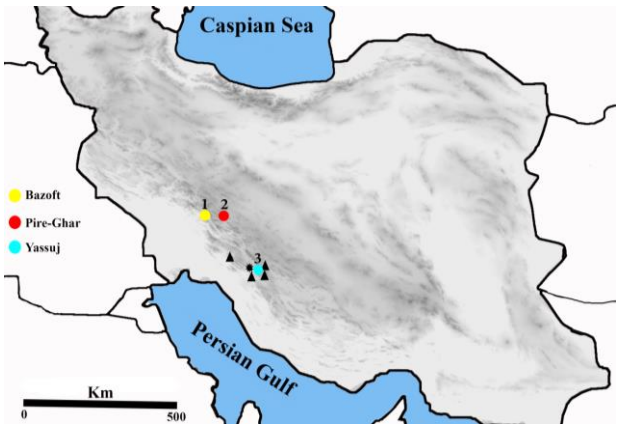

Figure 1. A- Distribution map of *Apathya yassujica* in Iran. The black star represents the type locality of *Apathya yassujica.* Circles represent the sites where specimens for the present study were caught, and black triangles represent previous records of *A. yassujica* in Iran. 1: Bazoft 2: Pire-Ghar; 3: 30 km SE of Yassuj. B- Habitat of *Apathya yassujica* in Bazoft, 15 km NW of Chaman-Goli, Kaft-Kachuz defile, Chaharmahal and Bakhtiari Province, Iran.

Low levels of genetic divergence among populations of *Apathya yassujica* (Squamata: Lacertidae) from Iran

The Genus *Apathya* Méhely, 1907 is distributed in southeastern Turkey, northern Iraq, Syria and western Iran with two taxa *A. cappadocica* and *A. yassujica* (Arnold et al. 2007, Nilson et al. 2003, Uetz & Hošek 2019). Molecular data showed an exceptionally high degree of intraspecific variability between populations of *A. cappadocica* (recognized as distinct subspecies) (Kapli et al. 2013). *Apathya yassujica* was originally described from 30 km South-West Yassuj, Kohgiluye-va-Boyerahmad Province, Iran (Nilson et al. 2003) and was reported from different locations in the Zagros Mountains (e.g. Rajabizadeh et al. 2010, Karamiani et al. 2015). However, the phylogenetic position of *A. yassujica* is even more complex, as the species is nestled within *A. cappadocica*, which makes it paraphyletic (Kapli et al. 2013). Despite this, until now there has been no attempt to study the genetic intraspecific variability in *A.yassujica* in Iran. Here, we employ partial sequences of the mitochondrial cytb gene in order to measure genetic divergence among three distant populations of *Apathya yassujica* in Iran.

The current study is a part of an ongoing project which aims to provide amphibian and reptile specimens for regional museum collections. Sampling was conducted under permission No 98/7554 of the Department of Environment of Chaharmahal and Bakhtiari Province. Seven specimens from Pire-Ghar (32°12.971' N, 50°32.502' E) and Bazoft (32° 16.818' N, 49° 56.552' E), were collected both located in Chaharmahaland Bakhtiari Province (Fig. 1). Cooling followed by freezing was used for euthanasia (Shine et al., 2015) and the specimens were deposited at Shahrekord University, Iran. We extracted whole genomic DNA from the liver of specimens using TNES buffer (400 mM NaCl, 100 mM EDTA, 50 mM Tris-HCl, pH 7.5, 0.5% SDS) and 5 µl of proteinase K (20 mg/ml) (Ahaniazad et al. 2018). Amplification of double-stranded fragments from the Cytochrome b (Cytb) was achieved by polymerase chain reaction (PCR) using the primers F1_Cytb (5'-TGA GGC CTG AAA AAC CAC CGT TG-3') (Oraie et al. 2018) and Ei700r (5′-GGGGTGAAA GGGGATTTTRTC-3′) (Rastegar-Pouyani et al. 2010). The PCR was carried out using WizPure™ PCR 2X (Wizbiosolution), 1µL of each primer (10 µM stock), and ~100 ng of DNA template in a 20 µL reaction volume. The PCR cycles consisted of an initial denaturation step at 95°C for 4 min, followed by 36 cycles of denaturation at 95°C for 40 s, annealing at 54°C for 40 s and extension at 72°C for 90 s, and a final extension at 72°C for 10 min. PCR products were then examined using gel electrophoresis on 1.3% Agarose gel. PCR products showing strong bands in gel electrophoresis were sequenced on an automated sequencer ABI 3730XL (Codon Genetic Group, Iran) according to standard protocols.

Published six sequences of *Apathya yassujica* (KF003351-KF003356) coming from 30 km South-East of Yassuj and five sequences of *A. cappadocica urmiana* (KF003346- KF003350)

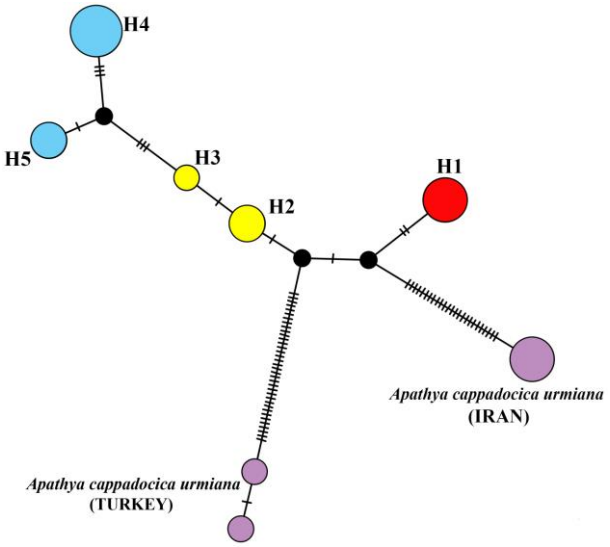

Figure 2. Statistical parsimony network analysis (TCS algorithm) based on 12 mtDNA *Cytb* sequences (280 bp) in three distinct populations of *Apathya yassujica*. Circle size corresponds to haplotype frequency. Dashes at branches indicate the number of mutational steps. The black circle represents the hypothetical haplotype. **H 1**: HAC858, HAC859, HAC860 (Pire-Ghar); **H 2**: HAC864, HAC866 (Bazoft); **H 3**: HAC867 (Bazoft); **H 4**: Ayus41, 42, 45, 46 (30 km SE of Yassuj); **H 5**: 44, 43 (30 km SE of Yassuj).

(Kapli et al. 2013) were downloaded from GenBank (http://www.ncbi.nlm.nih.gov) and included in the final dataset. Since our sequences (627 bp) were longer than those available on NCBI, we used a minor homologous region in the alignment of this gene. However, we deposited the full 627bp Cytb sequences in GenBank (HAC864:T007239; HAC866: MT007240; HAC867: MT007241; HAC858: MT007242; HAC859: MT007243; HAC860: MT007244). Multiple sequence alignments were generated using MAFFT v. 7 (Katoh & Standley 2013) with default parameters and FFT-NS-1algorithm. The mean genetic distances were estimated using the Mega X software (Kumar et al., 2018). A statistical parsimony network analysis was conducted using the TCS algorithm implemented in PopART v. 1.7.2 (Leigh & Bryant 2015).

A dataset with a final sequence length of 280 nucleotides from the mtDNA *Cytb* gene was generated from three distinct locations in central Zagros Mountain (Fig. 1). Of a total of 280 characters, 270 sites were conserved and 10 sites were variable (3.57% of the total length), including 10 parsimonious-informative sites. These sequences were biased towards A and T nucleotides, and averaged 30.48 % T, 29.17 % C, 26.70 % A, and 13.66% G. The highest number of haplotypes was found in Bazoft and SE Yassuj, where we registered two haplotypes in three and six specimens respectively. Our analysis does not recognize widespread haplotypes that are shared by at least two populations, so the results suggest that all recovered haplotypes are restricted to a particular geographical location (Figure 2). The minimum genetic distance (P-distance) between pairs of populations referred to Bazoft and SE Yassuj was 0.0133 while the maximum divergence between Pire-Ghar and SE Yassuj was 0.0212. The genetic distance between Pire-Ghar with Bazoft was 0.0143.

Our data provide first signs of genetic variability among three populations of *Apathya yassujica* based on partial sequences of mtDNA Cytb. Our findings lead us to the conclusion that all the specimens examined belong to *A. yassujica* as their evolutionary distances are less than the threshold (approximately 6%) for setting species boundaries of the Lacertidae family (e.g., Ahmadzadeh et al. 2013, Rastegar-Pouyani et al. 2010, Rastegar-Pouyani et al. 2012). A possible scenario to explain the current distribution pattern of haplotypes is the Pleistocene climatic oscillations, as was documented in some species of montane specialists (e.g. Shepard & Burbrink 2009, Taylor et al. 2009). Nonetheless, more sequences and microsatellite data from the entire range of this species in Iran should be used to check these implications.

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