

# Biosystematic study of *Calomyscus mystax* (Rodentia, Calomyscidae) from northeastern Iran

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We use a combination of traditional and geometric morphometric plus phylogenetic analyses of mitochondrial cytochrome b sequences to confirm the presence in the Kopetdagh Mts. of northeastern Iran (North Khorasan province) of the Great Balkhan brush-tailed mouse, *Calomyscus mystax* Kashkarov, 1925 previously regarded as endemic to the Nibit-Dag region of southwestern Turkmenistan. We further compare *C. mystax* to other brush-tailed mice species in eastern and northern Iran and provide diagnostic external and craniodental characters for each and to examine the affinities with *C. grandis* with which *C. mystax* shares a similar karyotype.

**Key words:** *Calomyscus mystax*; Kopetdagh Mts.; northeastern Iran.

## INTRODUCTION

The Great Balkhan brush-tailed mouse, *Calomyscus mystax* Kashkarov, 1925 is known from the Great Balkhan Mountains in southwestern Turkmenistan where it was first described from Bolshoi (=Greater) Balkhan Mts. (Nibit-Dag region), Bolshoi Murgul (Kashkarov, 1925). Vorontsov et al. (1979) extended its geographic range to include the eastern region of the Lesser Balkhan and Kopetdagh Mts. in southern Turkmenistan and the North Khorasan and Mazandaran provinces of northeastern Iran. Musser and Carleton (1993), Meyer and Malikov (1995, 2000) and Pavlinov and Rossilimo (1998) included the Lesser Balkhans and Kopetdagh Mts. within the distribution of *C. mystax*. However, Meyer and Malikov (2000) recognized the forms from the central Kopetdagh Mts. of Turkmenistan as *C. firiusaensis* and forms from the western and central Kopetdagh Mts. of Turkmenistan as *C. mystax zykovi*. Musser and Carleton (2005) included both *C. firiusaensis* and *C. mystax zykovi* in *C. elburzensis* and restricted *C. mystax* to southwestern Turkmenistan.

*C. mystax* was recognized as a distinct species by Vorontsov et al. (1979) based on its morphology and geographic distribution and its morphometric divergence was further confirmed by Lebedev et al. (1998). Specimens of *C. mystax* from the Great Balkhan region have a karyotype with a  $2N=44$  and  $FN_a=46$ , whereas most specimens currently recognized as *C. elburzensis* have a karyotype with a

2N=44 and FN<sub>a</sub>=58 (Graphodatsky et al., 2000). A different cytotype with a 2N=30, FN<sub>a</sub>=44 has been reported from central and western Kopetdagh Mts. (Graphodatsky et al., 2000), which was described as *C. mystax zykovi*. Partial reproductive isolation (Meyer & Malikov, 2000) and differences in cranial measurements (Lebedev et al., 1998) have been reported between *C. mystax zykovi* and *C. elburzensis*. Musser and Carleton (2005) included *C. mystax zykovi* in *C. elburzensis* and Norris et al. (2008) tentatively recognized it as a subspecies of this taxon.

*C. mystax* possess an identical karyotype with *C. grandis* from central Elburz Mts. in Tehran province. This similarity may be the result of having a common ancestor which was disturbed throughout the Koptdagh and Elburz Mts. Alpine glaciations in the Pleistocene may have isolated the northern populations in Turkmenistan from the southern ones in Iran (Malikov et al., 1999) facilitating morphological and genetic divergence. However, sharing similar cytotype can also be as a consequence of convergence (Graphodatsky et al., 2000).

The objectives of this paper were to determine the affinities of specimens recently collected from the Kopetdagh Mts. in northeastern Iran and to examine the phylogenetic affinities among the two taxa, *C. mystax* and *C. grandis*, that share a similar karyotype (2N=44; FN<sub>a</sub>=46).

## MATERIAL AND METHODS

Our analyses included data from 78 specimens of *Calomyscus* from Iran and a single sample of *C. mystax* from Turkmenistan (Table 1). The Iranian material includes 61 samples provided by Zoology Museum of Ferdowsi University of Mashhad (ZMFUM) and 17 newly captured specimens. These samples were collected from 14 localities and include specimens of *C. elburzensis*, *C. hotsoni*, *C. grandis* and six samples of *Calomyscus* sp. (Table 1 and Fig. 1). All of the specimens from Iran are deposited in ZMFUM (supplemental data). In addition, ethanol preserved tissue from a single specimen of *C. mystax* from voucher 83939 deposited in the Zoological Institute in St. Petersburg, Russia, was provided by Vladimar Malikov and was utilized in the molecular analysis (supplemental data)

**TABLE 1.** Locality, species and number of each species samples which were used in morphometric (first numbers) and molecular (second numbers in parentheses) analyses in this study.

N (sequenced samples)	Species	Locality City, province	Coordinates
2 (2)	<i>Calomyscus</i> sp.	Jodar, Kopetdagh Mts., North Khorasan	37.9533 N, 57.3656 E
3 (3)	<i>Calomyscus</i> sp.	Takale-ghoz, Kopetdagh Mts., North Khorasan	38.2164 N, 57.1847 E
1 (1)	<i>Calomyscus</i> sp.	Sambar, Kopetdagh Mts., North Khorasan	38.2108 N, 56.6894 E
9 (2)	<i>C. elburzensis</i>	KhajeMorad, Khorasan-e-Razavi	36.25 N, 59.5667 E
4 (1)	<i>C. elburzensis</i>	Torbat-e-Jam, Khorasan-e-Razavi	35.15 N, 60.4 E
4 (3)	<i>C. elburzensis</i>	Aghdarband, Khorasan-e-Razavi	36.5 N, 61.1167 E
2 (1)	<i>C. elburzensis</i>	Dargaz, Kopetdagh Mts., Khorasan-e-Razavi	37.4333 N, 58.7167 E
27 (2)	<i>C. elburzensis</i>	Neyshabur, Khorasan-e-Razavi	36.3167 N, 58.8831 E
4 (3)	<i>C. elburzensis</i>	Chenaran, North Khorasan	37.4219 N, 57.3764 E
3 (1)	<i>C. elburzensis</i>	Saluk, North Khorasan	37.1633 N, 57.1669 E
4 (2)	<i>C. elburzensis</i>	Kurkhud, North Khorasan	37.8167 N, 56.6833 E
5 (1)	<i>C. elburzensis</i>	FakhrAbad, Yazd	31.6667 N, 54.3167 E
5 (3)	<i>C. grandis</i>	Fasham, Tehran	35.9344 N, 51.5242 E
5 (4)	<i>C. hotsoni</i>	Saravan, Sistan-o-Baluchistan	27.3 N, 61.7667 E

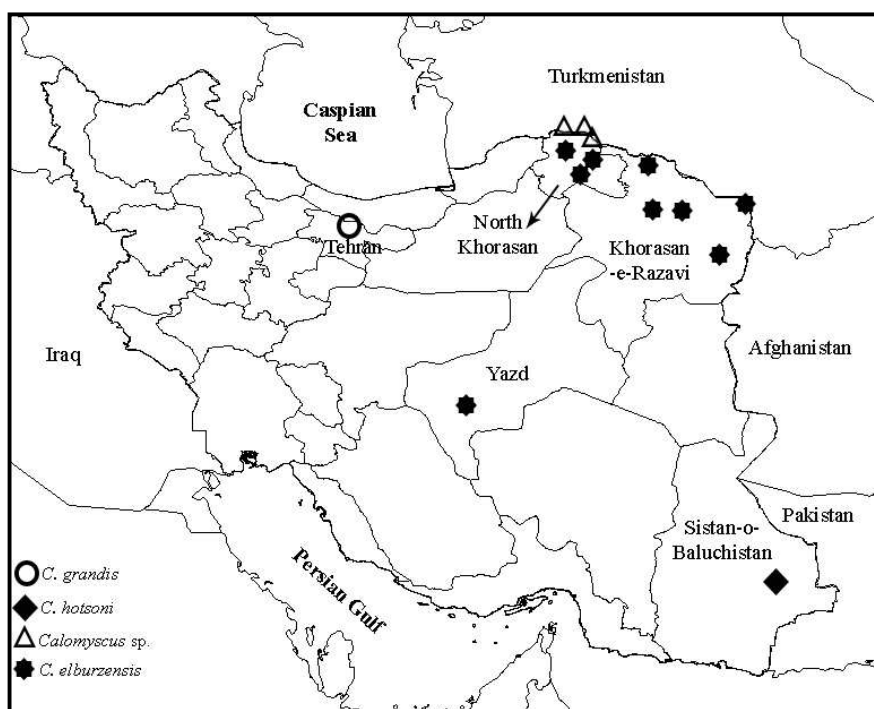


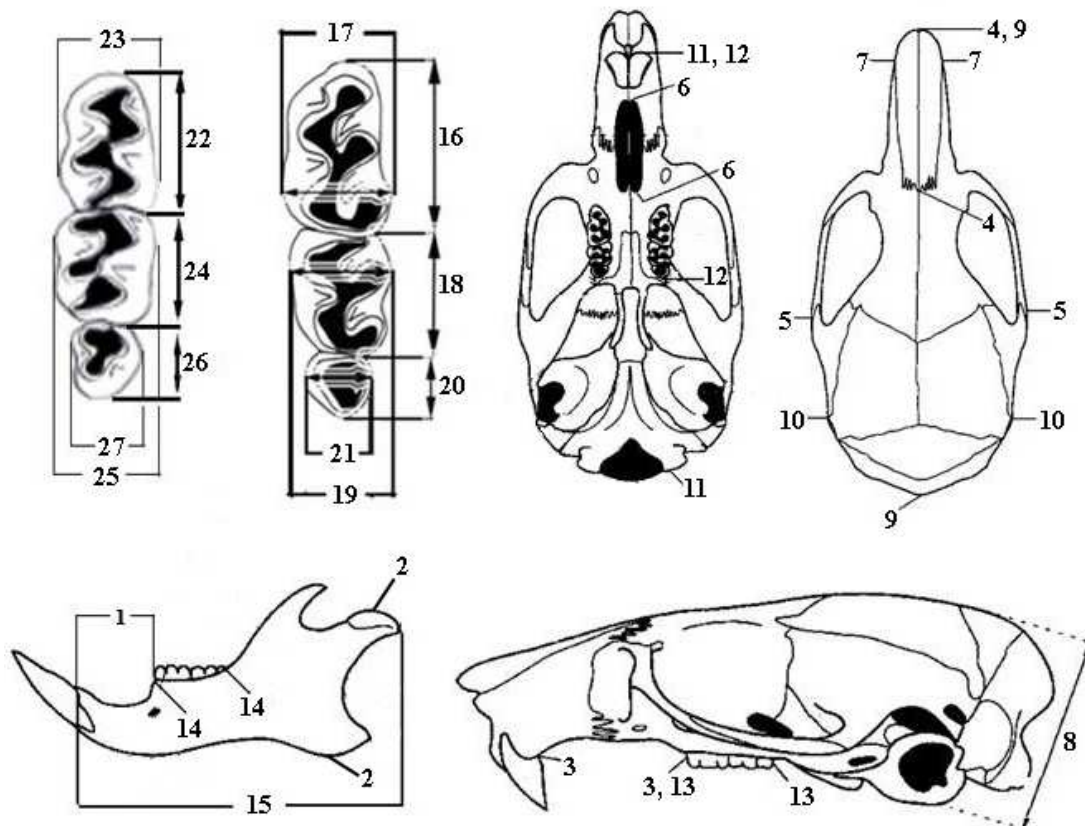
FIGURE 1. Sampling localities represented for four species of *Calomyscus*.

### Molecular analyses

Genomic DNA was extracted from fresh or 96% ethanol preserved muscle or liver tissue using a standard salt method extraction (Bruford et al., 1992). The mitochondrial cytochrome b gene was amplified using the modified universal L7 and H6 primers and protocol of Montgelard et al. (2002) or the primers and protocol used by Norris et al. (2008). PCR products were either sequenced at the MacroGen Company, Republic of South Korea or on an Applied Biosystem 373 automated DNA sequencer at the University of Vermont, USA. The sequences were aligned and edited by BioEdit v7.1.3.0 (Hall, 1999). Mean genetic distance (K2P) was calculated within and among pairwise comparisons of species using Mega5 (Tamura et al. 2011) and ExcaliBAR (Aliabadian et al., 2014). Bayesian analyses were conducted with MrBayes 3.1.1 (Ronquist & Huelsenbeck, 2003), using a TVM+I+G model of molecular evolution. Four Markov Chain Monte Carlo (MCMC) were run simultaneously for 6 million generations and the first 60,000 trees (as a conservative 'burn-in') were discarded. Posterior probabilities were calculated from the remaining trees using a majority-rule consensus analysis. The Bayesian tree was rooted with *Spalax ehrenbergi* from Spalacidae (Muroidea) and basal to the Eumorida (which included Calomyscidae) (Steppan et al., 2004). Cytb sequences of the outgroup, two additional *C. hotsoni* and three *C. baluchi* were retrieved from GenBank (supplementary data).

### Morphometric analyses

Four external (BL: body length, TL, tail length, EL: ear length and FL: hind foot length), 15 cranium and 12 dental variables were measured from adult specimens by a rule, digital caliper and measurescope, respectively (Fig. 2). Measurements were checked for normality with a Shapiro-Wilk test and the presence of sexual dimorphism and significant of differentiation of variables among species and groups were evaluated by ANOVAs. Eleven external and cranium measurements of type specimens of *C. mystax* recorded by Kashkarov (1925) were compared with four groups of brush-tailed mice.

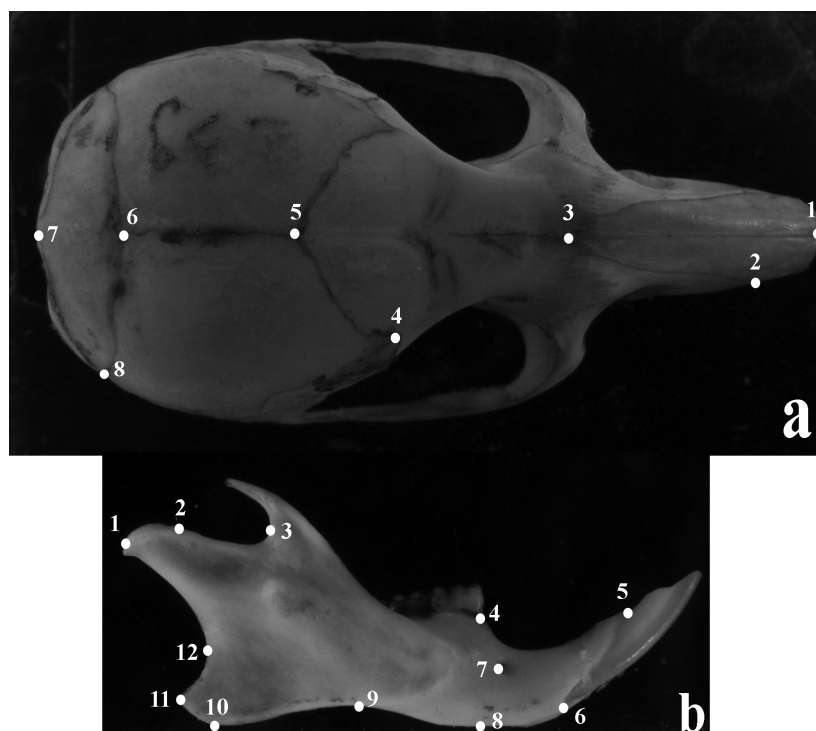


**FIGURE 2.** Description of cranial and dental measurements. 1-LLD (lower length of diastema); 2-MH (mandibular height); 3-UDL (upper diastema length); 4-NL (nasal length); 5-ZW (zygomatic width); 6-Forl (length of anterior palatine foramen); 7-NW (nasal width); 8-SH (skull height); 9-Occl (occipitonasal length); 10-CW (cranium width); 11-CBL (condylobasal length); 12-Patl (palatal length); 13-Mxl (maxillary tooth row); 14-Mnl (mandibular tooth row); 15-Mndl (mandible length); 16-M.1L (length of M1); 17-M.1W (width of M1); 18-M.2L (length of M2); 19-M.2W (width of M2); 20-M.3L (length of M3); 21-M.3W (width of M3); 22-m1.L (length of m1); 23-m1.W (width of m1); 24-m2.L (length of m2); 25-m2.W (width of m2); 26-m3.L (length of m3); 27-m3.W (width of m3).

Canonical Variate Analysis was conducted on four groups of brush-tailed mice. All the statistical analyses were performed using PAST v2.08 (Hammer et al., 2001) and SPSS Base 20 (SPSS Inc. 2011).

### Geometric-morphometric analyses

Shape variations in cranium and mandible were evaluated based on eight digitized landmarks on the dorsal side of the skull and 12 landmarks on the labial side of the mandible of each specimen (Fig. 3). Landmarks were digitized using the software TpsDig (Rohlf, 2001) and then significant differences among groups was calculated using ANOVA (in PAST). Landmark configurations were superimposed with procrustes method. Shape variations among superimposed configurations were visualized as a thin-plate spline deformation grid, and also through a canonical variate analysis of landmark coordinates or warp scores. Discriminant function analyses were then conducted to explore shape changes in pairs of groups.



**FIGURE 3.** Landmarks depicted on dorsal view of the studied skulls (a) and labial side of mandible (b).

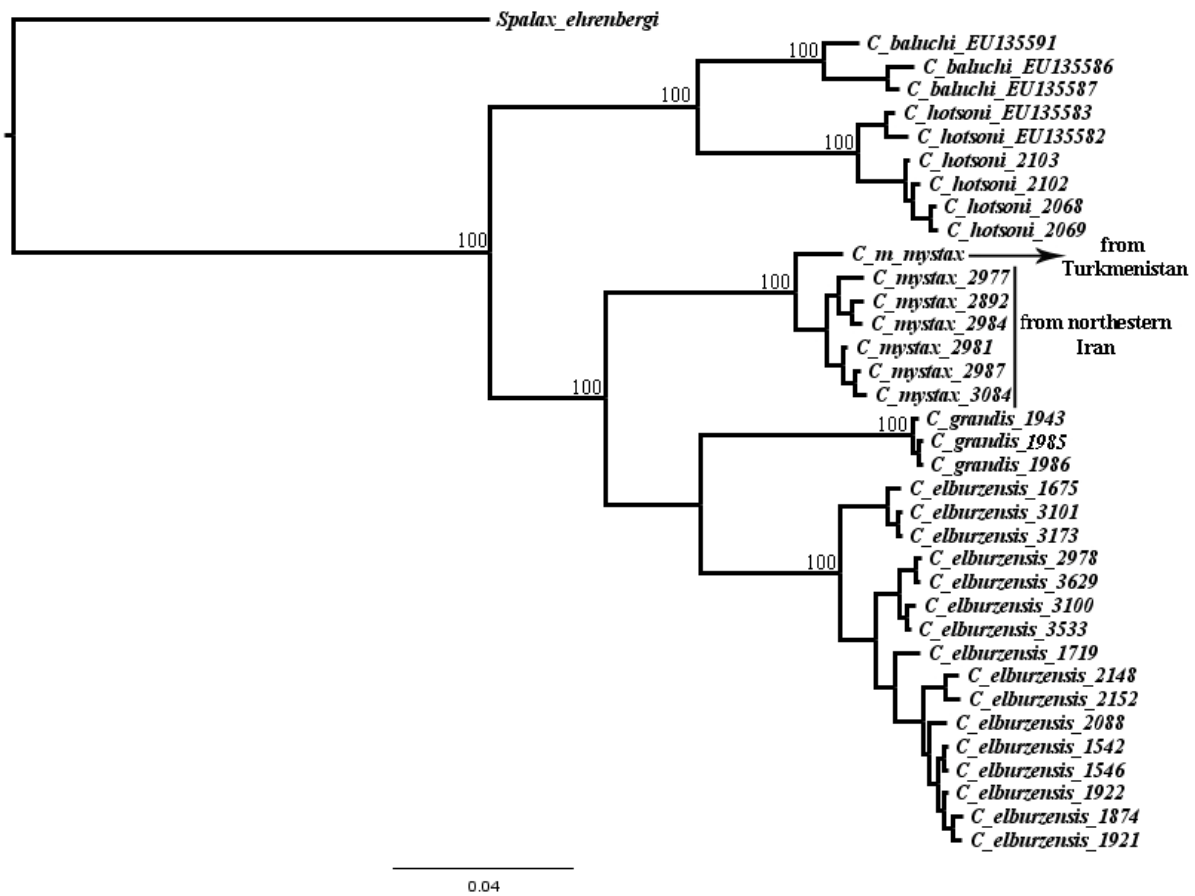
## RESULTS

### Molecular results

Cytochrome b sequences consisting of 988 bases without any stop codon were utilized for phylogenetic analysis. A total of 142 different mutations were observed among the sequenced examined and of these 988 bps, 117 sites were variable and 87 were parsimony informative. The phylogenetic tree (Fig. 4) constructed with Bayesian analysis placed the samples of brush-tailed mice captured from Kopetdagh Mountains in northeastern Iran, which had not previously been assigned to any species of *Calomyscus*, with *C. mystax* from Turkmenistan. This *C. mystax* clade containing sequences from both Turkmenistan and Iran was highly support with a posterior probability of 100% (Fig. 4). This *C. mystax* clade was the sister group to a *C. grandis* and *C. elburzensis* clade, forming a group of 3 species that all have a diploid number of 44. Low K2P genetic distance, 1.4%, was observed between individuals of *Calomyscus* sp. from northeastern Iran and *C. mystax* from Turkmenistan, in comparison to their mean genetic distances with other species (13.82%).

### Morphometric results

Most of the variables had a normal distribution and no sexual dimorphism was detected in our analyses ( $p > 0.05$ ). ANOVA and Tukey's test were conducted for identifying cranium and dental variables that differed significantly ( $p < 0.05$ ) among specimens from the Kopetdagh Mts. in northeastern Iran which were recognized as *C. mystax* based on their cyt b sequences, with other species of brush-tailed mice occurring in northern Iran. *C. mystax*, as well as specimens of *C. hotsoni* and *C. elburzensis*, showed significant shorter lower and upper diastem, shorter nasal, anterior palatine foramen, occipitonasal, condylobasal, palatal and mandible, with narrower nasal and cranium than *C. grandis* (Table 2). Specimens of *C. mystax* differed significantly from *C. elburzensis* in the neighboring regions in length of ear, lower diastem and third lower molar that were shorter in *C. mystax*. The only difference of *C. mystax* and *C. hotsoni* was in wider rostrum in the former species. In addition, *C.*



**FIGURE 4.** Phylogenetic tree based on the Bayesian analysis of *Calomyscus* species cytochrome b data. Posterior probability values are indicated for each node with significant level >95.

*hotsoni* had significantly shorter upper diastema, nasal, anterior palatine foramen and palatal with narrower nasal than *C. elburzensis*.

The mean of eleven external and cranial measurements of two type specimens of *C. mystax* recorded by Kashkarov (1925) were compared with external and cranium linear measurements of samples from northeastern Iran which clustered with *C. mystax* in phylogenetic tree. The mean of two individuals measures of Kashkarov' (1925) *C. mystax* are situated in the range of 5 specimens' external and cranium quantities of brush-tailed mice from northeastern Iran in the present study (Table 3) and confirmed belonging these 5 specimens to *C. mystax* species.

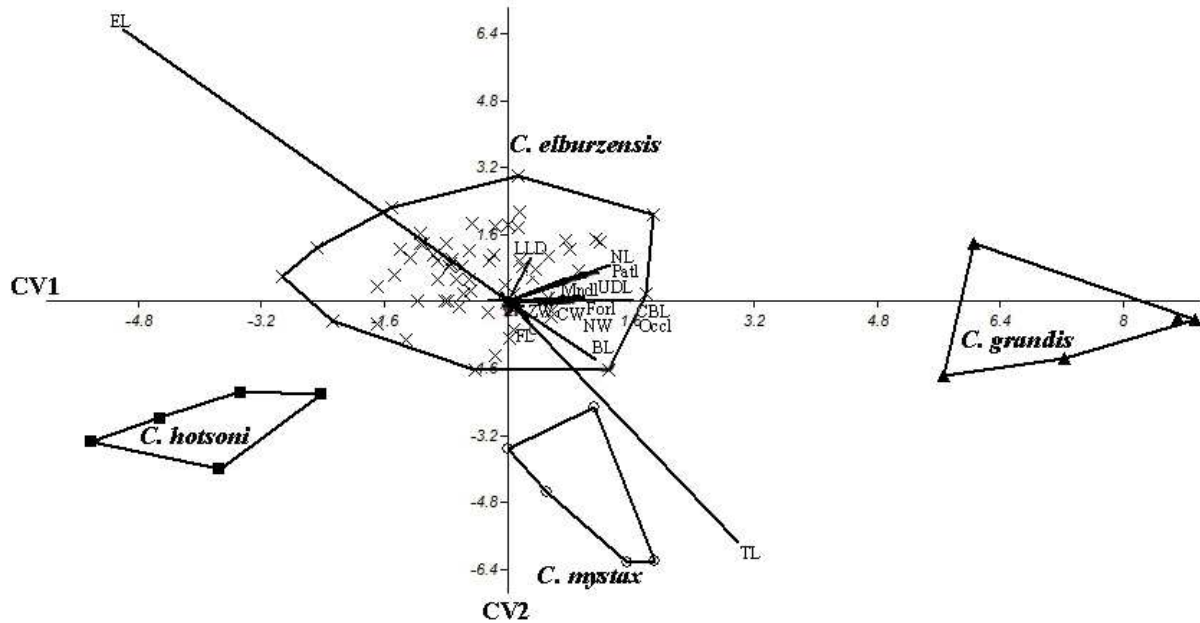
In the canonical variate analysis the first and second axes contained 56.67% and 28.02% of total variance, respectively (Table 4) and significant separation was observed among each species (Fig. 5). In the first CV, *C. elburzensis* and *C. mystax* overlapped with each other, but are separated from *C. grandis* and *C. hotsoni* (Fig. 5). The CV2 discriminated two species of *C. elburzensis* and *C. grandis* from two others, *C. hotsoni* and *C. mystax* (Fig. 5). Mahalanobis distances between group centroids were estimated which showed significant separation among these four species of brush-tailed mice (Table 5).

**TABLE 2.** Means, standard errors and range of variation for morphometric measurements in four species of *Calomyscus* ( $n$ = sample size).

Taxon Variables	<i>C. grandis</i> $n=5$		<i>C. hotsoni</i> $n=5$		<i>C. elburzensis</i> $n=63$		<i>C. mystax</i> $n=5$	
	Mean±Sd	Min-Max	Mean±Sd	Min-Max	Mean±Sd	Min-Max	Mean±Sd	Min-Max
BL	79.20±6.53	70-86	78.20±3.03	74-81	78.78±7.26	52-90	80.25±2.75	77-83
TL	91.20±4.54	85-97	88.50±6.45	80-94	90.08±5.40	75-100	95.25±3.40	92-100
FL	20.40±1.14	19-22	20.30±0.83	19-21	20.10±1.05	17-22	20.25±0.95	19-21
EL	14.60±3.57	10-18	17.80±2.25	15-20	18.39±2.08	11-22	14.50±1.73	12-16
LLD	3.56±0.12	3.5-3.79	3.15±0.24	2.84-3.41	3.40±0.21	2.87-3.85	2.93±0.23	2.6-3.24
HLD	2.17±0.05	2.1-2.23	2.25±0.11	2.1-2.38	2.15±0.10	1.72-2.44	2.10±0.04	2.04-2.16
MH	5.96±0.21	5.72-6.21	5.86±0.43	5.33-6.3	5.73±0.22	5.14-6.31	5.71±0.19	5.52-6.04
UDL	7.08±0.16	6.93-7.29	6.08±0.30	5.72-6.51	6.50±0.25	5.48-7.11	6.29±0.29	5.91-6.69
NL	10.54±0.30	10.22-10.98	9.13±0.50	8.69-9.75	9.73±0.39	8.6-10.81	9.42±0.37	9.03-9.89
ZW	12.84±0.20	12.61-13.02	12.36±0.45	11.72-12.8	12.61±0.37	11.33-13.84	12.55±0.28	12.17-12.92
Forl	5.15±0.25	4.94-5.55	4.25±0.24	3.97-4.54	4.57±0.25	3.71-5.03	4.66±0.27	4.26-4.93
NW	3.28±0.20	2.98-3.51	2.57±0.09	2.43-2.68	2.81±0.16	2.41-3.14	2.95±0.19	2.79-3.28
Intw	4.05±0.10	3.91-4.19	4.10±0.15	3.93-4.28	4.08±0.10	3.88-4.32	3.99±0.16	3.82-4.27
SH	8.00±0.18	7.86-8.32	8.22±0.08	8.14-8.33	8.22±0.19	7.8-8.64	8.25±0.11	8.14-8.42
Occl	26.33±0.42	25.65-26.81	25.18±0.40	24.8-25.6	25.06±0.49	23.64-26.18	24.84±0.63	24.25-25.58
CW	11.84±0.13	11.65-11.96	11.23±0.51	10.58-11.93	11.29±0.26	10.56-11.85	11.23±0.24	10.91-11.51
CBL	23.23±0.19	22.88-23.36	21.88±0.37	21.34-22.17	22.00±0.44	20.74-22.85	21.92±0.57	21.27-22.52
Patl	12.07±0.20	11.86-12.31	10.87±0.40	10.36-11.33	11.44±0.33	10.38-12.22	11.32±0.43	10.84-11.81
Mxl	3.41±0.10	3.28-3.51	3.47±0.08	3.36-3.58	3.48±0.12	3.19-3.76	3.38±0.07	3.32-3.5
Mnl	3.35±0.06	3.29-3.45	3.36±0.09	3.24-3.48	3.38±0.11	3.15-3.78	3.35±0.08	3.26-3.45
Mndl	13.42±0.19	13.18-13.65	12.69±0.51	12.13-13.35	12.91±0.38	11.63-13.8	12.64±0.26	12.33-12.98
M.1L	1.601±0.062	1.504-1.67	1.665±0.040	1.604-1.702	1.642±0.065	1.486-1.794	1.638±0.034	1.599-1.693
M.1W	1.070±0.026	1.052-1.114	1.098±0.044	1.045-1.145	1.090±0.042	1.016-1.187	1.083±0.030	1.044-1.122
M.2L	1.202±0.024	1.167-1.225	1.185±0.048	1.124-1.252	1.177±0.047	1.077-1.303	1.134±0.033	1.095-1.183
M.2W	1.103±0.032	1.079-1.140	1.069±0.041	1.03-1.135	1.064±0.031	1.012-1.138	1.056±0.019	1.044-1.089
M.3L	0.545±0.036	0.495-0.574	0.620±0.042	0.584-0.682	0.578±0.039	0.49-0.68	0.555±0.034	0.519-0.609
M.3W	0.746±0.036	0.693-0.791	0.716±0.712	0.658-0.817	0.720±0.034	0.642-0.801	0.719±0.054	0.663-0.795
m1.L	1.418±0.057	1.322-1.471	1.453±0.049	1.384-1.497	1.413±0.052	1.319-1.541	1.470±0.034	1.42-1.508
m1.W	0.926±0.014	0.909-0.947	0.980±0.020	0.947-1.004	0.966±0.038	0.897-1.081	0.980±0.014	0.961-0.998
m2.L	1.222±0.025	1.199-1.26	1.235±0.053	1.187-1.327	1.217±0.041	1.128-1.323	1.220±0.049	1.146-1.265
m2.W	1.004±0.038	0.957-1.055	1.026±0.026	1.005-1.07	1.033±0.031	0.977-1.123	1.019±0.013	0.999-1.035
m3.L	0.735±0.051	0.667-0.808	0.736±0.038	0.682-0.773	0.728±0.041	0.627-0.821	0.669±0.032	0.627-0.706
m3.W	0.677±0.042	0.611-0.708	0.685±0.030	0.661-0.73	0.701±0.032	0.628-0.766	0.704±0.050	0.65-0.768

**TABLE 3.** Comparison of eleven morphometric variables between two type specimens of *C. mystax* and five specimens from northeastern Iran.

Variables	Type specimens of <i>C. mystax</i> and Samples from northeastern Iran		
	Mean	Minimum	Maximum
BL	81	77	83
EL	15	12	16
FL	20	19	21
Occl	25.5	24.25	25.58
CW	11.35	10.91	11.51
NL	9.3	9.03	9.89
NW	2.9	2.79	3.28
Forl	4.4	4.26	4.93
Intw	4.05	3.82	4.27
Mxl	3.4	3.32	3.5
Mnl	3.45	3.26	3.45



**FIGURE 5.** Scatter plot of the first two canonical variates of morphometric variables with the most important character vectors on the first two CV axes.

**TABLE 4.** Eigenvalue, percentage of total variance and cumulative percentage of principal component loading in four species of *Calomyscus* for the first two CV axes.

Functions	Eigenvalues	%Variance	% Cumulative
1	4.821	56.67	58.561
2	2.384	28.02	84.220

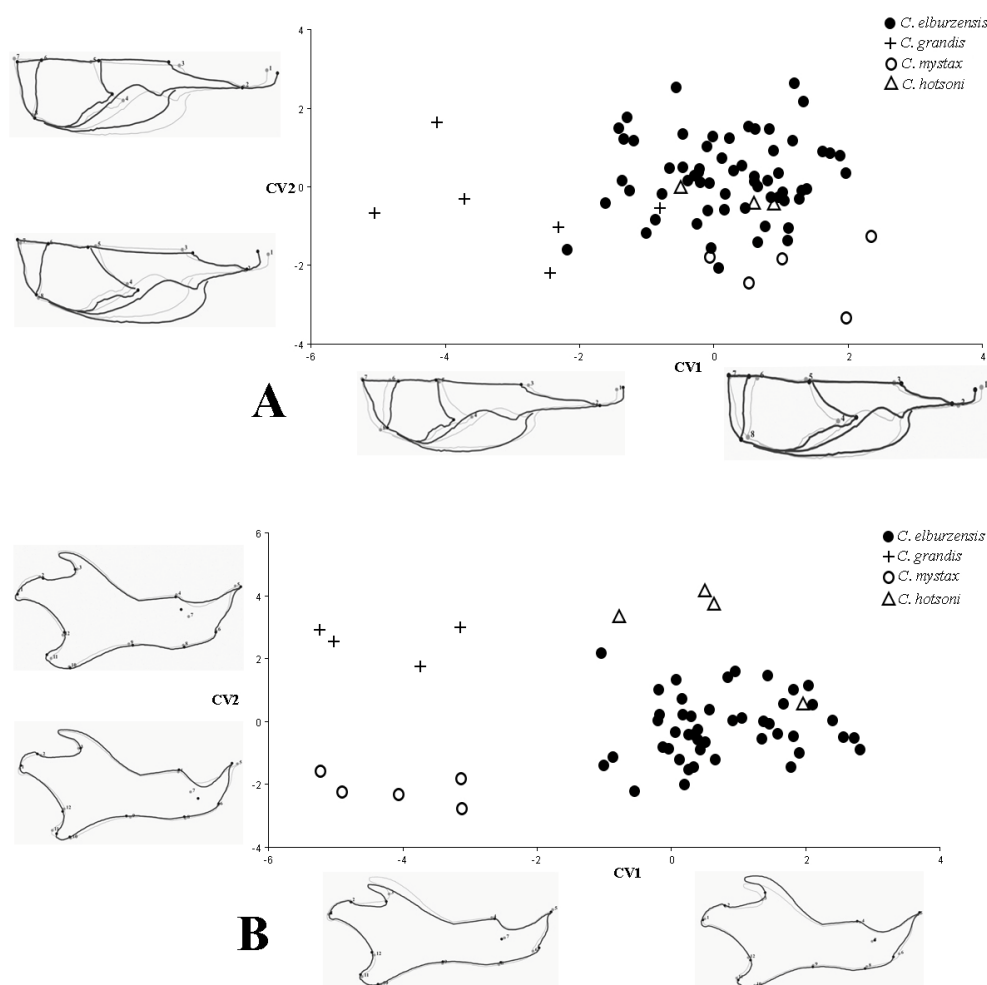
**TABLE 5.** Mahalanobis distances between pairs of centroid groups ( $p < 0.05$ ). \*: indicated the significant separation between groups.

	<i>C. grandis</i>	<i>C. hotsoni</i>	<i>C. elburzensis</i>	<i>C. mystax</i>
<i>C. grandis</i>				
<i>C. hotsoni</i>	12.447*			
<i>C. elburzensis</i>	11.261*	5.116*		
<i>C. mystax</i>	5.180*	8.081*	7.487*	

### Geometric-morphometric results

No sexual dimorphism in dorsal cranium and mandible shape analyses ( $p > 0.05$ ) was detected. The first two CVA factors for cranium and mandible explained 54.88% and 33.4% of total variance, respectively. Relative wraps analysis was conducted to explore shape variability along the axis of maximum covariation within the data. In the analysis of the cranium, the first axis separated *C. grandis* in the negative side with longer nasal and interparietal bones and shorter parietal than other three species by actual landmarks (Fig. 6A). This species had the widest zygomatic arc represented by semi-landmarks. Based on the second axis *C. mystax* and *C. hotsoni* have shorter nasal and parietal and longer frontal (actual landmarks) and wider zygomatic arc (semi-landmarks) than *C. elburzensis* (Fig. 6A). In the canonical variate analysis of mandibular shape, the first CV characterized *C. mystax* and *C. grandis* with lower position of coronoid process in comparison to the other two species. Whereas *C. mystax* and *C. elburzensis* showed shorter height of lower diastema and narrower angular process than *C. grandis* and *C. hotsoni* in the positive deviation of second CV (Fig. 6B).





**FIGURE 6.** Plot of canonical variate scores for landmarks on dorsal side of cranium of *Calomyscus* (A); Plot of canonical variate scores for landmarks on labial side of mandible of *Calomyscus* (B). The eight and twelve actual landmarks on cranium and mandible, respectively, have been joined by semi-landmarks.

## DISCUSSION

Vorontsov et al. (1997) first recognized the karyotypic difference between *C. mystax* ( $2N=44$ ) and *C. urartensis* ( $2N=32$ ). Meyer and Malikov (2000) reported two cytotypes of brush-tailed mice from the Kopetdagh Mts. in southwestern and southcentral Turkmenistan. One cytotype with a  $2N=30$ ,  $FN_a=40$  was recognized as a subspecies of *C. mystax* (*C. m. zykovi*) and the other with a  $2N=44$ ,  $FN_a=58$  was described as a new species, *C. firiusaensis* (Meyer & Malikov 2000). The later cytotype is also known from northeastern and eastern Iran (Esmaili et al. 2008) and both *C. firiusaensis* and *C. mystax zykovi* have been considered synonyms of *C. elburzensis* (Musser & Carleton, 2005; Norris et al., 2008). Karyotypes with a  $2N=44$  have been described from *C. mystax* (Meyer & Malikov 1995, 2000, Vorontsov et al. 1997, Malikov et al. 1999, Graphodatsky et al. 2000) and *C. grandis* (Graphodatsky et al. 2000) both with a  $FN_a=46$  and *C. elburzensis* as described above with a  $FN_a=58$ .

Although *C. grandis* and *C. mystax* share an identical karyotype, they were not found to share a common ancestor in our phylogenetic analysis.

Six specimens of *Calomyscus* from Kopetdagh Mts. in northeastern Iran were analyzed in this study. Cytochrome b sequences from these six samples form a well supported clade with the sequence

from a single specimen of *C. mystax* from Turkmenistan. These specimens from the Kopetdagh Mts. were recognized as *C. mystax* based on their placement in phylogenetic analysis of Cytb gene and the low genetic distance observed between them and a specimen of *C. mystax* from near the type locality. Discriminant analysis of cranial measurements has distinguished individuals of *C. mystax* from Turkmenistan (Lebedev et al., 1998). The present morphometric and geometric-morphometric studies also separated specimens of this species from three other species. *C. mystax* had shorter and narrower cranium and nasal than *C. grandis*; shorter ear, nasal, parietal, lower diastema and M3.L and wider zygomatic than *C. elburzensis* and wider nasal than *C. hotsoni* (Fig. 6A). The lower position of coronoid process of the mandible distinguished *C. mystax* from *C. elburzensis* and *C. hotsoni* (Fig. 6B). The range of *C. mystax* has been controversial with some workers (Graphodatsky et al. 2000, Musser & Carleton, 2005; Norris et al., 2008) restricting its distribution to the Great Balkhan Mountains, whereas other workers (Vorontsov et al. 1979, Musser and Carleton 1993, Meyer and Malikov 1995, 2000, Pavlinov and Rossilimo 1998) have expanded its distribution to include the Lesser Balkhans and Kopetdagh Mts. of Turkmenistan and northeastern Iran. We extended the known range of *C. mystax* from the area of the Greater Balkhan Mts. of Turkmenistan to include more southern regions in central Kopetdagh Mts. of northeastern Iran.

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#### LITERATURE CITED

- Aliabadian, M., Nijman, V., Mahmoudi, A., Naderi, M., Vonk, R., Vences, M. 2014. ExcaliBAR: a simple and fast software utility to calculate intra- and interspecific distances from DNA barcodes. *Contributions to Zoology* 83: 79-83.
- Bruford, M. W., Hanotte, O., Brokfield, J. F. Y., Burke, T. 1992. Single-locus and multilocus DNA fingerprinting. In Hoelzel, A. R. ed. *Molecular genetic analysis of populations: a practical approach*. Oxford University Press, New York, pp. 225-269.
- 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., Zima, J. 2000. Comparative cytogenetics of hamsters of the genus *Calomyscus*. *Cytogenetics and Cell Genetics* 88, 296-304.
- Hammer, Ø., Harper, D. A. T., Ryan, P. D. 2001. PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica* 4, 9pp.
- Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98NT. *Nucleic Acids Symposium Series* 41, 95-98.
- Kashkarov, D. 1925. [Materials to the knowledge of the rodents of the Turkestan.] Transactions of the SA scientific Society of Turkestan at Middle Asiatic University 2, 43-57 (in Russian).

- Lebedev, V. S., Pavlinov, I. Ya., Meyer, M. N., Malikov, V. G. 1998. [Cranio-metric analysis of mouse-like hamsters of the genus *Calomyscus* (Cricetidae)]. *Zoologicheskii Zhurnal* 77, 721-731 (in Russian).
- Malikov, V. G., Meyer, M. N., Graphodatsky, A. S., Polyakov, A. V., Sablina O. V., Vaziri, A. Sh., Nazari, F., Zima J. 1999. On a taxonomic position of some karyomorphs belong to genus *Calomyscus* (Rodentia, Cricetidae). *Proceedings of the Zoological Institute RAS* 281, 27-32.
- Meyer, M. N., Malikov, V. G. 1995. [On the distribution, taxonomic status and biology of mouse-like hamsters of the genus *Calomyscus* (Rodentia, Cricetidae)]. *Zoologicheskii Zhurnal* 74, 96-100 (in Russian).
- 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 Phylogenetic and Evolution* 22, 220-233.
- Musser, G. G., Carleton, M. D. 1993. Family Muridae. In Wilson, D. E., Reeder, D. M., eds. *Mammal species of the world: A taxonomic and geographic reference*. Smithsonian Institution Press, Washington D.C. second edition, pp. 501-755.
- Musser, G. G., Carleton, M. D. 2005. 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, third edition, volume 2, pp. 894-1531.
- Norris, R. W., Woods, C. A., Kilpatrick, C. W. 2008. Morphological and molecular definition of *Calomyscus hotsoni* (Rodentia: Muroidea: Calomyscidae). *Journal of Mammalogy* 89, 306-315.
- Pavlinov, I. Ya., Rossolimo, O. I. 1998. [Systematics of mammals of the USSR. Addenda M.] *Archives of the Zoological Museum, Moscow State University* 38, 190 pp (in Russian).
- Rohlf, F. J. 2001. TpsDig, version 1.30. Dept. Ecology and Evolution, State University of New York at Stony Brook.
- Ronquist, F., Huelsenbeck, J. P. 2003. MRBAYES3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-1574.
- 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.
- Tamura, K., Peterson, D., Peterson, N., Strecher, G., Nei, M., Kumar, S. 2011. Mega5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731-2739.

Vorontsov, N. N., Kartavtesva, I. V., Potapova, E. G. 1979. [Systematics of the genus *Calomyscus*. 1. Karyological differentiation of the sibling species from Transcaucasia and Turkmenia and a review of species of the genus *Calomyscus*.]. *Zoologicheskii Zhurnal* 58, 1215-1224 (in Russian).

### Supplementary TABLE 1

Details of sampled localities, tissue and voucher numbers and Cytb accession numbers of specimens from Iran, Pakistan and Turkmenistan.

Accession No.	Voucher No.	Locality	Species
	2987	Jodar, Kopetdagh Mts., North Khorasan, Iran	<i>Calomyscus</i> sp.
	2892	Jodar, Kopetdagh Mts., North Khorasan, Iran	<i>Calomyscus</i> sp.
	2981	Takale-ghoz, Kopetdagh Mts., North Khorasan, Iran	<i>Calomyscus</i> sp.
	2984	Takale-ghoz, Kopetdagh Mts., North Khorasan, Iran	<i>Calomyscus</i> sp.
	3084	Takale-ghoz, Kopetdagh Mts., North Khorasan, Iran	<i>Calomyscus</i> sp.
	2977	Sambar, Kopetdagh Mts., North Khorasan, Iran	<i>Calomyscus</i> sp.
	1542	KhajeMorad, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
	1543	KhajeMorad, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	1549	KhajeMorad, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2021	KhajeMorad, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2022	KhajeMorad, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2023	KhajeMorad, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2024	KhajeMorad, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2085	KhajeMorad, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2086	KhajeMorad, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2087	Torbat-e-Jam, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2088	Torbat-e-Jam, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2089	Torbat-e-Jam, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2090	Torbat-e-Jam, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
	1874	Aghdarband, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
	1921	Aghdarband, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
	1922	Aghdarband, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	1666	Dargaz, Kopetdagh Mts., Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
	1675	Dargaz, Kopetdagh Mts., Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2144	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2145	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2146	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2147	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2148	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2149	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2150	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2151	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2152	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2153	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2155	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2156	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2157	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2158	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2161	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2167	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2169	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2170	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2171	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2172	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2176	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2178	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2179	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2180	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2181	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2184	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
-	2186	Neyshabur, Khorasan-e-Razavi, Iran	<i>C. elburzensis</i>
	3100	Chenaran, North Khorasan, Iran	<i>C. elburzensis</i>

	3101	Chenaran, North Khorasan, Iran	<i>C. elburzensis</i>
	3173	Chenaran, North Khorasan, Iran	<i>C. elburzensis</i>
-	3240	Chenaran, North Khorasan, Iran	<i>C. elburzensis</i>
	2978	Saluk, North Khorasan, Iran	<i>C. elburzensis</i>
-	3216	Saluk, North Khorasan, Iran	<i>C. elburzensis</i>
-	3224	Saluk, North Khorasan, Iran	<i>C. elburzensis</i>
	3533	Kurkhud, North Khorasan, Iran	<i>C. elburzensis</i>
-	3536	Kurkhud, North Khorasan, Iran	<i>C. elburzensis</i>
	3629	Kurkhud, North Khorasan, Iran	<i>C. elburzensis</i>
-	3630	Kurkhud, North Khorasan, Iran	<i>C. elburzensis</i>
-	1677	FakhrAbad, Yazd, Iran	<i>C. elburzensis</i>
-	1683	FakhrAbad, Yazd, Iran	<i>C. elburzensis</i>
-	1714	FakhrAbad, Yazd, Iran	<i>C. elburzensis</i>
	1719	FakhrAbad, Yazd, Iran	<i>C. elburzensis</i>
-	1777	FakhrAbad, Yazd, Iran	<i>C. elburzensis</i>
	1943	Fasham, Tehran, Iran	<i>C. grandis</i>
	1985	Fasham, Tehran, Iran	<i>C. grandis</i>
	1986	Fasham, Tehran, Iran	<i>C. grandis</i>
	1987	Fasham, Tehran, Iran	<i>C. grandis</i>
-	3992	Fasham, Tehran, Iran	<i>C. grandis</i>
	2068	Saravan, Sistan-o-Baluchistan, Iran	<i>C. botsoni</i>
	2069	Saravan, Sistan-o-Baluchistan, Iran	<i>C. botsoni</i>
	2102	Saravan, Sistan-o-Baluchistan, Iran	<i>C. botsoni</i>
	2103	Saravan, Sistan-o-Baluchistan, Iran	<i>C. botsoni</i>
-	2104	Saravan, Sistan-o-Baluchistan, Iran	<i>C. botsoni</i>
EU135583		Pakistan, Balochistan, Khuzder Dist., Dancer, Village	<i>C. botsoni</i>
EU135582		Pakistan, Sindh, Dadu Dist., Rani Kot near Shergart Fort	<i>C. botsoni</i>
EU135586		Pakistan, Balochistan, Kalat Dist., Khan's Palace Bungalow	<i>C. baluchi</i>
EU135587		Pakistan, Balochistan, Kalat Dist., Kargaz, 4 km S Khan of Kalat's Bungalow	<i>C. baluchi</i>
EU135591		Pakistan, North Waziristan, Datta Khel, 55 km SW Miran Shaw	<i>C. baluchi</i>
	83939*	Turkmenistan, Great Balkhan Mts.	<i>C. mystax</i>
AJ389537			<i>Spalax ebrenbergi</i>

\* Deposited in Zoological Institute, St. Petersburg, Russia