Geometric morphometric analyses of four species of brush-tailed mice, genus *Calomyscus* (Rodentia: Calomyscidae), from the Iranian Plateau

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The brush-tailed mice of the genus *Calomyscus* are distributed in the mountains of central Asia and the Middle East. The genus *Calomyscus* represents five nominate species from Iran (*C. bailwardi, C. grandis, C. hotsoni, C. urartensis* and *C. elburzensis*). In total 64 specimens from four species of *Calomyscus* were studied for morphometric analyses The morphological differences among 10 populations of the genus *Calomyscus* were investigated using landmark-based geometric morphometrics of the left mandible and Fourier analysis of the first lower molar. The results suggested that four Iranian *Calomyscus* species, *C. bailwardi, C. grandis, C. hotsoni,* and *C. elburzensis* can be discriminated with a high probability based on their mandible and first lower molar shapes. *Calomyscus grandis,* however**,** was the most distinct species from others.
Key words: *Calomyscus,* Geometric morphometric, Mandible, Molar

INTRODUCTION

The genus *Calomyscus* covers a number of geographically isolated populations that inhabit arid, rocky hillsides and desert mountain ranges in Syria, Azerbaijan, Iran, Turkmenistan, Afghanistan, and Pakistan (Graphodatsky et al., 2000; Hassinger, 1973; Lay, 1967; Musser and Carleton, 1993, 2005; Peshev, 1989; Schlitter and Setzer, 1973; Vorontsov et al., 1979). This genus was first described by Thomas in 1905 and initially was considered as a monotypic genus consisting of *C. bailwardi* (Corbet and Hill, 1980; Ellerman and Morrison-Scott, 1951). Vorontsov and his colleagues in 1979 considered most subspecies of *C. bailwardi* as distinct species and recognized five allopatric species for the genus of *Calomyscus* (*C. hotsoni, C. mystax, C. urartensis, C. bailwardi, C. baluchi*) based on morphology and geographic isolation for Transcaucasia and Turkmenia. Whereas several studies (Graphodatsky et al., 1989, 2000; Meyer and Malikov, 2000; Esmaeili et al., 2008) have been focused on the geographical distribution of different chromosomal morphologies and their taxonomic significance, Lebedev and his colleagues (1998) using multivariate analyses on the genus *Calomyscus* demonstrated a distinct morphological clusters that correspond to the karyotypic differences. In recent revision, Musser and Carleton (2005) recognized eight species for the genus of *Calomyscus* including: *C. bailwardi, C. baluchi, C. elburzensis, C. grandis, C. hotsoni, C. mystax, C. tsolovi,* and *C .urartensis.* This classification has been evaluated subsequently with additional chromosomal data and multivariate analyses of cranial and dental measurements (Grophodatsky et al., 2000; Malikov et al., 1999; Meyer and Malikov, 1996; Lebedev et al., 1998).

Five species have been recognized for the genus of *Calomyscus* in Iran: Noble brush-tailed mouse (*C. grandis,* Schlitter and Setzer, 1973) from south foothills and ridge (8500 ft.) of Damavand, central Elburz mountain and on the north slopes of Mazandaran province at Abass-Abad; Urar brush-tailed mouse (*C. urartensis,* Vorontsov et al., 1979) from north west of Iran (north west of Azerbaijan province); Goodwin's brush-tailed mouse (*C. elburzensis,* Goodwin, 1938) from mountains of north and north east of Iran; Southern foothills of Elburz mountains in Semnan province, near Semnan and adjacent Sang-i-Sar eastward through north Khorasan province, in north east of Iran (Lebedev et al., 1998); Hotson's brush-tailed mouse (*C. hotsoni,* Thomas, 1920) from Baluchistan province of southeastern Iran (Musser and Carleton, 2005), and Zagros Mountains brush-tailed mouse (*C. Bailwardi,* Thomas, 1905) from the Zagros Mountains in western part of Iran Kurdistan, Ilam, west of Esfahan, east of Khuzistan, Luristan, Fars and west of Kerman provinces (Vorontsov et al., 1979). Since discrimination of species based on morphological characters of *Calomyscus* is difficult, the present investigation focused on using landmark-based geometric morphometrics of the left mandible and Fourier analysis of the first lower molar as a new method to resolve the taxonomic status of the 10 Iranian populations of *Calomyscus.*

MATERIAL AND METHODS
Sampling
In total 64 specimens from four species of *Calomyscus* were studied for morphometric analyses. The number of studied specimens per species, and further information about the locality of studied specimens are presented in Table 1. Specimens and morphometrical materials are deposited in the Rodents collection of the Zoological Museum of Ferdowsi University of Mashhad (ZMFUM).

Geometric morphometric analysis
Geometric morphometric analyses were performed separately for two-dimensional project of left mandibles landmark and lower cheek teeth using an Elliptic Fourier method. Since there was no significant sexual dimorphism in the size and shape of the mandible and lower molars of the examined populations (F= 0.3; P=0.7), all the data were combined in subsequent analyses.

Landmark method
Mandible images were taken by a Canon camera connected to a personal computer. Homologous landmarks were determined on a two-dimensional project of the left mandible by using the Tpsdig V2.12 program (Rohlf, 2003). Nine landmarks were determined on the lateral surface of the mandible (Fig. 1). The centroid size, the square root of the sum square distances of landmarks from their centroids was calculated for each species (Bookstein, 1991). Analysis of variance (ANOVA, Sokal and Rohlf, 1995) was used to test the significance of variation and construct variable characters box plot based on the centroid sizes of the lower molar. Homologous landmarks of the specimens were superimposed using a generalized least-squares algorithm. The consensus configuration as well as thin-plate spline parameters were also calculated (Bookstein, 1991; Rohlf, 1990, 1993; Rohlf and Slice, 1990). A multivariate analysis of variance (MANOVA) was carried out on the partial warp scores combined with uniform components to further examine the differentiation among four Iranian *Calomyscus* species (see for method Tarahomi, et al, 2010). Shape variables were used as input for canonical variate analyses (CVA) to evaluate the inter-specific variation among species of *Calomyscus.* Deformation grids of the shape were calculated through multivariate of the canonical scores on the thin plate spline parameters (Rohlf et al., 1996). Procrustean superimposition and centroid sizes were analysed using TpsRelw program (Rohlf, 2005).
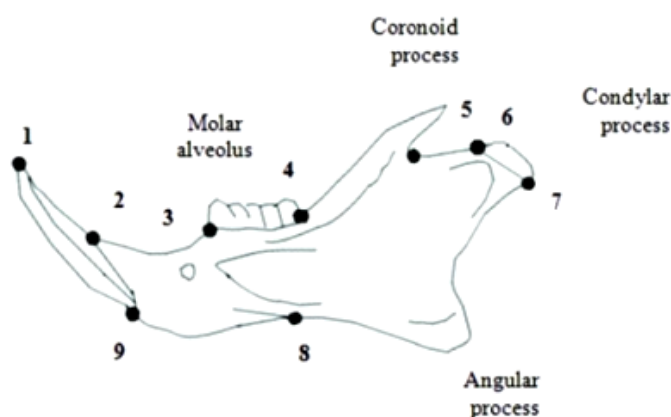
FIGURE 1. Nine landmarks captured on the mandible of *Calomyscus* (after Duarte et al. 2000).

Elliptic Fourier method

Outline-based geometric morphometric analysis in the shape of the first lower molar was performed using GMTP (Geometric Morphometric Tools Package) Program (Taravati, 2009). Digital images were captured with a DPVLI Olympus camera mounted to a BX51 stereo microscope. Differences in the centroid size of taxa were depicted by box plot and their significant differences were tested by an analysis of variance (ANOVA) (Taravati, et al., 2009). The Cartesian coordinates of the lower molar were computed with TPSdig 1.37 software (Rohlf, 2003). The data were extracted from a set of 150 points along the outline of the first lower molar. To convert the data from TPS into EFA format, we used Geometric Morphometric Tools Package (GMTP) Program (Taravati, 2009). Elliptic Fourier Analysis (EFA) was described by Kuhl and Giardinia (1982) and corresponds to the Elliptic Fourier Transform (EFT) performed using the EFAwin software (Ferson et al., 1985). Then the GMTP program was used to convert data to an identifiable format for the Past 1.98 software (Taravati, 2009). CVA was performed for classification of groups and analysis of the shape variability among taxa. The existence of allometric in the shape was tested using regression analysis between the CVA projection and the centroid size. Outline-based geometric morphometric analysis was undertaken using GMTP and past 1.98 softwares.

RESULTS
Landmark analyses

MANOVA analysis on the total shape space showed significant differences among taxa (p<0. 001). Multivariate regression of the relative warps against the centroid size showed significant correlation (P>0.007, Wilk's A). The first two (cv1 and cv2) axes of the CVA explained 68.25 and 31.75 of the variance of the shape matrix respectively (Fig. 2) in which *C. grandis* discriminated from *C. hotsoni* along the first axis and *C. bailwardi* from *C. elburzensis* on the second axis respectively. Shape differences are represented in (Fig. 2) as deformation mandible shapes implied by cv1 and cv2. The major deformation grids of the shape changes associated with the first canonical variate axis was localized in the head of the incisors, surface of condyle and incisor alveolus. The noble *Calomyscus*, *Calomyscus grandis,* with positive score on cv1 showed a longer head of the incisors, a slender condyle surface and a shorter incisor alveolus region, whereas *C. hotsoni* with negative score showed an opposite trend. The second canonical axis described a minor effect in the position of the head of the incisors, the surface of condyle, the innermost point of lower mandible and the incisor alveolus region. *Calomyscus bailwardi* showed a shorter head of the incisors, a more expended condyle surface, a longer innermost point of the lower mandible and a shorter incisor alveolus region.
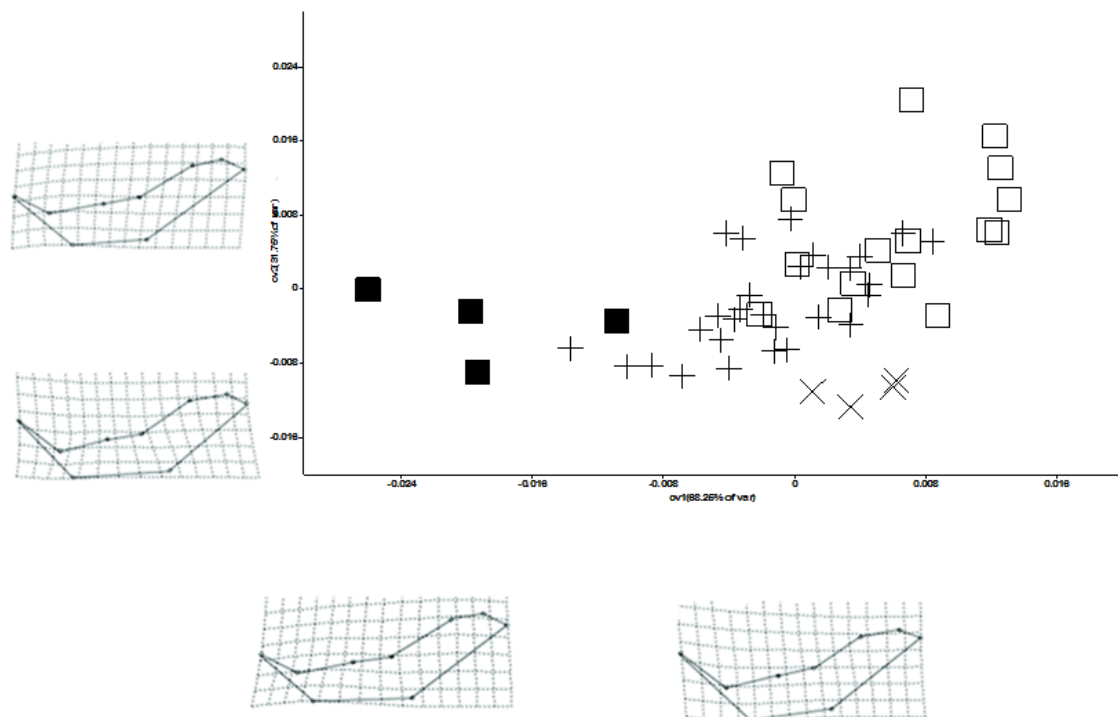
FIGURE 2. Ordination of eleven populations of brush-tailed mice *(Calomyscus)* in the space of canonical axes 1 and 2 based on the total shape matrix of the mandible. Deformation grids on the extremities of each axis are shown. Solid square: *C. hotsoni*, Open square: *C. bailwardi*, Multiply sign: C. *grandis*, Plus sign: *C. elburzensis*.

TABLE 1. list of l Localities for studied specimens.

Taxon	Collection sites	Specimens(n)
C. bailwardi	Arsanjan, Genu and Kerman	20
C. elburzensis	Bojnourd, Khajehmorad, Yazd, Tandureh and Aghdarband	35
C. hotsoni	Sistan and Bluchistan (saravan)	4
C. grandis	Tehran	5

Elliptic Fourier analysis

Single-classification ANOVA on centroid size showed significant differences among taxa (P <0.004). The Box plots of lower molar centroid size (Fig. 3) showed *C. bailwardi* had the smallest first lower molar.

The results of the MANOVA showed significant differences among taxa (P<0.04). CVA on Elliptic Fourier coefficients (Fig. 4) showed that the first two CV axes (cv1 and cv2) explained 99.2% and 0.6% of the shape variance respectively. The scatter plot (Fig. 4) showed similar results to that obtained from CVA of the mandible (Fig. 2). The CVA scatter plot of Elliptic Fourier coefficients indicated the shape differences between *C. grandis* and *C. hotsoni* and *C. bailwardi* and *C. elburzensis* on the first (cv1) axis. *C. grandis* on the first axis had a longer and a more slender first lower molar, whereas *C. hotsoni* had a long entoconid cusp. *C. bailwardi* had a wider first lower molar whereas *C .elburzensis* showed the opposite trend.
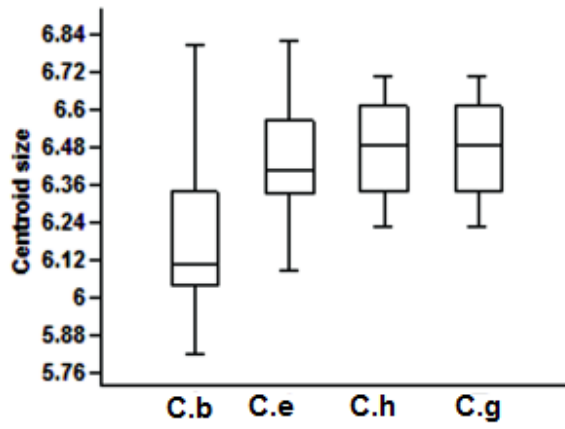
FIGURE 3. Boxplots for lower molar centroid size in the four species of *Calomyscus*, namely C.b: *C. bailwardi*, C.e: *C. elburzensis*, C.h: *C. hotsoni* and C.g: *C. grandis*. The mean and limits are indicated by the %95 confidence interval.
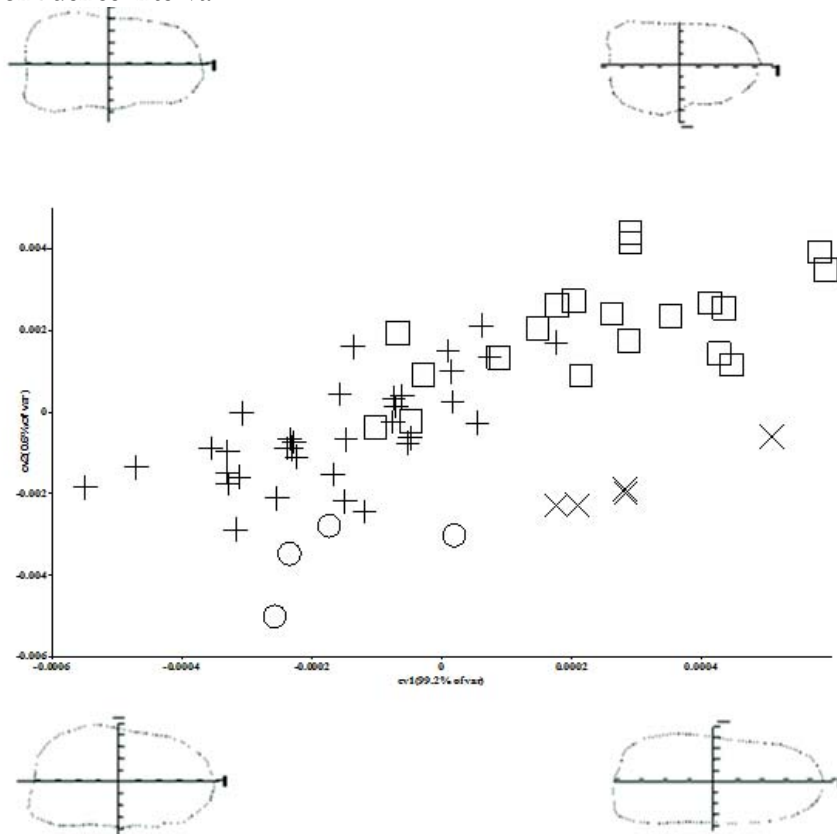

FIGURE 4. Ordination of eleven populations of *Calomyscus* in the space of canonical axes 1 and 2. Open circles: *C. hotsoni*, open square: *C. bailwardi*, multiply sign: C. *grandis*, plus sign: *C. elburzensis*.

In sum, on the basis of the geometric morphometric results presented here, the following description can be provided.

1- *Calomyscus grandis*: The Noble brush-tailed mouse has a divided fronto-parietal suture, a one cusp parital-occipital suture; a longer head of the incisors, a slender condyle surface and a shorter incisor alveolus region; a longer and slender first lower molar.

2- *Calomyscus hotsoni*: The Hotson's brush-tailed mouse has a shorter head of the incisors, a more expended condyle surface, and a longer incisor alveolus region; a long entoconid cusp in first lower molar**.**

3- *Calomyscus elburzensis*: The Goodwin's brush-tailed mouse has a longer head of the incisors, a slender condyle surface, a shorter innermost point of the lower mandible and a longer incisor alveolus region; a slender first lower molar.

4- *Calomyscus bailwardi*: The Zagros Mountain brush-tailed mouse also has a shorter head of the incisors, a more expended condyle surface, a longer innermost point of lower mandible and a shorter incisor alveolus region; a wider first lower molar**.**

DISCUSSION

Discrimination of four morpho-groups in this study is congruent with well distinguished clades of brush-tailed mice (*Calomyscus*) based on RFLP analysis by Sahebjam et al. (2009).

Geometric morphometrics analyses (GMA) demonstrates that *C. bailwardi* has the smallest first lower molar.The other three species (*C. elburzensis*, *C. grandis* and *C. hotsoni*) greatly overlap in the first lower molar centroid size character. Although mandible size was not significantly differ among the examined species, the results of GMA suggested that the four species can be discriminated with a high probability based on their mandible and first lower molar shapes. *Calomyscus grandis,* however**,** was the most distinct species from others. The distinction of *Calomyscus grandis* from *C. hotsoni* is more prominent based on the first axis of the left mandible.

Musser and Carleton (2005) stated that *C. grandis* is the largest, darkest species, with the longest tail within *Calomyscus.* The result of morphometrical analyses by Lebedev et al., (1998) demonstrated the great phenetic distance of *C. grandis* from *C. mystax*,*C. elburzensis* and *C. urartensis*. Our GMA results revealed that *C. hotsoni* and *C. elburzensis* show slightly overlap in first lower molar centroid size character. *C. hotsoni* is smaller than other species and is allied with *C. mystax* and *C. elburzensis* based on size. These results are in congruent with other studies in this group ((Vorontsov et al., 1979; (Musser and Carleton, 2005).

In sum, geometric morphometric methods and morphological patterns of the left mandible and fourier analysis of the first lower molar were able enough to allow the identification of these four species group from each other, more accurate interspecific correlations and resolution of the evolutionary differentiation level of these four species will require and further investigations involving molecular and cytogenetic studies.

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