Iranian Journal of Animal Biosystematics (IJAB)
Vol.19, No.1, 1-12, 2023
DOI: 10.22067/ijab.2023.81166.1051

IJAB
ISSN: 1735-434X (print); 2423-4222 (online)

RESEARCH ARTICLE Open access

Geometric morphometric analysis in nine species of genus *Hottentotta* (Birula 1908) (Arachnida: Scorpiones) from Iran

Masoumeh Amiri[1], Mansour Aliabadian[1,2], Roohollah Siahsarvie[1,3], Fereshteh Ghassemzadeh[1], Omid Mirshamsi[1,2*]

[1] *Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Iran*
[2] *Research Department of Zoological Innovations (RDZI), Institute of Applied Zoology, Faculty of Science, Ferdowsi University of Mashhad, Iran*
[3] *Rodentology Research Department (RRD), Institute of Applied Zoology, Faculty of Science, Ferdowsi University of Mashhad, Iran*

(Received: *16 February 2023*; Accepted: *10 April 2023*)

Abstract
Hottentotta Birula, 1908 is one of the most widely distributed buthid scorpions, with more than 40 described species from Africa, across the Middle East, to India. Currently, this genus is represented by ten morphological species in Iran (*H. akbarii*, *H. jayakari*, *H. juliae*, *H. khoozestanus*, *H. lorestanus*, *H. navidpouri*, *H. saulcyi*, *H. schach*, *H. sistanensis* and *H. zagrosensis*), all of which are endemic or subendemic in Iran. The members of this genus have not been properly studied from the taxonomic point of view. A tool that could contribute to scorpions' taxonomic studies is geometric morphometry, which is defined as the fusion between geometry and biology. In this study, the size and shape variations in sternocoxal structure in *Hottentotta* populations have been examined using the geometric morphometric method. The goal was to analyze the isometric size and conformation in nine species of *Hottentotta*. 100 individuals of *Hottentotta*, collected from different parts of Iran during 2018-2020, were photographed. Coordinate (x, y) configurations from landmarks were registered in sternocoxal structures. Geometric morphometric analyses were performed using R language. The results clearly showed divergence in the shape and size of sternocoxal structure among the studied taxa. However, the major shape changes were associated with *H. akbarii* which has a larger size of sternocoxal structure and a narrower sternum, shorter coxa II-III, and longer coxa IV.

Key words: *Coxae, Geometry, Hottentotta, Landmark, Shape variation, Sternum.*

INTRODUCTION

Geometric morphometrics (GM) is an alternative approach to the "traditional" method of measuring linear measurements and utilizing multivariate statistical analyses of these data. Landmark-based geometric morphometrics has been used frequently to quantify biological shape, shape variation, and covariation of shape with other variables (Torres & Miranda Esquivel, 2016; Nedeljković *et al*., 2015; Fox *et al*., 2020). The major aim of a morphometric analysis is to recover and recombine information in such a way as to obtain the most accurate biological insights on the variations in morphological structures (Navarro *et al*., 2004).

Corresponding Author: mirshams@um.ac.ir

A considerable number of studies have confirmed the significance of studies on shape variations in systematics and evolutionary biology (Pepinelli *et al*., 2013). Several studies revealed that GM is a good discriminator for the species in arthropods (Bechara & Liria, 2012; Chursina & Ruchin, 2018; Nedeljković *et al*., 2015; Torres & Miranda Esquivel, 2016). Few studies have been performed using GM for studying morphological structures of scorpions (Bechara & Liria, 2012).

Hottentotta is one of the most widely distributed genera of the family Buthidae, with several species distributed in Africa, the Arabian Peninsula, the Middle East, India, and Pakistan (Fet *et al*., 2000; Kovařík, 2007). This genus has ten taxonomically species in Iran, including: *H. akbarii* Yagmur *et al*., 2022, *H. jayakari* (Pocock, 1895), *H. juliae* Kovarik *et al*., 2019, *H. khoozestanus* Navidpour *et al*., 2008, *H. lorestanus* Navidpour *et al*., 2010, *H. navidpouri* Kovarik *et al*., 2018, *H. saulcyi* (Simon, 1880), *H. schach* (Birula, 1905), *H. sistanensis* Kovarik et al., 2018, and *H. zagrosensis* Kovarik, 1997.

The species delimitation in this taxon was mainly based on the traditional morphological characters, such as general coloration and carination of body segments (Barahoei *et al*., 2020). Members of the genus *Hottentotta* have not been so far comprehensively studied and the validity of some species, described based on singletons (e.g., *H. akbarii*, *H. khoozestanus* and *H. lorestanus*) needs further studies. Furthermore, some widely distributed species such as *H. saulcyi* shows extensive intraspecific variations in both morphological and morphometric characteristics, suggesting that this species might be a complex species. GM, with analyzing shape which reflects genetic variation more than what classic measurements do, is an approach that could contribute to scorpion taxonomy (Bookstein, 1982; Bechara & Liria, 2012). The importance of the coxosternal structure, as a useful attribute in discrimination of buthid scorpions, has been discussed by Bechara & Liria, (2012).

The aim of this study is to investigate variations in the shape and size of the sternocoxal structure for the nine species of the genus *Hottentotta* distributed in Iran including: *H. akbarii*, *H. jayakari*, *H. juliae*, *H. khoozestanus*, *H. navidpouri*, *H. saulcyi*, *H. schach*, *H. sistanensis*, and *H. zagrosensis*. The present investigation focused on using landmark-based geometric morphometrics of the sternocoxal structure as a new method to contribute to the knowledge of taxonomic studies.

MATERIAL AND METHODS

Specimens were collected by ultraviolet (UV) light detection at night between 2018-2020 from different localities in Iran (Fig. 1). Newly sampled material**,** were transferred to 75–96% ethanol, and deposited in the Zoological Museum of Ferdowsi University of Mashhad (ZMFUM). A total of 100 adult individuals belonging to nine species of *Hottentotta* from Iran were studied (Table 1).

Sternocoxal area (coxae II, III, and IV and sternum) was photographed using a Canon SX150 camera on a stand with a scale close to each specimen and at the same level as the sternocoxal structure to standardize the size digitization. Thirteen two-dimensional homologous landmarks (Bechara & Liria, 2012) were determined on the surface of the structure (Fig. 2), using Tpsdig 2.32 program (Rohlf, 2018). Landmarks configurations were scaled to unit centroid size and superimposed using the partial procrustes generalized least-squares method (Gower, 1975; Rohlf, 1990) using R language (R Development Core Team, 2019) following Claude, (2008). Two replicates of all individuals were photographed and digitized to calculate the measurement error. The mean of the two replicates of each individual was used in the subsequent analyses.

The size was computed as the centroid size, which is the square root of the sum of squares of the distances between each landmark and the centroid of the configuration of landmarks (Bookstein, 1991). The coordinates of the superimposed configurations were projected on the Euclidean tangent space and used as shape data for subsequent analyses. A one-way analysis of variance (ANOVA) using the specimen as a factor was performed on the centroid size of both replicates to estimate the measurement error in size (Yezerinac *et al*., 1992). Similarly, a Procrustes ANOVA (Klingenberg *et al*., 2002) was performed on the shape data taking the specimen effect into account to calculate the percent of measurement error relative to individual variation (Claude, 2008; Siahsarvie *et al*., 2012). To examine the interspecific and inter-sexual size differences, a type-II two-way analysis of variance (ANOVA) was

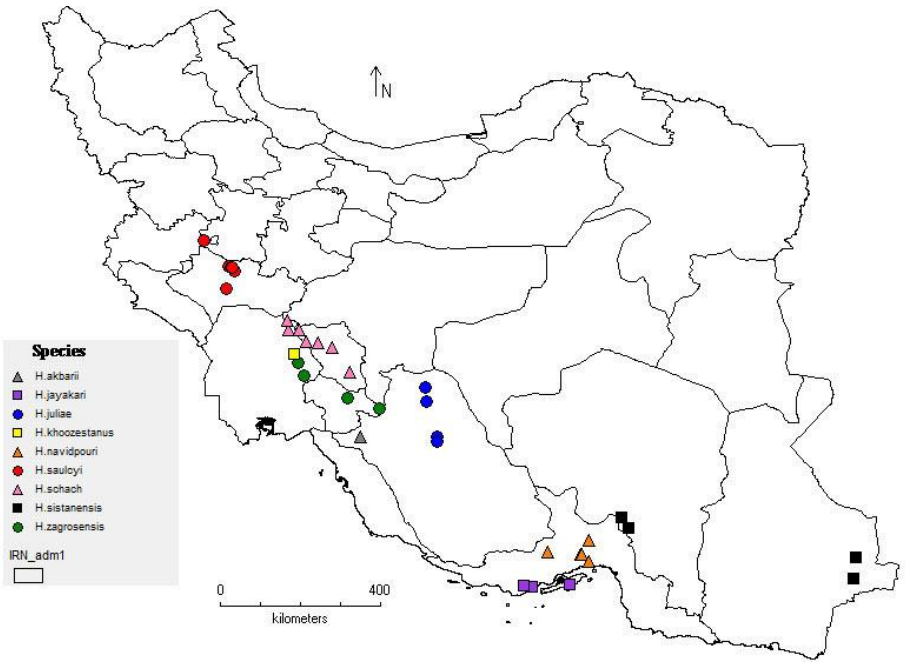


FIGURE 1. Map representing the localities of the Specimens studied in Iran.

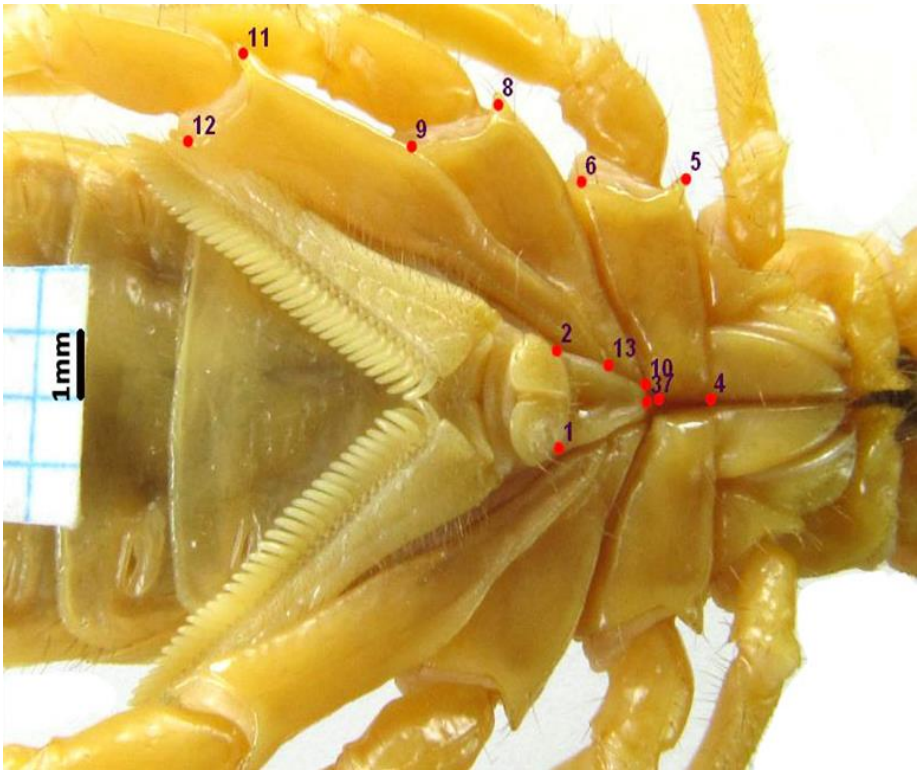


FIGURE 2. Position of 13 landmarks captured on the coxae and sternum.

carried out on the size based on two factors, sex and species, followed by Tukey's Post-Hoc Honestly Significant Difference (HSD) for pairwise comparisons. A box plot was performed on the centroid size to illustrate the size difference among species. For shape, the interspecific and inter-sexual variation was evaluated using two-way multivariate analysis of variance (MANOVA) on shape data using factors of species and sex. The analysis was followed by a pairwise MANOVA to determine the species that differ in the shape of the sternocoxal structure, significantly. As the sexual dimorphism was significant for shape, the procrustes data were orthogonally projected on the sex discriminant space, extending the Burnaby method (Burnaby, 1966). The data, hereafter called sex-corrected, were used for subsequent shape analyses. A linear discriminant analysis (LDA) was performed on the sex-corrected shape data. Species with less than five specimens were omitted from the discriminant analyze, and were, a posteriori, projected onto the discriminant axes. A neighbor-joining (NJ) tree based on Mahalanobis distance among species was estimated using the sex-corrected shape data to show the magnitude of the interspecific shape differences.

TABLE 1. Specimens, populations and provenance data (country, province or governate and abbreviated locality) of *Hottentotta* species deposited in the collections of the Zoological Museum of Ferdowsi University of Mashhad (ZMFUM), Iran, used for geometric morphometrics analysis.

Species	Locality	Sex	Museum code
H. akbarii	IRAN: Fars, Nurabad	1♂	ZMFUM-scr-2090
H. jayakari	IRAN: Hormozgan, Bandar Lengeh	1♂	ZMFUM-scr-1995
	IRAN: Hormozgan, Gheshm	1♂, 3♀	ZMFUM-scr-1972-1975
	IRAN: Hormozgan, Moalem	3♂, 6♀	ZMFUM-scr-1935-1942, 1951
H. juliae	IRAN: Fars, Abadeh	1♂, 1♀	ZMFUM-scr-1954-1955
	IRAN: Fars, Eghlid	5♂, 5♀	ZMFUM-scr-1956-1965
	IRAN: Fars, Shiraz, Marvdasht	1♂, 1♀	ZMFUM-scr-2037,2038
	IRAN: Fars, Shiraz, Sivand road	1♀	ZMFUM-scr-2050
H. khoozestanus	IRAN: Khuzestan, Behbahan	1♀	ZMFUM-scr-2015
H. navidpouri	IRAN: Hormozgan, Bandarabas, Chahchakor village	3♀	ZMFUM-scr-1952,1996,2043
	IRAN: Hormozgan, Bandarabas, Geno Mountain	2♂, 5♀	ZMFUM-scr-1966-1971,1997
	IRAN: Hormozgan, Bandarabas, Roydar	1♂	ZMFUM-scr-2071
	IRAN: Hormozgan, Bandarabas, Siahoo village	1♂	ZMFUM-scr-2072
H. saulcyi	IRAN: Lorestan, Aleshtar	8♂, 4♀	ZMFUM-scr-1907, 1908. 1910-1913, 1976, 2013, 2014, 2046, 2062, 2063
	IRAN: Kermanshah, Sahneh	3♂, 6♀	ZMFUM-scr-1921-1929
H. schach	IRAN: Chaharmahal and Bakhtiari, Ardal	2♀	ZMFUM-scr-1989,1990
	IRAN: Chaharmahal and Bakhtiari, Lordegan	1♂	ZMFUM-scr-1988
	IRAN: Chaharmahal and Bakhtiari, Shahrekord	6♂, 1♀	ZMFUM-scr-2031-2035, 2047,2070
H. sistanensis	IRAN: Kerman, Mourdan	4♂, 5♀	ZMFUM-scr-2073,2075, 2076,2077,2078,2080,2081,2082
	IRAN: Sistan and Baluchistan, Saravan	1♂, 5♀	ZMFUM-scr-1943-1947,1949
H. zagrosensis	IRAN: Khuzestan, Baghmalek, Baraftab village	2♂, 3♀	ZMFUM-scr-1983-1987
	IRAN: Khuzestan, Izeh	1♀	ZMFUM-scr-2036
	IRAN: Kohgiluyeh and Boyer-Ahmad, Yasouj, Cheshmeh Chenar village	2♀	ZMFUM-scr-1992,1993
	IRAN: Kohgiluyeh and Boyer-Ahmad, Yasouj, Lodab village	2♂, 1♀	ZMFUM-scr-1931,1933-1934

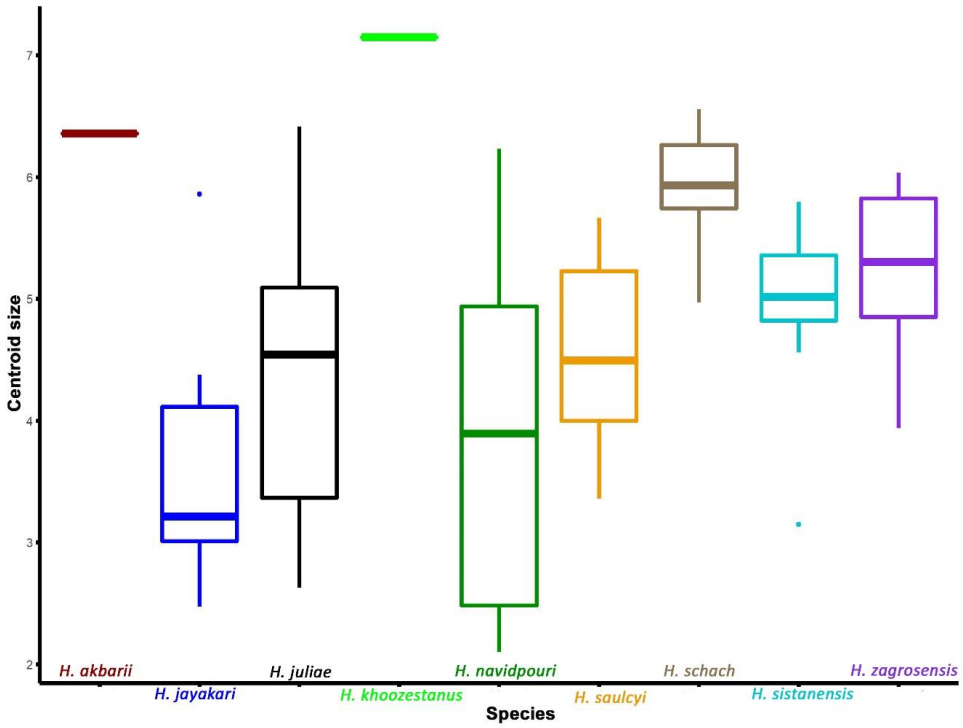


FIGURE 3. Boxplots of sex-corrected centroid size for species of *Hottentotta* occurring in Iran.

All analyses on landmarks were conducted using R 4.2.1 (R Development Core Team, 2022). The *MASS* package (Venables & Ripley, 2002) was used for LDA, the *ape* package (Paradis & Schliep, 2019) for NJ, the *ggplot2* package (Wickham, 2016) for plots, the *RVAideMemoire* package (Hervé & Hervé, 2020) for pairwise MANOVA, the *Agricolae* package (Mendiburu, 2021) for Tukey-HSD and *Pixmap* package (Bivand, 2022) for reading image files.

RESULTS

The measurement error was 4.4% for size and 9.8% for shape variation, which is significantly lower than inter-individual variation.

Size analysis

The type II two-way ANOVA suggests the studied species are significantly different in size (F=8.16; $P<10^{-7}$), but no sexual dimorphism in size of sternocoxal structure is evidenced. (F=0.01; P=0.932). The interaction between species and sex was not statistically significant, either (F=0.55; P=0.768) (Table 2). As a result, we kept both males and females together for size analyses).

Tukey's HSD and box plot analyses on the size of species (Table 3, Fig. 3) revealed that the greatest difference was between *H. jayakari* and *H. khoozestanus*. According to (Table 3), *H. jayakari* and *H. navidpouri* were smaller than *H. khoozestanus*, *H. schach H. sistanensis* and *H. zagrosensis.* Another difference was observed between *H. schach* with *H. juliae*, *H. saulcyi*, *H. navidpouri*, that *H. schach* was larger than other species.

The biggest sternocoxal structure belongs to *H. khoozestanus*, followed by *H. akbarii*, *H. schach*, *H. zagrosensis*, *H. sistanensis*, *H. saulcyi* and *H. juliae*, and the smallest sternocoxal belongs to *H. navidpouri* and *H. jayakari* (Fig. 3). *H. jayakari*, *H. navidpouri* and *H. schach* and other species, no differences have been observed between other species.

TABLE 2. Type II analysis of variance (ANOVA) on sternocoxal structure centroid size based on two factors, sex and species in *Hottentotta* populations occurring in Iran.

	Df	Sum Sq	Mean Sq	F value	**Pr (>F)**
species	8	55.50	6.938	8.160	**4.22e-8**
sex	1	0.01	0.006	0.007	0.932
Species * sex	6	2.81	0.468	0.550	0.768
Residuals	84	71.42	0.850		

TABLE 3. Statistically significant *P* values from pairwise comparisons among species of the buthid scorpion genus *Hottentotta* occurring in Iran, for size using Tukey's Post-Hoc HSD Test (N = 100 specimens). Asterisks indicate significant *P* values: * (0.05–0.01); ** (0.01–0.001); *** (< 0.001).

Species	***P*-value**	**Result**
jayakari-khoozestanus	**	*jayakari<khoozestanus*
jayakari- schach	***	*jayakari< schach*
jayakari- sistanensis	**	*jayakari< sistanensis*
jayakari- zagrosensis	***	*jayakari< zagrosensis*
juliae- schach	**	*juliae< schach*
khoozestanus- navidpouri	*	*navidpouri< khoozestanus*
schach-navidpouri	***	*navidpouri< schach*
sistanensis -navidpouri	*	*navidpouri< sistanensis*
zagrosensis -navidpouri	*	*navidpouri< zagrosensis*
saulcyi-schach	**	*saulcyi< schach*

TABLE 4. Two-way MANOVA of shape variation using sex and species factors on sternocoxal structure of *Hottentotta* occurring in Iran.

	Df	**Pillai**	**approx F**	**num Df**	**den Df**	**Pr (<F)**
Species	8	2.83590	2.6085	128	608	<e-14
Sex	1	0.35212	2.3438	16	69	<e-2
Species * sex	6	1.38105	1.3829	96	444	<e-1
Residuals	84					

TABLE 5. Pairwise MANOVA test on sternocoxal structure among species of the buthid scorpion genus *Hottentotta* occurring in Iran, using sex corrected shape variation. Significant *P* values indicated in boldface.

Species	***akbarii***	***jayakari***	***juliae***	***khoozestanus***	***navidpouri***	***saulcyi***	***schach***	***sistanensis***
jayakari	**0.0209**							
juliae	**0.0209**	**0.0018**						
khoozestanus	0.3750	**0.0471**	**0.0209**					
navidpouri	**0.0236**	**0.0418**	0.0946	**0.0367**				
saulcyi	**0.0172**	**0.0034**	**0.0418**	**0.0172**	**0.0172**			
schach	0.0604	0.1425	**0.0347**	0.4914	**0.0418**	0.0527		
sistanensis	**0.0275**	**0.0228**	**0.0182**	0.1538	**0.0209**	**0.0172**	0.5178	
zagrosensis	**0.0262**	**0.0073**	**0.0172**	0.1534	**0.0172**	**0.0182**	0.9854	0.5901

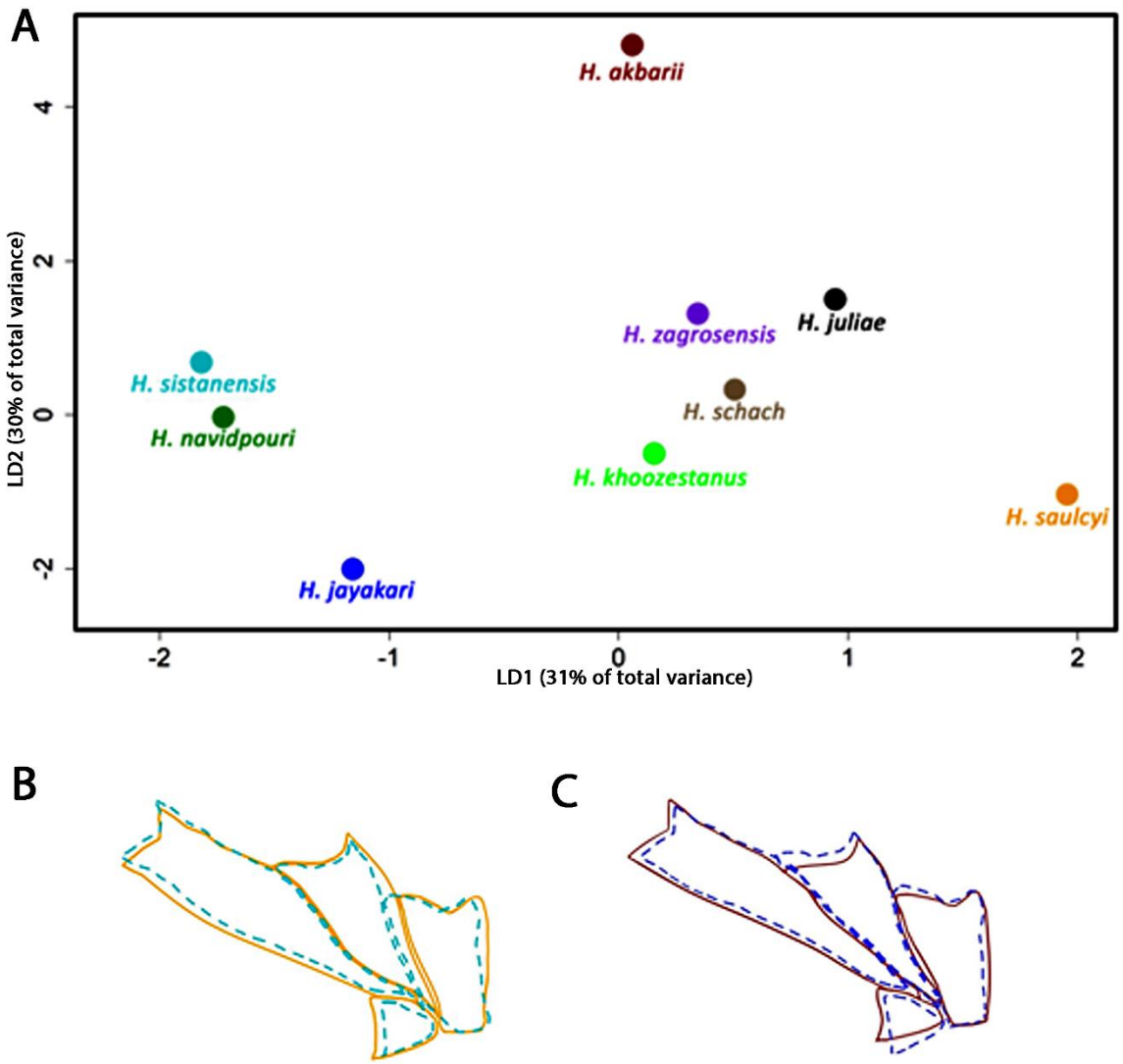


Figure 4- A) Linear discriminant analysis (LDA) of sternocoxal structure in nine species of genus *Hottentotta* in Iran based on sex corrected shape variables, B) Below of the graph is illustrated a schematic view of the sternocoxal structure and the deformation along the axes variation in the shape of the sternocoxal structure in LD1 (the orange continuous line indicates the change in the positive side and the dashed turquoise line indicates the change in the negative side of the first axes), C) Sternocoxal structural shape variation in LD2 (the brown continuous line indicates shape change on the positive side and blue dashed line indicates change on the negative side of second axes).

Shape analyzes

Type II two-way MANOVA species×sex on the shape of the sternocoxal structure (Table 4) showed significant differences both among species ($F=2.83$; $P<10^{-14}$), and between sexes ($F=2.34$; $P<10^{-2}$). The interaction between sex and species was also significant ($F=1.38$; $P<10^{-1}$), suggesting that shape sexual dimorphism does now show the same pattern in different species. As sexual dimorphism was significant and the sex ratios of the captured specimens were not similar in different species, the subsequent analyses were performed on corrected shape data. We could therefore analyze both sexes together.

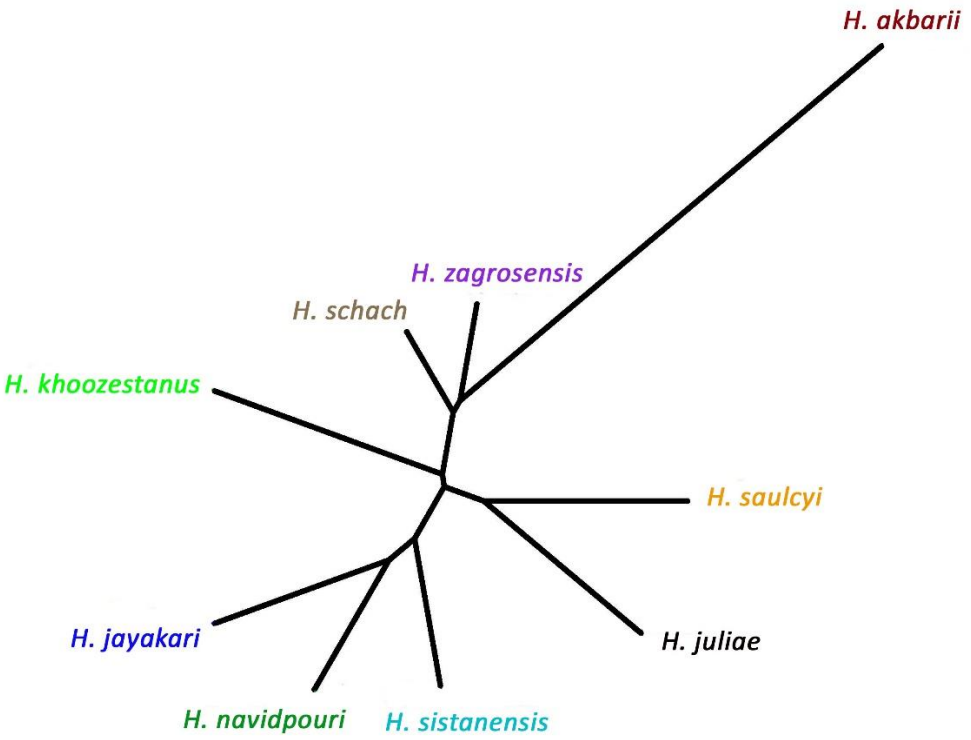


FIGURE 5. Neighbour-joining phenogram based on the Mahalanobis distance between species on the shape of sternocoxal structure in nine species of *Hottentotta*.

Pairwise MANOVA analysis (Table 5) revealed that *H. jayakari*, *H. juliae*, *H. navidpouri*, *H. saulcyi* had the most significant differences with other species. The least number of significant differences was observed between *H. schach* with other species, and it was only with *H. juliae* and *H. navidpouri*.

Figure 4 shows the pattern of shape variation among species in a linear discriminant space on species factor. The first two components of the LDA (LD1 and LD2) explained %31 and %30 of the variance of shape matrix, respectively (Fig. 4A). Figure extreme shape changes were amplified four times (Figs. 4B, C). This analysis was first performed on the species with a population of more than five samples, then the single specimen of *H. akbarii* and *H. khoozestanus* were projected in this space. The first axis discriminant (LD1) mainly distinguishes, *H. jayakari* and *H. saulcyi* from other species, and *H. khoozestanus*, *H. schach*, *H. zagrosensis*, *H. juliae* and *H. akbarii* were located together. Furthermore, *H. navidpouri* and *H. sistanensis* were in negative side of LD1. *H. akbarii* was separated from other species based on LD2.

In the analysis of the shape of the sternocoxal structure in (Fig. 4B), the continuous orange lines indicate the changes in the shape on the positive side of the LD1, and the dashed turquoise lines indicate the changes on the negative side. Based on this axis, *H. sistanensis*, *H. navidpouri* and *H. jayakari*, which are located at negative axes of LD1, the second coxae in these species are wider and the posterior apex is longer, as well as the third coxae is narrower than other species coxae. Moreover, the fourth coxa is shorter at the base and slightly longer at the apex, and in these species, the sternum is smaller than the species on the positive side of the axis (*H. saulcyi*). *H. juliae*, *H. khoozestanus*, *H. schach*, *H. zagrosensis* are intermediate in shape between these two groups.

In figure 4C, brown lines show the shape changes on the positive side of the LD2 axis, and the blue dashed lines show the changes on the negative side of the LD2 axis. *H. akbarii*, which is located at the positive side of LD2, the second coxa in this species is shorter and narrower than the base and middle

part, the third coxa is shorter, the fourth coxa is longer in the apex and base. The sternum is narrower and more elongated than other species. In addition, *H. jayakari* is on the negative side and other species are intermediate between these two groups in shape.

Neighbor-joining phenogram based on the Mahalanobis distance between the species on the shape showed that *H. akbarii* had the greatest distance. *H. khoozestanus* was separated from other species by a long distance. *H. schach* and *H. zagrosensis* were close together. *H. jayakari*, *H. navidpouri* and *H. sistanensis* were also separated by a short distance. Finally, the separation of *H. saulcyi* and *H. juliae* was observed with a greater distance. In general, discrimination is clearly shown in species for both size and shape (Fig. 5).

DISCUSSION

Sexual dimorphism is very common in arachnids as males are generally smaller than females (Prenter *et al*., 1999), which is affected by cannibalism and a higher rate of mortality (Polis, 1990). The results of this study reveal that although males may have smaller body size, but they have similar sternocoxal size as females, or in other words, bigger sternocoxal size compared to their body size. Males and females are, however, sexually dimorphic in terms of shape of this structure. Females have wider sternum and coxa than males.

The plots of LDA (Fig. 4) provide evidence of morphological differentiation in sternocoxal structure of the studied taxa, which is in line with the divergence between morphospecies of the genus *Hottentotta*. The results of geometric morphometric analysis on shape of the sternocoxal structure revealed that *H. akbarii* is distinguished from other species by the large size of the sternocoxal structure and having a narrower and more elongated sternum, shorter coxa II-III, and longer coxa IV. The LD1 axis (Fig. 4), discriminates *H. saulcyi* from other species, by having a wider and larger sternum, narrower coxa II, the wider coxa III, and shorter coxa IV at the apex. *H. jayakari* with the smallest size of the sternocoxal structure (Fig. 3), discriminates from other species by having a wider and shorter sternum, longer and narrower coxa II and III, and shorter coxa IV.

Although, *H. sistanensis* is larger than *H. navidpouri* (Fig. 3), but both species demonstrate similar shape pattern of sternocoxal structure and discriminate from other species on the first LDA axis. Morphologically, *H. navidpouri* and *H. sistanensis* are very close to *H. saulcyi*, and traditionally were considered as different forms of *H. saulcyi* (Navidpour *at al*., 2013; Kovařík *et al*., 2018). These two species can be easily distinguished from *H. saulcyi* based on the shape of chela which is significantly elongated in *H. navidpouri* and *H. sistanensis* (Kovařík *et al*., 2018). The shape of sternocoxal structure in *H. saulcyi* completely differs from other congeners by having a larger sternum and wider coaxes II-IV (Fig. 4). Additionally, movable fingers of pedipalps are longer in *H. sistanensis* than in *H. navidpouri* (Kovařík *et al*., 2018). As a result, the sternocoxal structure provides a reliable morphological feature, which could be applied to differentiating *navidpouri+sistanensis* from *H. saulcyi*.

According to the LDA plot, *H. schach* is the most similar to *H. zagrosensis*. Both species have entirely black coloration. However, *H. zagrosensis* is more densely hirsute and has deeper metasomal segments (Kovařík *et al*., 2019). The results of the current study showed that both species were similar in size (Fig. 3) and shape (Fig. 4).

The largest strenocoxal structure belongs to *H. khoozestanus* based on size (Fig. 3), which is congruent with the description of this species as the largest species in *Hottentotta* genus by Navidpour *et al*, (2008).

Neighbor-joining phenogram (Fig. 5) showed a similar result to the LDA plot (Fig. 4). species that demonstrate similar shape patterns on the LDA plot are close to each other by a short distance on the neighbor-joining phenogram.

In conclusion, the presented study showed a remarkable morphological variability in sternocoxal structure of the studied species. Our study revealed main novel insights into the importance of the interspecific variations of sternocoxal structure as diagnostic features for discrimination of different species of the genus *Hottentotta*.

ACKNOWLEDGEMENT
This study was supported by the Office of Research Affairs, Ferdowsi University of Mashhad, Iran (Project 3/50093).

LITERATURE CITED

Barahoei, H., Navidpour, S., & Aliabadian, M. (2020). Scorpiones of Iran (Arachnida: Scorpiones): Annotated checklist, DELTA database and identification key, *Journal of Insect Biodiversity and Systematics*. *06*(4), 391–492.

Bechara, W. Y., & Liria, J. (2012). Morfometría geométrica en cinco especies de Buthidae y Scorpionidae (Arachnida: Scorpiones) de Venezuela Geometric morphometrics in five species of Buthidae and Scorpionidae (Arachnida Morfometría geométrica en cinco especies de Buthidae y Scorpionida. *Revista Mexicana de Biodiversidad*. (*83*), 421–431.

Bivand, R. (2022). R packages for analyzing spatial data: A comparative case study with areal data. *Geographical Analysis*. *54*(3), 488-518.

Bookstein, F. L. (1982). Foundation of morphometrics. Annual Review of Ecology and Systematics 13, 451-470.

Burnaby T. P. (1966). Growth-invariant discriminant functions and generalized distances. *Biometrics.* 22, 96–110.

Chursina, M. A., & Ruchin, A. B. (2018). A checklist of Bombyliidae (Diptera) from Mordovia , Russia and variation of wing shape in *Bombylius* species. *Journal of Biological Diversity*. *19*(6), 2147–2156. https://doi.org/10.13057/biodiv/d190622

Claude, J. (2008). *Morphometrics with R*. (R. Gentleman, K. Hornik, & G. Parmigiani, Eds.). New York: Springer. https://doi.org/10.1007/978-0-387-77790-0

Fox, N. S., Veneracion, J. J., & Blois, J. L. (2020). Are geometric morphometric analyses replicable? Evaluating landmark measurement error and its impact on extant and fossil Microtus classification. *Ecology and Evolution*, *10*(7), 3260–3275. https://doi.org/10.1002/ece3.6063

Gower J. C. (1975). Generalized procrustes analysis. Psychometrika. 40, 33–50.

Hervé, M., & Hervé, M. M. (2020). Package 'RVAideMemoire'. *See https://CRANR-projectorg/package= RVAideMemoire*.

Klingenberg CP., Burluenga M., Meyer A. (2002). Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution.* 56, 1909– 1920.

Kovařík, F; Yağmur, E & Moradi, M. (2018). Two New *Hottentotta* Species from Iran, with a Review of *Hottentotta saulcyi* (Scorpiones: Buthidae). *Euscorpius*, *265*, 1–14.

https://doi.org/10.18590/euscorpius.2018.vol2018.iss265.1

Kovařík, F Yağmur, E & Fet, V. (2019). Review of *Hottentotta* described by A. A. Birula, with descriptions of two new species and comments on Birula's collection (Scorpiones: Buthidae). *Euscorpius*, *282*, 1–30.

Kovařík, F. (2007). A Revision of the Genus *Hottentotta* Birula, 1908, with Descriptions of Four New Species (Scorpiones, Buthidae). *Euscorpius*, *58*(58), 1–107.

de Mendiburu, F. (2021). agricolae tutorial (Version 1.3-5). *Universidad Nacional Agraria: La Molina, Peru.*

Navarro, N., Zatarain, X., & Montuire, S. (2004). Effects of morphometric descriptor changes on statistical classification and morphospaces. *Biological Journal of the Linnean Society*, *83*, 243–260. Without citation in the txt?

Navidpour, Sh., Kovařík, F., Soleglad, M. E., & Fet, V. (2008). Scorpions of Iran (Arachnida, Scorpiones). Part I. Khoozestan Province. *Euscorpius.* 65: 1–41.

Navidpour, Sh., Soleglad, M.E., Fet, V., & Kovařík, F. (2013). Scorpions of Iran (Arachnida, Scorpiones). Part IX. Hormozgan Province, with Description of *Odontobuthus Tavighiae* Sp. n. (Buthidae). *Euscorpius.* 170: 1–29. Paradis, E. and Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics, 35(3), 526-528.

Nedeljković, Z., Ačanski, J., Dan, M., Obreht-Vidaković, D., Ricarte, A., & Vujić, A. (2015). An integrated approach to delimiting species borders in the genus *Chrysotoxum Meigen*, 1803 (Diptera: Syrphidae), with description of two new species. *Contributions to Zoology*. *84*(4), 285-304.

Pepinelli, M., Spironello, M., & Currie, D. C. (2013). Geometric morphometrics as a tool for interpreting evolutionary transitions in the black fly wing (Diptera : Simuliidae). *Zoological Journal of the Linnean Society*. 169, 377–388. doi: 10.1111/zoj.12065.

Polis, G. A. 1990. *The Biology of Scorpions*. Stanford: Stanford University Press. Pp233.

Prenter, J., Elwood, R. W., & Montgomery, W. I. (1999). Sexual size dimorphism and reproductive investment by female spiders: a comparative analysis. *Evolution*. *53*(6), 1987-1994.

R Core Team. (2022). R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available at: https://www.R-project.org. Accessed 15 January 2022.

Rohlf, F. J. (1990). Rotational fit (Procrustes) methods. In: Rohlf FJ, Bookstein FL, eds. Proceedings of the Michigan morphometrics workshop. Ann Arbor, MI: Museum of Zoology, University of Michigan, 227–236.

Rohlf, F. J. (2018). *TpsDig version 2.31. Ecology and evolution*: (program). New York, NY: Suny at Stony Brook.

Siahsarvie, R., Auffray, J., Darvish, J., Rajabi-maham, H., Yu, H., Agret, S., Claude, J. (2012). Patterns of morphological evolution in the mandible of the house mouse *Mus musculus* (Rodentia : Muridae), 635–647.

Soleglad, M. E., & Fet, V. (2003). The Scorpion Sternum: Structure and Phylogeny (Scorpiones: Orthosterni). *Euscorpius* (5), 1–34. Retrieved from http://www.science.marshall.edu/fet/euscorpius/'

Torres, A., & Miranda-Esquivel, D. R. (2016). Wing shape variation in the taxonomic recognition of species of Diachlorus Osten-Sacken (Diptera: Tabanidae) from Colombia. *Neotropical entomology*. *45*, 180-191.

Wickham, H., Chang, W., & Wickham, M. H. (2016). Package 'ggplot2'. *Create elegant data visualisations using the grammar of graphics. Version, 2*(1), 1-189.

Yezerinac, SM., Loogheed, SC., Handford P. (1992). Measurement error and morphometric studies: statistical power and observer experience. *Systematic Biology*. 41, 471–482.

Iranian Journal of Animal Biosystematics (IJAB)
Vol.19, No.1, 13-21, 2023
DOI: 10.22067/ijab.2022.70517.1012

IJAB
ISSN: 1735-434X (print); 2423-4222 (online)

RESEARCH ARTICLE Open access

A preliminary molecular phylogeny of the genus *Pholcus* in Iran, with notes on taxonomy

Maryam. Hamidian[1], Alireza. Keikhosravi[1], Majid. Moradmand*[2]
[1] *Department of Biology, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran*
[2] *Department of Animal and Plant Biology, Faculty of Biological Sciences and Technology, University of Isfahan, Isfahan, Iran*

(Received: *29 May 2021*; Accepted: *05 October 2022*)

Abstract
Iran is a large country with diverse and unique climate and ecology; therefore, it is expected to discover an exceptional fauna with high species diversity by carefully examining the unknown areas. A few taxonomic studies have been so far conducted on the genus *Pholcus* in Iran. Taxonomic and preliminary phylogenetic evaluation of widespread species of the genus *Pholcus* from Iran is considered in the present study, based on specimens collected from northern and southwestern parts of the country. A molecular study was undertaken on some representatives of species of the *Pholcus phalangioides* species-group (cellar spiders) using newly designed primers with 350 bp of partial fragments of mtDNA gene, cytochrome oxidase subunit 1 (COI). These preliminary molecular data in line with morphological identifications using characters related to the copulatory organs presented a total of five distinct clades of *Pholcus* that four clades were contributed with formerly identified species and one represented a distinct lineage unknown for science.

Key words: *Species delimitation; DNA-based; CO1; morphology; Iran; Pholcus.*

INTRODUCTION

Pholcidae C. L. Koch, 1850 is one of the most species-rich spider families in the world (World Spider Catalog, 2021). This family, known as daddy long leg spiders, is distributed in a wide variety of habitats including urban areas, dark and humid places (e.g., caves, under rocks and in trees hole) (Huber, 2005). The genus *Pholcus* Walckenaer, 1805 with 329 species is the largest genus in this family (World Spider Catalog, 2021). Some of the species of *Pholcus* are either cosmopolitan or synanthropic species (e.g., *Pholcus phalangioides* (Fuesslin, 1775), *Ph. alticeps* Spassky, 1932 (Huber, 2013).

Iran is a country that its biodiversity is not well explored. In addition, due to climate diversity and topology (Amiri & Eslamian, 2010), a significant cryptic and undiscovered biodiversity is expected for the region. In general, study about spiders are limited in Iran, so that most study have been recently conducted on this widly distributed group (Zamani et al., 2021; Mirshamsi,2005). To date, 13 species of *Pholcus* have been reported from Iran, of which twelve were defined as new species or new records by Senglet (1974, 2008). Another species, recorded by Roewer (1955) as *Pholcus opilionoides* (Schrank, 1781), was probably a misidentification (Zamani et al., 2021). Huber (2011) partially revised the Iranian species and classified the listed species into four species-groups. The majority of the list consist of closely related species placed in the *Pholcus phalangioides* species-group. This group [except for the two widely distributed species *Ph. phalangioides* and *Ph. alticeps*] is largely restricted to Iran, and includes: *Ph.*

Corresponding Author: m.moradmand@sci.ui.ac.ir

armeniacus Senglet, 1974, *Ph. caspius* Senglet, 2008, *Ph. elymaeus* Senglet, 2008, *Ph. hyrcanus* Senglet, 1974, *Ph. hystaspus* Senglet, 2008, *Ph. medicus* Senglet, 1974 and, *Ph. persicus* Senglet, 1974.

Molecular data, in particular mtDNA markers has been applied from a couple of decades ago to in taxonomy of spiders and other arachnids (Barrett & Hebert, 2005). The barcode region of mtDNA, cytochrome oxidase 1 (COI), are being widely used in molecular identification, in combination with morphological data, to reconstruct the phylogeny of Pholcidae (Astrin et al., 2006; Dimitrov et al., 2013; Huber et al., 2018; Wang et al., 2018).

Some of the species of the genus *Pholcus*, recently described by Senglet (1974, 2008) from Iran, were based on a few specimens that showed subtle difference in their genital structure and its appendages such as procursus. Senglet (1974) did not access to a grand collection of the *Pholcus* from Iran and his finding were mainly based on the collection he gathered through his extrusion in 1973 from Iran. Therefore, it can be concluded that some species might be wrongly described or recorded from the region and a taxonomic revision using both, molecular and morphological method is demanded to discover the real taxonomy and phylogenetic relationship of the genus in the region. By applying a novel set of primers, we used the barcoding region (CO1) to get a preliminary phylogenetic perspective and DNA-based species delimitation of the Iranian representative of the genus. In addition. Morphological characters were examined to find out if the molecular finding are in line with morphological differences. In addition, intraspecific variability of the cosmopolitan species *Ph. phalangioides* was also considered.

MATERIAL AND METHODS

Morphological study

Specimen were collected from various habitats, including caves in northern and southwestern Iran, and were preserved in ethanol. Some specimens from Georgia were also added to the study. Two to four legs of each specimen were kept in absolute ethanol for molecular studies. The vouchers and tissue samples were deposited in the Zoological Museum, University of Isfahan (ZMUI). The taxonomic characters (male palp and procursus) of vouchers were examined using a stereomicroscope equipped with drawing tube. The morphological identification was based on Huber (2011).

Molecular analyses for species delimitation

Specimens used in the molecular analyses are listed in Table 1. Twenty specimens of five morphospecies were used for molecular examination. DNA was extracted from legs using Animal DNA Isolation Kit (DENA ZIST). Partial fragment of the gene cytochrome oxidase subunit 1 (CO1) was amplified using a couple of primer pairs; a newly designed for this study: Hcophol (5-AGTAAARTARGCDCGWGTRTCTACA-3) and Lcophol (5-GATATAGCTTTTCCTCGWATRAATA-3), and also C1j1718 (5-GGAGGATTTGGAAATTGATTAGTTCC-3) and C1N2191 (5-CCTGGWARAATYARAATATAHACTTC-3) (Simon et al., 1994). For PCR reaction in a total volume of 25 µl, 12 µl Red master mix (DENA ZIST), 5 µl non-diluted DNA, 1_1.25 µl primers and 5_6 µl dH_2O were used. The PCR program consist of one cycle with 36 repeats as follows: 45s denaturation at 95˚C, 60s annealing at 51ºC and 60s extension at 72ºC. The PCR products outsourced to Macrogen, South Korea and partial fragment of CO1 with readable sequences (i.e. 350 bp), was amplified (see Table 1).

Some sequences were retrieved from the GenBank (Table 1) for achievement of a better phylogenetic conclusion. Sequences were aligned and corrected manually using BioEdit ver7.2.5 (Hall, 1999). The Bayesian inference (BI) and maximum likelihood (ML) analyses were conducted using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) and RaxmlGUI 1.3 (Silvestro & Michalak, 2012), respectively. The best evolutionary model was estimated (K81uf+I), according to the Akaike's criterion with JMODELTEST 2.1.4, (Darriba et al., 2012). The BI was conducted with four Markov Chain Monte Carlo (MCMC) were run for = 10 000 000 generations, printing trees every 1000 generations. The first 25 percent of the total trees were excluded (burn-in phase) from the analysis. The ML trees were inferred from 1,000 bootstrap iterations. *Crossopriza* sp. was used as out-group for tree reconstruction.

The haplotype network for *Ph. phalangioides* was constructed using Pop ART ver1.7 (Leigh & Bryant, 2015) to show intraspecific variability the species.

TABLE1. Collected specimens used for DNA sequencing with locality, coordinates, GenBank accession number and collection number.

Species	Number	Locality	Geographic coordinates	Gen Bank Accession number	Voucher number
Ph. phalangioides	1♀	Iran, Mazandran Province- Noor	36.574º N 52.026° E	OQ872153	SD26
Ph. phalangioides	3♀	Iran, Mazandran Province- Nashtarood	36.749° N 51.015° E	OQ872153	SD82
Ph. phalangioides	1♀	Iran, Golestan Province- Alang dareh	36.791° N 54.451º E	OQ872153	SD71
Ph. alticeps	2♂, 1♀	Iran, Golestan Province- Tangerah	37.398º N 55.780º E	OQ872157	SD54
Ph. alticeps	1♀	Iran, Golestan Province- Alang dareh	36.791° N 54.451º E	OQ872157	SD72
Ph. alticeps	1♀	Iran, Golestan Province- Naharkhoran	36.681º N 52.459° E	OQ872157	SD73
Ph. alticeps	1♂, 3♀	Iran, Khorasan Razavi Province- Neyshabore	36.243° N 58.968° E	OQ872158	SD81
Ph. alticeps	1♂, 1♀	Georgia- Oni	42.577° N 43.434° E	OQ872157	SD86
Ph. persicus	1♂, 1♀	Iran, Kohgilluye va Boyer-Ahmad Province- Ankaboot cave	30.723° N 50.845º E	OQ872154	SD29
Ph. persicus	1♀	Iran, Kohgilluye va Boyer-Ahmad Province- Patave cave	30.957º N 51.264° E	OQ872159	SD90
Ph. cf medicus	1♀	Iran, Lorestan Province- Alashtar, Bozorg cave	33.943º N 48.315º E	OQ872155	SD33
Pholcus sp.	1♀	Iran, Fars Province- Palangan cave	35.090º N 46.602º E	OQ872156	SD40
Ph. phalangioides	-	GERMANY, Bonn, ZFMK, basement x. 2003 (J.J. Astrin)	-	DQ667926	pb05-G100
Ph. phalangioides	-	GERMANY, Bonn, ZFMK, basement ii. 2002 (B.A. Huber)	-	DQ667923	pb05-G55
Ph. phalangioides	-	China	-	MH382632	PH006
Ph. phalangioides		USA		EF537067	G526
Ph. phalangioides	-	Burgos, Spain	-	EU215671	CCRUB phalB
Ph. phalangioides	-	China	-	KY467210	PPH1
Ph. phalangioides	-	China	-	KY467211	PHH2
Ph. phalangioides	-	New Zealand	-	KF477299	Isolste9795
Ph. phalangioides	-	SPAIN, Teulada, Moraira, indoors xii. 2003 (J.J. Astrin)	-	DQ667921	pb05-J271
Ph. phalangioides	-	China	-	KY467212	PPH3
Ph. phalangioides	-	Germany	-	KY269460	ZFMK
Ph. phalangioides	-	Near Barcelona, Spain	-	EU215670	CCRUB phalC
Ph. phalangioides	-	USA, SC, Pickens Co., Clemson University iii. 2001 (W. Reeves)	-	DQ667922	pb05-G52
Ph. phalangioides	-	BRAZIL, Est. Alto da Serra xii. 2003 (B.A. Huber)	-	DQ667919	pb05-B26
Ph. phalangioides	-	South Korea	-	JN817071	LEGO5-2
Ph. phalangioides	-	MADEIRA/PORTUGAL, São Vicente, Laranjal ii. 2003 (J.J. Astrin)	-	DQ667924	pb05-J17
Ph. phalangioides	-	MADEIRA/PORTUGAL, São Vicente, Laranjal ii. 2003 (J.J. Astrin)	-	DQ667925	pb05-J17
Ph. alticeps	-	Germany	-	KY269003	ZFMK
Crossopriza lyoni	-	Maharashtra, India	-	KT383729	-

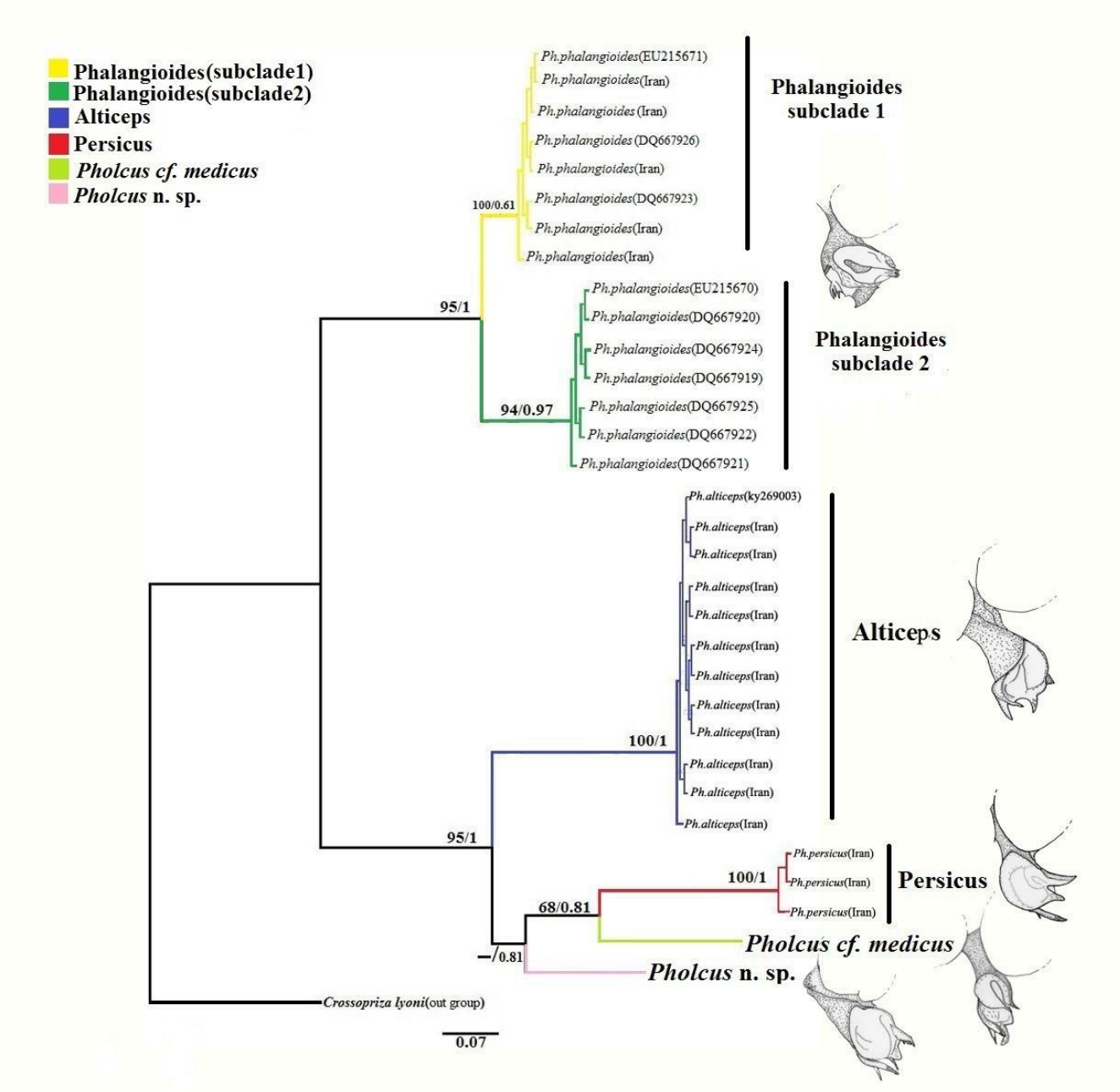
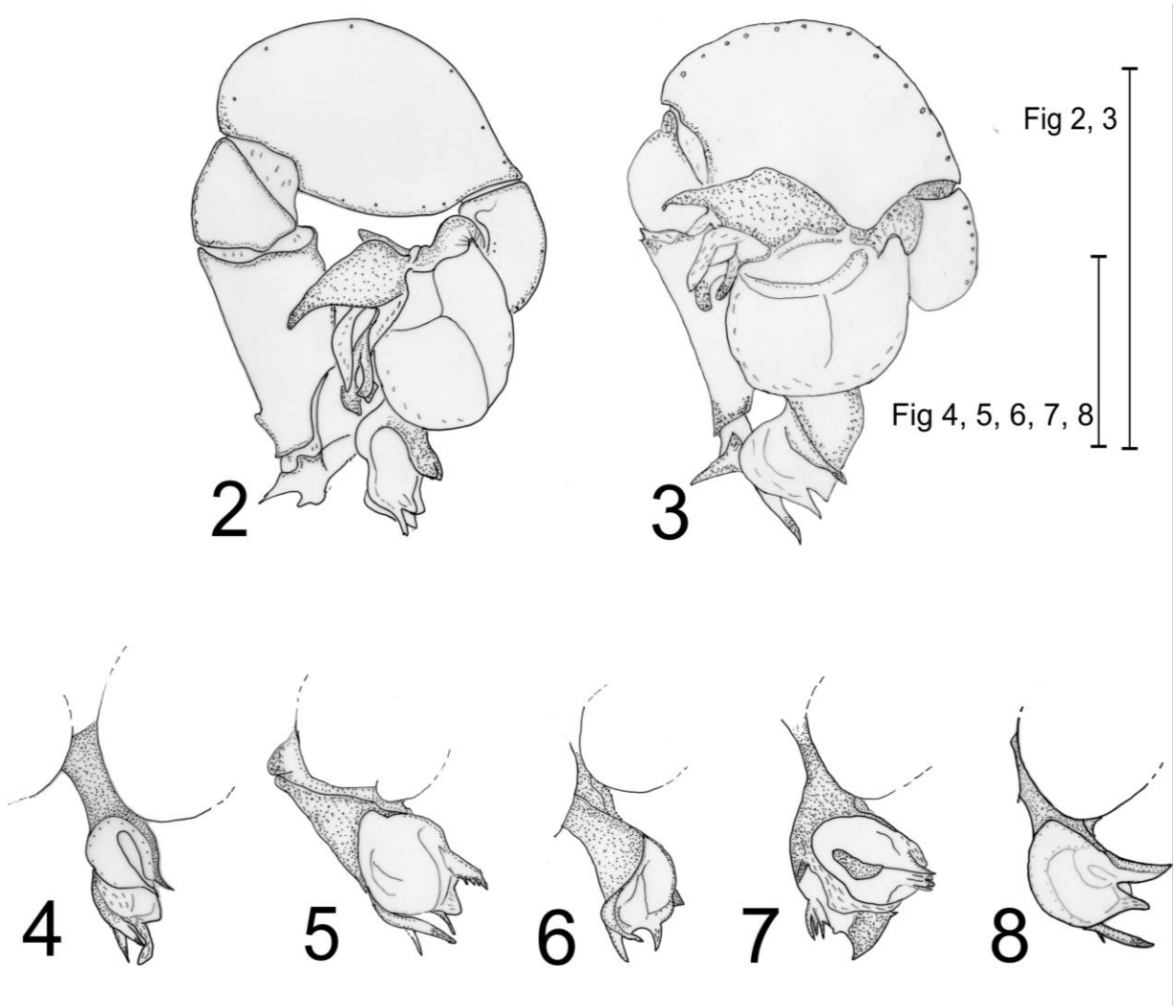


FIGURE 1. Phylogenetic reconstruction of the genus *Pholcus*, based on two mtDNA. Node values show bootstrap values and posterior probabilities from Bayesian and maximum likelihood analyses, respectively.

RESULTS

A phylogenetic tree assigned all specimens into five different clades (Figure 1) including: phalangioides, alticeps, persicus, pholcus sp., and medicus. The phalangioides clade is well supported statistically and holds a sister clade position to the other ingroups. This clade includes all specimens identified as *Ph. phalangioides* and consists of two subclades: subclade 1 contains specimens from Iran and Europe and subclade 2 consists of specimens from Europe, North and South America.



FIGURES 2-8. Prolateral view of the left male palps (2, 3) and prodorsal view of the procursus (4–8) of the *Pholcus* species from Iran. 2. *Pholcus* cf. *medicus*; 3. *Pholcus persicus*; 4. *Pholcus* cf. *medicus*; 5. *Pholcus* sp.; 6. *Pholcus alticeps*; 7. *Pholcus phalangioides*; 8. *Pholcus persicus*. Scale bars = 1 mm and 0.5 mm.

The second group includes four clades, of which the clade *alticeps* (Iran and Georgia) is well supported and the rest of distinctly separated clades belong to three endemic species from southwest of Iran including *Pholcus* cf. *medicus*, *Pholcus* sp. and *Ph. persicus*. In contrast to *Ph. phalangioides*, and *Ph. alticeps,* the other three species were collected from caves. *Pholcus* cf. *medicus* is relatively well supported group (since the value for posterior probability is low) as a sister to *persicus* clade which is a well-supported clade with noticeable intraspecific diversity. *Pholcus* sp. is a genetically distinct clade and holds a basal position to the clade including *Pholcus* cf. *medicus* and *Ph. persicus* with low supporting values which prompts for wider sampling. The molecular data corresponds finely with morphological identifications using characters of copulatory organs. Examining through different details of the male palp lead us to plot the prolateralo-dorsal view of procursus to identify different species. Five different morphospecies were identified and assigned to four previously known and probably one unknown *Pholcus* species for science (see Figure 2_8).

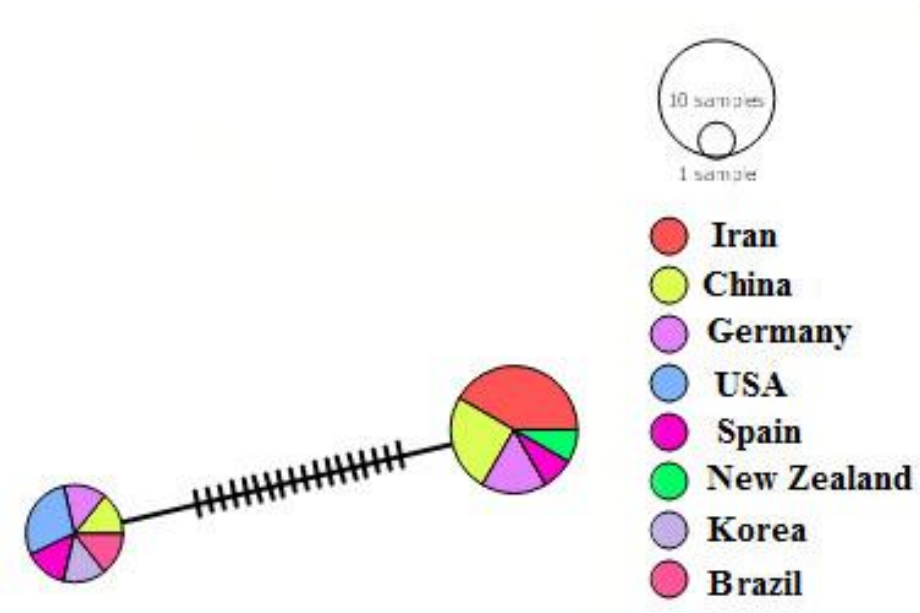


FIGURE 9. Haplotype networks of *Pholcus phalangioides*. Each line represents one substitution, and crossed lines indicating unsampled haplotypes. The circle size is associated with number of individuals in each haplotype.

A statistical parsimony network used to show intraspecific variation in *Ph. phalangioides*. The network (Figure 9) belongs to the cosmopolitan species; *Ph. phalangioides*, was defined by the existence of two main haplotypes with clear genetic partitioning between them. The distance between these two groups of *Ph. phalangioides* was recovered as 4.6%, more than the intraspecific p-distance recovered for other *Pholcus* species, (*Ph. pingtung* from Taiwan ranges between 2.9 and 3.1%; see Huber & Dimitrov, 2014).

DISSCUSSION

Despite the fact that *Pholcus* is the most specious genus of Pholcidae, studies on its taxonomy and phylogeny, particularly in the Middle East region, are limited. Additionally, the pholcid barcode sequence library is totally missing from Iran and other neighbor regions of the Middle East (Huber, per. comm.). As an example, in the main phylogenetic studies on the genus *Pholcus* (Dimitrov et al., 2013; Huber et al., 2018), just a single member of *Pholcus phalangioides* species group was included.

This study is a preliminary attempt to provide the first molecular data for *Pholcus phalangioides* species-group from Iran. Importantly, its molecular findings are in agreement with morphological studies on male characters. Phylogenetic results along with the pattern of haplotype network of *Ph. phalangioides* is probably due to synanthropic lifestyle of the species and its human-mediated dispersal (Astrin et al., 2006; Huber, 2011; Huber et al., 2018). However, a thorough study on the group with the aid of more molecular markers from an extensive sampling in its distribution range is necessary in the future studies.

Our probably undescribed species is collected from the southeastern region of the Zagros Mountains in Fars Province. Further examination and wider sampling is demanded to show the distribution and describe the real morphological and molecular diversity of the undescribed lineage. According to studies, west of Iran is known as a section of the Irano-Anatolian biodiversity hotspot as the Zagros Mountains is considered as a 20th global hotspot sector (Mittermeier *et al*., 2005; Kazemi et al., 2020). Such high degree of endemism and diversity is associated with heterogeneity of the environment, the complexity of the topography and the range of height of the mountain ranges (Frisch, 2006; Noroozi et al., 2008; Gholamifard, 2011; Esmaeili et al., 2016 & Noroozi et al., 2018). Some of the *Pholcus* species described by Senglet (1974, 2008), are morphologically extremely similar to each other. He ddditionally relied on a single specimen for new species' description (e.g. Senglet, 1973). Therefore, an

integrative analysis of the genus, for example by including molecular data, may discover the real diversity and additionally shed light on the evolutionary history and speciation processes of the genus in the Persian plateau.

ACKNOWLEDGMENTS

Authors are grateful to Dr. Bernard A. Huber (Zoological research Museum Alexander Koenig, Bonn) for his constructive comments on MSc thesis of M.H which resulted in the current work. The authors are also thankful to all friends who contributed in specimen collection: Mohamad J. Malek-Hosseini, Alireza Zamani and Farahnaz. Sheibak. The first author is thankful to Hasan Maddahi for his help on the analyses of the phylogenetic tree. Dr. Peter Castro (Polytechnic University, Pomona) and Dr. Rohollah Abbasi (University of Isfahan) kindly improved the English language of the manuscript, for this we are very thankful.

LITERATURE CITED

Amiri, M.J., Eslamian, S.S., 2010. Investigation of Climate Change in Iran. Journal of Environmental Science and Technology 3, 208–216.

Astrin, J.J., Huber, B.A., Misof, B., Klütsch, C.F.C., 2006. Molecular taxonomy in pholcid spiders (Pholcidae, Araneae): Evaluation of species identification methods using CO1 and 16S rRNA. Zoologica Scripta 35, 441–457.

Barrett, R.D.H, Hebert, P.D.N., 2005. Identifying spiders through DNA barcodes. Canadian Journal of Zoology 83, 481–491.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature methods*, 9(8), 772-772.

Dimitrov, D., Astrin, J.J., Huber, B.A., 2013. Pholcid spider molecular systematics revisited, with new insights into the biogeography and the evolution of the group. Cladistics 29, 132–146.

Esmaeili, H.R., Masoudi, M., ebrahimi, M., Elmi, A., 2016. Review of *Aphanius farsicus*: a critically endangered species (Teleostei: Cyprinodontidae) in Iran. Iranian Journal of Ichthyology 3, 1-18.

Frisch, J., 2006. The genus *Scopaeus* (Coleoptera, Staphylinidae, Paederinae) in Iran, with description of new species from the Zagros Mountains. Deutsche Entomologische Zeitschrift 53, 5-22.

Gholamifard, A., 2011. Endemism in the reptile fauna of Iran. Iranian Journal Of Animal Biosystematics 7(1).

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, in: Nucleic Acids Symposium Series. [London]: Information Retrieval Ltd., pp. 95–98.

Huber, B.A., 2005. The Pholcid Spiders of Africa (Araneae: Pholcidae): State of Knowledge and Directions for Future Research, in: African Biodiversity. Springer US, Boston, MA, pp. 181–186.

Huber, B.A., 2011. Phylogeny and classification of Pholcidae (Araneae): an update. The Journal of Arachnology 39, 211–222.

Huber, B.A., 2013. Revision and cladistic analysis of Pholcus and closely related taxa (Araneae, Pholcidae). Zootaxa 3713, 1–160.

Huber, B.A., Dimitrov, D., 2014. Slow genital and genetic but rapid non-genital and ecological differentiation in a pair of spider species (Araneae, Pholcidae). Zoologischer Anzeiger-A Journal of Comparative Zoology 253, 394–403.

Huber, B.A., Eberle, J., Dimitrov, D., 2018. The phylogeny of pholcid spiders: A critical evaluation of relationships suggested by molecular data (Araneae, Pholcidae). Zookeys 789, 51–101.

Kazemi, S.M. and Hosseinzadeh, M.S., 2020. High diversity and endemism of herpetofauna in the Zagros Mountains. *Ecopersia*, *8*(4), pp.221-229.

Leigh, J.W., Bryant, D., 2015. POPART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6, 1110–1116.

Mirshamsi, O., 2005. Faunistic study of spiders in Khorasan Province, Iran (Arachnida: Araneae). Iranian Journal of Animal Biosystematics, 1(1).

Mittermeier, R.A., Robles-Gil, P., Hoffmann, M., Pilgrim, J.D., Brooks, T.M., Mittermeier, C.G., Lamoreux, J.L. and Fonseca, G., 2005. Hotspots Revisited: Earth's Biologically Richest and Most Endangered Ecoregions (Cemex, Mexico City).

Noroozi, J., Akhani, H., Breckle, S.W., 2008. Biodiversity and phytogeography of the alpine flora of Iran. Biodiversity and Conservation 17, 493-521.

Noroozi, J., Talebi, A., Doostmohammadi, M., Rumpf, S.B., Linder, H.P., Schneeweiss, G.M., 2018. Hotspots within a global biodiversity hotspot-areas of endemism are associated with high mountain ranges. Scientific reports 8, 1-10.

Roewer, C.F. 1955. Die Araneen der osterreichischen Iran-Expedition 1949/50. Sitzungsberichte der Österreichischen Akademie der Wissenschaften (I) 164, 751-782.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Senglet, A., 1974. *Pholcus* nouveaux d'Iran (Araneae: Pholcidae). Revue suisse de zoologie 81, 803–812.

Senglet, A., 2008. New species of *Pholcus* and *Spermophora* (Pholcidae, Araneae) from Iran and Afghanistan, with notes on mating mechanisms. Revue suisse de Zoologie 115, 355–376.

Silvestro, D., Michalak, I., 2012. RaxmlGUI: A graphical front-end for RAxML. Organisms Diversity & Evolution 12, 335–337.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the entomological Society of America 87, 651–701.

Wang, Z.L., Yang, X.Q., Wang, T.Z., Yu, X., 2018. Assessing the effectiveness of mitochondrial COI and 16S rRNA genes for DNA barcoding of farmland spiders in China. Mitochondrial DNA Part A 29, 695–702.

World Spider Catalog, 2021. World Spider Catalog. Version 22.0. Natural History Museum Bern, online at http://wsc.nmbe.ch, accessed on (Dec 16, 2020)

Zamani, A., Mirshamsi, O., Marusik, Y.M., Moradmand, M. (2021). The Checklist of the Spiders of Iran. Version 2021, Online at http://www.spiders.ir

Iranian Journal of Animal Biosystematics (IJAB)
Vol.19, No.1, 23-32, 2023
DOI: 10.22067/ijab.2022.78445.1037

IJAB
ISSN: 1735-434X (print); 2423-4222 (online)

RESEARCH ARTICLE Open access

A comparative insight on Bombyliid's biodiversity indexes in grasslands in north of Iran

Shirin Aghavirdinezhad[1], Alireza Jalali zand[1]* and Hadi Ostovan[2]
[1] *Department of Plant Protection, Faculty of Agriculture, Isfahan (Khorasgan) branch, Islamic Azad University, Isfahan, Iran*
[2] *Department of Plant Protection, Faculty of Agriculture, Shiraz branch, Islamic Azad University, Shiraz, Iran*

(Received: *1 September 2022*; Accepted: *25 December 2022*)

Abstract
The Bombyliidae family with around 5382 described species is one of the most important families in order Diptera considering their remarkable roles in biological pest control. Species of the family Bombyliidae were monitored in Guilan province in 2019 and 2020. We aimed to evaluate their biodiversity in the grasslands along with the determining Simpson and Margalef indexes. Totally 20 species from 11 genera in 2019 and 17 species from 10 genera in 2020 were identified in eight selected stations. The highest numbers of Bombyliids individuals were observed in Heiran pass station in 2019. Surprisingly the lowest number of individuals also was present in Heiran pass station in 2020. The results of ecological indices also showed the highest Margalef index in Darestan station (2.18) in 2019 and the lowest observed in Bivazen station (0) and Rudbar- Loockhee (0) in 2019 and 2020 respectively. Also, the highest values for the Simpson index were recorded in Bivazen (1) in 2019 and Rudbar- Loockhee (1) in 2020. The lowest values of the Simpson index have been recorded for Amlash (0.13) stations as well. It seems that climate and ecological condition changes in different years clearly affect the insect biodiversity indexes via making changes in their population dynamics.

Key words: *Diversity, Bombyliidae, Simpson index, Margalef index, Guilan province.*

INTRODUCTION

Bombyliidae Latreille, 1802 (Asiloidea) with 5382 described species worldwide and more than 101 species in Iran is the eighth most diverse family within Diptera (Pape et al., 2011). They have a thick, hairy body and, as the name implies, are frequently confused with hymenoptera due to their bee-like appearance. Larvae stage with large, tong-like mandibles which are probably designed to help the parasitic life cycle (Merritt et al., 2009). All of the Bombyliid species feed on Coleoptera, Hymenoptera, Lepidoptera, Orthoptera, Neuroptera and Diptera's immature stages as parasitoid or predator (Yeates and Greathead, 1997; Boesi et al., 2009). The immature stages of Bombyliids facilitate the control of other insect populations, while the adult stages function as effective pollinators (Motten et al., 1981; Kearns, 2001).

Corresponding Author: arjalalizand@gmail.com

FIGURE 1. The sampling localities in Guilan province.

Biodiversity mainly studies diversity, population structure and many patterns. It is also used as an indicator to compare the ecological status of ecosystems. The goal is to achieve a single quantity to easily compare and evaluate communities and ecosystems (Jenkins and Parker, 1998). It is also important to note that biodiversity contributes to ecosystem stability and conservation, as more species in an area create a more complex structure, which suggests a greater likelihood of sustainability. As a result, more biodiversity implies greater sustainability of ecosystems. (Thompson et al., 2007).

Currently, there is little knowledge of the Bombyliidae family in northern Iran since only a few studies have been conducted in this area. The Bombyliids species richness and diversity in northern parts of Iran are probably high due to the temperate and humid climate conditions and the rich plants flora in the margin of the Caspian Sea (Gharali et al., 2010; Aghavirdinejad and Jalali Zand, 2021). The margalef Index expresses the presence of various species (Park et al., 2019), since the Simpson species diversity index, emphasizes the dominant species in the sample (Heidari Latibari et al., 2016).

Since Bombyliid species play such an important role in both pollination and pest management and considering the agronomic potential of the northern provinces of Iran, both investigation of Bombyliid fauna and their species diversity indexes may offer valuable insight, as a basis for further investigations related to Bombyliidae in similar ecological contexts (Aghavirdinejad and Jalali Zand, 2021). The present paper will examine the first comparative study between the species richness and diversity of Bombyliidae in two consecutive years in the grasslands of Guilan province, Iran.

MATERIAL AND METHODS

SAMPLING

Our sampling was conducted in the selected locations (Fig. 1), in the rangelands of Guilan province (Table 1). In order to collect larger species, sweeping nets were used.

TABLE 1. Sampling localities.

STATION	LOCATION
BIVAZAN	36°40’57.19”N, 49°34’39.57”E
HEIRAN PASS	36°42’12.85”N, 49°47’17.57”E
RUDBAR- LOOCKHEE	37°5’29.88”N, 50°11’12.98”E
ROSTAM ABAD- JOKIN	36°40’57.19”N, 49°34’39.57”E
DAMASH	38°26’32.45”N, 48°34’53.26”E
JIRANDEH	36°48’34.96”N, 49°24’57.98”E
RUDBAR-DARESTAN	3 6°48’34.96”N, 49°24’57.98”E
AMLASH	37°5’29.88”N, 50°11’12.98”E

SPECIES IDENTIFICATION

Species identification has been done using available keys (Greathead and Evenhuis, 2001; Yao et al., 2011). The identifications have been confirmed by Dr Rahim Abdollahi Mesbah (University of Tehran, IRAN).

STATISTICAL ANALYSIS

The collected Bombyliids were categorized based on their location and species. Their recorded data were analyzed based on Simpson and Margalef Indexes for comparing species diversity and richness between selected localities in Guilan province, Iran. SDR software (Hasanvand et al., 2015), was employed to calculate the amounts of mentioned indexes.

RESULTS

Twenty species of Bombyliids (Diptera, Bombyliidae) from eleven genera as well as seventeen species belong ten genera were collected from selected stations (Table 2).

Our findings in 2019 indicated the highest and lowest number of individuals were observed in Heiran pass and Rudbar- Loochkee station respectively (Figure 4). However, species richness differed among stations. Rudbar- Darestan was the richest station according to the Margalef richness indicator (2.18) while the lowest amount of Margalef richness was seen in the Bivazan (0) (Figure 2). Simpson index which is known as an indicator for species dominancy demonstrated the highest amount for the Bivazan station which included just one species (1) and the lowest amount was recorded for Rudbar-Darestan station (0.19) (Figure 3). According to the results of the investigation in 2020, Bivazan and Heiran pass stations were the most and least populated (Figure 7). According to the Margalef richness indicator, Rudbar- Darestan was the richest station (2.16), while Rudbar- Loockhee had the lowest amount (0) (Figure 5). Rudbar-Lockhee and Amlash stations recorded the highest (1) and the lowest (0.13) amount of Simpson index in 2020 respectively (Figure 6). It is surprising to note that, while Heiran station recorded the highest number of individuals in 2019, the lowest number of individuals in 2020 was recorded for this station, however, the number of species reported in 2019 was higher than in 2020.

TABLE 2. Information of collected species. A) 2019; B) 2020.

A)

Species	Bivazan	Heiran pass	RudbarLoockhee	Rostam abad- Jokin	Damash	Jirandeh	Rudbar- Darestan	Amlash	Sum
Exoprosopa amseli				3		3	7		13
E. kirgizorum				15		6			21
E. efflatounbeyi							4		4
E. pectoralis			3						3
E. grandis							1		1
E. dispar				2		2			4
Thyridanthrax punctum							1		1
T. elegans					4			8	12
T. griseolus							6		6
Lomatia belzebul					5				5
Usia bicolor		40					5	25	70
Parageron lutescens			1						1
Conophorus pseudaduncus		1							1
Heteralonia megerlie		1							1
H. suffuse			3						3
Callostoma soror								5	5
Veribubo misellus							3		3
Hemipenthes subvelutinus				6		10	2		18
Phthiria vagans	100	100	20	10	30	20	10	20	210
P. pulicaria					1				1
Sum	100	142	27	36	40	41	39	58	383

B)

Species	Bivazan	Heiran pass	RudbarLoockhee	Rostam abad- Jokin	Damash	Jirandeh	Rudbar- Darestan	Amlash	Sum
Exoprosopa amseli				14		3	2	22	41
E. kirgizorum				2		6			8
E. efflatounbeyi		1					8		9
E. grandis				9			24		33
E. dispar				2					2
Thyridanthrax punctum						2	7		7
T. griseolus					9		3		3
Lomatia belzebul				21					30
Usia bicolor		3					11		14
Conophorus pseudaduncus		1		13			9	2	25
Heteralonia megerlie		1							1
H. suffuse					48		1		49
Callostoma soror		4						44	48
Veribubo misellus							4		4
Hemipenthes subvelutinus						10	12		22
Phthiria vagans	86		44		30	20	20	13	213
P. pulicaria	18								18
Sum	104	10	44	59	87	41	101	58	527

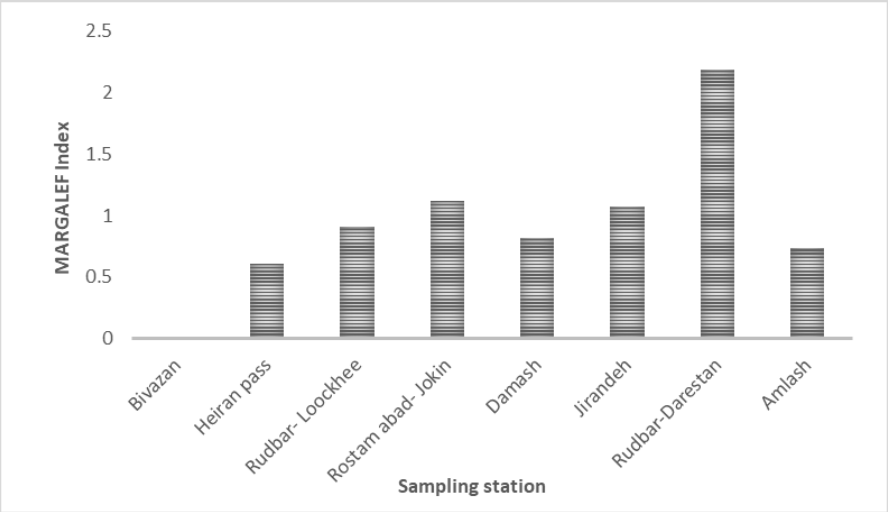


FIGURE 2. Margalef index in sampling stations 2019.

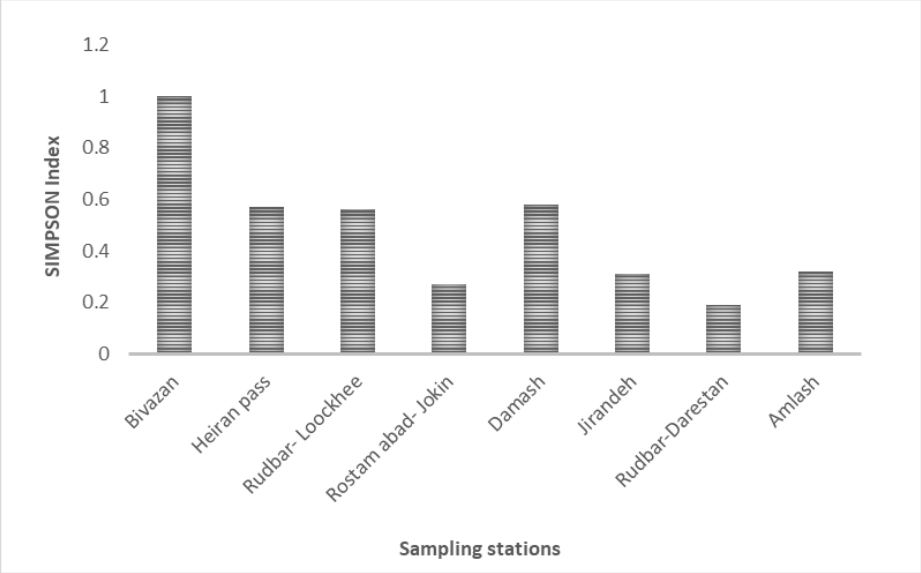


FIGURE 3. Simpson index in sampling stations 2019.

DISCUSSION

Dipterans are among the most common insects that visit flowers, and they also play an important role in pollinating them (Kevan and Baker, 1983). Adult dipterans feed on nectar to meet their energy requirements during flight, mate search, oviposition, finding mates, and mating (Larson et al., 2001). The Bombyliidae family is one of the flower-visiting dipteran families recorded in the present study. All Bombyliids species play a role as natural enemies in their larval stage (parasites of grasshopper eggs, solitary bees and wasps) (Aghavirdinejadand Jalili Zand, 2021).

The calculated biodiversity indexes in the present study, indicate the good diversity and richness of the Bombyliidae family in the grasslands of Guilan province. We concluded that the most numbers and richness of Bombyliids species were present in the mid of sampling seasons (summer and spring

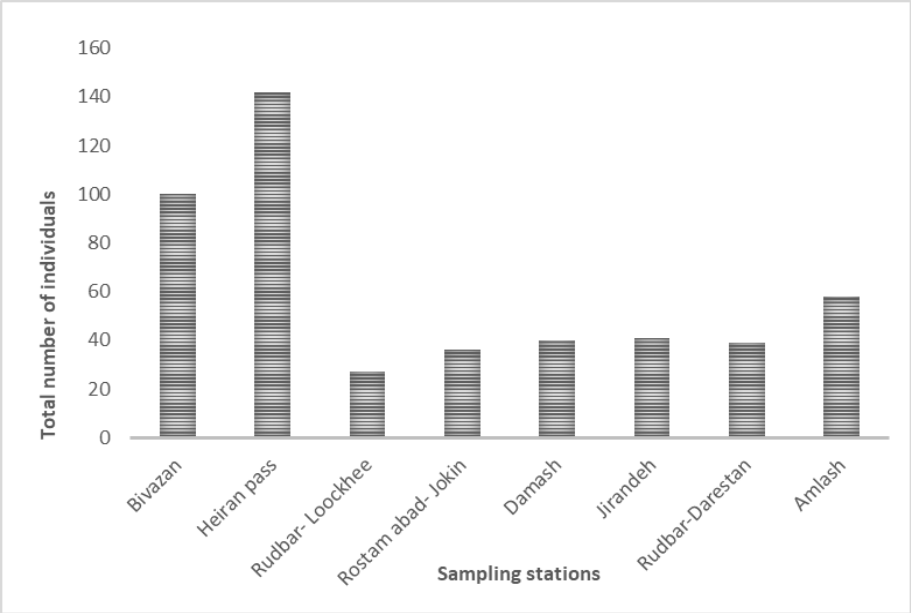


FIGURE 4. Number of individuals in sampling stations 2019.

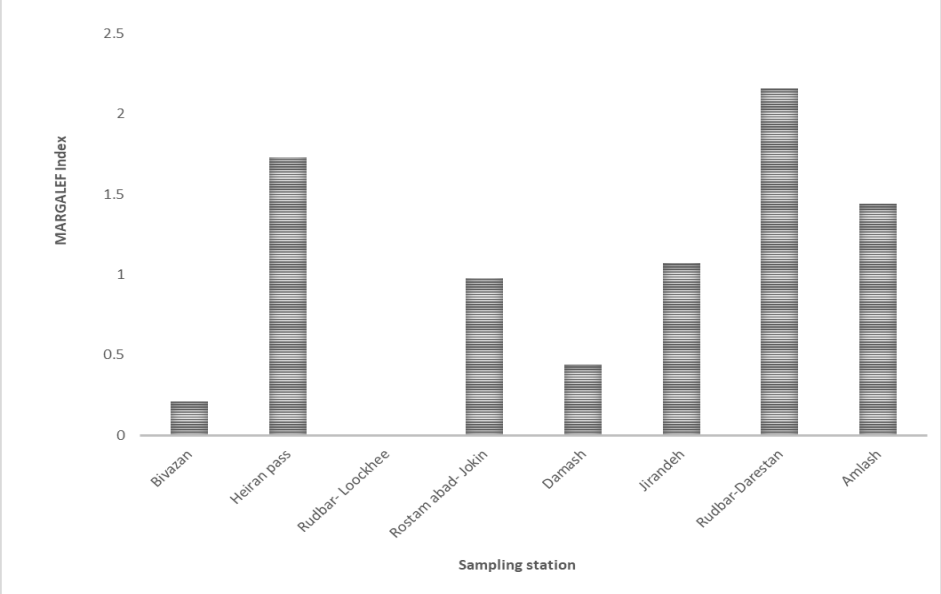


FIGURE 5. Margalef index in sampling stations 2020

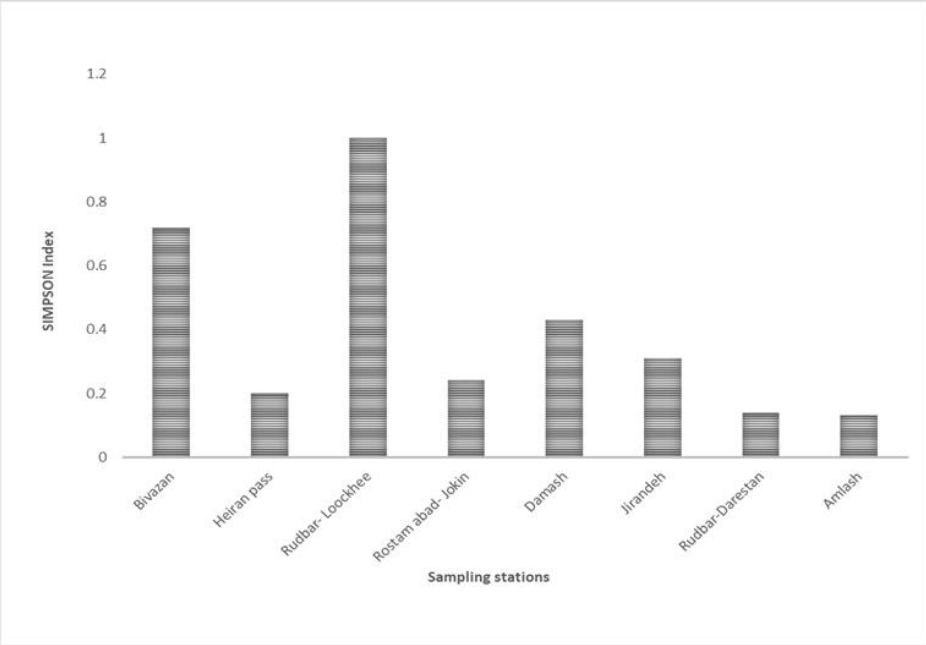


FIGURE 6. Simpson index in sampling stations 2020

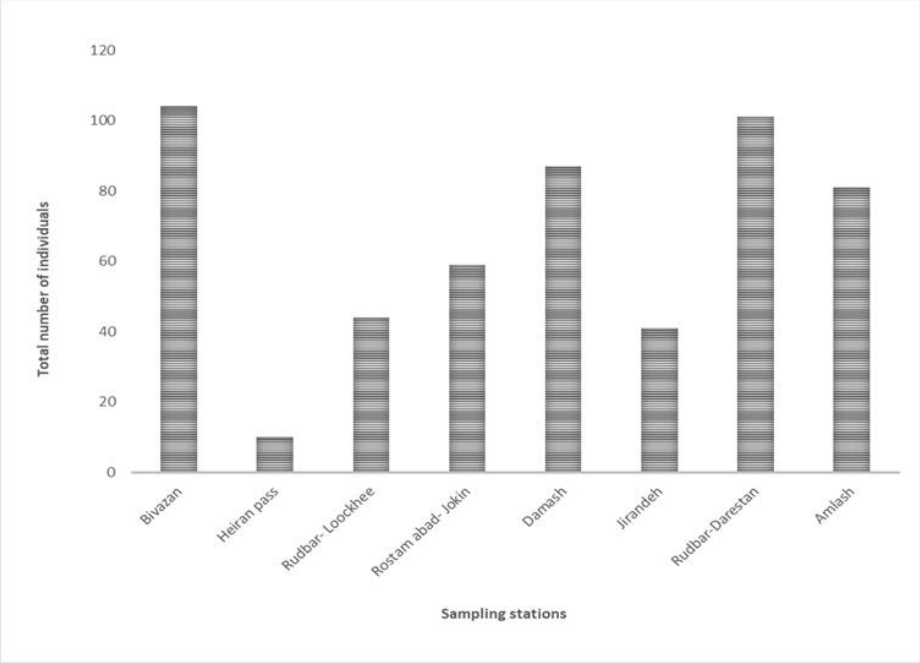


FIGURE 7. Number of individuals in sampling stations 2020.

respectively). The Bombyliidae showed an increase with increase in floristic biodiversity, which is probably due to the fact that they show specialization links with plants that are suitable for being visited by these pollinators (Benvenuti, 2022).

It should be noted that a peak in activity of most pest species, which are hosts of this family, is simultaneous with this event since it is a strong relationship between the population density of predators and their hosts (Stevens, 2010). However, the same sampling stations in different years showed different values of biodiversity indices for this family. We believe that climate changes and ecological conditions have caused these differences (Bellard et al., 2012). Guilan province on the southwest shore of the Caspian Sea is one of the most important agricultural cores of Iran. On the other hand, the environmental risks of using pesticides that are not hidden from anyone have doubled the importance of using biological control agents (Heidari Latibari et al., 2018). Therefore, the results of the present study have provided valuable information for the application of integrated pest control methods. Due to the rich flora and fauna of Guilan province, studies such as this study are strongly recommended for other families of insects that play a role as natural enemies.

ACKNOWLEDGMENTS

This research has been supported by the Islamic Azad University Isfahan (Khorasgan) Branch in the framework of the Ph.D. thesis, which is gratefully acknowledged. We wish to thank the anonymous reviewers for their constructive comments on the primary version of the manuscript. This work was financially supported by Islamic Azad University, Isfahan (Khorasgan) branch. The authors declare that there is no conflict of interest regarding the publication of this paper.

LITERATURE CITED

Aghavirdinejad, S., & Jalali-Zand, A. (2021). Study on humbleflies species biodiversity (Diptera: Bombyliidae) in the western margin of the Caspian Sea coastline. *Journal of the Entomological Research Society*, *23*(1), 61-67.

Benvenuti, S. (2022). Wildflowers-pollinator-crab spider predator food-web as indicator of the agroecosystem biodiversity. *Ecological Indicators*, *143*, 1-9.

Boesi, R., Polidori, C., & Andrietti, F. (2009). Searching for the right target: oviposition and feeding behavior in Bombylius bee flies (Diptera: Bombyliidae). *Zool Stud*, 48: 141-150.

Greathead, D. J., & Evenhuis, N. L. (2001). Annotated keys to the genera of African Bombylioidea (Diptera: Bombyliidae; Mythicomyiidae). *African Invertebrates*, *42*(1), 105-224.

Hasanvand, I., Jafari, S., Kazemi, S., & Shakarami, J. (2015). Fauna and species diversity of edaphic mesostigmatic mites of superfamilies Eviphidoidea and Ascoidea (Acari: Mesostigmata) in Khorramabad County, Lorestan Province (Text in Persian). *Plant Pests Research*, *4*(4), 25-34.

Heidari Latibari, M., Moravvej, G., Namaghi, H. S., & Khormizi, M. Z. (2016). Coccinellid biodiversity on the coniferous trees Thuja orientalis and Pinus mugo in urban landscape of Mashhad, Razavi Khorasan Province, Iran. *Egyptian Journal of Biological Pest Control*, *26*(2), 419.

Heidari Latibari, M., Zare Khormizi, M., Sahamian, E., Dehghan Dehnavi, L., & Moravvej, G. H. (2018). Faunistic survey of Aphidoidea (Hemiptera) and associated predatory ladybirds in orchards, Yazd Province, Iran. *EPPO Bulletin*, *48*(1), 160-163.

Jenkins, M. A., & Parker, G. R. (1998). Composition and diversity of woody vegetation in silvicultural openings of southern Indiana forests. *Forest ecology and management*, *109*(1-3), 57-74.

Kevan, P. G., & Baker, H. G. (1983). Insects as flower visitors and pollinators. *Annual review of entomology*, *28*(1), 407-453.

Kearns, C. A. (2001). North American dipteran pollinators: assessing their value and conservation status. *Conservation Ecology*, 5(1).

Larson, B. M. H., Kevan, P. G., & Inouye, D. W. (2001). Flies and flowers: taxonomic diversity of anthophiles and pollinators. *The Canadian Entomologist*, *133*(4), 439-465.

Merritt, R. W., Courtney, G. W., & Keiper, J. B. (2009). Diptera:(Flies, Mosquitoes, Midges, Gnats). In *Encyclopedia of insects* (pp. 284-297). Academic Press.

Motten, A. F., Campbell, D. R., Alexander, D. E., & Miller, H. L. (1981). Pollination effectiveness of specialist and generalist visitors to a North Carolina population of Claytonia virginica. *Ecology*, *62*(5), 1278-1287.

Pape, T., Blagoderov, V., & Mostovski, M. B. (2011). Order Diptera Linnaeus, 1758. In: Zhang, Z.-Q. (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. *Zootaxa*, *3148*(1), 222-229.

Park, J. H., Woo, S. Y., Kwak, M. J., Lee, J. K., Leti, S., & Soni, T. (2019). Assessment of the diverse roles of home gardens and their sustainable management for livelihood improvement in West Java, Indonesia. *Forests*, *10*(11), 970.

Thompson, I. D., Okabe, K., Tylianakis, J. M., Kumar, P., Brockerhoff, E. G., Schellhorn, N. A., Parrotta, J. A. & Nasi, R. (2011). Forest biodiversity and the delivery of ecosystem goods and services: translating science into policy. *BioScience*, *61*(12), 972-981.

Yao, G., Yang, D., & Evenhuis, N. L. (2011). Two new species of Tovlinius Zaitzev, from China, with a key to the genera of Bombyliinae from China and a second key to the world species (Diptera, Bombyliidae, Bombyliinae, Bombyliini). *ZooKeys*, (153), 73.

Yeates, D. K., & Greathead, D. (1997). The evolutionary pattern of host use in the Bombyliidae (Diptera): a diverse family of parasitoid flies. *Biological Journal of the Linnean Society*, *60*(2), 149-185

Iranian Journal of Animal Biosystematics (IJAB)
Vol.19, No.1, 33-45, 2023
DOI: 10.22067/ijab.2022.79924.1046

IJAB
ISSN: 1735-434X (print); 2423-4222 (online)

REVIEW ARTICLE Open access

Systematics and distribution of the Leopard Geckos *Eublepharis* Gray, 1827 (Sauria, Eublepharidae): A review

Rasoul Karamiani
Department of Biology, Faculty of Science, Razi University, 6714967346 Kermanshah, Iran

(Received: *5 December 2022*; Accepted: *29 December 2022*)

Abstract
The eyelid bearing geckos Eublepharidae including six widely disjunct genera and 45 species which are distributed in tropical to temperate habitats of Eurasia, North-Central America and Africa. One of the eublepharid genera is the fat-tailed geckos of the genus *Eublepharis* Gray, 1827. The specific characters of *Eublepharis* involves flat basioccipital bone, deep axial pockets (armpit), eyelids well developed and movable, pupil vertical, dorsal small scales juxtaposed larger tubercles. The fat-tailed geckos as a vicariant group, encompass seven secretive, and nocturnal species: *E. angramainyu* Anderson and Leviton, 1966, *E. fuscus* Börner, 1974, *E. hardwickii* Gray, 1827, *E. macularius* (Blyth, 1854), *E. satpuraensis* Mirza, Sanap, Raju, Gawai and Ghadekar, 2014, *E. pictus* Mirza and Gnaneswar, 2022), and *E. turcmenicus* Darevsky, 1977 which are distributed from Turkey through the Iranian Plateau to India as following: south-eastern Turkey, Syria, Iraq, Iran, Pakistan, Afghanistan, Turkmenistan, and north-eastern and central India in a variety of habitats from dry karst topography regions with gypsum deposits and clay-gravel soil to stony foothills. The data used for the distribution maps were based on all available bibliographic records, and personal field observations. The results showed that among the three species located in Iran, *E. angramainyu* has the most widespread distribution and habitat diversity, and *E. macularius* has a very limited distribution in the border of Iran and Afghanistan. The range of species distribution depends on various factors including behavioral mechanisms of superior habitat selection, interactions with other living organisms and physico-chemical factors.

Key words: *Eublepharis, Eyelid geckos, Eublepharidae, Distribution, Asia.*

INTRODUCTION

The eyelid geckos of the Family Eublepharidae encompass six widely disjunct genera and 45 species: the cat gecko, *Aeluroscalabotes* (1 sp.) from Indonesia (Kalimantan), Malaysia (Sabah, Sarawak, west Malaysia), Singapore, Thailand (Seufer et al., 2005), *Coleonyx* (8 sp.) from Central and western North America, *Eublepharis* (7 sp.), and *Goniurosaurus* (25 sp.) from Asia, *Hemitheconyx* (2 sp.) from east and west Africa, and *Holodactylus* (2 sp.) from east Africa (Vitt and Caldwell, 2013; Mirza et al., 2014; Agarwal et al., 2022; Mirza and Gnaneswar, 2022). Boulenger (1885) based on movable eyelids, smooth digit, procoelous vertebrae, a single parietal bone separated Eublepharidae from the Gekkota lizards. Then Gadow 1901 reduced taxonomic level to subfamily and believed that they did not form a monophyletic

Corresponding Author: rasoul.karamiani@gmail.com

group (Gadow, 1923); Werner (1912) in recognized Boulenger's classification added *Holodactylus* to the Eublepharidae; Camp (1923) believed procoelous vertebrae of eublepharid geckos was secondarily derived and placed them in the Gekkonidae. Kluge (1967) based on presence of eyelid, an unpaired parietal bone, and a supratemporal bone separated eublepharids from other geckos as subfamily Eublepharinae (Kluge, 1967).

Grismer (1988) separated eublepharids from other geckos on the basis of cladistic analysis of morphological characters (skeletal system, scale morphology, meristic characters, and color pattern) and raised them to the family level. Phylogenetic analyses based on mitochondrial DNA sequences 12S and 16S ribosomal RNA genes (Ota et al., 1999; Fig. 1) and NADH dehydrogenase subunit 2 gene (ND2) and parts of 12S and 16S rRNA genes (Jonniaux and Kumazawa, 2008; Fig. 2) confirmed that the Eublepharidae are sister taxon of Gekkonidae. Based on molecular phylogenetic analyses, Jonniaux and Kumazawa (2008) proposed the Laurasian (Asian) origin of Eublepharidae, hence concluded that the genus *Coleonyx* may have migrated to North America by Early Cretaceous (around 150 million years ago (MYA)); they also inferred that the ancestors of African genera (*Holodactylus* and *Hemitheconyx*) evolved outside of Africa and entered Africa (around 90 MYA) during the time Africa was connected to Eurasia. Pyron et al. (2013) inferred that the African genera (*Holodactylus* and *Hemitheconyx*) form a sister group with the South Asian genus *Eublepharis*. Numerous studies have been carried out on karyology, phylogeny and eco-physiological variations of eublepharid geckos (e.g., Murphy, 1974; Ota et al., 1987; Dial and Grismer, 1992; Mirza et al., 2014). The aim of this study is to review systematics and distribution of the genus *Eublapharis* based on all the available evidence so far obtained.

Systematics account

Order Squamata Oppel, 1811

Infraorder: Gekkota Cuvier, 1817

Family Eublepharidae Boulenger, 1883

Genus *Eublepharis* Gray, 1827

The genus *Eublepharis* ('Eu' = true 'blephar' = eyelid) was first described based on specimens from Chittagong, Penang (Chittagong, now in Bangladesh) by John Edward Gray in 1827. The anatomical and morphological characteristics in the genus *Eublepharis* are as follows: flat basioccipital bone; deep axial pockets (armpit); digits short, cylindrical, with transverse lamellae beneath, clawed, the claw partly concealed beneath two or four lateral scale and an upper scale; eyelids well developed and movable; pupil vertical; males with preanal pores; dorsum is covered with small juxtaposed scales and larger tubercles; tail shorter than their snout–vent length (Leviton et al., 1992; Anderson 1999). The genus *Eublepharis* encompasses seven secretive, and nocturnal species: *E. angramainyu*, *E. fuscus*, *E. hardwickii*, *E. macularius*, *E. satpuraensis*, *E. pictus*, and *E. turcmenicus* which sporadically occur from southeast Turkey, Syria, Iraq, Iran, Pakistan, Afghanistan, Turkmenistan, and northeast and central India (Fig. 3) in a variety of habitats from dry karst topography regions with gypsum deposits and clay-gravel soil to stony foothills (Grismer 1988, 1989; Jonniaux and Kumazawa 2008; Karamiani and Rastegar-Pouyani 2021; Mirza and Gnaneswar, 2022; Uetz et al., 2022). In Iran, the leopard geckos comprise three species with a vicariant distribution: the Iranian fat-tailed gecko, *E. angramainyu* Anderson and Leviton, 1966, which occurs west and southwest of the Zagros Mountains (Karamiani and Rastegar-Pouyani, 2010) to southwest Kerman Province (Moradi and Shafiei, 2011); *E. macularius* (Blyth, 1854), of which the only known locality is in the eastern region of South Khorasan Province (close to the Afghanistan–Iran border); and *E. turcmenicus* Darevsky, 1977 from the Turkmen borders in North Khorasan, Khorasan Razavi and Semnan Provinces (Auer et al., 2008).

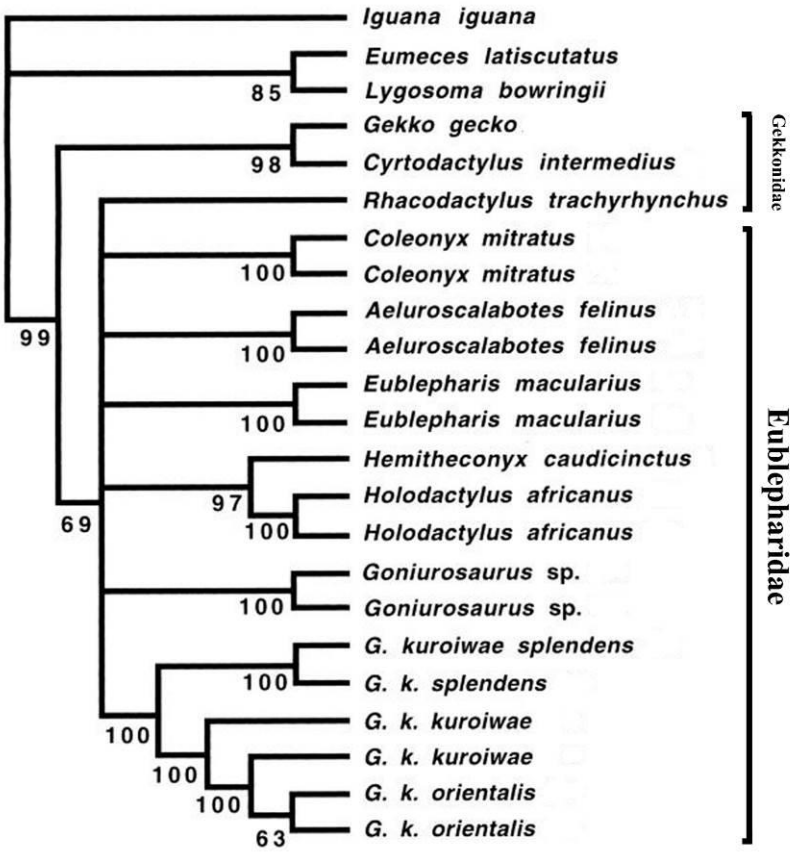


FIGURE 1. The majority-rule consensus tree deriving from parsimony analysis using nucleotide bases of 12S and 16S rRNA genes of the ingroup taxa, those belonging to the families Eublepharidae and Gekkonidae are shown (Ota et al., 1999).

***Eublepharis angramainyu* Anderson and Leviton, 1966**

The Iranian fat-tailed gecko, *E. angramainyu* (Fig. 4) was described from an old road between Masjed Soleyman and Batvand, Khuzestan Province, Iran in 1966 by Anderson and Leviton. The Iranian fat-tailed gecko is distributed in limestone and gypsum foothills (small caverns in the gypsum deposits; Fig. 5), arid grasslands, and steppes from southern Turkey, north-eastern Syria, and Iraq to southern and south-eastern regions of the Iranian Plateau (Anderson, 1999; Al-Sheikhly et al., 2020). The morphological characteristics for diagnosis in *E. angramainyu* as following: subdigital lamellae smooth; mid-dorsal tubercles not as large as intertubercular spaces; chin shields in contact with first lower labials; 41-48 eyelid fringe scales; 27-38 longitudinal rows hexagonal ventral scales; some elements of color pattern of head and body linearly arranged in adults*;* males with uninterrupted series of 11-17 preanal pores, pores discernible in females (Anderson, 1999; Grismer, 1991). Karamiani and Rastegar-Pouyani (2017) in the anatomical study showed that *E. angramainyu* has 18 paired bones (maxillae, nasal, prefrontal, jugal, lacrimal, vomer, septomaxilla, palatine, pterygoid, ectopterygoid, epipterygoid, postfrontal, stape, squamosal, supratemporal, quadrate, prootic, otooccipital) and a single parietal in skull. In Iran, the leopard gecko is distributed from Kermanshah, Ilam, Lorestan, Khuzestan, Kohgiluyeh Va Boyer Ahmad, Fars to Kerman Provinces. Conservation status in the IUCN Red List categories and criteria for the species is Data Deficient. In Iraq, the leopard gecko is distributed from the ruins of Nineveh, in the upper Mesopotamian Plain, Kirkuk Province to Khanaqain area (Boulenger, 1885; Leviton et al., 1992; Anderson, 1999). The species was also recorded from Al-Khatonia in Al-Hasakah northeastern (Syria) and southern Turkey (Martens and Kock 1991; Rosler 1995; Göçmen et al., 2002).

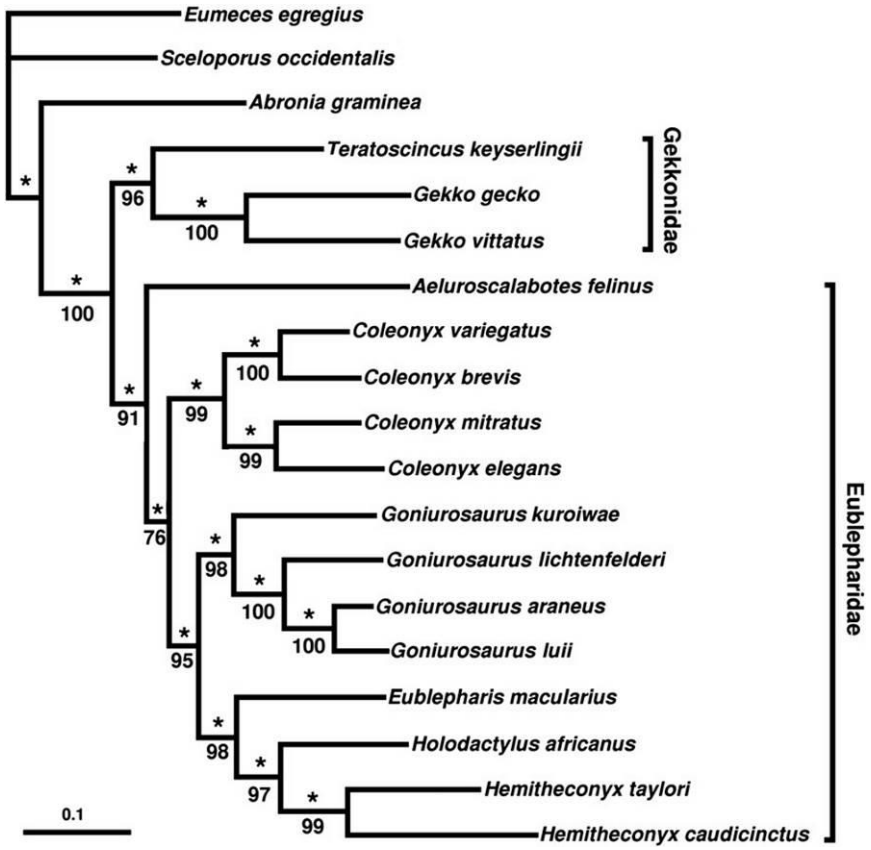


FIGURE 2. A Bayesian tree based on 2213 concatenated nucleotide sites from the ND2 region and two rRNA regions, showing relationships between Eublepharidae and Gekkonidae (Jonniaux and Kumazawa 2008).

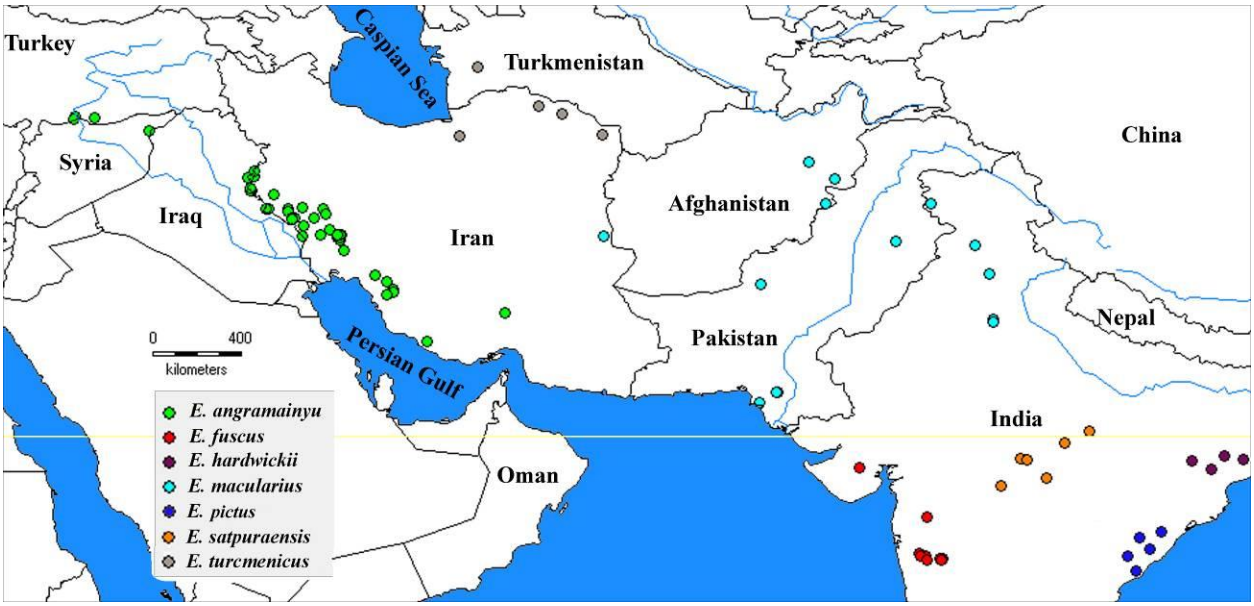


FIGURE 3. Distribution of the genus *Eublepharis*.

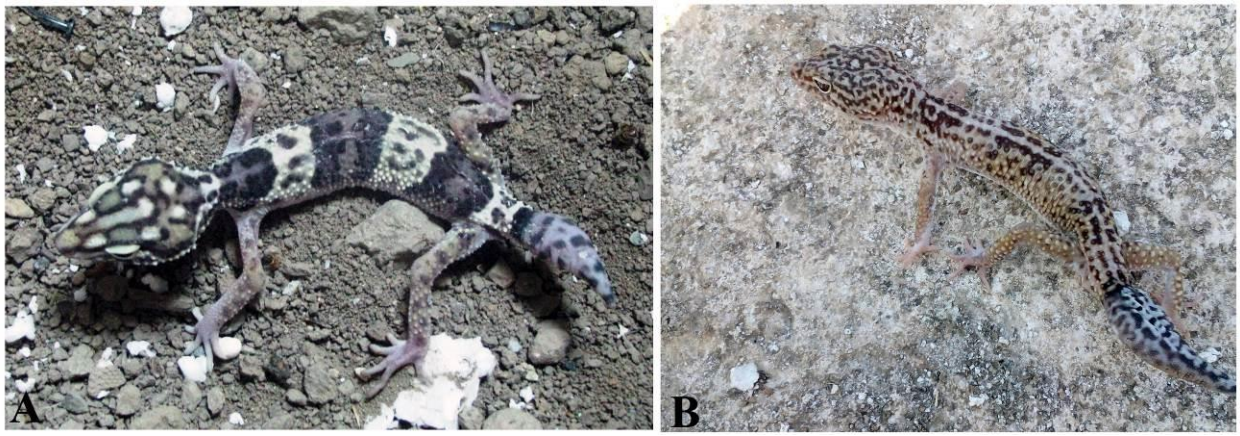


FIGURE 4. Alive subadult (A) adult (B) specimens of *Eublepharis angramainyu* with regenerated tail.

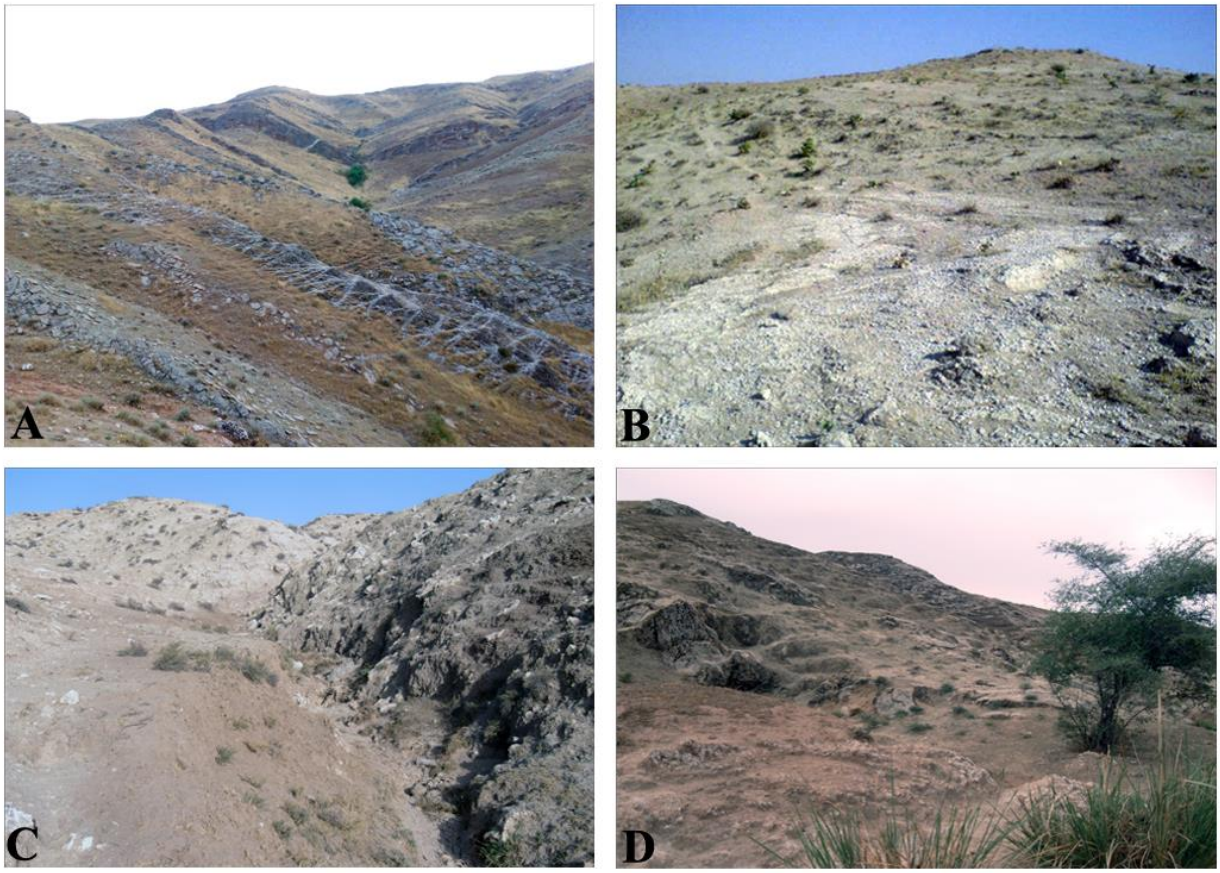


FIGURE 5. General aspect of habitat of *Eublepharis angramainyu* in Bazideraz Region, Kermanshah Province (A); Pol-e Dokhtar, Lorestan Province (B); Masjed-e-Suleiman (C), and Ramhormoz (D), Khuzestan Province (Karamiani and Rastegar-Pouyani, 2021).

***Eublepharis macularius* (Blyth, 1854)**

The spotted fat-tailed gecko was described as *Cyrtodactylus macularius* from the Salt Range, Punjab, Pakistan by Blyth in 1854. The species is distributed in Afghanistan, Pakistan, India, Iran, and Nepal (Anderson, 1999; Rawat et al., 2019); the leopard gecko is reported from Rajputana and Khandesh Districts of India. In Pakistan it has been recorded from Azad Kashmir, North Western Frontier Province,

FIGURE 6. An adult specimen of *Eublepharis macularius* (Photo by Rune Midtgaard).

Punjab, Balochistan, and Sindh. In Nepal it is recorded from Surkhet, Banke, Nawalparasi, Kipilvastu Districts (Rawat et al., 2019). The morphological characteristics for diagnosis in *E. macularius* (Fig. 6) as following: subdigital lamellae each with several distinct small tubercles; mid-dorsal tubercles generally larger than intertubercular spaces; chin shields usually in contact with first lower labials; elements of dorsal color pattern not linearly arranged; 46-47 eyelid fringe scales; 21-30 longitudinal rows rounded ventral scales (Anderson, 1999; Grismer, 1991). Conservation status in the IUCN Red List categories and criteria for the species is Least Concern. Distribution in Iran: South Khorasan Provinces.

***Eublepharis turcmenicus* Darevsky, 1977**

The Turkmenistan leopard gecko, *E. turcmenicus* (Fig. 7) was described by Darevsky in 1977 based on three individuals from Bakharden, Chandyr and near Ashkhabat, Turkmenistan. The species is reported from southern Turkmenistan and north-eastern Iran. The morphological characteristics for diagnosis in *E. turcmenicus* are the following: subdigital lamellae with weakly developed small tubercles; chin shields not in contact with first lower labials; 54-55 eyelid fringe scales; 20-22 longitudinal rows hexagonal ventral scales; males with uninterrupted series of 5-9 preanal pores (Anderson, 1999; Kaverkin and Orlov 1996). Conservation status in the IUCN Red List categories and criteria for the species is Least Concern. Distribution in Iran: North Khorasan and Khorasan Razavi and Semnan Provinces.

***Eublepharis fuscus* Börner, 1974**

The West Indian leopard gecko, *E. fuscus* (Fig. 8) was described in 1974 by Börner as a subspecies of *E. macularius* from 60 km north of Mumbai (Bombay) in the Maharashtra State, west India. Das (1997) redefined the taxon, and upgraded it to a full species. This upgrade was adopted by subsequent authors (e.g. Daniel, 1983, 2002; Vyas, 2000a, b; Starostová et al., 2005, 2009; Mirza et al., 2014; Harshil and Vyas, 2019). The species is recorded from northern Karnataka, Maharashtra and Gujarat in the western India. The specific name "*fuscus*" means dark or dusky. The morphological characteristics for diagnosis in *E. fuscus*: subdigital lamellae smooth; middorsal tubercles that are smaller than their interspaces, eight postnasals bordering the nasals; small tubercles on the dorsum (Das 1997). Conservation status in the IUCN Red List categories and criteria for the species is Least Concern.

FIGURE 7. An adult specimen of *Eublepharis turcmenicus* (Anderson, 1999).

***Eublepharis hardwickii* Gray, 1827**

The East Indian leopard gecko, *E. hardwickii* (Fig. 9) was first described by Gray in 1827, based on a single specimen from Chittagong, Penang (Chittagong, now in Bangladesh). The species is distributed from Aushgram in Burdwan District West Bengal (Chandra et al., 1997), Chaibasa (Cantor 1847, Smith 1935), and Barajamda (Jharkhand), Belpahari (West Bengal), (Samanta et al., 2021), Similipal (Dutta et al., 2009) to Balasore (Odisha) (Agarwal et al., 2022). The morphological characteristics for diagnosis in *E. hardwickii*: smooth subdigital lamellae; tubercle-like moderately keeled scales across the dorsum intermixed with much smaller scales, a single pale band between the nuchal loop and caudal constriction; 16 precloacal pores. Conservation status in the IUCN Red List categories and criteria for the species is Least Concern.

***Eublepharis pictus* Mirza and Gnaneswar, 2022**

A recent molecular phylogenetic study of the genus *Eublepharis* Gray, 1827 revealed a new species. The painted leopard gecko *E. pictus* (Fig. 10) is recorded by Mirza and Gnaneswar in 2022 from near a temple in Vishakhapatnam, Andhra Pradesh eastern India. The morphological characteristics for diagnosis in *E. pictus*: smooth subdigital lamellae; 17–18 precloacal pores; tubercle-like moderately keeled scales across the dorsum intermixed with much smaller scales, a single pale band between the nuchal loop and caudal constriction. Conservation status in the IUCN Red List categories and criteria for the species is not recorded.

***Eublepharis satpuraensis* Mirza, Sanap, Raju, Gawai and Ghadekar, 2014**

The Satpura leopard gecko, *E. satpuraensis* (Fig. 11) was discovered from Satpura Hills, Central India based on detailed comparison of morphological parameters with congeners by Mirza et al (2014). The distribution range of the species was described as Satpura Hills which included Panchmari and surrounding areas of Satpura Tiger Reserve, Bhopal, Melghat Tiger Reserve, Pench Tiger Reserve,

Bandhavgarh Tiger Reserve and Jabalpur. Also was recorded from Bhoramdeo Wildlife Sanctuary and its adjacent forest patches in Chhattisgarh India. The morphological characteristics for diagnosis in *E. satpuraensis*: dome shaped tubercles lacking keels arranged in 20 rows on dorsum, inter-tubercular space more than width of a tubercle; 46–48 ocular fringe scales, three pale bands between the nuchal loop and caudal constriction; median subdigital lamellae smooth; 13–14 preanal pores which may be interrupted medially by a single poreless scale. Conservation status in the IUCN Red List categories and criteria for the species is Least Concern (IUCN 2022).

FIGURE 8. An adult specimen of *Eublepharis fuscus* (Mirza and Upadhye, 2010).

DISCUSSION

The family Eublepharidae originated in the Cretaceous to Jurassic age (Grismer, 1988; Jonniaux and Kumazawa, 2008; Agarwal et. al., 2022). Grismer (1988) and Gamble et al. (2011) hypothesized a scenario for Asian or Laurasian ancestor for the eublepharid geckos, with *Coleonyx* dispersing to the New World through the Beringean land bridge. Agarwal et. al. (2022) analyzed the phylogenetic relationship among the genera of eublepharid geckos based on one mitochondrial gene (*ND2*) and two nuclear genes (*RAG1* and *PDC*), and showed that the Eublepharidae family is divided into two clades, the *Aeluroscalabotes* + *Coleonyx*, and the *Eublepharis* + *Goniurosaurus* + *Hemitheconyx* + *Holodactylus* clade. Prior studies reported *Aeluroscalabotes* as the sister to taxon to other eublepharids (e.g. Grismer, 1988; Kratochvíl and Frynta, 2002; Jonniaux and Kumazawa, 2008). The genus *Eublepharis* is distributed in the eastern and south western Asia and included the species *E. angramainyu*, *E. fuscus*, *E. hardwickii*, *E. macularius*, *E. satpuraensis*, *E. pictus*, and *E. turcmenicus*. The most important diagnostic characters for Identification of the genus are as follows: number of preanal pores, first lower labial scales status (contact with postmental chin shield or no), subdigital lamellae scales status (smooth/ with small tubercles), number of eyelid fringe scales, and size of dorsal tubercles. Additionally, pholidopsis features and anatomical pecularities (Grismer, 1988; Grismer and Ottley, 1998; Grismer, 1991; Anderson, 1999), molecular analyses (Ota et al., 1999; Jonniaux and Kumazawa, 2008; Agarwal et al., 2022; Mirza and Gnaneswar, 2022), and distribution pattern of species (Grismer, 1987; Auer et al., 2008; Mirza and Upadhye, 2010; Mirza et al., 2014; Basak et al., 2017; Naidu et al., 2020; Karamiani and Rastegar-Pouyani 2021) can separate species of the genus *Eublepharis*. The molecular phylogenetic study of the genus *Eublepharis* Gray, 1827 based on the ND2 gene revealed that *E. angramainyu* is sister to all other species (Agarwal et al, 2022). Karamiani and Rastegar-Pouyani (2021) modeled range distribution of *E.*

FIGURE 9. An adult specimen of *Eublepharis hardwickii* (Prakash et al., 2014).

FIGURE 10. An adult specimen of *Eublepharis pictus* (Mirza and Gnaneswar 2022).

angramainyu and showed that it is restricted to the western and south-western regions of the Iranian Plateau, north-eastern Iraq, and southern Turkey to the Levant had a wide variety of area suitability for *E. angramainyu*.

Eublepharis macularius has been the subject of numerous studies, including: physiology and behavior (Viets et al., 1993; Flores et al., 1994; Sakata et al., 2002; LaDage and Ferkin, 2006; Starostov´a et al., 2009; Pallotta et al., 2017); embryology (Thorogood and Whimster, 1979; Crews et al., 1998; De Vosjoli et al., 2005; Wise et al., 2009; Xiong et al., 2016); phenotypic evolution (Kiskowski et al., 2019). Few studies such as record reports (Ananjeva et al., 2006; Auer et al., 2008), and captive breeding (Kaverkin and Orlov, 1996) have been published for *Eublepharis turcmenicus*. As previously mentioned, *E. fuscus* was first introduced as a subspecies of *E. macularius* by Borner (1981), but due to its several morphological differences with known congeners, and it was elevated to a full species by Das (1977).

FIGURE 11. An adult *Eublepharis satpuraensis* (Naidu et al., 2020).

Although *E. hardwickii* is the type species of the genus *Eublepharis* Gray 1827, but it has not been subject of any further studies. *Eublepharis pictus* and *E. satpuraensis* have recently described based on molecular and morphological studies, however they need to be more studied in future, although recorded range extension of *E. satpuraensis* (Basak et al., 2017; Naidu et al., 2020).

ACKNOWLEDGMENTS.
The author thanks Ali Bazdar, Ali Permanoon, Erfan Permanoon, Akbar Fattahi, and Morad Besharati for assisting us with field work. I am grateful to the authorities of Razi University (Kermanshah, Iran) for financial support during the field work.

LITERATURE CITED

Al-Sheikhly, O.F., Haba, M.K., Fazaa, N.A., Al-Barazengy, A.N., Al-Haideri, M.L., Al- Joborey, A.D. 2020. New records of the Iraqi eyelid gecko, *Eublepharis angramainyu* Anderson and Leviton, 1966 (Sauria: Eublepharidae) from Iraq. Russian Journal of Herpetology 27, 240–244.

Ananjeva, N.B., Orlov, N.L., Roman, G.K., Darevsky, S.D., Ryabov, S.A., Barabanov, A.V. 2006. The reptiles of northern Eurasia-taxonomic diversity, distribution, conservation status. Sofia (Pensoft Publishers).

Anderson, S.C. 1999. The Lizards of Iran. Society for the Study of Amphibians and Reptiles, Oxford, Ohio.

Auer, M., Richter, S., Khani, A. 2008. A new record of the Turkmenian fat-tailed gecko, *Eublepharis turcmenicus* Darevsky, 1978, from north-eastern Iran (Squamata: Gekkonidae). Zoology in the Middle East 45, 107–110.

Basak, K., Ahmed, M. Suraj, M., Mondal, K. 2017. Satpura Leopard Gecko: Range extension record of a newly described species, *Eublepharis satpuraensis* from Bhoramdeo Wildlife Sanctuary, Chhattisgarh, central India. Reptile Rap #173. Zoos' Print Journal 32, 34–39.

Crews, D., Sakata, J., Rhen, T. 1998. Developmental effects on intersexual and intrasexual variation in growth and reproduction in a lizard with temperature-dependent sex determination. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 119, 229–241.

Daniel, J.C. 1983. The book of Indian reptiles. Bombay Natural History Society, Oxford University Press.

Daniel, J.C. 2002. The Book of Indian Reptiles and Amphibians. Bombay Natural History Society, Oxford University Press.

Das, I. 1997. Resolution of the systematic status of *Eublepharis macularius fuscus* Börner, 1981 (Eublepharidae: Sauria: Squamata). Hamadryad 22, 13–20.

De Vosjoli, P., Tremper, R., Klingenberg, R., 2005. The lerpetoculture of leopard geckos: twenty-seven generations of living art. Advanced Visions.

Flores, D., Tousignant, A., Crews, D., 1994. Incubation temperature affects the behavior of adult leopard geckos (*Eublepharis macularius*). Physiology and Behavior 55, 1067–1072.

Gamble, T., Bauer, A.M., Colli, G.R., Greenbaum, E., Jackman, T.R., Vitt, L.J. Simons, A.M. 2011. Coming to America: multiple origins of New World geckos. Journal of evolutionary biology 24, 231–244.

Grismer, L.L. 1987. Evidence for the resurrection of *Goniurosaurus* Barbour (Reptilia: Eublepharidae) with a discussion on geographic variation in *Goniurosaurus lichtenfelderi*. Acta Herpetologica Sinica 6, 43–47.

Grismer, L.L. 1988. Phylogeny, taxonomy, classification, and biogeography of eublepharid geckos. In: Estes, R., Pregill, G.K. (Eds.), Phylogenetic relationships of the lizard families. Stanford University Press, CA, 369–469.

Grismer, L.L. 1989. *Eublepharis ensafi* Baloutch and Thireau, 1986: A junior synonym of *E. angramainyu* Anderson and Leviton, 1966. Journal of herpetology 23, 94–95.

Grismer, L.L. 1991. Cladistic relationships of the lizard *Eublepharis turcmenicus* (Squamata: Eublepharidae). Journal of herpetology 25, 251–253.

Harshil, P., Vyas, R. 2019. Reptiles of Gujarat, India: updated checklist, distribution, and conservation status. Herpetology Notes 12, 765–777.

IUCN. 2022. The IUCN Red List of Threatened Species. Version 2022-1. https://www.iucnredlist.org. Accessed on 09 November 2022.

Jonniaux, P., Kumazawa, Y. 2008. Molecular phylogenetic and dating analyses using mitochondrial DNA sequences of eyelid geckos (Squamata: Eublepharidae). Gene 407, 105–115.

Karamiani, R., Rastegar-Pouyani, N. 2010. New specimens of *Eublepharis angramainyu* Anderson and Leviton, 1966 (Sauria: Eublepharidae), from south-western regions of the Iranian Plateau. Hamadryad 35, 116–121.

Karamiani, R., Rastegar-Pouyani, N. 2017. Skull anatomy and Comparative Cranial Osteology of *Eublepharis angramainyu* (Sauria: Eublepharidae) and *Asaccus elisae* (Sauria: Phyllodactylidae), Species 18, 117–132.

Karamiani, R., Rastegar-Pouyani, N. 2021. The effect of climate change on habitat suitability and a distribution model of the Iranian fat-tailed gecko, *Eublepharis angramainyu* Anderson and Leviton, 1966 (Sauria: Eublepharidae) since the Last Interglacial to 2050. Zoology and Ecology 31, 24–32.

Kaverkin, Y.I., Orlov, N.L. 1996. Experience of Captive Breeding of *Eublepharis turcmenicus* Darevsky, 1978 (Eublepharidae, Sauria). Russian Journal of Herpetology 3, 98–99.

Kiskowski, M., Glimm, T., Moreno, N., Gamble, T., Chiari, Y. 2019. Isolating and quantifying the role of developmental noise in generating phenotypic variation. PLOS Computational Biology 15, e1006943.

Kratochvíl, L., Frynta, D., 2002. Body size, male combat and the evolution of sexual dimorphism in eublepharid geckos (Squamata: Eublepharidae). Biological Journal of the Linnean Society 76, 303–314.

Leviton, A.E., Anderson, S.C. Adler, K. Minton, S.A. 1992. Handbook to Middle East amphibians and reptiles. Society for the Study of Amphibians and Reptiles, Oxford, Ohio.

Mirza, Z.A., Gnaneswar, C. 2022. Description of a new species of leopard geckos, *Eublepharis* Gray, 1827 from Eastern Ghats, India with notes on *Eublepharis hardwickii* Gray, 1827. Evolutionary Systematics 6, 77-88.

Mirza, Z.A., Sanap, R.V., Raju, D., Gawai, A., Ghadekar, P. 2014. A new species of lizard of the genus *Eublepharis* (Squamata: Eublepharidae) from India. Phyllomedusa 13, 75–90.

Mirza, Z.A., Upadhye, R. 2010. Zur Verbreitung und Lebensweise des in Indien endemischen Lidgeckos *Eublepharis fuscus* Börner, 1981. Sauria 32, 15–23.

Moradi, N., Shafiei, S. 2011. New record of the Western leopard gecko, *Eublepharis angramainyu* Anderson and Leviton, 1966 (Squamata: Eublepharidae) from southeastern Iran. Amphibian and Reptile Conservation 5, 88–91.

Murphy, R.W. 1974. A new genus and species of eublepharine gecko (Sauria: Gekkonidae) from from Baja California, Mexico. California Academy of Sciences 40, 87–92.

Naidu, R., Trivedi, K., Mandavia, A., Kalyar, R.K., Singh, S., Chouhan, A. 2020. Range Extension of the Satpura Leopard Gecko, *Eublepharis satpuraensis* (Squamata: Eublepharidae), from Jashpur District, Chhattisgarh, India. Reptiles and Amphibians, 27, 269–270.

Ota, H., Honda, M., Kobayashi, M., Sengoku, S., Hikida, T. 1999. Phylogenetic relationships of eublepharid geckos (Reptilia: Squamata): a molecular approach. Zoological Science 16, 659–666.

Prakash, S., Raziuddin, M., Mishra, A.K. 2014. Record of East Indian Leopard Gecko *Eublepharis hardwickii* Gray, 1827 (Squamata: Sauria: Eublepharidae) from Hazaribag, Jharkhand, India. *Reptile Rap* #16, 24-26.

Pyron, R.A., Burbrink, F.T., Wiens, J.J. 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC evolutionary biology 13, 1-54.

Seufer, H., Kaverkin, Y., Kirschner, A. 2005. Die Lidgeckos. Kirschner und Seufer Verlag, 238 pp.

Starostová, Z., Kratochvíl, L., Frynta, D. 2005. Dwarf and giant geckos from the cellular perspective: the bigger the animal, the bigger its erythrocytes? Functional Ecology, 744-749.

Starostová, Z., Kubička, L., Konarzewski, M., Kozłowski, J., Kratochvíl, L. 2009. Cell size but not genome size affects scaling of metabolic rate in eyelid geckos. The American Naturalist, 174, E100–E105.

Uetz, P., Freed, P, Hošek, J. 2022. The Reptile Database. Available at http://www.reptile-database.org. Accessed on 25 August 2022.

Vitt, L.J., Caldwell, J.P. 2013. Herpetology: an introductory biology of amphibians and reptiles. Academic press.

Vyas, R. 2000a. A review of reptile studies in Gujarat state. Zoos' Print Journal 15, 386–390.

Vyas, R. 2000b. Comments on A synopsis of the reptiles of Gujarat, India'. Hamadryad-Madras 25, 203–207.

Wise, P.A.D., Vickaryous, M.K., Russell, A.P. 2009. An embryonic staging table for in ovo development of *Eublepharis macularius*, the leopard gecko. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology: Advances in Integrative Anatomy and Evolutionary Biology 292, 1198–1212.

Xiong, Z., Li, F., Li, Q., Zhou, L., Gamble, T., Zheng, J., Kui, L., Li, C., Li, S., Yang, H., Zhang, G. 2016. Draft genome of the leopard gecko, *Eublepharis macularius*. Gigascience 5, s13742–s14016.

Iranian Journal of Animal Biosystematics (IJAB)
Vol.18, No.1, 47-70, 2023
DOI: 10.22067/ijab.2023.82443.1056

IJAB
ISSN: 1735-434X (print); 2423-4222 (online)

RESEARCH ARTICLE Open access

Interspecific variation within the genus *Ophiomorus* Duméril & Bibron, 1839 (Sauria: Scincidae) in Iran based on morphological characters

Hossein Nabizadeh[1], Nasrullah Rastegar-Pouyani[1,*], Eskandar Rastegar-Pouyani[2], Mehdi Rajabzadeh[3]

[1] *Department of Biology, Faculty of Sciences, Razi University, Kermanshah, Iran*
[2] *Department of Biology, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran*
[3] *Department of Biodiversity, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran*

(Received: *17 May 2023*; Accepted: *05 August 2023*)

Abstract
Many studies conducted on range of animals showed that morphology is related to habitat. In the present study, we aimed to examine the morphological characteristics of species assigned to the genus *Ophiomorus* in Iran. Seventy-one specimens from throughout the range of distribution in the central plateau of Iran were investigated. Eleven morphometric and four meristic variables were analyzed independently. Multivariate analyses were performed using canonical variate analysis (CVA) and principal component analysis (PCA). The results showed that there is significant morphological differentiation between three species groups *brevipes*, *tridactylus*, and *punctatissimus* in this genus in relation to habitat choice. ANOVA results showed that 14 morphological characters (SVL, HL, HW, HH, SL, LF, LA, LFL, LFH, LHF, NSL, NDSB, NMC, and NIL; 10 metric and four Meristic) were significantly different among all *Ophiomorus* species, which based on this, TL did not show a significant difference among species. Finally, we assigned three species groups based on limb reduction (especially fingers), and habitat surface (comparison based on habitat observations) for all *Ophiomorus* species in Iran. In addition, using newly applied morphological characteristics we proposed an updated identification key for the genus.

Key words: *Adaptation, Biogeography, Morphology, Ophiomorus, Iranian Plateau, Snake Skinks*

INTRODUCTION

Traditional taxonomy based on morphological diagnostics and the morphological species concept, is important to use accurate characteristics to determine the species (Mayr, 2000; Sites & Marshall, 2003; Bauer et al., 2011; Kornilios et al., 2018). However, it should be highlighted that each species may show geographic variation in morphology, and then in this case, the integrated taxonomic study will help the evaluation (Padial et al., 2010; Ford, 2018; Cicero et al., 2021). Reptiles are one of the ancient animal groups that live more than 300 million years on the earth, during this time adapted to different habitats and most diversified (Organ et al., 2008). Due to the low mobility of reptiles and select the microchabitat area as special habitat in some taxa, strict adaptations can be found in these types of animals (Cloudsley-Thompson, 1991). The Family Scincidae in Iran consists of six genera, one of which is the genus

Corresponding Author: nasrullah.r@gmail.com

Ophiomorus, which occurs in most parts of the central Iranian plateau (Nabizadeh et al., 2022). The recent molecular phylogenetic study showed that the genus *Ophiomorus* has a sister relationship with the genus *Mesoscincus* from Central America, which belongs to the Scincidae family (Pyron et al., 2013; Andrade et al., 2016). The genus *Ophiomorus* has 12 species, which show a considerable amount of morphological and ecological diversity. Three species of the genus inhabited the west of the Palearctic, namely in southeastern Europe, the Middle East, and the eastern Mediterranean region, and nine of them are distributed in south and southwest Asia (Nabizadeh et al., 2022). Seven species including endemic and non-endemic species are present in the Iranian plateau (Nabizadeh et al., 2022) (Fig. 1). Iranian endemic species comprise *Ophiomorus maranjabensis*, *Ophiomorus nuchalis*, *Ophiomorus persicus*, and *Ophiomorus streeti* (Anderson, 1999; Nabizadeh et al., 2022) (Fig. 2). Recently, two species of the genus *Ophiomorus* have been described. *Ophiomorus maranjabensis* and *Ophiomorus kardesi* were described in Iran in 2011 and southern Turkey in 2018, respectively (Kazemi et al., 2011; ŠMÍD et al., 2014; Eskandarzadeh et al., 2018; Kornilios et al., 2018). The genus dwells in different types of habitats and surfaces. The results obtained from the morphological studies showed that *O. maranjabensis*, *O. streeti*, and *O. tridactylus* are only distributed in completely sandy habitats or sand dunes, but *O. persicus* lives in hard and stony grounds. In addition, *O. nuchalis*, *O. brevipes*, and *O. blanfordii* live in habitats where the hard ground is a mixture of sand and gravel (Anderson, 1999; Nabizadeh et al., 2022) (Fig. 3). One of the main characteristics of skink members is the short limbs or no limbs, which means that this genus is a monophyletic group taxon (Pyron et al., 2013). Identification of all members of this genus except *Ophiomorus kardesi* was mainly based on morphological traits (Kornilios et al., 2018). The members of this genus choose different habitats based on their morphological characteristics (Greer & Wilson, 2001) and accordingly, Anderson and Leviton (1966) divided the members of this genus into three species groups, *brevipes*, *tridactylus*, and *punctatissimus*. These characters were defined based on their tendency to limb reduction (especially fingers) (Anderson & Leviton, 1966). Anderson (1999) divided the genus *Ophiomorus* into two western and eastern groups based on habitat preferences. The western group have a very cylindrical and long body and a conical snout, and they choose hard surfaces, such as under rocks or hard soils, as habitats which include *Ophiomorus punctatissimus*, *Ophiomorus latastii*, and *Ophiomorus persicus*. The eastern group consists of *Ophiomorus chernovi*, *Ophiomorus brevipes*, *Ophiomorus blanfordii*, *Ophiomorus nuchalis*, *Ophiomorus streeti*, *Ophiomorus raithmai*, and *Ophiomorus maranjabensis* that are dwelling in loose sand and sand dune regions. Due to the nocturnal activity and nest under the surface or being cryptic, the genus *Ophiomorus* has not been studied thoroughly. In the present study, we aimed to examine the morphological characteristics of species of the genus *Ophiomorus* from the Iranian Plateau. Meanwhile, the main goal of this study was to investigate the validity of the morphological characteristics of the *Ophiomorus* species in the Iranian Plateau.

MATERIAL AND METHODS

SAMPLING AND DEPOSITION

Ninety specimens of the genus *Ophiomorus* were collected during fieldwork in Central Iran from April 2019 to September 2021 (Table 1). Finally, we selected 71 male specimens belonging to eight taxonomic operational units (OTUs). The locality addresses of the collected population and their geographic coordinates are provided in Table 1. Elevation was obtained using a Garmin eTrex 30 GPS receiver. Specimens were photographed and then euthanized, and all specimens were preserved in 75% ethanol and deposited in the Sabzevar University Herpetological Collection (SUHC). The approval (no. 14968) for the study was provided by the ethical committee of Razi University of Kermanshah, Kermanshah, Iran.

MORPHOLOGY

Morphological characters were examined as body size and body shape, meristic characters of pholidosis, and the description of color patterns of adult specimens. All measurements were taken using digital calipers with 0.01 mm accuracy under the light loop. Furthermore, the heads of all species were drawn to compare the scales of the head. Morphometric and morphological descriptions followed Anderson (1999), Rastegar-Pouyani et al. (2001, 2007), Kazemi et al. (2011) and Nasrabadi et al. (2017).

FIGURE 1. Sampling localities of collected specimens of *Ophiomorus* in Iran.

For morphometric analysis, the following standard characters were used: SVL—snout-vent length (from snout to vent); TL—tail length (from the posterior edge of the cloaca to the tip of the tail); HL—head length (from the end of the snout to the angle of the jaw); HW—head width (at the widest point of the head); HH—head height (from lower edge infralabial to the tip of supraocular); SL—Snout Length (from the tip of snout to the anterior corner of the eye); LF—Length of femur; LA—Length of arm; LFL—Length of forelimb; LFH—Length of hindlimb; LHF—Length between hindlimb and forelimb. The following meristic characters were examined: NSL (R/L)—Number of supralabials; NIL (R/L)—Number of infralabials; NDSB—Number of dorsal scales around the body; NMC—Number of scales from mental to anterior edge of the cloaca. In addition to the characters mentioned above, we added body coloration and patterns for the specimen description.

MULTIVARIATE AND UNIVARIATE ANALYSES

The 11 morphometric and four meristic variables were analyzed independently (Table 2). Statistical analyses were used to investigate differences in shape and size among all *Ophiomorus* species and OTU (*Ophiomorus* cf. *nuchalis*) present in Iran.

FIGURE 2. Photos of the endemic species of the genus *Ophiomorus* in Iran, *O. streeti* (A), *O. maranjabensis* (B), *O. nuchalis* (C), *O. persicus* (D) and Non-endemic species *O. brevipes* (E), *O. blanfordii* (F), *O. tridactylus* (G).

SPSS Statistics V.26. was used for statistical analyses, and data normality was checked before analyses. Analysis was carried out separately for morphometric and meristic characters. All morphometric characters were $\log_{10}$-transformed to obtain data normality and increase the homogeneity of variance. After getting the normality test, a one-way analysis of variance (ANOVA) was used among the species. Multivariate analyses have been done using two popular approaches in morphological studies: Canonical Variate Analysis (CVA) and Principal Component Analysis (PCA). These multivariate approaches were done on the significant characters identified by ANOVA. Based on significant characters, the PCA was used to assess the variation among populations. Based on the population grouping, the CVA was used to determine the correct classification.

RESULTS

COLOR AND PATTERN

Based on data from all specimens examined, the color pattern in *Ophiomorus* is variable and may depend on ground color (pers. obs.). The members of the *tridactylus* group living in a completely sandy habitat have a light color (canary yellow color, cream or bright brown in preservative, sandy-beige in life) on the dorsal surface (Fig. 2 (A-B-G)). However, the dorsal color is cream or pale brown in the members of the *brevipes* group (Fig. 2 (C-E-F)).

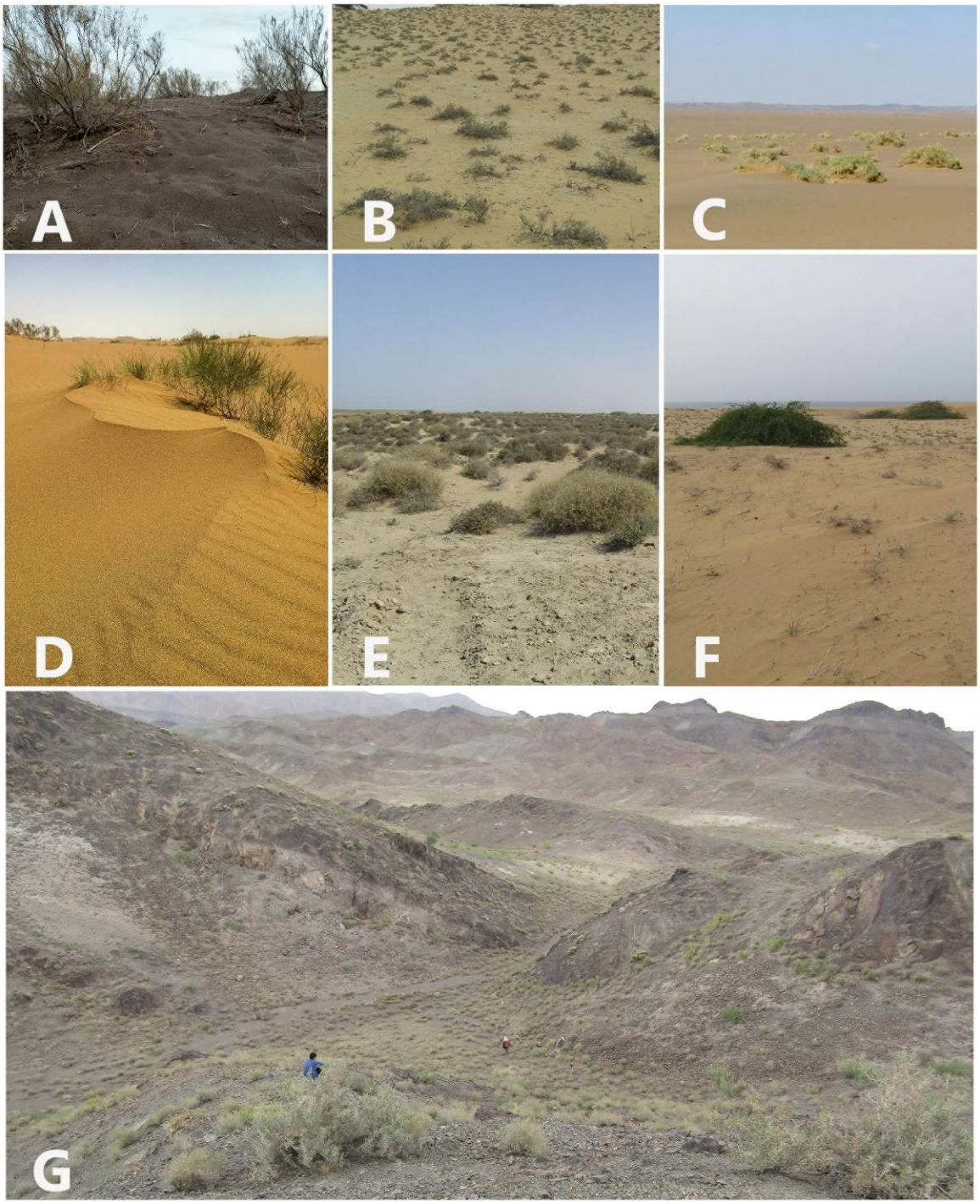


FIGURE 3. (A) One of the habitats of *Ophiomorus nuchalis*, Hoseynabad-e Mish Mast village, Qom province, Iran; (B) Type locality of *Ophiomorus blanfordii* is restricted to Chah Bahar, Iran; (C) Type locality of *Ophiomorus streeti*, Baluchistan, 11 miles west of Iranshahr, Iran; (D) Type locality of *Ophiomorus maranjabensis*, Maranjab, south of salt Lake, Iran; (E) Type locality of *Ophiomorus brevipes*, Sáadatabád (Now: Hajiabad, Hormozgan Province, Iran), S.W. of Karman; (F) East Zabol, one of the habitats of *Ophiomorus tridactylus*; (G) Rabor area in Kerman, one of the habitats of *Ophiomorus persicus*.

TABLE 1. Distribution records of the *Ophiomorus* used in this study.

Species	Latitude	Longitude	Locality
Ophiomorus blandfordii	25.121	61.228	Beris Village, Chabahar, Sistan and Baluchestan Province, Iran
Ophiomorus blandfordii	27.349	62.316	Saravan County, east of Sistan and Baluchestan Province, Iran
Ophiomorus brevipes	30.115	55.166	Shahrebabak city, Kerman Province, Iran
Ophiomorus brevipes	28.206	56.193	Vicinity of Hadjiabad (Sáadatabád) city, Kerman Province, Iran
Ophiomorus maranjabensis	34.311	51.863	Marnjab Desert, Aran o Bidgol, Isfahan Province, Iran
Ophiomorus nuchalis	35.097	51.855	Mobarakiyeh Village, Varamin, Tehran Province, Iran
Ophiomorus nuchalis	34.463	51.156	Hoseynabad-e Mish Mast Village, Qom Province, Iran
Ophiomorus cf. *nuchalis*	34.057	54.803	Mesr Desert,Khour va Biabanak County, Isfahan Province, Iran
Ophiomorus cf. *nuchalis*	33.519	53.855	Anarak-Khur road, Naein County, Isfahan Province, Iran
Ophiomorus cf. *nuchalis*	31.444	54.998	near Mehriz, 80 km SE form Yazd, Yazd Province, Iran
Ophiomorus persicus	29.262	56.979	Rābor, Baft, Kerman Province, Iran
Ophiomorus streeti	27.933	58.083	Roudbar Village, Kerman Province, Iran
Ophiomorus streeti	26.779	60.371	Espakeh-Chanf road, Sistan and Baluchestan Province, Iran
Ophiomorus streeti	27.172	60.735	Iranshahr, Sistan and Baluchestan Province, Iran
Ophiomorus tridactylus	25.796	57.815	Bandar-e-Jask, Hormozgan Province, Iran
Ophiomorus tridactylus	25.267	60.771	Chabahar, Sistan and Baluchestan Province, Iran
Ophiomorus tridactylus	26.128	60.109	Nikshahr, Sistan and Baluchestan Province, Iran
Ophiomorus tridactylus	27.628	62.779	Jālq, Sistan and Baluchestan Province, Iran
Ophiomorus tridactylus	27.112	63.222	Kuhak, Sistan and Baluchestan Province, Iran
Ophiomorus tridactylus	28.105	61.331	Khash, Sistan and Baluchestan Province, Iran
Ophiomorus tridactylus	29.068	61.385	Mirjaveh-Zahedan road, Sistan and Baluchestan Province, Iran
Ophiomorus tridactylus	31.066	61.652	Dust Mohammad, Zabol, Sistan and Baluchestan Province, Iran

TABLE 2. The main morphometric and meristic characters examined in the *Ophiomorus* specimens in this study.

	Character	Definition
Metric	SVL	Snout-Vent Length
	TL	Tail Length
	HL	Head Length (from end of snout to angle of jaw)
	HW	Head Width (at the widest point of head)
	HH	Head Height
	SL	Snout Length (from tip of snout to anterior corner of eye)
	LF	Length of eye (from anterior corner to posterior corner of eye)
	LA	Length of arm (Right)
	LFL	Length of forelimb (Right)
	LFH	Length of hindlimb (Right)
	LHF	Length between hindlimb and forelimb (Right)
Meristic	NSL	Number of supralabials (Right)
	NIL	Number of infralabials (Right)
	NDSB	Number of dorsal scales around body
	NMC	Number of scales from mental to anterior edge of cloaca

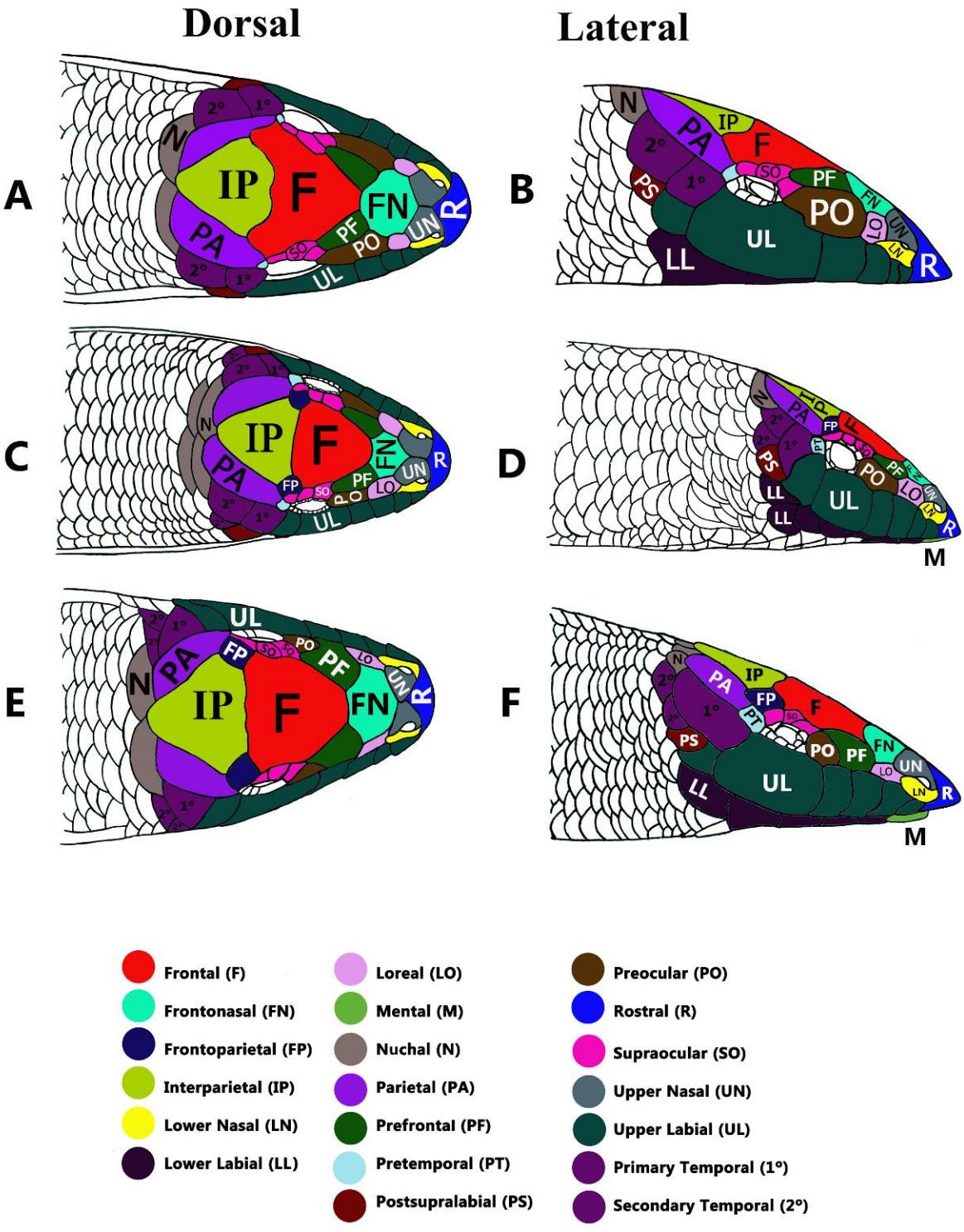


FIGURE 4. Head shape and types of head scales in members of the species group *tridactylus*. A-B: *Ophiomorus maranjabensis*; C-D: *Ophiomorus streeti*; E-F: *Ophiomorus tridactylus.*

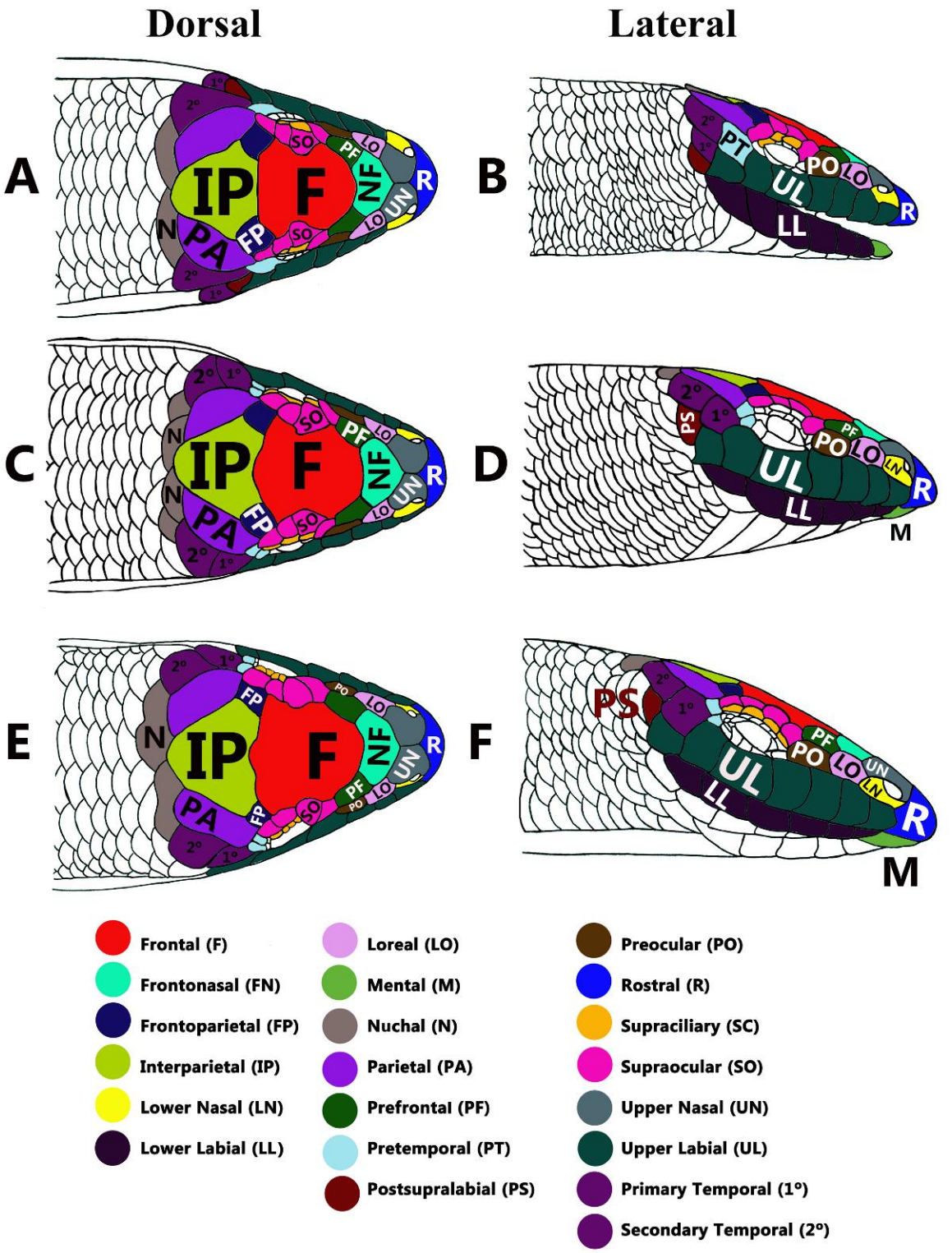


FIGURE 5. Head shape and types of head scales in members of the species group *brevipes*. A-B: *Ophiomorus blanfordii*; C-D: *Ophiomorus brevipes*; E-F: *Ophiomorus nuchalis.*

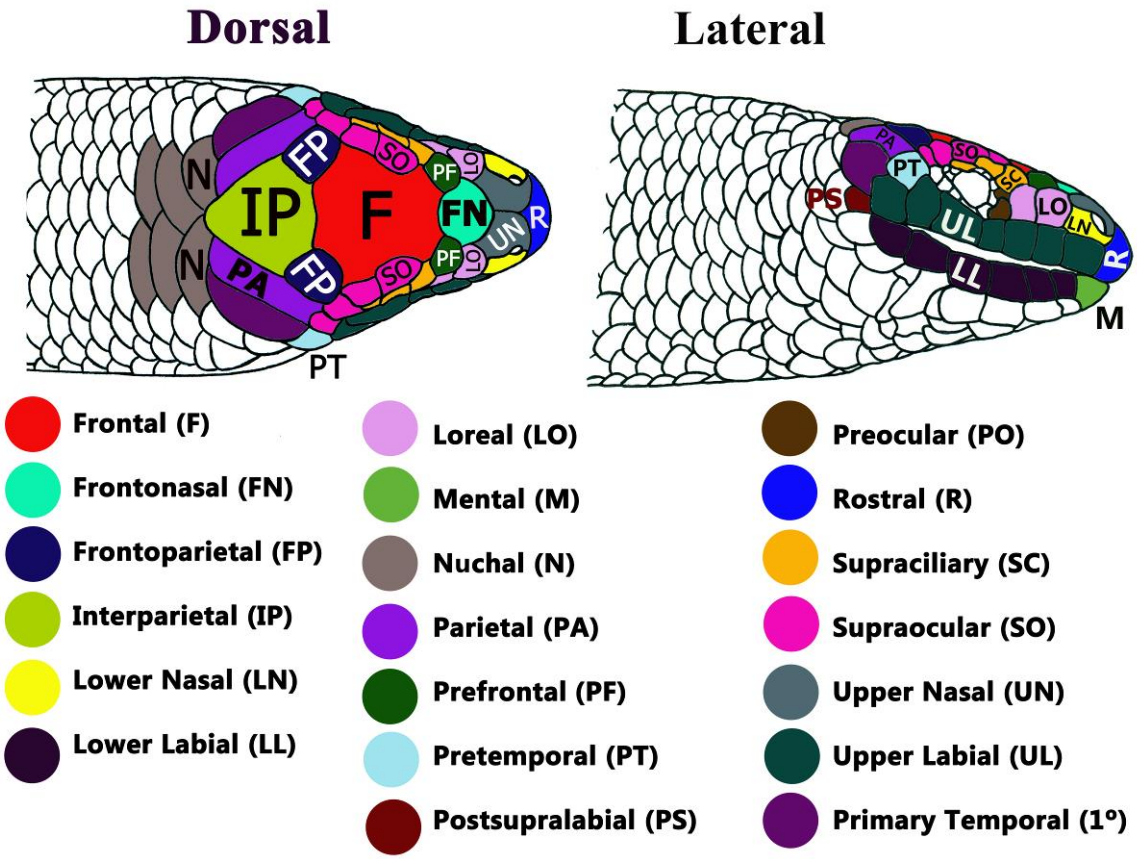


FIGURE 6. Head shape and types of head scales in *Ophiomorus persicus*.

SCALATION

The number of NSL and NIL scales is less in the *Ophiomorus* cf. *nuchalis* population than in the *Ophiomorus nuchalis*. There is an absent frontoparietal scale in the *Ophiomorus maramjabensis*, causing the frontal scale to be V-shaped (Fig. 4). In studies of the shape of the head scales, it was determined that the form of the parietals scale is differentiated between *Ophiomorus nuchalis* and *Ophiomorus brevipes* from species group's *Brevipes* and the population of *Ophiomorus brevipes* is semicircular, and all members of the *Ophiomorus nuchalis* population are rectangular (Fig. 5). Also, *Ophiomorus blanfordii* has a pretemporal scale compared to other members of the *brevipes* species group. The head shape of *Ophiomorus persicus* was smaller compared with the two other species groups (*tridactylus*: *O. maranjabensis*, *O. streeti*, and *O. tridactylus*; *brevipes*: *O. blanfordii*, *O. brevipes*, and *O. nuchalis*) (Fig. 6).

NORMALITY TEST

All characters have been examined for normality and distributed normally. Descriptive statistics of characters for each species and OTU are presented in Table 3. According to the descriptive statistics and ANOVA, the following characters were significantly differentiated between all species of the genus *Ophiomorus*: SVL, HL, HW, HH, SL, LF, LA, LFL, LFH, LHF, NSL, NIL, NDSB, and NMC (P <0.05) (Table 3). Based on the ANOVA, TL did not show a significant difference among species (Table 3). The meristic characters show zero variance at least between two OTU and then, we cannot use them in the PCA. In Principal Component Analysis (PCA) we used only the metric characters and the first three components explain 81.16% of the total variation.

TABLE 3. The mean, standard deviation, and range of 15 metric and meristic characters measured for *Ophiomorus* species on the entire distribution range in the Iranian Plateau.

	O. tridactylus (n = 12)		*O. streeti* (n = 20)		*O. persicus* (n = 4)		*Ophiomoru* cf. *nuchalis* (n = 4)		*O. nuchalis* (n = 7)		*O. maranjabensis* (n = 10)		*O. brevipes* (n = 11)		*O. blanfordii* (n = 3)		*P* value
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
SVL	81.49 ± 10.57	64.53-95.77	67.66 ± 9.55	46.85-90.39	67.18 ± 8.59	55.34-75.83	86.70 ± 6.66	80-95.90	92.05± 6.43	86.12-102.74	83.98 ± 6.24	74.40-92.98	82.23 ± 6.44	72.61-90.32	77.47 ± 7.07	70.47-84.62	0.000
TL	47.93 ± 9.1	32.07-61.48	45.10 ± 5.52	35.07-53.65	64.62 ± 11.01	56.87-77.23	76.06 ± 17.96	55.34-87.15	67.90 ± 13.41	46.87-81.29	43.38 ± 10.43	27.07-55.25	68.60 ± 8.23	58.51-83.07	66.41 ± 17.34	54.15-78.68	0.362
HL	6.90 ± 0.79	4.77-7.64	6.57 ± 0.98	5.46-9.93	4.60 ± 0.37	4.09-4.95	7.56 ± 0.61	7.06-8.33	8.00± 0.49	7.33-8.72	7.41 ± 0.56	6.38-8.08	6.90 ± 0.66	5.99-7.84	7.47 ± 0.31	7.17-7.79	0.000
HW	5.15 ± 0.44	4.37-5.84	4.74 ± 0.41	3.70-5.37	3.89 ± 0.37	3.41-4.28	5.44 ± 0.25	5.23-5.81	6.06 ± 0.30	5.7-6.51	5.08± 0.34	4.64-5.71	5.75 ± 0.40	5.26-6.36	5.23 ± 0.64	4.57-5.86	0.000
HH	3.5 ± 0.49	2.81-4.63	3.37 ± 0.48	2.26-4.01	3.19 ± 0.46	2.52-3.60	3.8 ± 0.54	3.10-4.24	4.47 ± 0.30	3.90-4.86	3.20 ± 0.36	2.89-4.10	4.30 ± 0.49	3.46-4.98	3.73 ± 0.47	3.44-4.29	0.000
SL	4.20 ± 0.29	3.51-4.55	3.89 ± 0.34	3.27-4.45	2.44 ± 0.21	2.20-2.70	4.38 ± 0.27	4.02-4.62	4.60 ± 0.34	4.22-5.18	4.44 ± 0.24	4.09-4.79	4.31 ± 0.36	3.44-4.77	4.43± 0.44	3.92-4.71	0.000
LF	5.45 ± 0.70	4.12-6.61	4.87 ± 0.44	3.84-5.52	1.59 ± 0.22	1.27-1.81	5.73 ± 0.71	5.06-6.68	6.00 ± 0.23	5.57-6.52	5.30 ± 0.44	4.43-5.83	5.45 ± 0.47	4.70-6.00	4.77 ± 0.31	4.55-5.13	0.000
LA	2.80 ± 0.17	1.87-2.41	2.39 ± 0.23	1.97-2.82	1.36 ± 0.13	1.21-1.54	3.35 ± 0.14	3.15-3.47	3.29 ± 0.39	2.69-3.83	2.05 ± 0.14	1.8-2.23	3.2 ± 0.36	2.50-3.90	2.7 ± 0.49	2.16-3.14	0.000
LFL	5.25 ± 0.84	4.17-6.75	6.03 ± 0.56	5.02-6.98	4.38 ± 0.33	3.89-4.61	7.47 ± 1.21	6.27-9.04	8.59 ± 0.83	7.54-9.64	5.36± 0.40	4.63-5.94	7.94 ± 0.58	6.86-9.21	7.22 ± 0.94	6.21-7.92	0.000
LFH	13.21 ± 2.96	5.37-17.23	12.47 ± 1.41	10.20-15.29	4.25 ± 0.29	3.86-4.58	13.38 ± 1.71	11.43-15.20	16.13 ± 0.23	15.63-16.33	13.53 ± 0.98	11.74-15.05	13.78 ± 1.15	11.26-15.93	12.95 ± 0.5	12.49-13.49	0.000
LHF	62 ± 8.94	47.67-74.11	49.81 ± 8.35	32.29-70.64	52.05 ± 6.5	43.15-58.78	65.03 ± 5.18	60.32-72.43	69.9 ± 6.63	62.30-80.93	65.10 ± 5.6	55.58-72.85	61.44 ± 5.85	51.86-69.72	55.35 ± 9.92	45-64.78	0.000
NSL	6.33 ± 0.000	6-6	7 ± 0.41	5-6	5.9 ± 0.000	7-7	7 ± 0.57	6-7	6.50± 0.000	7-7	7 ± 0.031	5-6	5.8 ± 0.000	7-7	6 ± 0.57	6-7	0.000
NIL	5.25 ± 0.45	5-6	5.35 ± 0.48	5-6	6 ± 0.000	6-6	5.5 ± 0.57	5-6	6 ± 0.000	6-6	5.9± 0.31	5-6	6 ± 0.000	6-6	5.33 ± 0.57	5-6	0.000
NDSB	21.17 ± 0.57	21-23	19.65 ± 0.48	19-20	20± 0.000	20-20	22 ± 0.000	22-22	22 ± 0.000	22-22	22.2 ± 0.63	22-24	21.19± 0.3	21-22	21 ± 1.7	20-23	0.000
NMC	124.25 ± 6.15	116-131	110.6 ± 4.24	103-118	124.75 ± 6.39	118-132	117.5 ± 3.78	115-123	117.14 ± 4.67	112-123	124.4 ± 7.66	112-137	116.27 ± 3.66	111-124	109.67 ± 9.5	100-119	0.000

TABLE 4. Factor loading of the first three principal components (PCs) from a correlation matrix of ten characters for the *Ophiomorus* populations used in this study.

Character	PC1	PC2	PC3
SVL	.885	.294	-.210
HL	.734	.198	.590
HW	.866	-.140	-.184
HH	.745	.020	-.216
SL	.521	.095	.832
LF	.724	.002	.003
LA	-.029	.876	-.261
LFL	.642	-.524	-.261
LFH	-.332	.876	.020
LHF	.853	.355	-.231
Eigenvalue	4.673	2.089	1.354
Accumulated percent of trace	46.732	67.625	81.161

TABLE 5. Factor loadings of the first three canonical variates of metric characters in the studied species of the genus *Ophiomorus* in Iran.

Character	CVA		
	1	**2**	**3**
SVL	.586	1.452	.870
HL	-.118	-.211	-.211
HW	.221	.359	.272
HH	-.271	.266	-.172
SL	.259	-.024	.259
LF	.546	-.819	-.078
LA	.367	.716	.149
LFL	.453	.559	-.474
LFH	.643	-.024	-.315
LHF	-1.140	-1.627	.197
Eigenvalue	18.287	5.458	1.019
Cumulative percent	71.6	93.0	97.0

Of this value, 46.73% was explained by PC1, in which SVL, HW, and LHF have the most weight; 67.62% was explained by PC2, which is mainly attributed to LA and LFH; and 13.53% was explained by PC3, which is mainly attributed to HL and SL (Table 4 and Fig. 7). Discriminant Function Analyses (DFA) has been done to provide more accurate discrimination between all species**.** The CVA between all species is highly significant, and the first component explained 97% of all variance (Table 5). Based on the CVA chart, species having four fingers in their forelimbs are separated from species having three fingers in their fore and hindlimb and finally, these species groups get differentiated completely from *Ophiomorus persicus* (Fig. 8). Based on table 6 and Appendix 1, metric characters that have shown the most differences between species have been determined. The results of all parametric tests of OTUs

based on all significant characters are presented in table 6 and Appendix 1. Furthermore, all morphological characters were measured on all specimens and are presented in Appendix 2. *Ophiomorus persicus* is the only extant representative of the *punctatissimus* group, which has shown the most morphological differences with the members of the *tridactylus* group**.** Therefore, *O. persicus* with 11 morphological characters has the most differences with *O. streeti*, nine morphological differences with *O. tridactylus*, and seven morphological differences with *O. maranjabensis*. These members of the *tridactylus* group have three fingers in both the forelimb and hindlimb (Table 6). *Ophiomorus persicus* has also shown a significant morphological difference with the members of the *brevipes* group, which have four fingers in the front limb, including the most differences with *O. bervipes*, *O. nuchalis* species in seven, and *O. blanfordii*, *O.* cf. *nuchalis* in six morphological characters (Appendix 1).

DISSCUSSION

Iran has a diversity of ecosystems from mountainous to plains, deserts, and forests, so the diversity of ecosystems in Iran has played a crucial role in the diversification of reptiles (Anderson, 1999). The Zagros Mountain in the west of Iran has caused changes in the shape of the center of the Iranian plateau. These changes have affected the isolation of the population and the process of speciation of reptiles in the plateau of Iran (Rajabizadeh, 2013). In the central plateau of Iran, lizards of the genus *Ophiomorus* have ecologically and morphologically diverse radiation that has successfully inhabited most of the terrestrial arid ecosystems. In the genus *Ophiomorus*, a line of specialization adapted to life in the wind-blown sand and consequently, the other one line of specialization resulted in the legless western species (*O. kardesi*, *O. latastii*, *O. persicus*, and *O. punctatissimus*) occupying an upland, under-rock habitat. Within living organisms, the relationship between morphological characters and functional capabilities has been studied

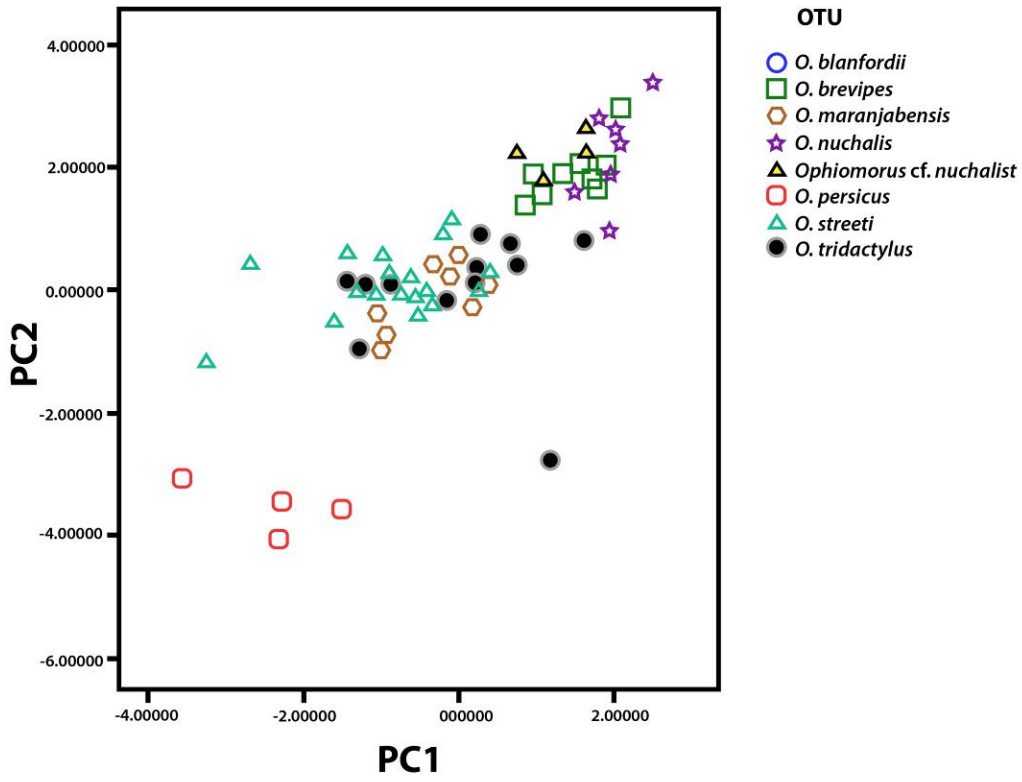


FIGURE 7. The PCA plot of inter-specific variation based on metric characters within the genus *Ophiomorus* in the Iranian Plateau.

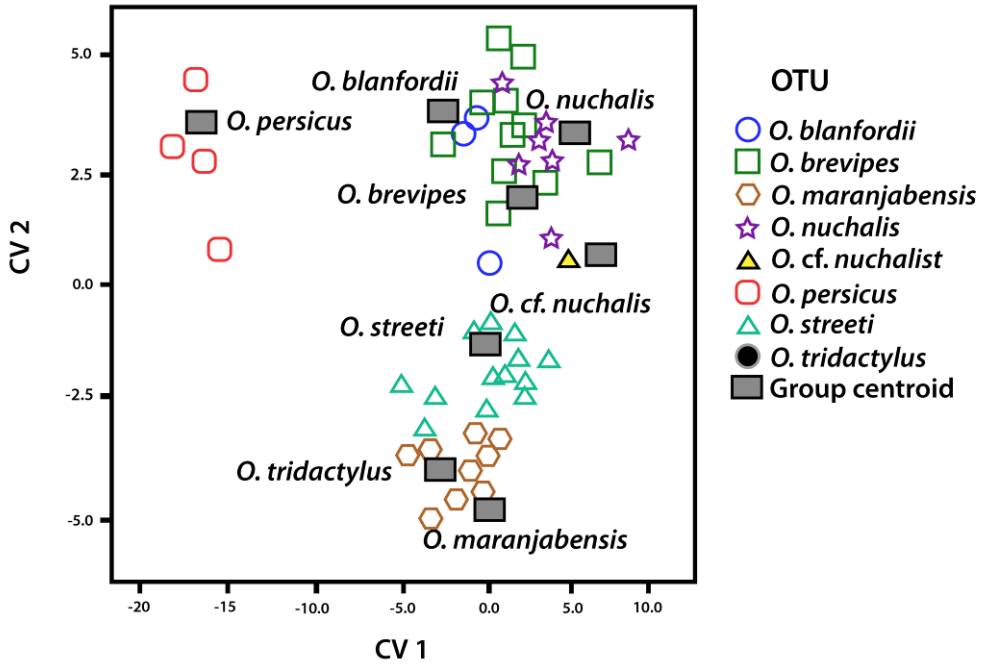


FIGURE 8. Ordination of the individuals of all species of the genus *Ophiomorus* in the Iranian Plateau on the first two canonical variates of metric characters.

by many researchers (Tulli et al., 2010). Accordingly, many studies on a range of animals have shown that morphology correlates to the type of habitat. (e.g. mammals: Norberg, 1994; birds: Collins & Paton, 1989; Miles & Ricklefs, 1987; fish: Douglas, 1987; McDowall, 1998; and reptiles: Moermond, 1979; Williams, 1983; Pounds, 1988; Losos & Sinervo, 1989; Losos, 1990). Scincid lizards probably constitute the most remarkable lizards to illustrate the high frequency of convergent limb loss (Miralles et al., 2012). Within this family, full limblessness as the complete absence of any external limbs is well observed in two genera, *Ophiomorus* and *Chalcides*. (Poulakakis et al. 2008; Brandley et al. 2008; Miralles et al., 2012). In *Ophiomorus* species, limbs can vary from slightly-developed to absent, trunks from short and stout to long and thin, and tails from long to short. The *Ophiomorus persicus*, have forelimbs that are much less reduced than the hindlimbs (Reduced in size or in number of fingers). In contrast, four other species of *Ophiomorus* (*O. maranjabensis*, *O. raithmai*, *O. streeti*, and *O. tridactylus*) have an equal number of fingers in the forelimbs and hindlimbs. Eventually, three species of *Ophiomorus* (*O. kardesi*, *O. latastii*, and *O. punctatissimus*) lack forelimbs and hindlimbs. Anderson and Leviton (1966) devided *Ophiomorus* in three groups include *brevipes*, *punctatissimus*, and *tridactylus* in terms of the tendency to degeneration of motor organs (especially fingers). In another grouping, Anderson (1999) states that the members of the *Ophiomorus* are classified into western and eastern groups based on habitat selection and morphology, respectively. Recent studies on geometric morphometrics showed that changes in the shape of the head scales in Iran's *Ophiomorus* species emphasized the division based on habitat selection and morphology (Nabizadeh et al., 2022). However, we have examined 71 specimens from throughout the range of distribution in the central plateau of Iran and have found that morphological differentiation among three groups' *brevipes*, *tridactylus*, and *punctatissimus* is distinctive. The results of our morphometric study showed that the Iranian *Ophiomorus* species emphasize division based on their tendency to limb reduction (especially fingers). Accordingly, ANOVA results showed that fourteen morphological characters (SVL, HL, HW, HH, SL, LF, LA, LFL, LFH, LHF, NSL, NDSB, NMC, and NIL; ten metric and four meristic) were significantly different between all *Ophiomorus* species.

TABLE 6. The results of all parametric tests of the OTUs based on all significant characters.

	O. tridactylus	*O. streeti*	*O. persicus*	*O.* cf. *nuchalis*	*O. nuchalis*	*O. maranjabensis*	*O. brevipes*
O. blanfordii	NSL- LFL/SVL	LF/SVL	HW/HL- HH/HL- LF/SVL- LA/SVL- LHF/SVL- LFL/SVL	**********	NSL- NIL	NIL- NMC- TL/SVL- LA/SVL- LFL/SVL- LHF/SVL	NSL- NIL- HH/HL
O. brevipes	NIL- NDSB- NMC- TL/SVL- HW/HL- HH/HL- LFL/SVL	NIL- NMC- TL/SVL- HW/HL- HH/HL- LA/SVL- LF/SVL	NDSB- NMC- SL/HL- LF/SVL- LA/SVL- LFL/SVL- LHF/SVL	NSL- NIL- HH/HL	HW/HL- SL/HL	NMC- TL/SVL- HW/HL- HH/HL- LA/SVL- LFL/SVL- LHF/SVL	
O. maranjabensis	NIL- NDSB- HH/HL- LA/SVL- LHF/SVL	NIL- NMC- TL/SVL- HH/HL- LF/SVL- LHF/SVL	NSL- NDSB- TL/SVL- HW/HL- HH/HL- SL/HL- LF/SVL	NIL- TL/SVL- LA/SVL- LFL/SVL- LHF/SVL	NIL- NMC- TL/SVL- HH/HL- LA/SVL- LFL/SVL		
O. nuchalis	NSL- NDSB- NMC- TL/SVL- LA/SVL- LFL/SVL	NIL- NMC- LF/SVL- LHF/SVL	NDSB- HW/HL- HH/HL- SL/HL- LF/SVL- LA/SVL- LFL/SVL	NSL- NIL			
O.* cf. *nuchalis	NSL-NDSB- NMC- TL/SVL- LA/SVL- LFL/SVL	NSL- NDSB- NMC- LA/SVL	NDSB- HW/HL- HH/HL- LF/SVL- LA/SVL- LHF/SVL				
O. persicus	NSL- NIL- TL/SVL- HW/HL- HH/HL-SL/HL- LF/SVL- LA/SVL- LHF/SVL	NSL- NIL- NMC- TL/SVL- HW/HL- HH/HL- SL/HL- LF/SVL- LA/SVL- LFL/SVL- LHF/SVL					
O. streeti	TL/SVL- LFL/SVL- LHF/SVL						

The results of PCA and CVA in this study approve this claim to a large extent. *Ophiomorus persicus* is the only extant representative of the *punctatissimus* group, which has shown the most morphological differences with the members of the *tridactylus* group**.** Also, we found that morphological differentiation between members of the two groups' *brevipes*, and *tridactylus* is distinctive**.** Based on Tables 4 and 5, the distribution of different species of *Ophiomorus* in Figs 7 and 8 shows that *O. persicus* from *punctatissimus* group is completely separated from the *brevipes* and *tridactylus* in the CV1 graph. This separation is based on the characters of the distance between LHF, LFH, and SVL. But, the members

of the *brevipes* group in the CV2, in one complex, are separated from the members of the *tridactylus* group, and this separation, which is the most effective factor between these groups, is based on the SVL, LHF, LF, and LA (Table 4-5). In conclusion, it can be said that this separation is based on choosing different habitats, as the habitat of *O. presicus* is completely separated and differentiated from other two groups**.** According to our results, species including *O. streeti*, *O. maranjabensis*, and *O. tridactylus* observed in sand habitats in which there are sand dunes, furthermore, this sand habitat is adaptive for their distribution, feeding, and procreation, and also, they never leave sand habitat naturally. But species like *O. blanfordii*, *O. brevipes*, *O. nuchalis*, and also *Ophiomorus* cf. *nuchalis* observed in habitats with steppe soils along with sandy microhabitates where they look for food, procreate, and shuttle between sand microclimates. But being diggers and Cryptozoic, they do feed in this microclimate. It is worth mentioning that the habitat of *O. persicus* is completely different from the members of the two other groups. They have distribution in the stony and rocky lands and spend their daytime under stones and holes. Choosing these habitats also has changed the shape of the head scales. According to the results of the morphological geometric done on the heads of all species existing on the Central Plateau of Iran, it is shown that members of the *tridactylus* group prefer sandy habitat with sand dunes**.** They have a bigger and triangular head shape, and the rostral (snout acutely cuneiform, with a sharp angular labial edge) is sharper to be able to burrow the sand easier and quicker (Fig. 5). Supraoculars also level with the eyes, which makes the head more triangular. But, the members of the *brevipes* group prefer habitats with steppe soils along with sandy microclimates making supraoculars more popped out than eyes, making supraoculars and eyes non-level (The supraoculars resemble the brim of a hat for the eyes) (Fig. 6). Consequently, the head compared to the prior group is slightly triangular to conical and rostral is also slightly conical (Fig. 6). Since, *O. persicus* distributed on the foothills (the transition zone between plains and low-relief hills), alluvial fans, and under stones, it leads to a completely conical head (snout acutely rounded, the rostral scarcely projecting beyond the lip) and rostral round (Fig. 7). According to Greer and Wilson (2001) and our morphological study on the head scale of *Ophiomorus* species in the current study, it is determined that 12 species except the *O. maranjabensis* species have a frontoparietal scale (Fig 5-6-7)**.** It is worth mentioning that Kazemi et al (2011) have not mentioned this significant trait for this species in identification keys, but having frontoparietal, is a key trait to determine this species from other *Ophiomorus***.** Given the morphological differences between the three species groups of *Ophiomorus* and according to the history of their description, we assigned these three species groups for all species of *Ophiomorus* in Iran. Finally, we suggest three species groups based on the tendency to limb reduction (especially fingers) and habitat preferences, which are explained as follow:

brevipes*: *O. blanfordii, O. brevipes, O. chernovi,* O. *nuchalis

Based on morphological compatibility with the type of steppe soil habitat with sand microhabitat, they have strong bodies compared to the two other groups, and based on four fingers on the forelimb and three on the hindlimb, they are able to move on steppe soil and burrow in sand microclimate.

tridactylus*: *O. maranjabensis, O. raitmai, O. streeti, O. tridactylus

The members of this group have their special morphological compatibility based on burrowing life and are extremely dependent on their own habitat. So, head and body shape are changed according to the sand habitat. Their specifications include a complete triangular head, wedge-shaped snouts, extremely smooth imbricate scales, and overlap. The members of this group never leave sand dunes and they are adapted to living in loose windblown dune sand, moving through this medium with strong lateral undulations**.**

punctatissimus*: *O. kardesi, O. latastii, O. persicus, O. punctatissimus

The members of this group have an extreme tendency to the reduction of hindlimbs or have no limbs. They have elongated cylindrical bodies, the head completely conical, and the snout acutely rounded. They are adapted to inhabit under stones and steppe ground with loose soil.

Finally, we suggest the following updated identification key for *Ophiomorus* species present in Iran:

Key to the species of *Ophiomorus* in Iran

1A. Fingers 4, toes 3.....2
1B. Fingers 3, toes 2 or 3.....5
2A. Scale rows 20 at midbody, 1 pretemporral scales…….*Ophiomorus blanfordii*
2B. Scale rows 22 or more at midbody, 2 pretemporral scales…….……..……3
3A. Scale rows 22…….……..4
3B. Scale rows 24…….… *Ophiomorus chernovi*
4A. Parietals semicircular shape ……...……*Ophiomorus brevipes*
4B. Parietals rectangular shape…….……...……...…*Ophiomorus nuchalis*
5A. Fingers 3, toes 2……..…….*Ophiomorus persicus*
5B. Fingers 3, toes 3…….…….……6
6A. Frontoparietals absent ……..…….*Ophiomorus maranjabensis*
6B. Frontoparietals present ……7
7A. Prefrontals not in contact with upper labials…….……..*Ophiomorus streeti*
7B. Prefrontals in contact with upper labials……..*Ophiomorus tridactylus*

Acknowledgments

Special thanks to Zohreh Ghanbarian for her kind collaboration in painting the samples. We thank Zahra Nabizadeh comments and suggestions to improve the quality of the manuscript. We also thank Massoumeh Gaeeini for reading an early draft of the manuscript and for providing valuable comments to improve the manuscript. We are grateful to Roman A. Nazarov, Morteza Modab, and Daniil Melnikov for their valuable assistance in collecting the specimens in Southeast Iran. Likewise, we would like to thank the Department of Environment (DOE) in Iran for issuing the permit letter for conducting the fieldwork. The Iran National Science Foundation (INSF) and the Russian Foundation of Fundamental Study (RFFS) under an international (Iran-Russian) project financially supported this study with proposal numbers INEF 99003440 and RFFS 20-54-56033, respectively.

LITERATURE CITED

Andrade, J.B., Lewis, R.P. and Senter, P., 2016. Appendicular skeletons of five Asian skink species of the genera Brachymeles and Ophiomorus, including species with vestigial appendicular structures. *Amphibia-Reptilia*, *37*(4), pp.337-344.

Anderson, S.C. and Leviton, A.E., 1966. *A Review of the Genus Ophiomorus:(Sauria: Scincidae), with Descriptions of Three New Forms*.

Andersson, S.C., 1999. The Lizards of Iran. Society for the study of Amphibians and Reptiles.

Bauer, A.M., Parham, J.F., Brown, R.M., Stuart, B.L., Grismer, L., Papenfuss, T.J., Böhme, W., Savage, J.M., Carranza, S., Grismer, J.L. and Wagner, P., 2011. Availability of new Bayesian-delimited gecko names and the importance of character-based species descriptions. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1705), pp.490-492.

Camaiti, M., Villa, A., Wencker, L.C., Bauer, A.M., Stanley, E.L. and Delfino, M., 2019. Descriptive osteology and patterns of limb loss of the European limbless skink Ophiomorus punctatissimus (Squamata, Scincidae). *Journal of Anatomy*, *235*(2), pp.313-345.

Cicero, C., Mason, N.A., Jiménez, R.A., Wait, D.R., Wang-Claypool, C.Y. and Bowie, R.C., 2021. Integrative taxonomy and geographic sampling underlie successful species delimitation. *The Auk*, *138*(2), p.ukab009.

Cloudsley-Thompson, J.L. and Cloudsley-Thompson, J.L., 1991. Interspecific relationships, feeding specializations and species diversity. *Ecophysiology of Desert Arthropods and Reptiles*, pp.147-168.

Collins, B.G. and PATON, D.C., 1989. Consequences of differences in body mass, wing length and leg morphology for nectar-feeding birds. *Australian Journal of Ecology*, *14*(3), pp.269-289.

Douglas, M.E., 1987. An ecomorphological analysis of niche packing and niche dispersion in stream fish clades. *Community and evolutionary ecology of North American stream fishes. University of Oklahoma Press, Norman*, pp.144-149.

Eskandarzadeh, N., Rastegar-Pouyani, N., Rastegar-Pouyani, E., Fathinia, B., Bahmani, Z., Hamidi, K. and Gholamifard, A., 2018. Annotated checklist of the endemic Tetrapoda species of Iran. *Zoosystema*, *40*(sp1), pp.507-537.

Ford, D.P., 2018. *The evolution and phylogeny of early amniotes* (Doctoral dissertation, University of Oxford).

Greer, A.E. and Wilson, G.D., 2001. Comments on the scincid lizard genus *Ophiomorus*, with a cladistic analysis of the species. *HAMADRYAD-MADRAS-*, *26*, pp.261-271.

Kazemi, S.M., Farhadi Qomi, M., Kami, H.G. and Anderson, S.C., 2011. A new species of Ophiomorus (Squamata: Scincidae) from Maranjab Desert, Isfahan Province, Iran, with a revised key to the genus. *Amphibian and Reptile Conservation*, *5*(1), pp.23-33.

Kornilios, P., Kumlutaş, Y., Lymberakis, P. and Ilgaz, C., 2018. Cryptic diversity and molecular systematics of the Aegean Ophiomorus skinks (Reptilia: Squamata), with the description of a new species. *Journal of Zoological Systematics and Evolutionary Research*, *56*(3), pp.364-381.

Losos, J.B., 1990. The evolution of form and function: morphology and locomotor performance in West Indian Anolis lizards. *Evolution*, *44*(5), pp.1189-1203.

Losos, J.B. and Sinervo, B., 1989. The effects of morphology and perch diameter on sprint performance of Anolis lizards. *Journal of Experimental Biology*, *145*(1), pp.23-30.

Mayr, E., 2000. The biological species concept. Species concepts and phylogenetic theory: a debate. *Columbia Univ*, pp.17-29.

McDowall, R.M., 1998. Phylogenetic relationships and ecomorphological divergence in sympatric and allopatric species of Paragalaxias (Teleostei: Galaxiidae) in high elevation Tasmanian lakes. *Environmental Biology of Fishes*, *53*, pp.235-257.

Miles, D.B., Ricklefs, R.E. and Travis, J., 1987. Concordance of ecomorphological relationships in three assemblages of passerine birds. *The American Naturalist*, *129*(3), pp.347-364.

Miralles, A., Anjeriniaina, M., Hipsley, C.A., Müller, J., Glaw, F. and Vences, M., 2012. Variations on a bauplan: description of a new Malagasy "mermaid skink" with flipper-like forelimbs only (Scincidae, Sirenoscincus Sakata & Hikida, 2003). *Zoosystema*, *34*(4), pp.701-719.

Moermond, T.C., 1979. The influence of habitat structure on a Nolis foraging behavior. *Behaviour*, *70*(1-2), pp.147-167.

Nabizadeh, H., Rastegar-pouyani, N., POUYANI, E.R., Rajabizadeh, M., Nazarov, R. and Mozaffari, O., 2022. Shape variation in head scales of species of the genus Ophiomorus DUMÉRIL & BIBRON, 1839 in Iran, a geometric morphometrics approach. *Turkish Journal of Zoology*, *46*(5), pp.423-433.

Nasrabadi, R., Rastegar-Pouyani, N., Rastegar-Pouyani, E. and Gharzi, A., 2017. A revised key to the lizards of Iran (Reptilia: Squamata: Lacertilia). *Zootaxa*, *4227*(3), pp.431-443.

Norberg, U.M., 1994. Wing design, flight performance and habitat use in bats In: Wainwright PC, Reilly SM, editors. Ecological Morphology: Integrative Organismal Biology.

Organ, C.L., Moreno, R.G. and Edwards, S.V., 2008. Three tiers of genome evolution in reptiles. *Integrative and Comparative Biology*, *48*(4), pp.494-504.

Padial, J.M., Miralles, A., De la Riva, I. and Vences, M., 2010. The integrative future of taxonomy. *Frontiers in zoology*, *7*(1), pp.1-14.

Poulakakis, N., Pakaki, V., Mylonas, M. and Lymberakis, P., 2008. Molecular phylogeny of the Greek legless skink Ophiomorus punctatissimus (Squamata: Scincidae): The impact of the Mid-Aegean trench in its phylogeography. *Molecular Phylogenetics and Evolution*, *47*(1), pp.396-402.

Pounds, J.A., 1988. Ecomorphology, locomotion, and microhabitat structure: patterns in a tropical mainland Anolis community. *Ecological Monographs*, *58*(4), pp.299-320.

Pyron, R.A., Burbrink, F.T. and Wiens, J.J., 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC evolutionary biology*, *13*(1), pp.1-54.

Rajabizadeh, M., 2013. *Biodiversity of the snakes in northern and western mountains of Iran, with special emphasis on biodiversity in colubroids* (Doctoral dissertation, Ghent University).

Rastegar-Pouyani, N., Johari, S.M. and Rastegar-Pouyani, E., 2007. Field guide to the reptiles of Iran.

Sites Jr, J.W. and Marshall, J.C., 2003. Delimiting species: a Renaissance issue in systematic biology. *Trends in Ecology & Evolution*, *18*(9), pp.462-470.

ŠMÍD, J., Moravec, J., Kodym, P., Kratochvíl, L., Yousefkhani, S.S.H. and Frynta, D., 2014. Annotated checklist and distribution of the lizards of Iran. *Zootaxa*, *3855*(1), pp.1-97.

Tulli, María José, V. Abdala, and Felix Benjamin Cruz. "Relationships among morphology, clinging performance and habitat use in Liolaemini lizards." *Journal of evolutionary biology* 24, no. 4 (2011): 843-855.

Wiens, J.J. and Slingluff, J.L., 2001. How lizards turn into snakes: a phylogenetic analysis of body-form evolution in anguid lizards. *Evolution*, *55*(11), pp.2303-2318.

Williams, E.E., 1983. Ecomorphs, faunas, island size, and diverse end points in island radiations of Anolis. In *Lizard ecology: studies of a model organism* (pp. 326-370). Harvard University Press.

APPENDIX

Appendix 1: Results of all parametric tests in numerical form for all the OTUs.

	O. blanfordii	*O. brevipes*	*O. maranjabensis*	*O. nuchalis*	*O.* cf. *nuchalis*	*O. persicus*	*O. streeti*	*O. tridactylus*
O. blanfordii	*	3	6	2	0	6	1	2
O. brevipes	3	*	7	2	4	7	7	7
O. maranjabensis	6	7	*	6	5	7	6	5
O. nuchalis	2	2	6	*	2	7	4	6
O.* cf. *nuchalis	0	3	5	2	*	6	4	6
O. persicus	6	7	7	7	6	*	11	9
O. streeti	1	7	6	4	2	11	*	3
O. tridactylus	2	7	5	6	6	9	3	*

Appendix 2: All measurements of *Ophiomorus* specimens were collected from Iran (metric characters were measured in mm).

NO.	SVL	TL	HL	HW	HH	SL	LF	LA	LFL	LFH	LHF	NSL	NIL	NDSB	NMC
RAN-3437- *O. blanfordii*	77.33	78.68	7.17	5.28	3.44	4.66	4.64	2.16	7.75	12.88	56.29	6	5	20	110
RAN-3436- *O. blanfordii*	70.47	54.15	7.79	4.57	3.48	3.92	4.55	2.8	6.21	12.49	45	6	5	20	100
HNH-65- *O. blanfordii*	84.62	99999	7.46	5.86	4.29	4.71	5.13	3.14	7.92	13.49	64.78	7	6	23	119
RAN- 4082- *O. brevipes*	72.61	62.09	6.38	5.26	3.86	4.22	4.78	2.5	7.5	13.45	51.86	7	6	21	116
RAN-4081- *O. brevipes*	89.66	74.11	7.71	6.23	4.98	4.77	5.75	3.2	7.89	15.93	66.73	7	6	22	114
RAN-4660- *O. brevipes*	77.27	63.91	6.2	6.02	3.56	4.21	6	3.15	7.78	14.53	57.03	7	6	22	111
RAN-4661- *O. brevipes*	87.61	34.02	6.93	6.36	4.42	4.57	5.16	3.23	9.21	13.91	64.93	7	6	22	115
RAN-4662- *O. brevipes*	80.5	68.84	7.26	6.1	4.39	4.55	5.96	3.46	8.31	13.81	59.18	7	6	22	117
RAN-4663- *O. brevipes*	73.4	58.51	6.27	5.41	4.41	4.17	5.43	3.36	7.62	13.43	53.65	7	6	22	113
RAN-4664- *O. brevipes*	90.32	49.88	7.64	5.44	4.52	4.67	5.8	3.69	8.33	14.42	69.72	7	6	22	124
RAN-4665- *O. brevipes*	87.28	83.07	7.1	6.02	4.36	4.22	5.12	3.9	8.04	13.24	66.81	7	6	22	119
RAN-4666- *O. brevipes*	82.68	37.22	6.57	5.34	4.48	4.14	5.36	3.22	7.77	13.21	60.83	7	6	22	113
RAN-4667- *O. brevipes*	86.18	69.64	7.84	5.74	4.96	4.48	5.97	3.11	8.07	14.44	66.11	7	6	22	119
HNH-192- *O. brevipes*	77.08	99999	5.99	5.34	3.46	3.44	4.7	3.04	6.86	11.26	59.04	7	6	22	118
ERP-7280- *O. maranjabensis*	87.38	40.83	7.59	5.59	4.1	4.79	4.94	2.11	5.23	14.14	69.36	5	5	22	125
HNH-99- *O. maranjabensis*	74.4	45.25	6.38	5.07	3.31	4.09	4.43	1.86	4.63	12.75	55.58	6	6	24	122
HNH-171- *O. maranjabensis*	81.15	53.77	7.56	5.08	3.32	4.14	5.82	2.15	5.23	15.05	63.33	6	6	22	120
HNH-172- *O. maranjabensis*	81.11	33.57	6.91	4.92	3.32	4.65	5.83	2.28	5.17	14.14	61.6	6	6	22	129
HNH-173- *O. maranjabensis*	91.65	44.82	7.56	5.71	2.93	4.41	5.77	2.03	5.75	14.04	70.61	6	6	22	133

NMC	NO.	SVL	TL	HL	HW	HH	SL	LF	LA	LFL	LFH	LHF	NSL	NIL	NDSB	NMC
121	HNH-174- *O. maranjabensis*	89.62	55.25	8.03	5.18	2.9	4.5	5.23	2.1	5.79	14.07	70.27	6	6	22	137
118	HNH-175- *O. maranjabensis*	80.91	29.22	8.08	4.64	2.97	4.15	5.08	1.8	4.99	11.74	61.03	6	6	22	127
112	HNH-176- *O. maranjabensis*	83.71	27.07	7.49	5.03	3.12	4.55	5.43	2.02	5.94	13.75	66.08	6	6	22	115
107	HNH-177- *O. maranjabensis*	92.98	53.82	6.73	4.76	3.2	4.5	5.15	2.21	5.62	13.3	72.85	6	6	22	124
108	HNH-178- *O. maranjabensis*	76.89	50.27	7.84	4.82	2.89	4.67	5.29	2.01	5.31	12.41	60.33	6	6	22	112
115	ERP-6662- *O. nuchalis*	95.91	81.29	8.17	5.82	4.44	4.52	5.57	3.34	7.54	16.24	74.69	7	6	22	123
116	ERP-6656- *O. nuchalis*	89.31	51.37	8.34	5.86	3.9	4.5	6.07	2.93	8.77	16.09	69.25	7	6	22	116
106	ERP-6657- *O. nuchalis*	96.63	79.01	8.06	5.96	4.32	4.68	5.87	3.35	9.08	16.13	72.7	7	6	22	123
107	ERP-6660- *O. nuchalis*	102.74	46.87	8.27	6.19	4.62	5.18	6.05	3.38	8	16.3	80.93	7	6	22	119
105	ERP-6661- *O. nuchalis*	86.3	73.73	8	6.51	4.67	4.86	6.52	3.25	9.39	16.33	64.14	7	6	22	115
107	RAN-4318- *O. nuchalis*	86.12	72.22	7.33	5.7	4.52	4.23	5.91	3.65	7.77	15.63	62.3	7	6	22	112
108	RAN-4066- *O. nuchalis*	87.34	70.84	7.43	6.4	4.86	4.22	6.01	2.69	9.64	16.2	65.36	7	6	22	112
108	ERP-6659- *O.* cf. *nuchalis*	95.9	99999	8.33	5.4	3.68	4.58	5.88	3.35	9.04	12.54	72.43	7	6	22	123
116	ERP-6658- *O.* cf. *nuchalis*	85.04	87.15	7.06	5.35	3.1	4.33	5.06	3.44	6.27	11.43	64.04	7	6	22	117
115	RAN-3317- *O.* cf. *nuchalis*	85.83	55.34	7.79	5.81	4.23	4.02	5.33	3.47	6.82	15.2	63.34	6	5	22	115
112	RAN-3318- *O.* cf. *nuchalis*	80	85.7	7.08	5.23	4.24	4.62	6.68	3.15	7.75	14.38	60.32	6	5	22	115
114	RAN-3080- *O. persicus*	69.59	59.78	4.61	3.79	3.38	2.51	1.81	1.21	4.61	4.58	53.4	7	6	20	132
113	RAN-3090- *O. persicus*	55.34	9.27	4.09	3.41	2.52	2.2	1.27	1.54	4.55	4.23	43.15	7	6	20	128
111	RAN-3326- *O. persicus*	75.83	77.23	4.79	4.09	3.6	2.35	1.65	1.35	4.5	3.86	58.78	7	6	20	118

NMC	NO.	SVL	TL	HL	HW	HH	SL	LF	LA	LFL	LFH	LHF	NSL	NIL	NDSB
111	ERP-3957- *O. persicus*	67.97	56.87	4.95	4.28	3.27	2.7	1.6	1.37	3.89	4.34	52.87	7	6	20
103	ERP-5294- *O. streeti*	90.39	99999	7.01	4.95	3.91	4.19	4.43	2.05	6.45	13.55	70.64	6	5	20
131	RAN-3347- *O. streeti*	53.65	47.43	6.28	4.44	3.33	3.48	4.43	2.8	5.7	11	37.75	5	5	20
129	RAN-3349- *O. streeti*	46.85	35.07	5.73	3.7	2.92	3.58	4.58	2.78	5.41	10.84	32.29	5	5	20
117	RAN-3348- *O. streeti*	50.56	45.79	5.46	4	2.38	3.69	3.84	1.97	5.02	11.11	37.08	5	5	20
118	RAN-3343- *O. streeti*	67.4	53.65	6.55	4.67	3.27	4.37	4.3	2.06	5.18	12.17	48.05	5	5	20
128	RAN-3342- *O. streeti*	71.67	50.56	5.88	4.62	3.39	3.71	4.94	2.3	5.68	10.2	55.56	6	5	20
118	RAN-3340- *O. streeti*	66.9	37.45	6.66	4.54	2.74	3.48	5.29	2.26	5.43	11.8	48.23	6	5	20
128	RAN-3338- *O. streeti*	64.83	41.95	5.88	5.07	3.6	3.94	5.33	2.37	5.41	11.85	46.1	6	5	20
118	RAN-3341- *O. streeti*	68.56	19.41	6.72	5.25	3.4	3.74	4.44	2.3	5.73	11.73	50.02	6	5	20
128	RAN-3346- *O. streeti*	63.29	39.68	6.28	4.45	3.33	4.32	5.25	2.34	6.39	12.93	45.37	6	5	20
131	RAN-3339- *O. streeti*	70.44	52.11	6.01	5.03	3.66	4.45	5.52	2.65	6.71	12.15	52.87	6	5	20
116	RAN-3344- *O. streeti*	75.09	44.85	7.23	4.79	3.59	4.09	4.89	2.64	6.61	14.09	56.25	6	5	20
129	RAN-3134- *O. streeti*	70.59	41.46	6.52	4.89	3.29	3.64	5.45	2.61	6.44	11.96	50.45	6	6	19
	RAN-3133- *O. streeti*	74.25	44.1	6.57	5.19	3.62	4.12	5.25	2.27	6.03	15.29	57.1	6	6	19
	RAN-3136- *O. streeti*	69.47	27.96	5.9	4.47	3.29	3.75	4.56	2.3	6.11	14.02	50.11	6	6	19
	RAN-3135- *O. streeti*	71.5	32.56	9.93	5.19	4.01	4.26	5.01	2.65	6.02	14.16	52.15	6	6	19
	RAN-3138- *O. streeti*	73.96	32.84	6.48	5.37	3.84	3.68	5.29	2.35	6.78	14.6	56.19	6	6	19
	RAN-3137- *O. streeti*	65.14	33.39	6.6	4.87	3.83	3.27	4.82	2.24	6.27	12.48	47.91	6	6	19

NO.	SVL	TL	HL	HW	HH	SL	LF	LA	LFL	LFH	LHF	NSL	NIL	NDSB
ERP-3958- *O. streeti*	75.09	49.23	7.87	4.85	2.26	4.34	5.08	2.5	6.98	12.65	56.06	6	6	19
RAN-3345- *O. streeti*	63.59	48.19	5.84	4.63	3.8	3.73	4.78	2.54	6.44	10.88	46.18	6	5	20
ERP-5969- *O. tridactylus*	92.27	99999	7.64	5.06	3.65	4.55	6.61	2.41	5.8	13.06	70.17	6	6	21
ERP-5972- *O. tridactylus*	92.37	51.52	7.44	5.16	3.17	4.39	5.7	2.12	5.72	13.95	69.52	6	6	21
RAN-3156- *O. tridactylus*	78.74	54.06	6.7	5.45	3.94	4.27	6.02	2.25	4.17	13.34	60.52	6	5	21
RAN-3431- *O. tridactylus*	77.71	34.89	7.6	5.44	3.79	4.51	5.85	2.12	4.63	12.57	57.71	6	5	21
RAN-3189- *O. tridactylus*	91.08	39.14	6.81	5.37	3.72	4.08	5.05	2.22	5.75	14.54	72.48	6	5	21
RAN-3155- *O. tridactylus*	66.52	51.54	6.24	4.99	3.34	4.14	5.35	1.92	4.46	12.46	48.27	6	5	21
RAN-3187- *O. tridactylus*	72.77	28.11	7.02	4.81	3.51	4.54	4.12	2.08	4.67	15.63	55.32	6	5	21
RAN-3157- *O. tridactylus*	64.53	32.07	4.77	4.37	2.81	3.51	4.53	2.24	4.36	13.01	47.67	6	5	21
RAN-3186- *O. tridactylus*	88.85	47.34	7.18	5.84	4	4.24	6.14	1.87	4.84	5.37	66.74	6	5	21
ERP-5971- *O. tridactylus*	81.84	46.35	7.19	5.09	3.23	4.1	5.69	2.41	6.75	17.23	63.5	6	5	21
HNH-82- *O. tridactylus*	75.43	99999	7.46	4.52	3.01	4.22	4.93	2.25	5.56	11.57	58.05	6	5	23
RAN-3188- *O. tridactylus*	95.77	61.48	6.67	5.71	4.63	3.92	5.46	2.32	6.35	15.9	74.11	6	6	21

Iranian Journal of Animal Biosystematics (IJAB)
Vol.19, No.1, 71-101, 2023
DOI: 10.22067/ijab.2023.79456.1045

IJAB
ISSN: 1735-434X (print); 2423-4222 (online)

RESEARCH ARTICLE **Open access**

A new species of brush-tailed mice of the genus *Calomyscus* from southern Iran (Calomyscidae: Rodentia)

Mehdi Dezhman[1], Safieh Akbarirad[1], Mansour Aliabadian[1,2], Roohollah Siahsarvie[1,3], Arya Shafaeipour[4], Omid Mirshamsi[1,2,*]

[1] *Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran*
[2] *Research Department of Zoological Innovations (RDZI), Institute of Applied Zoology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.*
[3] *Rodentology Research Department, Institute of Applied Zoology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.*
[4] *Department of Biology, Faculty of Science, Yasouj University, Yasouj, Iran*

(Received: *30 November 2022*; Accepted: *30 June 2023*)

Abstract

Calomyscidae is a monotypic family of muroid rodents with nine valid allopatric species distributed in southwestern Asia of which seven species have been so far recognized from Iran. The western and southern Zagros Mountains were thought to be home to a single species, *Calomyscus bailwardi*, but new researches revealed that the region is also home to four highly divergent molecular lineages. One of them was recently described as a new species (*C. behzadi*) but the taxonomic position of the other lineages remained unclear. Here we did an extensive sampling in southern Iran (2017-2018), during which 99 *Calomyscus* specimens were collected. Based on an integrated approach including karyotypic, mitochondrial as well as morphological data, we describe *Calomyscus kermanensis* sp. nov. as a new endemic species from the southern Zagros Mts (including Kohgiluyeh and Boyer-Ahmad, Fars, Kerman, Yazd and Hormozgan provinces), in the region that was previously thought to be occupied by *C. bailwardi*. The new species emerged as a new lineage with high intraspecific mtDNA and chromosome variations. The multivariate and univariate statistical analyses of craniodental measurements also separated *C. kermanensis* sp. nov. from other examined *Calomyscus* species with the highest maximum cranial height being the most distinctive of its craniodental features.

Key words: *Calomyscus, karyotype, mitochondrial genes, morphometry, taxonomy, phylogeny, Zagros Mountains, Iran.*

INTRODUCTION

Calomyscidae is one of the oldest monotypic families of the Eumuroida clade, which is the sister group of Nesomyidae, Cricetidae, and Muridae families (Steppan et al., 2004; Steppan & Schenk, 2017). The taxonomic position of the genus *Calomyscus* Thomas, 1905 is still not fully resolved and the exact number of species remained controversial and underwent many changes since its first description. *C. bailwardi* Thomas, 1905 is the type species of the genus which was described from Mala-Imir (Izeh), Khuzestan Province, the South-West of Iran. Subsequently, *C. hotsoni* Thomas, 1920 and *C. baluchi*

Corresponding Author: *mirshams@um.ac.ir*

HTTPS://ZOOBANK.ORG/ URN:LSID:ZOOBANK.ORG:PUB:B1964F3F-AC4B-4C65-ACD4-9ADAAA39825B

Thomas, 1920 were described both from Pakistan based on small differences in skull morphometric and external features. Later, *C. mystax* Kaschkarov, 1925 from Balkhan Mountain, Turkmenistan, *C. elburzensis* Goodwin, 1939 from the Kurkhud Mts, Bojnurd (Iran), *C. grandis* Schlitter and Setzer, 1973 from Fasham, the southern of Elburz, Iran, *C. urartensis* Vorontsov and Kartavseva, 1979 from Nakhichevanskaya, Azerbaijan, and *C. tsolovi* Peshev, 1991 from the southwest of Syria were described, respectively. Some researchers also considered *Calomyscus* as a monotypic genus including *C. bailwardi* and all its nominal forms were classified as subspecies (Corbet & Hill, 1980; Ellerman, 1948; Ellerman & Morrison-Scott, 1951; Lay, 1967; Roberts, 1997). Specific status of some of these forms has been subsequently tested using karyotype analyses (Graphodatsky et al., 2000; Malikov et al., 1999; Akbarirad et al., 2016*a, b, c*; Meyer and Malikov, 1995, 2000; Shahabi et al., 2010; Rezazadeh et al., 2020; Romanenko et al., 2021), hybridization experiments (Meyer & Malikov, 2000; Graphodatsky et al., 2000), molecular phylogenies (Morshed & Patton, 2002; Norris et al., 2008; Shahabi et al., 2013, Akbarirad et al., 2016*a, b*; Rezazadeh et al., 2020), multivariate analyses of craniodental measurements (Lebedev et al., 1998; Shahabi et al., 2011; Akbarirad et al., 2016*a*, Rezazadeh et al., 2020), geometric morphometric analysis (Zarei et al., 2013; Akbarirad et al., 2016), and ecological survey (Hamidi et al., 2017) and niche modeling (Hamidi et al., 2019). The main problems in the taxonomy of this genus are related to high morphologic resembalnce between species (e.g., *C. elburzensis* and *C. bailwardi*), intraspecific chromosomal variations (e.g., *C. elburzensis* and *C. hotsoni*), presence of natural hybrids between two different cytotypes of one species both in nature and in captivity (*C. elburzensis*), having the same karyotype in two distinct species (e.g., *C. grandis* and *C. mystax*) (Graphodatsky et al., 2000; Shahabi et al., 2011; Akbarirad et al., 2016*a, c*). Nevertheless, in the last published mammal checklists of the World (Musser and Carleton, 2005; Kilpatrick, 2017) eight allopatric species have been listed for this genus including: *C. bailwardi*, *C. hotsoni*, *C. baluchi*, *C. elburzensis*, *C. mystax*, *C. urartensis*, *C. grandis*, and *C. tsolovi*. The genus spread throughout the west of Asia in Turkmenistan, Iran, Pakistan, Afghanistan, and Syria (Musser and Carleton, 2005; Kilpatrick, 2017).

According to Akbarirad et al. (2015), six species of the genus *Calomyscus* occur in different mountainous areas in Iran including: *C. elburzensis*, *C. bailwardi*, *C. grandis*, *C. hotsoni*, *C. mystax* and *C. urartensis*. Recent studies have shown that molecular data have more information in determining species boundaries and phylogenetic relationships among the *Calomyscus* species (Norris et al., 2008; Shahabi et al., 2013; Akbarirad et al., 2016*a*; Rezazadeh et al., 2020).

According to Musser & Carletion (2005), *C. bailwardi* occupies a wide range in western Iran including Kurdistan, Ilam, Western Isfahan, Eastern Khuzistan, Lorestan, Fars to West of Kerman provinces, while the molecular data shown that its range is restricted only to Khuzestan province in Izeh (the type locality) and Behbahan (Akbarirad et al., 2016a). Previously, different cytotypes of *Calomyscus* from different localities along the Zagros Mountains (2N = 37 - 52) (Graphodatsky et al., 2000), led to the assumption that the Zagros mountains were inhabited by possible undescribed species of this genus. Cytogenetic analyses can play an important role in the identifying of morphologically similar species but karyotypes in *Calomyscus* genus serve as an ambiguous indicator of their species rank (Romanenko et al., 2021). More recent molecular phylogenies revealed that four additional distinct lineages of *C. bailwardi* exists in the Zagros Mountains that may consider as new species (i) *C. bailwardi* from east of Khuzestan province; (ii) *Calomyscus* sp. group B (*C.* cf. *bailwardi)* from the southeastern part of the Zagros; (iii) *Calomyscus* sp. group C, the unnamed from Songhor in Kermanshah Province; (iv) *Calomyscus* sp. group D from Saghez in Kurdistan province and (v) *Calomyscus* sp. group G from west of Isfahan province (Akbarirad et al., 2016*a*; Rezazadeh et al., 2020; Kilpatrick, 2017). The latest study based on integrative methods classified group C from Kermanshah and Ilam provinces (western Iran) as a new species named *C. behzadi* Akbarirad et al, 2021 (Dezhman et al., 2021). However, taxonomic rank, diversity, and distribution of the other unnamed lineages remained obscure due to the lack of diagnostic morphological features as well as inadequate sampling for proper comparisons. In the present study, we describe a new species of brush-tailed mice from the southern part of Iran based on an integrative approach including molecular, morphometric and karyological studies with a considerable geographical sampling.

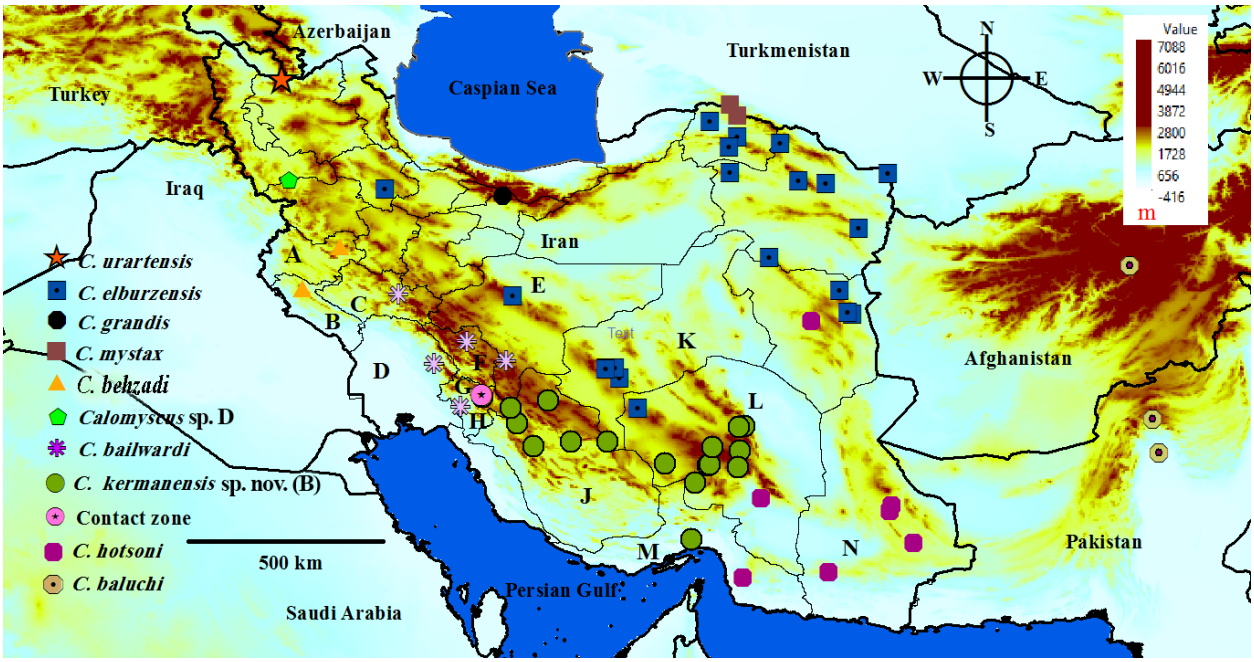


FIGURE 1. Map of *Calomyscus* specimens and localities in the present study was created using ArcGIS 10.7.1. A) Kurdistan, B) Ilam, C) Lorestan, D) Khuzistan, E) Isfahan, F) Chaharmahal and Bakhtiari, G) Contact zone H) Kohgiluyeh and Boyer-Ahmad, J) Fars, K) Yazd, L) Kerman and M) Hormozgan provinces.

MATERIAL AND METHODS

In total, 99 the brush-tailed mice were captured from 15 mountainous sites in western and southern Iran (2017-2018), 93 of which were included into the analyses and stored in ZMFUM (Zoological Museum of the Ferdowsi University of Mashhad) (Fig. 1, supplementary Table. 1). The collected specimens of this study are marked in bold. Because there is no useful morphological identification key for *Calomyscus* species, CYTB sequencing and karyotype analyses were used to identify the collected specimens (most of the specimens have CYTB sequences but here our molecular analyses were conducted on specimens that have sequences of both mitochondrial genes, CYTB and CO1. Complementary CYTB and CO1 sequences were downloaded from GenBank and used for phylogenetic analyses. Additional informations about examined specimens is presented in the supplementary Table. 1.

TABLE 1. Kimura-2-parameters genetic distance matrix on CYTB sequences (*Italics* are intraspecific distances).

	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
1. C. bailwardi	*0.018*									
***2. C. kermanensis* sp. nov. (B)**	0.105	*0.035*								
3. C. hotsoni	0.102	0.094	*0.012*							
4. C. behzadi	0.160	0.158	0.148	*0.016*						
5. C. elburzensis	0.155	0.143	0.146	0.105	*0.018*					
6. C. baluchi	0.105	0.090	0.085	0.158	0.144	*0.018*				
7. C. grandis	0.149	0.154	0.144	0.092	0.086	0.155	*0.000*			
8. C. urartensis	0.172	0.148	0.154	0.096	0.091	0.142	0.096	*0.000*		
***9. Calomyscus* sp. Group D**	0.158	0.139	0.140	0.106	0.095	0.135	0.092	0.069	*0.000*	
10. C. mystax	0.156	0.148	0.133	0.104	0.098	0.137	0.098	0.086	0.086	*0.022*

DNA extraction and phylogenetic analyses

Genomic DNA was extracted from 99% of ethanol-preserved tissues using DENAzist Asia's Animal DNA isolation kit (S-1033-1). Polymerase chain reaction (PCR) amplification was conducted for two mitochondrial genes: CYTB using primers L7: 5′-ACT AAT GAC ATG AAA AAT CAT CGT/T3′ and H6: 5′-TCT TCATTT TTG GTT TAC AAG AC-3′ (Montgelard et al., 2002) and CO1 using BatL5310 (5′ CCTACTCRGCCATTTTACCTATG 3′) and R6036R (5′ ACTTCTGGGTGTCCAAAGAATCA 3′) (Herbreteau et al., 2011; Robins et al., 2007). Amplified DNA fragments were sequenced by Macrogen Inc, South Korea. Ambiguous sequence sites were checked using the Chromas (2.6.6) program and aligned using the CLUSTAL W algorithm in BioEdit software (Hall, 1999). Phylogenetic analyses were performed on a single combined matrix of CYTB and CO1 sequences. Best-fit models of nucleotide substitution were found with Bayesian inference information criterion using jModelTest2 (Darriba et al., 2012). Bayesian inference was (BI) performed using MrBayes 3.2.7a (Ronquist and Huelsenbeck, 2003) on the CIPRES Science Gateway3.3 (available online: www.phylo.org). We used the GTR+G+I model, two simultaneous runs with four Markov Chain Monte Carlo (MCMC), 80 million generations, sampling after every 10000 generations and discarding of the first 25% trees as 'burn-in'. The Maximum Likelihood (ML) trees based on GTR+ G+ I model and the bootstrap method with 1000 replications was constructed using software PhyML (Guindon et al., 2010). The average Kimura 2-parameters genetic distances for within and between species were calculated by MEGA 6 (Tamura et al., 2013) and ExcaliBAR (Aliabadian et al., 2014). *Spalax* from Spalacidae and *Rhizomys* from Rhizomyidae were chosen as outgroups following Akbarirad et al. (2016*a*).

Karyology analyses

For karyological study, 21 specimens (indicated by asterisked in the S Table 1) from different localities were transported alive to the laboratory. The conventional cytogenetic study was conducted according to Yosida (1973) and Dutrillaux et al. (1982) methods. The brush-tailed mice were exposed for 45 min at a dose of 10% vinblastine solution (1 ml/100 g of body weight) by intraperitoneal injection. Metaphase chromosome spreads were obtained from bone marrow cells. For each specimen, 15 slides were prepared and stained with Giemsa. Spreads of each specimen were subsequently photographed using a 100× magnification digital CCD camera attached to the Olympus BX53 microscope. The Ideogram of each specimen was prepared with the best metaphase spread photo by the Chromosome Image Processing software (CIP) Programmed in the Rodentology Research Department of the Ferdowsi University of Mashhad to determine the diploid chromosome number (2n), the fundamental number of autosomal arms (FNa), and the number of all chromosomal arms (FN).

Morphometric analyses

In the present study, 181 specimens including 88 recently collected and 93 specimens deposited at ZMFUM Zoological Museum were studied (Fig. 1, S Table 1). We only used adults with fully erupted molars in morphometric analyses. Four external and thirty-one craniodental features were measured. We used a dissecting microscope equipped with an eyepiece graticule for dental measurements and a digital dial caliper (0.1 mm) for other measurements. The measurements were defined according to Peshev (1991) and Shahabi et al. (2011). For variables definition see S Fig. 1. Statistical analyses were performed on both uncorrected data (size + shape) and size-out data (shape) according to Navarro et al. (2004). In this size normalization, size is defined as the geometric mean between variables for every individual and shape is the log ratios of original variables divided by size. Data were checked for distribution normality and homogeneity of variance by Shapiro-Wilk (Shapiro and Wilk, 1965) and Levene's tests (Levene, 1960), respectively. Kruskal-Wallis ANOVA and Median test used for non-normal external measurement in univariate analyses. We used Welch's t-test and Student's t-test for normally distributed measurement with un-equal and equal variances, respectively. For pairwise comparison, one-way analysis of variance (ANOVA) and the Fisher LSD posthoc test were carried out to check for the statistically significant differences between groups. A Two-way multivariate analysis of variance (MANOVA) was performed to

evaluate sexual dimorphism and differences between species. A discriminant analysis (DA) was performed on the classified craniodental measurements to confirm between-group separation in multivariate space. All morphometric statistical analyses were conducted with the STATISTICA 12 software (Statsoft, 2015). We obtained a distance matrix derived from squared Mahalanobis distances of craniodental measurements among *Calomyscus* groups, as obtained in discriminant analysis and an unweighted UPGMA phenetic tree was constructed using MEGA 6 (Tamura et al., 2013).

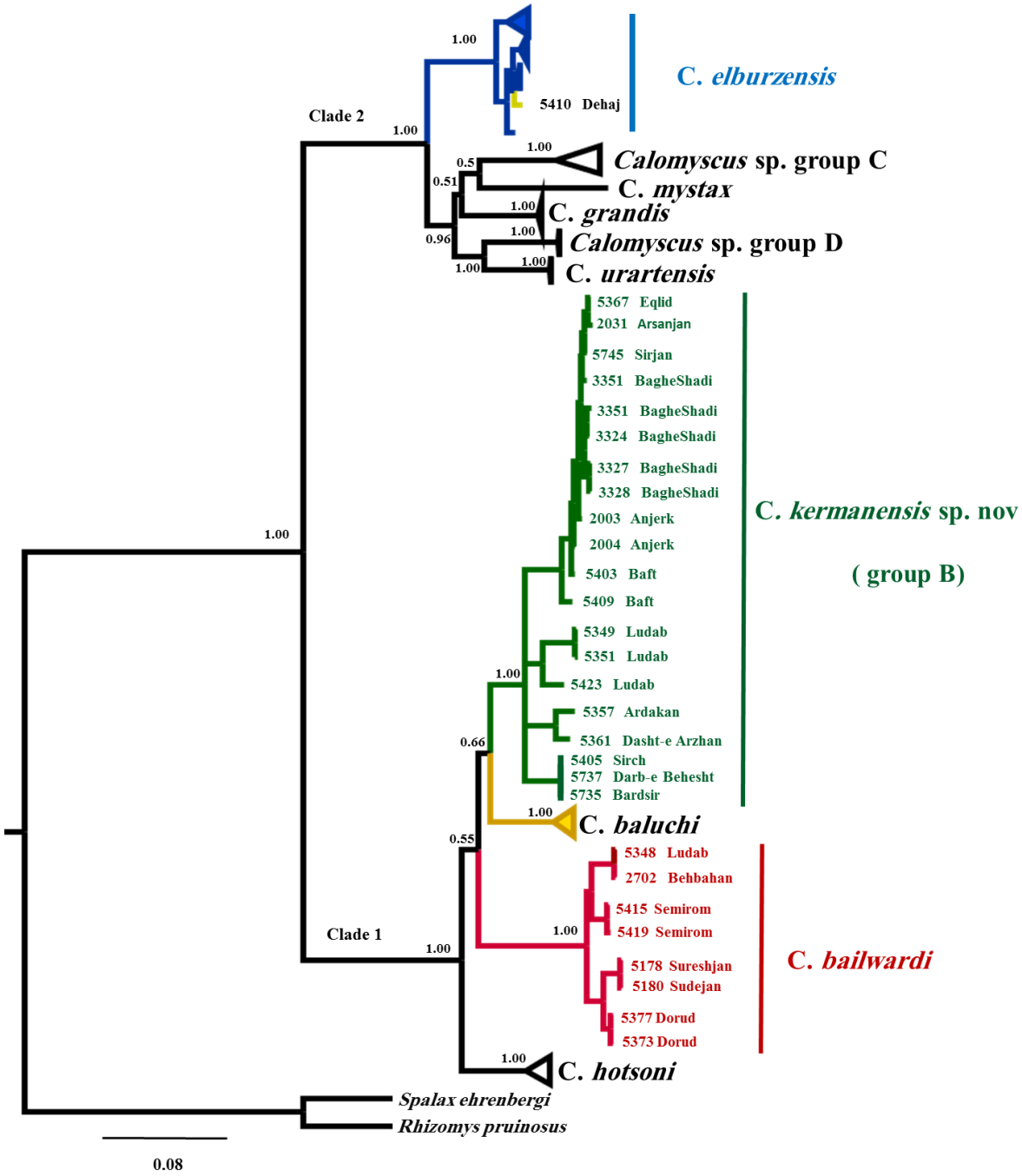


FIGURE 2. Combined tree resulted from a Bayesian analysis on CYTB and CO1 genes. The posterior probability is indicated with numbers on each node.

RESULTS

Phylogenetic analysis

We sequenced 1,534 bp of two concatenated mitochondrial genes (988 bp of CYTB and 546 bp of CO1), which was found to have 441 variable sites (28 % of total sequence) and 523 mutations of which 403 (26%) were parsimony informative. The phylogenetic tree resulting from a combined matrix of CYTB and CO1 sequences by Bayesian inference is presented in Fig. 2. Two major clades with high supporting posterior values (BPP=1) found in the BI analyses. Clade 1 is comprised of *C. elburzensis*, *C. mystax*, *C. urartensis*, *C. grandis*, *C. behzadi* and *Calomyscus* sp. group D and clade 2 consisted of *C. bailwardi*, group B as new lineage, *C. hotsoni* and *C. baluchi*. *Calomyscus* sp. group B was placed as a sister taxon to *C. baluchi* and groups B + *C. baluchi* which in turn are the closest relatives of *C. bailwardi*. Interspecies relationships among *Calomyscus* sp. group B, *C. baluchi* and *C. bailwardi* are not resolved rather showing a polytomic branching. The Maximum likelihood tree also showed a similar topology to the Bayesian tree, except in the ML tree four subclades of Group B retrieved with moderate to low statistical support (S Fig. 2). Phylogenetic analyses showed that specimens from Dorud (Lorestan Province), Semirom (Isfahan Province), Sudejan, Sureshjan (Chaharmahal and Bakhtiari Province) and Ludab (Kohgiluyeh and Boyer-Ahmad Province) belonged to *C. bailwardi* (Fig. 1(C, E, H, F), 2) and specimens of northwest of Kerman province were placed into *C. elburzensis* (Fig. 1L, 2). Group B contains the specimens from the southern part of the Zagros and the Jebal-Barez mountains chain, including brush-tailed mice from Ludab, Kakan (Kohgiluyeh and Boyer-Ahmad Province), Ardakan, Dashte Arzhan, Eqlid, Arsanjan (Fars Provinces), Sirch, Baft, Khabr, Darbe Behesht, Sirjan (Kerman Province), BagheShadi (Yazd Province) and Genu mountains (Hormozgan Province) (Fig. 1; points H, J, K, L, M and Fig. 2).

Genetic divergence

We found large genetic differences (Kimura 2-parameter) in the CYTB gene between all *Calomyscus* species groups ranging from 6.9% (between *C. urartensis* and *Calomyscus* sp. group D) to 17.2% (between *C. urartensis* and *C. bailwardi*) (Table 1). Group B was separated from other *Calomyscus* lineages by deep genetic K2P-distances (with C. *elburzensis* (14.3%), C. *mystax* (14.8%), *C. urartensis* (14.8%, C. *grandis* (15.4%), *C. behzadi* (15.8%), *Calomyscus* sp. group D (13.9%), *C. bailwardi* (10.5%), *C. hotsoni* (9.4%), and *C. baluchi* (9%). Among all examined brush-tailed mice, group B showed the highest mean (3.5%) of within lineage K2P-distance value (Table 1).

Karyological results

A female specimen from Dehaj (northwest of Kerman province) had a karyotype of 2n = 44 and FNa = 70 (Fig. 3A**)**. Cytogenetic analysis of specimens from Dorud (Lorestan Province) (n=2), Semirom (Isfahan Province) (n=2), Sureshjan (Chaharmahal and Bakhtiari Province) (n=1) and Ludab (Kohgiluyeh and Boyer-Ahmad Province) (n=1) showed 2n = 46, FNa = 44, FN = 48 (Fig. 3B, C, D, E, F, G**)**. This complement was composed of 22 pairs of acrocentric chromosomes and X and Y chromosomes were medium subtelocentric and short acrocentric, respectively. We found three different karyotypes for the populations belonging to the *Calomyscus* group B molecular clade: a specimen from Sirch (Kerman Province) presented a complement of 2n = 52, FNa = 56 (Fig. 3K**)**, specimens from Baft (n=3), Sirjan (Kerman Province) (n=1), and Eqlid (n=2) showed 2n = 50, FNa = 48 (Fig. 3L, M, N, O, P, Q**)** and one male and one female from Ludab (Kohgiluyeh and Boyer-Ahmad Province) indicated 2n = 46, FNa = 44, FN = 46 (Fig. 3H, J**)**, the latest complement comprised 22 pairs of acrocentric autosomal chromosomes and X chromosomes were large acrocentric while the Y chromosome was short acrocentric. The karyotype of specimens from Ardakanan and Dasht-e Arzhan (Fars Province) did not result in good chromosome spreads.

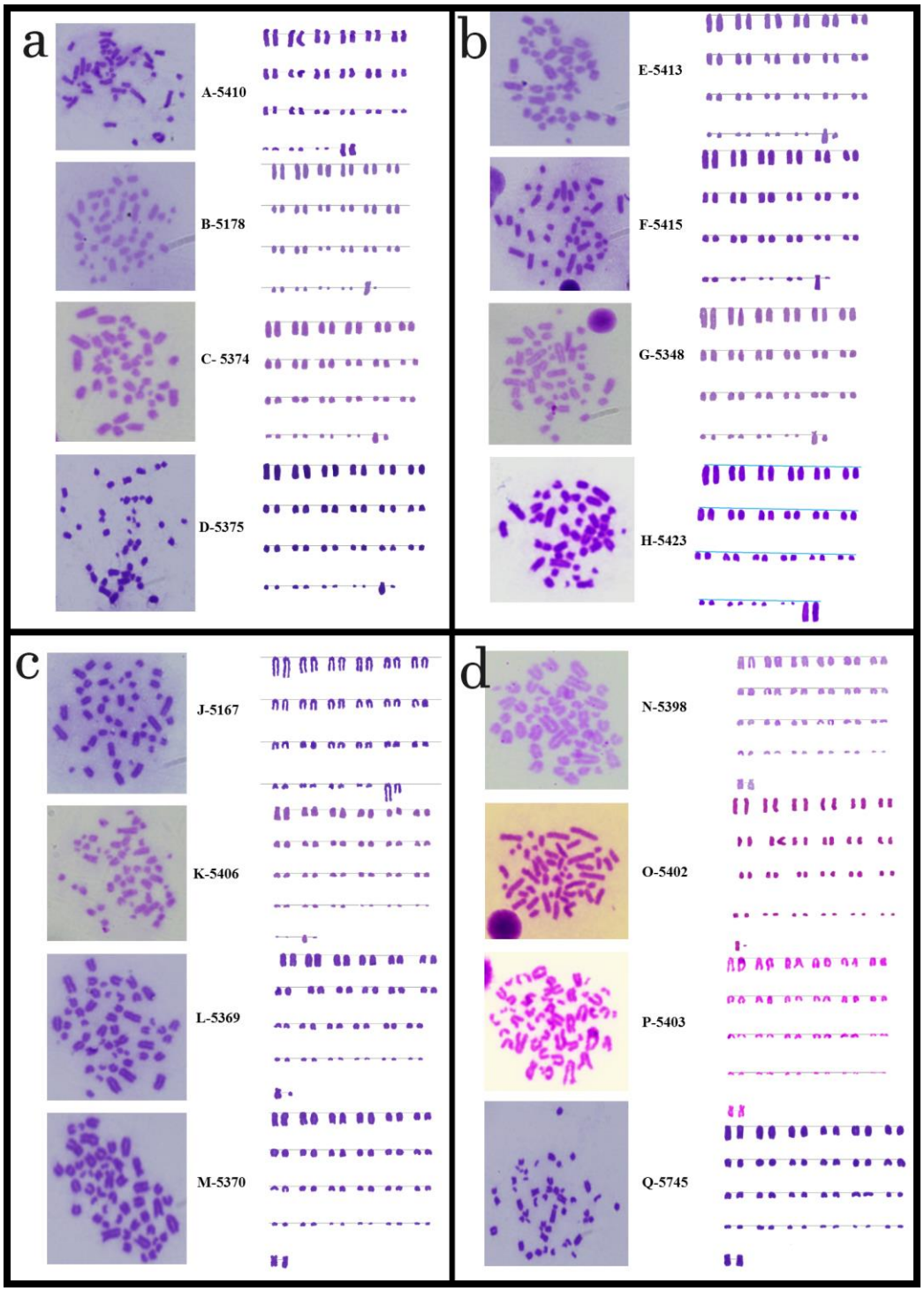


FIGURE 3. The karyotypes of *Calomyscus* specimens from different localities of southern Iran: A a female from Dehaj, 2n = 44 and FNa = 70 (*C. elburzensis isatissus*); B: a male from Sureshjan, C: a male from Dorud, D: a male from Dorud, E: a male from Semirom, F: a male from Semirom, G: a male from Ludab, 2n = 46, FNa = 44, FN = 48 (*C. bailwardi*); H: a female from Ludab, J: a male from Ludab, 2n = 46, FNa = 44, FN = 46, K: a male from Sirch, 2n = 52, FNa = 56, L: a male from Eqlid, M: a female from Eqlid, N: a female from Baft, O: a male from Baft, P: a female from Baft, Q: a female from Sirjan, 2n = 50, FNa = 48 (*Calomyscus* sp. group B). In each ideogram, sex chromosomes are placed at the end (XX or XY).

Morphometric results

Based on MANOVA results, interaction between "sex" and "species" was not significant in both uncorrected data (Wilk's = 0.48, F = 1.23, d.f. = 78, p = 0.09) and shape data (Wilk's = 0.41, F = 9, d.f. = 120, p = 0.6). Multivariate analysis of variance showed that *Calomyscus* group B presented a significant difference with all other groups (p-values < 0.000007). Discriminant analysis (DA) based on craniodental variables correctly identified 91.71% and 90% of examined specimens according to uncorrected data and shape data, respectively. This analysis demonstrated that the first and second canonical functions explained 59% and 58% of the whole variance, respectively. The cross-validation test indicated that 94.59% and 94% of specimens in group B were classified to the actual group in size and size-free data, respectively. The plot of *Calomyscus* specimens based on the first two functions showed that the samples belonging to group B were distinct from the other groups (Fig 4, 5**)**. Univariate analysis on skull size (geometric mean between variables) showed that group B had a significantly different value in comparison with all other groups (P < 0.05) (except with *C. behzadi*). The skull size is larger than *C. elburzensis* and *C. hotsoni* and smaller than *C. bailwardi*, *C. baluchi*, *C. grandis* and *Calomyscus* sp. group D. This group had significantly smaller dental size compared to *Calomyscus* sp. group D, *C. behzadi* and *C. baluchi* (S Table 2**)**. The UPGMA tree based on Squared Mahalanobis Distances of both uncorrected data and shape data revealed that group B and *C. bailwardi* are morphologically more similar (Fig 6**)**. The result of Welch's t-test and Student's t-test was similar to the result of a One-way analysis of variance (ANOVA) with the Fisher's LSD post-hoc test. The result of Welch's t-test and Student's t-test are presented in (S Table 2). These results show that group B is significantly different from other species in several craniodental measurements.

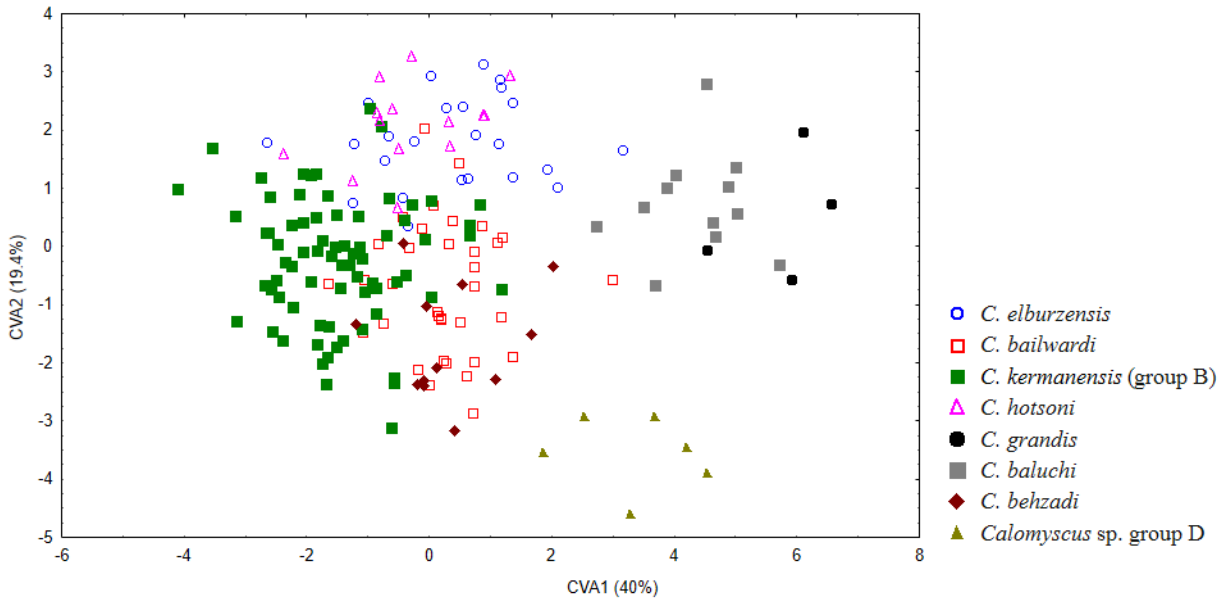


FIGURE 4. Scatter plot of the first two canonical functions for craniodental measurements (Size + shape)

DISCUSSION

Taxonomy

***Calomyscus kermanensis* sp. nov.**

Holotype

ZMFUM-5406, ♂, Sirch region of Kerman province (Fig. 8J), Iran (30˚11′60″N, 57˚32′60″E), Elevation of 1862 m, collected on 30 August 2017 by M. Dezhman. The holotype consists of skin skull (Fig. 7), karyotype slides and tissues preserved in 99% ethanol (all parts in good condition).

Holotype measurements: BL: 78; TL: 92; FL: 21; EL: 21; CBL: 22.67; SH: 8.23; Mndl: 13.37

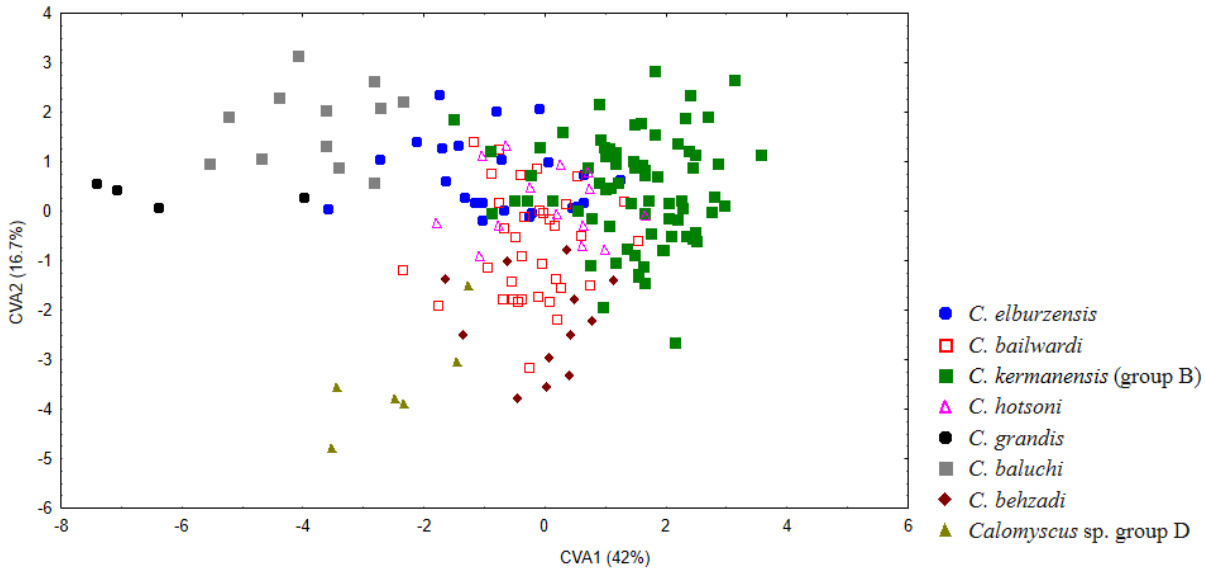


FIGURE 5. Scatter plot of the first two canonical functions for craniodental measurements (Shape).

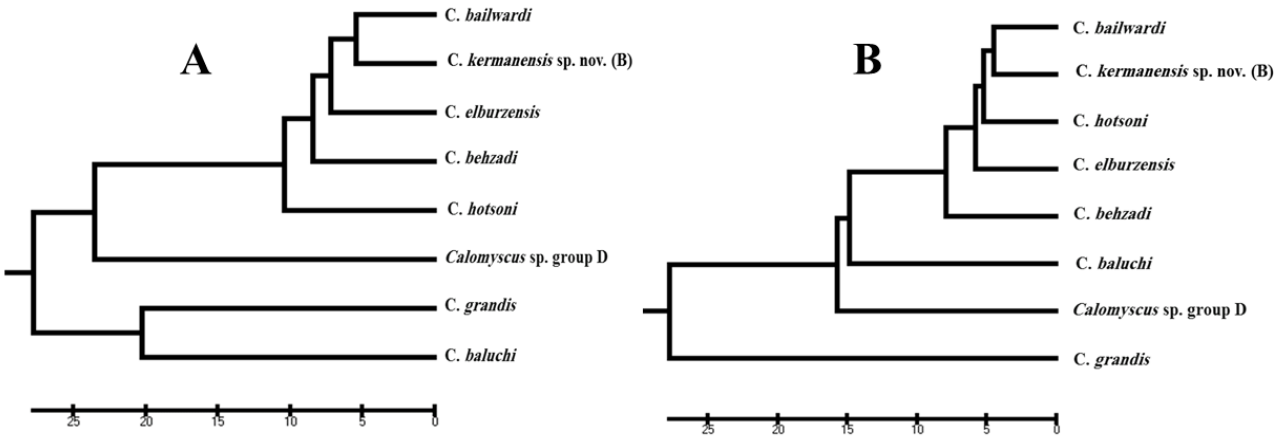


FIGURE 6. UPGMA tree derived from Mahalanobis distances of craniodental measurements between *Calomyscus* groups A: size+shape and B: shape.

Paratypes

ZMFUM-5407, ♀; ZMFUM-5389, ♂; ZMFUM-5391, ♀; ZMFUM-5392, ♂and ZMFUM-5394, an ♀ from Sirch in Kerman province, Iran (30° 11′ 60″N, 57° 32′ 60″E), other data same as holotype.

Paratype measurements

ZMFUM-5407: BL: 78; TL: 94; FL: 21; EL: 20; CBL: 22.60; SH: 8.36; Mndl: 13.53; ZMFUM-5389: BL: 78; TL: 87; FL: 21; EL: 19; CBL: 22.56 SH: 8.1; Mndl: 13.03; ZMFUM-5391: BL: 78; TL: 88; FL: 21; EL: 20; CBL: 23.25; SH: 8.08; Mndl: 13.06; ZMFUM-5392: BL: 78; TL: 89; FL: 21; EL: 20; CBL: 23.13; SH: 8.39; Mndl: 13.49; ZMFUM-5394: BL: 74; TL: 93; FL: 21; EL: 20; CBL: 22.45; SH: 8.2; Mndl: 13.1

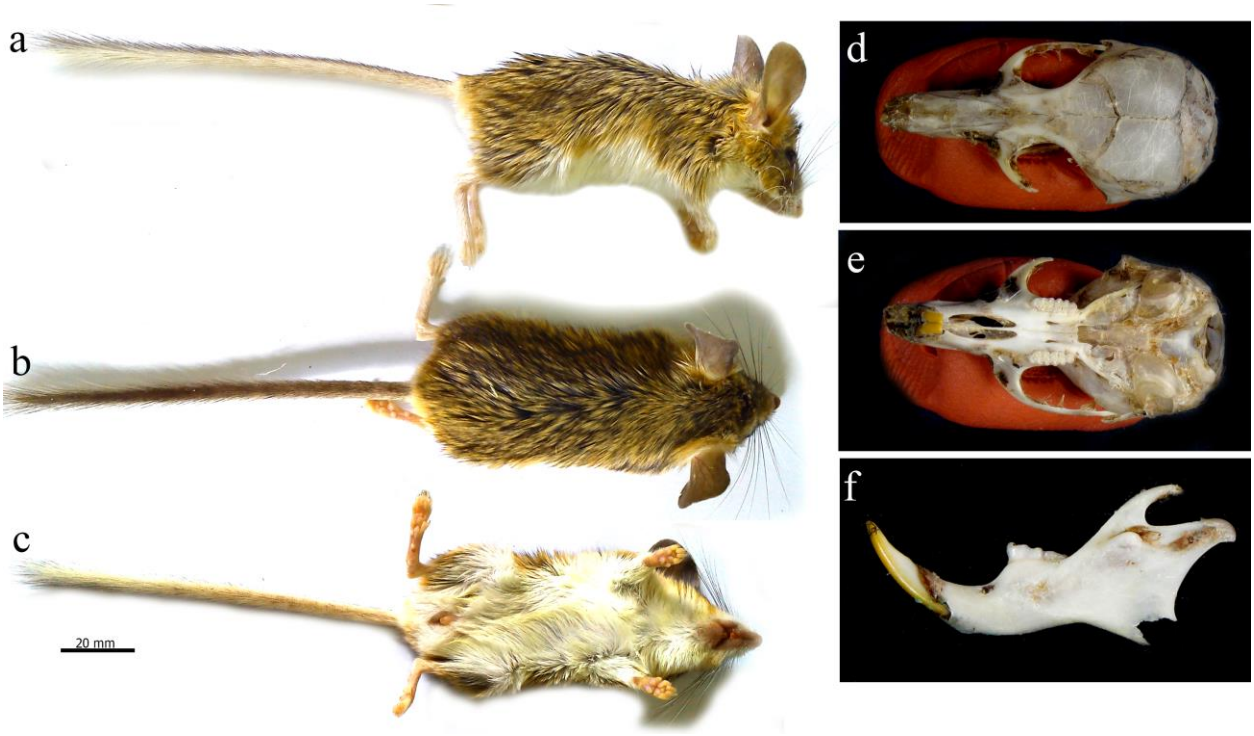


FIGURE 7. lateral (a), dorsal (b) and ventral (c) views of body, dorsal (d) and ventral (e) side of the cranium and external view of mandible (f) in holotype of *C. kermanensis* sp. nov. (Sirch region of Kerman province, Iran).

Etymology: The new species name refers to Kerman province where holotype and paratypes were collected.

Description: The medium-size brush-tailed mice with body length 65–91 mm, tail length 74–102 mm, ear length 17–21 mm, hindfoot length 18–23 mm. The coloration of the specimens is similar to C. *bailwardi*. Adult specimens have light brown color on their back. Hairs of the middle part of dorsal side are grey in the base and tip and were light brown in the middle. Ventral side is pure white. The ears are long and nearly naked. The tail is longer than the head and body length and is bicolored, greyish buff above and darkening terminally to blackish and covered by sparse hairs throughout the length with a slight brush at the tip. The skull is small and narrow. The rostrum is long and narrow and the braincase is rounded. The incisors are smooth and compressed. The first molar is the longest and the third molar is the shortest. The molars are brachyodont, with an asymmetrical bi-serial arrangement of five cusps on the first two molars and the third molar only with three cusps.

Diagnosis: Although the external morphology of *Calomyscus* species is so similar, several craniodental measurements can distinguish *C. kermanensis* sp. nov from the majority of others (S Table. 2). *C. kermanensis* sp. nov. has the highest cranial height in uncorrected data, but it does not differ significantly from *C. bailwardi*. The new species has a longer condylobasal than *C. hotsoni* and *C. elburzensis*, but it is shorter than *C. baluchi*, *C. behzadi*, *Calomyscus* sp. group D., and *C. bailwardi*. *C. kermanensis* sp. nov. has a longer mandible than *C. hotsoni* and *C. elburzensis*, but it is shorter than *C. baluchi*, *C. behzadi*, *Calomyscus* sp. group D., and *C. bailwardi*. The new species has longer ears than *C. grandis*, *C. behzadi*, *C. hotsoni* and *C. elburzensis*. *C. kermanensis* sp. nov. has shorter nasal than *C. hotsoni*, *C. behzadi*, *Calomyscus* sp. group D, *C. grandis* and *C. elburzensis* and narrower than *C. behzadi*, *Calomyscus* sp. group D, *C. bailwardi* and *C. baluchi* and *C. grandis*. This new species has shorter palatal than *Calomyscus* sp. group D, *C. behzadi*, *C. baluchi* and *C. grandis* and longer than *C. hotsoni*. *C.*

kermanensis sp. nov. has narrower interorbital than *C. behzadi*, *Calomyscus* sp. group D, *C. bailwardi* and longer interorbital than *C. elburzensis* and *C. baluchi*. Also, M2 is shorter than *C. baluchi*, *C. behzadi*, *Calomyscus* sp. group D and *C. elburzensis* and narrower than *C. baluchi*, *C. behzadi*, *Calomyscus* sp. group D, *C. grandis* and *C. hotsoni*. In addition to, zygomatic is wider than *C. hotsoni* and narrower than *C. behzadi*, *Calomyscus* sp. group D, *C. bailwardi* and *C. baluchi*.

According to size-free data, the new species has the highest cranium height of all the studied species, and nasal is wider than *C. grandis* but narrower than *C. elburzensis*, *C. bailwardi*, *C. baluchi*, *C. behzadi* and *Calomyscus* sp. group D., and nasal is shorter than *C. elburzensis*, *C. bailwardi*, *C. grandis* and *C. hotsoni*. Palatal is shorter than *C. elburzensis*, *C. baluchi* and *C. behzadi*. In this new species, occipitonasal is longer than *C. elburzensis*, *C. baluchi* and *C. grandis*. Bull is larger than *C. bailwardi*, *C. baluchi* and *C. behzadi*. *C. kermanensis* sp. nov. has longer M1 than *C. grandis*, *C. behzadi* and *Calomyscus* sp. group D and M1 is wider than *C. behzadi* and *Calomyscus* sp. group D and narrower than *C. hotsoni* and *C. baluchi*. M2 is shorter than *C. grandis*, *C. elburzensis* and *C baluchi* and narrower than *C. grandis*, *C. hotsoni* and *C. baluchi*.

Comparison: Based on uncorrected data, in comparison with *C. bailwardi*, the new species has a smaller hind foot, narrower cranium, shorter upper diastema, narrower zygomatic, shorter occipitonasal, narrower interorbital, shorter condylobasal, shorter nasal, shorter lower diastema, lower height of lower diastema, lower mandibular height, longer mandible, narrower m2 and in shape data higher cranium, longer tympanic bulla, wider distance between two meatus, shorter and narrower nasal, shorter lower diastema and narrower m2. Comparison with the other groups is shown in S Table 2. The new species, *C. kermanensis* sp. nov. has three different cytotypes (or karyotypes) including: (1) 2n = 52, FNa = 56, FN = 60; (2) 2n = 50, FNa = 48, FN = 52 (this karyotype is similar to one of the karyotypes belonging to *C. hotsoni*) and (3) 2n = 46, FNa = 44, FN = 46 (this karyotype is similar to the karyotype has already in *C. bailwardi* but they differ in X chromosome which is medium subtelocentric in *C. bailwardi* and large acrocentric in *C. kermanensis* sp. nov.

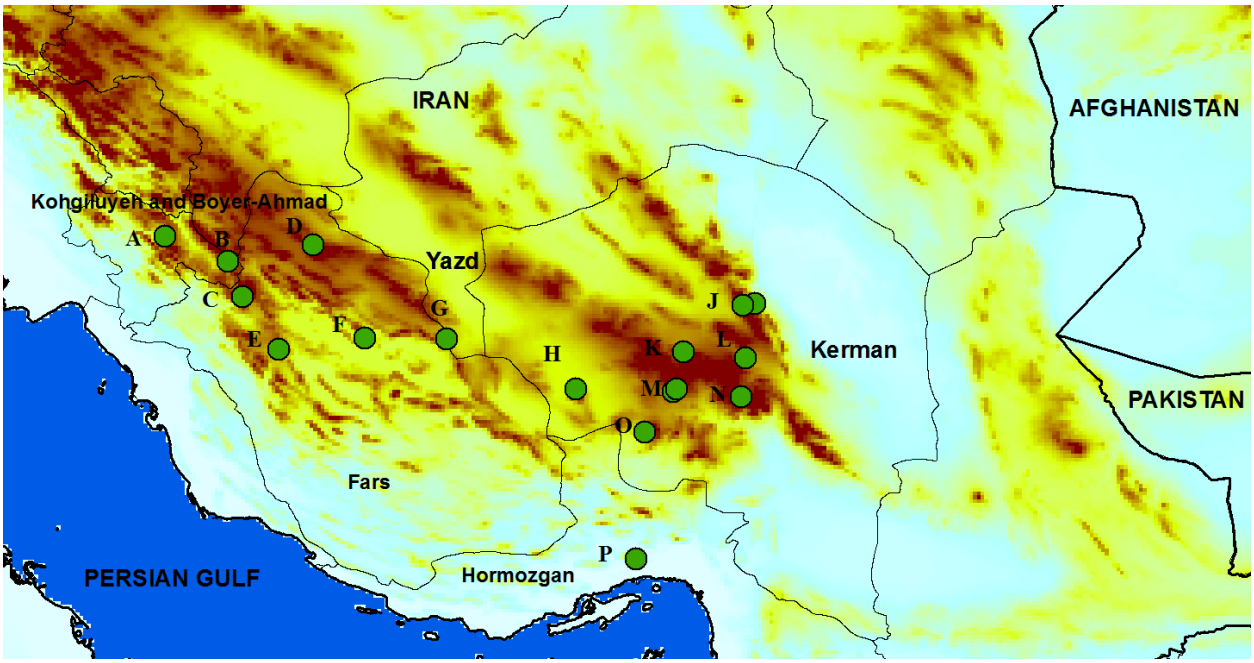


FIGURE 8. Sampling localities of C. *kermanensis* sp. nov. from the Zagros Mountains, south of Iran. A: Ludab; B: Kakan; C: Ardakan; E: Dasht-e Arzhan, D: Eqlid, F: Arsanjan; G: BagheShadi; J: Sirch, K: Bardsir L: Rayen, M: Baft, O: Khabr, N: Darb-e Behesht, H: Sirjan, and P: Genu Mountains.

Distribution: *C. kermanensis* sp. nov. is an endemic species in Iran and known from Ludab, Kakan (Kohgiluyeh and Boyer-Ahmad Province), Ardakan, Dasht-e Arzhan, Eqlid, Arsanjan (Fars Province),

Sirch, Baft, Khabr, Darb-e Behesht, Sirjan (Kerman Province), BagheShadi (Yazd Province) and Genu Mountains (Hormozgan province) in the northeast of Persian Gulf (Fig.8).

Habitat: *C. kermanensis* sp. nov. inhabits barren rocky and scarcely vegetated mountainsides with an altitude between 1773 to 2878 m (our own data) but reported only from 500 meters above sea level in Genu Mountains by Morshed & Patton (2002). One female gave birth to three offspring in captivity (June 2017). It seems that this new species has many fragmented populations and there are no major threats for its conservation.

The phylogenetic tree obtained from combined CYTB and COI sequences indicated two major clades within the genus *Calomyscus* which are consistent with recent studies based on nuclear and mitochondrial genes (Akbarirad et al., 2016*a*; Rezazadeh et al., 2020) . Clade 1 includes *C. hotsoni*, *C. bailwardi*, *C. baluchi* and *C. kermanensis* sp. nov., and clade 2 includes *C. elburzensis*, *C. mystax*, *C. urartensis*, *C. grandis*, *C. behzadi* and *Calomyscus* sp. group D. In clade 2, *C. hotsoni* is a basal clade and *C. bailwardi* is the sister taxon of *C. kermanensis* sp. nov. + *C. baluchi* clade, but there is a polyphyletic pattern within this major clade. Also, in previous molecular studies based on mitochondrial genes, the relationships of this major clade were not fully resolved (Akbarirad et al., 2016*a*; Rezazadeh et al., 2020) but the only Bayesian tree obtained from the nuclear gene Rbp3 for a few specimens of brush-tailed mice showed that relationships of species in clade 1 were fully resolved. *C. baluchi* was basal clade and *C. bailwardi* and group B (*C. kermanensis* sp. nov.) were sister taxa and then *C. hotsoni* joined them (Rezazadeh, 2020). Furthermore, the Rbp3 gene network and genomic analyses seperated specimens belong to group B (C. *kermanensis* sp. nov.) from *C. hotsoni* and *C. baluchi* and suggested that it could be a new species (Weller, 2019; Rawson, 2019).

Based on our molecular results, the range of *C. elburzensis isatissus* expanded to the northwest of Kerman Province. This subspecies occurs in Shirkuh Mountains in Yazd, Karkas Mt. in Isfahan and Qeydar Mts. in Zanjan Province (Akbarirad et al., 2016*b*). Also, one karyotype of our specimens (2n=44 and Fna=70) is the same with the complement reported for this subspecies (Graphodatsky et al., 2000; Shahabi et al., 2010; Akbarirad et al., 2016*b*). In previous studies, it was believed that *C. bailwardi* (with its type locality of Mala-i-Mir (or Izeh), Khuzistan Province) distributed in the Zagros Mountains from western and southwestern to east Iran, as far as Kerman and Hormozgan provinces (Thomas, 1905; Morshed and Patton, 2002; Musser and Carleton, 2005; Shahabi et al., 2013) with an unconfirmed report from Turkey only based on morphological features (Krystufek & Vohralik, 2009). While based on the molecular data, it has been confirmed so far from Izeh (type locality) and Behbahan (Khuzistan Province) (Akbarirad et al., 2016*a*). For the first time, our phylogenetic results and karyotypes data approve that *C. bailwardi* in addition to Izeh and Behbahan locality occurs in Dorud (Lorestan Province), Semirom (south of Isfahan), Sureshjan, Sudejan (Chaharmahal and Bakhtiari Province), and Ludab (Kohgiluyeh and Boyer-Ahmad Province) (Fig. 1, 2). The mean genetic distance based on CYTB sequences between *C. kermanensis* sp. nov. and all other examined *Calomyscus* groups is greater than 9% (9-15.8%), this range of distance values are commonly used to consider as interspecific divergences level in *Calomyscus* species (>6.9%) and other rodents (Bradley and Baker, 2001; Shahabi et al., 2013; Rosa et al., 2012). *C. kermanensis* sp. nov. has the highest interspecific K2P-distance value (3.5%), which is bigger than *C. elburzensis* (1.8%) with three known subspecies (Akbarirad et al., 2016*b*). This suggests that we need further analyses for a better understanding of intraspecific divergence in *C. kermanensis* sp. nov.

Our cytogenetic analysis, as well as Akbarirad et al. (2016*a*) and Romanenko et al. (2021) demonstrated that Zagros Mountains *Calomyscus* has only one karyotype, 2n = 46, FNa = 44, FN = 46. Our phylogenetic analyses showed that specimens from Ludab, Kakan (Kohgiluyeh and Boyer-Ahmad Province), Ardakan, Dasht-e Arzhan, Eqlid, Arsanjan (Fars Province), Sirch, Baft, Khabr, Darb-e Behesht, Sirjan (Kerman Province), BagheShadi (Yazd Province) and Genu Mountains (Hormozgan province) are forming a new species (*C. kermanensis* sp. nov.). This result is consistent with Akbarirad et al, (2016*a*). For this new species, three different karyotypes were observed: (A) 2n = 52, FNa = 56 for specimens from Sirch and Bardsir (Kerman Province), this karyotype has also been reported from Sirch

(Kerman Province) (Graphodatsky et al., 2000; Romanenko et al., 2021); (B) 2n = 50, FNa = 48 from Baft, Eqlid and Sirjan localities (Kerman Province). So far, two karyomorphs 2n = 50, FNa = 48 and 2n = 50, FNa = 50 have been reported from the same region (probably the same specimen) in Sivand (Fars Province) by Malikov et al. (1999) and Graphodatsky et al. (2000), respectively that according to the present study one of them is correct and a mistake has happened in sex chromosome recognition, so complement 2n = 50, FNa = 48 is correct. This karyotype of *C. kermanensis* sp. nov. is similar to one karyotype of *C. hotsoni* (2n = 50, FNa = 48) from Saravan (Shahabi et al., 2010). Another similar karyotype was found for *C. mystax* and *C. grandis* (2n = 44, FNa = 46) which can be explained by the existence of a shared ancestral cytotype in different region of the range or as an effect of convergent evolution (Grafodatsky et al., 2000); (C) 2n = 46, FNa = 44, FN = 46 for specimens from Ludab (Kohgiluyeh and Boyer-Ahmad Province), although this karyotype in 2n and FNa are similar to the karyotype reported for *C. bailwardi* from the same locality and all of *C. bailwardi*'s distribution range, they differ in FN (48 vs. 46). In *C. bailwardi*, the X chromosome was subtelocentric but specimens of the new lineage have a large acrocentric X chromosome. Cytogenetics analyses play an important role in identifying species with similar morphology (Romanenko et al., 2021)**.** In the previous works, there was an unanswered question whether the extensive karyotypic diversity in *Calomyscus* indicates recent speciation incidents or an appearance of intraspecific polymorphism (Shahabi et al., 2010; Graphodatsky et al., 2000, Romanenko et al., 2021). Our result shows that each different karyotype usually has a distinct geographical distribution. The brush-tailed mice have patchily distributed populations which some of them being geographically isolated and increase random genetic drift effect and it may be a substantial factor in the rapid evolution of karyotypes (Graphodatsky et al., 2000). However, karyotype of *Calomyscus* individuals is an ambiguous indicator of their species status (Romanenko et al., 2021).

Based on both karyological and molecular data from Ludab (Kohgiluyeh and Boyer-Ahmad Province), *C. bailwardi* and *C. kermanensis* sp. nov. are sympatric so this locality was identified as a new contact zone (Fig. 1G), but gene flow among these two divergent lineages is unknown and will require further nuclear gene sequencing. This contact zone probably resulted from population expansion events by one or both species during the warmer interglacial periods. Other contact zones and hybridization have been reported in this genus between two divergent clades of *C. elburzensis* in the central Iranian Plateau (Shirkooh, Yazd province) (Haddadian and Darvish, 2018) and another one in central Kopet Dagh Mountains (Meyer & Malikov, 2000). There is a report of male sterility in hybrids between two subspecies of *C. elburzensis* with two different cytotypes (Meyer & Malikov, 2000; Graphodatsky et al., 2000; Norris et al., 2008).

Forms (color, size, cranial and dental measurements) in the *Calomyscus* genus are so similar that they were often considered conspecific prior to investigations of karyotype, hybridization, and molecular analyses (Kilpatrick, 2017). Although *Calomyscus* species are similar in external morphology but univariate and multivariate analysis on craniodental measurements allowed us to separate *C. kermanensis* sp. nov. from the other *Calomyscus* groups in both uncorrected and shape data. Previous studies also showed that craniodental measurements can discriminate *Calomyscus* species while external features were not useful for species delimitation (Lebedev et al., 1998; Shahabi et al., 2011; Akbarirad et al., 2016*a*, Rezazadeh et al., 2020). This recommended new species share most of the morphometric characters with *C. bailwardi*.

In conclusion, we used an integrative taxonomic approach that included karyotypic, mitochondrial DNA and morphometric data and we found thet *C. kermanensis* sp. nov. is an endemic species in southern Iran with significant craniodental and molecular variations. So, this result emphasizes the importance of integrative taxonomy in resolving the systematics of this genus. Intraspecific relationships in *C. kermanensis* sp. nov. were not resolved in our phylogenetic tree with high genetic and karyotype diversity; therefore, further studies should include more specimens, nuclear and mitochondrial markers as well as population genetic methods to clarify diversity, evolutionary history and phylogeography of the newly described species. We must also underline that the taxonomic structure of the other new lineages in this genus is still unclear.

ACKNOWLEDGMENTS

Permission to the sampling of brush-tailed mice was authorized by the Iranian Department of Environment (Permission Number: 96/4735; 2017; 2th May). The authors are indebted to the late Professor Jamshid Darvish for his many year encouragements to us, proposing and supervising this project. We express our honest gratitude to Hadi Dezhman, Sadegh Salari, Mojtaba Salari, Milad Ebadian, Majid Vafaei nasab, Mehrdad Movahed and Dr. Ahmad Mahmoudi for their assistance in the sampling of this study. This research was supported by the office of research affairs, Ferdowsi University of Mashhad, Iran (Project No. 3-42645).

LITERATURE CITED

Akbarirad, S., Darvish, J., Aliabadian, M., & Kilpatrick, C. W. M. 2015. Biosystematic study of *Calomyscus mystax* (Rodentia, Calomyscidae) from northeastern Iran. *Iranian Journal of Animal Biosystematics*, 11: 65–77.

Akbarirad, S., Darvish, J., & Aliabadian, M. 2016a. Increased species diversity of brush-tailed mice, genus *Calomyscus* (Calomyscidae, Rodentia), in the Zagros Mountains, western Iran. *Mammalia*, 80: 549–562.

Akbarirad, S., Darvish, J., & Aliabadian, M. 2016b. Phylogeography of *Calomyscus elburzensis* (Calomyscidae, Rodentia) around the central Iranian Desert with description of a new subspecies in center of Iranian Plateau. *Journal of Science of Islamic Republic of Iran*, 27: 5–21.

Akbarirad, S., Darvish, J., & Aliabadian, M. 2016c. Molecular, chromosomal and morphometric variation of *Calomyscus hotsoni* and C. *elburzensis* (Calomyscidae, Rodentia) in the East Iran. *Folia Zoologica*, 65: 27–37.

Aliabadian, M., Nijman, N., Mahmoudi, A., Naderi, M., Vonk, R., & Vences, M. 2014. ExCaliBAR: A simple and fast software utility to calculate intra- and interspecific distances from DNA Barcoding. *Contribution of Zoology*, 83: 79-83.

Bradley, R. D., & Baker, R. J. 2001. A test of the genetic species concept: cytochrome-b sequences and mammals. *Journal of Mammalogy*, 82: 960–973.

Chauvier, G. 1982. Chromosomal phylogeny of forty-two species of Cerecopithecoids (*Primates Catarrhini*). *Annals of Géneral Psychiatry*, 25: 96–109.

Corbet, G. B., & Hill, J. E. 1980. A World List of Mammalian Species. British Museum (Natural History) and Cornell University Press, London and Ithaca (New York).

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, 9: 772.

Dezhman, M., Akbarirad, S., Aliabdian, M., Siahsarvie, R., Shafaeipour, A., & Mirshamsi, O. 2021. A new species of *Calomyscus* Thomas, 1905 (Calomyscidae: Rodentia) from western Iran. *Turkish Journal of Zoology*, 45: 585-593.

Dutrillaux, B., Couturier, J., Muleris, M., Lombard, M., & Chauvier, G. 1982. Chromosomal phylogeny of forty-two species of Cerecopithecoids (Primates Catarrhini). *Annals of Géneral Psychiatry*, 25: 96–109.

Ellerman, J. R. 1948. Key to the rodents of South-West Asia in the British Museum collection. *Proceeding of the Zoological Society of London*, 118: 765–817.

Ellerman, J. R., & Morrison-Scott, T. C. S. 1951. Checklist of Palearctic and Indian Mammals. London: British Museum (Natural History).

Goodwin, G. G. 1939. Five New Rodents from the Eastern Elburz Mountains and a New Race of Hare from Teheran. American Museum Novitates; The American Museum of Natural History: New York, NY, USA.

Graphodatsky, A. S., Sablina, O. V., Meyer, M. N., Malikov, V. G., Isakova, E. A., Trifonov, V. A., Polyakov, A. V., Lushnikova, T. P., Vorobieva, N. V., Serdyukova, N. A., Perelman, P. L., Borodin, P. M., Benda, P., Frynta, D., Leikepová, L., Munclinger, P., Piálek, J., Sádlová, J., & Zima, J. 2000. Comparative cytogenetics of hamsters of the genus *Calomyscus*. *Cytogenetics and Cell Genetics*, 88: 296–304.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimovam M., & Hordijk, W. et al. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 59: 307-321.

Hamidi, K., Matin, M. M., Kilpatrick, C. W., & Eskandarzadeh, N. 2019. Landscape and niche specialisation of two brush-tailed mice species *Calomyscus elburzensis* and C. *hotsoni* in Iran: a case of the role of ecological niche modelling in finding area (s) of contact. *Ethology Ecology & Evolution*, 31: 435-456.

Hamidi, K., Darvish, J., & Matin, M. M. 2017. Ecological survey of two Calomyscidae species; Goodwin's brush-tailed mouse and Hotson's brush-tailed mouse (Rodentia) in the eastern parts of Iran. *Acta Ecologica Sinica*, 37: 105-114.

Herbreteau, V., Jittapalapong, S., Rerkamnuaychoke, W., Chaval, Y., Cosson, J. F., Morand, S. 2011. Protocols for field and laboratory rodent studies. Bangkok, Thailand: Kasetsart University.

Hall, T. A. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41: 95–98.

Kilpatrick, C. W. 2017. Family Calomyscidae (brush-tailed mice). In: Wilson DE, Mittermeier RA, Lacher TE (ed) Handbook of mammals of the world, Vol. 7. Rodents II Lynx Edicions, Barcelona, Spain, Pp 144–155.

Krystufek, B., & Vohralik, V. 2009. Mammals of Turkey and Cyprus, Rodentia II: Cricetinae, Muridae, Spalacidae, Calomyscidae, Capromyidae, Hystricidae, Castoridae. 1st ed. Knjiznica Annales Majora, Koper.

Lay, D. M. 1967. A study of the mammals of Iran resulting from the Street expedition of 1962–63. *Fieldiana Zoology*, 54: 1-282.

Lebedev, V. S., Pavlinov, I. Ya., Meyer, M. N., & Malikov, V. G. 1998. Craniometric analysis of mouse-like hamsters of the genus *Calomyscus* (Critecidae). *Zoologicheskii Zhurnal*, 77: 721–731.

Levene, H. 1960. Robust tests for equality of variance. In Contributions to probability and statistics: essays in honor of Harold Hotelling. Stanford University Press.

Malikov, V. G., Meyer, M. N., Graphodatsky, A. S., Polyakov, A. V., Sablina, O. V., Vaziri, A. S., Nazari, F., & Zima, J. 1999. On a taxonomic position of some karyomorphs belonging to genus *Calomyscus* (Rodentia, Cricetidae). *Proceeding of the Zoological Institute of Russian Academy of Sciences*, 281: 27–32.

Meyer, M. N., & Malikov, V. G. 2000. New species and subspecies of mouse-like hamsters of the genus *Calomyscus* (Rodentia, Cricetidae) from southern Turkmenistan. *Zoologicheskii Zhurnal*, 79, 219–223 (in Russian).

Montgelard, C., Bentz, S., Tirard, C., Verneau, O., & Catzeflis, F. M. 2002. Molecular systematics of Sciurognathi (Rodentia): the mitochondrial cytochrome b and 12S rRNA genes support the Anomaluroidea (Peptidae and Anomaluridae). *Molecular Phylogenetics Evolution*, 22: 220–233.

Morshed, S., & Patton, J. 2002. New records of mammals from Iran with systematic comments on hedgehogs (Erinaceidae) and mouse-like hamsters (*Calomyscus*, Muridae). *Zoology in the Middle East*, 26: 49-58.

Musser, G. G., & Carleton, M. D. 2005. Subfamily Murinae. In Wilson DE, Reeder DM (Eds.), Mammal species of the world, a taxonomic and geographic reference. Baltimore: The Johns Hopkins University Press, Pp 894–1531.

Navarro, N., Zatarain, X., & Montuire, S. 2004. Effects of morphometric descriptor changes on statistical classification and morphospaces. *Biological Journal of the Linnean Society*, 83: 243–260.

Norris, R.W., Woods, C.A., & Kilpatrick, C. W. 2008. Morphological and molecular definition of *Calomyscus hotsoni* (Rodentia: Muroidea: *Calomyscus*). *Journal of Mammalogy*, 89: 306–315.

Peshev, D. 1991. On the systematic position of the mouse-like hamster *Calomyscus bailwardi* Thomas, 1905 (Cricetidae, Rodentia) from the Near East and Middle Asia. *Mammalia*, 55: 107-112.

Rawson, B. 2019. Phylogenomics and species delimitation in the brush-tailed mouse genus *Calomyscus* using ddRADSeq data. Doctoral dissertation. The Ohio State University. 17p.

Rosa, C. C., Flores, T., Pieczarka, J. C., Rossi, R. V., Sampaio, M. I. C., Rissino, J. D., Amaral, P. J. S., & Nagamachi, C. Y. 2012. Genetic and morphological variability in South American rodent *Oecomys* (Sigmodontinae, Rodentia): evidence for a complex of species. *Journal of Genetics*, 91: 265–277.

Robins, J. H., Hingston, M., Matisoo-Smith, E., & Ross, H. A. 2007. Identifying *Rattus* species using mitochondrial DNA. *Molecular Ecology Notes*, 7: 717–729.

Rezazadeh, E., Aliabadian, M. Darvish, J., & Ahmadzadeh, F. 2020. Diversification and Evolutionary History of Brush-tailed Mice, Calomyscidae (Rodentia), in Southwestern Asia. *Organism Diversity and Evolution*, 20: 155–170.

Rezazadeh, E. 2020. Taxonomic revision and phylogeny of family Calomyscidae Vorontsov and Potapova, 1979 from northwestern Iran. PhD Thesis. Ferdowsi University of Mashhad. 190 p (in persian).

Roberts, T. J. 1997. The mammals of Pakistan. Oxford University Press, Oxford.

Romanenko, S. A., Malikov, V. G., Mahmoudi, A., Golenishchev, F. N., Lemskaya, N. A., Pereira, J. C., Trifonov, V. A., Serdyukova, N. A., Ferguson-Smith, M. A., Aliabadian, M. & Graphodatsky, A. S. 2021. New Data on Comparative Cytogenetics of the Mouse-Like Hamsters (*Calomyscus* Thomas, 1905) from Iran and Turkmenistan. *Genes*, 12: 964.

Ronquist, F., & Huelsenbeck, J. P. 2003. Mrbayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572–1574.

Schlitter, D. A., & Setzer, H. W. 1973. New rodents (Mammalia: Cricetidae, Muridae) from Iran and Pakistan. Proceedings of the Biological Society of Washington, 86: 163–174.

Shapiro, S. S., & Wilk, M. B. 1965. An analysis of variance test for normality (complete samples). *Biometrika*, 52: 591–611.

Shahabi, S., Zarei, B., & Sahebjam, B. 2010. Karyologic study of three species of *Calomyscus* (Rodentia: Calomyscidae) from Iran. *Iranian Journal of Animal Biosystematics*, 6: 55–60.

Shahabi, S., Darvish, J., Aliabadian, M., Mirshamsi, O., & Mohammadi, Z. 2011. Cranial and dental analysis of mouse-like hamsters of the genus *Calomyscus* (Rodentia: Calomyscidae) from plateau of Iran. *Hystrix, Italian Journal of Mammalogy*, 22: 311-323.

Shahabi, S., Aliabadian, M., Darvish, J., & Kilpatrick, C. W. 2013. Molecular phylogeny of brush-tailed mice of the genus *Calomyscus* (Rodentia: Calomyscidae) inferred from mitochondrial DNA sequences. *Mammalia*, 77: 425–431.

Steppan, S. J., Adkins, R. M., & Anderson, J. 2004. Phylogeny and divergence date estimates of rapid radiation in muroid rodents based on multiple nuclear genes. *Systematic Biology*, 53: 533–553.

Steppan, S. J., & Schenk, J. J. 2017. Muroid rodent phylogenetics: 900- species tree reveals increasing diversification rates. *PLoS One*, 12: e0183070.

Thomas, O. 1905. On a collection of mammals from Persia and Armenia presented to the British Museum by Col. A. C. *bailwardi*. *Proceeding of the Zoological Society of London*, 2: 521.

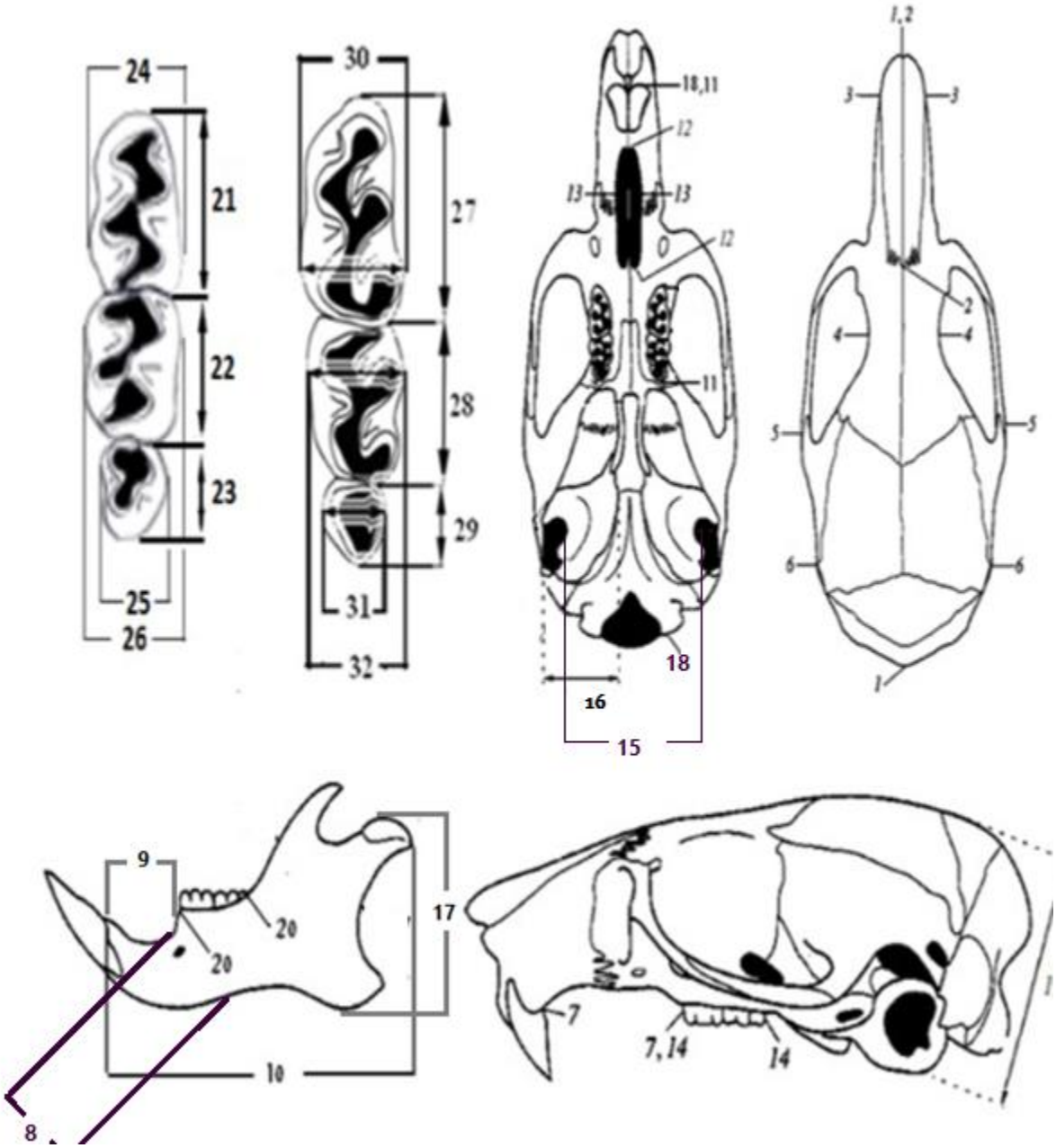
Thomas, O. 1920. Two new species of *Calomyscus*, (part B) scientific results from the mammal survey no. *Journal of the Bombay Natural History Society*, 26, 938–940.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30: 2725–2729.

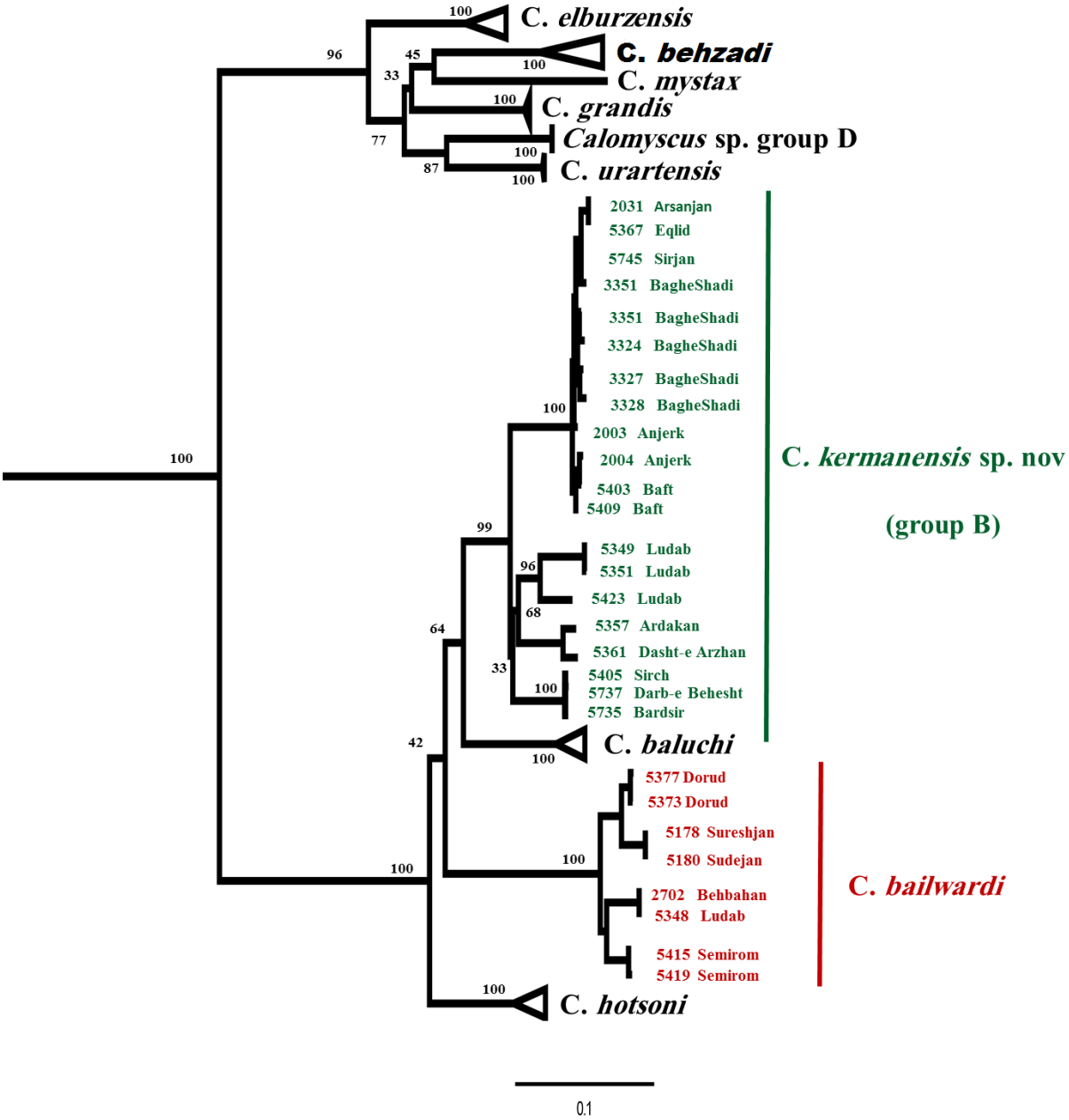
Vorontsov, N. N., Kartavtseva, I. V., Potapova, E. G. 1979. The systematic of mouse-like hamsters from *Calomyscus* (Cricetidae). The karyological differentiation of sibling species from Transcaucasia and Turkmenistan and review of species of *Calomyscus* genus. *Russian Journal of Zoology*, 58: 1213–1224.

Weller, A. 2019. Systematics and ecology of the brush-tailed mice, *Calomyscus*, in and around Pakistan based on the Rbp3 gene. Doctoral dissertation. The Ohio State University. 30p.

Yosida, T. H. 1973. Evolution of karyotypes and differentiation in 13 *Rattus* species. *Chromosoma*, 40: 285–297.



SUPPLEMENTARY FIGURE 1. Thirty two craniodental characters were measured in examined samples of *Calomyscus* (Shahabi et al., 2011): 1- occipitonasal length (Occl); 2- nasal length (NL); 3- nasal width (NW); 4- interorbital width (Intw); 5- zygomatic width (ZW); 6- cranium width (CW); 7- upper diastema length (UDL); 8- height of lower diastema (HLD); 9- length of lower diastema (LLD); 10- mandible length (Mndl); 11- palatal length (Patl); 12- length of anterior palatine foramen (Forl); 13- width of anterior palatine foramen (Forw); 14- maxillary tooth row length (Mxl); 15- distance between two meatus (DB2M); 16- length of tympanic bulla (BULL); 17- maximum mandibular height (MH); 18- condylobasal length (CBL); 19 - maximum cranial height (SH); 20- mandibular tooth row length (Mnl); 21- length of m1 (m1L); 22- length of m2 (m2L); 23- length of m3 (m3L); 24- width of m1 (m1W); 25- width of m2 (m2W); 26- width of m3 (m3W); 27- length of M1 (M1L); 28- length of M2 (M2L); 29- length of M3 (M3L); 30- width of M1 (M1W); 31- width of M2 (M2W); 32- width of M3 (M3W).



SUPPLEMENTARY FIGURE 2. Combined tree resulted from a Maximum Likelihood (ML) analysis on CYTB and CO1 genes. The bootstrap values are indicated with numbers on each node.

Supplemental Table 1: Details of sampled localities, tissue and voucher numbers and accession numbers of specimens examined in this study (for each sample the first accession number is for CYTB and the second is for CO1 genes). The specimens collected in this study are marked in **bold**. The specimens with successful karyotyping are indicated with an asterisk (Voucher no.).

Species	**Locality (city, province)**	**Voucher no.**	**Accession no.**	
			CYTB	CO1
Calomyscus sp. Group D	Saghez, Kurdistan	ZMFUM3869	KT878596	KT878556
Calomyscus sp. Group D	Saghez, Kurdistan	ZMFUM3870	KT878597	KT878557
Calomyscus sp. Group D	Saghez, Kurdistan	ZMFUM3878	KT878600	KT878560
Calomyscus sp. Group D	Saghez, Kurdistan	ZMFUM3881	KT878598	KT878558
Calomyscus sp. Group D	Saghez, Kurdistan	ZMFUM3882	KT878599	KT878559
Calomyscus sp. Group D	Saghez, Kurdistan	ZMFUM3897	KT878601	KT878561
Calomyscus sp. Group C	Songhor, Kermanshah	ZMFUM3871	KT878603	KT878563
Calomyscus sp. Group C	Songhor, Kermanshah	ZMFUM3880	KT878605	KT878565
Calomyscus sp. Group C	Songhor, Kermanshah	ZMFUM3891	KT878604	KT878564
Calomyscus sp. Group C	Songhor, Kermanshah	ZMFUM3896	KT878602	KT878562
C. behzadi	**Ilam, Ilam**	**ZMFUM 5380**	**MW888456**	
C. behzadi	**Ilam, Ilam**	**ZMFUM 5381**	**MW888461**	
C. behzadi	**Ilam, Ilam**	**ZMFUM 5382**	**MW888462**	**MW892625**
C. behzadi	**Ilam, Ilam**	**ZMFUM 5383**	**MW888457**	
C. behzadi	**Ilam, Ilam**	**ZMFUM 5384**	**MW888460**	**MW892623**
C. behzadi	**Ilam, Ilam**	**ZMFUM 5385**	**MW888459**	**MW892624**
C. behzadi	**Ilam, Ilam**	**ZMFUM 5386**	**MW888458**	
C. behzadi	**Ilam, Ilam**	**ZMFUM 5387**	**MW888463**	
C. behzadi	**Ilam, Ilam**	**ZMFUM 5388**		
C. behzadi	**Ilam, Ilam**	**ZMFUM 5379**		
C. grandis	Fasham, Tehran	ZMFUM 3992	KT884559	KT884587
C. grandis	Fasham, Tehran	ZMFUM1985	KT878591	KT878551
C. grandis	Fasham, Tehran	ZMFUM3992	KT878592	KT878552
C. grandis	Fasham, Tehran	ZMFUM1948	KT878593	KT878553
C. grandis	Fasham, Tehran	ZMFUM1943		
C. mystax	Taklah Quz, North Khorasan	ZMFUM 2984	KU129019	KU129021
C. mystax	Taklah Quz, North Khorasan	ZMFUM 3084	KU129020	
C. elburzensis	Mashhad, Khorasan-e-Razavi	ZMFUM1542	KT878581	KT878542
C. elburzensis	Mashhad, Khorasan-e-Razavi	ZMFUM2023		
C. elburzensis	Sarakhs, Khorasan-e-Razavi	ZMFUM1874	KT878585	KT878546
C. elburzensis	Sarakhs, Khorasan-e-Razavi	ZMFUM1922	KT878586	KT878547
C. elburzensis	Torbat, Khorasan-e-Razavi	ZMFUM2088	KT878587	KT878548
C. elburzensis	Saluk, North- Khorasan	ZMFUM2978	KT878588	KU043034
C. elburzensis	Shirvan, North- Khorasan	ZMFUM3533	KT878590	KT878550

C. elburzensis	Chenaran, North- Khorasan	ZMFUM3100		
C. elburzensis	Bijand, Gazik, South Khorasan	ZMFUM 4529	KT884557	KT884586
C. elburzensis	KhajeMor, Khorasan-e-Razavi	ZMFUM 1546	KT884547	KT884576
C. elburzensis	Neyshabur, Khorasan-e-Razavi	ZMFUM 2148	KT884550	KT884580
C. elburzensis	Neyshabur, Khorasan-e-Razavi	ZMFUM 2152	KT884551	KT884581
C. elburzensis	Ghaen, HajiAbad, South Khorasan	ZMFUM 3304	KT884553	KT884583
C. elburzensis	Kurkhud, North Khorasan	ZMFUM 3629	KT884555	KT884584
C. elburzensis	Sabzevar, Khorasan-e-Raza	ZMFUM 4490	KT884556	KT884585
C. elburzensis	Taft, Shirkuh, Godar-Nir, Yazd	ZMFUM 1	KU042999	KU043023
C. elburzensis	Taft, Shirkuh, Godar-Nir, Yazd	ZMFUM4	KU043000	KU043024
C. elburzensis	Taft, Shirkuh, Godar-Nir, Yazd	ZMFUM9	KU043001	KU043025
C. elburzensis	Taft, Shirkuh, Godar-Nir, Yazd	ZMFUM 10	KU043002	KU043026
C. elburzensis	Taft, Shirkuh, Yazd	ZMFUM 2932	KU043004	KU043027
C. elburzensis	Taft, Shirkuh, Cheshme, Yazd	ZMFUM 2948	KU043007	KU043029
C. elburzensis	Taft, Shirkuh, Yazd	ZMFUM 2952	KU043010	KU043030
C. elburzensis	Taft, Shirkuh, Tezerjan, Yazd	ZMFUM 3039	KU043015	KU043036
C. elburzensis	Qeidar, Zanjan	ZMFUM 3925	KU043020	KU043037
C. elburzensis	Qeidar, Zanjan	ZMFUM 3937	KU043021	KU043038
C. elburzensis	Karkas, Isfahan	ZMFUM 3938	KU043022	KU043039
C. elburzensis	Neyshabur, Khorasan-e-Razavi	ZMFUM 2144		
C. elburzensis	Neyshabur, Khorasan-e-Razavi	ZMFUM 2149		
C. elburzensis	Neyshabur, Khorasan-e-Razavi	ZMFUM 2172		
C. elburzensis	Neyshabur, Khorasan-e-Razavi	ZMFUM 2156		
C. elburzensis	Neyshabur, Khorasan-e-Razavi	ZMFUM 2169		
C. elburzensis	Neyshabur, Khorasan-e-Razavi	ZMFUM 2158		
C. elburzensis	Esfarayen, North Khorasan	ZMFUM 3224		
C. elburzensis	Saluk, Bojnurd,	ZMFUM 3085		
C. elburzensis	Bojnord, North Khorasan	ZMFUM 2616		
C. elburzensis	Esfaraien, North Khorasan	ZMFUM 3216		
C. elburzensis	Nishapur, Razavi Khorasan	ZMFUM 2176		
C. elburzensis	Shirkuh , Yazd	ZMFUM 2949		
C. elburzensis	Shirkuh , Yazd	ZMFUM 2968		
C. elburzensis	Shirkuh , Yazd	ZMFUM 2945		
C. elburzensis	Mahriz, Kuhe-Bakhtaki, Yazd	ZMFUM 3088		
C. elburzensis	Shirkuh , Yazd	ZMFUM 3057		
C. elburzensis	**Dehaj, Kerman**	**ZMFUM 5410***	**OP795973**	**OP784805**
C. elburzensis	**Dehaj, Kerman**	**ZMFUM 5411**		
C. elburzensis	**Dehaj, Kerman**	**ZMFUM 5414**		

C. elburzensis	**Dehaj, Kerman**	**ZMFUM 5416**		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM 4391		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM 4392		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM 4393		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM 4386		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM 5012		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM 4404		
C. kermanensis sp. nov	Arsanjan, Fars	ZMFUM 2045		
C. kermanensis sp. nov	Geno, Hormozgan	ZMFUM 26		
C. kermanensis sp. nov	Geno, Hormozgan	ZMFUM 36		
C. kermanensis sp. nov	Geno, Hormozgan	ZMFUM 309		
C. kermanensis sp. nov	Geno, Hormozgan	191929 MVZ	KT878615	
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM 5011		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM 4391		
C. kermanensis sp. nov	BagheShadi, Yazd	ZMFUM 3050		
C. kermanensis sp. nov	BagheShadi, Yazd	ZMFUM 3325		
C. kermanensis sp. nov	BagheShadi, Yazd	ZMFUM 3340		
C. kermanensis sp. nov	Arsanjan, Fars	ZMFUM2029	-	
C. kermanensis sp. nov	Arsanjan, Fars	ZMFUM2030	KT878606	KT878569
C. kermanensis sp. nov	Arsanjan, Fars	ZMFUM2031	KT878607	KT878570
C. kermanensis sp. nov	Arsanjan, Fars	ZMFUM2044		
C. kermanensis sp. nov	Arsanjan, Fars	ZMFUM2046		
C. kermanensis sp. nov	Arsanjan, Fars	ZMFUM2048		
***C. kermanensis* sp. nov**	**Ardakan, Fars**	**ZMFUM 5356**		
***C. kermanensis* sp. nov**	**Ardakan, Fars**	**ZMFUM 5357**	**OP795976**	**OP784808**
***C. kermanensis* sp. nov**	**Ardakan, Fars**	**ZMFUM 5358**		
***C. kermanensis* sp. nov**	**Ardakan, Fars**	**ZMFUM 5359**		
***C. kermanensis* sp. nov**	**Dasht-e Arzhan, Fars**	**ZMFUM 5360**		
***C. kermanensis* sp. nov**	**Dasht-e Arzhan, Fars**	**ZMFUM 5361**	**OP795968**	**OP784800**
***C. kermanensis* sp. nov**	**Dasht-e Arzhan, Fars**	**ZMFUM 5362**		
***C. kermanensis* sp. nov**	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM 5167***		

***C. kermanensis* sp. nov**	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM 5346**		
***C. kermanensis* sp. nov**	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM 5347**		
***C. kermanensis* sp. nov**	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM 5349**	**OP795975**	**OP784807**
***C. kermanensis* sp. nov**	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM 5351**	**OP795970**	**OP784802**
***C. kermanensis* sp. nov**	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM 5352**		
***C. kermanensis* sp. nov**	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM 5353**		
***C. kermanensis* sp. nov**	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM 5355**		
***C. kermanensis* sp. nov**	**Eqlid, Fars**	**ZMFUM 5363**		
***C. kermanensis* sp. nov**	**Eqlid, Fars**	**ZMFUM 5364**		
***C. kermanensis* sp. nov**	**Eqlid, Fars**	**ZMFUM 5365**		
***C. kermanensis* sp. nov**	**Eqlid, Fars**	**ZMFUM 5366**	**OP795972**	**OP784804**
***C. kermanensis* sp. nov**	**Eqlid, Fars**	**ZMFUM 5367**	**OP795977**	**OP784809**
***C. kermanensis* sp. nov**	**Eqlid, Fars**	**ZMFUM 5368**		
***C. kermanensis* sp. nov**	**Eqlid, Fars**	**ZMFUM 5369***		
***C. kermanensis* sp. nov**	**Eqlid, Fars**	**ZMFUM 5370**		
***C. kermanensis* sp. nov**	**Eqlid, Fars**	**ZMFUM 5371**		
***C. kermanensis* sp. nov**	**Sirch, Kerman**	**ZMFUM 5389**		
***C. kermanensis* sp. nov**	**Sirch, Kerman**	**ZMFUM 5390**		
***C. kermanensis* sp. nov**	**Sirch, Kerman**	**ZMFUM 5391**		
***C. kermanensis* sp. nov**	**Sirch, Kerman**	**ZMFUM 5392**		
***C. kermanensis* sp. nov**	**Sirch, Kerman**	**ZMFUM 5394**		
***C. kermanensis* sp. nov**	**Baft, Kerman**	**ZMFUM 5398***		
***C. kermanensis* sp. nov**	**Baft, Kerman**	**ZMFUM 5399**		
***C. kermanensis* sp. nov**	**Baft, Kerman**	**ZMFUM 5400**		
***C. kermanensis* sp. nov**	**Baft, Kerman**	**ZMFUM 5401**		
***C. kermanensis* sp. nov**	**Baft, Kerman**	**ZMFUM 5402***		
***C. kermanensis* sp. nov**	**Baft, Kerman**	**ZMFUM 5403***	**OP795966**	**OP784798**
C. *kermanensis* sp. nov	**Sirch, Kerman**	**ZMFUM 5404**		
***C. kermanensis* sp. nov**	**Sirch, Kerman**	**ZMFUM 5406***		
***C. kermanensis* sp. nov**	**Sirch, Kerman**	**ZMFUM 5407**		
***C. kermanensis* sp. nov**	**Baft, Kerman**	**ZMFUM 5408**		
***C. kermanensis* sp. nov**	**Baft, Kerman**	**ZMFUM 5409**	**OP795965**	**OP784797**

***C. kermanensis* sp. nov**	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM 5423***	**OP795979**	**OP784811**
***C. kermanensis* sp. nov**	**Bardsir, Kerman**	**ZMFUM 5731**		
***C. kermanensis* sp. nov**	**Bardsir, Kerman**	**ZMFUM 5732**		
C. *kermanensis* sp. nov	**Bardsir, Kerman**	**ZMFUM 5733**		
***C. kermanensis* sp. nov**	**Bardsir, Kerman**	**ZMFUM 5734**		
***C. kermanensis* sp. nov**	**Bardsir, Kerman**	**ZMFUM 5735**		
***C. kermanensis* sp. nov**	**Darbebehesht, Kerman**	**ZMFUM 5736**		
***C. kermanensis* sp. nov**	**Darbebehesht, Kerman**	**ZMFUM 5737**	**OP795980**	**OP784812**
***C. kermanensis* sp. nov**	**Darbebehesht, Kerman**	**ZMFUM 5738**		
***C. kermanensis* sp. nov**	**Darbebehesht, Kerman**	**ZMFUM 5739**		
***C. kermanensis* sp. nov**	**Darbebehesht, Kerman**	**ZMFUM 5740**		
***C. kermanensis* sp. nov**	**Sirjan, Kerman**	**ZMFUM 5741**		
***C. kermanensis* sp. nov**	**Sirjan, Kerman**	**ZMFUM 5742**		
***C. kermanensis* sp. nov**	**Sirjan, Kerman**	**ZMFUM 5743**		
***C. kermanensis* sp. nov**	**Sirjan, Kerman**	**ZMFUM 5746**		
***C. kermanensis* sp. nov**	**Sirjan, Kerman**	**ZMFUM 5744***		
***C. kermanensis* sp. nov**	**Sirjan, Kerman**	**ZMFUM 5745***		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM4392		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM4393		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM4386		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM5012		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM4404		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM5011		
C. kermanensis sp. nov	Kakan, Kohgiluyeh and Boyer-Ahmad	ZMFUM 391		
C. kermanensis sp. nov	BagheShadi, Yazd	ZMFUM3324	KT878608	KT878571
C. kermanensis sp. nov	BagheShadi, Yazd	ZMFUM3327	KT878609	KT878572
C. kermanensis sp. nov	BagheShadi, Yazd	ZMFUM3328	KT878610	KT878573
C. kermanensis sp. nov	BagheShadi, Yazd	ZMFUM3333	KT878611	KT878574
C. kermanensis sp. nov	BagheShadi, Yazd	ZMFUM3351	KT878612	KT878575
C. kermanensis sp. nov	BagheShadi, Yazd	ZMFUM3353		
C. kermanensis sp. nov	Anjerk, Kerman	ZMFUM2003	KT878613	KT878566
C. kermanensis sp. nov	Anjerk, Kerman	ZMFUM2004	KT878614	KT878567

***C. kermanensis* sp. nov**	**Eqlid, Fras**	**ZMFUM5370***		
***C. kermanensis* sp. nov**	**Sirch, Kerman**	**ZMFUM5405**	**OP795978**	**OP784810**
C. kermanensis sp. nov	Khabr, Kerman	192039 MVZ	KT878616	
C. kermanensis sp. nov	Sirch, Kerman	191923 MVZ	KT878617	
C. kermanensis sp. nov	Rayen, Kemran	191936 MVZ	KT878618	
C. kermanensis sp. nov	Rayen, Kemran	191938 MVZ	KT878619	
C. baluchi	Pakistan, Sibi Dist	980 (OUT-13)		KT884596
C. baluchi	FATA, North Waziristan, Pakistan		EU135591.1	
C. baluchi	Balochistan, Kalat Dist., Pakistan		EU135586.1	
C. baluchi	Balochistan, Kalat, Pakistan		AY288509.1	
C. baluchi	Ziarat, Pakistan	ZTNH897		KT878579
C. baluchi	Datta Khel, Tore Shore, Pakistan	ZTNH1262		KT878580
C. baluchi	Bamyan, Afghanistan	ZMFUM2798		
C. baluchi	Takhte Waras, Afghanistan	ZMFUM2782		
C. baluchi	Yakawlang, , Afghanistan	ZMFUM2786		
C. baluchi	Bamyan, Afghanistan	ZMFUM2800		
C. baluchi	Yakawlang, , Afghanistan	ZMFUM2787		
C. baluchi	Yakawlang, , Afghanistan	ZMFUM2820		
C. baluchi	Yakawlang, , Afghanistan	ZMFUM2789		
C. baluchi	Surkh Joy, Afghanistan	ZMFUM2813		
C. baluchi	Afghanistan	ZMFUM 2718		
C. baluchi	Afghanistan	ZMFUM 2821		
C. baluchi	Afghanistan	ZMFUM2810		
C. baluchi	Pitab-Joy, Afghanistan	ZMFUM2784		
C.hotsoni	Bashagard, Hormozgan	ZMFUM4739		
C.hotsoni	Khash, Sistan and Baluchestan	ZMFUM 3563		
C.hotsoni	Zahedan, Sistan and Baluchestan	ZMFUM3564		
C.hotsoni	Zahedan, Sistan and Baluchestan	ZMFUM3529	KT884569	
C.hotsoni	Balouchestan, Sistan and Baluchestan	ZMFUM4409		
C.hotsoni	Saravan, Sistan and Baluchestan	ZMFUM3287	KT884565	
C.hotsoni	Bashagard, Sistan and Baluchestan	ZMFUM19		
C.hotsoni	Bashagard, Sistan and Baluchestan	ZMFUM4785		

C.hotsoni	Bashagard, Sistan and Baluchestan	ZMFUM4761		
C.hotsoni	Birjand, South Khorasan	ZMFUM4024		
C.hotsoni	Birjand, South Khorasan	ZMFUM4012		
C.hotsoni	Birjand, South Khorasan	ZMFUM4013		
C. hotsoni	Saravan, Sistan-o-Baluchistan	ZMFUM2068	KT884560	KT878577
C. hotsoni	Saravan, Sistan-o-Baluchistan	ZMFUM2069	KT884561	KT878578
C. hotsoni	Saravan, Sistan-o-Baluchistan	ZMFUM2102	KT884562	KT884588
C. hotsoni	Saravan, Sistan-o-Baluchistan	ZMFUM2103	KT884563	KT884589
C. hotsoni	Saravan, Sistan-o-Baluchistan	ZMFUM3286	KT884564	KT884590
C. hotsoni	Khash, Sistan-o-Baluchistan	ZMFUM3306	KT884567	KT884591
C. hotsoni	Zahedan, Sistan-o-Baluchistan	ZMFUM3962	KT884571	KT884593
C. hotsoni	Bagheran, Bijand, South Khorasan	ZMFUM4013	KT884573	KT884594
C. bailwardi	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM5348***	**OP795982**	**OP784814**
C. bailwardi	**Ludab, Kohgiluyeh and Boyer-Ahmad**	**ZMFUM**	**OP795974**	**OP784806**
C. bailwardi	**Sureshjan, Chaharmahal and Bakhtiari**	**ZMFUM5174**		
C. bailwardi	**Sureshjan, Chaharmahal and Bakhtiari**	**ZMFUM5175**		
C. bailwardi	**Sureshjan, Chaharmahal and Bakhtiari**	**ZMFUM5176**		
C. bailwardi	**Sureshjan, Chaharmahal and Bakhtiari**	**ZMFUM5177**		
C. bailwardi	**Sureshjan, Chaharmahal and Bakhtiari**	**ZMFUM5178***	**OP795967**	**OP784799**
C. bailwardi	**Sureshjan, Chaharmahal and Bakhtiari**	**ZMFUM5179**		
C. bailwardi	**Sudejan, Chaharmahal and Bakhtiari**	**ZMFUM5180**	**OP795981**	**OP784813**
C. bailwardi	**Sudejan, Chaharmahal and Bakhtiari**	**ZMFUM5181**		
C. bailwardi	**Sureshjan, Chaharmahal and Bakhtiari**	**ZMFUM5158**		
C. bailwardi	**Dorud, Lorestan**	**ZMFUM5372**		
C. bailwardi	**Dorud, Lorestan**	**ZMFUM5373**	**OP795983**	**OP784815**
C. bailwardi	**Dorud, Lorestan**	**ZMFUM5374***		
C. bailwardi	**Dorud, Lorestan**	**ZMFUM5375***		
C. bailwardi	**Dorud, Lorestan**	**ZMFUM5376**		

C. bailwardi	**Dorud, Lorestan**	**ZMFUM5377**	**OP795984**	**OP784816**
C. bailwardi	**Dorud, Lorestan**	**ZMFUM5378**		
C. bailwardi	**Semirom, Isfahan**	**ZMFUM5412**		
C. bailwardi	**Semirom, Isfahan**	**ZMFUM5413***		
C. bailwardi	**Semirom, Isfahan**	**ZMFUM5415***	**OP795969**	**OP784801**
C. bailwardi	**Semirom, Isfahan**	**ZMFUM5417**		
C. bailwardi	**Semirom, Isfahan**	**ZMFUM5418**		
C. bailwardi	**Semirom, Isfahan**	**ZMFUM5419**	**OP795971**	**OP784803**
C. bailwardi	**Semirom, Isfahan**	**ZMFUM5420**		
C. bailwardi	**Semirom, Isfahan**	**ZMFUM5421**		
C. bailwardi	Behbahan, Khuzestan	ZMFUM2536		
C. bailwardi	Izeh, Khuzistan	ZMFUM3569	KT878621	
C. bailwardi	Izeh, Khuzistan	ZMFUM3570	KT878622	
C. bailwardi	Izeh, Khuzistan	ZMFUM3571	KT878623	
C. bailwardi	Izeh, Khuzistan	ZMFUM3578	KT878624	
C. bailwardi	Behbahan, Khuzistan	ZMFUM2514		
C. bailwardi	Behbahan, Khuzistan	ZMFUM2516		
C. bailwardi	Behbahan, Khuzistan	ZMFUM2522		
C. bailwardi	Behbahan, Khuzistan	ZMFUM2524		
C. bailwardi	Behbahan, Khuzistan	ZMFUM2529		
C. bailwardi	Behbahan, Khuzistan	ZMFUM2536		
C. bailwardi	Behbahan, Khuzistan	ZMFUM2700		
C. bailwardi	Behbahan, Khuzistan	ZMFUM2701		
C.bailwardi	Behbahan, Khuzistan	ZMFUM2702	KT878620	
C. urartensis	Kordasht, Eastern Azerbaijan	ZMFUM2253	KT878594	KT878554
C. uratensis	Kordasht, Eastern Azerbaijan	ZMFUM2908	KT878595	KT878555

Supplemental Table 2. Means and standard errors of craniodental measurements in *Calomyscus* groups: *C. kermanensis* sp. nov. (Ke), *C. elburzensis* (El), *C. grandis* (Gr), *C. behzadi* (C), *Calomyscus* sp. group D (D), *C. bailwardi* (Bai), *C. hotsoni* (Ho), and *C. baluchi* (BA). Statistically significant differences are marked with a greater or lesser sign (>, <).

Variables	**C. *elburzensis***	**C. *bailwardi***	**C. *kermanensis* sp. nov. (Group B)**	**C. *hotsoni***	**C. *grandis***	**C. *baluchi***	**C. *behzadi***	***Calomyscus* sp. Group D**	**T test and Welch T test result (size + shape)**	**Shape data**
BL	79.29±0.92	83.17±1.13	80.01±0.60	74.78±1.81	80.25±3.52	77.75±2.61	83.25±0.89	79.5±1.84		
TL	89.87±1.09	90.44±1.02	89.21±0.56	84.42±1.62	91.5±2.60	92.08±2.47	92.25±0.45	89.66±1.54		
El	17.54±0.39	19.64±0.26	19.34±0.10	17.5±0.59	13.75±1.75	17.5±0.66	18.08±0.23	18.5±0.43	Ke>El, Ke>C, Ke>Gr, Ke>Ho	
FL	20.16±0.19	21.92±0.26	20.80±0.12	19.07±0.35	20.75±0.48	20.66±0.33	21±0.28	20.83±0.48	Ke<Bai, Ke>Ho	
LLD	3.81±0.04	4.04±0.06	3.80±0.03	3.61±0.03	4.00±0.03	3.90±0.07	3.91±0.08	3.98±0.06	Ke<Gr, Ke>Ho, Ke<Bai	Ke <Bai, Ke<BA
HLD	2.29±0.02	2.37±0.03	2.27±0.01	2.29±0.02	2.37±0.05	2.4±0.02	2.23±0.04	2.38±0.02	BA>Ke, Ke<D, Ke<Bai	Ke<El, Ke<Ho, Ke>C
MH	5.86±0.04	6.08±0.04	5.91±0.03	6.01±0.07	6.12±0.07	6.07±0.03	6.00±0.06	6.30±0.07	BA>Ke, Ke<D, Ke<Bai	Ke<Ho
UDL	6.68±0.03	6.97±0.08	6.72±0.05	6.34±0.08	7.29±0.10	6.97±0.06	6.92±0.10	7.00±0.15	BA>Ke , Gr>Ke, Ke>Ho, Ke<Bai	Ke>Ho, Ke<Gr
NL	9.54±0.09	10.01±0.09	9.35±0.05	9.43±0.17	10.67±0.10	9.76±0.08	9.56±0.10	9.82±0.11	BA>Ke, Gr>Ke, Ke<D, Ke<El, Ke<Bai	Ke<El, Ke <Bai, Ke<Ho, Ke<Gr
ZW	12.35±0.06	12.69±0.10	12.32±0.05	11.96±0.09	12.67±0.09	12.96±0.09	12.61±0.015	12.91±0.13	BA>Ke, Ke>Ho, Ke<C, Ke<D, Ke<Bai	Ke<El, Ke<BA
Forl	4.81♀±0.04	4.89±0.06	4.79±0.03	4.64±0.07	5.31±0.04	5.00±0.05	4.71±0.09	4.79±0.09	BA>Ke, Gr>Ke	Ke<Gr, Ke>C
NW	2.92±0.03	3.09±0.03	2.89±0.03	2.87±0.06	3.41±0.06	3.44±0.07	3.12±0.08	3.57±0.07	BA>Ke, Gr>Ke, Ke<C, Ke<D,	Ke<El, Ke <Bai, Ke>Gr, Ke<BA,

									Ke<Bai	Ke<C, Ke<D
Patl	11.58±0.04	11.86±0.12	11.62±0.05	11.26±0.12	12.09±0.12	12.04±0.05	11.93±0.12	12.02±0.14	BA>Ke, Gr>Ke, Ke>Ho, Ke<C, Ke<D	Ke<El, Ke<BA, Ke<C
SH	7.89±0.04	8.07±0.04	8.15±0.02	7.76±0.07	7.76±0.12	7.91±0.05	8.02±0.07	7.95±0.11	BA<Ke, Gr<Ke, Ke>Ho, Ke>C, Ke>D, Ke>El	Ke>El, Ke >Bai, Ke>Ho, Ke>Gr, Ke>BA, Ke>C, Ke>D
Occl	25.05±0.10	26.31±0.22	25.55±0.10	24.80±0.21	26.01±0.23	25.98±0.07	25.98±0.19	26.25±0.29	BA>Ke, Ke>Ho, Ke>El, Ke<Bai	Ke>El, Ke>Gr, Ke>BA
CW	11.59±0.04	11.82±0.07	11.62±0.03	11.24±0.08	11.80±0.09	12.25±0.08	11.60±0.06	12.06±0.08	BA>Ke, Ke>Ho, Ke<D, Ke<Bai	Ke<BA, Ke>C
INTW	4.0±0.01	4.34±0.03	4.20±0.01	4.05±0.01	4.15±0.08	4.25±0.03	4.28±0.02	4.44±0.06	Ke>Ho, Ke<C, Ke<D, Ke>El, Ke<Bai	Ke>El, Ke>Gr
BULL	5.70±0.04	5.88±0.05	5.85±0.03	5.65±0.05	6.00±0.0	5.71±0.07	5.75±0.05	6.04±0.06	Gr>Ke, Ke>Ho, Ke>El	Ke >Bai, Ke>BA, Ke>C
CBL	22.09±0.10	23.09±0.17	22.55±0.09	21.69±0.18	23.24±0.17	23.22±0.08	22.91±0.12	23.45±0.24	BA>Ke, Ke>Ho, Ke<C, Ke<D, Ke>El, Ke<Bai	Ke<El, Ke>Ho
DB2M	8.10±0.06	8.26±0.06	8.20±0.04	7.99±0.08	8.89±0.11	8.81±0.04	8.16±0.06	8.41±0.14	BA>Ke, Gr>Ke	Ke >Bai, Ke<Gr, Ke<BA
Mxl	3.40±0.02	3.42±±0.03	3.40±0.01	3.41±0.03	3.32±0.04	3.51±0.04	3.50±0.04	3.45±0.07	BA>Ke, Ke<C	Ke>Ho, Ke>D
Mnl	3.37±0.02	3.33±0.03	3.34±0.01	3.37±0.04	3.33±04	3.47±0.06	3.47±0.02	3.54±0.04	BA>Ke, Ke<C, Ke<D	Ke<C, Ke<D
Mndl	12.92±0.06	13.42±0.09	13.10±0.05	12.61±0.08	13.51±0.14	13.43±0.04	13.50±0.05	13.70±0.08	BA>Ke, Ke>Ho, Ke<C , Ke<D, Ke>El, Ke<Bai	Ke<C
M1L	1.68±0.01	1.67±0.02	1.68±0.01	1.69±0.02	1.61±0.03	1.76±0.01	1.69±0.01	1.62±0.02	BA>Ke, Gr<Ke, Ke>D	Ke>Gr, Ke>C, Ke>D

M1W	1.13±0.01	1.11±0.01	1.11±0.01	1.15±0.01	1.13±0.01	1.19±0.01	1.11±0.01	1.10±0.01	BA>Ke, Ke<Ho	Ke<Ho, Ke<BA, Ke>C, Ke>D
M2L	1.27±0.01	1.23±0.01	1.22±0.01	1.24±0.02	1.27±0.02	1.32±0.02	1.28±0.02	1.27±0.04	BA>Ke, Ke<C, Ke<D, Ke<El	Ke<El, Ke<Gr, Ke<BA
M2W	1.07±0.01	1.07±0.01	1.06±0.01	1.09±0.01	1.12±0.01	1.15±0.01	1.09±0.01	1.12±0.01	BA>Ke, Gr>Ke, Ke<Ho, Ke<C, Ke<D	Ke<Ho, Ke<Gr, Ke<BA
M3L	0.62±0.01	0.64±0.01	0.64±0.01	0.64±0.01	0.61±0.03	0.66±0.01	0.66±0.02	0.71±0.02	Ke<D	
M3W	0.72±0.01	0.72±0.01	0.73±0.01	0.72±0.02	0.74±0.01	0.75±0.01	0.77±0.01	0.79±0.02	Ke<C, Ke<D	
m1l	1.44±0.01	1.46±0.02	1.46±0.01	1.49±0.02	1.47±0.03	1.48±0.01	1.49±0.02	1.50±0.02		Ke>El, Ke>BA,
m1W	0.96±0.01	1.00±0.01	1.00±001	1.01±0.01	0.97±0.01	1.01±0.01	0.99±0.01	1.00±0.01	Gr<Ke, Ke>El	Ke<El, Ke>BA, Ke>C
m2l	1.25±0.01	1.25±0.01	1.23±0.01	1.27±0.02	1.24±0.02	1.28±0.01	1.25±0.00	1.30±0.02	BA>Ke, Ke<Ho, Ke<C, Ke<D, Ke<El	Ke<El, Ke<Ho, Ke>C
m2W	1.05±0.01	1.10±0.01	1.06±0.00	1.08±0.01	1.06±0.01	1.11±0.01	1.07±0.01	1.06±0.02	BA>Ke, Ke<Bai	Ke>El, Ke <Bai, Ke>D
m3L	0.79±0.01	0.79±0.01	0.77±0.01	0.79±0.01	0.72±0.02	0.76±0.02	0.82±0.01	0.86±0.01	Gr<Ke, Ke<C, Ke<D	Ke>Gr, Ke>BA, Ke<C, Ke<D
m3w	0.71±0.01	0.72±0.01	0.71±0.00	0.71±0.01	0.70±0.02	0.74±0.01	0.72±0.02	0.75±0.01	BA>Ke, Ke<D	
dental size	6.14±0.029	6.13±0.045	6.11±0.020	6.17±0.059	6.06±0.063	6.35±0.045	6.28±0.048	6.3±0.080	C>Ke, BA>Ke, D>Ke	
Skull size	45.86±0.16	47.72±0.33	46.48±0.16	45.03±0.33	48±0.32	47.83±0.16	47.22±0.28	48.13±0.42	Ke>El, Ke<Bai, Ke>Ho, Ke<Gr, BA>Ke, Ke<D	