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A study of geographical changes of bullae characteristic in genus *Meriones* (Rodentia, Muridae) using Outline Method

MOMTAZI. F., GHASSEMZADEH.F.* AND ZAREIE. R.

Biology Department, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, IRAN

The characteristics of tympanic bullae have adaptation value. Based on the recent studies in the genus *Meriones*; meatus is the only part of tympanic bullae which has a taxonomic value. In this study, we examined interspecific changes of tympanic bullae in three species of this genus and also the relation of these changes to the climatic conditions of the regions was studied in two species. Three species: *M. persicus, M. libycus, M. crassus* were studied by Outline method using eigenshape analysis and elliptical Fourier analysis. Two populations of *M. libycus* from Birjand, Bardsir (Kerman) and Mashhad; three populations of *M. persicus* from Geno in Bandar abbas, from Varamin in Tehran and Birjand in Central Khorasan provinces. Our studies support the previous result for taxonomic value of meatus part based on result of elliptical Fourier analysis. They also indicate that hypertrophy on meatus is closely related to climatic and geographical conditions. It is related to the temperature changes and shows a cline with temperature between populations of one species.

Key words: Auditory meatus, Eigenshape analysis, Elliptical Fourier analysis, *Meriones*, outline.

INTRODUCTION

Subfamily Gerbillinae is distributed from north to South Africa and from west to central Asia (Musser & Carleton 2005, Chevret and Dobigny, 2005). Genus *Meriones,* which is distributed from north of Africa to China is characterized within the subfamily by slender rostrum, slender or elongated supraorbital bone and hypertrophied tympanic bullae (Yigit et, al. 1997). Seventeen species of this Genus have been so far recorded from the world of which eight species exist in Iran (Wilson & Reeder 2005). In this study, three species were studied. (*M. persicus*, *M. libycusand M. crassus*). However, two of which were included for intraspecific analyses (*M. libycus & M. persicus*). *M. libycus which is* distributed from North Africa to Middle East exists in desert and sandy areas except for the forest Caspian region and for the border of Persian Gulf. *M. persicus* is an endemic species of Iran and lives in mountainous steppes. *M. crassus* in comparison with two other species prefers desert environments and is found in central desert of Iran (Wilson & Reeder 2005).

Tympanic bullae is composed of two parts, tympanic and mastoid (Fig. 1) each one subdivided according to the genera and species. (Lay, 1972). One of the peculiarities of genus *Meriones* is well-marked enlarged tympanic bullae. Different studies show that tympanic bullae, as a median part of ear, play an important role in adaptation with environment. Lay (1972) considered this hypertrophy

*CORRESPONDING AUTHOR: ghasemzadeh@ferdowsi.um.ac.ir

as a possible way to escape from predators as it increases auditory ability especially in low frequencies. Peter (1961) stated that this hypertrophy causes the individuals equal chance for finding mates in desert populations with low density. Hatt's (1932) theory which has been currently rejected was insisting on changing center of gravity of skull toward posterior and facilitating its movement.
In this paper we studied the changes of tympanic bullae structures in three populations of *M. persicus* and *M. libycus* and examined geographical changes of two parts of tympanic bullae auditory meatus and mastoid.

MATERIAL AND METHODS

Tympanic bullae of 49 specimens belongs to three species of *Meriones* (*M. persicus*, *M. crassus* and *M. libycus*) and were studied using outline based geometric morphometric analyses. *M. persicus* specimens were collected from Birjand, Tehran and Geno. *M. libycus* specimens were collected from Mashhad, Birjand and Bardsir (Kerman). The study was based on 49 specimens that all were deposited in Rodentology Research Department of Ferdowsi University of Mashhad, Iran (ZMFUM). Some specimens were broken in one of these three parts of tympanic bullae; that's why in some analyses the specimens are less than 49 (Table 1). Also because of low specimens of *M. crassus*, study interspecies change on it was not possible.

OUTLINE ANALYSES:

DIGITALIZATION

Digital images were captured using Cannon, powershotA70 camera with magnification of 1 and the quality of 3.2 million pixels. The pictures were transferred to Photoshop software (cs 4) and were edited using magnetic lasso tool selecting the area of triangle suprameatal, mastoid, auditory meatus parts. Then each area was edited using brush tool (Fig.1). The Cartesian coordinates of each outline were calculated with TPSdig 1.37 (Rolf, 2001) software. Starting point of outline was defined by authors in the last study (Momtazi et al. 2008): in suprameatal triangle at closest part of the triangle towards rostral side of skull with 200 points per outline, in auditory meatus at the meeting point of zygomatic arch and bullae with 300 points per outline (Fig. 1).

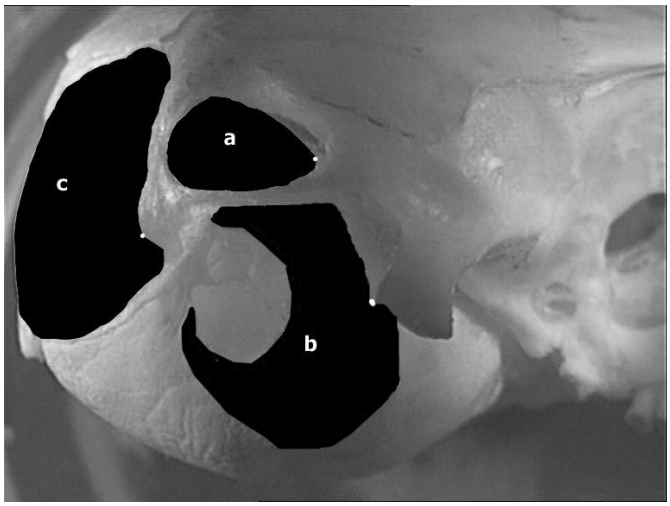

FIG.1.- Different parts of auditory bullae (a; suprameatal triangle, b: auditory meatus and c: mastoid) whit point demonstrated starting point of outline calculation.

EIGENSHAPE ANALYSIS

This method has been described by Lohmman (1983) (Renaud and Michaux, 2003). Eigenanalysis is a kind of PCA which is used for outline data. The function is constructed as a linear function of the observed data across one or more specimens. This analysis is based on the covariance matrix of the non-normalized turning angle increments around the outlines (Renaud and Michaux, 2003). Eigenanalysis was used for description shape changes in meatus and suprameatal triangle parts.

ELLIPTICAL FOURIER ANALYSIS:

The method was described by Kuhl and Giardinia (1982). It 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. 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 xy- 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 (Crampton, 1995). These coefficients therefore correspond to residuals after standardization, and should not be included in the following statistical analysis. Therefore these coefficients could be used as variables in other statistical analysis such as discriminate analysis (Momtazi et al.2008).

Data were analyzed with EFW software (Rohlf and Ferson1992) and coefficients of this method results were used as statistical variables in SPSS software (ver.11.5) for performing statistical analysis such as discriminate function analysis and t^2hotelling. In eagenshape analysis part we used of PAST software (Hammer et.al 2007).

RESULTS

A) INTERSPECIFIC ANALYSES

The interspecific auditory meatus changes were studied using eigenshape analysis (Fig. 2). In this part 40 eigenvalues were calculated and only first four values which had more portions in total variance has been shown in table 2. Significance of each value was determined by Jolliffe cut-off value. If the eingenvalue of each component is less than Jolliffe cut-off value thus the component would be meaningless (Hammer et.al 2007). First component in Fig. 2 indicated increasing meatus hypertrophy on positive score, in second half of this component ventral hypertrophy has been decreased although dorsal hypertrophy has not been increased more. This hypertrophy can be seen in *M. crassus* and *M. libycus*. Second component in this diagram indicates increasing in ventral part which becomes closed to zygomatic arch. *M. crassus* shows maximum value of this part and that of *M. libycus* has the second higher.

TABLE 2.- Eigenvalue and each portion total variance in eigenvalue analysis of two meatus parts

MEATUS	Value	Variance%
Eigenval 1	۰.۹۰۱۵۹۷	٦.۳۹۷٥
Eigenval 2	۰.۸٦۲۹۷	٦.۱۲۳٤
Eigenval 3	۰.۷۷۸۱۱	٥.٥۲۱۳
Eigenval 4	۰.۷۲۲۱۹	٥.۱۲٤٥
Jolliffe cut-off	۰.۳۳۱۰٤	
Standardized point number	300	

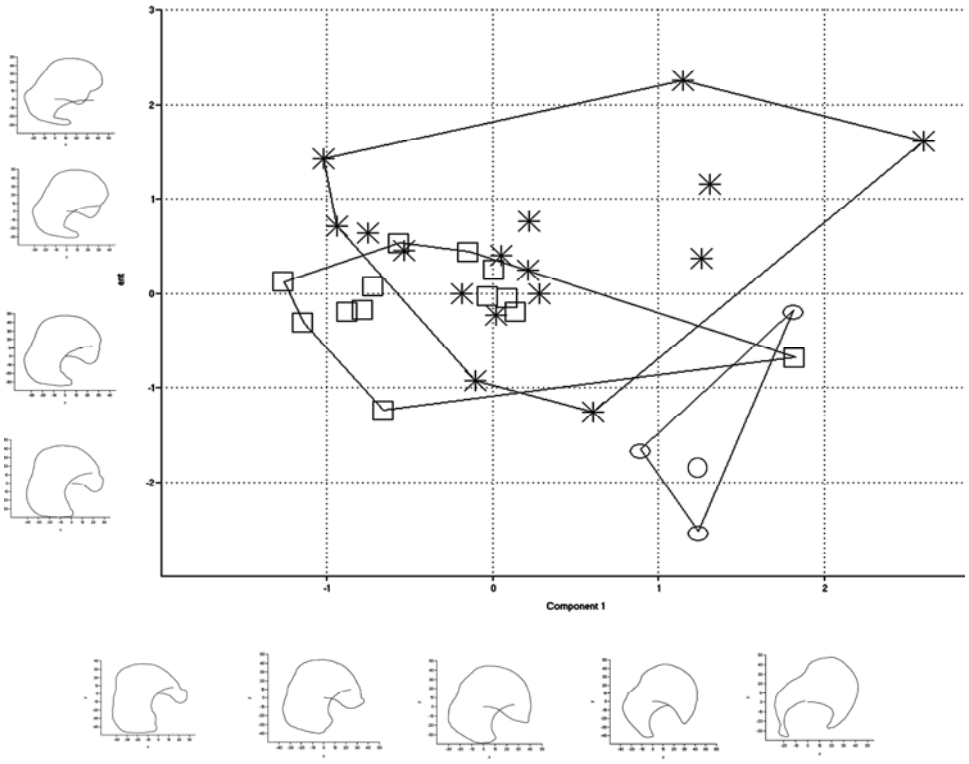

FIG.2.- Eigenshape analysis in auditory meatus part). In this figure the star shape (*) represents *M. persicus*, circle represents *M. crassus* and square represents *M. libycus*

B) INTRASPECIFIC ANALYSIS

Fig. 3 shows population changes in auditory meatus part of *M. persicus* and *M. libycus* based on eaigenshape analysis. First component in part A (*M. persicus*) shows gradual decrease of hypertrophy in posterior part and increase of hypertrophy in ventral part. Based on this analysis Geno population shows less increase in hypertrophy of dorsal part in comparison to the Birjand and Tehran populations. Second component shows decrease in hypertrophy symmetrically and increase in meatus opening. In Geno and Birjand populations it has less width than Tehran. In the part B of Fig. 3 (*M. libycus),* first component shows gradual increase in meatus hypertrophy and two populations have the same pattern but in the second component which shows an increase in meatus opening, Birjand population is to some extent located higher.

Based on T^2 Hotelling analysis on elliptical Fourier coefficients, in meatus part there was significant difference in different groups. Discriminate Function Analysis diagram of meatus part is shown in Fig 4.

DISCUSSION

Genus *Meriones* is considerable due to its talent to adaptation with desert areas (Wilson and Reeder, 2005).In desert area, the most important problem is distance between members of one species and also predator's threats and this fact that there is no place to hide. Both of these factors decreased power of existence and reproduction of species. In order to adapt with environmental conditions in this genus, bullae hypertrophy increased along with other adaptations, this adaptation also existed in carnivores (Prakash, 1959)

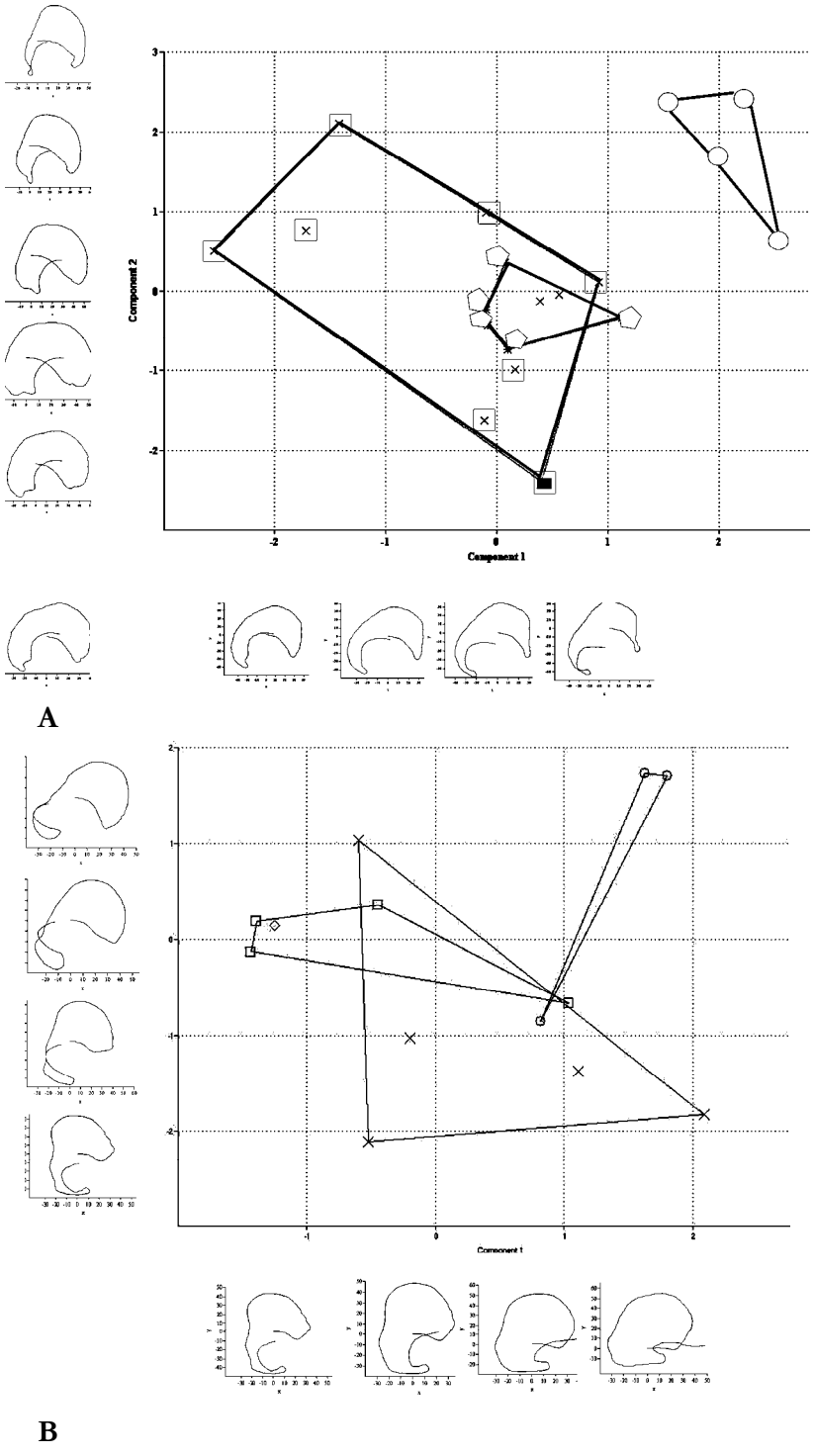

FIG.3. - Eigenshape analysis in auditory meatus part. A: *M.persicus*, Tehran, Birjand and Geno populations of *M.persicus* are shown with circle, polygonal and square respectively. B: *M.libycus*: Birjand and Mashhad populations of *M.libycus* are shown with square and lozenge, respectively.

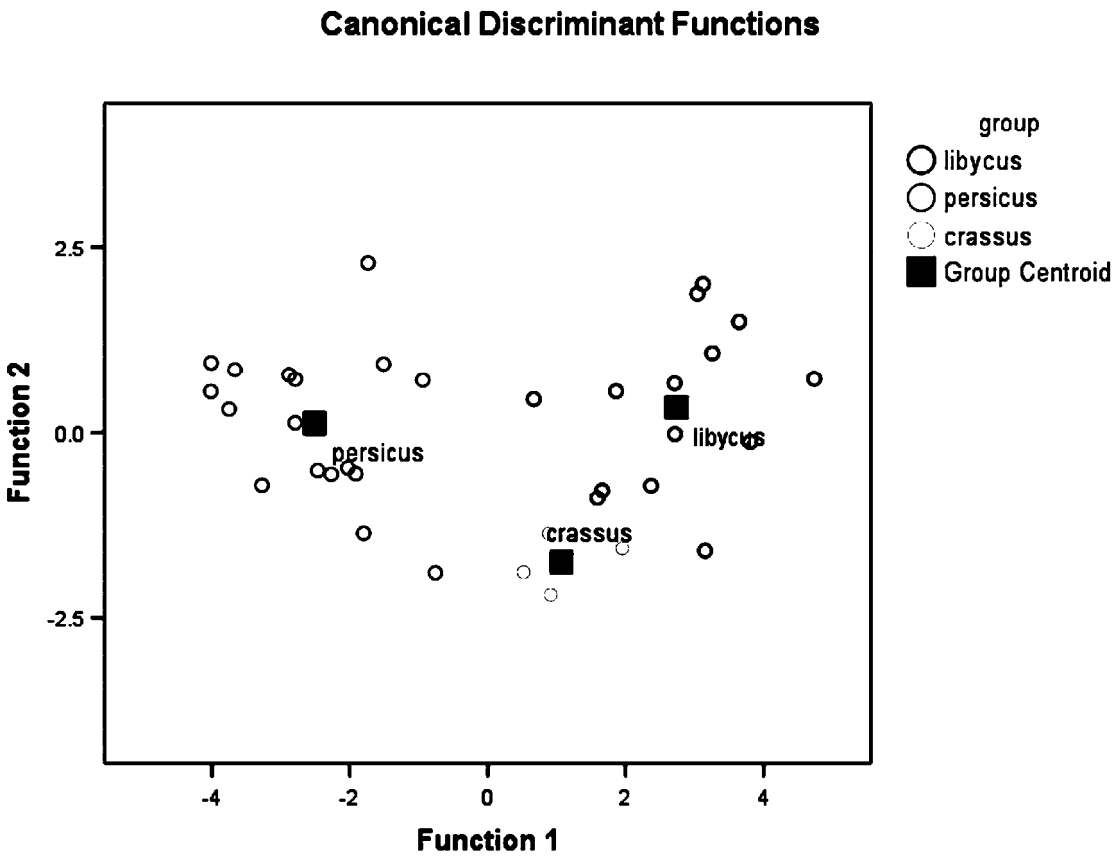

FIG. 4. - Discriminate Function Analysis of meatus part between species.

This characteristic is adaptive among *Meriones* species and also among populations of *M.libycus* and *M.persicus*. Based on Fig. 4 auditory meatus in Geno population of *M. persicus* has more hyperthrofy. Geno is at a distance of 29 kilometers from Bandar-Abas, in spite of its high height and its low temperature in comparison with Bandar-Abas, it is still a dried area. Birjand population also has bigger meatus than Tehran population of this species and it is proportionate to these 2 area temperatures. Similar interpretation is seen in population of *M.libycus*. Based on Pavlinov & Rogovin (2000), there is total tendency toward growth in structures of auditory, one is increasing auricles and tympanic bullae remains small and therefore cause increasing in auditory sensitivity and this would help in finding places. The other one is increasing tympanic bullae and it led to increasing in auditory sensitivity, this form is observed in the studied member of the genus. There are previous studies (Wilkins *et al.* 1999, Gardner and Louise, 1984) rodent classifications based on auditory structure characters (tympanic bullae along with other characters)in some families such as Giliridae, Echimydae and Geomyidae families are based on tympanic bullae along with other characters. As populations of each species do not show significant differences in characters statistically, this characteristic can be used in taxonomy. Also this study confirmed previous studies (Momtazi et al. 2008). It can also be concluded that this characteristic has a cline correspond to cline of temperature. A reason for this investigation is related to Iran plateau, in which Geno area has low height and latitude which provides similar condition as *Meriones libycus* lives and this character evolved towards adaptation with environment.

TABLE3: Eigenvalues and each portion in total variance of eigenshape analysis of two parts of meatus in *M.persicus* and *M.libycus*

M.libycus	**Value**	**Variance%**
Eigenval 1	۱.۵۸۹٤٤	۱۵.۰۵۱
Eigenval 2	۱.۵٤٦	۱٤.٦٤
Eigenval 3	۱.۱۰۰۵	۱۰.٤۲۱
Eigenval 4	۱.۰٤۹۳	۹.۹۳٦۵
Jolliffe cut-off	۰.۰۳۳٦۰۱	
Standardized point number	300	

M.persicus	**Value**	**Variance%**
Eigenval 1	۱.٦٤۵۲۵	۱۰.٦۲۲
Eigenval 2	۱.٤۳۱	۹.۲۳۹۱
Eigenval 3	۱.۲۹۸۳	۸.۳۸۲۱
Eigenval 4	۱.۲٤۷۹	۸.۰۵٦۷
Jolliffe cut-off	۰.۰۳٦۳۸۳	
Standardized point number	300	

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