

Genetic Variation in Populations of Field Mouse *Apodemus witherbyi* (Thomas, 1902) (Rodentia: Muridae) in Iran Inferred from Mitochondrial Cytochrome B Gene Sequences and Geometric Morphometrics

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Abstract: Alternating glacial and interglacial periods resulted in range shifts, persistence in distinct glacial refugia and extinction events in various temperate organisms. The integrative analysis of molecular markers and spatial distribution models carried out for multiple taxa allows the detection of phylogeographical patterns. In this study, molecular analysis of cytochrome b gene sequences from 40 individuals showed that there are some clues, indicating an initiating of the genetic differentiation among populations in Iran due to absence of gene flow among them. In addition, 133 specimens were studied using outline-based geometric morphometrics method on the first lower molar. The results revealed that populations of Central Iran, as an isolated area, are differentiated from other populations in both size and shape of the first lower molar. The study of spatial distribution model on 80 recorded points showed potential habitats where this species dispersed from 21ky to now. It seems that low intraspecific divergence in this species might be at least partly owing to its high tolerance to environmental factors that facilitate its dispersal ability. Therefore, it seems that climatic oscillations may not have very distinct influences on genetic structure of the species at the level of a taxonomic rank.

Key words: *Apodemus witherbyi*, cytochrome b, outline, Shirkooh, spatial distribution model

Introduction

Species change their ranges due to historical events and ecological factors. The geographic distribution of a species is shaped by geological and demographic events, as well as by climatic changes. The impact of these factors could be revealed through phylogeographic studies (TABERLET 1998). Phylogeographic methods (TABERLET 1998; AVISE 2000) allow the inference of barriers to gene flow and to estimate the time when these barriers were established. The effects of Pleistocene climate oscillations and consequential biogeographic barriers on genetic structure of popu-

lations have been reported by several researchers (AVISE 2000; LIU *et al.* 2012). Climatic fluctuations may play a principal role in determining the current geographical distribution of species. Extremes of oscillations may confine populations to areas called refugia (HEWITT 1996; TABERLET *et al.* 1998). Mountains are often considered as important refuge areas where fauna may be impacted by climatic fluctuations through moving up or down the altitude gradient (TOLLEFSRUD *et al.* 2008). Natural barriers, such as arid plains surrounding the mountain refugia,

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may contribute further to the isolation of the refugial populations (TOLLEFSRUD *et al.* 2008). HEWITT (1996) suggests that genetic diversity in expanding populations decreases, whereas populations in the refugia maintain their higher genetic diversity. Therefore, the genetic structure of populations is a reflection of the response to past climate oscillations and may leave detectable signs in their genome. Molecular genetics can describe intraspecific geographical structure by identifying lineages and consequently can reveal postglacial colonisation routes provided that the location of the refugia is known (TABERLET *et al.* 1998). In addition, spatial distributional models and molecular markers have been shown to be useful for detecting differentiation among populations as a response to climatic fluctuations (RAJAEI *et al.* 2013) in Iran.

Apodemus spp. are small rodents inhabiting woodlands and forests of the Palaearctic and Oriental region (Corbet 1978). Previous research conducted on *Apodemus* in Iran, has concentrated on interspecific relationships and geographic distributions using traditional and geometric morphometric, morphology and RFLP, PCR (NASERI *et al.* 2006; DARVISH *et al.* 2006a, 2006b, 2010, 2014, 2015; DARVISH 2014; JANGJOO 2010; JAVIDKAR *et al.* 2005, 2007). Intraspecific study on field mice (*A. witherbyi*) in North-western Iran has been conducted using RFLP (POUR FEIZI *et al.* 2009). Further examination of intraspecific variation is generally lacking.

The effects of Pleistocene climate oscillations on biota of Iran and its consequences have yet to be studied (AHMADZADEH *et al.* 2012). *Apodemus witherbyi* (Thomas, 1902) is the most widely distributed field mouse in Iran occurring in Central and Northern Iran and near the Zagros Mountains in the northwest (KARAMI *et al.* 2008). It inhabits agricultural fields, gardens, forest and woodlands, steppes and highlands. The type locality of *A. witherbyi* is in Southern Iran, Shul, Fars Province (THOMAS 1902) and its range extends from Northern and Southern Caucasus, the Anatolian Turkish steppe southward through most of Iran and South-western Turkmenistan eastwards to Western Central Pakistan (MUSSER & CARLETON 2005).

The aim of this study was to examine the sequences of mtDNA gene cytochrome b and outline-based geometric morphometrics of the first lower molar for detection of genetic structure among populations of *A. witherbyi* in Iran. According RAJAEI *et al.* (2013), the integrative analysis of molecular markers and spatial distribution models could allow the detection of phylogeographical patterns to further the understanding of the impact of the Pleistocene glacial cycles on the biodiversity of Iran. Moreover, suitable potential habitats were detected with spatial

distributional models for current and past climatic conditions in this species.

Material and Methods

One hundred and thirty-three specimens of field mice were collected from nine different localities (Table 1) including the Zagros and the Kopet Dagh Mountains, as well as Shirkooh in Central Iran (Fig. 1A).

DNA isolation, amplification and sequencing

Mitochondrial cytb (982 bp) from 40 individuals was used in molecular analysis and amplified by polymerase chain reaction (PCR) following the protocol of MONTGELARD *et al.* (2002). Sequences from *A. mystacinus* (n = 2), *A. ponticus* (n = 3) and *A. uralensis* (n = 1) were taken from GeneBank (for Accession numbers see Table 6) and used as outgroup taxa. Sequence alignment was performed using ClustalW (THOMPSON *et al.* 1994) as implemented in Bioedit 7.0.5 (HALL 1999) and verified visually. Levels of mtDNA diversity were evaluated by calculating haplotype diversity (h), nucleotide diversity (π) and the number of polymorphic and parsimony informative sites using DnaSP 5.10.01 (LIBRADO & ROZAS 2009).

Data analysis

Bayesian inference analysis was used to reconstruct phylogenetic relationships among haplotypes using MrBayes3.1 (HUELSENBECK & RONQUIST 2001). In Bayesian inference four Monte Carlo Markov chains were run simultaneously for 10,000,000 iterations with 1million generations of each run as the burn-in. In order to reveal the relationship among populations, a median joining network was plotted using Network v4.6.1.3 (available at: www.fluxus-engineering.com; BANDELIT *et al.* 1999). Genetic differences among populations were investigated by computing pair-wise F_{ST} values (analogue with Φ_{st}) in Arlequin 3.5.2 package (EXCOFFIER *et al.* 2005). Whether these values were significantly different from zero was estimated under 10,000 permutations at the alpha level of 0.05. Pair-wise F_{ST} value equal to or greater than 0.25 is characteristic of some restriction in gene flow between populations (LIU *et al.* 2012). A Neighbor Joining tree was constructed in PAST 2.06 (HAMMER *et al.* 2011) and Fig Tree 1.4.2 (ANDREW RAMBAUT; available at: <http://tree.bio.ed.ac.uk/>) using Slatkins's pair-wise distances among geographic areas (RAJABI *et al.* 2008).

Analysis of molecular variance (AMOVA) was used to examine the amount of genetic variability partitioned within and among populations using the software Arlequin 3.5.2 based on 10,100 permutations (EXCOFFIER *et al.* 2005). Population subdivision was es-

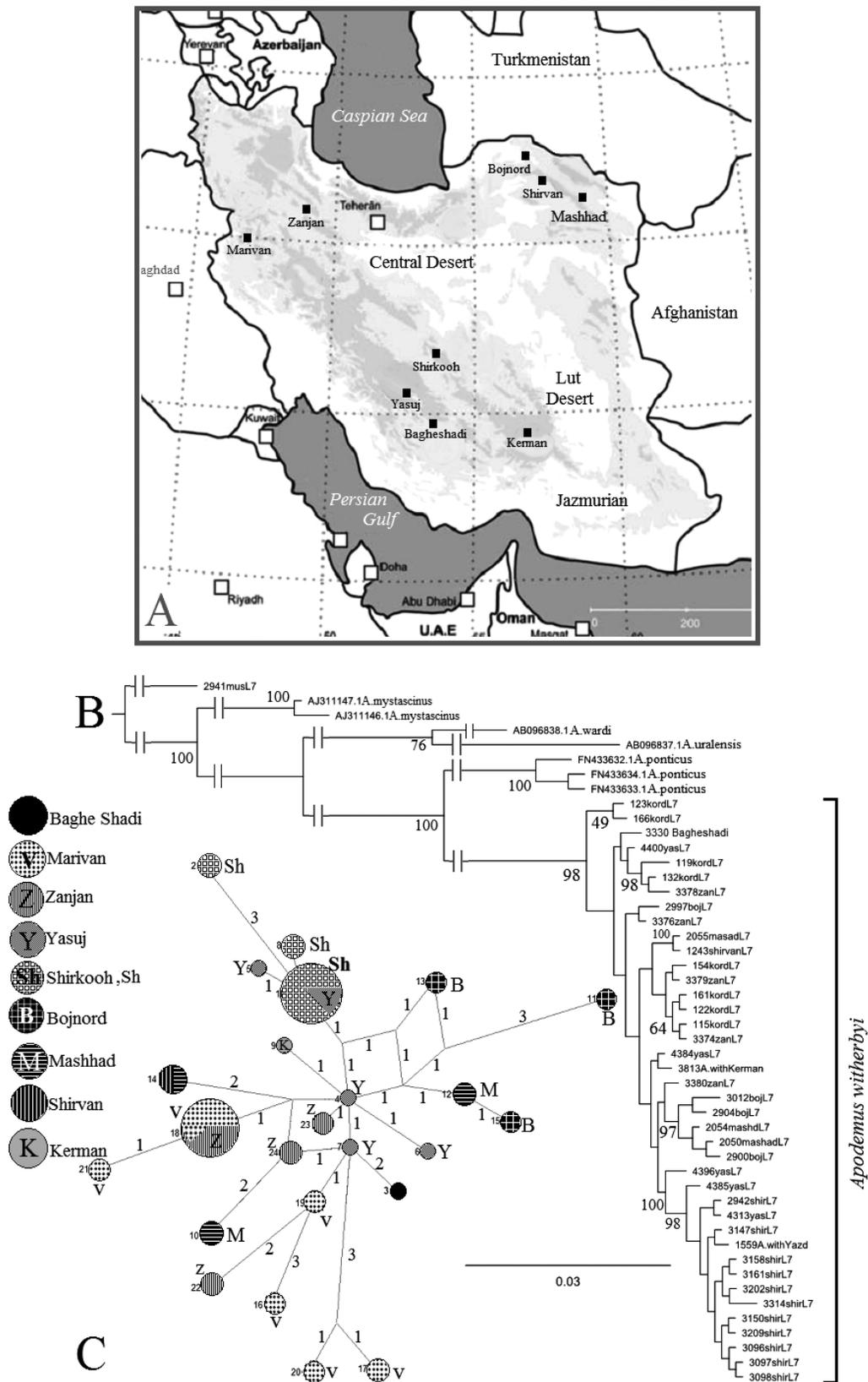


Fig. 1. A) Sampling localities of *Apodemus witherbyi* in Iran. B) Reconstructed molecular tree from haplotype of cytochrome *b* gene sequences. Bayesian posterior probabilities support for Bayesian inference indicated below each branch. C) Median joining analysis. Legend is for geographic areas. Size of each haplotype is proportional to frequency and the length of each branch is not corresponding to mutation step. The numbers on each branch indicate mutation changes between two haplotypes. See Table 6 for more details on haplotypes

estimated using SAMOVA 2.0 (DUPANLOUP *et al.* 2002), identifying groups of populations that are geographically homogeneous (low F_{sc}) and maximally differentiated (high F_{ct}) from each other (SALVI *et al.* 2010).

Spatial Distribution Model

Spatial Distribution Modelling (SDM) was used for evaluating influences of climate change on the historical distribution of *A. witherbyi*. Potential distribution of *A. witherbyi* was evaluated using Maxent (PHILIPS *et al.* 2006) algorithm for the last glacial maximum (LGM) and current conditions. A total of 80 locality records for the presence of this species were obtained from the literature (DARVISH *et al.* 2006a: 12 additional; DARVISH *et al.* 2010: ten ad-

ditional, JAVIDKAR *et al.* 2005: 14 additional), direct sampling of localities (nine localities), and museum records from the Mammal Networked Information (44 additional localities) System (MaNIS: <http://manisnet.org/manis>). Nineteen bioclimatic variables with spatial resolution of 150 arc s were downloaded at www.worldclim.org for current and 21k year BP. Pair-wise Pearson correlation was used for evaluating co-linearity among variables in SPSS (SPSS Corporation). Variables with $R^2 > 0.75$ were excluded and six biologically informative variables were included in subsequent analyses. Variables included were seasonality of precipitation (BIO15); driest quarter of precipitation (BIO17); isothermality (BIO3); seasonality of temperature (standard devia-

Table 1. The localities of sampling, geographic coordinate and sample size in outline-based geometric morphometric

Locality	Latitude	Longitude	n
Shirkoooh ,Yazd Province	31.64 N	54.14 E	62
Baghe Shadi, Yazd Province	29.80 N	54.13 E	5
Yasuj (Yasuj and vicinities) Kohkiloye va Boyer Ahmad Pov.	30.66 N	54.73 E	12
Bojnord, North Khorasan Province	37.57 N	57.33 E	13
Mashhad, Razavi Khorasan Province	36.12 N	59.37 E	11
Marivan, Kordestan Province	35.71 N	46.32 E	23
Shirvan	37.39 N	57.92 E	1
Kerman, Kerman Province	30.29 N	57.10 E	1
Zanjan, Zanjan Province	36.65 N	48.47 E	5
Total			133

Table 2. Localities of sampling with sample sizes (n), diversity of nucleotide (π), haplotypes (h) associated with standard deviations (SD), the number of Haplotypes (H) for the studied populations of *A. witherbyi*. Bold numbers in the first column show the highest nucleotide diversity and in last column show the shared haplotypes in different localities

Populations	n	π	SD	H	h	SD	Haplotypes
Shirkoooh(Shk)	14	0.00197	0.0008	5	0.505	0.158	1-2-3-8-9
Yasuj (Y)	5	0.00245	0.00052	5	1	0.126	1-4-5-6-7
Bojnord (B)	5	0.00672	0.00103	5	1	0.126	10-11-13- 14-15
Mashhad (M)	3	0.00407	0.00163	2	1	0.272	12-14
Marivan (Ma)	8	0.00473	0.00089	6	0.893	0.111	16-17- 18-19-20-21
Zanjan (Z)	5	0.00346	0.00091	4	0.9	0.161	18-22-23-24
Total	40	0.0043	0.00038	24	0.914	0.035	

Table 3. Comparison of different populations of field mice based on FST (above diagonal) and FST/ (1-FST) values (lower diagonal). Numbers in the parenthesis indicate geographic distances (Km)

	Shirkoooh	Yasuj	Bojnord	Mashhad	Marivan	Zanjan
Shirkoooh	0.00	0.34807*	0.91722*	0.94056*	0.91561*	0.93573*
Yasuj	0.53392(261)	0.00	0.86356*	0.89229*	0.87135*	0.89416*
Bojnord	11.08060(719)	6.32915(922)	0.00	0.00	0.12633*	0.06977
Mashhad	15.82308(695)	8.28395(933)	0(238)	0.00	0.15337	0.18397
Marivan	10.84965(815)	6.77280(715)	0.14459(983)	0.18115(1149)	0.00	0.00
Zanjan	14.55891(770)	8.44859(739)	0.07500(792)	0.22544(977)	0(214)	0.00

* indicates significant pair-wise FST values.

tion *100, BIO4); temperature annual range (BIO5-BIO6); and slope.

Model validity was surveyed by calculating the area under the curve (AUC) that reflects the ability of a model to discriminate between current presence records and random background points. AUC can range from 0.5 for inability of predicting to 1 for perfect predicting model. JESCHKE & STRAYER (2008) considered AUC of more than 0.9, 0.8 and 0.7 as “very good”, “good” and “useful”, respectively.

Outline analysis of m1

To obtain the shape descriptors of the external outline of the first lower molar (m1), an Elliptic Fourier Analysis (EFA) was performed using EFAWIN (ROHLF & FERSON 1992). EFA was conducted on 133 specimens (Fig. 1A). Molar outlines were digitised clockwise manually using tps.Dig 2.10 (ROHLF 2006). The program GMTP (TARAVATI & DARVISH 2010) was applied to adjust the tpsDig output file format directly opened in EFAWIN. The first 15 harmonics and 57 coefficients were used as variables in SPSS, version 16, and PAST, version 2.08 (HAMMER *et al.* 2011). Discriminant analysis was conducted to identify the main axes of differentiation among populations.

Results

Molecular analysis

The sequenced region for cytb contained 32 variable sites (3.3 %) and 24 different haplotypes. Nucleotide diversity (Table 2) varied from 0.00197 (0.0008) to 0.0067 (0.001). The highest nucleotide diversity (0.0067) was found in Bojnord, followed by Marivan and Mashhad populations. Shirkooh population had the largest sample size ($n = 14$) and was characterised by the lowest haplotype and nucleotide diversities (Table 2). The Bayesian tree (Fig. 1B) indicated that all of the sampled populations belonged to the same major clade, however, some of the subclades were well supported. The median joining network (Fig. 1C) indicated some shared haplotype among populations, such as among the populations from Yasuj and Shirkooh.

Significant pair-wise F_{ST} were observed between 2/3 of the populations of *A. witherbyi* (Table 3). The pair-wise F_{ST} between Mashhad - Marivan or between Mashhad-Zanjan populations was not significant. This might be owing to the small sample size, especially in Mashhad. In spite of a significant F_{ST} between Bojnord and Marivan populations ($P = 0.0338$, Table 3), this F_{ST} value was lower than 0.25. While the F_{ST} between Bojnord and Marivan might provided an indirect estimate of weak gene

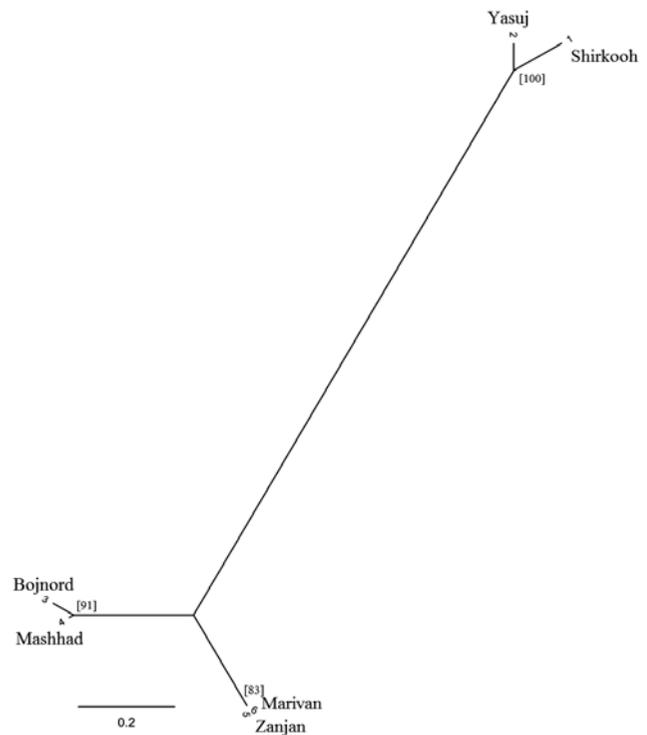


Fig. 2. Neighbor joining network based on Matrix of Slatkin linearized F_{ST} s as $F_{ST}/(1-F_{ST})$

flow, examination of the haplotypes present in these two populations failed to show any direct evidence of any gene flow (Table 2). Therefore, it should be taken into account that the unbiased estimates of F_{ST} might result in biased estimates (over estimates) of gene flow. Furthermore, significant F_{ST} between Shirkooh and Yasuj populations (0.34, $P < 0.05$) indicated no gene flow, regardless that these two localities have some shared haplotypes.

NJ tree (Fig.2) contained two main branches with one side including northern populations (Mashhad- Bojnord and Marivan- Zanjan) and another side - southern ones (Yasuj- Shirkooh).

AMOVA analysis was significant for two population clustering, showing genetic variation in the populations. This variation was confirmed by 80.98 % of the total variance ($\Phi_{ct} = 0.80982$, $P < 0.015$). In the SAMOVA (Table 4), clustering was examined for two to four groups. Although, all of the groups ($K = 2$ to $K = 4$) were supported at significant levels, new groups with three or four populations always contained a single population and with only single specimen (Bage shadi and Shirvan, Table 4). Thus, we concluded that the division of populations into two group best described the high level population structure in our dataset. These two groups included northern populations (Mashhad, Bojnord, Shirvan, Marivan and Zanjan) and southern ones (Shirkooh, Kerman, Yasuj and Baghe shadi).

Table 4. Results derived from AMOVA and SAMOVA analyses with proposed clusters. Group composition, fixation indices, and corresponding P values are shown. **B:** Bojnord, **M:** Mashhad, **Shv:** Shirvan, **Ma:** Marivan, **Z:** Zanjan, **Shk:** Shirkooh, **Y:** Yasuj, **Ke:** Kerman, **Bg:** Baghe shadi

Group composition as proposed by SAMOVA	% Total Variance	Φ_{CT}	Φ_{SC}	Φ_{ST}
K= 4 (Shk, Y, Ke)(Bg)(B, M, Ma, Z)(Shv)	88.7	0.88722**	0.15610*	0.90482**
K= 3 (Bg)(Y, Shk, Ke)(B, M, Shv, Z, Ma)	89.2	0.89280**	0.14883*	0.90876**
K= 2 (B, M, Shv, Z, Ma)(Y, Shk, Ke, Bg)	89.1	0.89167**	0.18772**	0.91201**

* P < 0.05 and ** P < 0.01

Table 5. The Wilk's Lambda values for teeth shape using discriminant analysis

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	0.391	116.091	39	P < 0.001
2 through 3	0.809	26.101	24	0.348
3	0.921	10.206	11	0.512

Table 6. Haplotypes numbers, museum voucher numbers used in molecular analysis based on cyt b (Fig. 1). The last column indicates sampling localities for each haplotype

Haplotype number	Museum voucher numbers	Geographic location
Hap_1: 11	2942- 3096- 3097- 3098- 3147- 3150- 3158- 3161- 3202-3209-4313	Shirkooh, Yasuj
Hap_2: 1	3314	Shirkooh
Hap_3: 1	3330	Baghe shadi
Hap_4: 1	4384	Yasuj
Hap_5: 1	4385	Yasuj
Hap_6: 1	4396	Yasuj
Hap_7: 1	4400	Yasuj
Hap_8: 1	1559	Shirkooh
Hap_9: 1	3813	Kerman
Hap_10: 1	2997	Bojnord
Hap_11: 1	3012	Bojnord
Hap_12: 2	2054- 2050	Mashhad
Hap_13: 1	2904	Bojnord
Hap_14: 2	2055- 1243	Mashhad, Shirvan
Hap_15: 1	2900	Bojnord
Hap_16: 1	119	Marivan
Hap_17: 1	123	Marivan
Hap_18: 5	161- 122- 115- 3379- 3374	Zanjan, Marivan
Hap_19: 1	132	Marivan
Hap_20: 1	166	Marivan
Hap_21: 1	154	Marivan
Hap_22: 1	3378	Zanjan
Hap_23: 1	3380	Zanjan
Hap_24: 1	3376	Zanjan
<i>A. mystacinus</i>	AJ311147, AJ 311146	Outgroup
<i>A. wardi</i>	AB096838	Outgroup
<i>A. uralensis</i>	AB096837	Outgroup
<i>A. ponticus</i>	FN433632, FN433634, FN433633	Outgroup
<i>Mus musculus</i>	2941	Shirkooh, Outgroup

Spatial Distribution Model

SDM estimated average AUC for current and past climatic conditions to 0.992 (SD = 0.009) and 0.973 (SD = 0.022; Fig. 3), respectively. Therefore, the reliability of the proposed model was evaluated as very good. For 21K year BP, relative importance of each

factor were 45.6%, 33.7%, 8.8%, 5.9% and 5.9% for Bio 4 (temperature seasonality), Bio 17 (precipitation during the driest quarter), Bio 15, Bio 7 and Bio 3, respectively. Comparison of current and past reconstructions revealed that the potential distribution of *A. witherbyi* had shifted from compacted areas to dis-

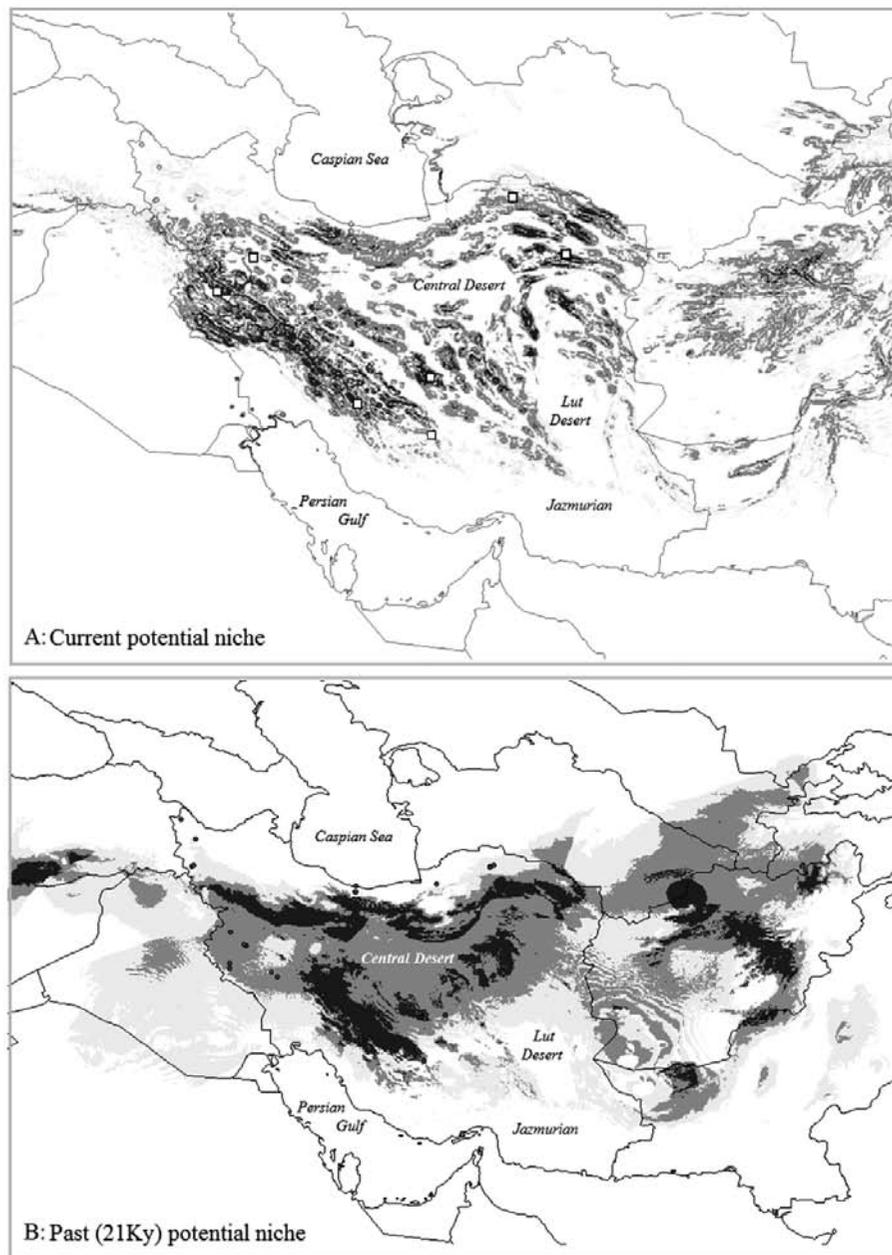


Fig. 3. Proposed species distribution models based on current (A) and the 21 K years BP (B). Solid squares in A represent the sampled localities in this study. Solid circles in B indicate the 80 localities used in Maxent which include mainly sites along Elborz and Zagros Mountains

persed regions (Fig. 3B). Precipitation and temperature were probably the two important environmental factors that could confine species distribution. SDM analysis based on current conditions indicated that *A. witherbyi* was distributed in most of the Iranian Plateau, Central Afghanistan and parts of Northern Pakistan (Fig. 3A). According to this model, no suitable climatic conditions were predicted for Central and Lut Deserts, and Jazmurian. The 21kyear BP distribution of the species was predicted as a Northern belt parallel to Alborz Mountains, Zagros Mountains and Shirkooh in Central Iran (Fig. 3B).

Geometric Morphometrics

The centroid size (CS) data was tested for normality ($P > 0.866$) and homogeneity of variances (Levene's test, $P > 0.645$). ANOVA tests found significant differences for CS ($P < 0.001$). Comparisons showed no differences between the sexes ($P > 0.05$), hence all data were pooled for subsequent analysis. Tukey's pair-wise comparison indicated that Shirkooh specimens were discriminated from other populations with broad molars ($P < 0.05$). The regression between centroid size and shape variables was significant ($P < 0.0014$). Therefore, differences in m1 shape could

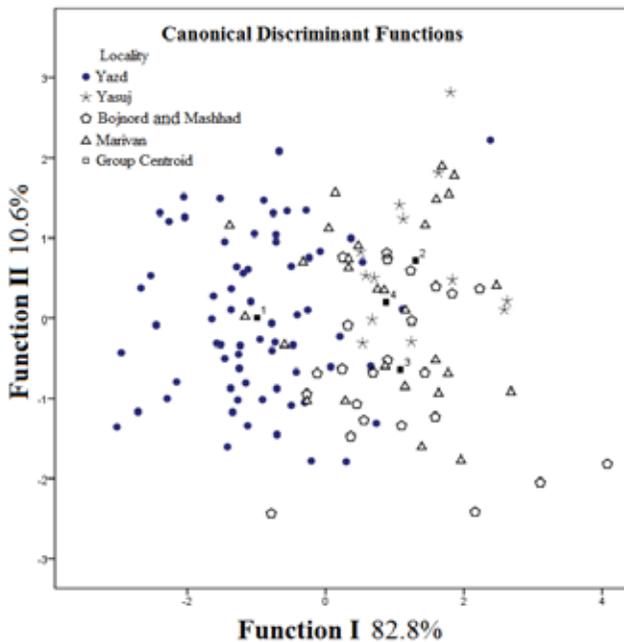


Fig. 4. The projection of six populations of *Apodemus witherbyi* on two first functions of CDA

be due to allometry. This allowed us to use the residuals of Fourier coefficients as shape variables. In discriminant function analysis, the first three components were responsible for 55.8, 20.4 and 12.1 % of the total variations. The Wilk's lambda value confirmed the significance of the first function (Table 5). Projection of the populations on the first two discriminant functions is showed in Fig. 4. The first function separated the Shirkooh population with positive scores, while it distinguished the other populations with negative scores.

Discussion

Molecular analysis revealed that the nucleotide diversity between the populations of *A. witherbyi* was low, which suggested a few divergences. However, some genetic differentiation could be identified among some populations especially between northern (Mashhad, Bojnord, Zanzan and Marivan) and southern (Yasuj and Shirkooh) populations. This differentiation was confirmed by AMOVA and SAMOVA analyses.

As a whole this little intraspecific divergence in the species might be at least partly due to its high tolerance to environmental elements that facilitate its dispersal ability. *Apodemus* spp. are considered very environmentally tolerant species and inhabiting different latitudes (RENAUD *et al.* 2005, GHORBANI *et al.* 2010). Therefore, it seems that climatic oscillations with limited gene flow led to the formation of endemic haplotypes but with no clear influences on

the genetic structure of the species at the level of a taxonomic rank.

In previous studies it was proposed that the field mice of Shirkooh should be separated as a new species on the basis of the geometric morphometrics method (DARVISH *et al.* 2006b) and RAPD-PCR (NASERI *et al.* 2006). However, our molecular studies did not show any differentiations at the specific level except for a low but significant F_{ST} between Shirkooh and Yasuj populations, and an additional separation of Shirkooh samples based on outline geometric morphometric.

There were indirect evidences that the field mice of Shirkooh have immigrated from Yasuj: the presence of higher genetic diversity in Yasuj population ($n = 5$ and $H = 5$, Table 2) and of the lowest genetic diversity in Shirkooh; the latter probably owing to the fact that the field mice of Shirkooh were probably fed from Yasuj population. Moreover, some of the haplotypes from Yasuj were found into the population from Shirkooh, while none of haplotypes of the Shirkooh population were shared with the one from Yasuj (Fig. 1C).

The Sanandaj- Sirjan plain, which is located between Shirkooh and Yasuj, has acted as a pluvial lake during climatic oscillation in quaternary. This plain has been affected by humid cold climate in the Late Pleistocene (TALEGHANI 2005, RAHIMPOUR BONAB & ABDI 2012), and from 6000 to 8000 and 3000 to 5000 BP (MOGHIMI 2008) by semi-arid warm climate, with higher evaporation in the Holocene. This immigration has occurred probably during the recent cold and humid climate. Since *A. witherbyi* is very dependant on the humidity (DARVISH *et al.* 2006a); the presence of an arid plain between the Yasuj and Shirkooh populations has probably prevented a recent exchange between these localities.

Genetic differentiation in Shirkooh population matches the topography of this region. The Shirkooh region has an altitude of more than 4050 m a.s.l. and is surrounded by neighbouring arid deserts, thus forming an isolated area in Central Iran. This area is confined from the west by the Sirjan- Sanandaj plain (Abarkooh arid desert), from the north - by the Central Desert, and from the east - by Lut and Jazmurian Deserts. The low nucleotide diversity and the few numbers of haplotypes were indicative of its recent colonisation and are a result from the founder effect, while the large size of the first lower molar could be explained by island gigantism (DARVISH 2014, MILLIEN & DAMUTH 2004, RENAUD *et al.* 2005). In Shirkooh region, being isolated, has initiated the genetic divergence of this field mouse; however, there was no enough time for the formation of a taxonomic rank.

The high and significant F_{ST} value between the field mice of Yasuj and Marivan suggested confined gene flow. These localities include two separate geographical areas, i.e., the humid and dry Zagros, respectively. The humid Zagros in the northwest consists of high mountains and is affected by Mediterranean winds, while the dry Zagros in the southeast has drier climate and wide parallel valleys (MOZAFFARIAN 2013 and references therein). Such gap between localities of populations was mentioned for lizard (AHMADZADEH *et al.* 2012) and for forest dormouse *Dryomys* sp. (HADDADIAN *et al.* in press). Populations of these areas might be separated by wide river valleys which may served as barriers against the immigrations of *Apodemus* species (LIU *et al.* 2012).-

It needs to be noted, that the environmental factors may impact differently the distinct species, depending on the special habitats, dispersal abilities and colonisation histories (LIU *et al.* 2012). Biota respond to climatic oscillations via changing their distribution

or occupying new habitats (LIU *et al.* 2012), since *A. witherbyi* use the same habitats thus it had to shift distribution responding to climate change. There are no records of *A. witherbyi* from the Central Desert (one of the most arid area on the world) and Lut Desert. Deserts of Lut, Jazmurian have probably acted as geographic barriers against dispersal of this species particularly from the central towards the south-east of the country. The species has not been reported from South-eastern Iran (DARVISH *et al.* 2015). Therefore, it is likely that Shirkooh is the easternmost border of field mouse distribution in Central Iran.

Acknowledgment: Special thanks to Professor C. William Kilpatrick, University of Vermont, USA, for reviewing the paper and for the many useful comments. This work has been supported by Center for International Research and Collaboration (ISMO) and French Embassy in Tehran: project number 8634, Department of Environment of Yazd Province, Iran (No.121-32217) and Northern Khorasan University of Medical Sciences, Bojnord, Iran (No. 1.19727).

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Received: 25.06.2015
Accepted: 25.05.2016