



Research article

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Phylogeography of the steppe field mouse, *Apodemus witherbyi* Thomas, 1902 in the Middle East (Rodentia: Muridae)

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Abstract. Geographic genetic variation at the regional level can be investigated using molecular methods by inferring phylogeographic structures within species. Small rodents are an interesting model for such kind of investigations due to their high variability. In this study, in order to assess the phylogeographic structure of the steppe field mouse, *Apodemus witherbyi*, DNA sequence data of the mitochondrial cytochrome b gene were analyzed from 100 individuals sampled at different localities across the Middle East region. Genetic variation among different population of this species is generally low. However, in certain areas like northeast Iran, Turkmenistan and northern, northeastern, and eastern Turkey, *A. witherbyi* shows higher genetic diversity indicating that these regions may have served as refugia for this species during the Quaternary. The effect of past climatic fluctuations on the genetic diversity and genetic structure of the steppe field mouse may be less pronounced than for other small mammals from the Western Palearctic region.

Keywords. *Apodemus witherbyi*, phylogeography, mitochondrial DNA, Middle East.

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Introduction

Wood mice of the genus *Apodemus* are one of the most common and widely distributed rodent groups across the Palearctic and Oriental regions, inhabiting a broad range of habitats (Corbet 1978). To date, 20 species have been recognized, divided into three subgenera: *Apodemus*, *Karstomys*, and *Sylvaemus* (Michaux *et al.* 2002; Suzuki *et al.* 2008). Among them, the steppe field mouse (*Apodemus witherbyi*

Thomas, 1902) inhabits plains, forests, gardens, woodlands, mountain and plateau steppes in the Northern and Southern Caucasus, the Anatolian Turkish steppe, and regions extending south to northern Israel and northwest Jordan, across most of Iran, southwestern Turkmenistan, and eastward to western-central Pakistan (Musser & Carleton 2005). Based on morphological studies, several new species of field mice were described over the last few decades but were later synonymized with *A. witherbyi* (e.g., *A. hermonensis*, *A. iconicus*). This synonymization was subsequently confirmed by genetic markers, suggesting significant morphological variation within this species (Musser & Carleton 2005; Hoofer *et al.* 2007; Siahsarvie & Darvish 2008). Darvish *et al.* (2006) described a new species (*A. avicennicus*) from the Shirkooch Mountains in the Central Iranian plateau based on morphological and morphometric analyses. This particular lineage was later confirmed by a preliminary RAPD-PCR analysis (Naseri *et al.* 2006). However, a subsequent phylogenetic study based on mitochondrial cytochrome b (*cytb*) sequences showed that *A. avicennicus* forms a monophyletic clade with *A. witherbyi*, with a genetic distance of 1.1%, and it was therefore considered synonymous with *A. witherbyi* (Darvish *et al.* 2015). Shad *et al.* (2016) assessed the genetic differentiation of a limited number of *A. witherbyi* populations in Iran by analyzing 40 *cytb* sequences, revealing a low level of genetic diversity in the species. A phylogenetic analysis of Armenian populations, using DNA sequence data from the mitochondrial control region, provided evidence of a recent population expansion in this species (Balasanyan *et al.* 2018). Stakheev *et al.* (2018) examined the genetic differentiation of populations from southern Russia, along with a limited number of specimens from Turkmenistan, Turkey, and Iran, analyzing the genetic diversity of the mitochondrial control region marker. This study suggested that the Cis-Manych region in southern Russia could be considered as a possible origin of *A. witherbyi*. However, the authors also noted that fossil evidence points to Southwest Asia as the more likely origin. While these previous studies have provided valuable insights into the phylogeographic patterns of *A. witherbyi*, information regarding its demographic history and potential refugia remains scarce. Pleistocene climatic oscillations led to inter- and intraspecific genetic divergence as well as population expansions and secondary contacts (Hewitt 2000). Many species became extinct across large parts of their distribution ranges, while others moved to new areas or survived in glacial refugia, later expanding their populations again during interglacial periods (Taberlet *et al.* 1998; Hewitt 2000; Liu *et al.* 2012). Small rodents are considered excellent subjects for phylogeographic studies, especially due to their suitability for investigating speciation and the effects of Quaternary climatic fluctuations (Michaux *et al.* 2004; Tougaard *et al.* 2013). Numerous phylogeographic studies on species of *Apodemus* have highlighted the importance of palaeoclimatic oscillations and biogeographic barriers in shaping the genetic structure of their populations (Michaux *et al.* 2003, 2005; Hoofer *et al.* 2007; Sakka *et al.* 2010; Liu *et al.* 2012; Latinne *et al.* 2020; Yalkovskaya *et al.* 2022; Chelomina *et al.* 2024). The Middle East, including the Anatolian Peninsula and the Iranian Plateau, has long been recognized as an important glacial refugium and a source for postglacial recolonization of European temperate species (Macholan *et al.* 2007). Despite its critical importance as a refugium during Quaternary climatic oscillations, the evolutionary processes and phylogeographic patterns of its biota remain largely understudied compared to North America and Europe (Mohammadi *et al.* 2020).

The present study analyses newly obtained sequence data of the mitochondrial cytochrome b gene, combined with those available in GenBank, from *A. witherbyi* populations in Iran, Turkey, Lebanon, Turkmenistan and Israel to answer the following questions: which populations within these studied regions show higher levels of genetic diversity and which regions could potentially have served as refugia during the quaternary glaciations? What was the demographic history of these populations during this period? What was the impact of past climate changes on the genetic structure of present-day species of *Apodemus*?

Material and methods

Sample collection

A total of 100 sequences of *A. witherbyi* were analyzed, each representing an individual sampled at one of 32 localities in Iran, Turkey, Turkmenistan, Lebanon and Israel.

The DNA sequences came from three sources: the present study ($n=45$), Shad *et al.* (2016) ($n=34$), and GenBank ($n=21$), (Table 1 and Figure 1). To obtain statistically meaningful sample sizes, the 32 original sampling localities were grouped into seven geographical units (Groups 1–7) based on proximity and major topographic features (e.g., mountain ranges). The composition of each group is as follows: Group 1 corresponds to the north, northwest, southwest, and west of Iran; Group 2 includes the populations from the northeast of Iran and Turkmenistan; Group 3 contains samples from central Iran; Group 4 corresponds to individuals from the north, northeast, and east of Turkey; Group 5 consists of samples from Israel and Lebanon; Group 6 contains samples from west and Group 7 from southeast Turkey. Groups 6 and 7 contained only two individuals each and were therefore only used for the haplotype network analysis (Figure 2) to visualize their genetic relationships with the other groups. All other population genetic analyses — estimates of nucleotide diversity (π) and haplotype diversity (H_d), pairwise F_{ST} , AMOVA, Tajima's D , Fu's F_s , and mismatch distribution — were performed on Groups 1–5, where sample sizes were sufficient for statistical analyses (Group 1: $n = 50$, Group 2: $n = 13$, Group 3: $n = 14$, Group 4: $n = 12$, Group 5: $n = 7$).

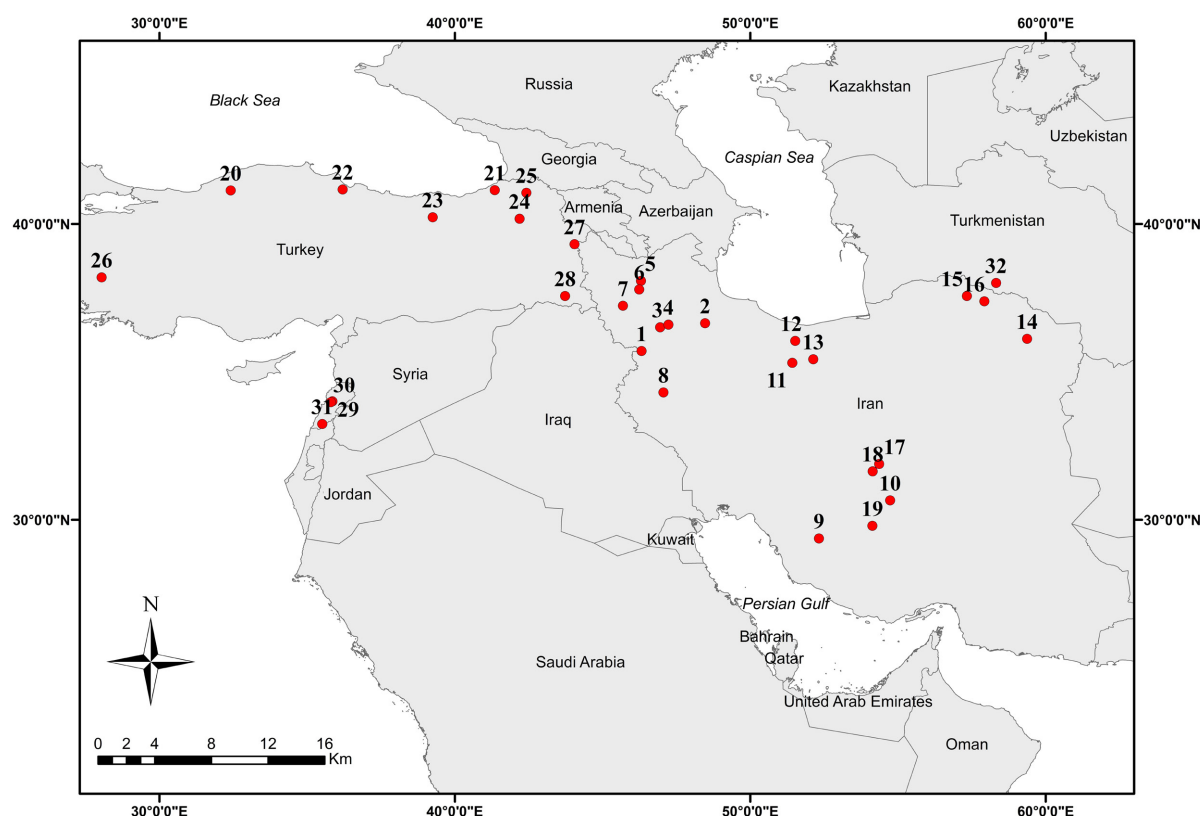


Figure 1 – Sampling localities of *Apodemus witherbyi*. The numbers correspond to those in Table 1.

TABLE 1 (continued on next page)

Overview of all samples. Sampling localities and their position in the map (Figure 1) are shown as “Map reference”, together with the country of origin, the name of the locality, the sample size and the matching GenBank accession numbers of the present study.

Map reference	Country	Locality	Total number of animals	GenBank accession numbers	Reference
1	Iran	Kordestan	12	QB095999, QB096000, QB096001, QB096028, QB096029, QB096030, QB096031, QB096032, KR003093, KR003094, KR003096, KR003102	Shad <i>et al.</i> (2016); GenBank
2	Iran	Zanjan	2	QB148850, KR003092	This study; GenBank
3	Iran	Choplu	1	QB148849	This study
4	Iran	Takhte soleyman	7	QB148820, QB148821, QB148822, QB148823, QB148825, QB148846, QB148858	This study
5	Iran	Tabriz	3	KR003087, KR003090, KR003091	GenBank
6	Iran	Kandovan	4	QB148824, QB148844, QB148845, QB148847	This study
7	Iran	Mohhamadyar	1	QB148848	This study
8	Iran	Kermanshah	2	KR003088, KR003095	GenBank
9	Iran	Shiraz	1	KR003100	GenBank
10	Iran	Yasooj	5	QB096023, QB096024, QB096025, QB096026, QB096027	Shad <i>et al.</i> (2016)
11	Iran	Abnik	7	QB148832, QB148837, QB148838, QB148840, QB148842, QB148843, QB148851	This study
12	Iran	Sorkhehesar	4	QB148834, QB148835, QB148836, QB148841	This study
13	Iran	Tehran	1	KY753945	GenBank
14	Iran	Mashhad	4	QB096003, QB096004, QB096005, KR003098	Shad <i>et al.</i> (2016); GenBank

TABLE 1 (continued)

Map reference	Country	Locality	Total number of animals	GenBank accession numbers	Reference
15	Iran	Bojnord	7	QB096006, QB096007, QB096008, QB096010, QB096011, KR003097, KR003099	Shad <i>et al.</i> (2016); GenBank
16	Iran	Shirvan	1	QB096002	Shad <i>et al.</i> (2016)
17	Iran	Yazd	2	KR003089, KR003101	GenBank
18	Iran	Shirkooch	11	QB096009, QB096012, QB096013, QB096014, QB096015, QB096016, QB096017, QB096018, QB096019, QB096020, QB096021	Shad <i>et al.</i> (2016)
19	Iran	Bagheshadi	1	QB096022	Shad <i>et al.</i> (2016)
20	Turkey	Safranbolu	2	QB148829, QB148856	This study
21	Turkey	Damar	1	QB148831	This study
22	Turkey	Samsun	1	QB148833	This study
23	Turkey	Guzyurdu	1	QB148863	This study
24	Turkey	Sirbasan	2	QB148828, QB148830	This study
25	Turkey	Ardahan	2	QB148853, QB148855	This study
26	Turkey	Bozdag	2	QB148852, QB148854	This study
27	Turkey	Dogubayasit	3	QB148827, QB148839, AJ311157	This study; GenBank
28	Turkey	Hakkari	2	QB148826, AJ311156	This study; GenBank
29	Lebanon	Faraya	4	QB148857, QB148859, QB148860, QB148861	This study
30	Lebanon	Faqra	1	QB148862	This study
31	Israel	Hermon	2	AB303227, AB303228	GenBank
32	Turkmenistan	Ashgabat	1	QB148864	This study

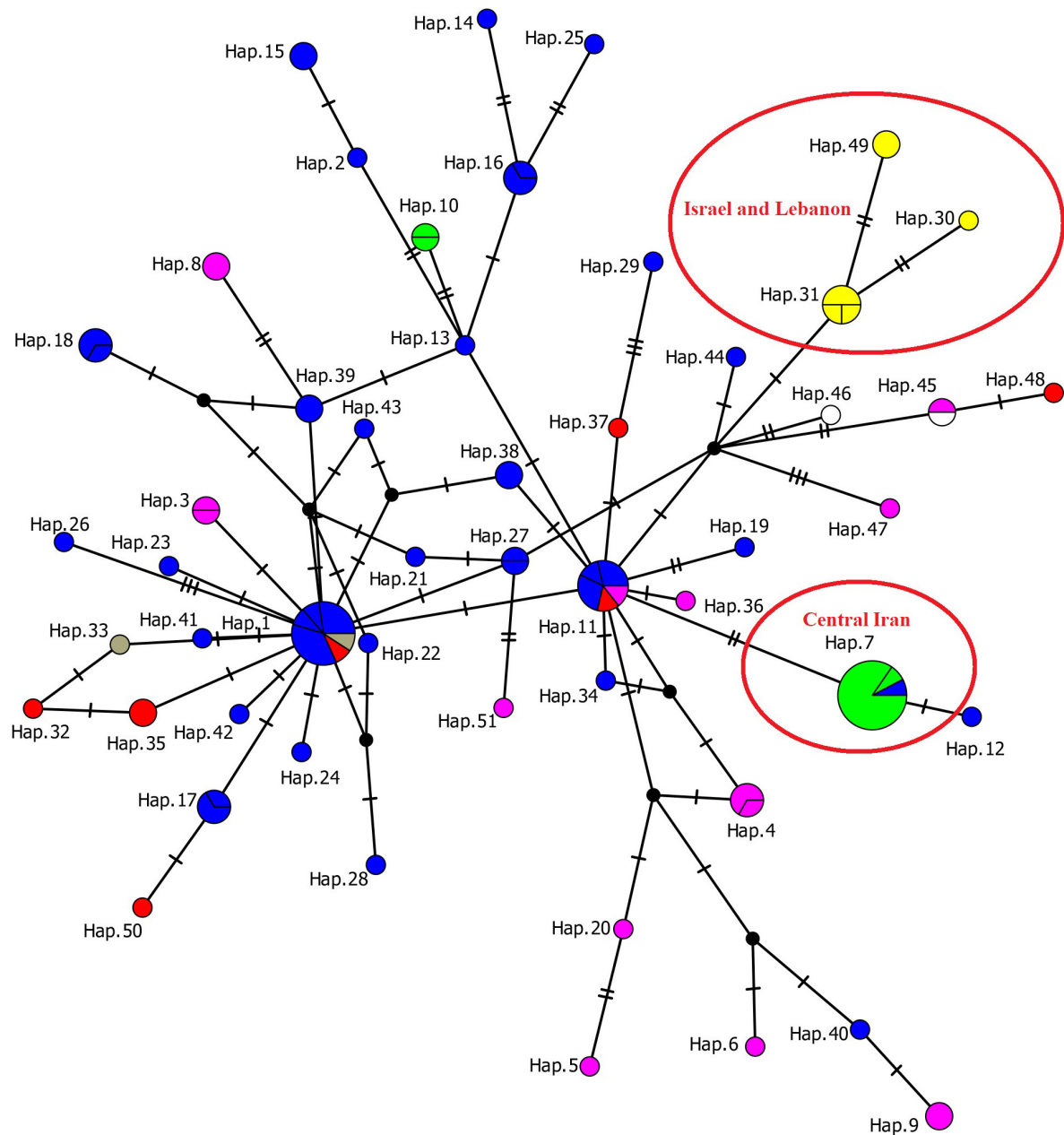


Figure 2 – Haplotype network of DNA sequences from part of the mitochondrial Cytb gene for *Apodemus witherbyi* populations from the Middle East. Colours correspond to the seven geographical groups: blue (Group 1), purple (Group 2), green (Group 3), red (Group 4), yellow (Group 5), white (Group 6), and gray (Group 7). Ticks (horizontal stripes) on individual branches indicate mutational steps; branches without ticks correspond to a single mutation, while each additional tick represents one additional mutational step. Red circles highlight haplotypes associated with Israel and Lebanon and the Shirkooh locality in central Iran.

Sequencing analysis

We extracted DNA from tissue samples (muscles and liver) using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany; Handbook version 02/2020), according to the manufacturer's protocol. Tissues were taken from the *Apodemus* tissue collection of J.R. Michaux. A large part of cytochrome b (965 bp) was amplified using the same primers and protocols as for *A. sylvaticus* (see Michaux *et al.* 2003).

Briefly, we used slightly modified versions of the universal polymerase chain reaction (PCR) primers L7 (5'-ACCAATGACATGAAAAATCATCGTT-3') and H16 (5'-ACATGAATYGGAGGYCAACCWG-3').

Amplification reactions were carried out in 50 μ L volumes including 25 μ L of each primer (2mM), 20 μ L of 1 mM dNTP, 10 μ L of 10 $\times$ reaction buffer, 10 μ L of purified water and 0.2 μ L of 5 U/ μ L Promega Taq DNA polymerase. Approximately 200 ng of DNA extract (10 μ L) was used per PCR amplification. Amplifications were performed in a Labover PTC100 thermal cycler with 33 cycles (20 s at 94°C, 30 s at 50°C and 1 min 30 s at 68°C) followed by a final extension step of 10 min at 68°C. PCR products were purified using the Ultra-free DA Amicon kit (Millipore) and directly sequenced. Both strands were sequenced using a Bigdye terminator (Applied Biosystems) sequencing kit on an ABI 310 (Applied Biosystems) automated sequencer.

The raw sequences were edited and prealigned using BioEdit version 7.2.5 (Hall 1999) and the ClustalW algorithm (Thompson *et al.* 1994) for the final alignment. After trimming primer regions and low-quality ends, the length of the final alignment was reduced from the original 965 bp to 721 bp.

Haplotype diversity and historical demography

For inferring the relationships among populations, a haplotype network was constructed using the median-joining algorithm (MJ) (Bandelt *et al.* 1999) implemented in PopART version 1.7 (Leigh & Bryant 2015 Leigh & Bryant). The analysis was performed on the Nexus file containing unique haplotypes using default settings, with the epsilon parameter set to 0 (the default value).

Haplotype diversity (Hd), and Nucleotide diversity (π) were calculated with DnaSP version 6 (Librado & Rozas 2009). To investigate the demographic history of the species, a mismatch distribution analysis between haplotype pairs was performed within Groups 1–5 and its fit compared to the Poisson model using DnaSP, version 6.

Neutrality tests, including Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997), were performed for the defined groups using Arlequin version 3.5.2.2 (Excoffier & Lischer 2010), where the significance of both tests was assessed with 10 000 replicates of coalescent simulations.

Population genetic structure

An analysis of molecular variance (AMOVA) was performed using Arlequin version 3.5.2.2 (Excoffier & Lischer 2010).

The five main geographical groups (Groups 1–5), defined according to their geographic distribution, were used in the population genetic analyses. For the hierarchical AMOVA, these five groups were further combined into two broader geographical groups. Group I comprised Groups 1–3, representing Iran and Turkmenistan, whereas Group II comprised Groups 4–5, representing Turkey, Israel, and Lebanon. This hierarchical grouping was used to test whether genetic variation was partitioned according to this broad geographical division.

The AMOVA was run with 10 000 permutations to assess the significance of variance components among groups, among populations within groups, and within populations. Additionally, pairwise F_{ST} values

TABLE 2

Parameters of genetic variability estimated within the main groups of *Apodemus witherbyi*.

Genetic Groups	Names of groups	Number of specimens	Number of haplotypes	Haplotype diversity $H_d \pm SD$	Nucleotide diversity $\pi \pm SD$
North, northwest, southwest and west Iran	Group 1	50	29	0.953 ± 0.018	0.00445 ± 0.00042
North-east Iran and Turkmenistan	Group 2	13	8	0.923 ± 0.050	0.00651 ± 0.00072
Central Iran	Group 3	14	2	0.264 ± 0.136	0.00183 ± 0.00094
North, northeast and east Turkey	Group 4	12	10	0.970 ± 0.044	0.00480 ± 0.00081
Israel and Lebanon	Group 5	7	3	0.667 ± 0.160	0.00211 ± 0.00065

were calculated with 10 000 permutations to evaluate genetic differentiation between all population pairs.

Results

Haplotype variation

The final alignment comprised 721 bp, of which 56 sites were variable and 24 were parsimony informative. A total of 51 haplotypes were detected among the 100 cytb sequences. Thirty-two of these were singletons. The haplotype network (Figure 2) revealed a lack of clear geographical structuring with the exception of haplotypes from Israel and Lebanon, which appeared separated, as well as those from the Shirkoooh locality in central Iran. Three haplotypes (Hap 1, Hap 7, and Hap 11) were represented by larger circles, corresponding to the most frequent haplotypes: Hap 1 ($n = 11$), Hap 7 ($n = 13$), and Hap 11 ($n = 7$). Of these, Hap 1 and Hap 11 exhibited star-like topologies. Hap 11 contained samples originating from southwestern and northwestern Iran, as well as from northern and northeastern Turkey. Samples belonging to Hap 1 originated from northern and northwestern Iran, as well as from northern and southeastern Turkey. The first star-like topology around Haplotype 11 comprised haplotypes from northwestern, southwestern, northeastern, and northern Iran, together with haplotypes from northern and northeastern Turkey. The second star-like topology around Hap1 comprised haplotypes from northern, northwestern, northeastern, and western Iran, along with haplotypes from eastern and southeastern Turkey.

Genetic diversity and demographic history

Nucleotide and Haplotype diversities were calculated for each group (Table 2). The highest nucleotide diversity (0.00651) was found in the northeast of Iran and Turkmenistan (Group 2), followed by the north, northeast and east Turkish group (Group 4) (0.00480). Genetic groups from central Iran and Israel/Lebanon (Groups 3 and 5) had the lowest nucleotide diversities (0.00183 and 0.00211), respectively. As mentioned above, specimens belonging to the west and southeast Turkey groups were not tested with this method because the number of the samples for these regions was too low ($n=2$). The mismatch distribution analyses (Figure 3) for Group 1 revealed a bell-shaped distribution suggesting a signal of population in expansion. The same signal was also observed for Group 4. In contrast, Groups 2, 3 and 5 presented more heterogeneous patterns suggesting that these populations were more stable or at least had not experienced population expansion. Given the relatively small sample size of Group 5 ($n=7$), the results for this group should be interpreted with caution. This analysis was not conducted for Groups 6 and 7 containing specimens from west and southeast Turkey as the numbers of samples for each group were too low ($n < 10$).

The Tajima's D and Fu's Fs values for Group 1 were significantly negative for both tests, indicating population expansion, Group 4 also showed a significantly negative Fu's Fs, although its Tajima's D

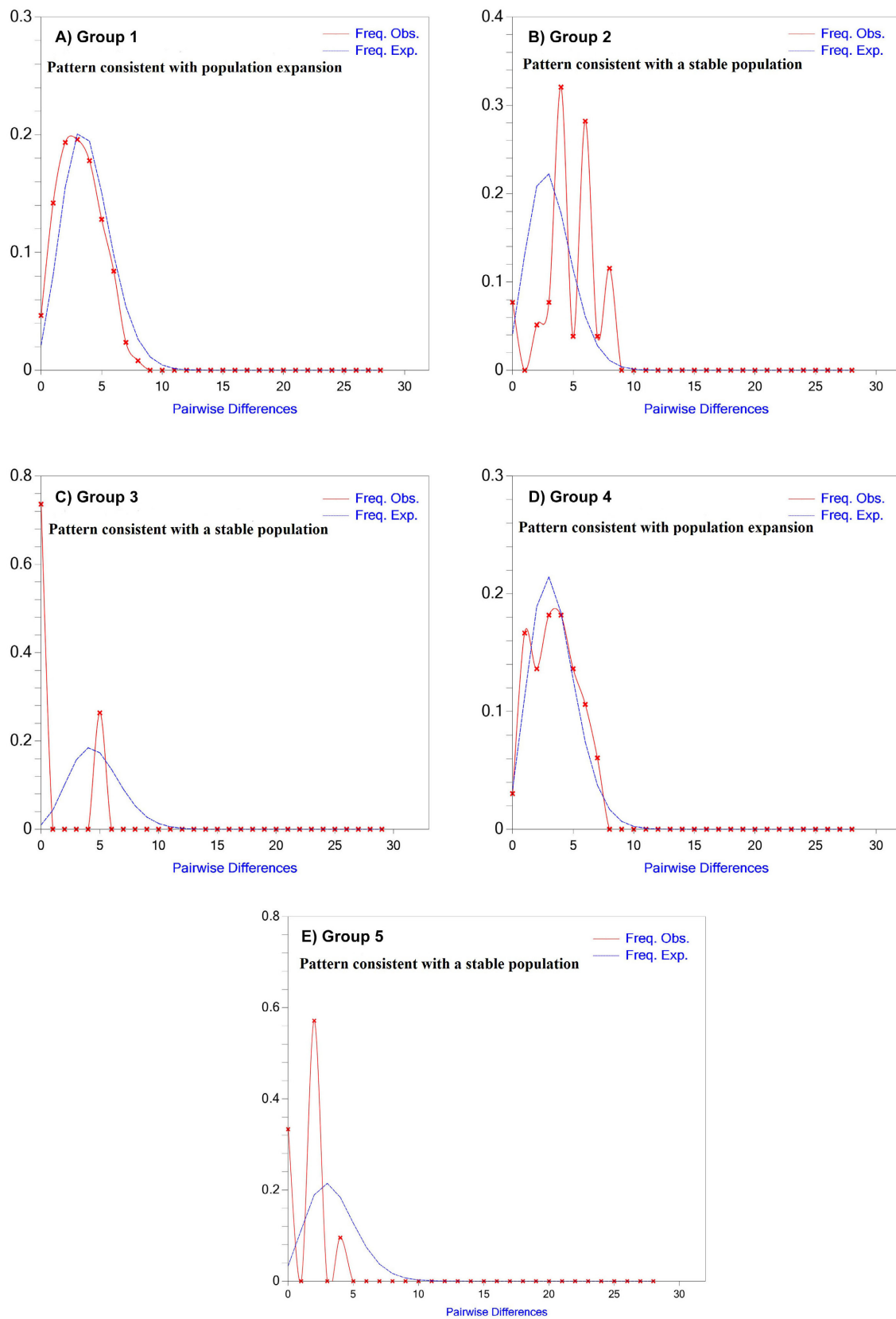


Figure 3 – Mismatch distribution analysis for the main groups of *Apodemus witherbyi*. A = Group 1; B = Group 2; C = Group 3; D = Group 4; E = Group 5.

TABLE 3

Neutrality analyses within the main groups of *Apodemus witherbyi*.

Names of groups	Number of specimens	Tajima's D	P-value (Tajima)	Fu's Fs	P-value (Fs)
Group 1	50	-1.89	0.0082	-24.84	0.0000
Group 2	13	-0.60	0.2926	-0.89	0.2956
Group 3	14	-0.55	0.3124	3.29	0.9322
Group 4	12	-1.08	0.1444	-5.18	0.0023
Group 5	7	-0.31	0.3835	0.78	0.6360

TABLE 4

Pairwise F_{ST} estimates between populations. F_{ST} values are shown below the diagonal, their matching P-values above the diagonal. Significant P-values ($P < 0.05$) are printed in bold.

Population	Group1	Group 2	Group 3	Group 4	Group 5
Group 1	–	<0.001	<0.001	0.029	<0.001
Group 2	0.125	–	<0.001	0.006	<0.001
Group 3	0.366	0.401	–	<0.001	<0.001
Group 4	0.045	0.111	0.435	–	<0.001
Group 5	0.424	0.406	0.722	0.394	–

was not significant, suggesting a weaker signal of expansion. The remaining Groups (2, 3, and 5) did not show significant negative values for either test implying demographic stability (Table 3).

Population genetic structure

Hierarchical AMOVA indicated that most of the genetic variation was distributed within populations (72.42%), while 30.79% was explained by differences among populations within groups ($F_{SC}=0.30$, $P<0.001$). The variation between the two predefined geographical groups (Group I: Group1–3; Group II: Group 4–5) was negative and statistically non-significant (-3.21%; $F_{CT}=-0.03$, $P=0.303$). In contrast, the overall F_{ST} across all five populations was 0.28 ($P<0.001$), indicating significant genetic structuring.

Pairwise F_{ST} values among the five populations ranged from 0.045 to 0.722, with all pairwise comparisons being statistically significant ($P<0.05$; Table 4). Moderate genetic differentiation was observed between Group 1 and Group 3 ($F_{ST}=0.366$, $P<0.001$). The lowest differentiation was found between Group 1 and Group 4 ($F_{ST}=0.045$, $P<0.05$), suggesting high genetic similarity and possible historical gene flow. The strongest differentiation was detected between Group 3 and Group 5 ($F_{ST}=0.722$, $P<0.001$), potentially reflecting long-term isolation or restricted gene flow. For the remaining population pairs, F_{ST} values ranged from 0.111 to 0.435 (Table 4).

Discussion

Haplotype variation

This study presents new insights into the phylogeographic structure of *Apodemus witherbyi* in the Middle East. Based on the haplotype network results, no specific geographical structure was observed among the haplotypes. Haplotypes from the northwest, northeast, and north of Iran as well as the southwest of Iran and various regions of Turkey were dispersed without an obvious geographic structure. In contrast,

haplotypes from the Shirkooh population (Central Iran) and from Israel and Lebanon formed distinct clusters. The genetic differentiation of the Shirkooh population from other groups is supported by high pairwise F_{ST} values ($F_{ST}=0.366\text{--}0.722$, $P < 0.001$), indicating significant but not complete genetic differentiation. This pattern may be related to the unique geographical position of this region. Shirkooh is one of the most prominent heights in central Iran, bordered to the north by the central desert, to the east by the Jazmurian and Lut deserts, and to the west by the Abarkooh desert. These natural geographical barriers likely play a significant role in isolating populations that have colonized this area. Field mice probably invaded Shirkooh from the Zagros Mountains in response to past climatic fluctuations (Darvish *et al.* 2006; Shad *et al.* 2016; Shad & Darvish 2018). The separation of the Shirkooh population is consistent with the results of the geometric morphometric analysis conducted by Shad *et al.* (2016), who found that this population in the Iranian central plateau is distinct from other populations in the shape and size of the first lower molar. Given the previous description of this population as *A. avicennicus* and its subsequent synonymization with *A. witherbyi*, the pronounced mitochondrial differentiation observed here, together with its previously reported morphological distinctiveness, raises the question of whether the Shirkooh population represents a distinct evolutionary lineage. This possibility should be further evaluated using broader sampling and additional nuclear markers before any taxonomic conclusions can be drawn. The role of biogeographic barriers such as mountains, deserts, and seas in isolating *Apodemus* species in the Asian Far East has been previously documented (Sakka *et al.* 2010). Two star-like patterns observed in the haplotype network suggest population expansion. These topologies indicate a rapid radiation from a small number of individuals (Avice 2000; Conroy & Cook 2000), leading to the emergence of many, closely related haplotypes now dispersed throughout Iran and Turkey. Similar star-like topologies have been reported for the main genetic sublineage (western Palearctic region) of the yellow-necked field mouse (*A. flavicollis*) and for Italo-Balkan populations of *A. sylvaticus* (Michaux *et al.* 2004, 2005). Two possible demographic histories for this *Apodemus* species can be imagined: a relatively stable one, suggesting that these regions did not significantly impact the genetic diversity of the species and may have served as refugia, and a second scenario represented by star-like topologies, indicating population decreases followed by rapid expansion in some areas.

Genetic diversity and demographic history

The analysis of nucleotide diversity (Table 2) indicated that Group 2, comprising populations from northeastern Iran and Turkmenistan, had the highest nucleotide diversity (0.00651), followed by Group 4, comprising specimens from northern, northeastern, and eastern Turkey.

The lowest nucleotide diversity (0.00183) was observed in the Shirkooh population from central Iran. This result aligns with the findings of Shad *et al.* (2016), who also reported the highest nucleotide diversity in the Bojnord population from northeastern Iran and the lowest in the Shirkooh population. High nucleotide diversity in northeastern Iran contrasts sharply with the low genetic diversity in the central Iranian population (i.e., Shirkooh), a similar pattern as reported for the brush-tailed mouse (*Calomyscus elburzensis*) populations (Shad & Darvish 2018). The findings of the current study indicate that genetic diversity values are generally low. However, when comparing different regions, populations from northeastern Iran and Turkmenistan, as well as northern, northeastern, and eastern Turkey, show higher genetic diversity than the other investigated regions. This suggests that these areas may have served as refugia for *A. witherbyi*. The role of northeastern Iran (Kopet-Dagh, Binaloud Mountains) as a refugium has been previously demonstrated for snow voles (*Chionomys nivalis*) and two moth species (Rajaei *et al.* 2013; Mahmoudi *et al.* 2017).

The slight intraspecific divergence observed in steppe field mouse populations may be associated with their tolerance to a wide range of environmental factors, which could facilitate their dispersal (Shad *et al.* 2016).

Similarly, low genetic variation in populations of the South American hystricognath rodent *Lagostomus maximus* in Argentina has been documented; this species inhabits a variety of environments and has shown potential to adapt to climate change (Gariboldi *et al.* 2019). The concordance among the three complementary analytical approaches (mismatch distribution, Tajima's D, and Fu's Fs) in the current study provides robust evidence for the demographic history of each group. Group 1 consistently showed signals of population expansion across all three analyses: a bell shaped mismatch distribution, a significantly negative Tajima's D (-1.89, $P=0.008$), and a significantly negative Fu's Fs (-24.84, $P<0.001$). Group 4 also exhibited a bell shaped mismatch distribution and a significantly negative Fu's Fs (-5.18, $P=0.002$), although its Tajima's D was not significant ($P=0.144$), suggesting a weaker or more recent expansion signal. In contrast, Groups 2, 3, and 5 displayed heterogeneous patterns with non-significant values for both neutrality tests ($P > 0.05$ in all cases), indicating demographic stability. The demographic expansion observed in Group 1 and Group 4 is consistent with findings in other taxa. For instance, in Eurasian populations of *Bombus terrestris*, a unimodal mismatch distribution together with a significantly negative Tajima's D was interpreted as evidence of recent population expansion following a glacial bottleneck (Ranjbaran *et al.* 2024).

Population genetic structure

The findings of the current study indicate that genetic diversity values are generally low across the studied populations of *A. witherbyi*. The variation between the two large geographical groups (Group I: Groups 1–3; Group II: Groups 4–5) was negative and non-significant (FCT = -0.03, $P=0.303$), indicating that this grouping does not explain the observed genetic structure.

In contrast, the pairwise F_{ST} values provide a more detailed picture, ranging from low differentiation between Group 1 and Group 4 (0.045), suggesting high genetic similarity and possible historical gene flow, to strong differentiation between Group 3 and Group 5 (0.722), indicating strong and long-term isolation.

Therefore, even in a species with low overall genetic diversity, significant population structure can arise through the combined effects of restricted gene flow and geographic barriers.

Conclusion

In conclusion, the presented results show that the genetic variation among different populations of the steppe field mouse occurring in the Near and Middle East region is low. But when comparing different regions, some mice populations exhibit higher genetic diversity than others, specifically, from northeast Iran, Turkmenistan, and northern, northeastern, and eastern Turkey. This pattern suggests that these areas may have served as refugia for *A. witherbyi*. It seems that the slight intraspecific divergence within different population of this species could possibly be attributed to their strong tolerance for environmental factors. The influence of climatic oscillations on the genetic structure of this species may be less pronounced than in other small mammals.

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