CRANIAL AND DENTAL ANALYSIS OF MOUSE-LIKE HAMSTERS OF THE GENUS *CALOMYSCUS* (RODENTIA: CALOMYSCIDAE) FROM PLATEAU OF IRAN

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ABSTRACT - To assess morphometric interspecific differences, we studied by multivariate and univariate analyses 32 cranial, mandibular and dental variables and four standard external measurements in 84 specimens of the genus *Calomyscus* (*C. elburzensis*, *C. bailwardi*, *C. hotsoni* and *C. grandis*) from Iran. Almost all mean cranial measurements in *C. grandis* were larger than in the other three species, while the opposite occurred for *C. hotsoni*. *C. bailwardi* showed the smallest dental measurement. Principal Components Analysis showed seven components with Eigen-Value > 1, explaining 77.08 % of total variance. PC1 explained 25.96 % of the total variance and clearly separated *C. grandis* from the other three species. The first two Canonical Discriminant Functions explained 84.4% of the total variance. CDF1 separated *C. grandis* from the other three species and *C. bailwardi* from *C. hotsoni*, while CDF2 separated *C. elburzensis* from *C. hotsoni* and, with a little overlap, *C. bailwardi.* In total, 93.7 % of cases was correctly classified. Cluster analysis showed that *C. bailwardi* and *C. elburzensis* formed a cluster, to which *C. hotsoni* was closer than *C. grandis*. We concluded that a combination of several cranial and dental measures may help to discriminate these four Iranian species of the genus *Calomyscus*.

INTRODUCTION

The genus *Calomyscus* had been considered monotypic and represented by the species *C. bailwardi* (type locality in Khuzestan Province, Izea, Iran) (Carleton and Musser 2005). Vorontsov et al. (1979) revised the genus and considered most former subspecies of *C. bailwardi* as separate species. The morphological and geographic integrity of some species has been then tested by chromosomal data (Malikov et al. 1999; Meyer and Malikov 1996, 2000; Graphodatsky et al. 2000), mitochondrial cytochrome b sequences (Morshed and Patton 2002; Ryan et al. 2008) and multivariate analyses of cranial and dental measurements (Lebedev et al. 1998). The genus includes a number of geographically isolated populations that occupy well-drained, barren,

rocky habitats in foothills and mountains (Musser and Carleton 1993; Carleton and Musser 2005). Populations appear to be patchily distributed and some are geographically isolated, promoting the effects of random genetic drift and, possibly, rapid karyotype evolution (Graphodatsky et al. 2000). Recently, Carleton and Musser (2005) recognized 8 allopatric species (*C. bailwardi*, *C. baluchi*, *C. elburzensis*, *C. grandis*, *C. hotsoni*, *C. mystax*, *C. tsolovi*, *C. urartensis*) present in Syria, Azerbaijan, Iran, Turkmenistan, Afghanistan and Pakistan and Turkey (Kryŝtufek and Voharlík 2009). Five out of these 8 species have been reported for Iran: *C. bailwardi,* from Zagros Mountains in southern and western Iran; *C. hotsoni* from SE Iran, Baluchistan Province; *C. urartensis* from NW Iran, Azerbaijan Province; *C. elburzensis* from NE Iran, Kopet Dag Mountains in Khorasan Province; and *C. grandis* from N Iran, Tehran and Mazandaran Provinces (Graphodatsky et al. 2000; Hassinger 1973; Lay 1967; Musser and Carleton 1993; Carleton and Musser 2005; Peshev 1989; Schlitter and Setzer 1973; Vorontsov et al. 1979). While much research has focused on the geographical distribution of different chromosomal morphologies and their taxonomic significance (reviewed in Graphodatsky et al. 2000), multivariate analysis showed that distinct morphological clusters correspond to karyotypic differences (Lebedev et al. 1998). More studies of this kind are required to better understand the taxonomy and geographic distribution of the genus (Musser and Carleton 1993; Carleton and Musser 2005).

We collected specimens from various localities of Iran with the aim of evaluating if external, cranial and dental measurements may help to differentiate the Iranian species of *Calomyscus.*

MATERIALS AND METHODS

Between March 2004 and August 2009, a total of 84 specimens of *Calomyscus* spp. were collected from 11 sites in Iran (*Fig. 1*), using several live- and snap traps. All specimens were deposited in the Zoological Museum of Ferdowsi University of Mashhad and Rodentology Research Department (ZMFUM). The specimens were from the following localities: site 1, Khorasan, Bojnurd (n = 6, 37º 26' N, 57º 17' E); site 2, Khorasan, 10 km east of Daregaz, Tandore (n = 3, 37º 29' N, 58º 43'E); site 3, Khorasan, Aghdarband, (n = 10, 36º 30' N, 61º 7' E); site 4, Khorasan, Mashhad, Khaje Morad (n = 15, 36º15' N, 59º 34' E); site 5, Khorasan, Torbat Jam, 15 km south of Nasr Abad (n = 6, 35º 9' N, 60º 24' E); site 6, Tehran, Fasham (n = 6, 35º 56' N, 51º 31' E); site 7, Yazd, Fakhr Abad village (n = 8, 31º 40' N, 54º 19' E); site 8, Kerman, Baft, Khabr Mountain (n = 7, 28º 47' N, 56º 20' E); site 9, Baluchistan, Paskoh, Birk Mountains (n = 7, 27º 18'N, 61º 46'E); site 10, Fars, 20 km west of Arsanjan, Ziad Abad village (n = 8, 29º 48' N, 53º 14' E); site 11, Hormozgan, Geno (n = 8, 27º 23' N, 56º 14' E). Four external measurements (HBL: head and body length, TL: tail length, FL: hindfoot length (claw excluded), EL: ear length) were taken to the nearest millimeter (mm) by a ruler. Furthermore, 32 cranial, mandibular and dental variables (*Fig. 2*) were measured on 84 skulls belonging to four species: *C. bailwardi* (sites 10, 8 and 11)*, C. hotsoni* (site 9)*, C. elburzensis* (sites 1, 2, 3, 4, 5 and 7), and *C. grandis* (site 6). Specimens were *a-priori* classified with reference to Graphodatsky et al. (2000), Carleton and Musser (2005) and Sahebjam et al. (2009). Dental and skull measurements were taken by both a measurescope and a digital caliper to the nearest 0.001 mm and 0.05 mm, respectively (Lebedev
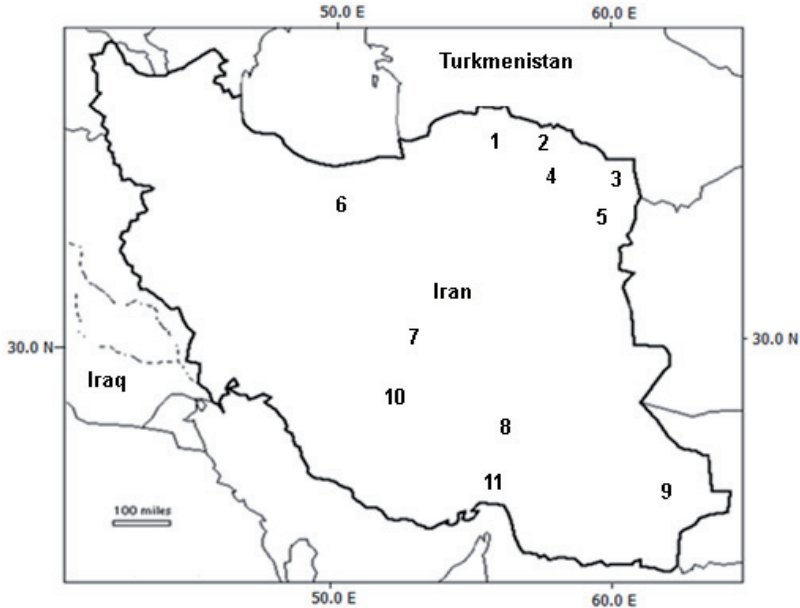
Figure 1 - Sampling areas: 1- Bojnord; 2- Daregaz; 3- Aghdarband; 4- Mashhad; 5- Torbat jam ; 6- Tehran (Fasham) ; 7- Yazd (Eslameye and Fakhrabad) ; 8- Kerman (Baft) ; 9- Saravan (Paskoh) ; 10- Fars (Arsanjan) ; 11- Geno.

et al. 1998; Javidkar et al. 2005).

Variables and abbreviations were as follows (*Fig. 2*): Occl: Occipitonasal length; Zygw: Zygomatic width; Patl: Palatal length; Nasl: Nasal length; Madl: Mandible lenght; Forl: Foramina incisive length; Forw: foramina incisive width; Cbl: Condylobasal length; Tbl: Length of tympanin bullae; Tbw: Width of tympanin bullae; Nasw: Nasal Width; Dial: Diastema length; Mxl: Maxillary toothrow length; Mnl: Mandibular toothrow length; Brh: Braincase height; Zygw: Zygomatic width; Intw: Interorbital width; Brb: Braincase breadth across bullae; M1M1: distance between the outer edges of the first left and right upper molars; M1co: distance between the front edges of the first upper molar and occipital condyle; Madh: height of the mandible ascending branch; M/1l: first lower molar length; M/2l; second lower molar length; M/3l: third lower molar length; M/1w: first lower molar width; M/2w: second lower molar width; M/3w: third lower molar width; M1/l: first upper molar length; M2/l: second upper molar length; M3/l: third upper molar length; M1/w: first upper molar width; M2/w: second upper molar width; M3/w: third upper molar width.

In order to minimize the effect of allometric variation associated with growth, only adult individuals were included in the analyses. Age determination was based on complete maxillary teeth and morphological measures (Frynta et al. 1992, 2001). ANOVA and pair-wise comparisons were used to compare mean measures among populations and between groups. Data were first checked for normality (Shapiro-Wilk test) and homogeneity of variances (Levence s test), and log-transformed whenever necessary. Univariate and bivariate analyses were performed to define phenetic differentiation between *Calomyscus* species. The significance level for all statistical tests was set at *p* = 0.05. Principal Components Analyses
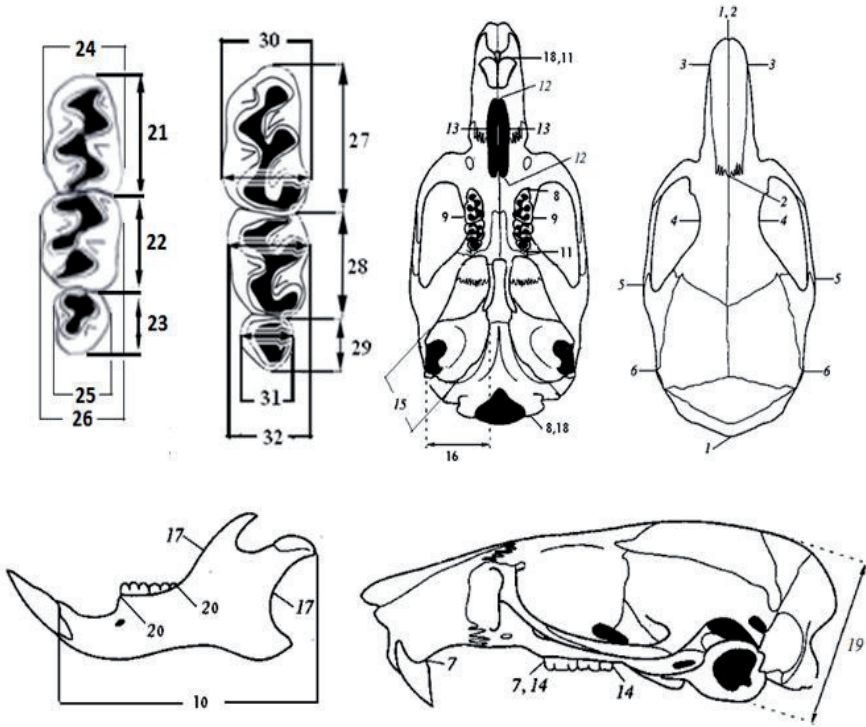
Figure 2 - Cranial, mandibular and dental measurements:1- Occl; 2- Nasl; 3- Nasw; 4- Intw; 5- Zygw; 6- Brb; 7- Dial; 8- M1co; 9- M1M1; 10- Madl; 11- Patl; 12- Forl; 13- Forw; 14- Mxl; 15- Tbw; 16- Tbl; 17- Madh; 18- Cbl; 19- Brh; 20- Mnl; 21- M/1l; 22- M/2l; 23- M/3l; 24- M/1w; 25- M/2w; 26- M/3w; 27- M1/l; 28- M2/l; 29- M3/l; 30- M1/w; 31- M2/w; 32- M3/w.

(PCA, with varimax rotation) and Canonical Discriminant Analyses (CDA) were applied to the 32 variables. Euclidian distances were computed and the corresponding distance matrix summarized as phenogram by UPGMA (Unweighted Pair-Group Arithmetic Average) algorithm. SPSS version 16 and PAST Packages (version 1.89) were used for all statistical procedures.

RESULTS

As no significant difference in external, cranial, mandibular and dental measures was found between males and females by One-Way ANOVA df=1 (p ≥ 0.247), the two sexes were combined for the successive statistical analyses. Except for Tbw, HBL, TL, FL, Intw, Brb, M1M1, Forw, Tbl, M3/l and M/3l, all variables (*Tab. 1*) varied significantly among the four species. Tukey tests showed which variables differed between pairs of species (*Tab. 2*).

On average, all cranial measurements were significantly larger in *C. grandis* than in the other species(p < 0.05), except for Brh (p =0.075), while the opposite oc curred for *C. hotsoni*, except for Zygw, Cbl and M1co (*Fig. 3*). *C. bailwardi* showed the smallest dental

Table 1 - Mean ± SE for 4 external, 17 cranial, 3 mandibular and 12 dental measurements of four *Calomyscus* species from Iran.

Variables	*C. hotsoni* n = 7	*C. bailwardi* n = 22	*C. elburzensis* n = 47	*C. grandis* n = 7
HBL	78.86±0.67	73.86±2.07	76.00±1.70	79.00±1.98
TL	93.14±1.51	90.29±1.55	91.42±0.60	89.17±0.54
EL	19.71±0.18	19.57±0.36	18.27±0.19	19.17±0.30
FL	20.71±0.42	19.71±0.42	20.00±0.12	20.17±0.30
Occl	24.70±0.30	24.65±0.15	25.00±0.07	26.40±0.29
Cbl	22.00±0.03	21.91±0.16	21.70±0.07	23.00±0.00
Zygw	12.20±0.17	12.16±0.08	12.40±0.05	12.80±0.11
Intw	4.11±0.08	4.11±0.04	4.16±0.02	4.09±0.03
Brb	11.00±0.01	10.91±0.06	10.90±0.04	11.00±0.01
Nasl	9.72±0.28	9.81±0.12	9.98±0.05	10.7±0.36
Dial	6.18±0.09	6.55±0.05	6.64±0.04	7.19±0.12
Forl	4.96±0.13	4.97±0.07	5.05±0.04	5.91±0.07
Tbl	6.42±0.07	6.35±0.06	6.34±0.04	6.47±0.03
Tbw	4.28±0.10	4.18±0.05	4.27±0.03	4.24±0.07
Maxl	3.46±0.02	3.33±0.03	3.41±0.02	3.38±0.02
Mnl	3.34±0.04	3.24±0.03	3.34±0.01	3.28±0.02
Brh	8.10±0.03	8.23±0.05	8.18±0.03	7.84±0.07
Nasw	3.14±0.04	3.30±0.03	3.29±0.03	3.58±0.01
Madl	13.88±0.14	13.51±0.09	13.60±0.04	14.50±0.19
M1M1	4.74±0.06	4.60±0.02	4.92±0.22	4.67±0.03
M1co	14.12±0.05	13.92±0.09	13.90±0.04	14.50±0.12
Patl	11.09±0.07	11.27±0.07	11.30±0.04	12.00±0.07
Madh	3.90±0.05	3.63±0.02	3.70±0.02	3.74±0.01
Forw	1.84±0.02	1.84±0.04	1.86±0.02	2.10±0.03
M/1w	1.01±0.01	0.96±0.01	1.01±0.02	0.96±0.01
M/2w	1.03±0.01	1.02±0.01	1.07±0.01	1.03±0.01
M/3w	0.70±0.02	0.68±0.01	0.72±0.01	0.69±0.01
M/1l	1.43±0.03	1.36±0.01	1.44±0.01	1.44±0.01
M/2l	1.22±0.02	1.15±0.01	1.20±0.01	1.18±0.01
M/3l	0.72±0.01	0.73±0.01	0.73±0.01	0.71±0.01
M1/w	1.08±0.01	1.04±0.01	1.12±0.01	1.06±0.01
M2/w	1.09±0.01	1.02±0.01	1.07±0.01	1.09±0.01
M3/w	0.73±0.01	0.71±0.01	0.74±0.01	0.78±0.02
M1/l	1.60±0.01	1.59±0.02	1.64±0.01	1.58±0.03
M2/l	1.15±0.00	1.11±0.01	1.16±0.01	1.21±0.01
M3/l	0.63±0.02	0.58±0.01	0.59±0.01	0.61±0.03

Table 2 - Significantly different variables between four *Calomyscus* species; pairwise Tukey test; $p < 0.05$; see the methods for abbreviations.

| Pairwise species	Different variables
C. bailwardi - C. grandis	All cranial characters, M/1l, M/1w, M2/l, M2/w, M3/w
C. bailwardi - C. hotsoni	M/1l, M/2l, M1/w, M2/w, Nasl, Dial
C. bailwardi - C. elburzensis	All dental characters, El, Zygw, Nasl, Dial, Madh
C. grandis - C. hotsoni	Forl, Brh, Nasw, Madl, Patl, Occl, Nasl, Dial
C. grandis- C. elburzensis	Zygw, M1/l, M2/l, M3/w, Nasl, Dial
C. hotsoni - C. elburzensis	M1/w, Madh, Dial, Nasl, El
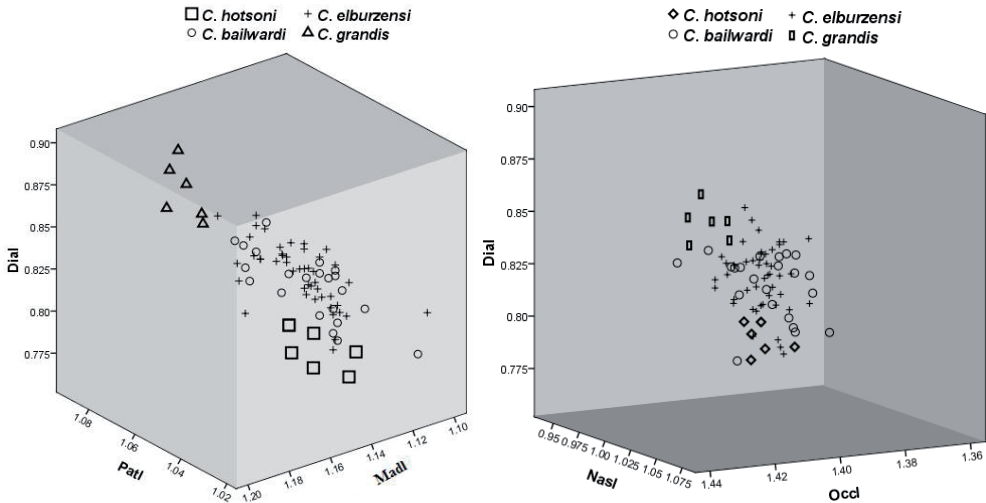
Figure 3 - Three-dimensional plots of Dial, Patl, Madl (left) and Dial, Nasl, Occl (right).

measurements. External measures did not differ among the four species, except for ear length, which was significantly ($p < 0.05$) smaller in *C. elburzensis* than in the other three species.

Through the application of PCA seven components were selected based on their eigenvalues. These components explained 77.08 % of total variance. PC1 explained 25.96 % of the total variance and was positively associated to dental variables (M1/w, M/3w, M/2w, M/1l) and, secondarily, to maxillary and mandibular lengths (Mxl, Mnl). PC1 clearly separated *C. grandis* from the other three species (*Fig. 4*). PC2 explained 19.04 % with the highest positive loading for M/1w, while 11.41 % of variance was explained by PC3.

Canonical Discriminant Functions (CDF) 1 and 2 explained 50,1% and 34.3%, respectively, of the total variance (*Tab. 3*). Wilks lambda was significant for all functions (*Tab. 4*). CDF1 separated *C. grandis* from the other species and also *C. bailwardi*

Table 3 - Within-groups correlations between discriminating variables and standardized canonical discriminant functions in four species of *Calomyscus*; *highest absolute correlation; only the highest values have been used for stepwise discriminant analyses; see the methods for abbreviations.

	Function		
	1	2	3
Eigen-Value	3.706	2.538	1.152
% of variance	50.1	34.3	15.6
Canonica correlation	0.887	0.847	0.732
Madl	0.360*	0.001	-0.020
Forl	0.337*	0.125	-0.212
Occl	0.331*	0.165	-0.162
M1co	0.253*	-0.090	-0.078
Brh	-0.252*	-0.041	0.013
Col	0.226*	-0.066	-0.170
M3/w	0.208*	0.168	0.078
Nasl	0.191*	0.154	-0.129
M/2w	-0.059	0.366*	0.297
M2/l	0.278	0.308*	0.226
Zygw	0.159	0.213*	0.004
M1/l	-0.057	0.173*	0.162
M2/w	0.194	0.222	0.430*
Madh	0.154	-0.040	0.416*
M/1w	-0.033	0.145	0.412*
Dial	0.212	0.285	-0.411*
M1/w	-0.041	0.337	0.389*
M/2l	0.064	0.139	0.378*
M/1l	0.122	0.315	0.369*
Nasw	0.177	0.098	-0.342*
Patl	0.271	0.153	-0.308*
Mnl	0.012	0.183	0.304*
Maxl	0.044	0.102	0.302*
M/3w	-0.035	0.179	0.251*

Table 4 - Wilks statistics for each canonical discriminant function.

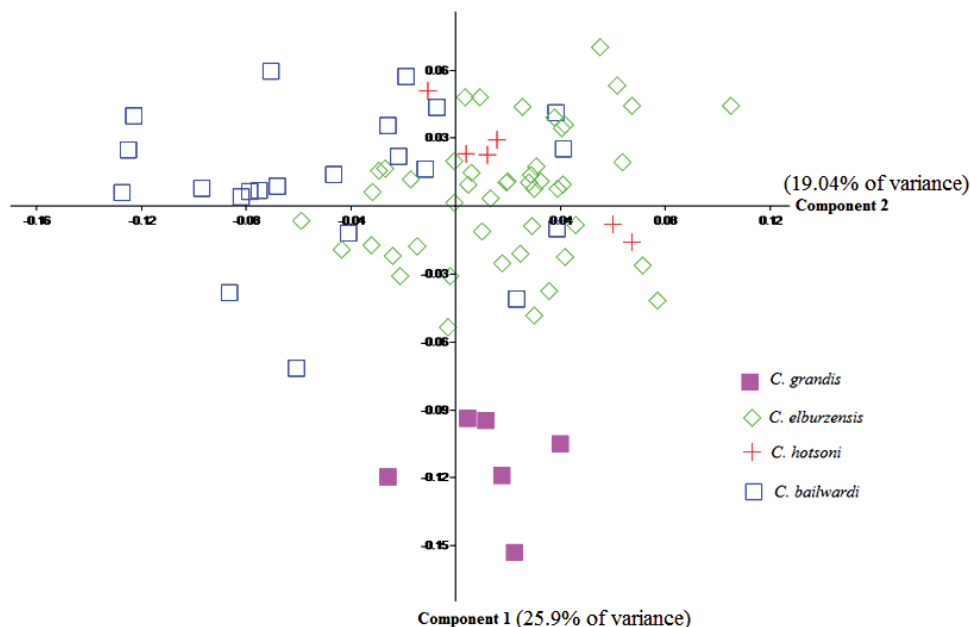| Test of function	Wilks' Lambda	χ^2	df	*p*
1 through 3	0.028	229.039	72	0.000
2 through 3	0.131	129.910	46	0.000
3	0.465	49.045	22	0.001

Figure 4 - Plot of the four *Calomyscus* species in relation two the first two Principal Components extracted from 32 morphometric variables.

from *C. hotsoni*. CDF2 separated *C. elburzensis* from *C. hotsoni* and, with a little overlap, *C. bailwardi* (*Fig. 5*). In total, 93.7 % of cases was correctly classified (Tab. 5). Based on skull and dental characters, the phenogram built from Euclidean distances showed that *C. bailwardi* and *C. elburzensis* are close morphometrically and formed a single cluster, to which *C. hotsoni* was closer than *C. grandis*. (*Fig. 6*).

The scatter plot of DF1 *vs*. DF2 showed overlap between *C. bailwardi* and *C. elburzensis* through Yazd and Kerman populations (*Fig. 7a*), while removing those specimens from the analysis no overlap occurred (*Fig. 7b*). CDA performed with Yazd population as a separate group showed that the specimens of this province were closer to *C. elburzensis* than to any other group (Fig. 8).

DISCUSSION

Our results show that a combination of several cranial and dental measures can discriminate four Iranian *Calomyscus* species, while neither the use of only a few measures, nor external features do help to separate their populations. Considering the geographical range of *Calomyscus* species (Carleton and Musser 2005), we expected Yazd specimens to belong to *C. bailwardi*. Nonetheless, according to the results of PCR-RFLP analyses, Sahebjam et al. (2009) recommended that Yazd specimens should be included in *C. elburzensis*. The chromosome number of specimens from Yazd is 44 (author's unpublished data), the same as of *C. elburzensis* individuals from Kopet Dag Mountains (Carleton and Musser 2005).
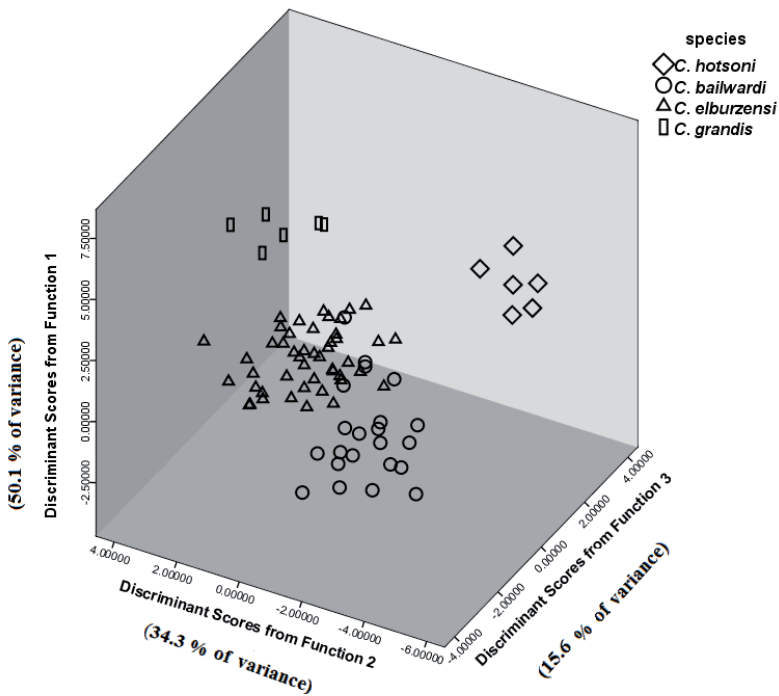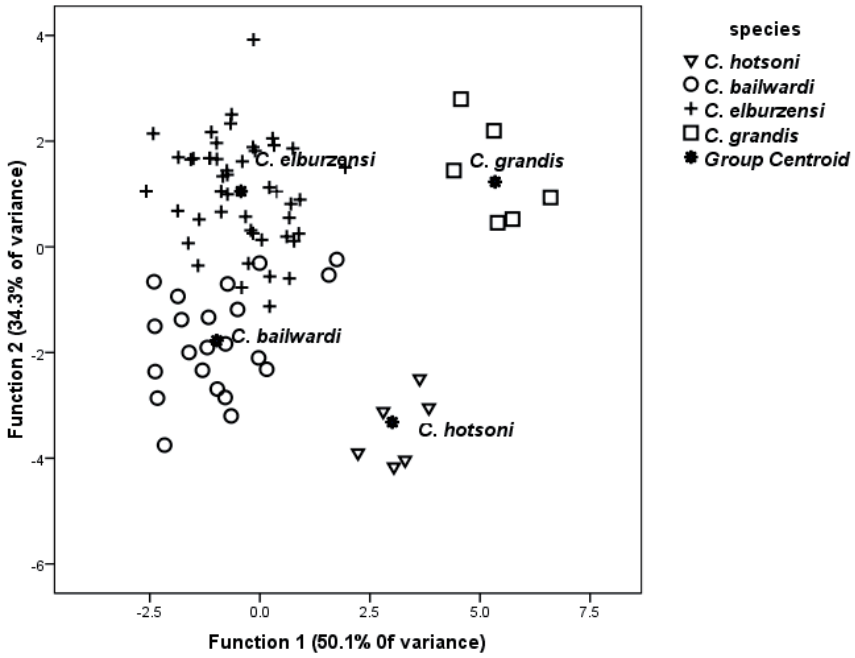
Figure 5 - Plots of the four *Calomyscus* species in relation to the two first canonical discriminant functions (top) and Discriminant Scores from Function1, 2 and 3 (bottom).

Table 5 - Percentage of cases correctly classified by canonical discriminant functions.

	Predicted group membership			
Species	*C. hotsoni*	*C. bailwardi*	*C. elburzensis*	*C. grandis*
C. hotsoni (n = 5)	100.0%	0	0	0
C. bailwardi (n = 22)	0	86.4%	13.6%	0
C. elburzensis (n =47)	0	4.3%	95.7%	0
C. grandis (n = 5)	0	0	0	100.0%

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          0         5        10        15        20        25
          +---------+---------+---------+---------+---------+

C. elburzensis    ———————
                         |————————————————————————————————
   C. bailwardi    ———————                                |
   C. hotsoni      ——————————————————————————————————     |
                                                     |—————
   C. grandis      ——————————————————————————————————
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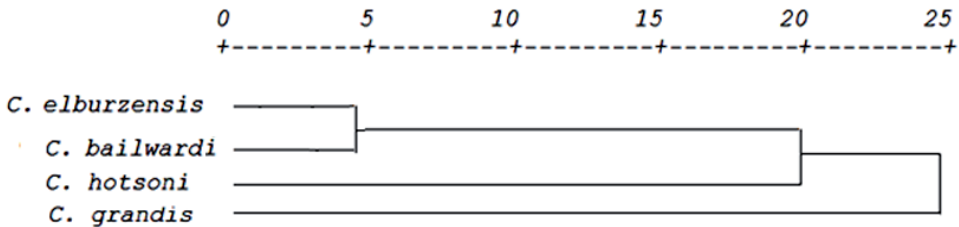
Figure 6 - Morphometric relationship of the four *Calomyscus* species based on Euclidean distances.

Also considering, we argue that specimens from Yazd may be assigned to *C. elburzensis.* As a consequence, the known range of *C. elburzensis* in Iran may expand from the mountains of northern and north-eastern Iran to Yazd Province.

Within the genus *Calomyscus,* several distinct karyotypes have been identified in Iran (Graphodatsky et al. 2000; Malikov et al. 2001; Esmaeili et al. 2008; unpublished data). Lebedev et al. (1998) demonstrated that distinct morphological clusters correspond to these karyotypic differences. Our results disagree with those of Lebedev and collaborators. As an example, despite *C. grandis* was clearly separated from the other three species, its chromosome number (2n = 44) is the same of *C. elburzensis* from Iran, *C. mystax* from the Balkan Region of Turkmenistan (Graphodatsky et al. 2000; Malikov et al. 2001) and *C. hotsoni* from southeastern Iran (author's unpublished data). On the other hand, despite the wide variation in the chromosome number of *C. elburzensis* and *C. bailwardi,* this two species are morphologically closer than other species.

We conclude that morphometric studies can be useful to investigate the taxonomy of this interesting genus. Nonetheless, molecular and cytogenetic studies would be advisable to answer to many questions about the origin and phylogeography of *Calomyscus* species.
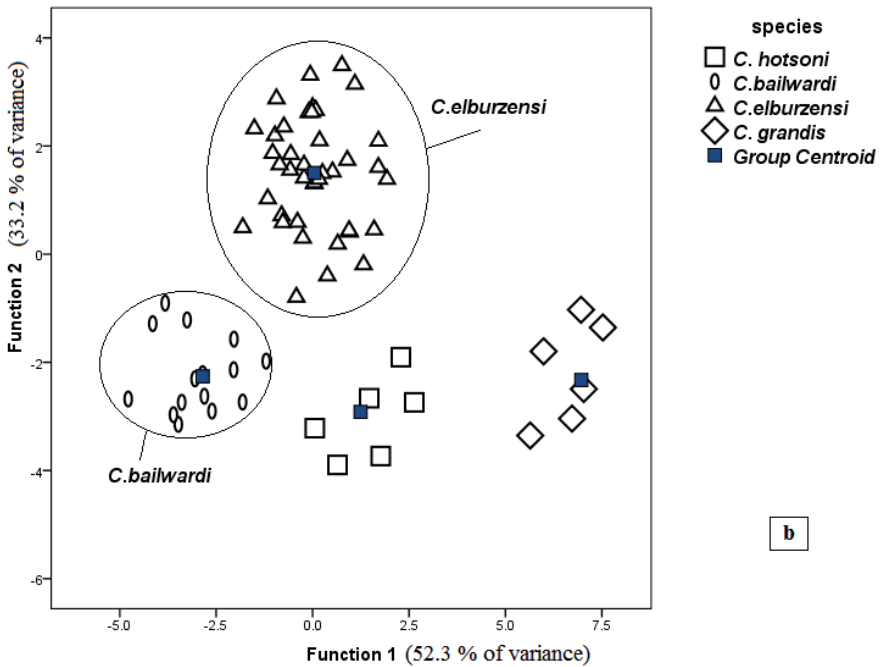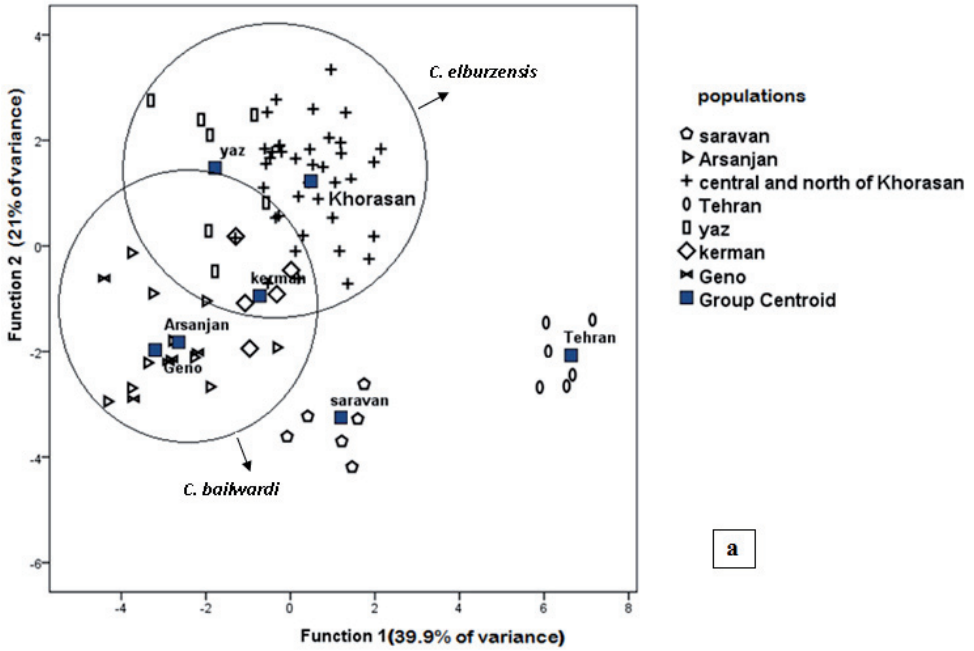
ACKNOWLEDGEMENTS

Figure 7 - Plot of *Calomyscus* populations in relation to two canonical discriminant functions for individuals of seven populations of *Calomyscus* species before (a) and after (b) removing Yazd and Kerman specimens from the analysis.
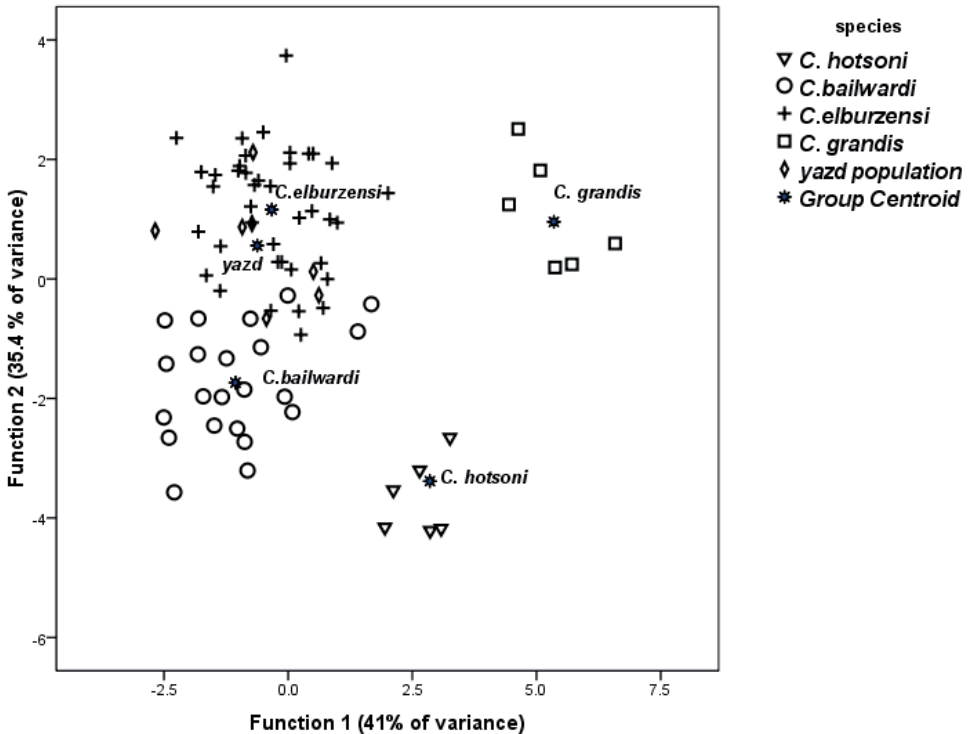
Figure 8 - Plot of *Calomyscus* species and Yazd population (as a separate group) in relation to the two first canonical discriminant functions.

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