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# **Research Article**

# Two new species of brush-tailed mouse, genus *Calomyscus* (Rodentia: Calomyscidae), from the Iranian Plateau

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#### Abstract

The monogeneric family Calomyscidae includes the brush-tailed mice, genus *Calomyscus*, which have a Palearctic distribution ranging from the Hindu Kush Mountains in western Pakistan to the eastern Mediterranean region. Zagros Mountains—stretching from northwest to south of Iran—was assumed for long as the range of a single species, *Calomyscus bailwardi*; however, recent studies revealed considerable heterogeneities among the geographical populations presumably referring to the persistence of undescribed diversity. One such group from the western Zagros Mountains has recently been recognized as *C. behzadi* Akbarirad, Dezhman, Aliabadian, Siahsarvie, Shafaeipour, and Mirshamsi, 2021. Using molecular, morphometric, geometric morphometric, and karyotypic data, we examine the divergence of 2 additional groups from western Iran and name these as new species. The first of these species is distributed in the northern Zagros and western Alborz Mountains in northwestern Iran was recovered as the sister species to *C. urartensis* from the South Caucuses. The second species from a single locality in the central Zagros Mountains was clustered closely with *C. grandis* from the western Alborz Mountains. Morphologic analyses, karyological features, and genetic distances disentangled these 2 new species from their sister species.

Key words: Cytb, Cox1, geometric morphometrics, Iran, karyology, molecular phylogenetics, morphometrics, Rbp3, Zagros Mountains.

از فلات ایران Calomyscus توصیف دو گونه جدید از زیباموشیان جنس

#### چکیدہ

خانواده زیبا موشیان Calomyscidae دربرگیرندهی تکجنس Calomyscus است که در پالنارکتیک از کوههای هندوکش در غرب پاکستان تا شرق مدیترانه پراکنش دارد. رشته کوههای زاگرس که از شمال غرب تا جنوب ایران امتداد یافته است، به مدت طولانی به عنوان منطقه پراکنش تنها یک گونه، Calomyscus bailwardi در نظر گرفته میشد، اما مطالعات آخیر توع قابلتوجهی میان جمعیتهای جغرافیایی این منطقه نشان داده است که احتمالاً به تنوع توصیف نشده در این کوهستان اشاره دارد. یکی از این گروهها، که اخیراً از رشته کوههای زاگرس غربی به عنوان گونه جدید معرفی شد، اما مطالعات آخیر توع قابلتوجهی میان جمعیتهای جغرافیایی این منطقه نشان داده است که احتمالاً به تنوع توصیف نشده در این کوهستان اشاره دارد. یکی از این گروهها، که اخیراً از رشته کوههای زاگرس غربی به عنوان گونه جدید معرفی شد، و گروموزومی، واگرایی دو گروه دیگر از غرب ایران را بررسی و اثبت کرده و به عنوان گونه می و کروموزومی، واگرایی در عنوان گونه خواهی ریخت سنجی هندسی و گروموزومی، واگرایی دو گروه دیگر از غرب ایران را بررسی و اثبت کرده و به عنوان گونههای جدید توصیف میکنیم. اولین گونه که در شمال زاگرس و رشته کوههای ایرز غربی در شمال غرب ایران پراکنش دارد. نجزیه و تعلیراهی مونولوژیی، ویژگیهای کروهون دو میگر از غرب ایران را بررسی و اثبت کرده و به عنوان گونههای جدید توصیف میکنیم. اولین گونه که در شمال زاگرس و رشته کوههای ایرز غربی داره نور گونه خواهری زیبا موش ایرانی، کره موزومی و یواکی ژنیکی، این دو گرونه جدید را از گونههای کوههای زاگرس مرکزی معرفی شد که خویشاوندی نزدیکی با زیبا موش فشمی، C. grandis، در رشته کوههای ایرز غربی دارد. نجزیه و تحلیلهای موزفولوژیکی، ویژگیهای کروموزومی و فواصل ژنتیکی، این دو گونه جدید را از گونههای خواهریشان مجزا کرده است.

Extant forms of the family Calomyscidae (Rodentia) are endemic to southwestern Asia and generally inhabit barren and rocky mountainous habitats (Morshed and Patton 2002; Kilpatrick 2017). Molecular phylogenies have recovered the Calomyscidae within the Eumuroida as the sister group to a clade containing the Nesomyidae, Cricetidae, and Muridae (Steppan et al. 2004; Steppan and Schenk 2017). The geographic distribution of this monogeneric family ranges from Afghanistan and western Pakistan to the eastern Mediterranean region (Musser and Carleton 2005; Kilpatrick 2017) and consists of a number of geographically isolated populations. Nine species are currently recognized (Musser and Carleton 2005; Kilpatrick 2017; Dezhman

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et al. 2021), which are divided into at least 2 clades, referred to as "Clade 1" and "Clade 2" by Akbarirad et al. (2016a). Seven of these species occur in Iran: Calomyscus bailwardi Thomas, 1905, from southwestern Iran and perhaps Turkey; C. hotsoni Thomas, 1920, from southeastern Iran and southwestern Pakistan; C. mystax Kashkarov, 1925, from northeastern Iran and southwestern Turkmenistan; C. elburzensis Goodwin, 1938, ranging from northwestern Afghanistan, and southern Turkmenistan to central Iran; C. grandis Schlitter and Setzer, 1973, which is endemic to northern Iran; C. urartensis Vorontsov and Kartavseva, 1979, from northwestern Iran and southern Azerbaijan; and C. behzadi Akbarirad, Dezhman, Aliabadian, Siahsarvie, Shafaeipour, and Mirshamsi, 2021, which is endemic to the Kermanshah and Ilam provinces of western Iran (Kryštufek and Vohralik 2009). Defining species boundaries in the calomyscids has been primarily based on morphology (Vorontsov et al. 1979; Meyer and Malikov 1995; Lebedev et al. 1998), and karyology (Malikov et al. 1999; Graphodatsky et al. 2000; Shahabi et al. 2010). A number of molecular studies have tried to explore species diversity in Calomyscus (Norris et al. 2008; Shahabi et al. 2013; Dezhman et al. 2021); however, the exact number of species remains unclear.

The presence of undescribed species within Calomyscus is likely arising from their relatively insular and patchy distribution in rocky landscapes across the Iranian Plateau (Morshed and Patton 2002; Karami et al. 2008). If populations were isolated during past climate oscillations, genetic divergence and possibly speciation may have occurred. Recently, based on integrative methods such as molecular, morphometric, and karyological studies, 4 distinct evolutionary units have been discovered from the Zagros Mountains in western Iran. The lineage Calomyscus sp. Group C (sensu Akbarirad et al. 2016a) has recently been described as a valid species namely C. behzadi (see Dezhman et al. 2021) and its distribution range and karyotype were uncovered. The other 3, Calomyscus sp. Group D from the northern Zagros (Akbarirad et al. 2016a), C. cf. bailwardi Group B from the southern Zagros (Akbarirad et al. 2016a, 2016b), and Calomyscus sp. Group G from the central Zagros (Rezazadeh et al. 2020), have also been proposed as potentially distinct species. Consequently, there exists great need for further investigation of the taxonomic status of the unnamed molecular lineages of Calomyscus from the Zagros Mountains which are currently treated as part of C. bailwardi (Musser and Carleton 2005; Kilpatrick 2017; Yusefi et al. 2019). We focus here on 2 of these potential new species, Calomyscus sp. Group D from the northern Zagros, and Calomyscus sp. Group G from the central Zagros. Although historically treated as part of C. bailwardi, which is part of "Clade 1," both of these potential new species have been shown to be a part of "Clade 2" alongside C. mystax, C. elburzensis, C. grandis, C. urartensis, and C. behzadi (Akbarirad et al. 2016a; Rezazadeh et al. 2020; Dezhman et al. 2021).

Calomyscus sp. Group D was previously reported only from Saqez County, Kurdistan Province, Iran (Akbarirad et al. 2016a). We expanded the surveys for brush-tailed mice in areas of the northern Zagros and western Alborz Mountains in this study and expand the known geographic distribution of *Calomyscus* sp. Group D. Molecular studies have suggested that this taxon is most closely related to *C. urartensis* from northwestern Iran and southern Azerbaijan (Akbarirad et al. 2016a; Rezazadeh et al. 2020, 2022; Dezhman et al. 2021), but species-level differentiation may be suggested based on genetic distance and morphometrics. *Calomyscus* sp. Group G from the central Zagros was identified by Rezazadeh et al. (2020) as a sister taxon of *C. grandis*, but distinct in terms of genetic distance, morphometrics, and karyotype. Herein, we describe these taxa as 2 new species of *Calomyscus* based on molecular, morphological, and karyological data.

# Materials and methods Fieldwork

Sampling was carried out in western and northwestern Iran using box traps (250 mm × 150 mm × 150 mm) baited with walnuts, cucumbers, and Cheetos. Traps were set and baited in the afternoon and checked the following morning. Samples from Hir, Ardabil (n = 8) were captured from a semi-vegetated rocky area, near a stream and fruit gardens, on the westernmost edge of the Talysh Mountains at an elevation of 1,615 m. Calomyscus from Piranshahr, West Azerbaijan (n = 5) were sampled from dry and barren rocky mountains that were scarcely vegetated with barberry and pistachio trees at an elevation of 1,647 m (Fig. 1). Animals were handled in accordance with ASM guidelines (Sikes et al. 2016). Sampling was performed after approval by the Department of Environment of Iran (agreement number: 93/340). All experiments were carried out according to the directive 2010/63/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes (protocol available on http://www. ceropath.org/references/rodent\_protocols\_book).

### Molecular methods and phylogenetic analyses

Total genomic DNA from 21 individuals (Table 1) was extracted using a standard saline method (Sambrook et al. 1989). Two mitochondrial, cytochrome b (Cytb) and cytochrome oxidase subunit 1 (Cox1), and 1 nuclear, retinol-binding protein 3 (Rbp3), genes were amplified using primers L7 and H6 (Montgelard et al. 2002) for Cytb, Vf1d and Vr1d (Ivanova et al. 2006) for CoxI, and IRBP-F and IRBP-R (Dianat et al. 2017) for Rbp3. Polymerase chain reaction (PCR) conditions for Cytb and CoxI followed Chevret et al. (2005) and Aliabadian et al. (2007), respectively, and Dianat et al. (2016) for Rbp3. PCR products were purified using QIAGEN quick PCR purification Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and commercially sequenced using dye-labeled dideoxy terminator cycle sequencing with Big Dye V.3.1 (Applied Biosystems, Inc.) by Macrogen Company (South Korea). Sequences were checked and edited with the Codon Code Aligner V. 6.0.2 (Codon Code Corp) and BioEdit (Hall 1999). In total, 27 sequences of 3 genes have been deposited in GenBank. These data sets were complemented with an additional 110 sequences downloaded from GenBank (Table 1).

The best-fit partitioning scheme and the associated nucleotide substitution models for each partition were determined using PartitionFinder v.2 (Lanfear et al. 2017). Using the "greedy" algorithm (Lanfear et al. 2012), the best models identified for codon positions 1, 2, and 3 are as follows, respectively: K80+I+G, TRN+I, and GTR for Cytb; GTR+I, TIM, and TIM+G for CoxI; and HKY for Rbp3. Four outgroup taxa, Mus musculus (Murinae), Meriones unguiculatus (Gerbillinae), Nannospalax ehrenbergi (Spalacinae), and Rhizomys pruinosus (Rhizomyinae) were used for tree rooting following the results from Steppan and Schenk (2017). Bayesian inference (BI) trees were constructed using MrBayes V.3 (Ronquist and Huelsenbeck 2003) from a concatenated data set of the 2 mitochondrial and 1 nuclear gene. The analysis was run for 1 × 10<sup>6</sup> generations, saving 1 tree in each 1,000 generations. The log likelihood values of the sample points were plotted against the generation time, and all trees were discarded prior to reaching a stationary phase. A majority rule consensus tree was generated from the remaining trees. Phylogenetic trees were also inferred



**Fig. 1.** Map showing the geographic distribution of *Calomyscus* in Iran and adjacent countries. *Calomyscus* tsolovi from Syria and unidentified specimens from Turkey (Kryštufek and Vohralík 2009) not shown. "Shapes" indicate localities of samples used in this study.

by maximum likelihood (ML) using RaxML V. 8.2 (Stamatakis 2006) for this 3 gene concatenated data set. Maximum likelihood bootstrap values were determined with RaxML (1,000 replicates). Nodal support of  $\geq$ 80% for the bootstrap proportion (BP) and Bayesian posterior probabilities (BPP) > 95% were considered to be "good" support. According to literature (see Bradley and Baker 2001), Kimura 2 parameter (K2P) genetic distances for Cytb were calculated using MEGA6 (Tamura et al. 2013).

### Geometric morphometric analyses

A total of 94 adult specimens (Table 1) with fully erupted molars were photographed using a CANON S5IS 8 mega-pixel digital camera. We defined 10 groups based on the range of species according to bibliographical data, genetic results, and geographical origin of specimens (Fig. 1): 7 known species included C. elburzensis (n = 16), C. grandis (n = 11), C. urartensis (n = 4), C. mystax (n = 4), C. behzadi (n = 4), C. bailwardi (n = 14), and C. hotsoni (n = 4); and 3 molecular lineages included Calomyscus sp. Group D (n = 17), C. cf. bailwardi Group B (n = 10), and Calomyscus sp. Group G (n = 10). Twenty 2D landmarks were digitized on images of the dorsal and ventral view of skulls using TpsDig 2.17 software (Rohlf 2013). These landmarks have been previously used by Tong (1989) and Stoetzel et al. (2017) in studies of the Gerbillinae. In our study, a classical geometric morphometrics approach was followed (Zelditch et al. 2012). Generalized Procrustes analyses were performed in MorphoJ. V.1.06d (Klingenberg 2011) to remove the effect of orientation and scale from coordinate configuration. Results of these superimpositions were extracted as shape variables (Rohlf and Slice 1990). In size and shape analyses, we retained only the ventral view because it includes most of the taxonomically informative structures (Tabatabaei Yazdi and Adriaens 2013). Centroid sizes of the ventral side were extracted using MorphoJ. To clarify size difference between groups, an analysis of variance (ANOVA) was performed on log-transformed centroid size data (P = 0.05). To highlight the overall shape differences between groups, Mahalanobis distances of shape variables were compared between groups using MANOVA (10,000 random permutations, P = 0.0001). Canonical variate analysis (CVA) was conducted to clarify shape variability among samples, using MorphoJ V.1.04a (Klingenberg 2011). Skull shape variables among the studied groups were shown as a scatter plot depicting the first 2 canonical axes. Shape differences were visualized on thin-plate spline deformation grids generated along the first 4 CV axes for dorsal and ventral views. Secondary sexual dimorphism (SSD) was evaluated by nonparametric ANOVA on log-transformed centroid size and of shape variables. All statistical analyses were conducted using IBM SPSS Statistics V.24 (IBM Crop. 2016) and PAST V.2.7 (Hammer et al. 2001).

### Morphometric analyses

A total of 122 specimens (Table 1) including those captured during this survey and museum specimens deposited in the Zoological Museum of Ferdowsi University of Mashhad (ZMFUM) were included in craniometric analyses. Measurements of the characters examined by Peshev (1991) were taken from adults with fully erupted molars. Four external characters measured with **Table 1.** Information on specimens used in this study, with voucher numbers, collecting localities (for specimens included in genetic analyses), and GenBank accession number. *n* = numbers of analyzed specimens in each group used in traditional craniometrics and geometric morphometrics, respectively. ZMFUM = Zoology Museum of Ferdowsi University of Mashhad, Mashhad, Iran; UF = Florida Museum of Natural History, Gainesville, United States. New sequences generated in this study are shown in bold.

				GenBank accession numbers					
Species	Museum number/ field code	n	Locality	Cytb	Cox1	Rbp3	Lat (N)	Long (E)	
Calomyscus sp. D	4978ZMFUM	13 (16)	Hir, Ardabil, Iran	OR786660	-	-	38.0528	48.3129	
Calomyscus sp. D	5030ZMFUM		Hir, Ardabil, Iran	OR786661	-	-	38.0528	48.3129	
Calomyscus sp. D	5031ZMFUM		Hir, Ardabil, Iran	OR786662	-	_	38.0528	48.3129	
Calomyscus sp. D	5032ZMFUM		Hir, Ardabil, Iran	-	OR780556	_	38.0528	48.3129	
Calomyscus sp. D	5033ZMFUM		Hir, Ardabil, Iran	-	OR780557	_	38.0528	48.3129	
Calomyscus sp. D	5034ZMFUM		Hir, Ardabil, Iran	OR786663	OR780559	-	38.0528	48.3129	
Calomyscus sp. D	5035ZMFUM		Hir, Ardabil, Iran	OR786664	OR780558	-	38.0528	48.3129	
Calomyscus sp. D	5088ZMFUM		Hir, Ardabil, Iran	OR786665	-	OR972408	38.0528	48.3129	
Calomyscus sp. D	5133ZMFUM		Piranshahr, West Azerbaijan, Iran	OR786666	-		36.5159	45.1041	
Calomyscus sp. D	5136ZMFUM		Piranshahr, West Azerbaijan, Iran	OR786667	-		36.5159	45.1041	
Calomyscus sp. D	5171ZMFUM		Piranshahr, West Azerbaijan, Iran	OR786668	OR780560	OR972409	36.5159	45.1041	
Calomyscus sp. D	5172ZMFUM		Piranshahr, West Azerbaijan, Iran	OR786669	OR780561	_	36.5159	45.1041	
Calomyscus sp. D	5173ZMFUM		Piranshahr, West Azerbaijan, Iran	OR786670	-	_	36.5159	45.1041	
Calomyscus sp. D	3869ZMFUM		Saghez, Kurdistan, Iran	KT878596	KT878556	_	36.3544	46.1872	
Calomyscus sp. D	3870ZMFUM		Saghez, Kurdistan, Iran	KT878597	KT878557	MK211770	36.3544	46.1872	
Calomyscus sp. D	3878ZMFUM		Saghez, Kurdistan, Iran	KT878600	KT878560	_	36.3544	46.1872	
Calomyscus sp. D	3881ZMFUM		Saghez, Kurdistan, Iran	KT878598	KT878558	_	36.3544	46.1872	
Calomyscus sp. D	3882ZMFUM		Saghez, Kurdistan, Iran	KT878599	KT878559	_	36.3544	46.1872	
Calomyscus sp. D	3897ZMFUM		Saghez, Kurdistan, Iran	KT878601	KT878561	_	36.3544	46.1872	
Calomyscus sp. G	5182ZMFUM	11 (11)	Fereydun shahr,Isfahan, Iran	MK205402	-	-	32.5207	50.0453	
Calomyscus sp. G	5184ZMFUM		Fereydun shahr,Isfahan, Iran	MK205403	MK211761	MK211769	32.5207	50.0453	
Calomyscus sp. G	5282ZMFUM		Fereydun shahr,Isfahan, Iran	OR786671	-	-	32.5207	50.0453	
Calomyscus sp. G	5203ZMFUM		Fereydun shahr,Isfahan, Iran	MK205404	MK211762	_	32.5207	50.0453	
Calomyscus sp. G	5278ZMFUM		Fereydun shahr,Isfahan, Iran	MK205411	MK211765	OR972406	32.6209	50.2411	
Calomyscus sp. G	5279ZMFUM		Fereydun shahr,Isfahan, Iran	MK205412	MK211764	OR972407	32.6209	50.2411	
C. grandis	4976ZMFUM	13 (10)	North Alamut Mts., Qazvin,Iran	MK205405	MK211758	_	36.3345	50.13	

# Table 1. Continued

				GenBank accession numbers					
Species	Museum number/ field code	n	Locality	Cytb	Cox1	Rbp3	Lat (N)	Long (E)	
C. grandis	5117ZMFUM		Miyaneh, East Azerbaijan, Iran	MK205407	MK211756	MK211768	37.1909	48.0847	
C. grandis	5118ZMFUM		Miyaneh, East Azerbaijan, Iran	MK205408	MK211757	-	37.195	48.1026	
C. grandis	1986ZMFUM		Fasham, Tehran, Iran	KT878592	KT878552	MK211767	36.93	52.52	
C. grandis	3992ZMFUM		Fasham, Tehran, Iran	KT884559	KT884587	OR972405	38.93	54.52	
C. behzadi	3871ZMFUM	4 (4)	Songhor, Kermanshah, Iran	KT878603	KT878563	MK211772	34.42	47.28	
C. behzadi	3880ZMFUM		Songhor, Kermanshah, Iran	KT878605	KT878565	-	34.42	47.28	
C. behzadi	3891ZMFUM		Songhor, Kermanshah, Iran	KT878604	KT878564	-	34.42	47.28	
C. behzadi	3896ZMFUM		Songhor, Kermanshah, Iran	KT878602	KT878562	-	34.42	47.28	
C. elburzensis	5267ZMFUM	16 (15)	Toure, Markazi Province, Iran	MK205398	-	-	34.0358	49.2403	
C. elburzensis	1546ZMFUM		Khaje Morad, Mashhad, Iran	KT884547	KT884576	-	36 15	59.34	
C. elburzensis	1922ZMFUM		Aqdarband, Khorasan, Iran	KT878586	KT878547	-	36.3	61.7	
C. elburzensis	3533ZMFUM		Shirvan, NorthKhorasan, Iran	KT878590	KT878550	MK211766	37.81	56.68	
C. elburzensis	1777ZMFUM		Taft, Yazd, Iran	KT878584	KT878545	-	31.4	54.19	
C. urartensis	5134ZMFUM	4 (4)	Khoy, West Azerbaijan, Iran	MK205413	MK211753	MK211771	38.4337	44.5451	
C. urartensis	2253ZMFUM		Kordasht, East Azerbaijan, Iran	KT878594	KT878554	-	38.87	46.01	
C. urartensis	2908ZMFUM		Kordasht, East Azerbaijan, Iran	KT878595	KT878555	-	38.87	46.01	
C. mystax	3084ZMFUM	4 (4)	Kopetdagh Mts.,North Khorasan, Iran	KU129019	KU129022	MK211773	38.21	57.18	
C. mystax	2984ZMFUM		Kopetdagh Mts.,North Khorasan, Iran	KU129018	KU129021	-	38.21	57.18	
C. mystax	83939		Balkhan Mts., Bolshoi, Turkmenistan:	KU745283	-	-	-	-	
C. bailwardi	3569ZMFUM	15 (10)	Izeh, Khuzistan, Iran	KT878621	_	MK211775	31.46	49.48	
C. bailwardi	3570ZMFUM		Izeh, Khuzistan, Iran	KT878622	_	-	31.46	49.48	
C. bailwardi	2702ZMFUM		Behbahan, Khuzistan, Iran	KT878620	KT878576	MK211774	30.38	50.23	
C. cf. bailwardi (B)	2030ZMFUM	9 (14)	Arsanjan, Fars, Iran	KT878606	KT878569	OR972401	29.48	53.14	
C. cf. bailwardi (B)	2031ZMFUM		Arsanjan, Fars, Iran	KT878607	KT878570	-	29.48	53.14	
C. cf. bailwardi (B)	3333ZMFUM		BagheShadi area, Yazd, Iran	KT878611	KT878574	MK211776	30.11	53.52	
C. hotsoni	3529ZMFUM	8 (4)	Zahedan, Sistan- Baluchistan, Iran	KT884569	KT884593	OR972402	29.74	60.78	
C. hotsoni	2069ZMFUM		Saravan, Sistan- Baluchistan, Iran	KT884561	KT878578	-	27.3	61.7	
C. hotsoni	2102ZMFUM		Saravan, Sistan- Baluchistan, Iran	KT884562	KT884588	-	27.3	61.7	

#### Table 1. Continued

Species	Museum number/ field code	n	Locality	Cytb	Cox1	Rbp3	Lat (N)	Long (E)
C. hotsoni	4024ZMFUM		Saravan, Sistan- Baluchistan, Iran	KT884574	KT884595	-	27.3	61.7
C. hotsoni	3306ZMFUM		Khash, Sistan- Baluchistan, Iran	KT884567	KT884591	OR972403	28.15	61.17
C. hotsoni	2069ZMFUM		Saravan, Sistan- Baluchistan, Iran	KT884561	KT878578	_	27.3	61.76
C. baluchi	2810ZMFUM		Sorkhkuk, Bamyan, Afghanistan		_	OR972404		
C. baluchi	UF28993		Datta Khel, SW Miran Shaw, Pakistan	EU135591	_	_	33.08	69.73
C. baluchi	UF29596		Kharan Dist., Balochistan, Pakistan	EU135586	_	_	29.26	66.71
Nannospalax ehrenbergi				AJ416891	AJ416891	KC953405		
Rhizomys pruinosus				NC021478	NC021478	AY326107		
Mus musculus				AJ512208	AJ512208	AF126968		
Meriones unguiculatus				KC762224	KC762224	FN984755		
Acomys cahirinus				JN571139	JN571139	AJ698898		

dial caliper include body length (BL), tail length (TL), hind foot length to the base of claw (FL), and ear length (EL). Cranial and mandibular measurements (Supplementary Data SD2) including length of lower diastema (LLD), height of lower diastema (HLD), maximal mandibular height (MH), upper diastema length (UDL), nasal length (NL), zygomatic width (ZW), anterior foramina incisive length (Forl), nasal width (NW), maximum cranial height (SH), occipitonasal length (Occl), cranium width (CW), condylobasal length (CBL), maxillary tooth row length (Mxl), mandibular tooth row length (Mnl), and mandible length (Mndl) were measured with dial calipers (0.1 mm). Dental measurements (Supplementary Data SD2) for upper molars include length of M1 (M1L), width of M1 (M1W), length of M2 (M2L), width of M2 (M2W), length of M3 (M3L), width of M3 (M3W), and for lower molars include length of m1 (m1L), width of m1 (m1W), length of m2 (m2L), width of m2 (m2W), length of m3 (m3L), width of m3 (m3W). All measurements were scored using a dissecting microscope fitted with an eyepiece graticule (0.01 mm). Analyses were conducted on the data from only complete skulls (n = 94) sorted to the same 10 groups as in the geometric morphometric analyses. Craniodental measurements were tested for normality and homogeneity by Levene's test (P < 0.05). Measurements were log<sub>10</sub>-transformed to reduce variance among variables. Because of an unequal sample size of groups, Kruskal-Wallis nonparametric ANOVA was performed to test significant differences among groups using SPSS Statistics V. 24 (IBM Corp. 2016). Principal components analyses (PCA) were conducted to determine morphological variations (Lemen1983). The first PC is considered to be a size factor with the largest proportion of variance, and remaining components are size-out functions (Kryšufek et al. 2015). CVA and PCA were conducted using PAST V. 2.7 (Hammer et al.2001).

### Karyology analyses

In this study, karyotypes of 2 *Calomyscus* sp. Group D individuals, female (ZMFUM 5030) from Hir and male (ZMFUM-5133) from Piranshahr, were obtained. Before extraction of bone marrow from femoral and tibial bones, mice were treated for 45 min with an intraperitoneal injection of a 10% vinblastin solution at a dose of 1 ml/100 g of body weight (Dutrilaux et al. 1982). Chromosome spreads from bone marrow cells were obtained by the methods of Yosida (1973). Ten slides for each individual were made and were stained with Giemsa and 10 spreads of each individual were photographed for determining the diploid chromosome number. Diploid number (2n) and fundamental number of autosomal arms (FNa) were determined from photographs of well-spread cells using the Chromosome Processing software (CIP) developed in the Rodentology Research Department of Ferdowsi University of Mashhad (http://metsofts.ir/download-mipstudent/).

## Results

#### Molecular analyses

The BI and ML analyses of the concatenated 3 gene data set recovered similar topologies with 2 well-supported main clades (BP = 99, BPP = 1.00) labeled Clade 1 and Clade 2 (Fig. 2) consistent with the terminology used in prior studies (Akbarirad et al. 2016a; Rezazadeh et al. 2020; Dezhman et al. 2021). Clade 1 consists of 5 entities (*C. hotsoni*, *C. baluchi*, *C. bailwardi*, and *C. cf. bailwardi* Group B), while Clade 2 comprises 7 entities (*C. elburzensis*, *C. mystax*, *C. urartensis*, *C. grandis*, *C. behzadi*, Calomyscus sp. Group D, and Calomyscus sp. Group G). All lineages obtained high values of BP and BPP (BP = 82–100, BPP = 0.78–0.99), except C. cf. bailwardi



**Fig. 2.** Consensus tree resulting from concatenated analysis of mitochondrial Cytb, Cox1, and nuclear Rbp3 genes. Posterior probability values from the Bayesian analysis are indicated by asterisk at the > 99% (\*\*) > 95% (\*) significance levels. Numbers represent maximum likelihood bootstrap values (only values > 70% are shown).

Group B which retrieved moderate values (BP = 82, BPP = 0.87; Fig. 2).

The phylogenetic trees showed a close relationship between Calomyscus sp. Group D and C. urartensis in Clade 2 (BP = 92, BPP = 0.99), which clustered subsequently with C. elburzensis, albeit with low values (BP = 65, BPP = 0.86). Calomyscus sp. Group G and C. grandis appeared as well-supported (BP = 96, BPP = 0.96) sister taxa. Additional relationships among taxa in Clade 2 were not well-resolved (Fig. 2).

The mean nucleotide divergence (K2P) of Cytb between Calomyscus sp. Group D and other species of Calomyscus ranged from 7.8% to 18.3% (Table 2). The least divergence was with C. *urartensis* (7.8%), and the greatest divergences were with taxa of Clade 1 (16.2%–18.3%). The genetic divergence of Calomyscus sp. Group G and other taxa ranged from 7.5% to 18.4%, with the least divergence with C. grandis (7.5%) and the greatest with taxa of Clade 1 (15.5% to 18.4%).

#### Geometric morphometric analyses

There was allometry both in the ventral and dorsal side of examined samples (P =  $4.6 \times 10^{-6}$ ). Hence, all analyses were conducted on size-corrected data (Residuals). No sexual dimorphism was observed in our samples. The result of Kruskal–Wallis nonparametric (NP) ANOVA showed significant differences (P < 0.05) in the ventral side of cranium landmarks between *Calomyscus* species and groups examined. According to centroid size data, *Calomyscus* sp. Group G had significantly high values compared to other calomyscids (P < 0.001) except for *Calomyscus* sp. Group C. Mahalanobis distances were compared between groups to

determine shape differences (Supplementary Data SD3)— Calomyscus sp. Group G was significantly different with other groups in shape ( $P \le 0.0001$ ) and Calomyscus sp. Group D was significantly separated in shape from members of Clade 2 ( $P \le 0.0001$ ) except with C. behzadi.

In a CVA of the shape data of the dorsal side of the cranium, the first and second CVs explained 49.94% and 15.42% of the total variance, respectively (Fig. 3). CV1 had the largest amount of variance, sharply separating C. grandis, Calomyscus sp. Group G, and Calomyscus sp. Group D on the positive side of the axis from all other species of Calomyscus and C. cf. bailwardi on the negative side. Specimens in the positive direction—including both new species (Group D and Group G) and C. grandis-have a longer and wider cranium, longer and wider nasals, shorter frontal, and wider interparietal bones (Fig. 3). However, the second factor of CV—with much lesser contribution to total variance—was able to differentiate Calomyscus sp. Group D from Calomyscus sp. Group G, C. elburzensis, C. behzadi, and C. hotsoni. Calomyscus sp. Group D has shorter and narrower nasals and a narrower cranium than Calomyscus sp. Group G. Calomyscus sp. Group G can be differentiated from C. grandis along CV2 by its wider braincase, narrower zygomatic plate, and longer nasals.

PCA performed on the dorsal side mostly confirmed the results of CVA in the case of *Calomyscus* sp. Group G and *Calomyscus* sp. Group D. PC1, PC2, and PC3 explained 26%, 13%, and 10% of total variance, respectively. *Calomyscus* sp. Group G can be distinguished from other groups examined along PC1, and from *C. grandis* along PC2. *Calomyscus* sp. Group D overlaps with taxa of *Calomyscus* examined other than *Calomyscus* sp. Group G along PCA1 and PCA2 (Supplementary Data SD4).

	1	2	3	4	5	6	7	8	9	10	11
C. cf. bailwardi 1	0.3										
C. bailwardi 2	11.3	4.4									
C. baluchi 3	10.0	10.4	1.9								
C. hotsoni 4	10.8	11.2	8.7	1.0							
C. mystax 5	16.8	18.2	15.9	15.6	2.4						
C. behzadi 6	18.3	18.4	18.4	17.5	11.4	0.0					
C. grandis 7	17.4	17.3	17.7	16.4	11.1	10.8	0.4				
C. Group G 8	17.0	18.4	15.7	15.5	10.1	10.3	7.5	0.7			
C. Group D 9	16.8	18.3	16.2	16.9	10.5	12.6	10.9	9.6	1.8		
C. urartensis 10	17.5	19.8	16.4	18.0	10.4	11.5	10.6	8.3	7.8	1.2	
C. elburzensis 11	16.9	17.9	16.4	16.9	11.2	11.5	9.4	11.0	10.2	10.2	1.9

Table 2. Mean pairwise K2P Cytb distances between taxa of Calomyscus with maximum intraspecific distances along the diagonal.



Fig. 3. Canonical variate analyses of shape variations in the dorsal side of cranium in Calomyscus.

From analysis of the ventral surface of specimens, factors CV1 and CV2 explained 33.49% and 18.89% of total variance, respectively (Fig. 4). *Calomyscus* sp. Group G, *Calomyscus* sp. Group D, and C. *behzadi* were located on the positive side of the first axis, with Group G clearly differentiated. Positive deviation of the first CV was associated with longer nasals, a shorter posterior palatine foramen, and a shorter lower tooth row. The zygomatic plate of C. grandis was wider than Calomyscus sp. Group D has a wider zygomatic plate than Calomyscus sp. Group G. CV2 separated C. grandis and C. urartensis from other taxa. A positive deviation of CV2 found in Calomyscus sp. Group D, C. behzadi, C.

mystax, C. bailwardi, and C. cf. bailwardi Group B samples was characterized with a shorter cranium and a shorter posterior palatine foramen (Figs. 4 and 5).

A PCA analysis conducted on the ventral surface found that PC1, PC2, and PC3 explained 16.28%, 14.55%, and 11.23% of total variance, respectively. *Calomyscus* sp. Group G is differentiated from all groups along PC1 and PC2. *Calomyscus* sp. Group D can be distinguished from *Calomyscus* sp. Group G, C. *elburzensis*, C. *mystax*, C. *urartensis*, C. cf. *bailwardi* Group B, C. *hotsoni*, and C. *grandis* along PC1. Neither C. *behzadi*, nor C. *bailwardi* could be differentiated from *Calomyscus* sp. Group D along either axis (Supplementary Data SD5).



Fig. 4. Plot of canonical variate scores for landmarks on the ventral side of the cranium of Calomyscus.



**Fig. 5.** Comparison of skulls of Calomyscus species; C. bailwardi (A), C. cf. bailwardi (B), C. hotsoni (C), C. kiabii **sp. nov.** (D), C. darvishii **sp. nov.** (E), C. grandis (F), C. elburzensis (G), C. mystax (H), Calomyscus behzadi (I), C. urartensis (J).

### Morphometric analyses

Sexes were pooled in all analyses since the results of NP ANOVA revealed no significant difference between males and females (P > 0.05). In total, the 3 principal components explained 61.23% of the variance. The scatterplot of the individuals in the space of first and second components (PC1 vs. PC2) showed a separation of *Calomyscus* sp. Group G from other species and groups (Fig. 6; Supplementary Data SD1). Within *Calomyscus* sp. Group D, individuals of the Zagros population (Saqez and Piranshahr) morphologically were larger than individuals of the Alborz population (Ardabil) along PC1 (Fig. 6). The Zagros population of Group D was smaller than *Calomyscus* sp. Group G, but was generally larger than other taxa examined.

CV1 explains 45% and CV2 explains 22% of total variance (Fig. 7). The largest body and longest tail both belonged to *Calomyscus* sp. Group G, which was were significantly larger or longer than those of *C. hotsoni*, *C. bailwardi*, *C. cf. bailwardi*, *C. grandis*, *Calomyscus* sp. Group D, and *C. urartensis*. Group D individuals had significantly greater lower and upper diastema lengths, condylobasal

length, cranium length, and mandibular length than all taxa with the exception of *Calomyscus* sp. Group G, *C. grandis*, and *C. behzadi*. *Calomyscus* sp. Group D was significantly smaller than *Calomyscus* sp. Group G for these characters. *Calomyscus* sp. Group D possessed significantly wider nasal bones and greater zygomatic breadth than all taxa except for *C. grandis*, *C. behzadi*, and *Calomyscus* sp. Group G. *Calomyscus* sp. Group G has a wide cranium, although that of *C. behzadi* is wider. The maxillary tooth row was significantly longer in *Calomyscus* sp. Group D than in *Calomyscus* sp. Group G. *Calomyscus behzadi* had significantly larger upper second molars than other taxa, with *C. behzadi* being slightly larger than Group D.

# Karyology

Specimens of *Calomyscus* sp. Group D from Ardabil (female) and Piranshahr (male) showed 2N = 44, FNa = 56 (Fig. 8). The karyotype is composed of 7 pairs of subtelocentric and 14 pairs of acrocentric autosomes. The Y chromosome is a small acrocentric (Fig. 8A) and the X chromosome is a submetacentric (Fig. 8B). While several other species of *Calomyscus* have a diploid number of 44, the karyotype of 2n = 44 with FNa = 56 of *Calomyscus* sp. Group D is unique among the Calomyscidae (Table 3).

# Calomyscus kiabii, new species Rezazadeh, Ahmadzadeh and Aliabadian

# Atropatene Brush-tailed Mouse

Calomyscus sp. Group D: Akbarirad et al. 2016a; Rezazadeh et al. 2020; Dezhman et al. 2021; Rezazadeh et al. 2022. Calomyscus bailwardi: Kilpatrick 2017; part, not C. bailwardi Thomas, 1905.

### Holotype

A specimen, identified as an adult male (ZMFUM-5133) with skin and skull (Figs. 9 and 10), along with tissues preserved in 90% ethanol, has been placed in the collections of the Zoological Museum at Ferdowsi University of Mashhad. A Cytb sequence (Accession number: OR786666) was obtained from the holotype.



Fig. 6. Projection of specimens onto the morphological principal components PCA1 and PCA2 based on morphometric measurements.



Fig. 7. Plot of the first and second canonical variate axes of the Calomyscus studied based on morphometric measurements.

#### Type locality

Iran, West Azerbaijan Province, Piranshahr, 1,647 m (a.s.l.) (UTM S38-514858-4078246; 36°51′N, 45°10′E), collected in June 2016 by E. Rezazadeh.

#### Paratypes

Skins, skulls, postcranial skeletons, and ethanol-preserved tissues of 4 female specimens collected by E. Rezazadeh from the type locality (5136 ZMFUM, 5171 ZMFUM, 5172 ZMFUM) deposited in the Zoological Museum of Ferdowsi University of Mashhad. Sequences of Cytb, Cox1, and Rbp3 were obtained from the paratypes (Table 1).

#### Other specimens

Additional specimens of the Atropatene Brush-tailed Mouse were examined from the following locations: Hir, Ardabil Province (geometric morphometrics, n = 7; morphometrics, n = 6; and molecular, n = 8); and from Saghez, Kurdistan Province (geomorphometric, n = 5; morphometric, n = 4; and molecular, n = 6).

# Distribution

Known from 3 localities in northwestern Iran including Piranshahr, the type locality (West Azerbaijan Province), Saghez, county (Kurdistan Province), and a single locality from Hir



**Fig. 8.** Karyotype of *Calomyscus* sp. Group D: (A) Male specimen (5133ZMFUM) from Zagros Mountain, (B) Female specimen (5090ZMFUM) from Alborz Mountain (2N = 44, FNa = 56).

(Ardabil Province). This is the first report of *Calomyscus* occurring in the Ardabil Province of Iran.

#### Etymology

The name is a tribute to honor of Dr. Bahram Hassanzadeh Kiabi (Shahid Beheshti University of Tehran, Iran) in appreciation of his indefatigable work as an ecologist, who contributed greatly to the knowledge of Iranian wildlife. The common name Atropatene Brush-tailed Mouse is recommended. Atropatene is a region in antiquity that served as a precursor to modern-day Azerbaijan (sensu lato) and was found more southerly and chronologically later than Urartu. The geographic scope of Atropatene aligns reasonably well with the known range of *C. kiabii*.

#### Diagnosis

Calomyscus kiabii is a large brush-tailed mouse (head and body length 67-90 mm and tail length 73-100 mm) with large hind feet (17-22 mm), and variable ear height (11-23 mm; Table 4). Pelage is soft and dense with yellowish light brown color on dorsal surface. Dorsal hairs are light gray at tips, gray at the base, and yellowish light brown in the middle. Dorsal side of the fore and hind feet are pinkish white and that of the tail is light brown (Fig. 9). The ventral surface of the tail is yellowish white, and that of the fore and hind feet is light brown. There is a distinct border between the color patterns of the dorsal and ventral pelage. Tail is thick and well-haired along the total length with a distinct terminal brush. Rostrum is long, approximately one-third of the skull length (about 30% of OCCL). Premaxilla is narrow and frontal has low width between orbits. Maxillary bone is wide while auditory bulla is small. Foramen magnum is almost small and occipital bone is enlarged. M2 is large and its morphology is very similar to the main cuspides of M1. M3 is the smallest upper tooth about 40% of M1 in length.

Moreover, C. kiabii sp. nov. has wide nasal, large zygomatic breadth, long skull, long maxillary and mandibular tooth rows, and long upper diastema (Table 5). *Calomyscus kiabii* sp. nov. possess a distinct karyotype with 2n = 44 and FNa = 56, and showed a reasonable K2P divergence (>9.6%) in Cytb gene with other *Calomyscus* taxa.

#### Measurements

External measurements of the holotype and paratypes (in mm) are as follows: head-body length, 76.85 ± 6.5; tail length, 93.62 ± 4.03; ear height, 14.25 ± 2.46; hind foot, 21.17 ± 0.3. Mean measurements and standard errors of craniodental characters are as follows: HLD,  $1.77 \pm 0.11$ ; MH,  $6.33 \pm 0.12$ ; UDL,  $7.44 \pm 0.08$ ; NL,  $10.16 \pm 0.23$ ; ZW,  $13.39 \pm 0.06$ ; Forl,  $5.05 \pm 0.09$ ; NW,  $3.85 \pm 0.06$ ; SH,  $8.5 \pm 0.7$ ; OCCL,  $27.76 \pm 0.21$ ; CW,  $11.9 \pm 0.11$ ; CBL,  $25.30 \pm 0.15$ ; Mxl,  $3.62 \pm 0.1$ ; Mnl,  $3.64 \pm 0.02$ ; Mndl,  $14.7 \pm 0.26$ ; M1L,  $1.57 \pm 0.01$ ; M1W,  $1.11 \pm 0.0$ ; M2L,  $1.22 \pm <0.01$ ; M2W,  $1.13 \pm <0.01$ ; M3L,  $0.64 \pm 0.02$ ; M3W,  $0.85 \pm 0.01$ ; m1L,  $1.48 \pm 0.01$ ; m1W,  $1 \pm 0.01$ ; m2L,  $1.2 \pm 0.01$ ; m2W,  $1.07 \pm 0.01$ ; m3L,  $0.82 \pm 0.0$ ; m3W,  $0.75 \pm <0.01$ .

#### Description and comparisons

Calomyscus kiabii sp. nov. differs from C. bailwardi by its longer, higher, and wider cranium; and longer condylobasal length, anterior palatine foramen, lower diastema, mandibular and maxillary tooth rows, mandible, occipital length, M2, M3, m1, m2, and m3; and wider nasal, zygomatic breadth, M1, M2, M3, m1, m2, and m3.

In comparison with *C. elburzensis*, and *C. urartensis*, *C. kiabii* sp. nov. has wider nasal, longer upper and lower diastema, longer CBL, longer Occl, larger mandible, and longer tooth row. *Calomyscus kiabii* sp. nov. has a longer maxillary tooth row than *C. elburzensis*; and a longer head and body, tail and hind foot, a wider zygomatic arch, a greater mandibular height, and wider M3 and m1 than *C. urartensis*. This new species has both wider M3 and longer M2, M3, and m3, as well as shorter M1 than *C. elburzensis*.

Calomyscus kiabii has a greater height of the lower diastema; shorter and wider nasal; wider M3, m1; and larger M3, m2, m3, but a m1 that is shorter in length than C. grandis (Table 5). Compared to C. behzadi, C. kiabii has a wider cranium, a lower height of the lower diastema, a longer mandible and mandibular tooth row, a wider M1, a narrower m2, and a m1 that was shorter in length.

The karyotype of C. kiabii (2N = 44, FNa = 56) is unique among the Calomyscidae. *Calomyscus bailwardi*, C. *behzadi*, C. *elburzensis*, *C. grandis*, C. *mystax*, and *Calomyscus* sp. Group G have the same diploid number (2N = 44). The chromosome number of C. *kiabii* is distinct from other species of *Calomyscus* including *C. hotsoni*, *C.* cf. *bailwardi*, and *C. urartensis* (Table 3). The number of autosomal arms (FNa) in C. *kiabii* of 56 is unique among taxa of *Calomyscus* with a diploid number of 44, where the FNa ranges from 44 to 46 in *C. bailwardi*; 58 to 62, 68 to 72, and 76 in *C. elburzensis*; and is 46 in *C. grandis* and *C. mystax*, 48 in *C. behzadi*, and 62 in *Calomyscus* sp. Group G.

#### Habitat

Found in semi-dry rocky mountains, often with large boulders and sparse vegetation. Steppe vegetation is in association with shrubland including *Amygdalus* lycioides (Rosaceae) and *Lycium* sp. (Solanaceae) at an elevation of 1,647 m in northern Piranshahr (Supplementary Data SD6), and semi-dense shrubland is in association with thorny bushes including *Astragalus*, *Acanthophyllum* 

Taxon	2N	FNa	Reference
C. bailwardi	37	44	Malikov et al. 1999; Graphodatsky et al. 2000
	44	45	Romanenko et al. 2021
	44	46	Akbarirad et al. 2016a
C. cf.	50	48	Graphodatsky et al. 2000
bailwardi	50	50	Malikov et al. 1999
	51	57	Romanenko et al. 2021
	52	56	Malikov et al. 1999; Graphodatsky et al. 2000; Romanenko et al. 2021
C. baluchi	?	?	
C. behzadi	44	48	Dezhman et al. 2021
С. е.	44	58	Malikov et al. 1999
elburzensis	44	60	Esmaeili et al. 2008; Akbarirad et al. 2016b
	44	61	Romanenko et al. 2021
	44	62	Shahabi et al. 2010; Akbarirad et al. 2016b; Romanenko et al. 2021
	44	68	Mohammadi et al. 2013
	44	70	Mohammadi et al. 2013
	44	72	Shahabi et al. 2010; Mohammadi et al. 2013
	44	76	Mohammadi et al. 2013
C. e isatissus	44	70	Shahabi et al. 2010; Akbarirad et al. 2016b
C. e. zykovi	30	44	Meyer and Malikov 1995; Malikov et al. 1999; Graphodatsky et al. 2000; Meyer and Malikov 2000
C. grandis	44	46	Malikov et al. 1999; Graphodatsky et al. 2000; Shahabi et al. 2010; Mohammadi et al. 2013; Rezazadeh et al. 2020
C. hotsoni	48	46	Akbarirad et al. 2016a
	48	48	Shahabi et al. 2010; Akbarirad et al. 2016a, 2016b
	50	48	Shahabi et al. 2010; Mohammadi et al. 2013
C. mystax	44	46	Graphodatsky et al. 1989; Meyer and Malikov 1995; Malikov et al. 1999; Graphodatsky et al. 2000; Meyer and Malikov 2000; Romanenko et al. 2021
C. tsolovi	?	?	
C. urartensis	28	44	Mohammadi et al. 2013
	32	42	Graphodatsky et al. 1989; Meyer and Malikov 1995; Malikov et al. 1999
	34	42	Graphodatsky et al. 2000
Calomyscus sp. Group D	44	56	This study
Calomyscus sp. Group G	44	62	Rezazadeh et al. 2020
Calomyscus	46	44	Romanenko et al. 2021
sp. 2	46	45	Romanenko et al. 2021
Calomyscus sp. 3	44	67	Romanenko et al. 2021

(Fabaceae), Acantho, and Caryophyllaceae at an elevation of 1,615 m in temperate mountains in southwestern Ardabil. The Atropatene Brush-tailed Mouse is commonly found in rock walls surrounded by grain fields and fruit gardens. Other rodents in the same habitat include *Meriones* sp. (subfamily Gerbillinae), captured in Piranshahr, and *Nothocricetulus migratorius* (Cricetinae), captured at the Ardabil location. Sampling of this species from Ardabil was successful in both cold weather of late March and moderate weather of June in the same location. A female captured from Piranshahr had active mammary glands during sampling in June 2016. No endoparasites were found in the digestive system of any captured individuals.

# Calomyscus darvishi, new species Rezazadeh, Zali, Ahmadzadeh, Siahsarvie, and Aliabadian

# Darvish's Brush-tailed Mouse

Calomyscus sp. Group G: Rezazadeh et al. 2020.

#### Holotype

A specimen, identified as an adult male (ZMFUM-5282) with skin and skull (Figs. 11 and 12), along with tissues preserved in 90% ethanol, has been placed in the collections of the Zoological Museum at Ferdowsi University of Mashhad (ZMFUM). A Cytb sequence (Accession number: OR786671) was obtained from the holotype.

# Type locality

20 Km north of Fereydun Shahr, Isfahan Province (32°59′N, 50°06′E), 2,800 m (a.s.l.) in the central Zagros Mountains, collected in July 2016 by H. Zali.

# Paratypes

Skins, skulls, and ethanol-preserved tissue of 4 female specimens collected by H. Zali (5182 ZMFUM, 5203 ZMFUM, 5272 ZMFUM, and 5274 ZMFUM) deposited in the Zoological Museum of Ferdowsi University of Mashhad. Sequences of Cytb, Cox1, and Rbp3 were obtained from the paratypes (Table 1).



**Fig. 9.** Dorsal and ventral views of the holotype of *C. kiabii* (ZMFUM-5133).

# Other specimens

In addition to the holotype and paratypes (n = 4), additional materials from Fereydun Shahr, Isfahan Province, housed in the Zoological Museum of Ferdowsi University of Mashhad were examined in geomorphometric (n = 6), morphometric (n = 6), and molecular (n = 3) analyses.

# Distribution

Known only from the type locality, Fereydun Shahr, Isfahan Province, in the western part of the central Zagros Mountains of Iran.

# Etymology

The species name is a patronym honoring the late Prof. Dr. Jamshid Darvish (1952–2017), the head of the rodentology research group at Ferdowsi University of Mashhad (Iran), for his countless contributions to zoology in general, and rodentology of Iran, in particular. The suggested common name is Darvish's Brush-tailed Mouse.

# Diagnosis

Calomyscus darvishi is a large brush-tailed mouse (head and body 75–99 mm) with a very long tail (100–115 mm) and large hind foot (21–22 mm) and ear (19-21 mm) (Table 4). Pelage is soft and dense with gray color on dorsal surface and a sharp demarcation between the dorsal and ventral pelage colors. Dorsal hairs are gray at tips and dark gray at base. Ventral pelage is pure white. Dorsal surface of feet and hands is pinkish white. Ventral surface of fore, hind feet, and tail is yellowish white. Tail is covered with light gray hair on the dorsal surface with a distinct terminal brush. Calomyscus darvishi has a large braincase (26.58–28.56 mm in length; 11.94-12.74 mm in width), a long mandible (14.24–19.32 mm), but a narrow zygomatic breadth (13.3 ± 0.08) (Fig. 4). Nasal is long (approximately 40% of OCCL) and frontal bone has low width between orbits. Premaxilla is narrow and maxillary



Fig. 10. Holotype of C. kiabii showing dorsal (A) and ventral (B) views of the skull, labial (C) and lingual (D) views of mandible, and upper (E) and lower (F) tooth rows.

Character/species	Calomyscus kiabii sp. nov.	Calomyscus darvishi sp. nov. Holotype and paratypes (mean ± SE)			
	Holotype and paratypes (mean ± SE)				
BL	76.85 ± 6.5	86 ± 4.1			
TL	93.62 ± 4.03	$109 \pm 4$			
FL	21.17 ± 0.3	$21.60 \pm 0.2$			
EL	$14.25 \pm 2.46$	$20.4 \pm 0.5$			
LLD	$4.12 \pm 0.13$	4.25 ± 0.31			
HLD	$1.77 \pm 0.11$	2.17 ± 0.11			
MH	$6.33 \pm 0.12$	6.07 ± 0.21			
UDL	$7.44 \pm 0.08$	7.8 ± 0.07			
NL	$10.16 \pm 0.23$	$10.78 \pm 0.18$			
ZW	$13.39 \pm 0.06$	$13.09 \pm 0.17$			
Forl	5.05 ± 0.09	5.02 ± 0.9			
NW	$3.85 \pm 0.06$	$3.2 \pm 0.08$			
SH	8.5 ± 0.7	8.33 ± 0.08			
Occl	27.76 ± 0.21	27.63 ± 0.21			
CW	$11.9 \pm 0.11$	$12.27 \pm 0.09$			
CBL	25.30 ± 0.15	24.79 ± 0.4			
Mxl	$3.62 \pm 0.1$	3.44 ± 0.05			
Mnl	$3.64 \pm 0.02$	3.79 ± 0.25			
Mndl	$14.7 \pm 0.26$	$14.99 \pm 0.35$			
M1L	$1.57 \pm 0.01$	$1.57 \pm 0.01$			
M1W	$1.11 \pm 0.0$	$1.03 \pm 0.0$			
M2L	1.22 ± <0.01	$1.14 \pm 0.01$			
M2W	$1.13 \pm < 0.01$	1.02 ± <0.01			
M3L	$0.64 \pm 0.02$	$0.6 \pm 0.01$			
M3W	$0.85 \pm 0.01$	0.75 ± 0.01			
m1L	$1.48 \pm 0.01$	$1.44 \pm 0.01$			
m1W	$1 \pm 0.01$	0.98 ± <0.01			
m2L	$1.2 \pm 0.01$	$1.16 \pm 0.01$			
m2W	$1.07 \pm 0.01$	1.03 ± <0.01			
m3L	$0.82 \pm 0.0$	0.71 ± 0.02			
m3W	0.75 ± <0.01	0.72 ± <0.01			

Table 4. Means and standard errors of craniodental measurements of holotype and paratypes of Calomyscus kiabii **sp. nov.** and Calomyscus darvishi **sp. nov.** 

bone is wide. Palatal region has a long incisive foramen (approximately 64% of UDL). Tympanic bulla and foramen magnum are large and occipital bone is slightly small (Fig. 4). M2 is large and its morphology is very similar to the main cuspides of M1. M3 is the smallest upper tooth, 38% of M1 in length. The karyotype of *C. darvishi* with a 2N = 44 and a FNa = 62 is only shared with *C. elburzensis* (Table 3) sampled from Bojnord (Shahabi et al. 2010; Romanenko et al. 2021) and the Kurkhud Mountains (Akbarirad et al. 2016b) in the North Khorasan Province. Both the karyotype of *C. darvishi* and *C. elburzensis* from North Khorasan contain 10 pairs of subtelocentric and 11 pairs of acrocentric autosomes and a subtelocentric X chromosome and an acrocentric Y chromosome.

Distribution: Known only from the type locality, Fereydun Shahr, Isfahan Province, in the western part of the central Zagros Mountains of Iran.

#### Measurements

External measurements of the holotype and paratypes (in mm) are: head-body length,  $86 \pm 4.1$ ; tail length,  $109 \pm 4$ ; ear height,  $20.4 \pm 0.5$ ; hind foot,  $21.60 \pm 0.2$ . Mean measurements and standard errors of cranial and dental features are as follows: HLD,  $2.17 \pm 0.11$ ; MH,  $6.07 \pm 0.21$ ; UDL,  $7.8 \pm 0.07$ ; NL,  $10.78 \pm 0.18$ ; ZW,  $13.09 \pm 0.17$ ; Forl,  $5.02 \pm 0.9$ ; NW,  $3.2 \pm 0.08$ ; SH,  $8.33 \pm 0.08$ ; Occl,  $27.63 \pm 0.21$ ; CW,  $12.27 \pm 0.09$ ; CBL,  $24.79 \pm 0.4$ ; Mxl,  $3.44 \pm 0.05$ ; Mnl,  $3.79 \pm 0.25$ ; Mndl,  $14.99 \pm 0.35$ ; M1L,  $1.57 \pm 0.01$ ; M1W,  $1.03 \pm 0.0$ ; M2L,  $1.14 \pm 0.01$ ; M2W,  $1.02 \pm <0.01$ ; M3L,  $0.6 \pm 0.01$ ; M3W,  $0.75 \pm 0.01$ ; m1L,  $1.44 \pm 0.01$ ; m1W,  $0.98 \pm <0.01$ ; m2L,  $1.16 \pm 0.01$ ; m2W,  $1.03 \pm <0.01$ ; m3L,  $0.71 \pm 0.02$ ; m3W,  $0.72 \pm <0.01$ .

#### Description and comparisons

Based on morphometric results, *C. darvishi* is distinguished from *C. bailwardi* by its longer body, tail, hind foot, anterior palatine

Table 5.	Means and	standard e	error of mor	phometric	: measurements	s in 10	grou	ps of g	genus	Calomy	scus.

	C. bailwardi	C. cf. bailwardi	C. hotsoni	C. elburzensis	C. mystax	C. urartensis	C. grandis	C. behzadi	Calomyscus sp. G	Calomyscus sp. D
BL	78.1 ± 1.10	80.33 ± 2.04	75.18 ± 2.50	79.43 ± 0.82	81.25 ± 0.85	67.85 ± 1.41	77.46 ± 2.27	81.75 ± 1.65	88.2 ± 1.69	76.63 ± 1.92
TL	85.34 ± 0.85	83.33 ± 1.64	$92.64 \pm 0.52$	91.08 ± 0.90	$96.25 \pm 1.3$	77.63 ± 1.61	86.80 ± 3.00	93.5 ± 1.30	$100 \pm 2.90$	89.21 ± 2.07
FL	$19.15 \pm 0.08$	19.66 ± 0.23	$19.55 \pm 0.38$	$19.70\pm0.18$	$20 \pm 0.40$	$18.73\pm0.53$	$19.41\pm0.46$	$20.5 \pm 0.28$	$21.82\pm0.12$	$20.42\pm0.46$
EL	$15.5 \pm 0.50$	$18.55 \pm 0.41$	$16.81\pm0.78$	$17.13\pm0.41$	$14.25\pm0.85$	$13.3 \pm 1.55$	$13.8 \pm 0.94$	$17.5 \pm 0.28$	$20.81\pm0.22$	$16.42 \pm 1.28$
LLD	$3.39 \pm 0.04$	$3.41 \pm 0.03$	$3.42\pm0.04$	$3.35 \pm 0.03$	$2.10\pm0.13$	$3.43 \pm 0.07$	$3.68 \pm 0.05$	$3.68 \pm 0.03$	$4.43\pm0.15$	$3.83 \pm 0.09$
HLD	$2.17\pm0.02$	$2.20\pm0.04$	$2.25 \pm 0.01$	$2.11\pm0.02$	$2.95 \pm 0.02$	$1.49\pm0.03$	$1.88 \pm 0.09$	$2.27\pm0.04$	$2.25 \pm 0.05$	$2.03 \pm 0.05$
MH	$5.79 \pm 0.08$	5.66 ± 0.09	$6.03 \pm 0.08$	$5.72 \pm 0.04$	$5.76 \pm 0.09$	$5.24 \pm 0.14$	$5.84 \pm 0.07$	$6.24 \pm 0.15$	$6.17 \pm 0.10$	$5.98 \pm 0.15$
UDL	$6.37\pm0.04$	6.35 ± 1.45	$6.38\pm0.09$	$6.48\pm0.04$	$6.33 \pm 0.16$	$6.18 \pm 0.15$	$6.87\pm0.08$	$7.12\pm0.09$	7.77 ± 0.07	$6.94\pm0.08$
NL	$9.55 \pm 0.09$	$9.35 \pm 0.17$	$9.66 \pm 0.13$	9.82 ± 0.08	$9.52 \pm 0.17$	8.95 ± 0.28	$10.10\pm0.14$	$10.08\pm0.18$	$10.73 \pm 0.12$	9.57 ± 0.18
ZW	$12.50 \pm 0.08$	$12.43 \pm 0.16$	$12.33 \pm 0.12$	$12.49 \pm 0.05$	$12.65 \pm 0.10$	$12.40\pm0.11$	$12.81\pm0.09$	$13.15 \pm 0.29$	$13.06 \pm 0.08$	13.06 ± 0.07
Forl	$4.37\pm0.07$	$4.54 \pm 0.08$	$4.58\pm0.12$	$4.67\pm0.04$	$4.69\pm0.15$	$4.83\pm0.09$	$4.96\pm0.08$	$4.71\pm0.08$	$5.03 \pm 0.05$	$4.76\pm0.09$
NW	$2.74 \pm 0.06$	$2.84\pm0.08$	$2.77 \pm 0.07$	$2.84\pm0.02$	$2.99\pm0.09$	$3.11 \pm 0.03$	$3.32 \pm 0.06$	$3.35 \pm 0.12$	$3.46 \pm 0.05$	$3.48 \pm 0.06$
SH	8.19 ± 0.05	8.52 ± 0.07	8.29 ± 0.05	$8.32\pm0.04$	$8.57 \pm 0.05$	8.28 ± 0.09	$8.17 \pm 0.07$	$8.55 \pm 0.07$	8.38 ± 0.07	$8.40\pm0.06$
Occl	$24.88 \pm 0.13$	25.07 ± 0.29	$25.30\pm0.16$	$25.20 \pm 0.08$	25.9 ± 0.29	24.93 ± 0.29	$26.15 \pm 0.15$	26.36 ± 0.15	$27.62 \pm 0.16$	$26.44 \pm 0.30$
CW	$11.38\pm0.07$	$11.49\pm0.07$	$11.21\pm0.09$	$11.36\pm0.03$	$11.31\pm0.09$	$11.91\pm0.16$	$11.89\pm0.08$	$11.49\pm0.10$	$12.28\pm0.07$	$11.89\pm0.09$
CBL	$22.56 \pm 0.11$	$22.05 \pm 0.33$	$22.16 \pm 0.11$	$22.13\pm0.12$	$22.03\pm0.30$	$22.81\pm0.28$	$23.69 \pm 0.15$	$23.36 \pm 0.11$	$25.00 \pm 0.22$	$24.00 \pm 0.23$
Mxl	$3.38 \pm 0.02$	$3.43 \pm 0.03$	$3.56 \pm 0.02$	$3.52 \pm 0.02$	$3.38 \pm 0.08$	$3.63 \pm 0.06$	$3.52 \pm 0.04$	$3.68 \pm 0.04$	$3.44 \pm 0.04$	$3.60 \pm 0.03$
Mnl	$3.26 \pm 0.02$	$3.28 \pm 0.03$	$3.38\pm0.03$	$3.43 \pm 0.02$	$3.34 \pm 0.04$	$3.48 \pm 0.02$	$3.53 \pm 0.05$	$3.46 \pm 0.02$	$3.60 \pm 0.13$	$3.60\pm0.04$
Mndl	$12.60\pm0.07$	$12.64 \pm 0.15$	$12.91\pm0.11$	$12.92\pm0.04$	$12.72\pm0.04$	$13.11\pm0.18$	$13.66\pm0.09$	$13.69\pm0.07$	$15.25 \pm 0.41$	$14.16\pm0.14$
M1L	$1.60\pm0.01$	$1.62 \pm 0.02$	$1.67\pm0.01$	$1.65 \pm 0.00$	$1.63 \pm 0.02$	$1.61\pm0.02$	$1.60\pm0.01$	$1.69\pm0.00$	$1.58 \pm 0.01$	$1.60 \pm 0.01$
M1W	$1.00\pm0.01$	$1.05 \pm 0.00$	$1.12\pm0.00$	$1.10\pm0.00$	$1.08\pm0.01$	$1.08\pm0.01$	$1.05 \pm 0.01$	$1.03\pm0.00$	$1.04\pm0.00$	$1.06 \pm 0.01$
M2L	$1.10\pm0.01$	$1.12\pm0.00$	$1.17\pm0.01$	$1.17\pm0.00$	$1.13\pm0.02$	$1.16 \pm 0.01$	$1.21 \pm 0.01$	$1.25 \pm 0.01$	$1.14\pm0.01$	$1.23 \pm 0.01$
M2W	$0.99\pm0.00$	$1.03\pm0.01$	$1.09\pm0.00$	$1.06\pm0.00$	$1.05\pm0.01$	$1.08\pm0.00$	$1.08\pm0.01$	$1.08\pm0.00$	$1.02\pm0.00$	$1.08 \pm 0.01$
M3L	$0.58 \pm 0.00$	$0.58 \pm 0.01$	$0.62\pm0.01$	$0.58 \pm 0.00$	$0.55 \pm 0.02$	$0.61 \pm 0.02$	$0.58 \pm 0.01$	$0.61 \pm 0.01$	$0.60 \pm 0.01$	$0.64\pm0.01$
M3W	$0.69 \pm 0.01$	$0.72 \pm 0.01$	$0.70\pm0.01$	$0.71 \pm 0.00$	$0.72 \pm 0.02$	$0.73 \pm 0.01$	$0.74 \pm 0.01$	$0.78 \pm 0.00$	$0.74 \pm 0.01$	$0.79 \pm 0.01$
m1L	$1.37\pm0.01$	$1.39\pm0.01$	$1.46\pm0.01$	$1.42\pm0.00$	$1.47\pm0.01$	$1.43\pm0.00$	$1.460\pm0.01$	$1.49\pm0.01$	$1.45\pm0.01$	$1.42\pm0.0\ 2$
m1W	$0.93\pm0.01$	$0.98\pm0.00$	$1.00\pm0.00$	$0.96\pm0.00$	$0.97\pm0.00$	$0.92\pm0.00$	$0.94\pm0.01$	$0.99\pm0.01$	$0.99\pm0.00$	$0.98\pm0.01$
m2L	$1.11 \pm 0.01$	$1.19\pm0.01$	$1.23 \pm 0.01$	$1.21 \pm 0.00$	$1.21\pm0.02$	$1.18\pm0.02$	$1.24 \pm 0.01$	$1.24 \pm 0.01$	$1.16\pm0.01$	$1.24\pm0.00$
m2W	$0.98\pm0.01$	$1.00\pm0.01$	$1.05\pm0.00$	$1.03\pm0.00$	$1.01\pm0.00$	$1.01\pm0.00$	$1.03\pm0.00$	$1.05\pm0.00$	$1.02\pm0.00$	$1.03 \pm 0.01$
m3L	$0.69\pm0.00$	$0.73\pm0.01$	$0.74\pm0.01$	$0.74\pm0.00$	$0.66 \pm 0.01$	$0.78\pm0.01$	$0.72\pm0.01$	$0.78\pm0.00$	$0.71\pm0.01$	$0.79\pm0.00$
m3W	$0.67 \pm 0.00$	$0.68\pm0.01$	$0.68 \pm 0.00$	$0.70\pm0.00$	0.70 ± 0.02	$0.71\pm0.00$	$0.69\pm0.01$	$0.71\pm0.00$	$0.71\pm0.00$	$0.72\pm0.01$

foramen, diastema, mandible and mandibular tooth row, M2, m1, m2, and m3, and occipitonasal and condylobasal lengths; wider cranium, zygomatic breadth, M1, M2, M3, m1, m2, and m3; and both longer and wider nasals.

Calomyscus darvishi has a longer body, ear, hind foot, diastema, and nasals; greater height of the lower diastema and mandible, and occipitonasal and condylobasal lengths; wider cranium; shorter and narrower M2; wider m1 and longer m2 than it sister species, *C. grandis*. This new species has longer diastema and mandible; a wider cranium; greater condylobasal length; and shorter maxillary tooth row, M1, and M2 than *C behzadi*.

In comparison with *C. elburzensis*, *C. darvishi* has longer body, tail, ear, and hind foot, longer diastema, nasal, and mandible; a wider cranium; and greater condylobasal and occipitonasal lengths. It also has a shorter and narrower M1 than *C. elburzensis*.

The karyotype of *C. darvishi* (2N = 44, FNa = 62) has the same chromosome number as *C. bailwardi*, *C. behzadi*, *C. elburzensis*, *C. grandis*, *C. mystax*, and *C. kiabii*, but a different diploid number

from C. cf. bailwardi, C. hotsoni, and C. urartensis (Table 3). An autosomal arm number of 62 found in this new taxon has only been reported from populations of C. elburzensis (Table 3) from North Khorasan Province.

#### Habitat

Rocky mountaintops with sparse vegetation at elevations of 2,800–3,110 m a.s.l. (Supplementary Data SD7). Vegetation included low-lying shrubs (mountain almond, *Amygdalus scoparia*, Rosacea; and *Astragalus*, Fabaceae), grasses (*Stipa*, Poaceae), flowering plants such as chervil (*Chaerophyllum*, Apiaceae), lettuce (*Launaea*, Asteraceae), and mullein (*Verbascum*, Scropullariaceae), and common thistle (*Cirsium* sp., Asteraceae).

# Discussion

Recent revisions of the Calomyscidae by Musser and Carleton (2005) and Kilpatrick (2017) have both recognized 8 extant

species. Dezhman et al. (2021) recently described a new species, *C. behzadi*, from the Zagros Mountains in western Iran which formerly had been referred to as *Calomyscus* sp. Group C based on morphological and mtDNA comparisons with other *Calomyscus* from the Zagros Mountains (Akbarirad et al. 2016a). Herein we described 2 new species. *Calomyscus kiabii* from West Azerbaijan, Kurdistan, and Ardabil corresponds to *Calomyscus* sp. Group D of Akbarirad et al. (2016a). *Calomyscus darvishi* from the western central Zagros Mountains of Isfahan represents the lineage identified as *Calomyscus* sp. Group G in Rezazadeh et al. (2020). Additional distinct mitochondrial and/or cytological lineages of *Calomyscus* have been identified, but remain unnamed including, *C. cf. bailwardi* Group B (Akbarirad et al. 2016a, 2016b), and cytological species 1, 2, and 3 (Romananeko et al. 2021).

Our phylogenetic analyses of sequence data from 2 mitochondrial and 1 nuclear gene from the 3 mitochondrial lineages proposed by Akabarirad et al. (2016a) and Rezazadeh et al. (2020) and all the described species of *Calomyscus*, except *C.* tsolovi, recovered Group D and Group G as 2 distinct clades which we described as 2 valid species, *C.* kiabii and *C. darvishi*,



Fig. 11. Dorsal and ventral view of the holotype of C. darvishi (ZMFUM-5282).

respectively (Fig. 2). Calomyscus kiabii occurs in the northern Zagros and western Alborz (Talysh) Mts., appeared as the sister taxon of C. urartensis from the Lesser Caucasus with strong nodal support; however, the samples in these 2 clades have a mean genetic divergence (K2P) of Cytb of 7.8% (Table 2). A well-supported sister relationship was also recovered between C. darvishi from the central Zagros Mountains and C. grandis from the central and western Alborz Mountains, a relationship that was previously proposed based only on Cytb data (Rezazadeh et al. 2020). The mean genetic divergence of Cytb between these 2 lineages equals 7.5%. These genetic distances are beyond the putative K2P thresholds suggested for intraspecific variation for Cytb gene in rodents (Bradley and Baker 2001). Furthermore, these 2 new species and their sister taxa were recovered in a well-supported larger clade ("Clade 2") with C. elburzensis, C. behzadi, and C. mystax to the exclusion of C. bailwardi. The genetic divergence of Cytb between these 2 new species and C. bailwardi is greater than 18%. It has been estimated that the major Clade 2 (including C. kiabii and C. darvishi) and major Clade 1 (including C. bailwardi) diverged from each other around 9 Mya on the eastern Iranian Plateau (Rezazadeh et al. 2020).

Although there are high levels of genetic divergence among species of Calomyscus (Table 2), most of the species occur in similar rocky and barren habitats, and resemble each other morphologically. However, craniodental assessments separated specimens from the Zagros Mountains into 5 groups (C. cf. bailwardi, C. behzadi, C. kiabii, C. darvishi), in addition to C. bailwardi. Cranial shapes in geometric morphometric analyses (Akbarirad et al. 2016a; Rezazadeh et al. 2020, and the present study) clearly distinguish these groups. While morphological results revealed that C. kiabii and C. darvishi are more similar to C. grandis and C. behzadi, all 4 species could be differentiated by a number of cranial and dental characters. Karyotypes of both C. kiabii and C. darvishi have a 2N = 44 which is identical with 4 taxa (C. behzadi, C. elburzensis, C. grandis, C. mystax) of molecular Clade 2 but only taxon C. bailwardi of molecular Clade 1 (Table 3). Calomyscus urartensis as sister to C. kiabii has karyotypes with diploid numbers of 28, 32, and 34 with fundamental arm numbers (FNa) of 44, 42, and 42, respectively (Table 3). Calomyscus kiabii has a FNa = 56 and the only other Calomyscus with the same fundamental autosomal arms belongs to C. cf. bailwardi, reported from 30 km SE Kerman City in the vicinity of the Shahdad Tunnel in the Kerman Province (Malikov et al. 1999; Graphodatsky et al. 2000; Romanenko et al.



Fig. 12. Holotype of C. darvishi showing dorsal (A) and ventral (B) views of the skull, labial (C) and lingual (D) views of mandible, and upper (E) and lower (F) tooth rows.

2021). However, this population of *C*. cf. *bailwardi* has a diploid number of 52 (Table 3), with unknown taxonomic position. The karyotype of *C*. *kiabii* contains 7 pairs of subtelocentric and 14 pairs of acrocentric autosomes, a submetacentric X and a small acrocentric Y. The karyotype of *C*. cf. *bailwardi* with a 2n = 52 and a FNa = 56 contains 3 pairs of submetacentric and 22 pairs of acrocentric autosomes (Romanenko et al. 2007). Thus, *C*. *kiabii* has a karyotype distinct from its sister taxon and unique among the Calomyscidae.

The karyotypes of *C. darvishi* (2N = 44, FNa = 62) was previously reported for *Calomyscus* sp. Group G by Rezazadeh et al. (2020). The sister taxon of *C. darvishi*—*C. grandis*—has a karyotype of 2n = 44 and FNa = 46. A karyotype with the same diploid number (2n = 44) and fundamental arm number (FNa = 62) were reported from *C. elburzensis* from Bojnord and the Kurkhud Mountains of the North Khorasan Province in eastern Iran (Shahabi et al. 2010; Akbarirad et al. 2016b; Romanenko et al. 2021). The karyotypes of *C. darvishi* and *C. elburzensis* from the Kurkhud Mountains both contain 10 pairs of subtelocentric and 11 pairs of acrocentric autosomes, a subtelocentric X and a small acrocentric Y. While the karyotype of *C. elburzensis* from Kurkhud Mountains was examined by higher resolution banding approaches (Romanenko et al. 2021), such studies have not been conducted on *C. darvishi*.

Considerable chromosomal variation has been reported in the Calomyscidae (Table 3); however, only 3 karyotypes appear to be found in more than a single taxon: 2n = 44 and FNa = 46 reported in C. bailwardi, C. grandis, and C, mystax; 2n = 44 and FNa = 62 in C. darvishi and C. elburzensis; and 2n = 50 and FNa = 48 in C. bailwardi and C. hotsoni. While simple cytological studies are helpful in delineating species of calomyscids, given the likelihood of convergence of identical diploid and fundamental arm numbers in geographically isolated populations more detailed banding studies are needed to better infer homology of chromosomal characters. Although the present detail of the chromosome morphology of C. darvishi does not allow its karyotype to be differentiated from the karyotypes of some C. elburzensis, an integrative approach including molecular and morphological data in addition to karyotype demonstrates that these shared diploid and fundamental numbers are unlikely to be synapomorphic characters.

Calomyscus kiabii from West Azerbaijan, Kurdistan, and Ardabil provinces in western Iran and C. darvishi from the Isfahan Province were described from regions previously considered occupied by C. bailwardi (Graphodatsky et al. 2000; Morshed and Patton 2002; Musser and Carleton 2005; Karami et al. 2008). Kryštufek and Vohralik (2009) provides reports of Calomyscus from 2 localities in Turkey (Hakkari and Gaziantep Provinces). They discussed the probability that C. bailwardi occurs in the southeastern and southern Turkey, while mentioning that these 2 localities may represent 2 different species. It is geographically improbable that these populations are allied to C. bailwardi. According to our findings, because C. kiabii occurs on the western Iranian border, close to Hakkari Province in Turkey, we hypothesize that the distribution of this new species likely extends into the mountainous regions of southeastern Turkey. Intraspecific variation in size was observed in C. kiabii in that the Zagros population was morphologically larger than the Alborz population. The Cytb genetic divergence between these 2 populations of C. kiabii was 3.1%. Additional field work is needed to further examine both the range and diversity of C. kiabii.

The current distribution pattern of calomyscids likely has been shaped by Late Pliocene aridification and subsequent climate fluctuations on the Iranian Plateau. The family Calomyscidae split from the rest of eumuroids at about 20 Mya (Steppan and Schenk 2017) and diversification within the family has been estimated to have begun about 9.5 Mya (Rezazadeh et al. 2020). According to Rezazadeh et al. (2020) it seems that C. kiabii and C. darvishi diverged from their sister taxa in the late Pliocene (~3.3 Mya). Aridification events around the Iranian Plateau contributed to the emergence of the Lut and Central deserts during the late Pliocene (Girdler 1984; Sun et al. 2015) that could have isolated populations of Calomyscus in the central and northern Zagros, resulting in the split of C. darvishi in the central Zagros and C. kiabii in the northern Zagros from their sister species. Habitat fragmentation due to Pliocene climatic incidents and subsequent Pleistocene climate cycles, which was previously emphasized in several phylogeographical surveys in the study area (Homke et al. 2004; Agard et al. 2005; Dubey et al. 2007; Rajaei et al. 2013; Shahabi et al. 2013; Alaei Kakhki et al. 2016; Haddadian-Shad et al. 2016; Dianat et al. 2017), appears to be the primary isolating mechanism that has led to the divergence of the Calomyscidae in Iran.

# Supplementary data

Supplementary data are available at *Journal of Mammalogy* online. **Supplementary Data SD1.**—Results of ANOVA of external and craniodental measurments: 1—Result of ANOVA between *Calomyscus* sp. Group G and other groups. 2—Result of ANOVA between *Calomyscus* sp. Group D and other groups. Bold numbers indicate significant variables (P > 0.05).

Supplementary Data SD2.—Twenty-seven craniodental characters that were measured in samples of genus Calomyscus based on (Peshev 1991): Length of lower diastema (LLD), 2-Maximal mandibular height (MH), 3—Upper diastema length (UDL), 4— Nasal length (NL), 5—Zygomatic width (ZW), 6—Anterior foramina incisive length (Forl), 7—Nasal width (NW), 8—Maximum cranial height (SH), 9—Occipitonasal length (Occl), 10—Cranium width (CW), 11-Condylobasal length (CBL), 12-Height of lower diastema (HLD), 13-Maxillary tooth row length (Mxl), 14-Mandibular tooth row length (Mnl), 15—Mandible length (Mndl). Dental measurements; Upper molars: 16-M1L (length of M1), 17—M1W (width of M1), 18—M2L (length of M2), 19—M2W (width of M2), 20-M3L (length of M3), 21-M3W (width of M3), Lower molars: 22-m1L (length of m1), 23-m1W (width of m1), 24m2L (length of m2), 25—m2W (width of m2), 26—m3L (length of m3), 27—m3W (width of m3).

**Supplemental Data SD3.**—Mahalanobis distances between pairs of groups obtained from MorphoJ. Star mark indicated the significant separation between groups (P-value < 0.0001).

**Supplementary Data SD4.**—Plot of principle component analyses (PCA) on dorsal view of skull of genus *Calomyscus*.

**Supplementary Data SD5.**—Plot of principle component analyses (PCA) on ventral view of skull of genus *Calomyscus*.

**Supplementary Data SD6.**—The habitat of *Calomyscus kiabii* **sp. nov.** in Piranshahr, northern Zagros Mountains.

**Supplementary Data SD7.**—The habitat of Calomyscus darvishi **sp. nov.** in Fereydun shahr, central Zagros Mountains.

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# Author contributions

Conceptualization: ER, MA, and FA; methodology: ER, FA, CWK, and HZ; formal analysis: ER; investigation: ER; resources: ER, HZ, RS, and MA; writing—original draft preparation: ER; writing—review and editing: ER, CWK, and RWN; supervision: MA, FA, and ER; funding acquisition: MA.

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# **Conflict of interest**

None declared.

# Data availability

The data sets generated or analyzed during this study are included within the article and its supplementary information files.

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