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NEW RECORD OF PIGMY FIELD MICE (GENUS *APODEMUS*, MURIDAE, RODENTIA) FROM NORTHEASTERN IRAN

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ABSTRACT - Field mice from eastern Elborz, Golestan province, were examined using molecular, morphologic and morphometric characters. RFLP analysis showed the presence of three different haplotypes, corresponding to as many species: *Apodemus* cf. *uralensis*, *A*. cf. *hyrcanicus* and *A. witherbeyi*. While the two latter species have been previously reported from the studied area, the discovery of *A*. cf. *uralensis* in this region extends the eastern border of this species' distribution.

Key words: RFLP, new records, step field mouse, hyrcanian field mouse, pigmy field mouse, northeastern Iran

RIASSUNTO - ***Nuova segnalazione di topo selvatico pigmeo (genere* Apodemus*) in Iran nord orientale***. Topi selvatici dell'Elborz orientale, provincia di Golestan (Iran nord orientale) erano esaminati usando caratteri molecolari, morfologici e morfometrici. L'analisi RFLP ha rilevato la presenza di tre aplotipi, corrispondenti a altrettante specie: *Apodemus* cf. *uralensis*, *A.* cf. *hyrcanicus* e *A. witherbeyi*. Mentre le due ultime specie sono già state segnalate per l'area di studio, la scoperta di *Apodemus* cf. *uralensis* in questa regione amplia i confini orientali dell'area di distribuzione.

Parole chiave: RFLP, topi selvatici, genere *Apodemus*, nuove segnalazioni, Iran nord orientale

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INTRODUCTION

So far 79 species of rodents have been reported from Iran (Karami *et al*., 2008). Among them, the field mice of the genus *Apodemus* Kaup 1829, which is widespread in the western Palaearctic, are remnants of post glacial refugees. Available information concerning the taxonomy and range of *Apodemus*

species in Iran is scarce (Filippucci, 1992; Mezhzherin, 1997; Frynta *et al*., 2001; Filippucci *et al*., 2002; Michaux *et al*., 2002; Darvish *et al*., 2006). Currently, five species have been reported from Iran (Musser and Carleton, 2005; Karami *et al*., 2008): *A. flavicollis* (Melchior, 1834) from the Zagros Mountains (W Iran); *A. hyrcanicus* (Vorontsov *et al*., 1992), from the Hyrcanian forests along the southern border of the Caspian Sea and, eastward, up to Dasht (Bulatova *et al*., 1991; Musser and Carleton, 2005; Javidkar *et al*., 2007); *A. uralensis* Palas1811, from Makidi in Arasbaran, NW Iran (Kryštufek and Hutterer, 2006); *A. witherbyi* (Thomas, 1902), from plains, mountain and plateau steppes and highland semi-deserts of north-eastern Iraq and most of the central and northern Iranian Plateau, including Azerbaijan, Kurdistan, Lorestaan, Isfahan, Fars, Semnan, Tehran, central and eastern Mazandaaran, northern and eastern Khorasan and Kopet Dagh Mountain (Zagros and Elburz provinces; Macholan *et al*., 2001; Musser and Carleton, 2005; Javidkar *et al*., 2005 and 2007; Darvish *et al*., 2006; Siahsarvie and Darvish, 2008); *A. avicennicus* (Darvish *et al*., 2006), recently described from Yazd province, central Iran (Darvish *et al*., 2006).

With the aim of improving the knowledge about the geographic distribution of field mice in northeastern Iran, we i) applied a PCR-based restriction fragment length polymorphism (RLFP) method for distinguishing the species of the genus *Apodemus* by the analysis of cytochrome *b* (mtDNA), and ii) examined 39 morphological and morphometric diagnostic characters from 13 specimens trapped in Golestan province.

STUDY AREA AND METHODS

Sampling was performed in three different localities of Golestan, north-eastern Iran: Touskahestan (36º 42' 35N, 54º 35' 19 E), Garmabdasht (36º 42' 12 N, 54º 35' 06 E) and Gachian (36 º 43' 36N, 54 º 34' 60 E) (Fig. 1).

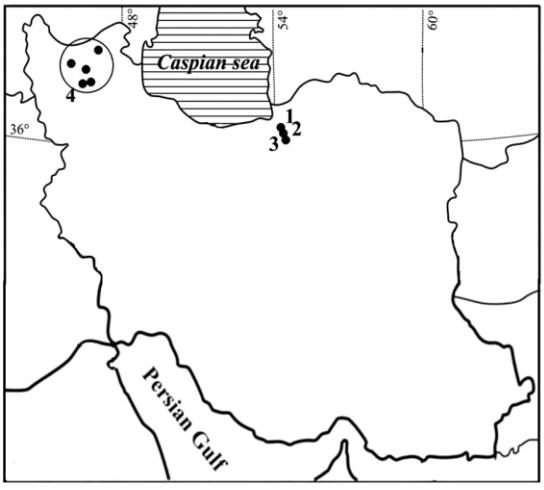

Figure 1 - Study area: 1- Tuskahestan, 2- Garmabdasht, 3- Gachian, 4- Azarbaijan.

Table 1 - Museum code (ZMFUM), haplotype and sampling locality of the three studied species of the genus *Apodemus*.

Species	Museum code	Haplotype	Locality
A. witherbyi	1816, 1820, 1822, 1823, 1824, 1825, 1826, 1830, 1832, 1839, 1840, 1842, 1855, 1857, 1896, 1897, 1898, 1899, 1900, 1914	C	Azarbaijan
	1935		Gachian
A. hyrcanicus	1797	B	Garmabdasht
	1860, 1865, 1910		Tuskahestan
	1926, 1931		Gachian
A. uralensis	1880, 1885, 1906, 1911, 1929, 1932	A	Tuskahestan

The first two areas are woodland, with oaks (*Quercus* spp.), beech (*Fagus sylvatica*) and maples (*Acer* spp.), at an altitude of, respectively, 895 and 1045 m a.s.l. Gachian is a steppic region at 1888 m a.s.l. with junipers (*Juniperus* sp.) and *Sedum* sp.

Molecular, morphological and morphometric analyses were carried out on 13 specimens. A further 20 individuals from Azerbaijan, NW Iran, were included as a reference for *A. witherbyi* (Hossein Pour Feizi *et al*., 2009) (Tab. 1).

Genomic DNA was extracted from liver or muscle tissues preserved in 98% ethanol, using Genetbio DNA extraction kit. Standard voucher specimens (skin and skull) were deposited at the Zoology Museum of Ferdowsi University of Mashhad (ZMFUM), Iran. Complete cytochrome *b* gene was amplified using modified universal primers L7 (5′-ACTAATGACATGAAAAATCATCGTT-3′) and H6 (5′-TCTTCATTTTTGGTTTACAAGAC-3′) (Montgelard *et al*., 2002). Amplifications were carried out in a Primus 96 thermal cycler with an initial denaturation step at 94°C for 2 minutes followed by 35 cycles (45s at 94°C, 45s at 50°C and 90s at 68°C) and a final extension time of 10 minutes at 68°C (Chevret *et al*., 2005). The PCR products were incubated at 37° C for 3-4 hours to be completely digested by two restriction enzymes: *Alu*I and *Hin*fI. As different species produce as many patterns, to distinguish among species results were analysed by electrophoresis on 1% agarose gels.

Overall 39 morphological traits and morphometric measurements were considered (after Filippucci *et al*., 1996 and Frynta *et al*., 2001), including in the analyses only adult specimens so as to rule out growth and evolutionary effects (Frynta and Zizkova, 1992).

The following dental (N = 12), cranial (N = 9) and external (N = 4) measures were filed: M1L: first upper molar length, M1W: first upper molar width, M2L: second upper molar length, M2W: second upper molar width, M3L: third upper molar length, M3W: third upper molar width, LM1L: first lower molar length, LM1W: first lower molar width, LM2L: second lower molar length, LM2W: second lower molar width, LM3L: third lower molar length, LM3W: third lower molar width, RH: rostrum height, ZYGW: zygomatic width, RW: rostrum width, IOW: interorbital length, BCW: braincase width, CBL: condylobasal length, BULL: bulla length, UML: upper

molar tooth row length, LML: lower molar tooth row length, BL: body length, TL: tail length, FL: foot length, EL: ear length.

Moreover, the variation in size, shape or position of 14 further parameters was also considered (Tab. 2).

Table 2 - Variation in size, shape or position of the 14 parameters analysed.

1. Bulla	a: massive and well developed		b: medium size	
2. Angular Process of the Mandible (Fig. 2A)	a: well developed and wide		b: tender and blade shaped	
3. Fronto-Parietal Suture	a: V shaped and angled		b: U shaped and curved	
4. Posterior edge of the Palatine (Fig. 2B)	a: curved		b: rather straight	
5. Position of the Incisors (Fig. 2C)	a: orthodont	b: semiorthodont	c: opisthodont	
6. Connective plan of the labial Anterocone (t3) and Anterostyle (t1) to the protocone (t5) (Fig. 3A).	a: no connection	b: t3 or t1 with a short enamel horn towards t5	c: t3 or t1 with a long enamel horn towards t5	d: t3 or t1 are connected to the side of t5
7. Relative position of the Enterostyle (t4) and paracone (t6) in upper M1 (Fig. 3B)	a: both in a row	b: t4 is upper	c: t4 is lower	
8. Size of the Anteroconule (t1bis-t3bis) in the upper M1 (Fig. 3C).	a: absent	b present	c: well developed and similar to a real cusp	
9. Position of the Metacone (t9) in the upper M1.	a: massive, large and similar to the paracone (t6)		b: relatively smaller than t6; straightly connected to the hypocone (t8)	
10. Position of the median Anteroconid (tma) in the lower M1 (Fig. 3D).	a: well developed and similar to lower cusps		b: medium size	c: Tiny
11. Relative position of tma and paired Anteroconid cusps (Fig. 3E).	a: tma is connected to paired labial and lingual Anteroconid		b: no connection	
12. Number of Cingula in the lower M1 (Fig. 3F).	a: 1	b: 2	c: 3	d: 4
13. Number of Cingula in the lower M2 (Fig. 3G).	a: 0	b: 1	c: 2	
14. Size of C1 in the lower M2 (Fig. 3H).	a: large/medium	b: small/tiny	c: absent	

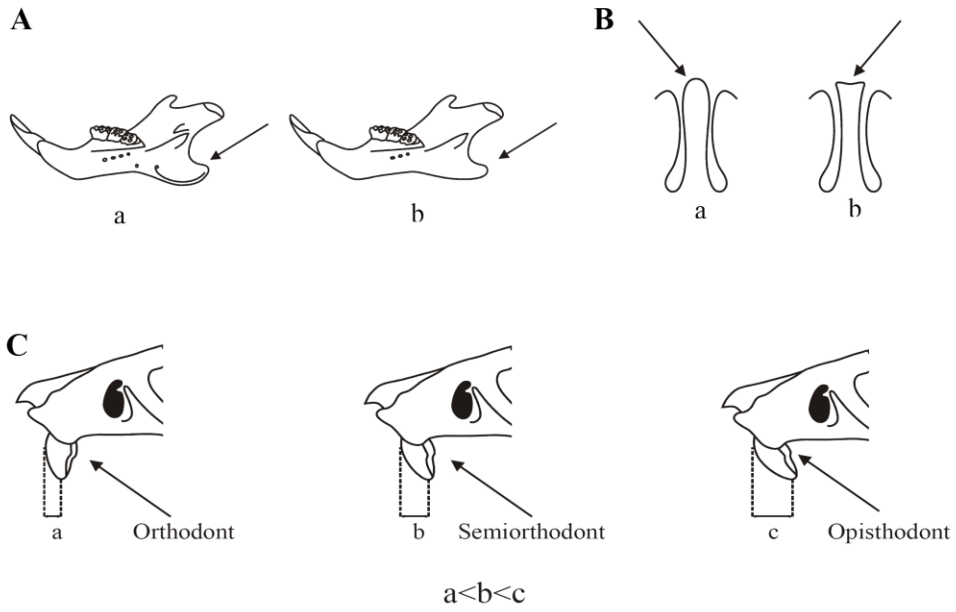

Figure 2 - Morphological states of cranial characters (see the text for explanations).

Dental characters were measured by a stereomicroscope accurate to the nearest 0.01 mm. Cranial measurements were taken using a caliper accurate to the nearest 0.05 mm. Standard external measurements were taken by a ruler accurate to the nearest 1 mm.

Having tested the normality and homogeneity of the variance of each variable, Univariate Analysis of Variance (ANOVA) and Multivariate Analysis of Variance (MANOVA) were performed to test for significant differences among the populations. Tukey's test was used to test the significance of all pairwise comparisons between groups. Patterns of interspecific differences were appraised by performing a Discriminant Function Analysis (DFA) on the characters defined as significant, through the MANOVA, among the *taxa* studied. The linear discriminant coefficients were defined from the non-null eigenvectors of the betweengroup variance-covariance scaled by the within-group variance-covariance. The original data were projected onto the functions defined by the standardized linear discriminant coefficients to obtain individual scores (Claude, 2008). The only specimen of Golestan (Gachian) attributed to *A. witherbyi* was excluded from the analysis and was posteriorly projected on the discriminant space. SPSS version 11.5 was used to perform all statistical procedures.

RESULTS

1. Molecular analyses

The results of RFLP analyses showed that the specimens belonged to three different haplotypes of restriction patterns, hereafter called A, B and C (Tab. 1; Fig. 4).

Six specimens captured in Touskahestan, near Gorgan, belonged to haplotype A. Their dorsal fur was gray with

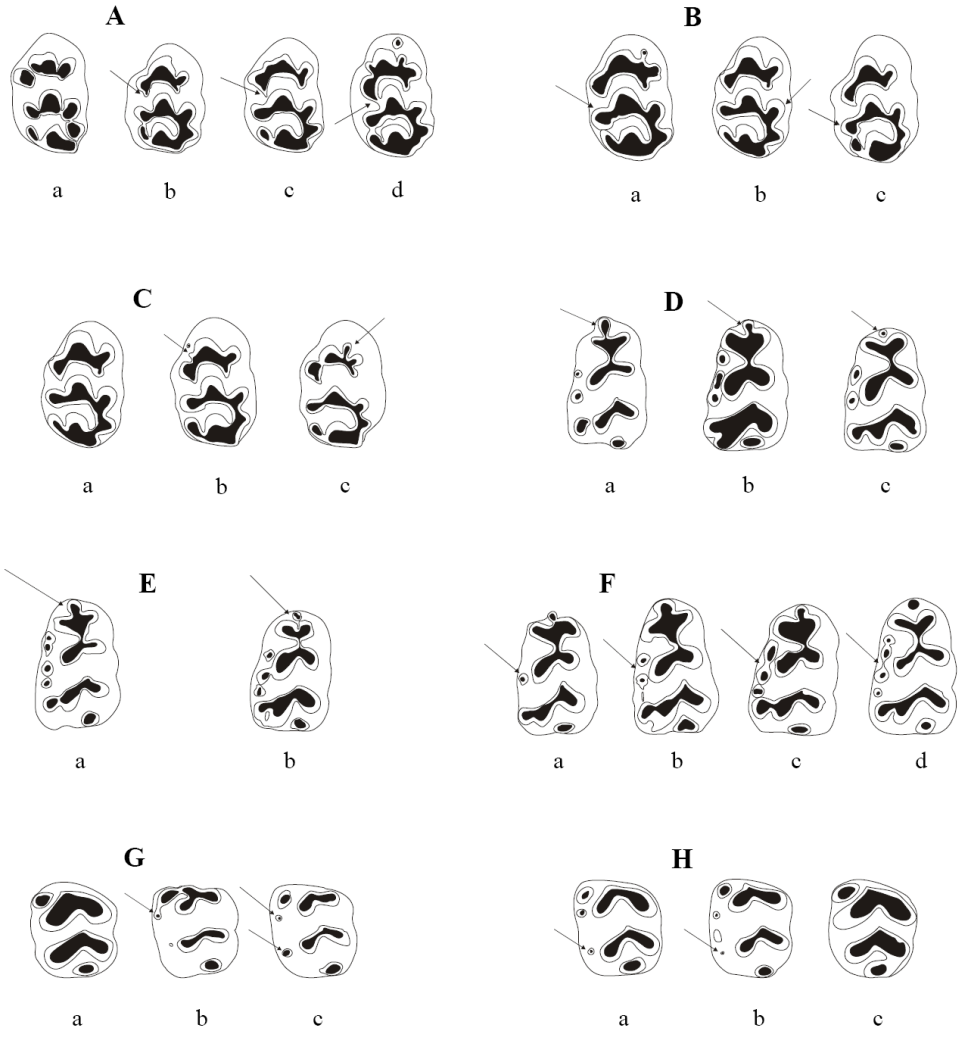

Figure 3 - Morphological states of dental characters in upper M1/ (A-C), lower M/1 (D-F) and lower M/2 (G, H). See the text for explanations.

brown tips, while the ventral fur was white with a gray base. The pectoral yellow spot was absent in three specimens, small in two and large in the last one. The tail was slightly shorter than body length.

The posterior edge of the Palatine was rather straight and large in four specimens and curved in the other two. The fronto-parietal suture was almost angular (N = 4) or curved (N = 2). In the first upper molar t4 and t6 were located in a row in all 6 specimens; t1bis was present in one, t3bis in three, t1-t3 showed no connection to t5 in all specimens; t9 was straight and ridge-like in three specimens and with a protrusion in the other three; the median ante-

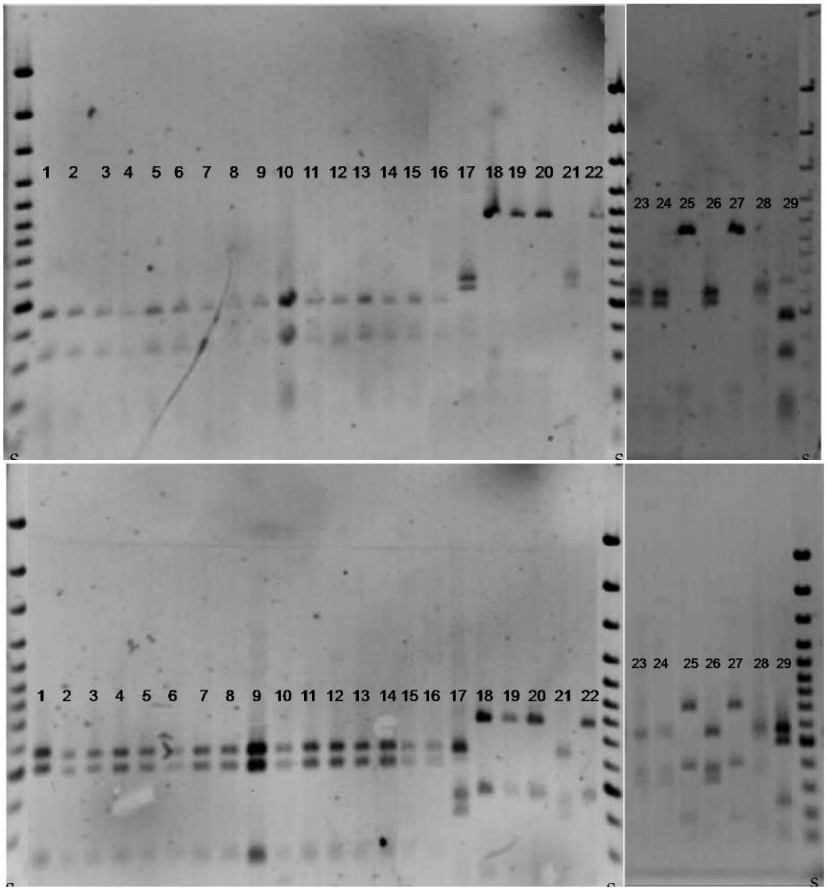

Figure 4 - Restriction patterns of cytochrome *b* PCR product by *Alu*I (top) and *Hin*fI (down). 1-16: specimens from Azarbaijan and C haplotype; 17, 21, 23, 24, 26, 28: specimens from Golestan and B haplotype; 18, 19, 20, 22, 25, 27: specimens from Golestan and A haplotype; 29: specimen from Golestan and C haplotype; S: 100 bps size marker.

roconid was well developed in four mice, median sized in one and tiny in the last specimen; it also was isolated from the paired anteroconid cusps in four specimens and connected to the lingual cusp in the other two. The number of cingula of the first lower molar ranged from 2 to 4, while in the second lower molar only one cingulum was recorded for five specimens and none for the last one.

The dental and cranial measurements of this haplotype were rather in accord with those reported by Vorontsov *et al*. (1992) and Kryštufek and Hutterer (2006) for *A. uralensis*, although our specimens have larger auditory bulla, longer occipito-nasal and tooth-row than the *A. uralensis* described by these authors. Anyway the taxonomic status of haplotype A should be confirmed through the examination of type speci-

mens or its comparison with *A. uralensis* from Arasbaran forests (NW Iran) or Turkish Anatolia.

Haplotype B was recorded for Touskahestan (N = 3), Garmabdasht (N = 1) Gachian (N = 2). The tail of these six specimens was sharply bicolored and approximately as long as the body length. The dorsal fur was dark brown, while the ventral fur varied from dark to white. A pectoral spot was seen only in one specimen. The fronto-parietal suture was always angular. Incisors were opistodont in four specimens and orthodont in one. The posterior edge of the palatine was curved in three and rather straight with small protrusion in one of the specimens (it was broken in the other two specimens). The inferior foramen of the palatine was slightly bypassing the alveolar of M1. The upper M1 was clearly stephanodont. In this molar, t1 bis was absent in three specimens and present in the others, t3 bis was present only in two specimens; t1 was disconnected from t2 and t3 in two specimens, while it was connected in three; connection of t1-t3 to t5 showed a short edge in two and no edge in three of the specimens; The metacone (t9) was large in three and pretty small in two of the specimens; t7 was small in five specimens; the median antroconid was medium sized in two and developed in three of the specimens; it was connected to the posterior tubercle in five specimens. The number of labial cingula in the lower M1 was 3 in three specimens and 4 in the other two. The number of cingula in the lower M2 was one in two specimens (with small C1 tubercles), whereas they were absent in the other three individuals. Most of the characters of this taxon were in accord with those reported for *A. hyrcanicus* (Vorontsov *et al*., 1992; Javidkar *et al*., 2007).

Haplotype C was recorded for only one specimen from Gachian, near Gorgan (1888 masl). The posterior edge of palatine was rounded and relatively short (4.7-5.1mm) and narrow (1.7-2 mm). The fronto-parietal suture was angled. Although the characters of this specimen slightly differed from those of *A. witherbyi* (as *A. fulvipectus*) as described by Voronsov *et al*. (1992), they sharply agreed with those reported by Javidkar *et al*. (2007) and Hossein Pour Feizi *et al*. (2009) for Iranian steppe field mice.

2 Morphometric results

The Univariate Analysis of Variance (ANOVA) showed that the specimens belonging to the three haplotypes were significantly different for 11 measured variables (Tab. 3). Haplotype B was the largest taxon, being larger than haplotype A for all characters except the length of the bulla and than *A. witherbyi* for 6/11measures (Tab. 3). Multivariate Analysis of Variance (MANOVA) on the 11 abovementioned significant characters indicates that the taxa representing haplotype A and haplotype B and *A. witherbyi* of NW Iran are significantly different (Wilks' Lambda value = 0.836, $P < 0.001$). A Discriminant Function Analysis (DFA) on the 11 significant characters was performed. Classification results were 100% correct for all the populations studied. The first two discriminant functions make up 100% of the variance (71.9% and 28.1% respectively).

Table 3 - Standard descriptive statistics in mm (mean ± SD) for 25 variables in *A.* cf. *hyrcanicus* (hy), *A. witherbyi* (wi) and *A.* cf *uralensis* (ur).

Variable	Haplotype A (*A.* cf. *uralensis*) N = 6		Haplotype B (*A.* cf. *hyrcanicus*) N = 6		*A. witherbyi* of Azarbaijan N = 21		Haplotype C (*A. witherbyi*) N = 1	Tukey's test, P<0.05	P-value ANOVA
	Mean	SD	Mean	SD	Mean	SD			
BL	86.50	10.15	93.80	12.70	85.70	10.94	81.00	---	
TL	81.83	3.31	90.40	11.10	91.70	13.19	97.00	---	
FL	21.50	1.05	22.50	0.55	21.10	1.17	22.00	hy>ur, wi	P<0.03
EL	14.67	1.37	15.17	1.47	13.80	1.85	15.00	---	
M1/L	1.79	0.03	1.83	0.05	1.83	0.08	1.78	---	
M2/L	1.17	0.03	1.18	0.04	1.15	0.05	1.13	---	
M3/L	0.81	0.04	0.91	0.06	0.87	0.04	0.82	hy, wi>ur	P<0.003
M1/W	1.17	0.03	1.19	0.03	1.18	0.04	1.10	---	
M2/W	1.12	0.03	1.17	0.04	1.13	0.04	1.14	hy>wi, ur	P<0.05
M3/W	0.82	0.05	0.89	0.03	0.83	0.54	0.81	hy>ur	P<0.04
M/1L	1.63	0.04	1.72	0.05	1.70	0.06	1.69	hy, wi>ur	P<0.03
M/2L	1.16	0.03	1.22	0.04	1.14	0.04	1.10	hy>ur, wi	P<0.001
M/3L	0.92	0.04	1.03	0.05	0.91	0.05	0.95	hy>ur, wi	P<0.001
M/1W	1.05	0.03	1.08	0.02	1.04	0.06	1.03	---	
M/2W	1.05	0.02	1.09	0.02	1.06	0.05	1.06	---	
M/3W	0.86	0.02	0.96	0.02	0.87	0.06	0.87	hy>ur, wi	P<0.002
RH	4.18	0.16	4.28	0.29	3.98	0.33	3.95	---	
ZYGW	12.5	---	13.6	0.61	13.06	0.65	12.10	---	
RW	4.27	0.18	4.75	0.32	4.48	0.30	4.32	hy>ur	P<0.03
IOW	4.20	0.06	4.23	0.12	4.18	0.14	4.30	---	
BCW	11.60	0.13	11.74	0.37	11.67	0.21	11.60	---	
CBL	24.15	1.09	25.43	1.30	24.73	1.22	23.90	---	
BULL	5.66	0.28	5.93	0.29	4.91	0.32	5.30	hy, ur>wi	P<0.001
UML	3.90	0.21	4.18	0.16	3.87	0.17	3.80	hy>wi, ur	P<0.008
LML	3.77	0.16	4.10	0.20	3.74	0.31	3.80	---	

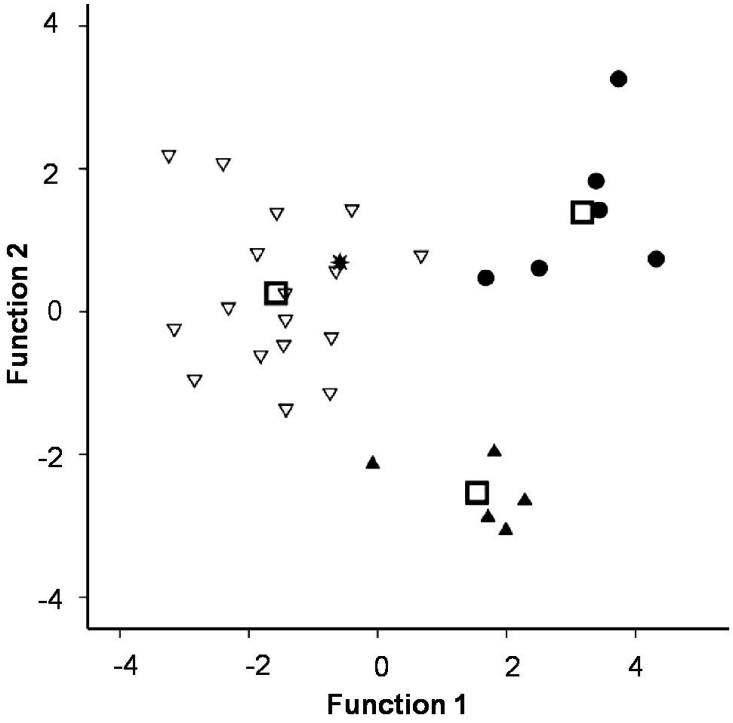

Figure 5 - Projection of specimens of four populations on the first two canonical variete. Solid circles: *A. cf. hyrcanicus*, solid triangles: *A. cf. uralensis*, open triangles: *A. witherby* of Azarbaijan, asterisks: *A. witherbyi* of Golestan province.

The only specimen representing haplotype C, which was considered as an ungrouped case in the analysis, was very close to *A. witherbyi* from Azarbaijan (Fig. 5).

DISCUSSION

Field mice have a patchy distribution in Iran, as a consequence of to the topographic and climatic conditions of this area. Considering the known range of both *A. witherbyi* and *A. hyrcanicus* (Musser and Carleton, 2005; Karami *et al*., 2008), the presence of these two species in the northern foothills of Elburz Mountains, Golestan province, is not unexpected. *A. uralensis* had been previously reported only from Arasbaran forests, where it is sympatric with *A. witherbyi* (Kryštufek and Hutterer, 2006). The recording of *A. uralensis* in upper Pleistocene deposits of Yuzhnoosetinskoy cave, Kudar region (Gromov and Fakanov, 1980), confirms the ancient presence of this species in the Armano-kurd zone of tension and speciation (Misonne, 1959). Consequently, *A. uralensis* may have been introduced by men to the Elburz Mountains from northwestern Iran. Currently, the Caucasus and eastern Elburz are the two extremities of the distribution range of *A. uralensis*, which occurs in sympatry with *A. hyrcanicus* in the Gorgan Mountains.

Lay (1967) described two different field mice from northern Iran, a bright and small species and a larger, darker one. He considered them as two ecological subspecies. Musser and Carleton (2005) argued that the first was *A. witherbyi* and the latter *A. hyrcanicus*. They considered them as parapatric species, selecting for different altitudes. In this study, the only *A. witherbyi* was trapped at about 1900 m a.s.l. Four out of six *A.* cf. *hyrcanicus* were trapped between 900-1050 m a.s.l., whereas the other two individuals were captured in the same area of *A. witherbyi*. Finally, the six *A.* cf. *uralensis* were captured at about 900 m a.s.l. All things considered, we can suppose that *A.* cf. *uralensis* is distributed in woodlands (*Parotica persica*, *Acer* sp. and *Quercus* sp.), *A. hyrcanicus* in deciduous forests with oaks (*Quercus* sp.) and beech (*Fagus sylvatica*) between 900 to 1900 m a.s.l. and *A. witherbyi* in the steppes and rocky regions above the tree-line.

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