

Phylogeography of *Calomyscus elburzensis* (Calomyscidae, Rodentia) around the Central Iranian Desert with Description of a New Subspecies in Center of Iranian Plateau

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Abstract

Calomyscus elburzensis Goodwin, 1938, was previously known from central and northern Iran to southern and southwestern Turkmenistan and northwestern Afghanistan. Its type locality is at altitude of 1200m elevation in the Kurkhud Mts., North Khorasan province in northeastern Iran. This study provides new evidence that its range is more extensive than thought previously and central Iranian Mountains, Shirkuh (Yazd province) and Karkas Mts. in Isfahan and Zanjan provinces in the northwest were recognized as its distribution areas. Two major clades were detected within *C. elburzensis* from the analysis of two mitochondrial genes *Cytb* and *CoI*. The outline on m2 and karyotypes analyses also supported these two molecular clades; one northeast with $2n=44$ and $FN_a=58, 60$ and 62 and the other west and northwest of central Iranian desert with $2n=44$ and $FN_a=70$. The Statistical Dispersal-Vicariance Analysis (S-DIVA) suggested dispersal events from north east to the center of Iran occurred in two separate occasions. As 2.5% was the rather low level of sequence divergence between these two major clades, we assigned the central-northwestern brush-tailed mice as a subspecies of *C. elburzensis* and therefore we named as *C. e. isatissus* ssp. novo.

Keywords: Goodwin's brush-tailed mouse; *C. e. isatissus* ssp. novo; mtDNA; Shirkuh.

Introduction

The taxonomy of *Calomyscus*, brush-tailed mouse, has undergone substantial change in recent years. Structure of auditory bullae resembles that of New

World deer mice *Peromyscus* (Cricetidae: Neotominae; Osgood 1947) whereas that of the glans penis is similar to the Old World hamster *Cricetulus migratorius* (Cricetidae: Cricetinae) (Krystufek and Vohralik 2009). However, recent molecular phylogenetic studies

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employing both nuclear and mitochondrial gene sequences place *Calomyscus* as a member of a monophyletic Calomyscidae, basal to both Cricetidae and Muridae and diagnosable by several distinctive morphological traits (Jansa and Weksler 2004; Michaux et al. 2001; Steppan et al. 2004). Thus, while the phylogenetic position of the genus is now rather stable, the number and diagnostic characterization of species within the genus remains a taxonomic challenge, with all known species sharing similar morphological characters (Musser and Carleton 2005).

Here we focus on Goodwin's brush-tailed mouse, *Calomyscus elburzensis* Goodwin, 1938, which is known from central and northern Iran to southern and southwestern Turkmenistan and northwestern Afghanistan (Karami et al. 2008; Musser and Carleton 2005; Norris et al. 2003). In Iran, this species occurs apparently in the Elburz Mts. in Semnan province and the Khorasan provinces (North and South Khorasan and Khorasan-e-Razavi). The type locality is at 1200m elevation in the Kurkhud Mts., North Khorasan province in northeastern Iran, a barren rocky range where temperatures fall below zero and snow is prevalent during winter months (Goodwin 1938; Karami et al. 2008). Recent work has extended the range into central Iran to the Shirkuh Mts. in Yazd province (Sahebjam et al. 2009; Shahabi et al. 2010, 2011, 2013).

The Elburz Mts., the main distributional area of *C. elburzensis*, are rocky mountains with many crags and bare cliffs (Krustufek and Vohralik 2009). The Elburz tectonic unit, which includes the entire mountain range from Azerbaijan in the west to northern Khorasan, and the eastern Binalud Mts. are part of the north of Iranian plateau south of the Caspian Sea (Taleghani 2005). The southern-most part of the known range of *C. elburzensis*, the Shirkuh Mts. in central Iran, was usually regarded as a geological part of the Zagros Mountains (Ghorbani 2013). Central Iranian mountains border the largest desert of Iran, the Great Kavir or Central Iranian desert, which is between the Elburz Mts. on the north and the mountainous region of Kashan-Anarak-Khur in the south (Jackson et al. 1990).

Across the known range of *C. elburzensis* karyotypic diversity is extensive. In Khorasan-e-Razavi province in northeastern Iran four karyotypes are known: $2n=44$, $FN_a=60$ (Esmaeili et al. 2008); $2n=44$, $FN_a=62$ and $2N=44$, $FN_a=72$ (Shahabi et al. 2010); and $2n=44$, $FN_a=58$ (Meyer and Malikov 1995; Graphodatsky et al. 2000). Karyotypes with $2n=44$, $FN_a=58$ and $2n=30$, $FN_a=44$ have been recorded from localities in the Kopetdag Mts. in Turkmenistan (Meyer and Malikov 1995; Graphodatsky et al. 2000). Also a cytotype of $2N=44$, $FN_a=70$ was recorded from Yazd province in

central Iran.

Our goal here is to evaluate the taxonomy of Goodwin's brush-tailed mouse, *Calomyscus elburzensis*, which has been complicated in prior studies by limited sampling and species allocations based on sparse chromosomal data. We document the distribution and phylogeny of this species using mitochondrial DNA sequences from the cytochrome b (*Cytb*) and cytochrome oxidase I (*COI*) genes. In addition, we characterize this and related species by geometric morphometric analyses of molar tooth characters.

Materials and Methods

Samples

We obtained 60 specimens of four species of brush-tailed mice in Iran (Table 1, Appendix 1 and Fig. 1), including *C. elburzensis* from 19 localities, *C. mystax* and *C. urartensis* from one locality, *C. grandis* from its type locality and *Calomyscus* sp. Group B from two locations. We also included two *Cytb* sequences each from *C. hotsoni* (EU135579.1 and EU135583.1) and *C. baluchi* (EU135586.1 and EU135587.1), obtained from Genbank, two *CoI* sequences of each of these species from Shahabi et al. (2013), and three *Cytb* sequences of samples from Kerman province (Sirch and Rayen) reported by Morshed and Patton (2002). Voucher specimens for these data are deposited in the Museum of Vertebrate Zoology, University of California, Berkeley, CA, USA; these samples were not included in our geometric-morphometric analyses. We mapped our sample localities in DIVA-GIS 7.5 (Hijmans et al. 2012) (Fig. 1).

DNA extraction

We extracted genomic DNA from fresh and 96% ethanol-preserved tissues using standard salt-extraction methods described by Bruford et al. (1992). DNA amplification employed primer pairs L7 and H6 for *Cytb* (Montgelard et al. 2002) and VF1d and VR1d for *CoI* mtDNA genes (Ivanova et al. 2006); thermal reactions followed conditions given by Montgelard et al. (2002) for *Cytb* and Ivanova et al. (2006) for *CoI*. Amplification of cytochrome *b* was carried out with an initial denaturation step at 94°C for 2 min., followed by 35 cycles of 45s at 94°C, 45s at 50°C and 90s at 68°C, with a final extension time of 10 min. at 68°C (Montgelard et al. 2002). While *CoI* proliferation was performed in an initial denaturation step at 95°C for 5 min., followed by 4 cycles of 30s at 95°C, 30s at 54°C and 60s at 72°C; 4 cycles of 30s at 95°C, 30s at 52°C and 60s at 72°C; 36 cycles of 30s at 95°C, 30s at 50°C and 60s at 72°C with a final extension time of 8 min. at

Table 1. Samples localities (numbered as in Fig. 1), number of specimens and geographic coordinates. Samples from each locality were used for geometric-morphometric analyses are in parenthesis.

Location				Coordinates
City	Village	Species	N	
Dasht (1)		<i>C. elburzensis</i>	1	37° 29' N, 57° 17' E
Bojnord	Chenaran (2)	<i>C. elburzensis</i>	3(2)	37° 25' 19" N, 57° 22' 35" E
Esfaraien	Saluk (3)	<i>C. elburzensis</i>	1(1)	37° 20' 42" N, 57° 54' 14" E
Daregaz	Tandure (4)	<i>C. elburzensis</i>	2(2)	37° 26' N, 58° 43' E
Neyshabur	Binalud (5)	<i>C. elburzensis</i>	2(2)	36° 18' 56" N, 58° 52' 59" E
Mashhad	KhajeMorad (6)	<i>C. elburzensis</i>	2(2)	36° 15' N, 59° 34' E
Sarakhs	Aghdarband (7)	<i>C. elburzensis</i>	3(3)	36° 30' N, 61° 7' E
Torbat-e-Jam	NasrAbad (8)	<i>C. elburzensis</i>	1(2)	35° 9' N, 60° 24' E
Bajestan (9)	Siahkuh Mts.	<i>C. elburzensis</i>	1(1)	34° 24' 29" N, 58° 10' 20" E
Sabzevar	Zarghan (10)	<i>C. elburzensis</i>	1(1)	36° 31' 33" N, 57° 11' 10" E
Ghaen	HajiAbad (11)	<i>C. elburzensis</i>	2(2)	33° 36' 15" N, 59° 54' 36" E
Birjand	Darmiyan (12)	<i>C. elburzensis</i>	1(1)	33° 2' 26" N, 60° 7' 7" E
Birjand	Gezik (13)	<i>C. elburzensis</i>	1(2)	32° 59' 59" N, 60° 13' 36" E
Mehriz (14)	Kuhe-Bakhtaki	<i>C. elburzensis</i>	1(2)	31° 24' 53" N, 54° 25' 48" E
FakhrAbad (15)		<i>C. elburzensis</i>	3(2)	31° 40' N, 54° 19' E
Taft, Shirkuh (16)	Ab-Mazrae	<i>C. elburzensis</i>	4(4)	31° 38' 26" N, 54° 5' 7" E
	Cheshme	<i>C. elburzensis</i>	3(3)	31° 39' 19" N, 54° 9' 32" E
	Dare-Bidun	<i>C. elburzensis</i>	3(4)	31° 39' 23" N, 54° 5' 23" E
	Tezerjan	<i>C. elburzensis</i>	3(3)	31° 34' 3" N, 54° 9' 11" E
	Godar-Nir	<i>C. elburzensis</i>	5(2)	31° 33' 19" N, 54° 7' 50" E
	Ab-Shaghan	<i>C. elburzensis</i>	1(1)	31° 40' 17" N, 54° 4' 52" E
Karkas (17)		<i>C. elburzensis</i>	1(1)	33° 27' 21" N, 51° 47' 59" E
Qeidar (18)		<i>C. elburzensis</i>	2(1)	36° 7' 47" N, 48° 35' 33" E
Kurkhud Mts. (type locality)		<i>C. elburzensis</i>	2(3)	37° 49' 40" N, 56° 41' 14" E
Tehran	Fasham	<i>C. grandis</i>	4(5)	35° 56' 4" N, 51° 31' 27" E
Bojnord	TakaleGhoze	<i>C. mystax</i>	3	38° 12' 59" N, 57° 11' 5" E
Kordasht		<i>C. urartensis</i>	2	38° 52' 1" N, 46° 0' 36" E
Anjerk		<i>Calomyscus sp. Group B</i>	2	28° 47' N, 56° 20' E

We analyzed separate alignment files for both *Cytb* and *CoI* sequences using DnaSP v5.10.01 (Librado and Rozas 2009), and constructed haplotype networks with NETWORK 4.6.1.3 (Brandelt et al. 1999) to visualize the relationship among all haplotypes for each gene. We also used DnaSP to estimate haplotype diversity for both genes.

Geometric morphometrics analysis

We digitized the outline of second lower molar outlines for 52 adult specimens belong to *C. elburzensis* and *C. grandis* (Table 1), as x and y coordinates of one hundred and fifty points equally spaced along the tooth outline using TPSDig2 v.2.12 (Rohlf 2008). The initial digitized point of each tooth outline began at the most anterior part of m2. Elliptic Fourier Analysis (EFA) was performed by EFAwin software (Rohlf and Ferson 1992). Using GMTP program (Taravati 2010), the TPSDig output file was converted to an adjusted format to be opened in EFAwin from which we could extract Fourier coefficients from original outline and normalize shape variable. After conversion, the centroid size (CS)

was calculated (by GMTP software) for each specimen. We retained the first 12 harmonics to compare variation between m2 shapes of our samples. We then performed a Canonical Variate Analysis (CVA) analysis on output file of EFAwin, in PAST v2.06 (Hammer et al. 2001) to visualize the pattern of morphological differentiation in *a priori* determined molecular clades and species. Finally, we examined the linear correlation between the size of m2 in all individuals and their corresponding CV1 and CV2 shape estimators.

Karyological analysis

We prepared karyotypes from femoral and tibial bone marrow cells of *C. elburzensis* specimens that were transported to laboratory alive. Before the extraction of bone marrow mice were treated 45 min with 10% vinblastin solution at a dose of 1ml/100g of body weight using the method of Dutrillaux et al. (1982). We made 10 slides for each individual and stained with giemsa. About 15 spreads were examined in slides of each sample for determining the diploid chromosome number. Diploid number of chromosomes (2n) and

number of autosomal arms (FNa) were assigned from photographs of well-spread cells by the chromosome processing software (CIP) developed in Rodentology Research Department of Ferdowsi University of Mashhad. We compared the prepared karyotypes for *C. elburzensis* with those reported in the literature by other authors.

S-DIVA analysis

We used the S-DIVA (Statistical Dispersal-Vicariance Analysis, version 1.9b, Yan et al. 2010) program implemented in RASP (Yan et al. 2011) to determine statistical support for ancestral range reconstruction. The distribution range of *C. elburzensis* that we used included five areas (Fig. 1): (A) Kopetdagh and east of Elburz Mts. in the northeast (localities 1, 2, 3, 4 and type locality), (B) Binalud Mts. and southern parts in east of central desert (localities of 5, 6, 7, 8, 9, 11, 12 and 13 in Fig. 1), (C) Shirkuh Mts. in central Iran (localities 14, 15 and 16), (D) Qeidar Mts. (locality 18), and (E) Karkas Mts. (locality 17). We used the output tree file of Mr. Bayes the S-DIVA analysis, with 200,000 trees from the MCMC (Markov Chain Monte Carlo) output and S-DIVA run on all of them. The possible ancestral range for *C. elburzensis* was then acquired for each node of this species tree and dispersal or vicariance probability was determined in each node information.

Results

Phylogenetic analysis

A total of 1616 bps were available for analysis after editing each gene, 988 bps for *Cytb* and 628 bps for *CoI*. There were no stop codons or gaps in the alignments of either gene. Of 988 sites of *Cytb*, 76 sites were variable or polymorphic, 68 were parsimony informative, and the total number of mutations was 86. These values for the 628 sites of *CoI* gene were 91 variable and 81 parsimony informative sites, with 102 mutations.

The topologies of all three Bayesian, ML and MP trees were congruent for both each separate gene analysis and for the combined data set (Fig. 2). Each analysis resulted in the same gene tree, with all specimens of *C. elburzensis* from a monophyletic group with a very high support.

With available data, *C. elburzensis* is the sister group of a group consists of three species: *C. grandis*, *C. urartensis* and *C. mystax* (Fig. 2). A number of well-supported clades are apparent within the monophyletic assemblage of *C. elburzensis* samples depicted in Fig. 2. The basal clade, identified as Clade 1 (Fig. 2) is strongly

supported by a posterior probability > 99, includes those samples from Tandure (locality 4) and Chenaran (locality 2), localities in the middle of the Kopetdagh Mts. in Khorasan-e-Razavi and North Khorasan provinces (Fig. 2). The complementary, or sister, clade to these samples encompasses all other samples of the species from the remainder of its range in Iran. Nevertheless, this second large group is divisible into additional subclades we identify as Clade 2 through Clade 7 (Fig. 2). This large assemblage of subclades is also divisible into two global units, one comprised of Clades 2-4 and the second by Clades 5-7.

Clade 2 is well supported (posterior probability > 99) and includes specimens from type locality of *C. elburzensis* in the Kurkhud Mts. (North Khorasan) as well as those from, Saluk (locality 3), Dasht (locality 1), a single individual from Chenaran (locality 2), and Sabzevar (locality 10). All of the specimens in Clade 2 are from the central Kopetdagh Mts. (North Khorasan province) or near-by sample from Sabzevar in the eastern Elburz Mt. in Khorasan-e-Razavi province (Fig. 2). Clade 3 is not supported in ML analysis but well supported in BI and MP ones is consistently present in all analyses. It includes samples collected from Taft (locality 16) and FakhrAbad (locality 15) from the Shirkuh Mts. (Yazd province) as well as one individual from Gazik (locality 13) in South Khorasan. Finally Clade 4 is well supported and contains our samples from KhajeMorad in Mashhad (locality 6), Binalud Mountain in Neyshabur (locality 5), Sarakhs in the eastern Kopetdagh Mts. (locality 7), Siahkuh Mountain in Bajestan (locality 9), and Shahnesh in Mountain in Torabt, all in Khorasan-e-Razavi Province, and both Ghaen (locality 12) and Darmiyan (locality 12) in the Shaskuh Mountain in South Khorasan Province. Clade 1 plus all members of clades 2 through 4, with the exception of individuals of Clade 3 from Shirkuh in Yazd Province, are located in the northeastern part of the Iranian range for the species (Fig. 2).

The second branch of the major group of clades of *C. elburzensis* contained most of the specimens from Taft (locality 16), Mehriz (locality 14), and FakhrAbad (locality 15) in the Shirkuh Mountains of Yazd province (Clade 7) along with those from Karkas (locality 17, Isfahan province; Clade 5) and Qeidar mountains (locality 18, Zanjan province, Clade 6). These sets of clades are well supported and distributed along the central mountainous region of Iran.

For the most part, each clade of *C. elburzensis* contains specimens from a unique set of geographically adjacent localities. However, in two cases individuals from single localities are clustered in different clades. For example, the population sample from Chenaran

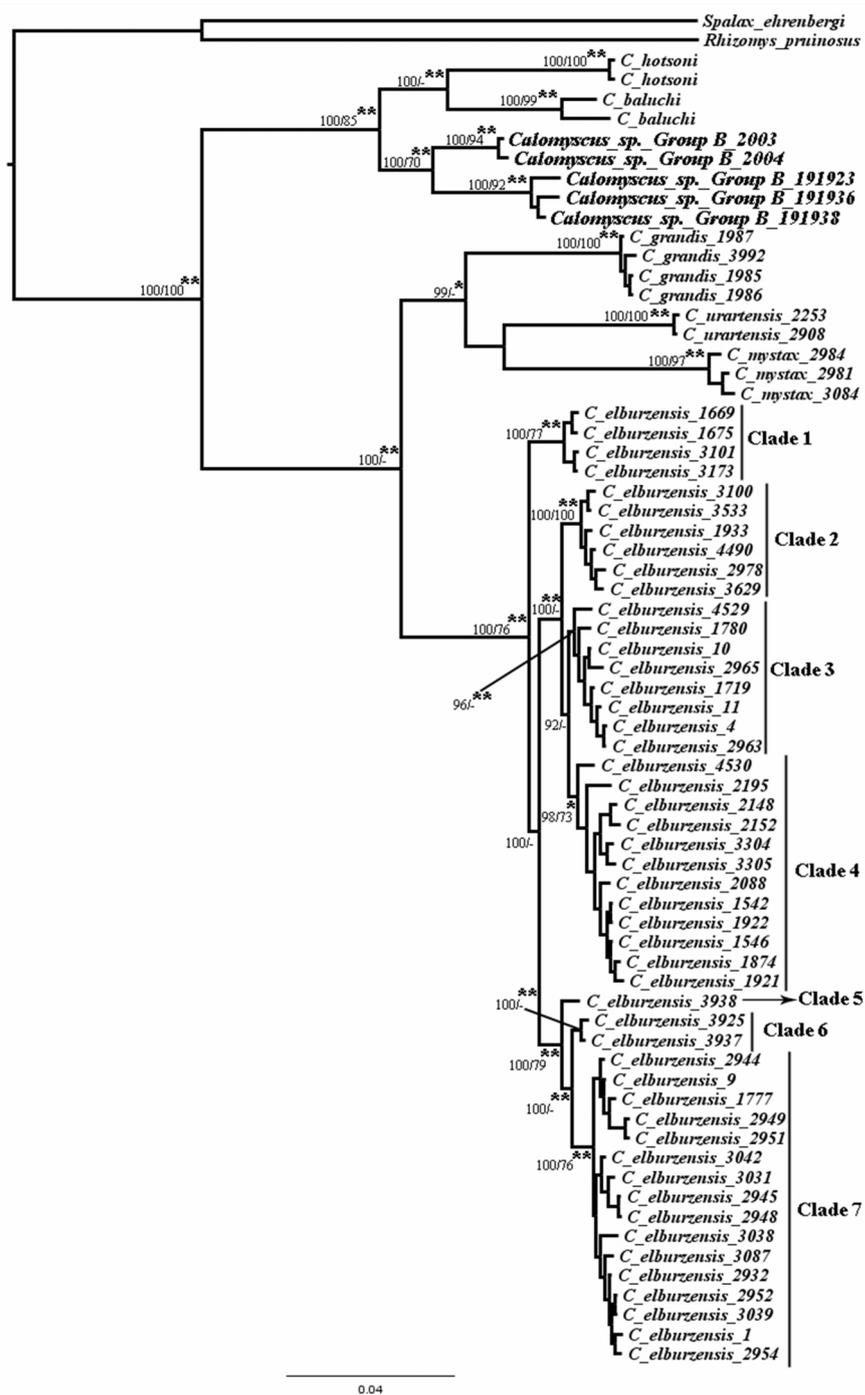


Figure 2. Consensus tree of the combined data set of *Cytb* and *CoI* genes. Bayesian posterior probabilities are indicated by asterisks (>99% =**, >90% =*) and numbers on branches are bootstrap values derived from the maximum parsimony (first value) and maximum likelihood (second value) analysis (give only if >70% which is the level of significance).

(locality 2) contains individuals belonging to both Clades 1 and 2, and, of more interest, specimens from the Shirkuh Mts. in Yazd Province assort to both Clades 3 and 7 (Fig. 2 and 3). This region between the Shirkuh Mts. (locality 16) and Gazik (locality 13) thus provides

a nexus geographically connecting the two major clades that otherwise sort samples from northeastern Iran in the Koptedagh and Elburz mountains from those from central Iranian ranges.

K2P distances estimated for both genes separately are

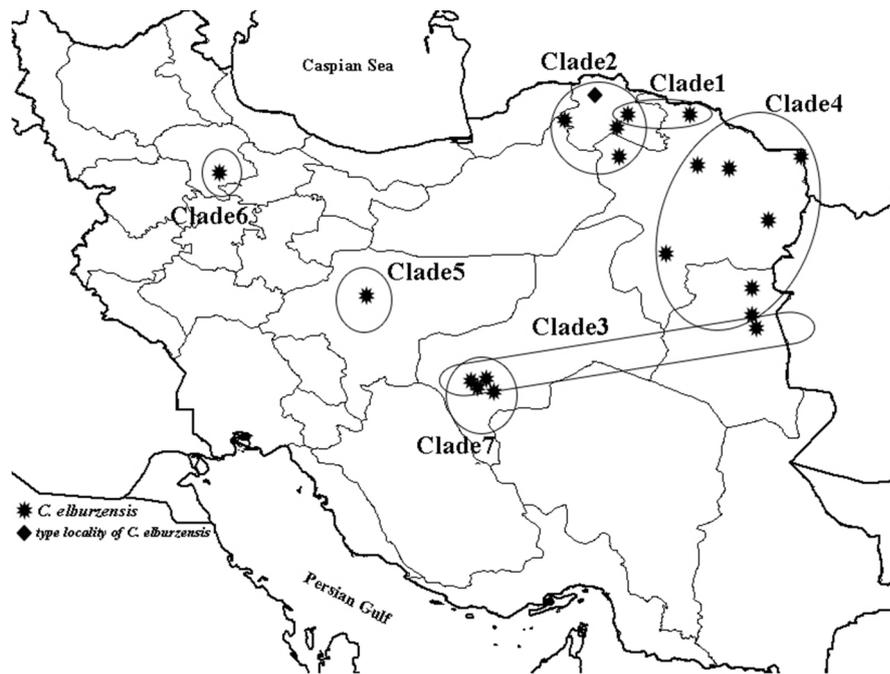


Figure 3. Geographical distribution of seven clades in *C. elburzensis* around Central Iranian desert.

given in Table 2. For both *CoI* and *Cytb*, respectively, these values ranged from 0 to 1.2% and 0-3% within species and 4.7-19.2% and 7.9-18.9% among them. The mean distances within our samples of *C. elburzensis* were 1.2% for *CoI* and 1.9% for *Cytb*.

Also comparative genetic distances between three sets of clades are provided in Table 3. The Mantel correlation between geographic distance and genetic divergence for two *Cytb* and *CoI* genes in *C. elburzensis* populations was 0.508 ($R^2=0.258$) for *Cytb* and 0.432 ($R^2=0.187$) for *CoI*, so showed a linear relationship between these two elements (Fig. 4). Thus genetic differences increased linearly with geographic distances.

Haplotype analysis

Fourteen of a total of 25 *Cytb* haplotypes and 18 of 25

CoI haplotypes, respectively, were unique to *C. elburzensis*. The group of Clade 5-7 haplotypes from central and northwestern Iran included four unique *Cytb* and six unique *CoI* (Fig. 5A and B). As depicted in Fig. 4, haplotypes from these two geographic regions are apportioned to separate parts of the overall network, in complete accordance with the phylogenetic analysis (Fig. 2). Haplotypes in each *Cytb* and *CoI* analyses form a single network (Fig. 5A).

Estimated haplotype diversity for the two regions of Iran on two different sides of central desert for the two mitochondrial genes was higher in the northeastern Iranian samples belonging to Clades 1-4. These values for *Cytb* and *CoI* sequences, respectively, were 0.96 for northeast and 0.92 for west of the central desert, and 0.90 versus 0.84.

Table 2. Within and between group Kimura2-parameter distances for the *CoI* (above the diagonal) and *Cytb* (below the diagonal) mitochondrial genes; numbers on the diagonal are values within species related to distances within species, with *Cytb* distances in bold and underline number for *CoI* distances.

	<i>C. elburzensis</i>	<i>C. grandis</i>	<i>C. mystax</i>	<i>C. urartensis</i>	<i>Calomyscus sp. Group B</i>	<i>C. hotsoni</i>	<i>C. baluchi</i>
<i>C. elburzensis</i>	1.9/1.2	8.8	10.8	9.7	16.3	15.8	18.1
<i>C. grandis</i>	10.1	0.0/0.3	8.7	6.8	15.5	14.0	17.0
<i>C. mystax</i>	11.2	11.4	0.6/0.5	10.7	15.9	14.9	19.2
<i>C. urartensis</i>	10.1	10.5	9.9	0.0/0.2	17.5	16.7	18.4
<i>Calomyscus sp. Group B</i>	15.6	17.6	15.5	15.8	3.0/0.3	4.7	5.6
<i>C. hotsoni</i>	17.2	17.6	15.8	18.9	10.0	0.3/0.0	5.7
<i>C. baluchi</i>	16.5	18.2	15.0	15.8	9.6	7.9	0.3/0.3

Table 3. K2P genetic distances between clades of *C. elburzensis*. Between-clade values for the *Col* data are above the diagonal, and complementary values for the *Cytb* data are below the diagonal; numbers on the diagonal correspond to distances within each sets of clade (Bold numbers: *Cytb* and underline numbers: *Col*).

	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	Clade 6	Clade 7
Clade 1	0.2/0.4	1.7	1.1	1.6	1.8	1.5	1.8
Clade 2	2.3	0.2/0.5	0.7	1.0	2.0	1.8	2.0
Clade 3	2.3	1.3	0.4/0.3	0.6	1.5	1.2	1.4
Clade 4	3.0	1.7	1.3	0.7/0.4	1.5	1.6	1.7
Clade 5	2.1	2.1	1.7	2.5	-/-	0.7	1.0
Clade 6	2.3	2.0	1.9	2.4	0.8	0.1/0.2	0.6
Clade 7	2.7	1.2	2.5	3.1	1.2	0.9	0.3/0.4

Geometric morphometric results

Allometry analysis showed no correlation between size and shape in m2 ($df=49$, $p_{(reg)}=0.108$). There was also no sexual dimorphism in shape of m2. The first two CVA axes accounted for 99% of total variance and Pairwise Group Comparisons confirmed the

significance of these functions. We illustrate the dispersion of each specimen we examined on the first two CVA axes in Fig. 6. Individuals of *C. grandis* are strongly separated from those of all *C. elburzensis* clades on the first axis (CV1, which explains 95.3% of the pooled variance). The second axis (CV2) separates

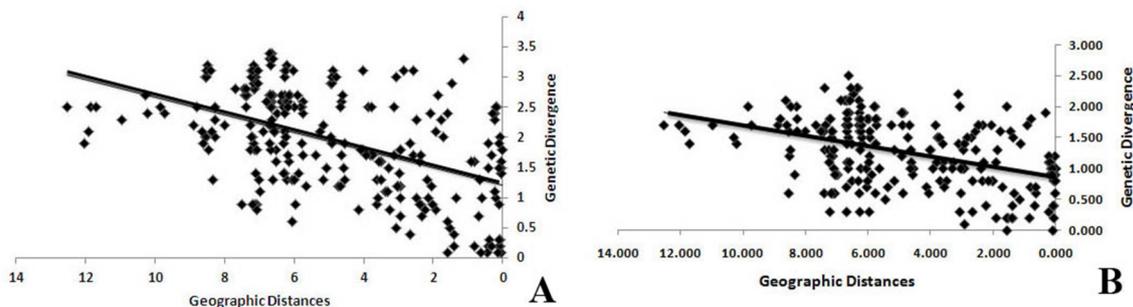


Figure 4. Pairwise differentiation estimates from *Cytb* and *Col* genes between different localities which *C. elburzensis* were collected are plotted against geographical distances.

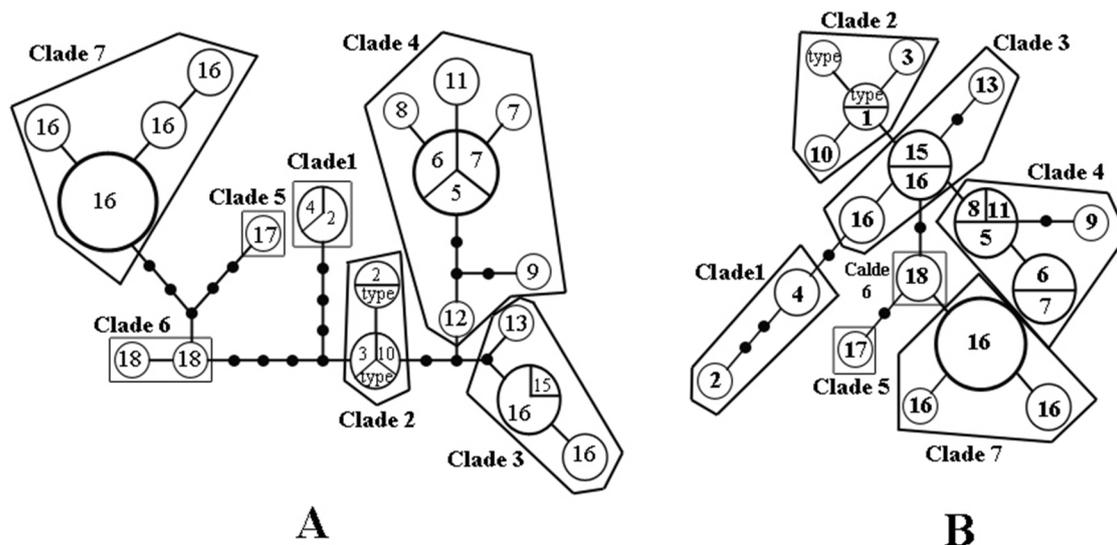


Figure 5. Median-joining of mtDNA *Cytb* (A) and *Col* (B) haplotype networks of *C. elburzensis*. Circle sizes are proportional to total number of samples in each haplotype. The numbers are corresponded to locality numbers in Table 1 (type: type locality of *C. elburzensis*). Dots on connection lines are mutational steps between neighbouring haplotypes.

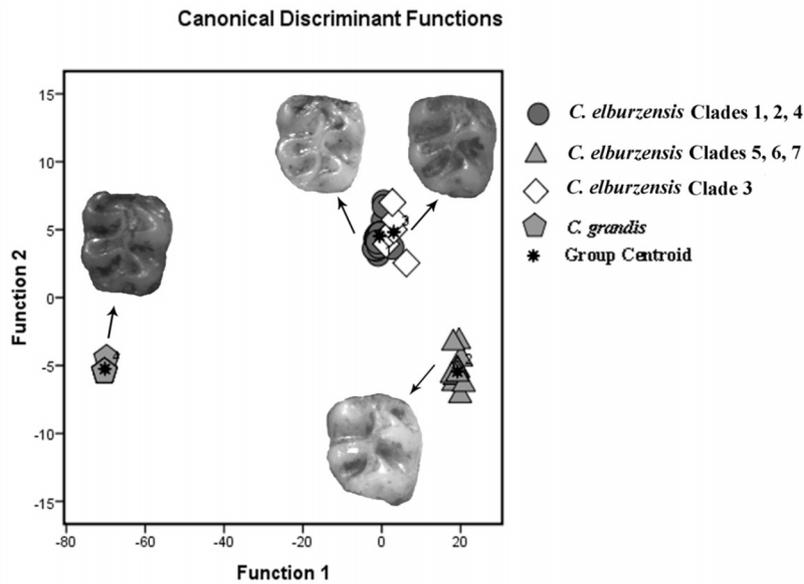


Figure 6. CVA scatter plot on shape variable of second lower molar.

those samples of *C. elburzensis* from northeastern Iran (those belonging to mtDNA clades 1-4) from those from central to northwestern Iran (Clades 5-7). Critically important, Clade 3 specimens from the Shirkuh Mts. in Yazd Province share the same molar pattern as all northeastern specimens belonging to Clades 1, 2, and 4 as well as other Clade 3 members.

Karyological results

We found three different karyotypes among the samples we examined herein, consistent with intraspecific chromosomal diversity detailed by previous workers (Esmaeili et al. 2008; Shahabi et al. 2010). In each case, the diploid number of the three

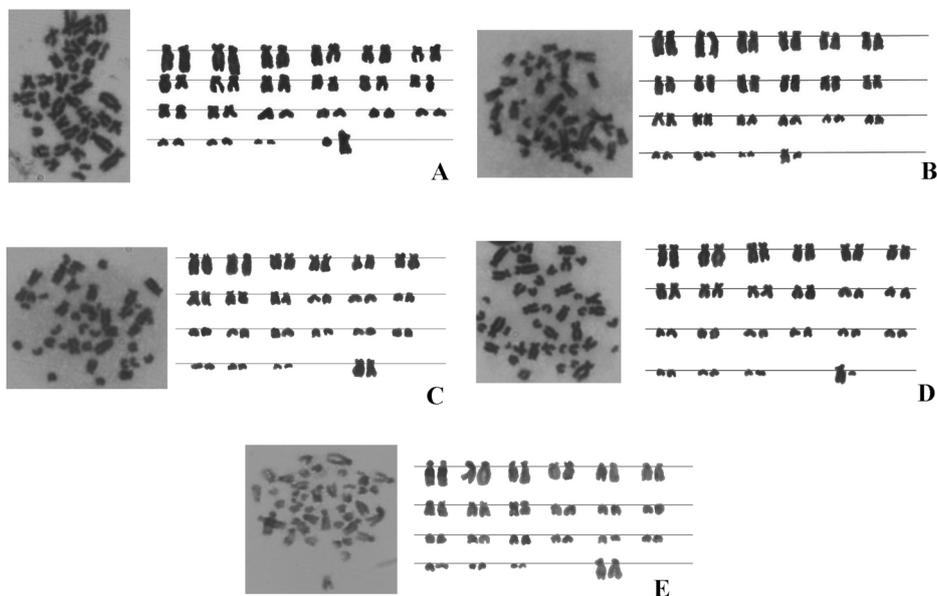


Figure 7. Karyotypes from five individuals of *C. elburzensis*. A: a male sample (voucher number: 2932-ZMFUM) from Shirkuh Mts. (locality 16, Clade 7) and B: a male sample (3087-ZMFUM) from Mehriz (locality 14, Clade 7); C: a female sample from Bojnord in Kopetdag Mts. (3100-ZMFUM) and D: a male individual from type locality of *C. elburzensis* (3630-ZMFUM) (Clade 2); E: a female sample from Gazik in South Khorasan (4529-ZMFUM) (Clade 3).

cytotypes was 44, but numbers of autosomal arms differed. Three males and two females from the Shirkuh Mts. (in Clade 7, locality 16) had karyotypes of $2n=44$ and $FN_a=70$ (Fig. 7A and 7B). A single male (from Clade 1, locality 2) (not shown) and female from the Kopetdagh Mts. in northeastern Iran (Clade 2, locality 2) and one female from the eastern part of the Iran

(Clade 3, locality 13) had $2n=44$ and $FN_a=60$ (Fig. 7C and E), the same as one previously reported from this geographic area (Esmaeili et al. 2008). And, a karyotype of a male from the type locality (Clade 2) also exhibited the previously described karyotype of $2n=44$, $FN_a=62$ (Shahabi et al. 2010) (Fig. 7D).

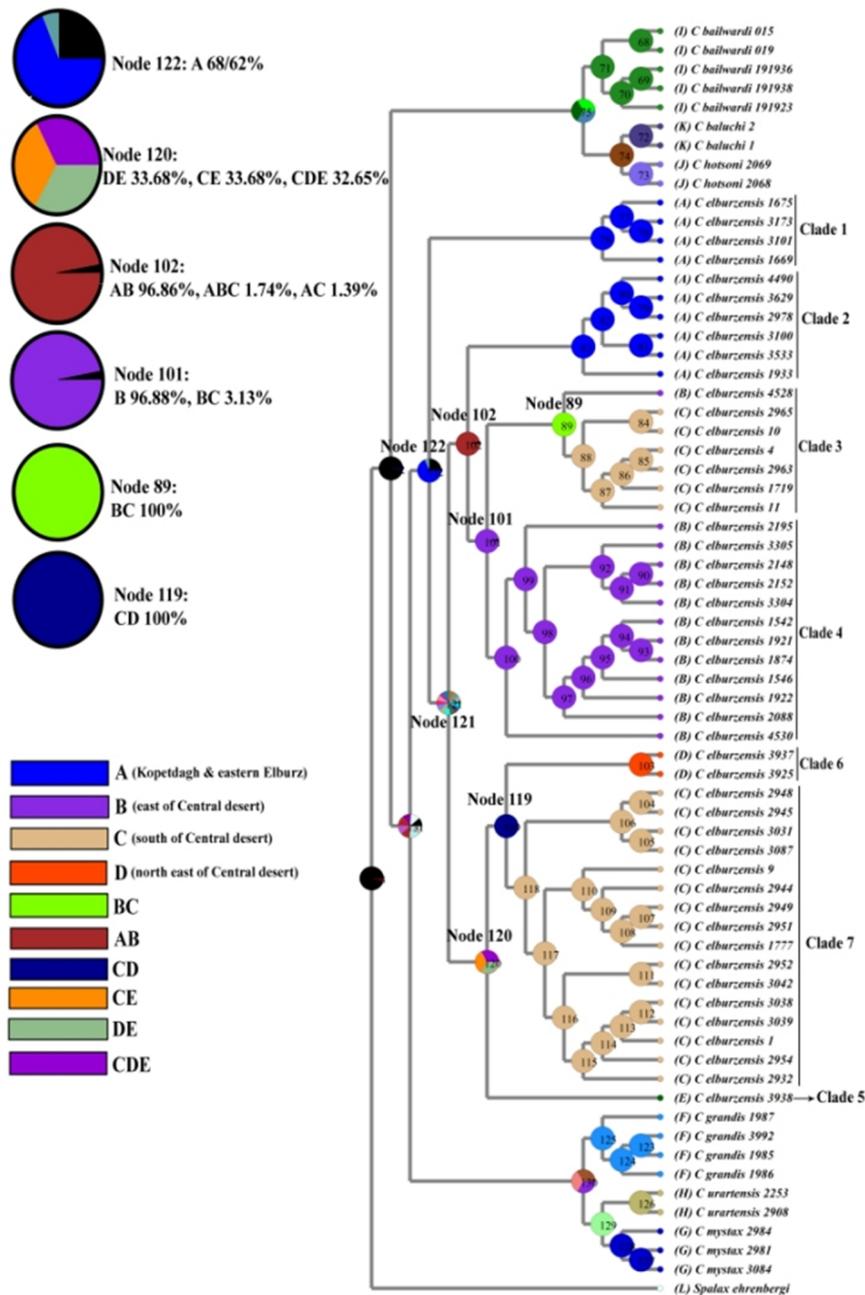


Figure 8. Graphical output of S-DIVA implemented in RASP. Different selected areas were explained (see text for more information).

S-DIVA results

The sequences analysis of combined *Cytb* and *CoI* with S-DIVA revealed that the ancestral range at node 122, which contained all the samples of *C. elburzensis*, included the Kpoetdagh mountainous region in northeastern Iran, with a 68.62% marginal probability. Thus, the S-DIVA postulates that the ancestral stock of the species originated in the Koptedagh Mts. and eastern parts of the Elburz Mts., an area encompassing northeastern Iran and both adjacent Turkmenistan and Afghanistan. From this ancestral region, *C. elburzensis* then spread into the central mountainous region of Iran to the south and west of the low-lying Great Kavir Basin.

Possible ancestral range of node 102 consists of Clades 2 and 4 from the northeast and Clade 3 from Shirkuh, was AB with 96.86% marginal probabilities. This node supposed a vicariance event of Goodwin's brush-tailed mice between two north east clades (Clade 2 and 4). Afterwards in node 101 it was obvious that dispersal was occurred from ancestor region of B (96.88%) in Clade 4 in north east and east of central desert to the south of it in Shirkuh mountain (Fig. 8).

In node 120, CE (Shirkuh and Karkas Mts.) and DE (Shirkuh and Qeidar Mts.) both with 33.68% marginal probabilities was ancestor region of *C. elburzensis* in south and west of central Iranian desert, and with equal probability of dispersal or vicariance events from Karkas to Shirkuh and Qeidar mountains in center and north west of central desert. Then vicariance event was followed between Qeidar and Shirkuh region as is evident in node 119 (CD=100%).

Discussion

Based on the present and previous studies, *C. elburzensis* has one of the widest ranges of all currently known species of *Calomyscus* genus, occurring throughout northwestern Afghanistan, southern Turkmenistan, and northeastern Iran in the Kopetdag Mts. and into the central Iranian Mountains, Shirkuh and Karkas Mts. in Yazd and Isfahan provinces to Zanjan province in the northwest (Graphodatsky et al. 2000; Norris et al. 2003; Shahabi et al. 2013).

Center or origin and historical spread of *C. elburzensis*

Herein we discover the expanding of distribution area of *C. elburzensis* into the central Iranian Shirkuh Mts. in Yazd province and, in combination with samples from throughout the rest of the known range of the species in Iran (our sampling localities), demonstrate substantial phylogeographic structure using two mitochondrial

genes, *Cytb* and *CoI*, in which most nearby populations are relatively more similar to each other possibly as a result of considerable gene flow between them. Three major molecular clades are found in phylogenetic analysis, one of them in northern and eastern part of the *C. elburzensis* range (Clades 1, 2, and 4), the other in its west and south (Clades 5-7) and the last clade (Clade 3) shares members between these two global regions (Fig. 3). Furthermore, mice belonging to both Clade 7 and Clade 3 were found together in the Shirkuh Mts. in Yazd province. We thus argue that the Shirkuh region must have received migrants on at least two separate occasions from northeastern Iran probably via mountainous areas between these two regions. One migration likely constituted Clade 7 individuals and then populations of northwest in Isfahan and Zanjan provinces, and once formed Clade 3 individuals.

Alternatively, our Dispersal-Vicariance analysis in RASP also suggested two dispersal events into Shirkuh, both from the northeast (nodes 122 and 101). At the first invasion, it constructed populations in Taft, FakhrAbad and Mehriz (Shirkuh Mts., Clade 7), Karkas in Isfahan and Qeidar Mts. in northwest in Zanjan province (Clade 5; node 122). This spread from central to northwestern Iran eventually led to the set of populations uniquely definable by clade structure, karyotype, and molar morphology that we describe formally below. We propose that the second dispersal event from northeast into the center (node 101) occurred more recent than the previous one, because this expansion formed two other populations in Taft and FakhrAbad in Shirkuh (Clade 3), which has a closer genetic relationship to populations in northeast and have had lesser time than previous dispersed populations to diverge (Table 3).

In addition to the S-DIVA results that support the northeast as the ancestor region of *C. elburzensis*, this area also contains higher haplotype diversity than those populations to the south and west of central desert areas (Fig 5A and B). Moreover genetic distances within populations of Shirkuh Mts. and geographically distant populations from Karkas Mts. in Isfahan and Qeidar Mountain in Zanjan were less than this value within *C. elburzensis* populations and clades in a close area in Kopetdagh and its southern parts in north east, so this area demonstrated more genetic divergence (Table 3).

Cytogenetic studies revealed four different cytotypes of *C. elburzensis* from Kopetdagh mountains in south east of Turkmenistan and north east of Iran, one of these showed $2N=30$, $Fn_a=44$ and three had 44 chromosomes with 58, 60 and 62 autosomal arms. However only a single cytotype was recognized for Shirkuh region with $2n=44$ and 70 autosomal arms (Graphodatsky et al. 2000; Shahabi et al. 2010 and the present study).

Thereupon, Kopetdagh region showed greater karyological diversification. The first cytotypes of Kopetdagh ($2n=30$, $Fn_a=44$) which is sympatric in central Kopetdagh with individuals with $2n=44$, $Fn_a=58$, had natural hybrids but their males were sterile (Meyer and Malikov 2000). Furthermore, there was morphometric differentiation between these (Lebedev et al. 1998). So based on the mentioned studies these two cytotypes were reported as two different sympatric subspecies; *C. e. elburzensis* ($2n=44$), and *C. e. zykovi* ($2n=30$) (Norris et al. 2008).

Taxonomy

We provide evidence of shallow, but well-supported clade structure in *C. elburzensis* that divides the species into two major groups of clades in the phylogeographic analysis of two mtDNA genes. Each major grouping is distributed in separate regions of Iran: the northeastern quadrate (Clades 1-4, Figs. 2 and 4), which includes the type locality of the species in the Elburz Mts. and those samples from the central Iranian ranges stretching from Yazd province in the center of the country to Zanjan province in the northwest (Clades 5-7, Fig. 2 and 5). These separate groups of populations are quite distinct in the morphology of the second lower molar (Fig. 5), have different sets of karyotypes (Fig. 7), and are posited to have separate and unique geographic histories (Fig. 8). We believe the combined data and analyses

warrant the recognition of those samples of *C. elburzensis* from the central and northwestern regions of Iran as a distinct taxon, but one obviously closely related to the nomino-typical members of the species distributed across the northeastern parts of the country and extending into Turkmenistan and northwestern Afghanistan. While members of the two major clades overlap geographically in Yazd province (Fig. 3), it is not possible with present data to determine if they interbreed or are reproductively isolated. Given the rather low level of sequence divergence between them (Table 3), we choose the conservative path and allocate the central-northwestern brush-tailed mice as a subspecies of *C. elburzensis*, which we name, and Shirkuh Mts. is probably a contact zone of two subspecies.

Calomyscus elburzensis isatissus Subsp. novo

Holotype: ZMFUM (Zoological Museum, Ferdowsi University of Mashhad, Mashhad, Iran) 2952, adult female, collected in 2012, by J. Darvish; prepared skin, skull with mandibles, all in good condition; liver and muscle tissues preserved in 96% ethanol and maintained at $-20\text{ }^{\circ}\text{C}$

Type Locality: Ab-Mazrae, Taft, Shirkuh, Yazd Province, Iran (31.6406 N , 54.0853 E ; Table 1; Fig. 1). Collected at an altitude of 3200 m in North Slope of Shirkuh, with 43% moisture, temperature of $11\text{ }^{\circ}\text{C}$ and

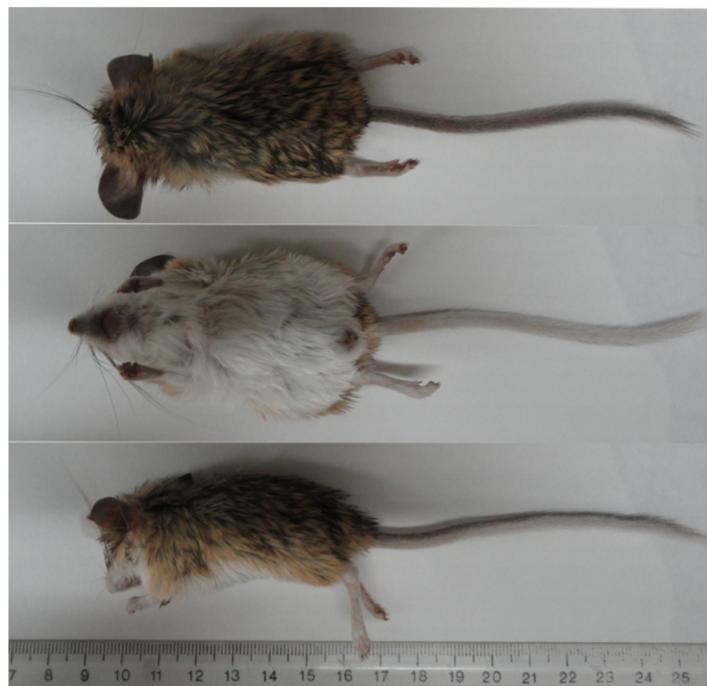


Figure 9. Holotype of *Calomyscus elburzensis isatissus* subsp. novo prior to preparation.



Figure 10. Dorsal (A) and ventral (B) side of cranium, and left mandible (C) of Holotype of *Calomyscus elburzensis isatissus* subsp. novo.

grassland vegetation.

Paratypes: ZMFUM: 3925 and 3937 from Qeidar mountain in Zanjan province (36.1147 N, 48.5911 E), 3938 from Karkas mountain in Isfahan province (33.45 N, 51.7667 E), 3087 Bakhtaky mountain in Mehriz of Yazd province (31.4147 N, 54.43 E), 1777 from FakhrAbad in Shirkuh (31.6667 N, 54.3167 E), 2932, 2949 and 2954 from Ab-Mazraein Shirkuh (31.6404 N, 54.0853 E), 2944, 2945 and 2948 from Cheshme in Shirkuh (31.6553 N, 54.1589 E), 2933 and 2951 from Dare-Bidun in Shirkuh (31.6564 N, 54.0897 E), 3038, 3039 and 3042 from Tezerjan in Shirkuh (31.5675 N, 54.1531 E), 1 and 9 from Godar-Nir in Shirkuh (31.6528 N, 54.1186 E) and 3031 from Ab-Shaghan in Shirkuh Mts. (31.6714 N, 54.0811 E) in Yazd province.

External measurements (in mm) of the holotype: BL: 76, TL: 94, FL: 19, EL: 15.

External measurements (in mm) of paratypes (mean and standard errors): BL: 78.78 ± 4.32 , TL: 90.61 ± 6.26 , FL: 19.39 ± 0.91 , EL: 15.78 ± 2.60 .

Etymology: The subspecies name is referred to the *Isatis*, the ancient name for Yazd.

Description: Adult specimens had light brown color on their back. Hairs of the middle part of dorsal side were grey in the base and tip and were light brown in the middle. Ventral side was yellowish white (Fig. 9). Mean of hind foot length and ear length were shorter than *C. e. elburzensis* samples and wider mean of cranium width from that subspecies. Second lower molar had oval-shaped and posterior part of hypoconid (posterior cusp of labial side) was bulgy (Fig. 6). Chromosome number was 44 and the numbers of autosomal arms were 70 (Fig. 7). Dorsal and ventral side of cranium and labial side of mandible were depicted in Fig. 10.

Distribution: Shirkuh Mountains in Yazd, Karkas Mt. in Isfahan and Qeidar Mts. in Zanjan provinces.

Therefore, in addition to two subspecies of *C. e. elburzensis* and *C. e. zykovi* in north east of Iran and south east of Turkmenistan, the present study describe another subspecies of *C. e. isatissus* subsp. novo in the south and west of central Iranian desert. Future studies should examine hybridization between two different subspecies of *C. elburzensis* especially in Shirkuh Mts. where both of these co-occur.

Acknowledgement

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References

1. Brandelt H-J., Foster P. and Rohlf A. Median joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**: 37-48 (1999).
2. Bruford M. W., Hanotte O., Brookfield J. F. Y., and Burke T. Single locus and multilocus DNA fingerprinting. In: Hoelzel A. R. (Eds.), *Molecular Genetic Analysis of Populations. A Practical Approach*, Oxford, Oxford University Press, pp. 225-269 (1992).

3. Clement M., Posada D., and Crandall K. A. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**: 1657-1660 (2000).
4. Dutrillaux B., Couturier J., Muleris M., Lombard M., and Chauvier G. Chromosomal phylogeny of forty-two species of Cercopithecoidea (Primates Catarrhini). *Ann. Genet-Paris.* **25**: 96-109 (1982).
5. Esmacili R. S., Darvish J., Hadad F., and Ghasemzade F. A new karyotype of *Calomyscus* from the Khorasan Province, Iran. *Hystrix Ital J Mamm.* **19**: 67-71 (2008).
6. Ghorbani M. *The economic geology of Iran, mineral deposits and natural resources*. Springer Dordrecht Heidelberg, New York, 569 p. (2013).
7. Goodwin G. G. Five new rodents from the eastern Elburz mountains and a new race of hare from Tehran. *American Museum Novitates.* **1950**: 1-5 (1938).
8. 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., Leikepova L., Munclinger P., Pialek J., Sadlova J., and Zima J. Comparative cytogenetics of hamsters of the genus *Calomyscus*. *Cytogenet. Cell. Genet.* **88**: 296-304 (2000).
9. Hall T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98NT. *Nucl. Acid. S.* **41**: 95-98 (1999).
10. Hammer Ø., Harper D. A. T., and Ryan P. D. PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica* **4**: 9 pp (2001).
11. Hijmans R. J., Guarino L., and Mathur P. DIVA-GIS. Version 7.5, Manual. Available at: http://www.diva-gis.org/docs/DIVA-GIS_manual.pdf. (2012).
12. Ivanova N. V., Dewaard J. R., and Herbert P. D. N. An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Mol. Ecol. Notes.* **6**: 998-1002 (2006).
13. Jackson M. P. A., Cornelius R. R., Craig C. H., Gansser A., Stocklin J., and Talbot C. J. *Salt Diapirs of the Great Kavir, Central Iran*. Geological Society of America 139 p. (1990).
14. Jansa S. A., and Weksler M. Phylogeny of muroid rodents: relationship within and among major lineages as determined by IRBP gene sequences. *Mol. Phylogenet. Evol.* **31**: 256-276 (2004).
15. Karami M., Hutterer R., Benda P., Siahsharvie R., and Krystufek B. Annotated check-list of the mammals of Iran. *Lynx.* **39**: 63-102 (2008).
16. Krystufek B., and Vohralik V. *Mammals of Turkey and Cyprus, Rodentia II: Cricetinae, Muridae, Spalacidae, Calomyscidae, Capromyidae, Hystricidae, Castoridae*. Univerzita Primorskem, first ed. Knjiznica Annales Majora, Koper 372 p. (2009).
17. Lebedev V. S., Pavlinov I. Ya., Meyer M. N., and Malikov V. G. Craniometric analysis of mouse-like hamsters of the genus *Calomyscus* (Cricetidae). *Zool. Zhurnal.* **6**: 721-731 (1998).
18. Librado P., and Rozas J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451-1452 (2009).
19. Meyer M. N., and Malikov V. G. New species and subspecies of mouse-like hamsters of the genus *Calomyscus* (Rodentia, Cricetidae) from southern Turkmenistan. *Zool. Zhurnal.* **79**: 219-223 (2000) (in Russian).
20. Michaux J., Reyes A., and Catzefflis F. Evolutionary history of the most speciose mammals: molecular phylogeny of muroid rodents. *Mol. Biol. Evol.* **18**: 2017-2031 (2001).
21. Montgelard C., Bentz S., Tirard C., Verneau O., and Catzefflis F. M. Molecular systematics of Sciurognathi (Rodentia): the mitochondrial cytochrome b and 12S rRNA genes support the Anomaluroidea (Peptidae and Anomaluridae). *Mol. Phylogenet. Evol.* **22**: 220-233 (2002).
22. Morshed S., and Patton J. L. New records of mammals from Iran with systematic comments on hedgehogs (Erinaceidae) and mouse-like hamsters (*Calomyscus*, Muridae). *Zool. Middle East.* **26**: 49-58 (2002).
23. Musser G. G., and Carleton M. D. Subfamily Murinae. In: Wilson D.E., Reeder D.M. (Eds.), *mammal species of the world, A taxonomic and geographic reference*, The Johns Hopkins University Press Baltimore, 3rd Ed. volume 2 (2005).
24. Norris R. W., Morshed S., Kilpatrick C. W., Woods C. A., Polina P., Romanenko S. A., and Malikov V. G. The new data on diversity of *Calomyscus* Thomas, 1905 (Rodentia, Calomyscinae). Proceedings of International Conference devoted to the 90-th anniversary of Prof. I. M. Gromov, Saint Petersburg p: 166-169 (2003).
25. Norris R. W., Woods C. A., and Kilpatrick C. W. Morphological and molecular definition of *Calomyscus hotsoni* (Rodentia: Muroidea: *Calomyscus*). *J. Mammal.* **89**: 306-315 (2008).
26. Osgood W. H. Cricetine rodents allied to *Phyllotis*. *J. Mammal.* **28**: 165-174 (1947).
27. Peakall R., and Smouse P. E. GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**: 288-295 (2006).
28. Posada D., and Crandall K. A. Modeltest: Testing the model of DNA substitution. *Bioinformatics* **13**: 817-818 (1998).
29. Rohlf F. J., and Ferson S. EFAW. Version 11794. N. Y. Stat Univ. At Stony Brook (program) (1992).
30. Rohlf F. L. tpsDig version 2.11. SUNY, Stony Brook. New York (2008).
31. Ronquist F., and Huelsenbeck J. P. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574 (2003).
32. Sahebjam B., Darvish J., Rastegar-Pouyani E., Siahsharvie R., and Akbarirad S. A preliminary molecular study of the Iranian species of *Calomyscus* (Rodentia-Calomyscidae) using RFLP. *Iranian Journal of Animal Biosystematics* **5**: 33-41 (2009).
33. Shahabi S., Zarei B., and Sahebjam B. Karyologic study of three species of *Calomyscus* (Rodentia: Calomyscidae) from Iran. *Iranian Journal of Animal Biosystematic* **6**: 55-60 (2010).
34. Shahabi S., Darvish J., Aliabadian M., Mirshamsi O., and Mohammadi, Z. 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 (2011).

35. Shahabi S., Aliabadian M., Darvish J., and Kilpatrick C. W. Molecular phylogeny of brush-tailed mice of the genus *Calomyscus* (Rodentia: Calomyscidae) inferred from mitochondrial DNA sequences. *Mammalia* **77**: 425-431 (2013).
36. Stepan S. J., Adkins R. M., and Anderson J. Phylogeny and divergence-date estimates of rapid radiation in muroid rodents based on multiple nuclear genes. *Systematic Boil.* **53**: 533-553 (2004).
37. Swofford D. L. PAUP. Phylogenetic Analysis Using Parsimony (and other methods), Version 4b10. Sinauer Associates, Sunderland, MA. (2002).
38. Taleghani M. A. *Geomorphology of Iran*. Ghomes Publishing Company Ltd, Tehran p. 404 (2005).
39. Tamura K., Peterson D., Peterson N., Strecher G., Nei M., and Kumar S. Mega5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**: 2731-2739 (2011).
40. Taravati S. GMTTP: Geometric morphometric tools package version 2.0 beta (program) (2010).
41. Thompson J. D., Higgins D. G., and Gibson T. J. CLUSTAL W: improving the sensitivity of progressive multiple sequences alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673-4680 (1994).
42. Yan Y., Harris A. J., and Xingjin H. S-DIVA (Statistical Dispersal-Vicariance Analysis): A tool for inferring biogeographic histories. *Mol. Phylogenet. Evol.* **56**: 848-850 (2010).
43. Yan Y., Harris A. J., and Xingjin H. RASP (Reconstruct Ancestral State in Phylogenies) 1.1. Available at: <http://mnh.scu.edu.cn/soft.blog.RASP> (2011).

Supplementary file 1. Details of sampled localities, tissue and voucher numbers and accession numbers of specimens from Iran (for each sample the first accession number is for *Cytb* and the second is for *CoI* genes).

Species	Locality	Voucher number	Accession number	
			<i>Cytb</i>	<i>CoI</i>
<i>C. elburzensis</i>	Dasht, North Khorasan	ZMFUM 1933	-	KT884579
<i>C. elburzensis</i>	Bojnord, Chenaran, North Khorasan	ZMFUM 3100	KT878589	-
<i>C. elburzensis</i>	Bojnord, Chenaran, North Khorasan	ZMFUM 3101	KU043018	KT878549
<i>C. elburzensis</i>	Bojnord, Chenaran, North Khorasan	ZMFUM 3173	KU043019	-
<i>C. elburzensis</i>	Esfaraïen, Saluk, North Khorasan	ZMFUM 2978	KT878588	KU043034
<i>C. elburzensis</i>	Daregaz, Tandure, Khorasan-e-Razavi	ZMFUM 1669	-	KT884577
<i>C. elburzensis</i>	Daregaz, Tandure, Khorasan-e-Razavi	ZMFUM 1675	KT884548	KT884578
<i>C. elburzensis</i>	Neyshabur, Binalud, Khorasan-e-Razavi	ZMFUM 2148	KT884550	KT884580
<i>C. elburzensis</i>	Neyshabur, Binalud, Khorasan-e-Razavi	ZMFUM 2152	KT884551	KT884581
<i>C. elburzensis</i>	Mashhad, KhajeMorad, Neyshabur, Binalud, Khorasan-e-Razavi	ZMFUM 1542	KT878581	KT878542
<i>C. elburzensis</i>	Mashhad, KhajeMorad, Neyshabur, Binalud, Khorasan-e-Razavi	ZMFUM 1546	KT884547	KT884576
<i>C. elburzensis</i>	Sarakhs, Aghdarband, Khorasan-e-Razavi	ZMFUM 1921	KT884549	-
<i>C. elburzensis</i>	Sarakhs, Aghdarband, Khorasan-e-Razavi	ZMFUM 1922	KT878586	KT878547
<i>C. elburzensis</i>	Sarakhs, Aghdarband, Khorasan-e-Razavi	ZMFUM 1874	KT878585	KT878546
<i>C. elburzensis</i>	Torbate-Jam, NasrAbad, Khorasan-e-Razavi	ZMFUM 2088	KT878587	KT878548
<i>C. elburzensis</i>	Bajestan, Siahkuh, Khorasan-e-Razavi	ZMFUM 2195	KT884552	KT884582
<i>C. elburzensis</i>	Sabzevar, Zarghan, Khorasan-e-Razavi	ZMFUM 4490	KT884556	KT884585
<i>C. elburzensis</i>	Ghaen, HajiAbad, South Khorasan	ZMFUM 3304	KT884553	KT884583
<i>C. elburzensis</i>	Ghaen, HajiAbad, South Khorasan	ZMFUM 3305	KT884554	-
<i>C. elburzensis</i>	Birjand, Darmiyan, South Khorasan	ZMFUM 4530	KT884558	-
<i>C. elburzensis</i>	Bijand, Gazik, South Khorasan	ZMFUM 4529	KT884557	KT884586
<i>C. elburzensis</i>	Mahriz, Kuhe-Bakhtaki, Yazd	ZMFUM 3087	KU043017	-
<i>C. elburzensis</i>	FakhrAbad, Yazd	ZMFUM 1719	KT878582	KT878544
<i>C. elburzensis</i>	FakhrAbad, Yazd	ZMFUM 1777	KT878584	KT878545
<i>C. elburzensis</i>	FakhrAbad, Yazd	ZMFUM 1780	KT878583	KT878543
<i>C. elburzensis</i>	Taft, Shirkuh, Ab-Mazrae, Yazd	ZMFUM 2932	KU043004	KU043027
<i>C. elburzensis</i>	Taft, Shirkuh, Ab-Mazrae, Yazd	ZMFUM 2949	KU043008	-
<i>C. elburzensis</i>	Taft, Shirkuh, Ab-Mazrae, Yazd	ZMFUM 2952	KU043010	KU043030
<i>C. elburzensis</i>	Taft, Shirkuh, Ab-Mazrae, Yazd	ZMFUM 2954	-	KU043031
<i>C. elburzensis</i>	Taft, Shirkuh, Cheshme, Yazd	ZMFUM 2944	KU043005	-
<i>C. elburzensis</i>	Taft, Shirkuh, Cheshme, Yazd	ZMFUM 2945	KU043006	KU043028
<i>C. elburzensis</i>	Taft, Shirkuh, Cheshme, Yazd	ZMFUM 2948	KU043007	KU043029
<i>C. elburzensis</i>	Taft, Shirkuh, Dare-Bidun, Yazd	ZMFUM 2951	KU043009	-
<i>C. elburzensis</i>	Taft, Shirkuh, Dare-Bidun, Yazd	ZMFUM 2963	KU043011	KU043032

Phylogeography of *Calomyscus elburzensis* (Calomyscidae, Rodentia) around...

Cntd.

<i>C. elburzensis</i>	Taft, Shirkuh, Dare-Bidun, Yazd	ZMFUM 2965	KU043012	KU043033
<i>C. elburzensis</i>	Taft, Shirkuh, Tezerjan, Yazd	ZMFUM 3038	KU043014	-
<i>C. elburzensis</i>	Taft, Shirkuh, Tezerjan, Yazd	ZMFUM 3039	KU043015	KU043036
<i>C. elburzensis</i>	Taft, Shirkuh, Tezerjan, Yazd	ZMFUM 3042	KU043016	-
<i>C. elburzensis</i>	Taft, Shirkuh, Godar-Nir, Yazd	ZMFUM 1	KU042999	KU043023
<i>C. elburzensis</i>	Taft, Shirkuh, Godar-Nir, Yazd	ZMFUM 4	KU043000	KU043024
<i>C. elburzensis</i>	Taft, Shirkuh, Godar-Nir, Yazd	ZMFUM 9	KU043001	KU043025
<i>C. elburzensis</i>	Taft, Shirkuh, Godar-Nir, Yazd	ZMFUM 10	KU043002	KU043026
<i>C. elburzensis</i>	Taft, Shirkuh, Godar-Nir, Yazd	ZMFUM 11	KU043003	-
<i>C. elburzensis</i>	Taft, Shirkuh, Ab-Shaghan, Yazd	ZMFUM 3031	KU043013	KU043035
<i>C. elburzensis</i>	Karkas, Isfahan	ZMFUM 3938	KU043022	KU043039
<i>C. elburzensis</i>	Qeidar, Zanjan	ZMFUM 3925	KU043020	KU043037
<i>C. elburzensis</i>	Qeidar, Zanjan	ZMFUM 3937	KU043021	KU043038
<i>C. elburzensis</i>	Kurkhud, North Khorasan	ZMFUM 3629	KT884555	KT884584
<i>C. elburzensis</i>	Kurkhud, North Khorasan	ZMFUM 3533	KT878590	KT878550
<i>C. grandis</i>	Fasham, Tehran	ZMFUM 1985	KT878591	KT878551
<i>C. grandis</i>	Fasham, Tehran	ZMFUM 1986	KT878592	KT878552
<i>C. grandis</i>	Fasham, Tehran	ZMFUM 1987	KT878593	KT878553
<i>C. grandis</i>	Fasham, Tehran	ZMFUM 3992	KT884559	KT884587
<i>C. mystax</i>	Bojnord, TakaleGhoze, North Khorasan	ZMFUM 2981	KU129018	-
<i>C. mystax</i>	Bojnord, TakaleGhoze, North Khorasan	ZMFUM 2984	KU129019	KU129021
<i>C. mystax</i>	Bojnord, TakaleGhoze, North Khorasan	ZMFUM 3084	KU129020	KU129022
<i>C. urartensis</i>	Kordasht, Eastern Azerbaijan	ZMFUM 2253	KT878594	KT878554
<i>C. urartensis</i>	Kordasht, Eastern Azerbaijan	ZMFUM 2908	KT878595	KT878555