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Elliptic Fourier analysis on the tympanic bullae in three *Meriones* species (Rodentia, Mammalia): its application in biosystematics

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Abstract. The size and shape of the auditory bullae are investigated across three species of *Meriones,* in order to determine the biosystematic value of bulla characteristics in species recognition. This study is based on outline (geometric morphometric) with elliptical Fourier and eigenshape analysis of tympanic bullae in: (a) the suprameatal triangle, (b) the mastoid, (c) the auditory meatus, and (d) the ventral view of bullae. The results show that characters of the auditory meatus separate these three species from each other. The shape of different parts of the bullae within each species is also shown to vary such as in two populations of *M. persicus*. The shape of organs is important in the interaction between the organism and its environment. Tympanic bullae variation is similar in the Geno population of *M. persicus* (Geno is located in south of Iran), and in *M. libycus* which lives in similar climates. The results of this analysis of tympanic bullae differ from the characters described by CORBET (1978).

Key words: auditory bullae, outline, eigenshape, elliptical Fourier analysis, *Meriones,* Rodentia.

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I. INTRODUCTION

The genus *Meriones* ILLIGER, 1811 (subfamily Gerbillinae, Muridae, Rodentia), has a broad distribution in the desert and semi-desert areas of North Africa, Middle East, and Asia Minor (MUSSER & CARLETON 2005; CHEVRET & DOBIGNY 2005). The species *M. persicus* (BLANFORD, 1875)*, M. libycus* LICHTENSTEIN, 1823 and *M. crassus* SUNDEVALL, 1842 are found in semi-arid rocky areas, warm low lands and hot deserts respectively (SHENBROT *et al*. 2002; YIGIT & COLAK 1999).

The anatomy, physiology, function, and evolution of specialized hearing organs in Gerbillinae was studied by LAY (1972). Rodent classifications based on auditory structure characteristics have been achieved for the Gliridae, Echimydae and Geomyidae families based on the tympanic bulla along with other characteristics (WILKINS et al. 1999; GARDNER & LOUISE 1984). The tympanic bulla is composed of 2 parts, the tympanic and the mastoid, which are subdivided according to the genera and species. Three primary mastoid air chambers occur in the Gerbillinae: anterior, posterior superior; and posterior inferior (LAY 1972).

Taxonomic differentiation is commonly associated with morphological divergence, but patterns of interspecific variation are less well investigated. Paleontologists and biologists frequently encounter situations in which a homologous region can be identified in different specimens but homologous points cannot be specified. In these situations an outline method is recommended (ROHLF 1990). Therefore, in the case of the auditory bulla of the *Meriones*, where homologous points cannot be identified, an outline method was used. We studied the differences in the shape of the tympanic bullae of three species (*M. persicus*, *M. libycus*, *M. crassus*) in four parts (the suprameatal triangle, the mastoid, the auditory meatus and the ventral view of bullae). Shape variability was analyzed using the elliptical Fourier approximation of contours method (ROBERTS et al. 1983). Fourier decomposition allows for a detailed analysis of complex structures as a whole, and has been used for a wide variety of organisms ranging from invertebrates to parts of vertebrate anatomy (MONTI et al. 2001).

II. MATERIALS AND METHODS

Sampling

Tympanic bullae from 3 species of *Meriones* (*M. persicus*, *M. crassus* and *M. libycus*) were studied in four parts: the suprameatal triangle, the mastoid, the meatus and the ventral part (Figure 1).

Forty-one specimens were used in this research and are stored in the Rodentology Research Department, Ferdowsi University, Mashhad, Iran. Some specimens were damaged in one of these four parts so that in some analyses less than 41 skulls were used (Table I). Samples of *M. libycus* and *M. crassus* come from the desert environment of the Khorasan Province. Fewer *M. crassus* specimens were available due to the climatic conditions of the hot desert and low population densities. *M. persicus* samples were divided into two groups: the Tehran samples were collected from an area south of Tehran at 51° 25’ E and 35° 29’ N associated with temperate conditions; and the Geno samples were collected from Geno, 20 kilometers north of Bandar Abas (in the center of the Hormozgan province) at 56° 7’ E and 27° 6’ N. Geno has a semi desert environment and its lowest annual temperatures never reach below zero degrees.

Table I

Number of specimens studied in each species for every anatomical part

Part	*M. persicus*	*M. crassus*	*M. libycus*
Ventral	18	4	14
Meatus	11	3	18
Mastoid	14	5	16
Triangle	17	4	18

Digitalization

Digital images were prepared using a Cannon Powershot A70 camera with a magnification of 1 and a quality of 3.2 million pixels. The images were edited with the Photoshop software. The magnetic lasso tool in Photoshop was used to select the areas of the triangle, the mastoid, the auditory meatus and the ventral part of the bullae and then each area was edited with the brush tool (Figure 1). The x and y coordinates of each outline were calculated with TPSdig 1.37 (ROHLF 2001) software. The starting point of the outlines were defined as follows:

1 – The suprameatal triangle from the closest part towards the rostral side of the skull with 200 points per outline.

2 – The auditory meatus from the meeting point of the zygomatic arch and the bullae with 300 points per outline.

3 – The mastoid from the meeting point of the infrasuprameatal apophysis with 400 points per outline.

4 – The ventral view of the inferior edge of the meatus with 400 points per outline (Figure 1).

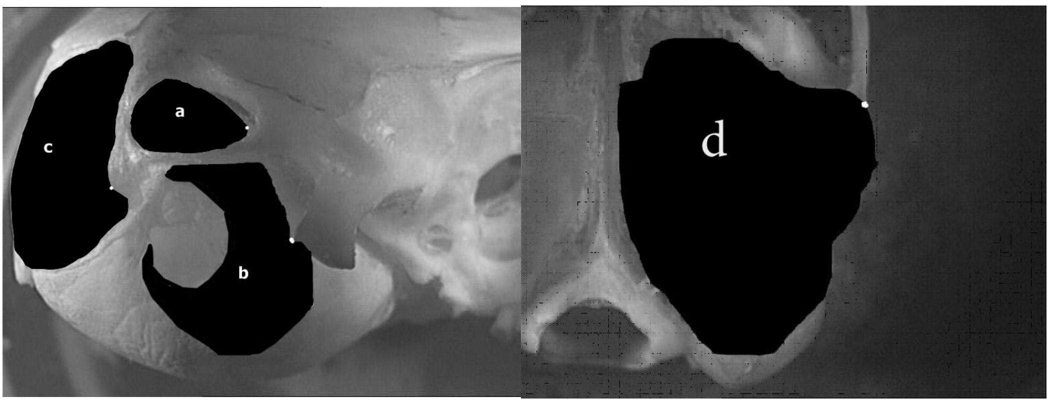


Fig. 1. Different parts of auditory bullae. a – suprameatal triangle; b – auditory meatus; c – mastoid; d – ventral view of bullae with the white point demonstrating the starting point of the outline calculation.

Eigenshape analysis

This method was described in 1983 by LOHMMAN. Eigenanalysis is a type of PCA which is used for outline data. In this case it was difficult to fit the curve to the data. The function is constructed as a linear function of the observed data across one or more specimens (RENAUD & MICHAUX 2003). This analysis is based on the co-variance matrix of the non-normalized turning angle increments around the outlines. Eigenanalysis was used for the description of shape variations in the meatus and suprameatal triangle areas.

Elliptical Fourier analysis

This method was described in 1982 by KUHL and GIARDINA and applied to our research. The method consists of decomposing a curve into a sum of harmonically related ellipses. This method is based on the separate Fourier decompositions of the incremental changes of the x and y coordinates as a function of the cumulative length along the outline (KUHL & GIARDINA 1982). Any harmonic corresponds to four coefficients: A_n and B_n for x, and C_n and D_n for y, defining on ellipse in the x-y plane. The coefficients of the first harmonic, describing the best- fitting ellipse of any outline, are used to standardize the size and orientation of the object. These coefficients therefore correspond to residuals after standardization, and should not be included in following statistical analysis (CRAMPTON 1995).

In this research the first 20 harmonics were used to describe shape variations in bulla parts. Coefficients were used for discriminate analysis and T^2 Hotelling in order to show group separation. For Eigenshape analysis PAST 1.67 (HAMMER et al. 2007) software, achieving Fourier coefficients EFAW (ROHLF & FERSON 1992) software and for discriminate analysis and T^2 Hotelling SPSS software was used.

Table II

Eigenvalues of components in eigenshape analysis in the meatus and mastoid parts. Significance of these values is determined with Jolliffe cut-off value. Values that are less than the Jolliffe cut-off are not meaningful. All values in Table 2 are meaningful ($p<0.05$). Standardized point numbers were shown and the number of each part that it was calculated from. Variance % was shown as the proportion of each component in total variance

A: meatus part

	Value	Variance %
Eigenval 1	1.05046	6.89277
Eigenval 2	0.97708	6.4112
Eigenval 3	0.93957	6.1651
Eigenval 4	0.82973	5.4444
Jolliffe cut-off	0.035799	
Standardized point number	300	

B: suprameatal triangle part

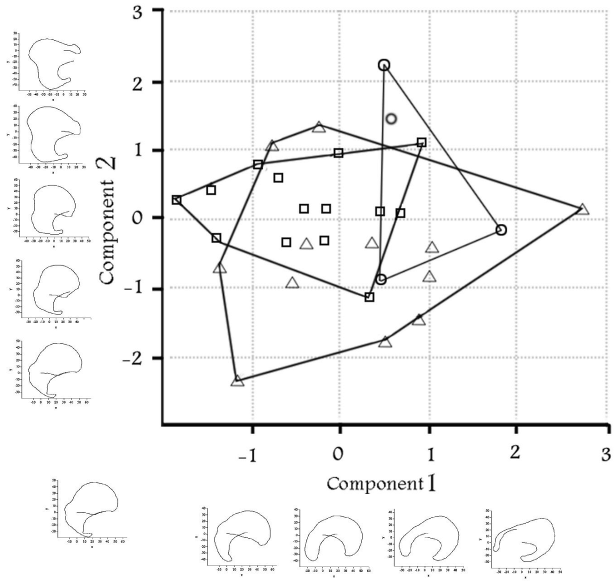
	Value	Variance %
Eigenval 1	1.26323	7.5139
Eigenval 2	1.1654	6.9321
Eigenval 3	1.0537	6.2674
Eigenval 4	0.91119	5.4199
Jolliffe cut-off	0.059436	
Standardized point number	200	

III. RESULTS

Eigenanalysis

In this section 4 diagrams were achieved. Figure 2 demonstrates shape changes in the meatus section. For this analysis 40 eigenvalues were calculated but only 4 were demonstrated as they had the largest proportion of the total variance. The significance of each value is determined by the Jolliffe cut-off value. It was found that if the Eingenvalue of each component is less than the Jolliffe cut-off value then the component would be meaningless. In the meatus part the first 4 eigenvalues

A



B

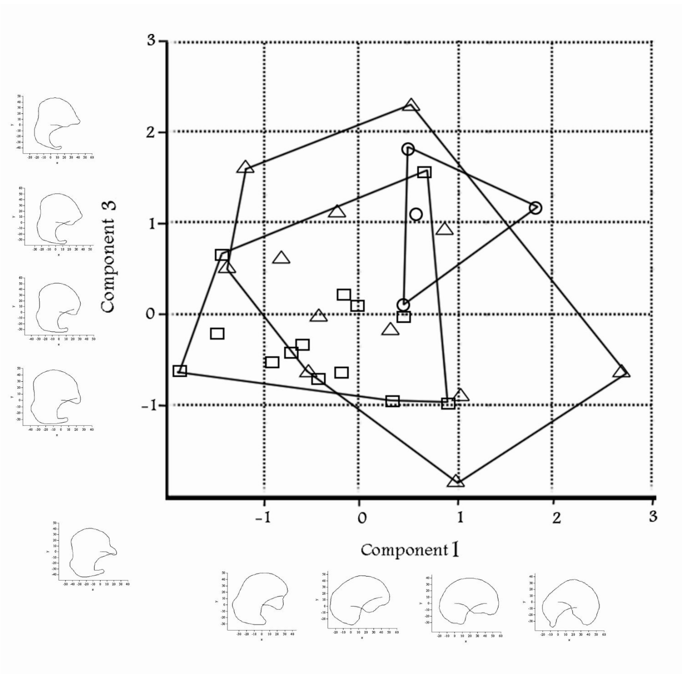


Fig. 2. Diagram based on eigenanalysis in the meatus part. A – Diagram based on Components 1 and 2 (Component 1 indicates meatus hypertrophization. In this component ventral hypertrophization decreased in the second half of the axis, however the dorsal hypertrophization indicates little increase and Component 2 demonstrates an increase of the tendency in the ventral part of meatus towards the zygomatic arch); B – Diagram based on Components 1 and 3 (Component 1 is similarly defined to part a, and Component 3 indicates the degrees of hypertrophy in the dorsal part of the meatus). In this Figure the triangle represents *M. persicus*, circle represents *M. crassus* and square represents *M. libycus*.

had a maximum proportion and were significant (Table IIA). Figure 2A demonstrated shape changes based on components 1 and 2 that had maximum eigenvalues. Component 1 indicated meatus hypertrophization. In this component the ventral hypertrophization decreased in the second half of the axis. However, the dorsal hypertrophization indicated little increase. The greatest degree of symmetrical hypertrophy (in the ventral and dorsal sections) of meatus was seen in *M. crassus* and there is also a significant hypertrophy in the *M. libycus* meatus which, in comparison with *M. crassus,* had a lesser degree of hypertrophy. Component 2 demonstrated an increase of the tendency in the degree of hypertrophy in the ventral part of the meatus towards the zygomatic arch. In this diagram the maximum value on Component 2 belongs to the *M. crassus* group and generally this group is situated at the top of the diagram. After this group, the *M. libycus* group shows the greatest value of this component. Also Component 3 indicated in Figure 2A shows a decrease in the dorsal part hypertrophy of the meatus therefore in this axis generally the *M. crassus* group is located at the top of diagram.

In the suprameatal triangle part, the first 4 eigenvalues had the highest eigenvalues and were significant (Table II). The total variance was divided between 40 eigenvalues and therefore each eigenvalue had a low value. Figure 3 demonstrated shape changes in the suprameatal triangle part based on Components 1, 2 and 3. Component 1 in this figure, displayed the existence of a process, or a 90 degree rotation of the head of the triangle. Component 2 displays changes from equilateral to isosceles triangles, and Component 3 displays distances from the triangle shape. According to Figure 3A, which was based on Components 1 and 2, *M. persicus* has a wide distribution and both isosceles and equilateral triangle shapes could be assigned. It should be mentioned that *M. persicus* of the Tehran group mostly had a process in the suprameatal triangle. In other words, it had the highest value in component 1. *M. libycus* in this figure is located in the top lefthand position. Consequently any process is not seen and this group and the *M. crassus* group are mostly located in the isosceles triangle position. However, the dispersal of *M. libycus* was contained in some equilateral part. These two groups have a complete overlap in this figure. According to Figure 3B all groups are not seen in more detail and all of them that tend to be non-triangle shape were located on the top of figure.

Elliptical Fourier analysis

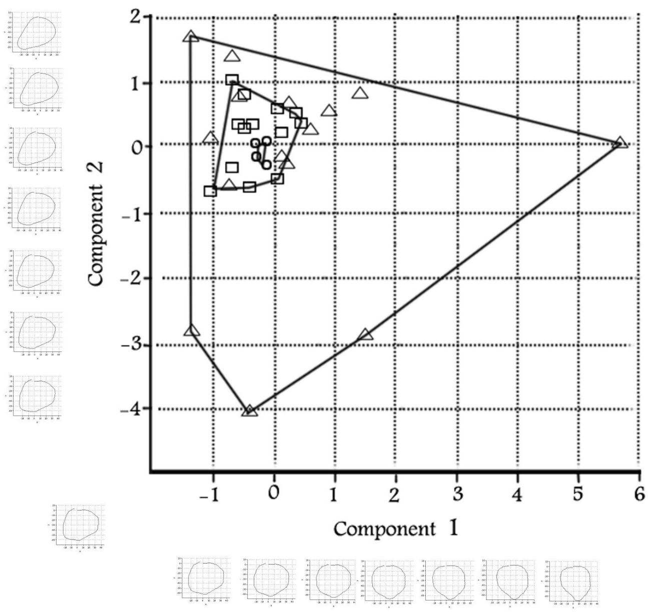
T^2 Hotelling analysis based on Fourier coefficients showed that only the meatus part could separate all the groups from each other significantly. The results are shown in Table III. The two groups of *M. persicus* are not separated according to any anatomical part, and also the meatus part separated the three species meaningfully ($p<0.05$).

Table III

Results of T^2 Hotelling in four parts based on Fourier coefficients. 1, 2, 3 and 4 are separating meaningfully in meatus, suprameatal triangle, mastoid and ventral part respectively ($p<0.05$). Two groups of *M. persicus* are not separated by any part. Also meatus part separated three species meaningfully

Species	*M. libycus*	*M. crassus*
M. libycus	1, 3, 4	
M. persicus (*Tehran*)	1, 3	1, 2, 4
M. persicus (*Geno*)	1, 4	1, 4

A



B

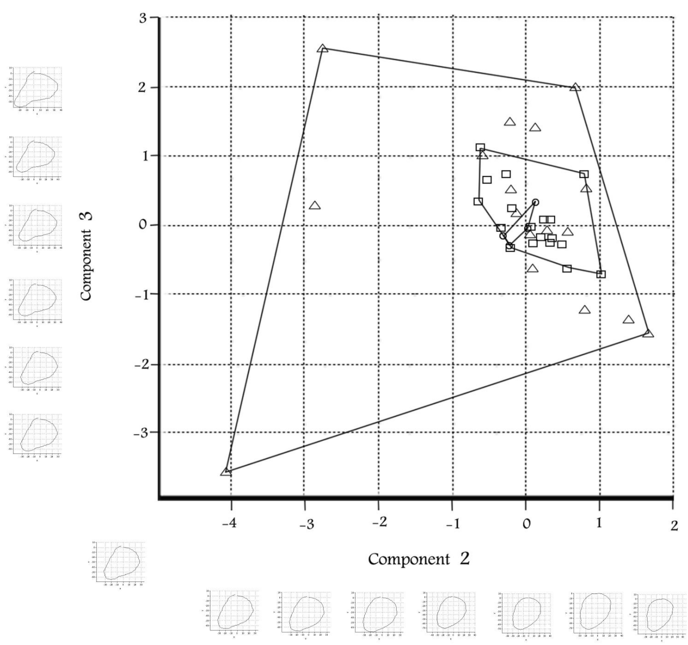
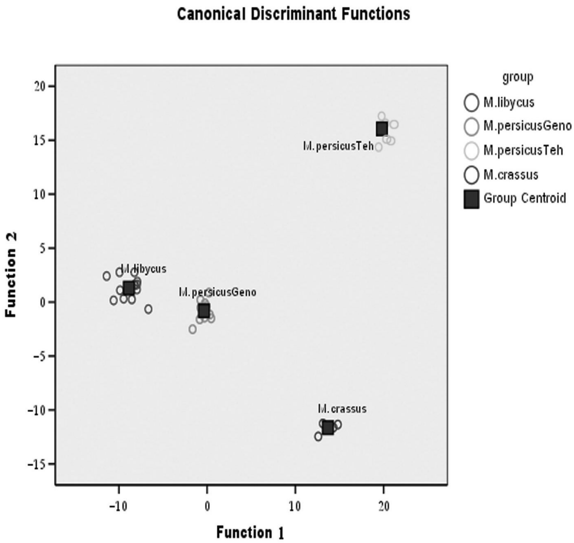


Fig. 3. Diagram based on eigenanalysis in the suprameatal triangle part (Component 1 displays existence of a process or a 90 degree rotation of the head of the triangle. Component 2 displays changes from equilateral to isosceles, and Component 3 displays distances from triangle shape). A – Diagram based on Components 1 and 2;. B – Diagram based on Components 2 and 3. In this figure triangle represents *M. persicus*, circle represents *M. crassus* and square represents *M. libycus.*

Figure 4 demonstrates discriminate analysis of the meatus and mastoid parts based on Fourier coefficients. In Figure 4A which belonged to mastoid *M. libycus* was closer than *M. persicus* Tehran to *M. persicus* Geno. In Figure 4B which belonged to meatus part all groups based on two functions were separated from each other. *M. crassus* totally was separated from other groups and also *M. libycus* was separated from complex of *M. persicus* group based on function 1.

A



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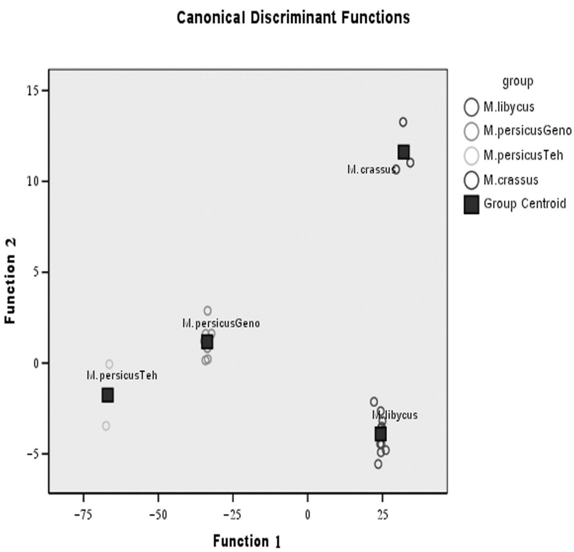

Fig. 4. Discriminate analysis based on Fourier coefficient. A – Discriminate analysis for mastoid part. Proportion of Function 1 in total variance is 57.4 % and proportion of Function 2 is 34.4; B – Discriminate analysis for meatus part. Proportion of Function 1 in total variance is 96.7 % and proportion of Function 2 is 2.9.

IV. DISCUSSION AND CONCLUSION

The genus *Meriones* is impressive due to its ability to adapt to desert areas, desert belt areas, and has demonstrated an ability to live in a variety of areas with different temperature regimes. No other genus is as well adapted to desert conditions (WILSON & REEDER 2005). A morphological description of *Meriones* species was published by MOMENZADEH (2001). However, in our study, the four parts of the bulla were, for the first time, investigated using the outline method.

In spite of various variations in the structure of different parts of the tympanic bullae, our studies have demonstrated that among the four parts studied, the auditory meatus can be used to separate three species from each other and could be considered as a taxonomic character. Other studied parts were valuable for shape variation investigations within species and subspecies but could not be used as taxonomic characters.

Our studies indicated that the greatest hypertrophization of the auditory meatus is in *Meriones crassus* which has been attributed to the desert conditions of its habitat. According to HEIM DE BALSAC (1936), the dryness of the environment is the only progressive and stable factor that concords with the increasing volume of the tympanic bulla. PETTER (1961) also considered the increasing volume of the tympanic bulla as one of the most important adaptations of desert animals. Although the existence of *Meriones crassus* in desert areas was noted by DALY and_DALY (1975), they only considered it in relation to competition with *Meriones libycus* and did not consider the hyperthrophy of the auditory bulla.

Hypertrophization of tympanic bullae in Gerbillinae was considered by PAVLINOV and ROGOVIN (2000) as contributing to increased auditory sensitivity at low frequencies. Several theories exist regarding increasing sensitivity to low frequencies. HATT (1932) considered the theory of tympanic hypertrophization bullae in relation to equilibrium during running. HEIM DE BALSAC and PETTER discussed population density and considered hyperthrophization as a factor in finding a mate in a population with a high density (PETTER 1961). LAY (1972) explained the hypertrophization as an adaptation to predator avoidance.

Results in this study in the suprameatal triangle changes for *M. crassus* contrast with CORBET (1978). Our results demonstrate that the suprameatal triangle in *M. libycus* was not isosceles triangle shaped, and according to our results with *M. libycus* ranges from equilateral to isosceles triangle, and for *M. crassus* the shape is considered isosceles.

Our results show that there are more similarities between the Geno population of *M. persicus* and *M. libycus* than between the Geno and the Tehran population of *M. persicus*. The reason for this is related to the Iran plateau, in which the Geno area is low in height and latitude thus providing a similar habitat to that of *Meriones libycus,* and this has caused adaptation according to the environment.

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