

Integrative taxonomy of *Meriones persicus* (Rodentia, Gerbillinae) in Iran

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Five subspecies of the Persian Jird, *Meriones persicus*, were reported from Iran. These subspecies were described based on morphological characters and coat colors of few specimens. The question of the validity of these subspecies and their geographic distribution is raised. In this study, genetic markers (Cox1, Cytb and IRBP genes), morphometric (external and skull measurements), morphologic (coloration), and karyotypic data were combined to study intraspecific variation in *M. persicus* in the Iranian Plateau. Three distinct genetic lineages can be recognized in Iran (I, IIA, IIB), and these genetic lineages are separated by natural geographic barriers (Abarkooh, Central and Lut deserts). Our morphometric results also showed significant differences between these three lineages, and emphasized morphometric variability within clade IIA, where two subgroups could be recognized. However one of these subgroup was represented by only two individuals in our analyses, and additional morphometric data are needed to confirm this result. Fur coloration vary greatly among Iranian specimens and does not seem to be a reliable taxonomic character. Variation in FNa and morphology of the sex chromosomes was observed between populations, but the determinants of this variation and its significance for taxonomy needs to be investigated. To conclude, this study suggest that three to four subspecies should probably be recognized in Iran, but additional data with more specimens are needed to confirm this result.

Key words: Morphology, Morphometry, Karyology, Molecular, *Meriones persicus*, Iranian Plateau.

INTRODUCTION

Iran has a diverse mammal fauna due to its location in crossroad of several biogeographic regions (Karami et al., 2008). This is particularly true for the gerbil rodent genus *Meriones* (Illiger, 1811). This genus is confined to the Palearctic region (Corbet, 1978) and contains 17 currently recognized species, eight being present in Iran (Harrison & Bates, 1991; Lay, 1967; Misonne, 1957; Musser & Carleton, 2005).

Meriones persicus, the Persian Jird (Blanford, 1875), is distributed in Eastern Minor Asia and Transcaucasia through north-eastern Iraq and Iran, to southwestern Turkmenistan, Afghanistan, Pakistan (West of Indus River) and in the northeast and eastern part of Turkey (Lay, 1967; Harrison & Bates, 1991; Yigit & Colak, 1999; Vinogradov & Argyropulo, 1941; Darvish et al., 2006; Karami et al., 2008; Misonne, 1957; Musser & Carleton, 2005; Goodwin, 1940; Brown, 1980). The type locality of this species is Kohrud, 72 miles (115 Km) North of Esfahan, Iran (Musser & Carleton, 2005). In Iran, this species is widely distributed, being present everywhere, with the exception of the

Caspian forests and the border of the Persian Gulf. It inhabits rocky habitats including mountain slopes, talus and rocky outcrops (Pavlinov et al., 1990; Lay, 1967; Misonne, 1959; Darvish, 2009). This species is characterized by its small suprameatal triangle and tympanic bulla compared to other *Meriones* species. Its zygomatic plates are less projected forwards and the external bony auditory meatus does not come into contact with the posterior root of the zygoma (Darvish, 2009; Darvish, 2011; Yigit & Colak, 1999; Momenzadeh et al., 2008).

Up to now, five subspecies were reported from Iran (Ellerman & Morrison-Scott, 1951) (Figure 1):

1- *Meriones p. persicus* Blanford, 1875 was described from Kohrud, Esfahan and is distributed from north of Esfahan to Pakistan (Boudet, 2010; Etemad, 1978; Ellerman, 1966). 2- *Meriones p. baptistae* Thomas, 1920 was described from PashtKuh, southwest of Baluchistan, and is distributed in Pakistan (from Kelat, Kuldur, Pasht Kuh and Turbat in Baluchistan Province) and south east of Iran (Kerman, probably from East of Zagros to south east of Iranian Baluchistan and south of Khorasan province) (Boudet, 2010; Etemad, 1978; Ellerman & Morrison-Scott, 1951; Ellerman, 1966). 3- *M. p. gurganensis* Goodwin, 1939 was described from Bujnord district and is only known from Gorgan River to the Kurkhud Mountains (North Khorasan province) (Boudet, 2010; Goodwin, 1939; Etemad, 1978; Ellerman & Morrison-Scott, 1951). Lawrence (1993) claimed that *M. p. gurganensis* is the synonym of *M. p. persicus*. 4- *M. p. rossicus* Heptner, 1931 was described from Arzni (20 km north of Eriwan, Transcaucasia) and is distributed from Turkey to Armenia (Boudet, 2010; Ellerman & Morrison-Scott, 1951; Ellerman, 1966). Darvish et al. (2014) mentioned that the specimens of *M. p. persicus* in the northwest of Iran in Kordasht village, Sufian, Tabriz and Zanjan are belonging to *M. p. rossicus*. 5- *M. p. suschkini* Kashkarov, 1925 was described from Arshevi les (Bashi-Mgur, Great Balkhan Mountains, Turkmenistan), and is only distributed in the Great Balkhan Mountains in the west of Turkmenistan (Boudet, 2010; Ellerman & Morrison-Scott, 1951; Ellerman, 1966). 6- *M. p. ambrosius* Thomas, 1919 was described from Dopolan Mountains (Bakhtiari, 150 miles (241 Km) northeast of Ahwaz, Iran) and is only known from the southeast of Iranian Baluchistan (Boudet, 2010; Ellerman, 1966; Corbet, 1978).

Until now, few studies have been published about intraspecific variation in Iranian Persian Jirds. Momtazi et al. (2008) showed differences in the shape of meatus and mastoid parts of the tympanic bulla between populations from the Geno area (in the south of Iran, Hormozgan province) and from Tehran. Tabatabae and Adriaens (2011) showed that the shape of the skull is correlated to geoclimatic factors: the Persian Jirds from the southern populations living in lower, warmer and drier localities are characterized by bulla hypertrophy, less convex zygomatic arch, narrower zygomatic plate, longer incisive foramen and a slightly shorter nasal. Persian Jird subspecies were described based on morphological characters and coat colors, and they were described on few specimens. The question of the validity of these subspecies and their geographic distribution is raised.

In this study, we combined genetic markers, morphometric and morphologic data, and karyotypic features to study intraspecific variation in *M. persicus* in the Iranian Plateau. The aim of this paper is to test the validity of the five subspecies reported from Iran.

MATERIAL AND METHODS

MORPHOLOGIC AND MORPHOMETRIC ANALYSES

A total of 50 and 43 individuals of Persian Jird were compared morphometrically and morphologically, respectively. They come from different parts of Iran, including Zagros and Alborz Mountain chains, the Mountains of the eastern of Iran, and Kohrud Mountains (Figure 2, Table 1).

Only adult specimens with fully erupted and worn molars were incorporated into the morphometric analyses. These analyses were carried out based on four standard external measurements, fourteen cranial and six dental measurements. They were taken with a ruler to the nearest millimeter, a vernier

TABLE 1. List of specimens examined in this study, with geographic origin.

ID	locality	Samples used in morphometric analysis	Samples used in morphology	Samples used in Karyology	Samples used in molecular analysis	Morphometric groups	locality number in Figure 2
3750	Yazd-Shirkooh, Darreh Gahan	√				3	1
3749	Yazd-Shirkooh, Darreh Gahan	√	√			3	1
3751	Yazd-Shirkooh, Darreh Gahan	√	√		√-cladeIIA	3	1
3834	Yazd-Shirkooh, Baghe Mahdi	√				3	1
1519	Yazd-Shirkooh, Tezerjan	√	√			3	1
1710	Yazd-Shirkooh, Tezerjan	√				3	1
1619	Yazd-Shirkooh, Tezerjan	√	√			3	1
1376	Yazd-Kharanagh	√				3	1
3546	Yazd-Baghe Shadi	√	√			5	13
3535	Yazd-Baghe Shadi	√	√	√	√-cladeI	5	13
4516	Esfahan-Kohrud, Type locality	√	√			3	2
4517	Esfahan-Kohrud, Type locality	√	√		√-cladeIIA	3	2
4522	Esfahan-Kohrud, Type locality	√	√	√		3	2
4527	Esfahan-Kohrud, Type locality	√	√			3	2
4515	Hormozgan-Geno, Bandar Abbas	√				6	3
4514	Hormozgan-Geno, Bandar Abbas	√			√-cladeIIA	6	3
3695	Sistan Baluchistan-Zahedan, Mirjave	√				4	4
3694	Sistan Baluchistan-Zahedan, Mirjave	√	√			4	4
3685	Sistan Baluchistan-Zahedan, Mirjave	√	√			4	4
3526	Sistan Baluchistan Zahedan, Koole Sangi	√	√			4	4
3525	Sistan Baluchistan Zahedan, Koole Sangi	√	√			4	4
3279	Sistan Baluchistan Khash, Ab khaan	√	√			4	4
3280	Sistan Baluchistan Khash, Ab khaan	√	√			4	4
3301	Sistan Baluchistan-Taftan	√				4	4
3972	Sistan Baluchistan Zahedan, Manzel ab	√				4	4
3973	Sistan Baluchistan Zahedan, Manzel ab	√				4	4
3985	Sistan Baluchistan Zahedan, Manzel ab	√		√	√-cladeIIB	4	4
4066	Markazi-Arak, Namak koor	√				2	5
4558	Kurdistan -Marivan	√				2	6
3921	Kurdistan -Saghez	√			√-cladeI	2	6
3922	Kurdistan -Saghez	√				2	6
1638	Zanjan-Soltanieh	√				2	7
3902	Kermanshah-Songhor, Jamishan	√				2	8
3928	Kermanshah-Songhor, Jamishan	√				2	8
4347	Western Azerbaijan-Orumic	√	√			1	9

TABLE 1. Continued.

4348	Kurdistan Bijar	√	√	√	√-cladeI	2	6
4349	Kurdistan Bijar	√				2	6
2224	Eastern Azerbaijan-Tabriz	√	√			1	10
2223	Eastern Azerbaijan -Tabriz	√				1	10
2174	Razavi Khorasan-Neyshaboor, Soomee	√			√-cladeIIB	7	11
2177	Razavi Khorasan-Neyshaboor, Soomee	√				7	11
2852	Razavi Khorasan-Chenaran	√				7	11
1109	Razavi Khorasan-Dargaz, Tandure	√	√			7	11
1113	Razavi Khorasan-Dargaz, Tandure	√				7	11
1237	Razavi Khorasan-Khajeh Morad	√				7	11
2924	North Khorasan-Bojnord, Ayoob	√	√			7	12
2888	North Khorasan-Bojnord, Ayoob	√				7	12
2916	North Khorasan-Bojnord, Ayoob	√			√-cladeIIB	7	12
2925	North Khorasan-Bojnord, Ayoob	√	√			7	12
2843	North Khorasan-Bojnord, Ayoob	√				7	12
2909	Eastern Azerbaijan -Tabriz		√	√		1	10
2910	Eastern Azerbaijan -Tabriz		√			1	10
4351	Kurdistan-Bijar		√			2	6
4350	Kurdistan -Bijar		√			2	6
3811	Yazd-Sange Deraz		√			3	1
3055	Yazd		√			3	1
3810	Yazd-Mehriz		√			3	1
3731	Yazd-Mehriz		√			3	1
3035	Yazd		√			3	1
3684	Sistan Baluchistan-Zahedan, Mirjave		√			4	4
3680	Sistan Baluchistan-Zahedan, Mirjave		√			4	4
3755	North Khorasan-Bojnord, Ayoob		√			7	12
3183	North Khorasan-Bojnord, Ayoob		√			7	12
2655	North Khorasan-Bojnord		√			7	12
3193	North Khorasan-Bojnord		√			7	12
3001	North Khorasan-Bojnord		√			7	12
3222	North Khorasan-Bojnord		√			7	12
1455	Razavi Khorasan-Zoshk		√			7	11
3188	North Khorasan-Jajarm		√			7	12
3174	North Khorasan-Jajarm		√			7	12
3859	North Khorasan-Bojnord		√			7	12

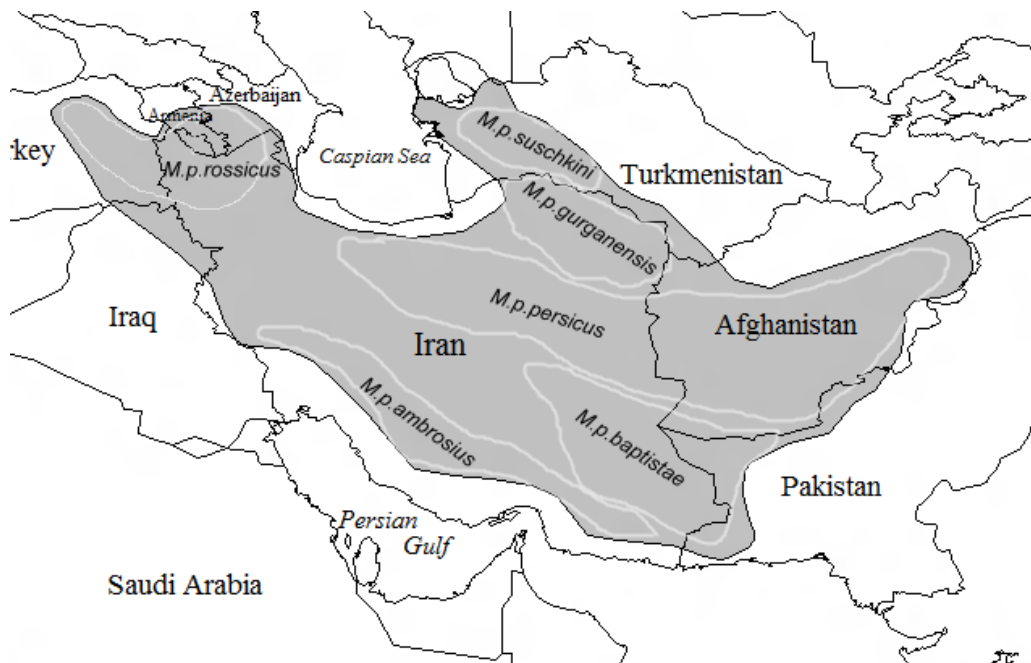


FIGURE 1. Map showing the actual geographic distribution of *M. persicus* (in grey) and its subspecies based on Boudet, 2010; Ellerman & Morrison-Scott, 1951; Krystufek and Vohralik 2009; Ellerman, 1966; Corbet, 1978.

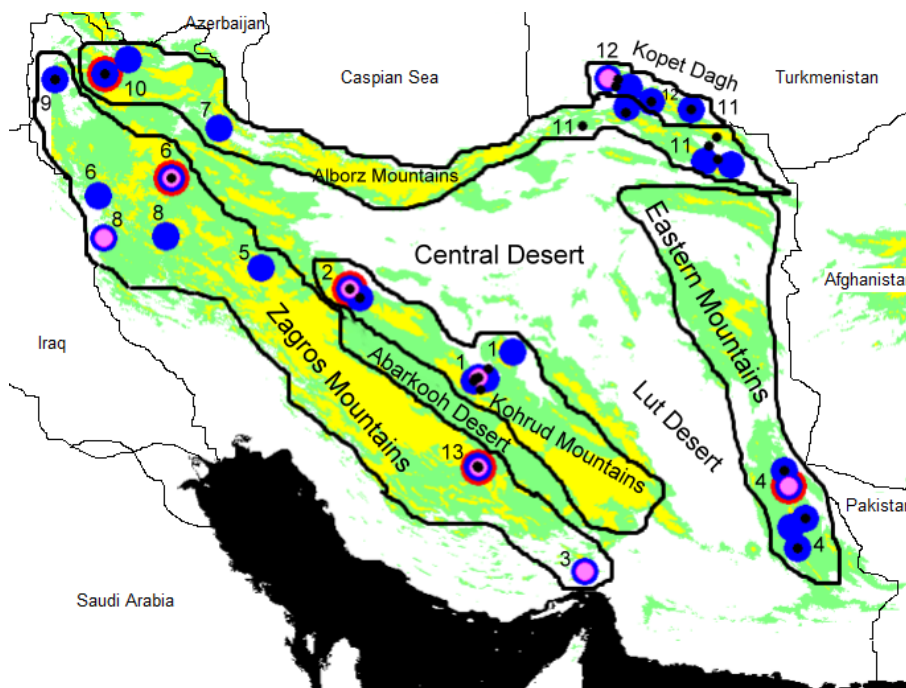


FIGURE 2. GeoRef map of sampling localities. Black circles indicate specimens used in morphologic analysis; pink circles indicate specimens used in molecular analysis; blue circles indicate specimens used in morphometric analysis, and red circles indicate specimens used in karyology (numbers refer to sampling localities see Table 1 for more information).

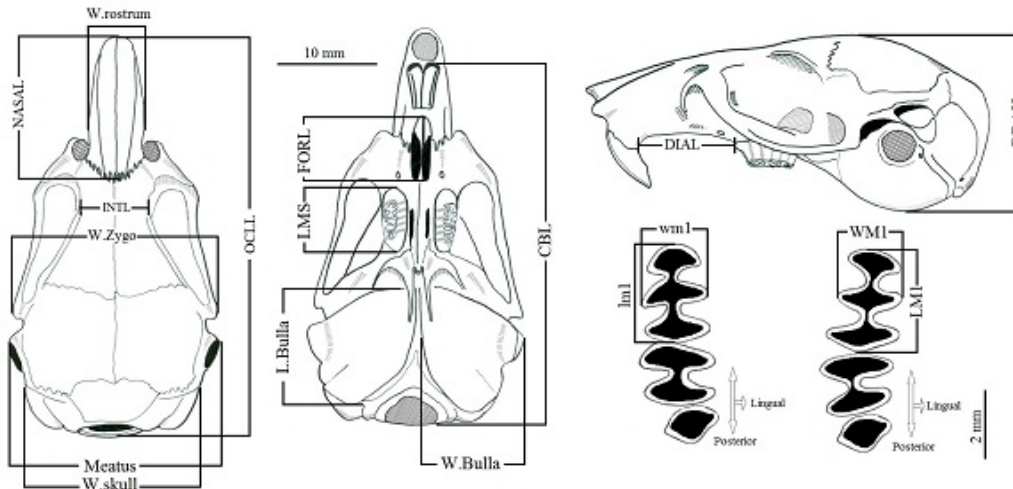


FIGURE 3. Definition of measured cranial and dental variables. See text for abbreviations.

caliper to the nearest 0.1 mm and an Olympus measurescope to the nearest 0.001 mm, respectively. The measurements abbreviation are as follows: **BL**: Body length, **TL**: Tail length, **FL**: Foot length, **EL**: Ear length, **DIAL**: upper diastem length, **NASAL**: Nasal length, **W.Zygo**: Zygomatic width at the auditory meatus level, **FORL**: Length of anterior foramen of palatine, **INTL**: Interorbital constriction width, **OCLL**: Occipitonasal length, **L.bulla**: Tympanic bulla length, **W.bulla**: Tympanic bulla width, **BRAH**: Braincase height on tympanic bulla, **W.skull**: width of skull at the back of meatus, **Meatus**: distance of two tympanic meatus from the lateral edges, **CBL**: Condylobasal length, **W.rostrum**: width of rostrum at the base of zygomatic arch, **L.Bulla/W.Bulla**: the proportion of Tympanic bulla length to Tympanic bulla width, **LMS**: Length of raw upper molars at the alveoli level, **LM1**: Length of first upper molar, **WM1**: width of first upper molar, **lm1**: Length of first lower molar, **wm1**: width of first lower molar, **Lms**: Length of raw lower molars at the alveoli level (Darvish, 2009; Darvish, 2011) (Figure 3).

We defined seven groups based on the range of subspecies according to bibliographical data (Figure 4, Table 1).

Groups 1, 3, 4, 6 and 7 include specimens that come from the geographic range of *M. p. rossicus* (specimens from Eastern Azerbaijan and Western Azerbaijan), *M. p. persicus* (Esfahan and Yazd (Kohrud)), *M. p. baptistae* (southeast Iran: Sistan Baluchistan), *M. p. ambrosius* (specimens from Bandar Abbas) and *M. p. gurganensis* (specimens from Northeast Iran: Razavi Khorasan and North Khorasan), respectively. Group 2 consists of specimens from Kurdistan, Kermanshah, Markazi and Zanjan provinces, and Group 5 includes two specimens from BagheShadi. No subspecies name is available in the literature for these two last groups. Sexual dimorphism was never reported in this species (Darvish, 2009; Tabatabaei & Adrians, 2013). Therefore, specimens of both sexes were pooled in our analyses. Statistical procedures were performed using SPSS (version 16, SPSS Corporation) and PAST 2.08 (Hammer et al., 2011). Descriptive statistics (mean and standard deviation) were calculated for each group (Table 2). Normality and homogeneity of variance was tested by Shapiro and Leven tests, respectively. ANOVA and Principal Component Analysis (PCA) were performed to reveal if the populations differ morphologically. In PCA, KMO (Kaiser-Meyer-Olkin Measure) and Bartlett test were used to test adequate sample size and suitability of the data for PCA, respectively (Yigit et al., 2011; Sadeghpour & Moradi, 2011). Significant value was set at 0.05.

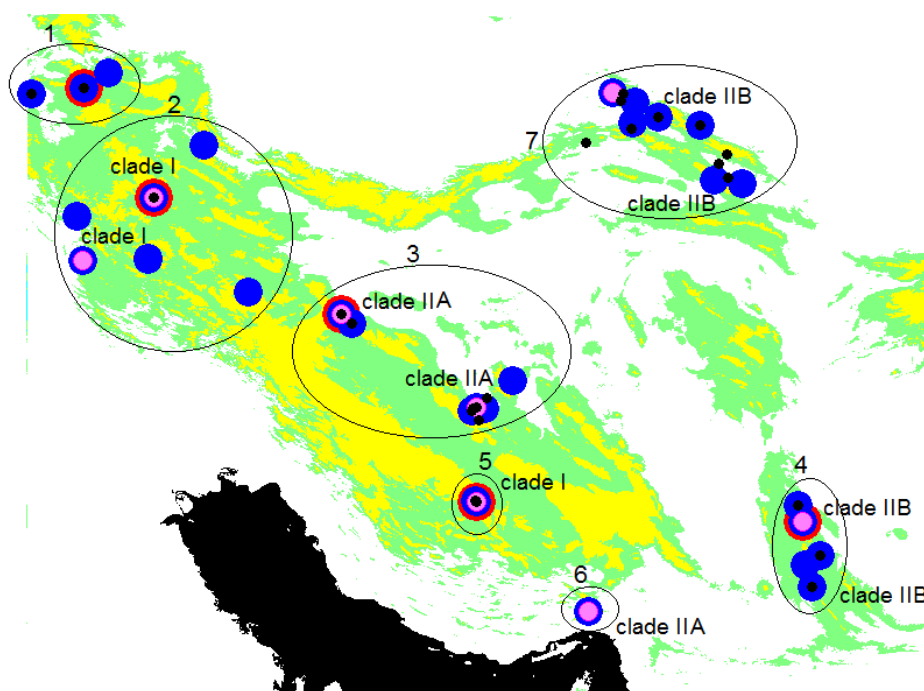


FIGURE 4. Map showing the sampling points (see figure 2 for more information), the 7 groups used for morphometric analyses and the distribution of the three main phylogenetic lineages.

TABLE 2. Comparison of 24 morphometric variables among all groups of *M. persicus*, means with standard Deviation (SD). Numbers 1 to 7 refer to the studied groups.

	1 (Mean)±SD	2 (Mean)±SD	3 (Mean)±SD	4 (Mean)±SD	5 (Mean)±SD	6 (Mean)±SD	7 (Mean)±SD
BL	138.7 ±13.8	145 ± 21.1	131.6 ± 19.6	129.6±10.1	151 ± 2.1	-	146.5 ± 31.3
TL	39.7 ± 2.5	39.2 ± 0.7	162.8 ± 10.8	139.5±41.7	180	-	150.6 ± 22.1
FL	19.5 ± 4.8	22.1 ± 2.2	37.2 ± 1.5	36 ± 1.3	39.5 ± 0.7	-	36 ± 3.3
EL	6.2 ± 0.2	6.3 ± 0.4	20.8 ± 2.8	19 ± 3.2	25	-	21.5 ± 3.4
Lms	2.55 ± 0.14	2.47 ± 0.19	6.318 ± 0.194	6.025±0.578	6.25 ± 0.032	6.33 ± 0.085	6.289 ± 0.289
lm1	1.56 ± 0.1637	1.52 ± 0.356	2.334 ± 0.203	2.527±0.204	2.50 ± 0.132	2.35 ± 0.009	2.478 ± 0.220
wm1	1.568±0.163	1.528±0.356	1.453±0.262	1.702±0.120	1.705±0.035	1.679±0.0192	1.576±0.222
WM1	5.987 ± 0.187	5.90 ± 0.303	1.453 ± 0.262	1.702±0.120	1.70 ± 0.035	1.67 ± 0.019	1.576 ± 0.222
LMS	2.62 ± 0.2244	2.55 ± 0.258	5.990 ± 0.672	5.869±0.420	6.08 ± 0.028	6.00 ± 0.048	5.604 ± 0.197
LM1	1.673 ± 0.204	1.59 ± 0.319	2.467 ± 0.323	2.751±0.283	2.67 ± 0.024	2.49 ± 0.088	2.620 ± 0.336
DIAL	11.3 ± 0.9	11.2 ± 1.2	10.7 ± 1.1	10.6±0.6	12.5 ± 1.3	9.8 ± 0.5	10.5 ± 1.7
NASAL	15.9 ± 1.1	15.6 ± 2.1	15.2 ± 0.9	15.4±1	17.7 ± 0.6	15.5 ± 0.3	15.3 ± 1.6
W.bulla	9.4 ± 0.2	9.5 ± 0.6	10.0 ± 0.5	9.9±0.4	10.3 ± 0.1	10.6 ± 0.1	10.0 ± 0.6
L.bulla	10.8 ± 0.5	10.9 ± 0.9	12.7 ± 0.7	12.5±0.4	12.6 ± 0.5	14.9 ± 0.3	12.3 ± 0.9
OCLL	41.3 ± 1.6	40.6 ± 3.4	39.8 ± 2.7	39.7±1.5	44.0 ± 1.5	41.2 ± 0.1	40.2 ± 3.3
W.Zygo	21.3 ± 1.4	20.9 ± 2.7	20.2 ± 1.6	20.3±0.8	21.9 ± 0.5	20.7 ± 0.6	21.4 ± 2.0
CBL	36.8 ± 0.9	36.4 ± 3.4	36.1 ± 2.7	36.1±1.4	39.7 ± 1.7	37.1 ± 0.2	36.4 ± 3.1
INTL	6.9 ± 0.2	7.2 ± 0.4	6.8 ± 0.5	6.8±0.7	7.5 ± 0.2	7.2 ± 0.4	7.1 ± 0.4
FORL	7.2 ± 0.3	7.1 ± 0.8	7.1 ± 0.7	7.5±0.7	7.9 ± 0.7	7.2 ± 0.4	7.5 ± 0.7
W.skull	16.7 ± 0.7	16.4 ± 0.7	18.3 ± 1.2	17.6±0.7	18.0 ± 1.1	-	17.5 ± 0.9
BRAH	14.6 ± 0.3	14.5 ± 0.56	15.0 ± 0.4	15.1±0.2	15.4 ± 0.1	-	14.7 ± 0.5
Meatus	20.3 ± 0.6	20.2 ± 1.4	20.7 ± 0.9	20.6±0.6	21.7 ± 0.2	-	20.7 ± 1.1
W.rostrum	6.1 ± 0.6	6.1 ± 0.6	5.6 ± 0.3	5.3±0.5	6.7 ± 0.4	5.8 ± 0.0	5.8 ± 0.7
L.bulla/W.bulla	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.0	1.3±0.0	1.2 ± 0.0	1.4 ± 0.0	1.2 ± 0.1

MOLECULAR ANALYSIS

The whole genomic DNA was extracted from frozen and preserved (90% Ethanol) tissues (liver or muscle) of 9 specimens from several localities (Table 1) using salt methods (Bruford et al., 1992). Two mitochondrial (Cox1, Cytb) and one nuclear (IRBP) genes, were amplified using primers VF1d 5'-TTC TCA ACC AAC CAC AAR GAY ATY GG-3' and VR1d 5'-TAG ACTTCT GGG TGG CCR AAR AAY CA-3' (Ivanova et al., 2006), L7: 5'-ACT AAT GAC ATG AAA AATCAT CGT T-3' and H6: 5'-TCT TCA TTT TTG GTT TAC AAGAC-3' (Montgelard et al. 2002) and IRBP-F 5'-GAATGCAAGCAGCCATTGAGC-3' and IRBP-R 5'-CACGGCTGAGTAGTCCATGC-3' (this study), respectively. PCR conditions are described in Aliabadian et al. (2007) and Chevret et al. (2005), for Cox1 and Cytb, respectively. PCR conditions for IRBP were: 94°C for 3 min, followed by 38 cycles at 94°C for 30 sec, 56°C for 40 sec and 72°C for 1 min and 30 sec; and a final extension at 72°C for 5 min.

These three genes were sequenced with Sanger method. Alignment was performed by BioEdit (Hall, 1999). Tree was rooted with *Meriones vinogradovi*. We combined all genes (mtDNA+IRBP) in our phylogenetic analyses. A partition for each gene was defined. We chose for each marker the mutation model that best fitted the data according to the Akaike information criterion (Cox1: TIM+I, Cytb: TVM+G, IRBP: HKY+I, for combine data: GTR+I+G) using MrModeltest 3.7 (Posada & Crandall, 2001). Phylogenetic relationships were inferred using Bayesian inference (BI), as implemented in Bayes v.3.2 (Ronquist et al., 2012) using the Markov Chains Monte Carlo method (MCMC). For BI, in the MCMC process, four chains were simultaneously run for 1,000,000 generations, with trees sampled every 100th generations (resulting in 10,000 trees), using default priors. The analyses began on a random starting tree. The first 5000 trees were discarded as a conservative "burn in", and the posterior probability (Pp) values were calculated from the remaining trees. Stationarity was assumed when the cumulative Pp values of all

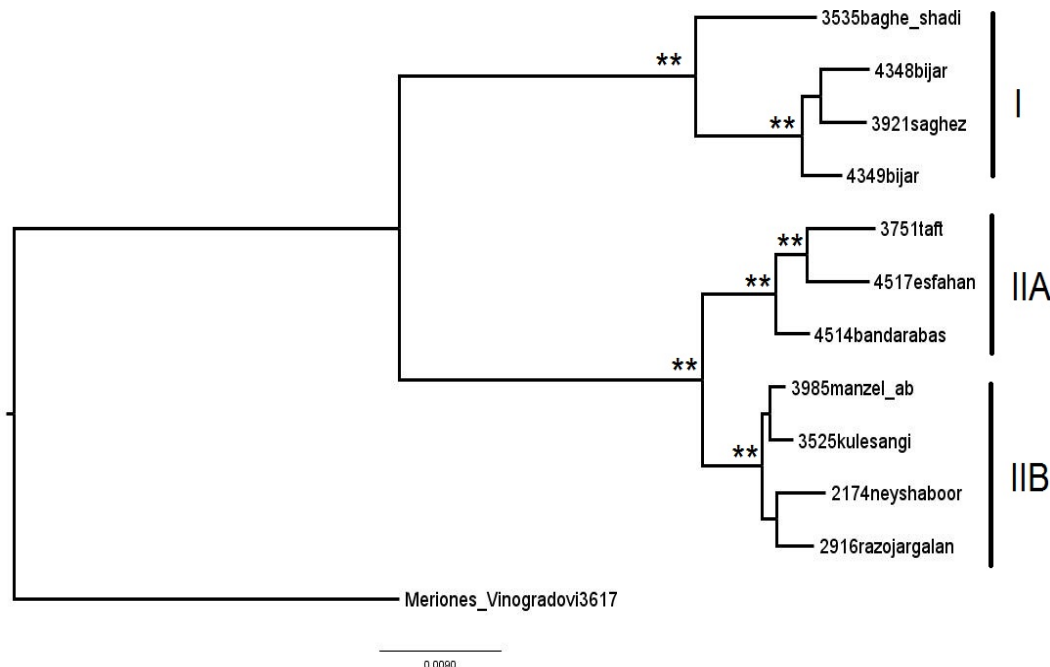


FIGURE 5. Ninety-percent majority-rule consensus tree sampled from the posterior distribution of the most-partitioned analysis. Posterior probability values from the Bayesian analysis are indicated at the >99% (**), >95% (*) significance levels.

TABLE 3. Results of the ANOVA analysis. The posterior Tukey test was used to evaluate significant differences between groups. Only variables showing significant differences among groups are reported in the table.

Pairwise groups	Different measurements	P < 0.05
1,3	L.Bulla, L.Bulla/W.Bulla	1 < 3
1,4	L.Bulla, L.Bulla/W.Bulla	1 < 4
1,6	L.Bulla, L.Bulla/W.Bulla	1 < 6
1,7	L.Bulla, L.Bulla/W.Bulla	1 < 7
2,3	L.Bulla, L.Bulla/W.Bulla, W.skull	2 < 3
2,4	L.Bulla, L.Bulla/W.Bulla, W.rostrum (2 > 4)	2 < 4
2,6	L.Bulla, L.Bulla/W.Bulla	2 < 6
2,7	L.Bulla, L.Bulla/W.Bulla	2 < 7
3,5	W.rostrum	3 < 5
3,6	L.Bulla, L.Bulla/W.Bulla	3 < 6
4,6	L.Bulla, L.Bulla/W.Bulla	4 < 6
5,6	L.Bulla/W.Bulla	5 < 6
6,7	L.Bulla/W.Bulla, L.Bulla	6 > 7

TABLE 4. Relative contribution of each variable to the first four principal components.
*Variables explaining the greatest percentage of variation on each PC.

	components	
	1	2
Eigen-Value	11.732	2.95
% of variance	51.008	12.826
BL	0.019	-0.021
FL	-0.043	-0.051
EL	-0.096	0.019
Lms	-0.047	-0.008
lm1	0.211*	-0.094
wm1	0.180*	0.007
LMS	0.084	-0.047
LM1	0.198*	-0.039
WM1	0.194*	0.008
DIAL	0.035	-0.013
NASAL	0.043	0.020
W.bulla	0.004	0.157
L.bulla	-0.035	0.264*
OCLL	0.025	0.033
W.Zygo	0.067	0.000
CBL	0.035	0.053
INTL	-0.029	-0.025
FORL	0.085	0.049
W.skull	-0.047	0.220*
BRAH	0.056	0.161
Meatus	0.037	0.100
W.rostrum	-0.015	-0.064
Lbulla/Wbulla	-0.061	0.270*

clades stabilized. In the Bayesian approach, clades with $P_p \geq 0.95$ were considered to be strongly supported (Figure 5).

Genetic distances between groups were calculated using the Kimura 2-parameter (K2P) model of evolution (Kimura, 1980), as implemented in MEGA 4.0 (Tamura et al., 2007) and ExcaliBAR (Aliabadian et al., 2014).

KARYOLOGIC STUDIES

Karyotypes of five specimens were prepared: 2909-Eastern Azerbaijan-Tabriz, 4348-Kurdistan-Bijar, 3535-BagheShadi, 4522-Esfahan-Murchehort, 3985-Sistan Baluchistan-Manzelaab. Chromosome preparations were obtained from bone marrow cells according to Yosida (1973). About 12 metaphase plates from three specimens were examined and at least 10 of the best chromosomal spreads were photographed at x100 magnification using digital CCD camera. The ideograms of all specimens were prepared. Chromosomes were classified according to Levan protocol and each was placed next to its presumed homologue to determine the diploid chromosome number (2n) and autosomal fundamental number (FNa) (Figures 6, 7, 8, 9 and 10).

RESULTS

MORPHOMETRIC ANALYSIS

All measurements indicated normal distributions and homogeneity of variances ($P\text{-value} > 0.05$). Mean and standard deviation (SD) of all measurements are given in Table 2. According to ANOVA and Tukey pairwise comparisons (Table 3), the bulla dimensions (L.Bulla and L.Bulla/W.Bulla) are the most discriminant measurements among groups. The external and dental measurements are not significantly different among groups. No significant difference is recorded between groups 7 and 4, and between groups 1, 2 and 5.

In PCA (Principle Component Analysis), Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy showed a high level of sampling adequacy with the value of 0.832 and Bartlett test was statistically significant ($p < 0.0001$). The scatter plot of the first two PCs is presented in Figure 11. PC1 and PC2 explain 51.0% and 12.8% of the total variance, respectively. The first PC is strongly positively correlated to the dental variables (lm1, wm1, LM1 and WM1; Table 4). This PC does not allow a good distinction between the 7 studied groups. The second PC is strongly positively correlated to bulla length, width of skull and the ratio of L.bulla/W.bulla (Table 4). PC2 separates group 3 from group 6, and from groups 1 and 2 (Figure11).

EXTERNAL MORPHOLOGY

Differences could be seen among populations with respect to claws and fur coloration in dorsal and ventral parts of the body and tail. We studied the morphology of 43 specimens coming from localities for which morphometric and/or genetic data were available (Table 1).

WESTERN POPULATIONS (GROUPS 1, 2 AND 5)

Museum ID of studied specimens: 2224, 2909, 2910 (Tabriz); 4348, 4351, 4350 (Bijar); 4347 (Orumieh), 3535-3546 (BagheShadi).

The underbelly is white. The tail is totally light brown in the upper and lower parts (it is bicolor in some samples) without any distinct dark line in the upper part. The tuft of the tail is blond to dark. Claws are white and a little dark at their base. The proximal part of the claws of the hind foot is partially covered with fur. Dorsal hairs are gray at their base, metallic light ochre to yellowish in the median and black at the tip. There is no black tip on the hairs of the flanks. Interestingly, the specimens of BagheShadi in Yazd Province (3535-3546; group 5) have longer tail than the others, and the tail is externally articulated.

CENTRAL POPULATIONS (GROUP 3)

Museum ID of studied specimens: 3811 (Yazd-Sange Deraz); 1519, 3055, 3749, 1619, 3035 (Yazd); 3810, 3731 (Yazd-Mehriz); 4516, 4517, 4522, 4527 (Esfahan), 3751 (Yazd-Taft).

The underbelly is white yellowish. The tail is long: its upper part is dark with a short black line (the hairs of this black line are yellow at the base and black at the tip), and its lower part is light brown. The tuft of the tail is long and light brown yellowish to black. Dorsal hairs are dark gray (coal) to sandy. Overall, the colors are not shiny. Claws are dark reddish at their base.

EASTERN POPULATIONS (GROUPS 4 AND 7)

Due to the presence of variation in the eastern populations, we described the population of south east and north east separately:

Southeast (group 4): Museum ID of studied specimens: 3280, 3279 (khash); 3684, 3680, 3694 (Zahedan, Mirjave); 3525, 3526, 3685 (Zahedan, Koole Sangi).

The underbelly is light white. There is a black line in the upper part of the tail. The dark line is continued into the tuft in the population from Khash. The color of the tail varies, but generally, the upper part is darker and the lower part is brown. The color of the upper part of the body is sandy gray to coal. Claws are yellow and their base is dark reddish.

Northeast (group 7): Museum ID of studied specimens: 3755, 3183, 2655, 3193, 3001, 2925, 2924, 3222, 3859 (North Khorasan); 1455 (Zoshk); 1109 (Tandure); 3188, 3174 (Jajarm).

The underbelly is snow white. There is a pale dark line in the upper side of the tail in some specimens. The dorsal part of the tail is covered with pale gray fur, while the ventral side is dusty white to pale brown. The tuft of the tail is pale gray to dark. The upper part of the body is coal to ochre. The claws are pale yellow.

MOLECULAR STUDIES

The final aligned dataset included 2358bp ((Cox1 (n=9): 624 bp; Cytb (n=11): 877 bp; and IRBP (n=10): 857 bp)). A strict consensus tree inferred from the three genes indicates that all the nodes are well supported (Posterior probabilities > 0.95). The specimens from the west of Iran and BagheShadi formed well supported clade (Clade I, posterior probability=1) (Figure 5). Clade II (pp=1) is divided into two subclades, Clade IIA and Clade IIB (pp=1 for both). Subclade IIB groups all individuals from north east and south east of Iran, and Subclade IIA includes individuals from Central Iran.

The mtDNA mean genetic distance (K2P) between Clades I and II is about 8-9% (between clade I and subclade IIA= 8.3% and 9.8%, between clade I and subclade IIB=8.3% and 9.1%, for Cox1 and Cytb, respectively). The mean genetic distance is about 2% between subclades IIA and IIB (1.9% and 2.6% for Cox1 and Cytb, respectively).

KARYOLOGIC STUDIES**3535-BagheShadi (correspond to Clade I-Group 5)-male**

Diploid number of chromosomes in this specimen is $2n=42$ and $FNa=74$. The autosomal set contains 16 pairs of metacentric, 3 pairs of acrocentric and 1 pair of sub-metacentric chromosomes. The X chromosome is sub-metacentric and the Y chromosome is acrocentric (Figure 6).

4348-Bijar (Correspond to Clade I-Group 2)-male (Figure 7) and 2909-Tabriz (corresponds to Clade I-Group 1)-male (Figure 8)

These two specimens have exactly the same karyotype as specimen 3535, except that the X chromosome is metacentric and the Y chromosome is sub-metacentric.



FIGURE 6. The karyotype of *M. persicus* specimen from BagheShadi (3535).



FIGURE 7. The karyotype of *M. persicus* specimen from Bijar (4348).



FIGURE 8. The karyotype of *M. persicus* specimen from Tabriz (2909).

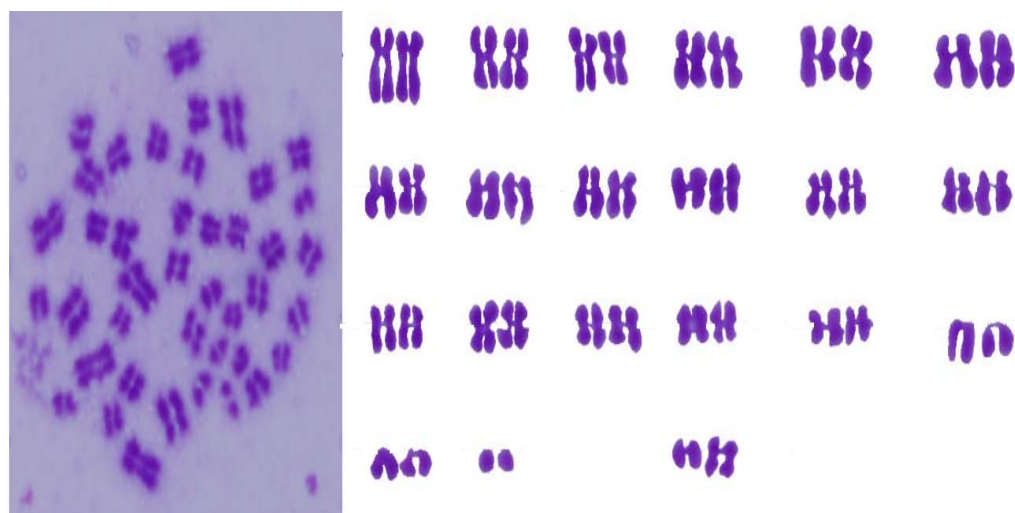


FIGURE 9. The karyotype of *M. persicus* specimen from Esfahan (4522).

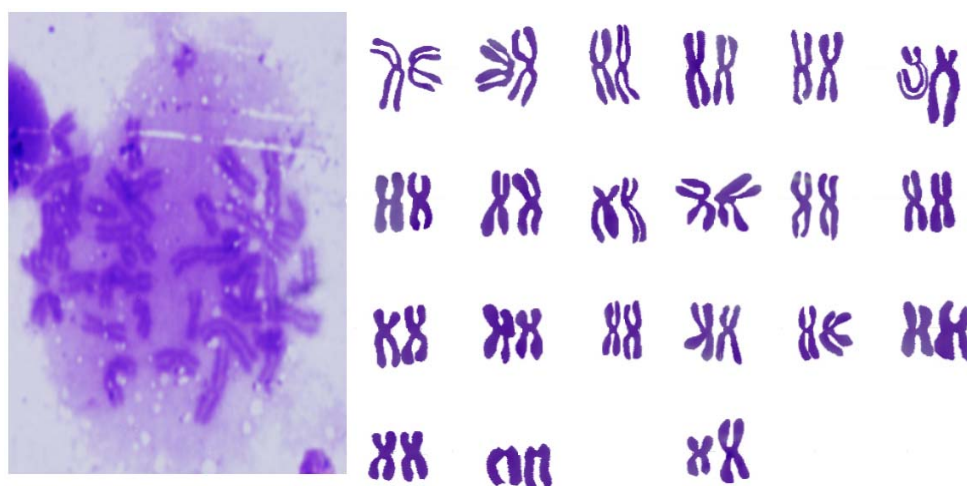


FIGURE 10. The karyotype of *M. persicus* specimen from Sistan Baluchistan (3985).

4522-Esfahan MurcheKhort (correspond to Clade IIB-Group 3)-male

$2n=42$ and $FN_a=74$. This specimen has 15 pairs of metacentric, 2 pairs of sub-metacentric and 3 pairs of acrocentric autosomal chromosomes. Both sex chromosomes are sub-metacentric but the Y chromosome is smaller than the X chromosome (Figure 9).

3985-Sistan Baluchistan Zahedan (correspond to Clade IIA-Group 4)-female

$2n=42$ and $FN_a=76$. This specimen has 17 pairs of metacentric, 2 pairs of sub-metacentric and 1 pair of acrocentric chromosomes. X and Y chromosomes are metacentric (Figure 10).

DISCUSSION

At least five subspecies of *M. persicus* have been identified in the literature in the Iranian Plateau, based on morphological and morphometrical characteristics (Ellerman & Morrison-Scott, 1951; Darvish, 2011). In this paper we combined molecular, cytogenetic, morphometric and morphologic data to investigate intra-specific variability in *M. persicus* in Iran, and to test the validity of the proposed subspecies.

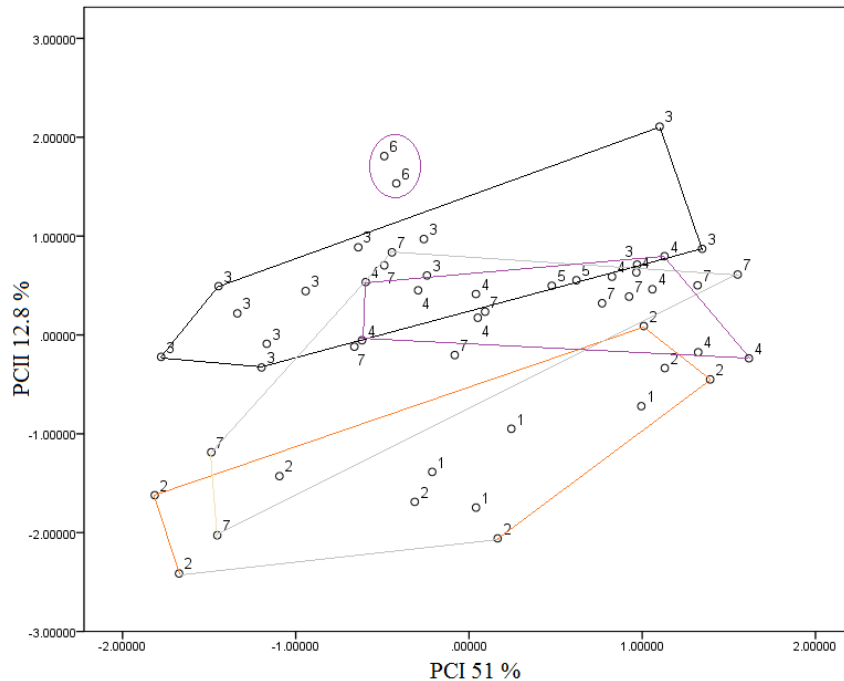


FIGURE 11. Principle Component Analysis of morphometric measurements based on PCI and PCII.

Our genetic data identified three well supported clades (I, IIA, IIB). These lineages are separated by natural geographic barriers: Sirjan-Sanandaj plain including Abarkooh desert is located between lineages I (Zagros Mountains) and IIA (Kohrud), Central and Lut deserts are located between lineages IIB (Eastern Mountains) and IIA.

Clade I is present in the west of Iran and BagheShadi. Our genetic, morphometric, morphologic and cytogenetic data are congruent and identify only one entity in this part of Iran (groups 1, 2 and 5 are similar to each other and different from other groups). The only sub-species known from this geographical area is *M. p. rossicus* (Boudet, 2010; Darvish et al., 2014). This subspecies was described based on its elongated rostrum, small cranium and bulla, narrow zygomatic arch, and by its yellowish red dorsal color and white belly (Heptner, 1931). The small tympanic bulla of our groups 1 and 2 fits this description. The dorsal hair color of our specimens is only partly congruent with this description since their dorsal hairs are gray at their base, metallic light ochre to yellowish in the median and black at the tip. Our cytogenetic results for groups 1, 2 and 5 are in accordance with previous reports by Yigit and Colak (1999) for *M. p. rossicus* from Oltu, Erzurum in Turkey, except for sex chromosomes which show some variability.

Clade IIB is mainly distributed in the east of Iran, including Alborz Mountains (Binalood) and Kopet Dagh in the northeast, and the mountain chains in the southeast (Taftan Mountains, Maleksiahkooh and Abkhan Mountains). Our morphometric analyses show no significant differences between specimens from the southeast (group 4) and the northeast (group 7). Moreover, our morphometric analyses show that these specimens have smaller tympanic bulla and a smaller L.Bulla/W.Bulla ratio than specimens from group 6 (genetic clade IIA, Bandar Abbas in south-central Iran), and a longer tympanic bulla and a higher L.Bulla/W.Bulla ratio than specimens from groups 1, 2, 3 and 5 (clades I and IIA, western and central Iran). Our genetic and morphometric data are congruent in identifying one distinct entity in eastern Iran. These results suggest that *M. p. gurganensis* (group 7) could be considered as a junior synonym of *M. p. baptistae* (group 4). Both subspecies were described based on their larger and/or inflated bulla (Goodwin 1939; Thomas

1920), which is concordant with our results. However the fur and tail coloration of the type specimens differ from what we observed in our specimens: in *M. p. gurganensis* dorsal hairs have a pinkish buff color, the upper side of the tail is blackish and the lower side cinnamon-buff (Goodwin, 1939); in *M. p. baptistae* the dorsal part of the tail is ochraceous lined with black and the under part is whitish (Thomas, 1920). We found several differences in coloration between specimens from the southeast (group 4) and the northeast (group 7): the underbelly is light white in group 4 and snow white in group 7; the upper part of the body is sandy gray to coal in group 4 and coal to ochre in group 7; and the claws are yellow with a dark reddish base in group 4 while they are pale yellow in group 7. More data on the determinants of fur coloration in jirds are needed to understand the significance of the observed differences. In the southeast of Iran we found a different karyotype ($2n=42$ and $FNa=76$, 17 pairs of metacentric, 2 pairs of sub-metacentric and 1 pair of acrocentric chromosomes; X and Y chromosomes metacentric), than those found by Mohammadi et al. (2012) in northeast of Iran. In fact, a karyotype of $2n=44$ and $FNa=78$ and a sub-metacentric X chromosome was reported from Birjand and $2n=44$ and $FNa=80$ with a sub-metacentric X chromosome and a telocentric Y chromosome was reported from Neyshabour.

Clade IIA geographically overlaps with the range of *M. p. persicus* (group 3) and *M. p. ambrosius* (group 6). *M. p. persicus* was described based on its narrow nasal, curved upwards zygomatic arch, rufous fur in the upper parts and white in the lower parts, rufous brown tail above and white below, without any dark line along the lower surface (Blanford, 1875). *M. p. ambrosius* was recognized by its small skull and bullae, narrow braincase, cinnamon-buff hairs in upper side, sharply white hairs in under surface and buffy tail. Contrary to expectations, our morphometric data show that specimens of group 3 are characterized by smaller tympanic bulla and narrower skull than those of group 6. Only two specimens of group 6 were included in our morphometric analyses and this result needs to be confirmed by additional data. The external coloration of our specimens of group 3 does not fit the description of *M. p. persicus*: in our specimens the upper part of the tail is dark with a short black line, and its lower part is light brown; and dorsal hairs are dark gray (coal) to sandy. Unfortunately no specimens of group 6 could be included in our external morphologic study. The karyotype of the specimen 4522 from Esfahan (group 3) is exactly the same as those found by Shirani Bidabadi et al. (2009) for 17 specimens of *M. p. persicus* from Esfahan, Mobarakeh. No specimen of group 6 could be karyotyped. More data are needed to conclude on the taxonomical validity of the subspecies *M. p. persicus* and *M. p. ambrosius*.

To conclude, this study is the first integrative taxonomic study carried out on *M. persicus*. It shows that three distinct genetic lineages can be recognized in Iran. Our morphometric results also show significant differences between these three groups, and emphasized morphometric variability within clade IIA, where two subgroups could be recognized. However one of these subgroups was represented by only two individuals in our analyses, and additional morphometric data are needed to confirm this result. Fur coloration vary greatly among Iranian specimens, and the determinants of fur coloration in Jirds are needed to understand the significance of the observed differences. In rodents, pelage coloration can evolve rapidly and is sometimes correlated with habitat color and not with phylogeny (Hoekstra et al., 2005). Thus it can be an unreliable taxonomic character. Cytogenetic results show that the diploid number of chromosomes, the FNa and the morphology of the sex chromosomes vary between populations. More precise cytogenetic data (banding and Fish techniques) are needed to understand the origin of the observed differences in chromosomal numbers among these populations, and their significance for taxonomy. In fact polymorphism in $2n$ or FNa is sometimes observed within rodent species (Britton-Davidian, 2012; Hima et al., 2011).

Acknowledgments

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