	3,189	0,000304	3,613	0,000257	1,985
9	0,000134	2,820	0,000270	3,210		
10	0,000122	2,557	0,000171	2,033		
11	7,21E-05	1,515	0,000125	1,485		
12	6,18E-05	1,299	7,91E-05	0,939		
13	4,65E-05	0,977				
14	4,15E-05	0,872				
15	2,53E-05	0,531				
16	2,48E-05	0,521				

Table 3: Results of the principal component analysis, PC: principal component

Figure 1: Graphical distribution of samples according to PC1 in terms of race factor, a. Malleus, b. Incus, c. Stapes, Red: Akkaraman, Blue: Morkaraman

Figure 2: Graphical distribution of samples according to PC1 in terms of gender factor, a. Malleus, b. Incus, c. Stapes, Red: Female, Blue: Male

The graphs showing the landmarks at which the shape differences were concentrated according to PC1 are shown in Fig. 3. Accordingly, shape differences became evident in the medial surface of the corpus mallei and the processus muscularis in the malleus, in the peripheral corners in the incus, and in the caput and crus rostrale in the stapes.

Figure 3: Wireframe graphical representation of shape differences with respect to PC1 and PC2. Blue represents the average shape with respect to the principal component.

As a result of the discriminant function analysis, the data according to race and gender and cross-validation grouping characteristics are presented in the Table 4. According to these results, the weakest and strongest grouping was seen in the stapes in terms of gender factor.

Table 4
Discriminant function analysis results by breed and gender

	Breed				Gender			
	Mahalanobis	**Procrustes**	**p-value**	**Correct Grouping (%)**	**Mahalanobis**	**Procrustes**	**p-value**	**Correct Grouping (%)**
Malleus	2,2270	0,04360684	0,1231	47,37	2,2220	0,02324383	0,1238	60,52
Incus	1,3426	0,03170360	0,3155	52,27	1,3057	0,03444697	0,3582	52,27
Stapes	0,7483	0,03741243	0,7944	50	0,7105	0,04051386	0,8360	40

Table 4: Discriminant function analysis results by breed and gender

The shape variations of the bones according to the groups in the wire-frame warp graph are shown in Figs. 4 and 5. In terms of breed, shape variation between groups was only partially seen in LM9 in malleus, LM6 and 7 in incus. In terms of gender, a significant shape variation was observed in LM1 and 2 in the stapes compared to the others. Accordingly, the stapes was more conical in sheep than in rams.

Figure 4: Wire-frame warp graph of ossicula auditus shape in terms of race factor according to discriminant function analysis. Red: Akkaraman, Blue: Morkaraman

Figure 5: Wire-frame warp graph of ossicula auditus shape in terms of sex factor according to discriminant function analysis. Red: Female, Blue: Male

Discussion

The most important factor in differences in bone shape is genetic structure (Seeman 2003). It has been reported that the morphological characteristics of auditory structures are closely related to different environmental and behavioral patterns (Huang et al. 2000; Mason 2016). Furthermore, Rosowki (1992), suggested that hearing ability can be predicted from anatomical structures. The bones of intraspecific, interspecific and family subgroup members have similar structures. However, there are many studies investigating anatomical differences in bone shape and size between members of a family subgroup, within or between species of different species of the same breed (Gürbüz et al. 2020; Demircioğlu et al. 2021; Duro et al. 2021; Gürbüz and Demiraslan 2023; Hadžiomerović et al. 2023; Demiraslan et al. 2024). The aim of this study was to determine the anatomical features, similarities and differences of ossicula auditus in Akkaraman and Morkaraman sheep breeds by geometric morphometric method. It was determined that the shape of the ossicula auditus differed from each other in some anatomical formations according to the breed and gender factors.

As in most mammals (Gürbüz et al. 2016; Gürbüz et al. 2019; Hadžiomerović et al. 2023; Arpaçay et al. 2024), the ossicula auditus ossicles of Akkaraman and Morkaraman sheep consist of the malleus, incus and stapes. The general shape of the malleus is in agreement with ruminants (Botti et al. 2006; Dalga 2019; Martonos et al. 2021; Arpaçay et al. 2024). Stoessel et al. (2016b) reported that all the taxa they examined in their study had significant shape variation in the shape of the malleus, especially on the articular face. In the present study, it was observed that the shape variation in terms of race was at the level of proc. muscularis.

The geometric shape of the second bone, the incus, was found to have a large body and two projections separated from the body in accordance with the literature (Demiraslan et al. 2015; Mason 2016; Dalga 2019; Martonos et al. 2021; Gürbüz and Demiraslan 2023; Arpaçay et al. 2024) Similar dimensions of the two incus limbs have been reported in goats and buffaloes (Martonos et al. 2021; Nourinezhad et al. 2021). In this study, the geometric shape shows similar findings in the sheep breeds examined. In a previously presented study, it was reported that although horse and donkey incus are anatomically similar, they are geometrically morphometrically different (Gürbüz and Demiraslan 2023). In the present study, geometric shape variation was determined on the incudomalleolar articular surface and the left corner of the corpus incudis in the sheep breeds examined.

It is stated that the stapes consists of a head, two crura and the base of the stapes and is triangular in many species (Gürbüz et al. 2019; Martonos et al. 2019; Stoessel et al. 2016a). In addition, its distinctive rectangular shape has been recorded in ruminants (Botti et al. 2006). In this study, it was found that the stapes was more ovoid in sheep than in rams. Stoessel et al. (2016b) reported statistically significant differences in the shape of the stapes between most of the species they examined. In the present study, shape differences were observed in caput stapedis and crus rostrale. As re Geometric morphometric methods have been used in various species in recent years to contribute to the taxonomic classification of animals and to determine sexual dimorphism (Gündemir et al. 2020; Demircioğlu et al. 2021; Szara et al. 2022; Demiraslan et al. 2024; Karaavci et al. 2024). In this study, it was determined that the effect of the breed factor on the shape of the ossicula auditus in the sheep breeds examined in this study was higher than the gender. For this reason, it was revealed that the classification of the ossicula auditus of closely related species can be made with the geometric analysis method. In addition, since there is a certain ratio between sheep and human inner ear sizes, sheep are used as models in human medicine in areas such as hearing aids, cochlear implantation, stapedotomy (Gocer et al. 2007; Cordero et al. 2011; Mantokoudis et al. 2016; Péus et al. 2020). Lavinsky et al. (1997) explained that the middle ear of sheep is anatomically and histologically remarkably similar to that of humans, hence how to use sheep in experimental procedures. Anatomical similarities between human and sheep breeds have also been presented by different researchers and it has been stated that it would be useful to use for education and applications (Seibel et al. 2006; Arpaçay et al. 2024;). We believe that the results obtained in this study will be a reference for studies on animal models and will contribute to the literature in terms of geometric morphometry.

Declarations

Authors' contributions

FAK and YD participated in the design of this study, and FAK and İD performed dissection of the study materials. BK and AK performed the geometric morphometric analyses. FAK and AK completed the writing of the article with the contribution of other authors. All authors read and approved the final manuscript.

Data Availability Statement

The data that support the findings of this study are available from thecorresponding author upon reasonable request

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical approval

The materials used in our study were collected as waste material from the local slaughterhouse in QQQ province and according to the Regulation on the Working Procedures and Principles of Animal Experimentation Ethics Committees

published in the Official Gazette published on February 15, 'Processing with dead animals or tissues, slaughterhouse materials, waste fetuses are not subject to the permission of HADYEK (Animal Experiments Local Ethics Committee).

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Figures

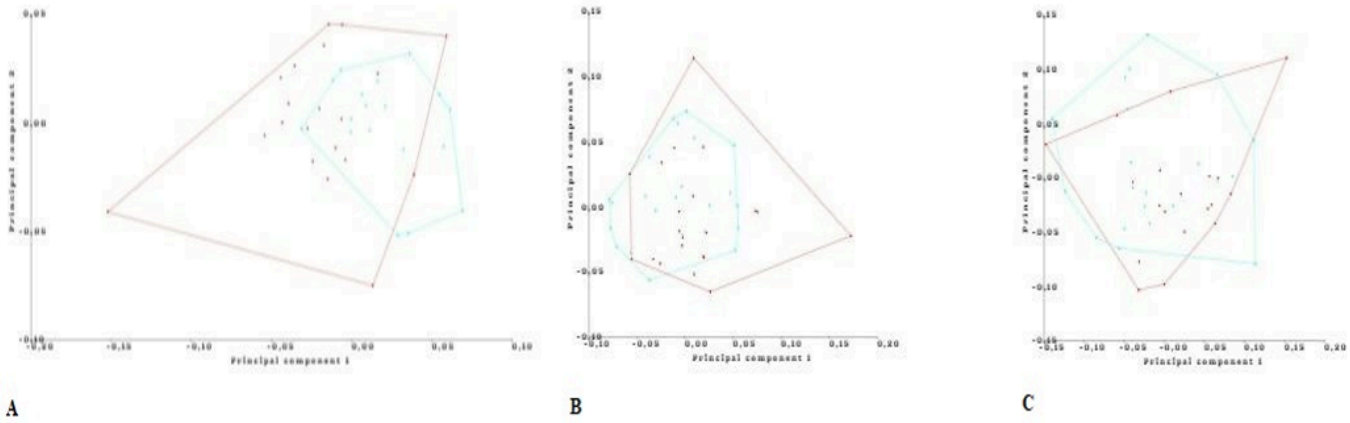


Figure 1

Graphical distribution of samples according to PC1 in terms of race factor, a. Malleus, b. Incus, c. Stapes, Red: Akkaraman, Blue: Morkaraman

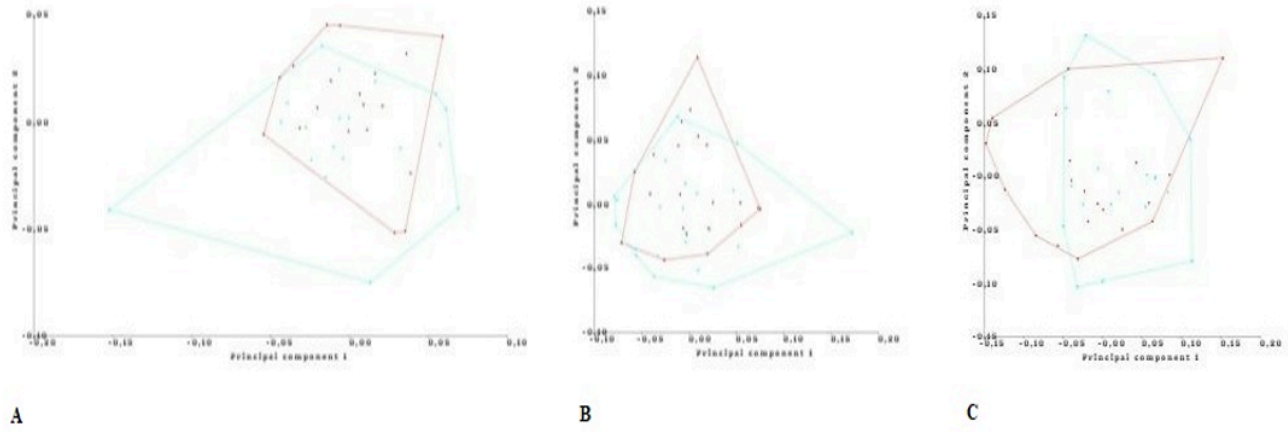


Figure 2

Graphical distribution of samples according to PC1 in terms of gender factor, a. Malleus, b. Incus, c. Stapes, Red: Female, Blue: Male

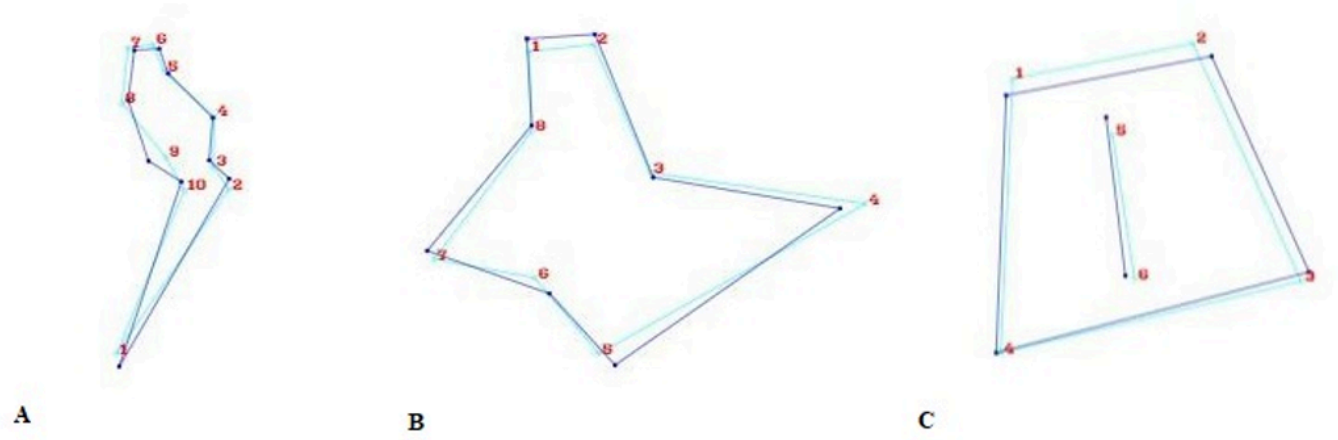


Figure 3

Wireframe graphical representation of shape differences with respect to PC1 and PC2. Blue represents the average shape with respect to the principal component.

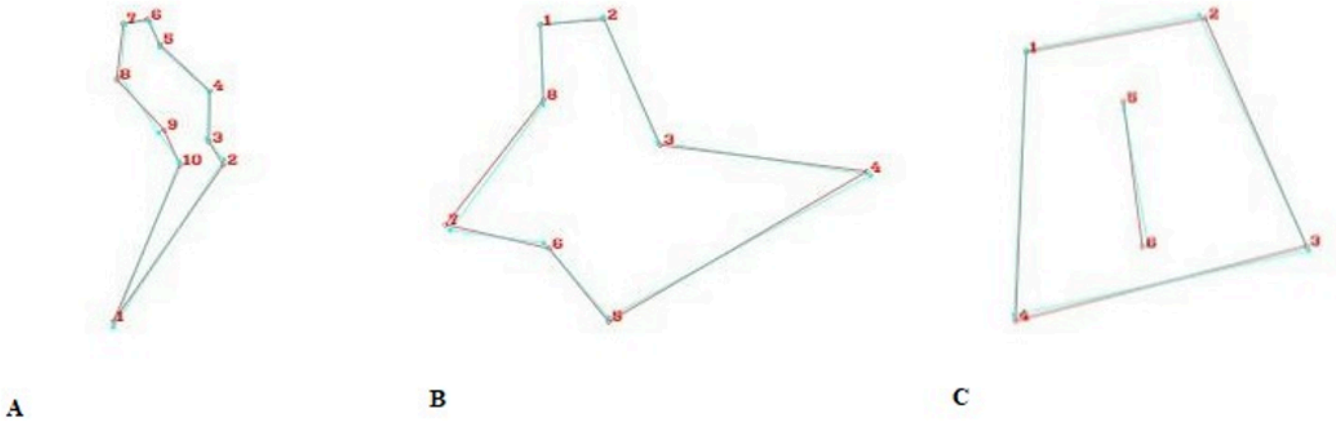


Figure 4

Wire-frame warp graph of ossicula auditus shape in terms of race factor according to discriminant function analysis. Red: Akkaraman, Blue: Morkaraman

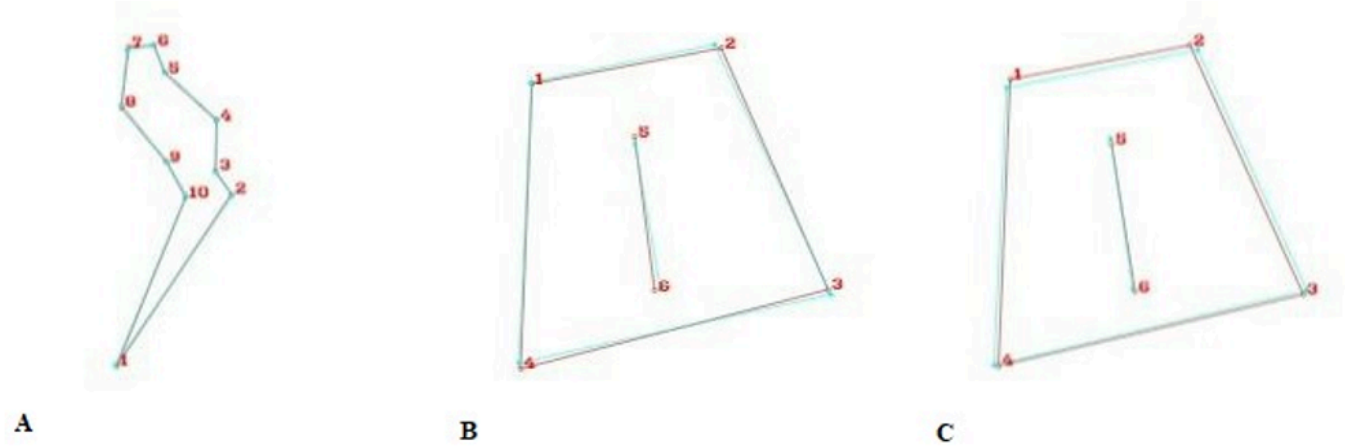


Figure 5

Wire-frame warp graph ossicula auditus bone shape in terms of sex factor according to discriminant function analysis. Red: Female, Blue: Male

Supplementary Files

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