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Ecological survey of two Calomyscidae species; Goodwin's brush-tailed mouse and Hotson's brush-tailed mouse (Rodentia) in the eastern parts of Iran

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ABSTRACT

Calomyscus elburzensis and *C. hotsoni* are two species of the rodents family Calomyscidae which are distributed mainly in Iran. Herein, we evaluated the habitat and ecological differences of these two brush-tailed mice in order to test the credibility of a hypothesis stating that species from habitats with different climates and vegetation show greater intraspecific differentiation than those from areas with more similar climates and vegetation. This study was carried out in four rocky regions in Iran between 2013 and 2015. Totally 52 brush-tailed mice were captured from Kopet-Dag, Khaje-Morad, Ark, and Shadan and Olang during the field studies. Maximum parsimony analysis inferred from mitochondrial DNA sequences (*Cytb*) was used for species identification, and also comparison of mean Kimura 2-paramater distances was performed. According to the molecular studies, specimens from the first two regions were assigned to *C. elburzensis* and samples from Ark, and Shadan and Olang belonged to *C. hotsoni*. The mean distances within all examined Iranian samples of both *C. elburzensis* and *C. hotsoni* were 2.3% and 0.9%, respectively. Based on our field studies, *C. elburzensis* were captured either from "cold mountainous" climate zone with *Juniperus excelsa* as main vegetation cover or from "Mediterranean" in which *Pistacia atlantica* is predominant vegetation. *C. hotsoni* were found in "hot dry desert" and "cold semi-desert" regions both characterized by *Ephedra* sp. and *Avena sativa* vegetation cover. Dog rose seeds were the main food of *C. elburzensis* in Kopet-Dag, whereas brush-tailed mice in the other three regions fed mainly on Mount Atlas pistache fruits. *C. elburzensis* inhabited concealed rock crevices, but *C. hotsoni* were found living in burrows which were digged in the soft soils. Individuals of *C. hotsoni* showed more calm behavior as compared with *C. elburzensis*. *Meriones persicus* and *Cricetulus migratorius* (Rodentia) as well as *Ochotona rufescence* (Lagomorpha) were the most abundant small mammals encountered with *C. elburzensis*, while *M. persicus* was the most successful rodent with sympatric colonies with *C. hotsoni*. In conclusion, in accordance with the greater genetic distances observed in *C. elburzensis* populations as compared with *C. hotsoni*, more significant differences in the habitat structure were also found for the first species in different parts of its distribution range.

1. Introduction

Brush-tailed mice are a family of small rodents found in rocky outcrops and semimountainous areas in desert regions of Iran, Turkmenistan, Afghanistan, Pakistan, Azerbaijan, and Syria. Although they were traditionally known as "mouse-like hamsters", they are not true hamsters [1]. They represent an early divergence from the rest of the mouse-like rodents (such as mice and rats), thus brush-tailed mice have been placed in a separate family, Calomyscidae Vorontsov and Potapova, 1979 [2–5].

Calomyscidae consists of eight known species which all belong to a single genus, *Calomyscus* Thomas, 1905 [2]. Despite several studies on morphological [2,6–13], biological [14], molecular [1,15–21] and karyologic [22–28] traits of this genus, relatively little is known about habitat and ecology of any species of *Calomyscus* [29–39]. The favorite habitat of *Calomyscus* species is considered as well-drained rocky slopes in arid steppes, semi-deserts and desert regions [30,34]. There is a record of this rodent nesting in a narrow horizontal crevice in rock strata in Iran with storing fine grasses and sheep wools [29]. In few literatures there are descriptive notes on vegetation covers in the habitats of most of

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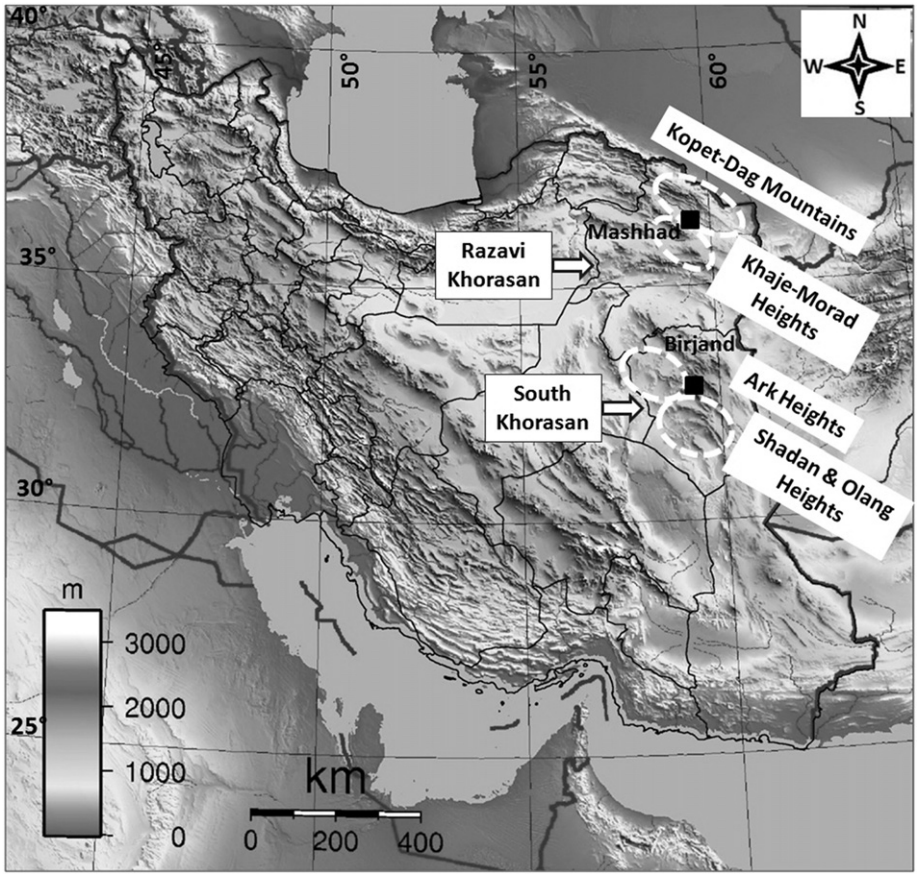

Fig. 1. Study areas and sampling localities. "Kopet-Dag Mountains" and "Khaje-Morad" heights are located in Razavi Khorasan province. "Ark" and "Shadan and Olang" heights are situated in South Khorasan province (reproduced from iranpoliticsclub.net/maps/maps14).

Calomyscus species which consist of scant vegetation of grass clumps, nettle weed, legumes, thistle, and wild rose bushes [29,33,37]. They feed mainly on seeds, leaves, buds and flowers of wild plants and are used to storing their food for consuming in cold months [31]. They also willingly eat animal matter [36] and are cannibalistic in captivity [35].

Three species of the genus *Calomyscus* are distributed in the eastern parts of Iran including Afghan mouse-like hamster (*C. mystax* Kashkarov, 1925), Goodwin's brush-tailed mouse (*C. elburzensis* Goodwin, 1938) and Hotson's brush-tailed mouse (*C. hotsoni* Thomas, 1920). However, the distribution ranges of these species are not completely known [2]. *C. elburzensis* has been reported from mountains of north and northeast of Iran [10], eastern parts of Yazd province (Iran) [28], southwest and south of Turkmenistan and also northwest of Afghanistan [2]. This species is found in barren, dry and rocky mountain sides with little vegetation. It nests in concealed rock crevices, and feeds on brome grasses (Poaceae; *Bromus* Scop.) [40]. *C. hotsoni* has been recorded from southeastern parts of Iran and southwestern regions of Pakistan [1,2]. Recently we reported this species from South Khorasan province, in the northeast of Iran (e.g. [21]). This species is found in arid rocky habitats in association with dwarf mazari palm (Arecaceae; *Nannorrhops ritchieana* (Griff.) Aitch.) and dry rocky mountain tops with sparse shrubby vegetation [1].

In our recent molecular study (analysis of *Cytb* and *COI* gene sequences) [19], one major clade for *C. elburzensis* in the northeast of Iran was considered to consist of four subclades as follows: 1) a subclade which includes samples from the middle of the Kopet-Dag Mountains in Razavi Khorasan province (Tandure, Chenaran), 2) a subclade which contains those samples captured from the type locality (Kurkhud; North Khorasan province) and the central Kopet-Dag (Saluk and Dasht; North Khorasan province, and a single individual from Chenaran; Razavi Khorasan province) or nearby (Sabzevar), 3) a subclade which includes samples from Khaje-Morad, Binalud Mountains (Neyshabur), eastern Kopet-Dag (Sarakhs), southern parts of Razavi Khorasan province (Bajestan, Torbat) and northeast of South Khorasan province (Ghaen, Darmian), and finally 4) a subclade consisting of one individual from Gazik (South Khorasan province) and samples from central Iran (Taft, Fakhrabad; Yazd province). Moreover, in this study the mean genetic distances within samples of *C. elburzensis* from Iran were recorded as 1.9% for *Cytb* and 1.2% for *COI*. Comparative genetic distances (for both *Cytb* and *COI* genes) between different sets of subclades in *C. elburzensis* populations showed that genetic differences increased linearly with geographic distances [19]. Furthermore, based on our phylogenetic trees obtained from *Cytb* and *COI* data set [21], only two subclades of *C. hotsoni* were identified in Iran: 1) a subclade including samples from Saravan (the southernmost known locality for *C. hotsoni* in Iran), and 2) a subclade containing samples from Birjand, Zahedan, and Khash (in Iran) and also samples of a site near the type locality (in Pakistan). These two molecular subclades along with a subclade containing samples from the vicinity of the type locality and also its western parts in southern Pakistan, exhibited low mean genetic divergence (1.8% for *Cytb*) [21].

Herein, we aimed to study the climatological, geological and ecological characteristics of *C. elburzensis* and *C. hotsoni* in details. We hypothesize that differences observed in the habitat and ecological characteristics within the distribution range of these two species are in accordance with divergence at intraspecies level and the topology of molecular trees. In other words, we assume that species which are distributed in areas with different climates and vegetation might show greater intraspecific differentiation than species which inhabit areas with more similar climates and vegetation. Thus, integration of ecological surveys with molecular studies may bring about novel findings.

2. Materials and methods

2.1. Study area, field work and trapping method

This study was carried out in four distinct regions (Fig. 1) including: 1) Kopet-Dag Mountains, Razavi Khorasan province, northeastern Iran and near the Iran-Turkmenistan frontier (36°45′–37°10′N and 59°05′–59°50′E) at an average elevation of approximately 2251 m. The topography of this high plain is characterized by high mounds and rocky habitats. 2) Khaje-Morad heights, approximately 12 km southcenter of Mashhad, Razavi Khorasan province (36°08′–37°03′N and 59°13′-59°42′E, 1146 m in average). The topography of this area is generally flat with some mounds, and barren rocky habitats in the foothills. Some lands in this region were under cultivation but patches of trees remain on the steepest foothills. 3) Ark heights in the northwest of Birjand, South Khorasan province and near the central Iranian desert (33°01′–33°08′N and 58°45′–58°39′E, 2421 m in average), and 4) Shadan and Olang heights, in the southcenter of Birjand (32°14′–32°21′N and 58°93′–59°29′E, 2526 m in average). The southern parts of the South Khorasan province primarily consist of deserts and alluvial plains with small mounds.

Trapping was performed between January 2013 and August 2015. Custom-made mesh live traps (designed for single captures), measuring 25 × 9 × 9 cm, baited with mainly scorched sunflower and gourd seeds were used. Traps were set in the late afternoons and checked the following mornings. The precise geographical position and elevation as well as climatic conditions, geological and ecological characteristics (such as habitat and soil type, vegetation cover and food habits) of each sampling locality were recorded. Data on burrow and food habits of the target rodents were also collected. Sampled plants were transferred to the lab and identified based on Mozaffarian [41] and the field guide of Eskandari [42]. Captured brush-tailed mice were transferred to the animal house and kept a while for further investigations (such as behavioral study in captivity). Finally, their identification was carried out by molecular studies. Animal care and experimental procedures were performed in compliance with the "Guideline for the care and use of laboratory and experimental animals, Rodentology Research Group, Ferdowsi University of Mashhad" [43].

2.2. Molecular identification

Genomic DNA was isolated from fresh or 96% ethanol-preserved muscle tissue samples by an overnight incubation at 45 °C in a lysis buffer and proteinase K, using a standard salt extraction protocol after Bruford et al. [44]. Slightly modified universal primers L7 (5′-ACTAATGACATGAAAAATCATCGTT-3′) and H6 (5′-TCTTCATTTTTGGTTTACAAGAC-3′) [45] were used for the amplification of the complete mitochondrial cytochrome *b* (*Cytb*) gene via polymerase chain reaction (PCR) [1]. To confirm the success of the amplification, PCR products were separated on a 1.2% agarose gel. Due to presence of some non-specific bands, gel recovery kit (DENA Zist Asia, Iran) was used for recovery of nucleic acids from agarose gel according to manufacturer's instruction. After sequencing of purified DNA samples at Macrogen Company (South Korea), nucleotide sequences were aligned and edited manually using BioEdit7 [46], and checked for internal gaps or stop codons using MEGA7 program [47]. Maximum parsimony (MP) tree was generated in PAUP4.0b10 [48] with a heuristic search algorithm. The robustness of the nodes was evaluated with 5000 random addition sequence replicates. The bootstrap values ≥70% were considered as "significant" for each branch in MP tree. Mean genetic distances based on the Kimura 2-parameter (K2P) [49] were calculated within and among species with 10,000 bootstrap using MEGA7.

Totally, twenty nine sequences representing five *Calomyscus* species were analyzed for variations in the *Cytb* gene. All specimens from Iran are deposited in Zoology Museum of Ferdowsi University of Mashhad (ZMFUM). GenBank accession numbers of reference sequences and also the sequences obtained in the present study are given in Tables 1 and 2, respectively. Two sequences related to the Middle East blind mole-rat, *Spalax ehrenbergi* (Nehring, 1898) (Spalacinae), and hoary bamboo rat, *Rhizomys pruinosus* Blyth, 1851 (Rhizomyinae) were used as outgroups. Spalacinae together with Rhizomyinae have been determined as a basal clade of Muroidea in several phylogenetic analyses (e.g. [3–5]).

Table 1
Localitites and GenBank accession numbers of twenty nine *Cytb* sequences for five *Calomyscus* species and two for *Rhizomys pruinosus* and *Spalax ehrenbergi* (as outgroups) used in the present study.

Species	Locality	*Cytb* accession no.
C. cf. *bailwardi*	Bagh-e-Shadi, Yazd, Iran	KT878608
C. cf. *bailwardi*	Bagh-e-Shadi, Yazd, Iran	KT878609
C. bailwardi	Izeh, Khuzistan, Iran	KT878621
C. bailwardi	Izeh, Khuzistan, Iran	KT878622
C. bailwardi	Izeh, Khuzistan, Iran	KT878624
C. baluchi	Kalat District, Balochistan, Pakistan	EU135586.1
C. baluchi	Datta Khel, North Waziristan, FATA, Pakistan	EU135591.1
C. elburzensis	Khaje-Morad Mts., Mashhad, Razavi Khorasan, Iran	KT878581
C. elburzensis	Kurkhud, North Khorasan, Iran	KT878590
C. elburzensis	Dargaz, Razavi Khorasan, Iran	KT884548
C. elburzensis	Aghdarband, Razavi Khorasan, Iran	KT884549
C. elburzensis	Kurkhud, North Khorasan, Iran	KT884555
C. elburzensis	Sabzevar, Razavi Khorasan, Iran	KT884556
C. elburzensis	Gazik, South Khorasan, Iran	KT884557
C. elburzensis	Darmian, South Khorasan, Iran	KT884558
C. elburzensis	Taft, Yazd, Iran	KU043007
C. elburzensis	Taft, Yazd, Iran	KU043013
C. elburzensis	Taft, Yazd, Iran	KU043014
C. elburzensis	Mehriz, Yazd, Iran	KU043017
C. elburzensis	Qeidar, Zanjan, Iran	KU043020
C. elburzensis	Qeidar, Zanjan, Iran	KU043021
C. elburzensis	Karkas, Isfahan, Iran	KU043022
C. hotsoni	Saravan, Sistan-o-Baluchistan, Iran	KT884564
C. hotsoni	Saravan, Sistan-o-Baluchistan, Iran	KT884565
C. hotsoni	Khash, Sistan-o-Baluchistan, Iran	KT884567
C. hotsoni	Malek Siahkooh, Sistan-o-Baluchistan, Iran	KT884571
C. hotsoni	Bagheran, South Khorasan, Iran	KT884572
C. mystax	Bojnord, North Khorasan, Iran	KU129019
C. mystax	Turkmenistan	KU745283
Rhizomys pruinosus	south of China	KC789518.1
Spalax ehrenbergi	Diyarbakir, Turkey	AJ311138.1

3. Results

Totally 52 brush-tailed mice were captured from Kopet-Dag (2), Khaje-Morad (45), Ark (3), and Shadan and Olang (2) during the field studies. It is worth noting that based on our previous studies samples from Khaje-Morad were known as *C. elburzensis*, according to their cranial and dental characters [12,50], and also molecular analysis of *COI* gene [17]. Hence, they were excluded from molecular identification. These samples were used for parallel developmental studies (unpublished data). After editing sequences and checking for the presence of

Table 2
Localitites, voucher and GenBank accession numbers of four *Cytb* sequences for four brush-tailed mice captured from eastern parts of Iran during this study (ZMFUM: Zoology Museum of Ferdowsi University of Mashhad).

Locality	Voucher no. (ZMFUM)	*Cytb* accession no.
Kopet-Dag Mts., Kalat, Razavi Khorasan, Iran	ZMFUM5014	KY039480
Ark, Ark heights, South Khorasan, Iran	ZMFUM5024	KY039481
Tejg, Ark heights, South Khorasan, Iran	ZMFUM5025	KY039482
Hamech, Shadan and Olang heights, South Khorasan, Iran	ZMFUM5026	KY039483

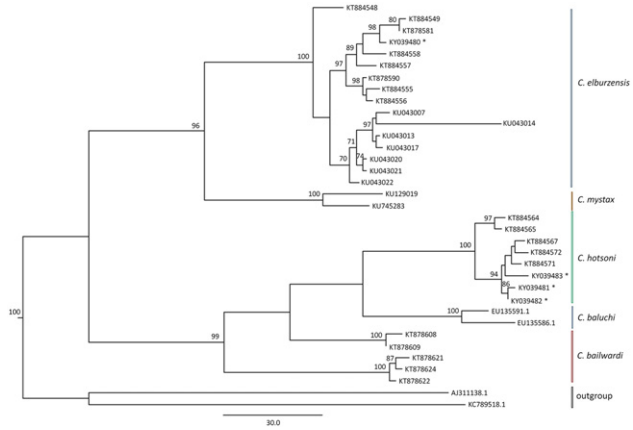

Fig. 2. Phylogenetic tree resulted from Maximum Parsimony (MP) analysis of *Cytb* gene for five *Calomyscus* species rooted with sequences of *Rhizomys pruinosus* and *Spalax ehrenbergi*. Numbers above branches represent bootstrap values (only those ≥70% which is the level of significance are represented). Samples related to the present study are indicated with *.

gaps or stop codons, a total of 1043 bps of *Cytb* gene were used for phylogenetic analyses. Totally, 602 characters were constant among the examined sequences, 299 were parsimony-informative characters, and 142 variable characters were parsimony-uninformative. Based on the maximum parsimony analysis (Fig. 2), the specimens from Kopet-Dag were identified as *C. elburzensis* with a bootstrap value of 100%. The specimens from South Khorasan (both Ark, and Shadan and Olang heights) were identified as *C. hotsoni* according to the molecular data. The whole clade has a bootstrap value of 100% in MP tree (Fig. 2).

The genetic distance (K2P) between our captured specimen from Kopet-Dag and studied samples of *C. elburzensis* was 2.1%. Furthermore, low mean genetic distance (0.8%) was observed between our three samples from South Khorasan and those examined of *C. hotsoni*. Kimura 2-parameter distances among all studied *Calomyscus* species are given in Table 3. These values ranged from 0.9% (*C. hotsoni*)-6.5% (*C. bailwardi*) within species and 8.7% (*C. hotsoni* with *C. baluchi*)-16.6% (*C. bailwardi* with *C. mystax*) between species. The mean genetic distances within all studied samples of *C. elburzensis* and *C. hotsoni* were 2.3% and 0.9%, respectively. The mean K2P distances between *C. elburzensis* with other species was 14.5%. This value was 12.4% for *C. hotsoni*.

Ecological characteristics, habitat details, food habits and small notes on the behavior of captured specimens were also recorded as follows:

***C. elburzensis* captured from Iran-Turkmenistan frontier** - The climatic condition of Kopet-Dag region is mainly "Mediterranean with spring rains" and in some parts has "cold mountainous" climate. During 2013 to 2015, the maximum and minimum annual temperatures were between +19 °C and −7 °C in summer and winter, respectively. The average annual precipitation was about 272 mm and the average annual relative humidity was 34% [51]. The study area was part of "Kopet-Dag" structural zone, which mainly consists of sedimentary rocks and lime formation. Animals were observed to live in the areas having firm soil (due to the great content of lime and carbonate) [52] and they inhabited crevices and natural voids among the rocks in this region.

The typical vegetation on Kopet-Dag region was identified as Greek juniper (Cupressaceae; *Juniperus excelsa* M. Bieb.), dog rose (Rosaceae; *Rosa canina* L.) and berberry shrubs (Berberidaceae; *Berberis integerrima* Bunge). There was also massy cover of *Acanthophyllum glandulosum* Bunge ex Boiss. (Caryophyllaceae), blessed milk thistle (Asteraceae; *Silybum marianum* (L.) Gaertn.), harmel peganum (Zygophyllaceae; *Peganum harmala* L.), and sparse cover of yellow mingnonette (Resedaceae; *Reseda lutea* L.), great mullein (Scrophulariaceae; *Verbascum thapsus* L.) and *Aegopordon* sp. (Asteraceae) (Fig. 3). Individuals of *C. elburzensis* likely consumed *R. canina* and *B. integerrima* flowers and seeds as their main food based on the traces of these materials at the opening (mouth) of the burrows.

Brush-tailed mice in this region seemed to live solitary and rarely found in large colonies. They rarely showed pugnacious tendencies when kept in captivity. However, due to our inadequate sample size, precise statement about their behavior is not possible. *Ochotona rufescence* (Lagomorpha) was the most abundant small mammal encountered with *C. elburzensis* in this region, however other small mammals such as *Meriones persicus* and *Cricetulus migratorius* or their traces (e.g. feces) were also observed during field expeditions.

***C. elburzensis* captured from southcenter of Mashhad** - Khaje-Morad region is located in "Mediterranean with spring rains" climatic zone. During the study period, the maximum and minimum annual temperatures were between +35 °C and −3 °C in summer and winter, respectively. Approximately, 237 mm was recorded as an average annual precipitation and the average annual relative humidity was 25% [51]. The study area was part of "Elburz" structural zone, which mainly consists of acidic plutonic igneous rocks with high amounts of silica. In this region as in Kopet-Dag, the soil type is so firm (due to the clay and silica contents in the soil structure) [52] which does not allow Goodwin's brush-tailed mice to build burrows, so this might be a reason which confines this small rodent to use only rock clifts and natural crevices.

The typical vegetation on this site was mostly Mount Atlas pistache (Anacardiaceae; *Pistacia atlantica* Desf.), shrubby horsetail (Ephedraceae; *Ephedra* sp.) and sun spurge (Euphorbiaceae; *Euphorbia helioscopia* L.). There were also a massy cover of *S. marianum* and *P. harmala*, and a sparse cover of plum trees species *Prunus domestica* L. (Rosaceae), *A. glandulosum* and *V. thapsus* (Fig. 4). These rodents fed mainly on *P. atlantica* fruits, which their remains were found near and/or in the entrance of their burrows.

Goodwin's mice of this area rarely showed aggressive behavior when encountered with their congenerics in captivity. They were social rodents (but not highly) and could be housed in a cage with other individuals of the same species easily. Female *C. elburzensis* might become aggressive toward the males if kept together for too long after mating. In some rare cases, male was dead after being attacked by the female (intersexual aggression) (unpublished data). Cannibalism was rarely observed among these rodents. In the study area, other rodents species such as *M. persicus, C. migratorius*, *Mus musculus* and also *O. rufescence*, were encountered.

Table 3
Between groups mean Kimura 2-parameter distance matrix for studied *Cytb* sequences of *Calomyscus* species. Bold numbers on the diagonal indicate the mean distances within each species.

	1	2	3	4	5
C. elburzensis (1)	**0.023**				
C. mystax (2)	0.104	**0.032**			
C. hotsoni (3)	0.159	0.151	**0.009**		
C. bailwardi (4)	0.163	0.166	0.102	**0.065**	
C. baluchi (5)	0.154	0.153	0.087	0.100	**0.025**

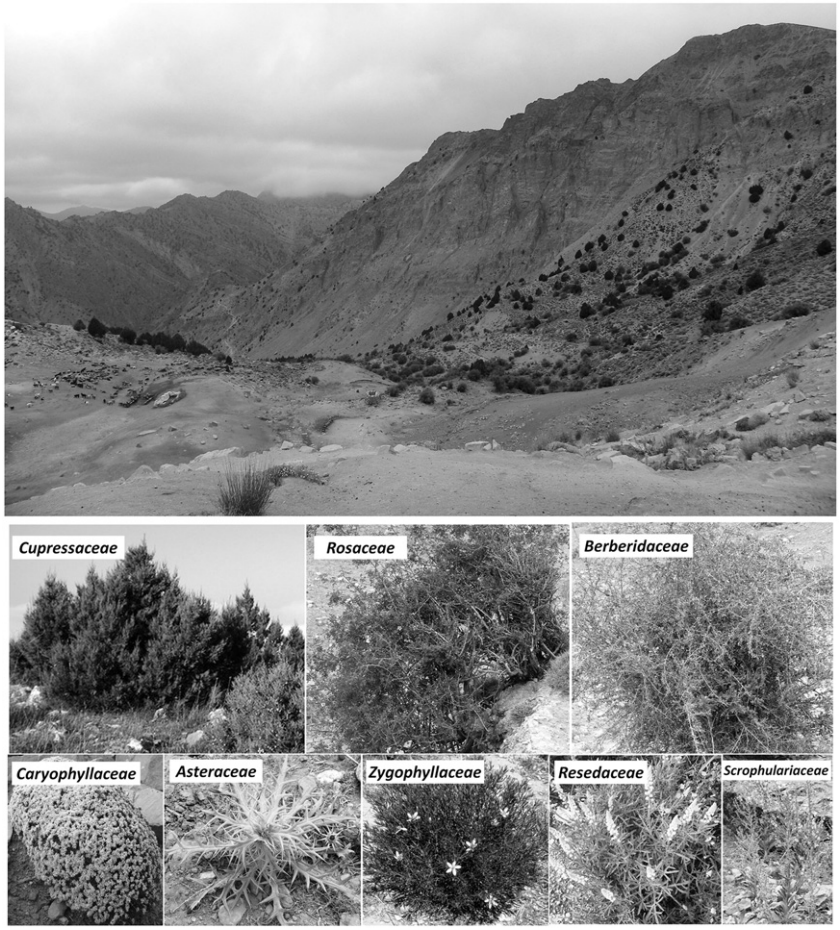

Fig. 3. Habitat and vegetation cover of *Calomyscus elburzensis* in Kopet-Dag Mountain, Razavi Khorasan province, near Iran-Turkmenistan border.

***C. hotsoni* captured from northwest of Birjand** - Ark heights are located in "hot dry desert" climatic zone and hence have completely dry climatic conditions. During field expedition, the annual maximum and minimum temperatures were between +41 °C (summer) and +4 °C (winter), respectively. The average annual precipitation and relative humidity were about 107 mm and 9% [51]. The study area was part of "eastern Iran belt" and "eastern Iran magmatic assemblage" zones, which mainly consist of igneous and magmatic rocks. Animals were observed to live in the areas having very soft soil (due to the great clay content) [52] which allow them to dig and make burrows.

The typical vegetation on this locality was mostly common fig (Moraceae; *Ficus carica* L.), bushes of *Ephedra* sp. and also common oat (Poaceae; *Avena sativa* L.). There were massy cover of *P. atlantica* and *S. marianum*, and sparse cover of *P. harmala* and other Asteraceae members (Fig. 5). Hotson's mice fed mainly on *F. carica* seeds and *P. atlantica* fruits.

They had more calm behavior in comparison with *C. elburzensis* and seemed to be highly social rodents similar to *C. bailwardi*. These rodents did not show any aggression toward their congenerics in captivity. In this area, *M. persicus* was the most successful rodent with sympatric colonies with *C. hotsoni*. During our study, in some areas in which the soil type was firmer, Hotson's brush tailed mice were found in burrows with traces (e.g. feces) of Persian jird. It seemed that *C. hotsoni* uses the old and empty burrows of jirds.

***C. hotsoni* captured from southcenter of Birjand** - The climatic condition of Shadan and Olang areas is relatively dry due to their location in "cold semi-desert" climatic zone. Temperatures of +36 °C in summer and +2 °C in winter were recorded as the annual maximum and minimum temperatures, respectively. The average annual precipitation was about 134 mm and value of 13% was recorded as the average annual relative humidity [51]. The study area was mainly part of "eastern Iran belt" zone. Similar to Ark heights, these regions mainly consist of igneous rocks [52] and animals were also found living in burrows in the areas having great clay content and soft soil.

The typical vegetation on this site was very similar to Ark region. *Ephedra* sp. and *A. sativa* were intensely dispersed in most of this area, also massy cover of *P. atlantica* and very sparse cover of *P. harmala* could be found (Fig. 6). Brush-tailed mice living in this area had deposited large amounts of *P. atlantica* seeds in their burrows as their food materials. These Hotson's mice had gentel behavior similar to those individuals which were captured from Ark heights. Similarly, they made considerable successful sympatric colonies with *M. persicus*.

4. Discussion

Brush-tailed mice genus *Calomyscus* are small rodents with the Near East and Middle Asia distribution which favor mountainous steppe regions between 400 and 3500 m, and are typically absent from low valleys. They are both granivorous (mainly eat seeds) and herbivorous (eating seeds, grasses, flowers, and leaves) [2,35,37,39]. Geographically isolated populations of this genus occupy rocky habitats in foothills and mountains [2]. There are two views regarding contingency of *Calomyscus* populations: 1) according to Lay [29] habitat availability allows brush-tailed mice to exist throughout Iran. 2) Graphodatsky and colleagues [23] believe that populations of this genus are distributed in a patchy, mosaic pattern. Patch-like distribution of the populations promotes the effects of random genetic drift and possibly, rapid karyotype evolution (e.g. [21–28]). Roberts [35] noted that differences regarding habitat selection among various species are hardly recognized. For example, both *C. baluchi* and *C. hotsoni* in Pakistan favor mountain

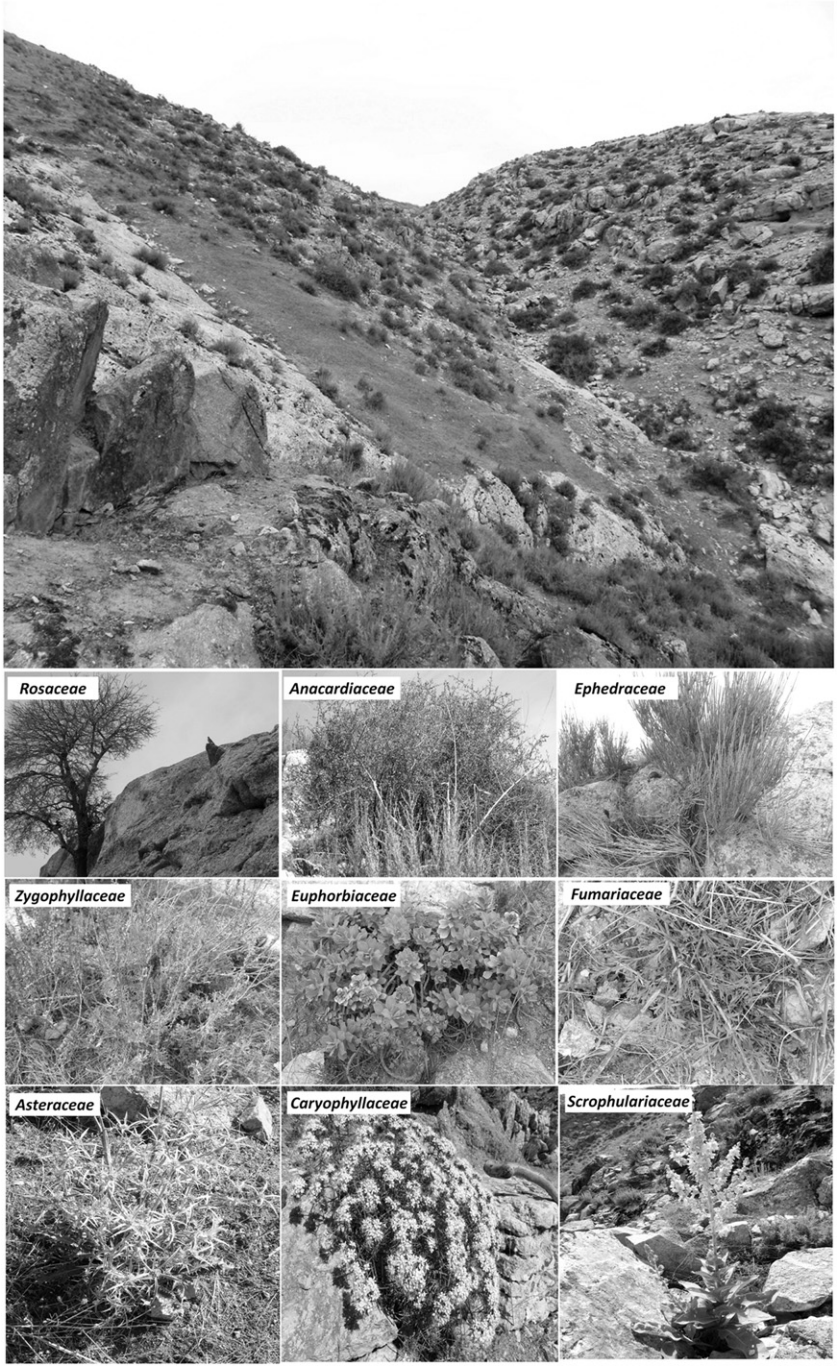

Fig. 4. Habitat and vegetation cover of *C. elburzensis* in Khaje-Morad heights, southcenter of Mashhad, Razavi Khorasan province.

steppe regions and the driest rocky hillsides, and are absent from low elevation valleys [35]. Moreover, no obvious correlation between the type or amount of vegetation and the distribution of *Calomyscus* species is recorded in Afghanistan [53]. In spite of the importance of biological, ecological and behavioral (especially sexual manner) findings in giving better insights on speciation mode in newly evolved species, such as Calomyscidae members, a limited number of studies have been done as reviewed earlier [14,29–40,53].

According to our observations, Goodwin's brush-tailed mice in Iran are distributed in a broad range of different biotopes including mountainous and desert regions (e.g. “Mediterranean with spring rains”, “cold mountainous”, “cold semi-desert”, “hot semi-desert” and “hot dry desert” climatic zones), while the main distribution areas of Hotson's brush-tailed mice are in hot and dry regions (e.g. “hot dry desert”, “hot semi-desert” and “cold semi-desert” climatic zones). The great differences in the climatic conditions which individuals of *C. elburzensis,* as compared with those of *C. hotsoni,* have encountered with, as well as the considerable elevation range of the first species (1146–2251 m in this study and 1703–3700 m in previous records), are correlated with significant differences in the temperature, precipitation and humidity of *C. elburzensis* habitats. These factors along with diversity in geological characteristics have resulted in variation of vegetation cover among different regions of *C. elburzensis* distribution range. For example, based on geologic structural zone maps, *C. elburzensis* in Iran are mainly distributed in “Kopet-Dag”, “Elborz” and “eastern Iran belt” zones, however they could be found in “Tabriz-Saveh” zone, “Elborz magmatic assemblage”, and also “Yazd” and “Lut” blocks, while *C. hotsoni* are found in “eastern Iran belt” and somehow

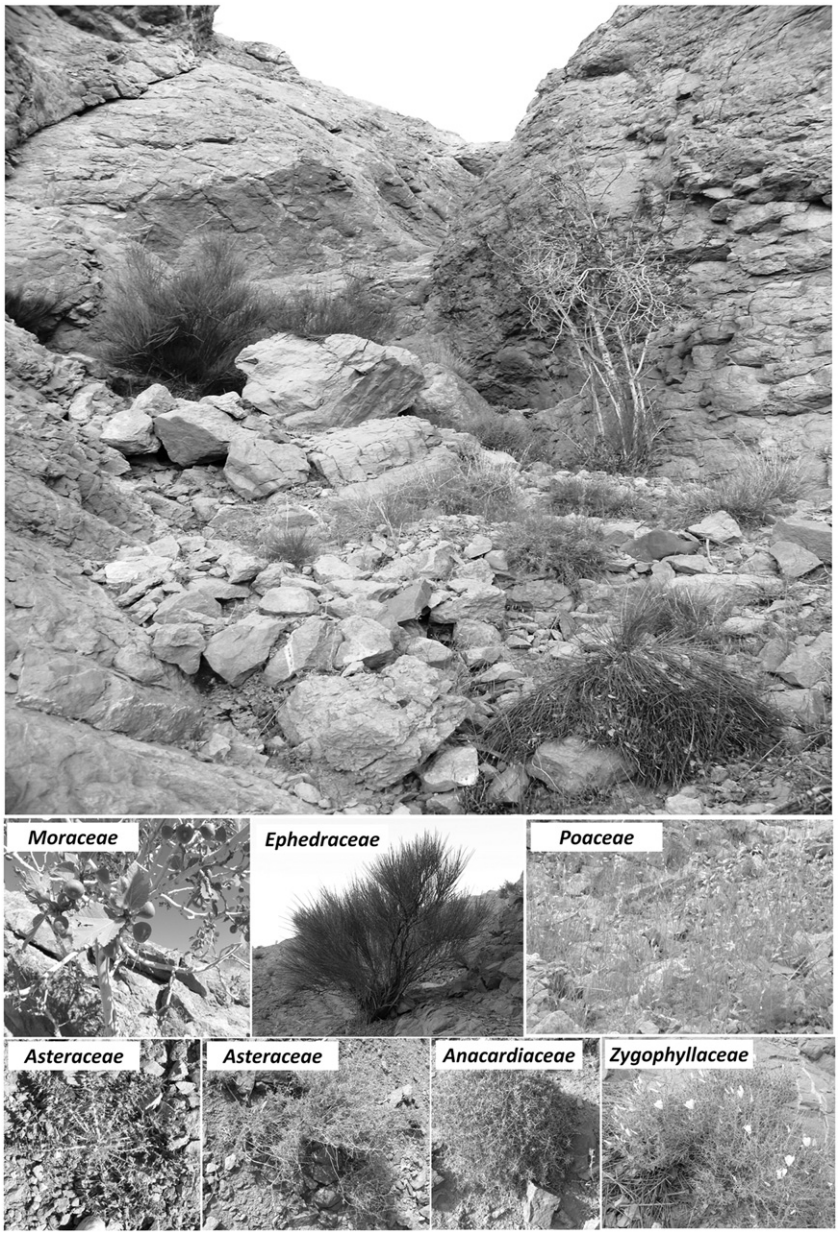

Fig. 5. Habitat and vegetation cover of *C. hotsoni* in Ark heights, northwest of Birjand, South Khorasan province, near the central Iranian desert.

in "eastern Iran magmatic assemblage". Furthermore, considering our previous records, it can be concluded that populations of *C. elburzensis* in Iran are living in "forest steppes" and "semi-desert" biotopes with "limestone juniperus mountainous forests", "pistachio" and "oak" forests and those of *C. hotsoni* inhabit "forest steppes" and "desert lowlands" biotopes with low diversity of vegetation cover (such as "pistachio forests" and "shrubs of the Kavir"). Furthermore, although massy cover of plants such as *Aegopordon* sp. and *Reseda* sp., which grow mainly in hard soils (calcareous sedimentary and limy rocks), were found in Kopet-Dag region but in its close geographic proximity, in Khaje-Morad (with igneous rocks), this vegetation cover changes to semi-sparse cover of sacculent plants mainly bushes of *Euphorbia* sp. This may in part be due to the presence of Binalud Mountains between these two regions, which is the probable cause of habitat differences between the most northeastern distribution ranges of *C. elburzensis* in Iran (Kopet-Dag) as compared with the lower geographical latitude of its range. On the other hand, little differences in the habitat structure were observed among different distribution regions of *C. hotsoni* in the present study. This might be due to the fact that Ark highlands and Shadan and Olang heights are two neighboring areas located in a linear pattern. Generally, Hotson's brush-tailed mice in the upper latitudes of their distribution range in the northeast of Iran (South Khorasan province) inhabit in humid areas of dry regions with a vegetation cover of draught resistant plants such as *Ficus* sp. and *Avena* sp. Therefore, these new findings show that habitat suitability has an important role in distribution pattern of brush-tailed mice and hence habitat selection may be occurring among various species (at least in the two studied species). Nevertheless, *Calomyscus* species exhibit a common ecological feature; they select rocky areas of humid foothills in high heights.

There are notes on the vegetation cover within the distribution range of *C. elburzensis* in the Elborz Mountains which is known as scant vegetation of yarrow (Asteraceae; *Achillea* sp.), locoweed (Fabaceae; *Astragalus* sp.), chicory (Asteraceae; *Cichorium* sp.), *Cousinia* sp. (Asteraceae), sage (Lamiaceae; *Salvia* sp.), thyme (Lamiaceae; *Thymus* sp.), and occasionally scattered Christ's thorn species *Paliurus aculeatus* Lam. (Rhamnaceae). Sagebrush (Asteraceae; *Artemisia* sp.), knapweed (Asteraceae; *Centaurea* sp.), thistle (Asteraceae; *Cirsium* sp.), *Varthemia persica* DC. (Asteraceae) and jujube (Rhamnaceae;

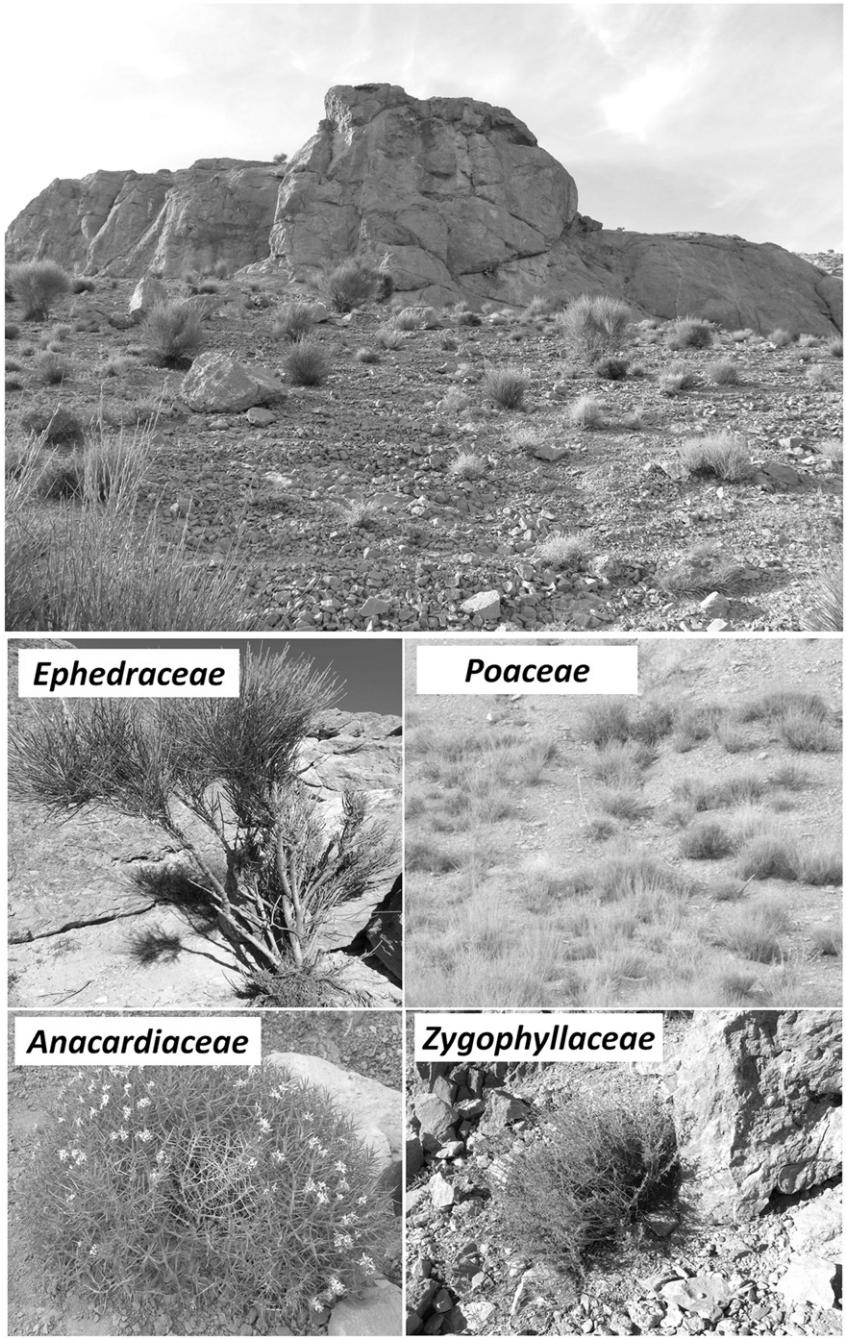

Fig. 6. Habitat and vegetation cover of *C. hotsoni* in Shadan and Olang heights, southcenter of Birjand, South Khorasan province.

Zizyphus sp.) are rarely found [29]. Nevertheless, no plants description was found for the habitat of *C. hotsoni*. Furthermore, there are records of food habits of *C. mystax* [34], *C. urartensis* [30] and some unknown species of *Calomyscus* in Iran and neighboring countries [29,35,53]. Lay [29] noted that individuals of *C. elburzensis* share their habitat with *Apodemus* sp., *C. migratorius*, *Chionomys nivalis*, *M. persicus*, and *Hemiechinus auritus* in Iran. Although there is one record on the association of *Gerbillus nanus* with probably *C. hotsoni* and/or *C. baluchi* in Pakistan [35], but no more clear notes were found in this regard for Hotson's brush-tailed mice. With respect to these issues, our new data concerning the habitat's vegetation cover, food habits and coexistence of each of the two examined species of brush-tailed mice, collated with previously published records, might be helpful to infer on the divergence at lower taxonomic levels (microevolution).

In our recent study [21], no significant geographical variation was observed in cranial and dental variables in different distribution localities of *C. elburzensis* in northeast of Iran (from northern to the southern localities in Kurkhud and Darmiyan, respectively). Moreover, in the related phylogenetic analysis, the mean genetic distance between studied samples of this species in the northeast of Iran showed very low intraspecific values [21]. In a parallel study [19] this distance within the populations of *C. elburzensis* throughout Iran was reported as 1.9% for *Cytb* and 1.2% for *COI*, and also it was noted that geographical distances and genetic differences show linear correlation [19]. Low genetic divergence was also recorded between the presented subclades 2 and 3 (1.2% for *COI*). Nevertheless examined *C. elburzensis* of northeast of Iran showed much more variation in their morphology than was seen in *C. hotsoni* [21]. In the present study, we showed that the mean distances (for

Cytb gene) within all examined samples of both *C. elburzensis* and *C. hotsoni* throughout Iran were 2.3% and 0.9%, respectively. With regards to the greater genetic distance in *C. elburzensis* as compared with that of the second species, we could assume that genetic distance has been affected by the geographical distances and maybe the differences observed in some habitats of the first species. *C. elburzensis* in northeast of Iran is distributed in three main parallel mountains including Kopet-Dag, Binalud and highlands of southcenter of Mashhad with innermost planes, but the distribution range of *C. hotsoni* in Iran is mainly continuous heights surrounded by lowlands. Furthermore, significant variations were observed in the habitat structure of *C. elburzensis*, as compared with very small differences in that of *C. hotsoni* as mentioned above.

Finally, in concordance with our hypothesis, samples of *C. elburzensis* from the two different climates and vegetation types showed greater intraspecific differentiation than the samples of *C. hotsoni* from very similar climates and vegetation. These findings are in agreement with the divergence at the intraspecific level observed in the topology of provided molecular trees (e.g. [19,21]). It is worth noting that although this hypothesis may account for some of the intraspecific differentiations, but it does not explain all of the intraspecific variations observed for all populations of these two species (7 subclades in *C. elburzensis* [19] and 3 subclades in *C. hotsoni* [21]) due to the limitation of the data used.

5. Conclusion

In short, with respect to the widest range of *C. elburzensis* among all known species of family Calomyscidae and the diversity observed in its habitat structure, it is assumed that scattered (non-linear) distribution pattern of *C. elburzensis* in the northeast of Iran might have been resulted from the presence of more significant differences in the habitat structure within its range. From this point, *C. hotsoni* with most linear dispersal pattern, show lower intraspecific differentiation. Provided data in present study include only small parts of the distribution range of both *C. elburzensis* and *C. hotsoni*. Further surveys to characterize climatic conditions and vegetation cover of all sampling localities in which these two species have been recorded, are needed for precise evaluating the present hypothesis. Furthermore, regarding the geographic status of northeast of Iran and the presence of topographic barriers such as the position of Kopet-Dag mountain in the north and two large deserts, Dasht-e-Lut in the south and Central Kavir in the west [54], more studies on the general ecological features, habitat and its relevants (e.g. microecological characteristics, niche specification, micro-climatic conditions) and also monitoring of the populations are needed for providing precise inferences regarding the dispersal and diversification patterns of this genus in Iranian plateau and its neighborhood.

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