Mitochondrial evidence indicates a shallow phylogeographic structure for *Jaculus blanfordi* (Murray, 1884) populations (Rodentia: Dipodidae)

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**Abstract:** Our study was performed on the phylogeographic structure of Blanford's jerboa (*Jaculus blanfordi* (Murray, 1884)) collected from nine localities in Iran, Turkmenistan, and Uzbekistan and was based on mitochondrial evidence indicating a slight phylogeographic divergence among the populations. We aimed to amplify two frequently used mitochondrial markers, cytochrome *b* (cyt *b*) and cytochrome *c* oxidase subunit I (COI) fragments, from 33 specimens obtained from the abovementioned countries. Our phylogeographic analyses uncovered two distinct groups, thus supporting the presence of two subspecies: *J. b. blanfordi* in Iran and *J. b. turcmenicus* in northern Turkmenistan and Uzbekistan. Finally, we discuss the intraspecies genetic structure of Blanford's jerboa in relation to the biogeography of the Middle East and Middle Asia.

**Key words:** Blanford’s jerboa, *Jaculus*, Middle Asia, Middle East, phylogeography

1. **Introduction**

The arid areas of the Middle East and Middle Asia are some of the oldest known desert regions and host their own specific fauna (Geptner, 1938; Korovin, 1961; Velichko, 2009). Despite the short glacial and interglacial periods in eastern Eurasia, these regions remained extremely cold and arid after those periods (Elenga et al., 2000; Tarasov et al., 2000; Velichko, 2009). The phylogenetic history of animals within these areas has not been thoroughly studied (Melville et al., 2009; Chelomina and Atopkin, 2010; Dianat et al., 2013). Jerboas from the genus *Jaculus* (Erxleben, 1777, Dipodidae) are widely distributed in the desert and semiarid habitats across the Palearctic biogeographic region (northern Africa, Arabia, the Middle East, and Middle Asia) (Holden and Musser, 2005). The main morphological criterion that is employed to distinguish *Jaculus* species from other jerboas (such as members of the genus *Allactaga*) is the reduced number of digits; i.e., there are only three functional digits in *Jaculus* members (Ellerman, 1941). The genus contains six species: *J. jaculus* (Linnaeus, 1758), *J. orientalis* (Erxleben, 1777), *J. deserti* Loche 1867, *J. thaleri* (Darvish and Hosseinie, 2005), *J. hirtipes* (Lichtenstein, 1823), and *J. blanfordi* (Murray, 1884) (Darvish and Hosseinie, 2005; Holden and Musser, 2005; Ben Faleh et al., 2011, 2012, 2016; Boratynski et al., 2012; Shenbrot et al., 2016).

Previous phylogeographic studies have focused only on African *Jaculus* species populations, i.e. *J. orientalis*, which have been reported in Morocco to Egypt and eastwards to Sinai and the Negev (Harrison and Bates, 1991). Ben Faleh et al. (2011) showed that *J. orientalis* was subdivided into three lineages, corresponding to the following: Morocco and western Algeria; eastern Algeria, Tunisia, and western Libya; and eastern Libya and Egypt. Ben Faleh et al. (2016) studied the genetic diversity distribution patterns in greater Egyptian jerboa (*J. orientalis*) from Tunisia based on mitochondrial markers and concluded that the populations sampled from the northern and central regions were grouped into one distinct and well-supported clade from the southern population. Such evolutionary divergence had been related to geographical barriers, such as being located in the middle zone. The authors also concluded that the clear distinction present between the clades was probably due to a Libyan origin for the southern population. *J. jaculus* ranges across the whole of northern Africa from Mauritania and Morocco to Egypt, Sudan, and Somalia. It also occurs in the Arabian Peninsula and southwestern Iran (Harrison and Bates, 1991). Based on mitochondrial and nuclear gene amplification and morphometric and cytogenetic data, Ben Faleh et al. (2012) showed that *J. jaculus* populations from Tunisia possibly constitute two cryptic species, *J.
deserti and J. jaculus. Middle Pleistocene climatic changes and environmental consequences were mentioned as the causal factor for the diversification of these two clades. Another study (Boratynski et al., 2012) appeared to confirm the monophyly of these cryptic species. The reevaluation of the taxonomic status of the Egyptian jerboa, Jaculus jaculus from North Africa and the Middle East, performed by Shenbrot et al. (2016) resulted in two species: Jaculus jaculus (Linnaeus 1758) and Jaculus hirtipes (Lichtenstein, 1823). These species showed high niche divergence between them that coincided with genetic differences. The eastern boundaries of the range of J. jaculus have not been explored. Some authors have described a new member of the genus Jaculus in Iran, J. thaleri, which is closely related to Blanford’s jerboa based on skull characteristics and the complex structure of the penis (Darvish and Hosseini, 2005). Our study can be considered the first investigation to focus on the phylogenetic and phylogeographic status of Blanford’s jerboa in the Middle East and Middle Asia.

Jaculus blanfordi is the only species from the genus that can be found in the Middle East and Middle Asia and is reported from Afghanistan, Iran, Pakistan, Turkmenistan, and Uzbekistan (Figure 1). This burrowing species is found in desert and semidesert habitats with sparse vegetation (Naderi et al., 2014). In Turkmenistan, they stick to clay-sandy areas and avoid large sand dunes. It appears that the geographic clines are reflected in the species’ body size and cranial characteristics, although the coat color patterns have remained unchanged. Based on morphological characteristics, including body size and cranial measurements, three subspecies have been described to date (Shenbrot et al., 1995): J. b. blanfordi from central, eastern, and southern Iran, southern Afghanistan, and western Pakistan (Murray, 1884); J. b. margianus (Shenbrot, 1989) from southern Turkmenistan (the ancient delta of the Murghab and Tedjen); and J. b. turcmenicus (Vinogradov and Bondar, 1949; Gepner, 1984; Shenbrot et al., 1995) from western, northern, and northeastern Turkmenistan (Kyzyl Kum). We aimed to investigate the intraspecific genetic variations in different Blanford’s jerboa populations based on the COI and cyt b gene fragments and to analyze the phylogeographic patterns occurring in the Middle East and Middle Asia.

2. Materials and methods
2.1. Sampling
Both museum and fresh samples were used in this investigation. All tissue samples were obtained by applying
noninvasive sampling methods, such as taking tissue from ear pads or hair samples, with regards to the animal care protocol issued by the DOE (certificate number: 95/102-1825). In total, 33 specimens were obtained from nine populations in Iran, Turkmenistan, and Uzbekistan (Figure 1; Table 1); six samples from Turkmenistan and Uzbekistan were obtained from museum skin collections at the Zoological Institute of the Russian Academy of Science (Saint Petersburg, ZIN RAS) and the Museum of Vertebrate Zoology (Berkeley, California, MVZ).

2.2. Laboratory procedures
Genomic DNA was extracted from the ethanol-preserved tissues using a salt extraction method protocol (Miller et al., 1988). DNA from museum specimens was isolated using a genomic DNA isolation kit (Omnix, Russia), which included a 5-h digestion with lysis buffer supplemented with proteinase K at 60 °C. Negative extraction (no tissue) and PCR controls (no template DNA) were used in each experiment. To amplify cyt b and COI markers, two pairs of primers, i.e. H15915 (5´–TTCCATTTCTGGTTT ACAAGAC–3´), L14723 (5´–ACCAATGACAT GAAAAATCATGGTT–3´) (Ben Faleh et al., 2012) and VUTF (5´–TGTAAGCAGCGG CCAGTTTCAACCAAYCAYAARGAYATYG–3´), VUTR (5´–CAGGAACAGCTATGACTARACTTCTGG RTGKCCRAARAAYC–3´) (Melnikov et al., 2013), were used, respectively. To amplify and sequence the DNA extracted from the old skin samples, new primers were designed, including VUTR–COI2F (5´–TCATYW ATARTT GAAGCAGGCG–3´) for the short COI fragment and H15915–H4F (5´–AACAAACTYGGYGG YGTAGTAG–3´) for the short cyt b fragment. Cycling conditions included an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of 30 s of denaturation at 94 °C and 35 s at an annealing temperature of 61 °C for the cyt b fragment and 58 °C for the COI fragment, with a 50-s elongation step at 72 °C. The thermocycling program ended with a final elongation step at 72 °C for 5 min. Amplification was conducted in 20-µL reaction volumes containing 2 µL of DNA (50 ng), 20 pmol of each primer, 0.4 mM of each dNTP , 3 mM MgCl2, 2 μL 10X PCR buffer (0.01 M Tris-HCl, 0.05 M KCl, and 0.1% Triton X-100, pH 9.0), and 0.6 U/µL Taq polymerase (Helicon, USA). The PCR products were further purified using columns.
(Omnix). Sequencing was carried out on an ABI 3130 automated DNA analyzer sequencer (Applied Biosystems, USA) according to the manufacturer’s instructions.

2.3. Phylogenetic analyses

The sequences were aligned using the Clustal W algorithm (Thompson et al., 1994) by running BIOEDIT 7.0.5.3 software (Hall, 1999). The sequencing data were translated into amino acids to confirm the absence of premature stop codons. We used *J. jaculus* and *J. orientalis* as outgroups (the GenBank reference numbers for the cyt *b* fragment were JN652640, JN652658, GU433411, GU433439, and AJ416890, and that for the COI fragment was AJ416890). We also used original *J. jaculus* (AJ416890, mk8, mk9) and *J. orientalis* (mk16, mk17) sequences for the COI fragments from Egypt.

Phylogenetic relationships were reconstructed using Bayesian inference (BI) and maximum-likelihood (ML) approaches for the long COI and cyt *b* fragments (obtained from fresh samples) and combined data for the two COI and cyt *b* gene short fragments. Tree reconstruction and bootstrapping (1000 replicates) based on the ML criteria were performed using Treefinder (Jonn et al., 2008). To choose the best molecular evolution model, we used the AIC (Akaike, 1974) and BIC criteria in jModeltest (Posada, 2008). The evolutionary relationships between haplotypes were also estimated by the Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analyses in MrBayes v.3.1 using the default priors (Huelsenbeck et al., 2001; Huelsenbeck and Ronquist, 2003). Three heated chains and a single cold chain were used in all MCMC analyses, and the runs were initiated with random trees. Two independent MCMC runs were conducted (with 2,000,000 and 500 generations per run). The trees and parameters were sampled every 100 generations. For each run, the first 25% of the sampled trees were discarded as burn-in. Bayesian posterior probabilities were used to assess the branch support of the MCMC tree.

Haplotype (*h*) and nucleotide (*π* in percentage) diversities were estimated using DNASP 5.10.01 (Rozas et al., 2003). Genetic distances and average nucleotide divergences within phylogeographic groups were created in MEGA 6 (Tamura et al., 2013). To evaluate the recent demographic histories within the geographic groups, we calculated the mismatch distributions using DNASP (*θ* initial = 2, *θ* final = 200, and τ = 5; Rozas et al., 2003). Multimodal distributions were consistent with demographic stability, whereas sudden demographic expansions (Slatkin and Hudson, 1991) or range expansion with high levels of migration (Excoffier, 2004) generated unimodal patterns. Median networks are a special class of split networks that are commonly used in the study of intraspecific and population data. Relationships between the *J. blanfordi* haplotypes were estimated using the median-joining (MJ) approach in NETWORK 4.6.1.3 (Bandelt et al., 1999) with an equal transition/transversion rate ratio.

3. Results

Thirty-three field samples were sequenced for their cyt *b* and COI gene fragments (1110 bp and 618 bp, respectively; Table 1). For museum specimens, short segments of both markers were obtained. In total, three datasets for *J. blanfordi* including long cyt *b* fragments (1110 bp) for 24 samples from 6 localities (GenBank accession numbers KU291617–KU291640), long COI fragments (618 bp) for 27 samples from 6 localities (GenBank accession numbers KU291585–KU291611), and the combined data for the two short COI (313 bp) and cyt *b* (284 bp) fragments for 29 samples from 8 localities (GenBank accession numbers KU291579–KU291584 and KU291612–KU291616) were collected.

All of the *J. blanfordi* samples studied formed a well-supported monophyletic group, as underlined by high bootstrap values (99%) and high posterior probabilities (1.0). However, the COI + cyt *b* dataset showed a low-supported monophyletic group. For the long COI fragment, long cyt *b* fragment, and COI + cyt *b* datasets, the J2 (G:5), GTR (G1:5), and TN (I) models were chosen, respectively. The different phylogenetic analyses (ML and BI) performed on all of the generated datasets essentially demonstrated the same topology; for simplicity, we present only the phylogenetic tree corresponding to the ML analyses and the information produced by the Bayesian posterior probabilities (Figure 2). Phylogenetic analyses of *J. blanfordi* for the long COI fragment and combined dataset showed the existence of two genetic groups that corresponded to specific geographical regions. Group I comprised haplotypes from Iran (localities 1–4 in Figure 1 and Table 1), whereas group II comprised haplotypes from northern and central Turkmenistan and from Uzbekistan (localities 5–9 in Figure 1 and Table 1). The two clades were not well supported by the bootstrap values (Figure 2; bootstrap values of 55% and 89% for groups I and II for the COI fragment, respectively, and of 74% and 56% for groups I and II for COI + cyt *b*, respectively) or posterior probabilities (0.53 and 0.63 for groups I and II for COI, respectively). For all types of data obtained for Iranian and Middle Asian populations, no groups were identified (Figures 2 and 3). *J. blanfordi* phylogenetic analysis for the cyt *b* dataset showed polytomy and the lack of groups I and II. The MJ haplotype network showed the genealogical relationships between the different *J. blanfordi* datasets (Figure 3). The phylogenetic structure was also evident due to the MJ network, which generated two haplotype groups. Groups I and II showed marked differences (5 or 6 mutation steps). Our results showed...
that the genetic diversity in the Iranian populations was much higher than in the other populations in Middle Asia, whereas the haplotypes in group II showed lower genetic diversity in Middle Asian territory. The haplotypes of this group differed by 1–3 mutation steps compared to the other groups. Comparison of the haplotypes from the Iranian group differed by 1–9 mutation steps. The main genetic parameters represented by these sequences are summarized in Table 2. The $J. blanfordi$ COI haplotypes showed higher genetic diversity than did the cyt b haplotypes. The genetic distance between the two main groups was 0.9% for COI (618 bp), 0.5% for cyt b (1110 bp), and 1.2% for the combined dataset (313 bp COI and 284 bp cyt b). The nucleotide divergence $d$ within these groups was 0.1%, 0.2%, and 0.3% (for COI, cyt b, and the combined datasets, respectively; group I) and 0.2%, 0.5%, and 0.2% (for COI, cyt b, and the combined data, respectively; group II). Central Iranian $J. b. blanfordi$ populations showed the highest genetic diversity ($H = 0.95 \pm 0.09$, $\pi = 0.33 \pm 0.06\%$ for COI + cyt b; $H = 0.73 \pm 0.1$, $\pi = 0.21 \pm 0.03\%$ for COI; and $H = 1.0 \pm 0.07$, $\pi = 0.42 \pm 0.06\%$ for cyt b). Samples from Sistan and Baluchestan Province near the Afghanistan border demonstrated diversity parameters with lower degrees ($H = 0.76 \pm 0.1$, $\pi = 0.33 \pm 0.08\%$ for COI + cyt b; $H = 0.60 \pm 0.1$, $\pi = 0.17 \pm 0.05\%$ for COI; and $H = 0.98 \pm 0.04$, $\pi = 0.46 \pm 0.06\%$ for cyt b). The hiatus observed between the haplotypes suggested a low degree of genetic discontinuity between the compared population groups (Figure 4). The cyt b fragment dataset showed a multimodal distribution without a genetic hiatus between the groups (Figure 4).

### 4. Discussion

The monophyly of the genus *Jaculus* has been previously demonstrated (Pisano et al., 2015); it has been shown that *J. blanfordi* is phylogenetically closer to *J. jaculus* than *J. orientalis* (Pisano et al., 2015). The original analyses on *J. blanfordi* mtDNA data are characterized by a low rate of mitochondrial and genetic (COI and cyt b) evolution compared with other *Jaculus* members. The phylogeography of Blanford's jerboa is consistent with Category V, as defined by Avise (2000), which corresponds to closely related and geographically localized haplotypes with high gene flow and an effective population size whose populations have not been isolated by ancient geographic barriers. In Blanford's jerboa, the phylogeographic patterns are consistent with a division into two mtDNA lineages (Figures 2 and 3); however, the genetic distances between them are not marked (0.5%–1.2% with K2P). It appears that the Iranian and Middle Asian groups were isolated from others and began to diverge, in spite of the relatively low genetic differences between these groups. A star-like pattern MJ network with a widespread haplotype from different Iranian populations was recovered (Figure 3 for the long COI fragment and for the combined dataset). Comparison of nucleotide and gene diversity can give insights into the demographic history of the populations and allow us to speculate on past demographic events. The
Figure 3. Median-joining networks for *J. blanfordi* mitochondrial DNA haplotypes in the different datasets for cyt b (1110 bp), COI (618 bp) and COI + cyt b (313 bp + 284 bp). The numbers of mutations (greater than 1) between the haplotypes are indicated near the branches and circle sizes are proportional to the number of similar haplotypes. See Table 1 for the haplotype designations.
relatively higher genetic diversity associated with lower nucleotide diversity (as observed in the Iranian and Middle Asian groups) could have resulted from a period of long isolation with a relatively small population (Avise, 2000). Similar genetic diversity values are also characteristic of some groups of *Jaculus orientalis* (Ben Faleh et al., 2011). Such isolation from other parts of the Middle East has also been reported previously for other species, such as the fat dormouse (*Glis glis*, Linnaeus, 1766) (Naderi et al., 2013).

The two phylogenetic groups correspond to the subspecies *J. b. blanfordi* (group I) and *J. b. turcmenicus* (group II). In this paper, we examined five *J. b. turcmenicus* populations, which showed no differences in sequence. In Turkmenistan, two populations were studied. One was from the northeastern part of the country and the second was from near Ashgabat (Figure 1; Table 1). In Uzbekistan, we sampled three populations whose haplotypes also had a low degree of divergence in the Kyzyl Kum Desert (Figure 1; Table 1). Our study did not include *J. b. margianus* specimens from southern Turkmenistan. Those populations were isolated from other Turkmenian *J. blanfordi* populations and are probably quite distinctive. The faunal diversity of Iran is far greater than what has been reported by naturalists to date (Darvish et al., 2006). Recently, several new species of jerboas, including *Allactaga toussi* (Darvish et al., 2008) and *Jaculus thaleri* (Darvish and Hosseinie, 2005), have been described in Iran. Dianat et al. (2013) and Mohammadi et al. (2016) reported that the Iranian populations of *Allactaga elater* could be considered a complex species. These closely related species also displayed high genetic diversity in Iranian populations in comparison to Blanford’s jerboa. We examined four populations from Isfahan, Yazd, and Sistan and Baluchestan provinces (Figure 1; Table 1). However, we did not find any distinct genetic groups corresponding to specific geographical regions in Iran (Figures 2 and 3). Nevertheless, some regional data (Afghanistan and Pakistan) are absent in the present study (Figure 1). Genetically isolated *J. blanfordi* groups were expected to be found in this area. Homogeneity of the Iranian and Middle Asian populations was also observed in the gerbil *Meriones penicilliger* (Pallas, 1773) (Nanova, 2014). *J. blanfordi* populations from Iran were studied previously, but the mtDNA sequences were not deposited in GenBank (Darvish et al., 2016). Jerboa populations create one clade of molecular research, but morphological and morphometric studies have shown that the populations from Southeast Iran differ from other populations (Darvish et al., 2016). The adaptive radiation of *Jaculus* species in Central Asia occurred at the end of the late Miocene epoch, leading to the *J. blanfordi* lineage during the early Pliocene epoch (Pisano et al., 2015). The low genetic diversity and shallow phylogeographic structure recovered for *J. blanfordi* suggest that gene flow among populations was not restricted by biogeographic barriers, such as the Iranian Plateau or the Kopet Dag.
Figure 4. Observed and expected mismatch distribution of haplotypes for the cyt $b$ (1110 bp), COI (618 bp), and COI + cyt $b$ (313 bp + 284 bp) datasets.
mountain range. Determining the species’ center of origin can be helpful in interpreting the results of this study. Our genetic diversity data for *J. blanfordi* indicate a strong possibility that populations expanded from that territory in relatively recent times and that populations migrated from west to east. The genetic differences between the Iranian and Middle Asian populations are below the subspecies level, but these populations were not isolated for a long time.

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**References**


