

# Intraspecific variability in *Teratoscincus bedriagai* Nikolsky, 1900 (Squamata: Sphaerodactylidae) from Iran

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The Bedraiga's skink gecko, *Teratoscincus bedriagai*, is one of the fairly known lizards of Iran. Phylogenetic relationships among five distant populations of *Teratoscincus bedriagai* were estimated based on the mitochondrial COI partial fragment. This study highlights very low genetic divergences among examined populations despite the noticeable geographic distance between them. We assumed that the current genetic structure of haplotypes is shaped as a consequence of climate changes and glaciations in the Quaternary.

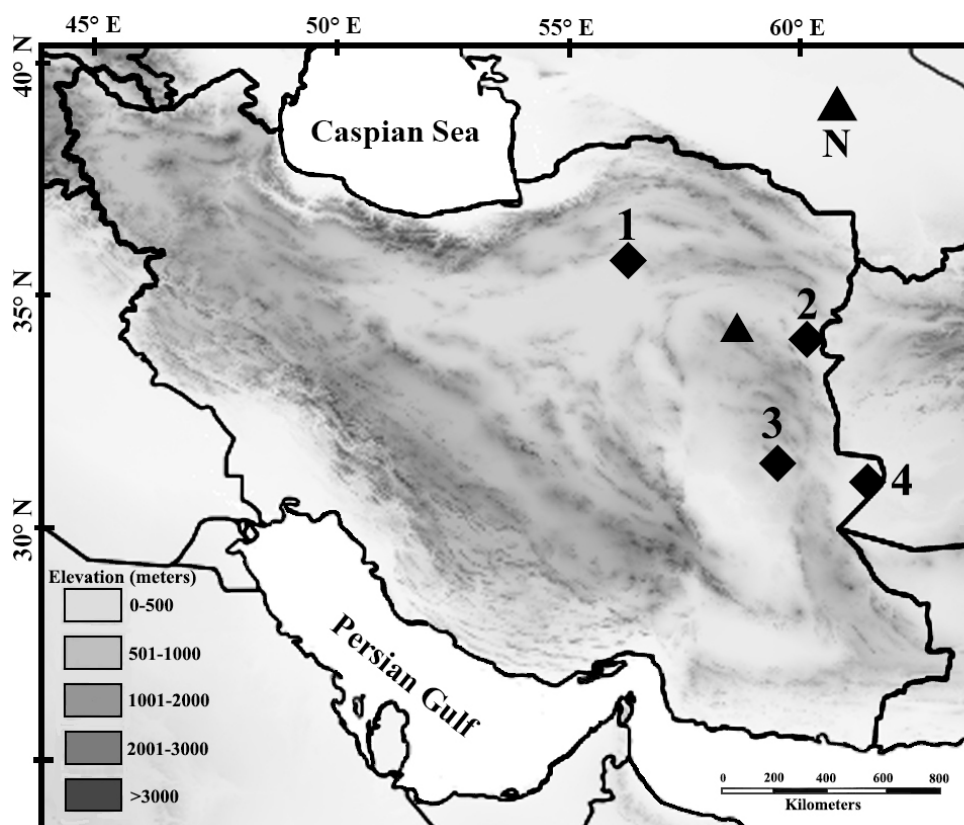
**Key words:** Genetic distance, *Teratoscincus bedriagai*, Iran, Sphaerodactylidae, Quaternary.

## INTRODUCTION

The frog-eyed geckos of the genus *Teratoscincus* Strauch, 1863, have a wide distribution range along the arid belt of Middle and Central Asia (Szczerbak & Golubev, 1996; Anderson, 1999; Macey *et al.*, 1999, 2005). Iran with five out of eight described species (Uetz & Hošek, 2017), reflects a unique biogeographic condition among the inhabited countries. However, for quite a while, the Iranian populations of the genus *Teratoscincus* haven't been subject to taxonomic research, with the recent exception resulting in the description of new taxa (Nazarov *et al.*, 2017; Akbarpour *et al.*, 2017).

*Teratoscincus bedriagai* is one of the slightest well-known reptiles that occurred in Iran (Szczerbak & Golubev, 1996; Anderson, 1999). There have been few studies on *T. bedriagai* in Iran over the previous few years. Only morphological data (Hojati *et al.*, 2009) and something on its biology (Hojati *et al.*, 2014), both from a confined range in Semnan Province are available. Therefore, there is yet no sufficient data on the geographic variation in morphological characters in *T. bedriagai*, and also its genetic structure in Iran. One of the most recent molecular studies show that the phylogenetic position of *T. bedriagai* is poorly resolved among other species of *Teratoscincus* (Nazarov *et al.*, 2017).

This study provides a preliminary assessment of the intraspecific variability among distant populations of *T. bedriagai* in Iran. For this purpose, phylogenetic analyses were conducted based on the partial COI sequences from five populations within its distribution range in Iran.



**FIGURE 1.** Map of the sampling localities for *Teratoscincus bedriagai* in Iran. Black lozenges represent the sites where specimens of present study coming from while black triangle represent a locality near Gonabad, Razavi Khorasan Province, used in Nazarov *et al.* (2017). For more details of numbers, refer to Table 1.

#### MATERIAL AND METHODS

As a source of DNA, we used 15 ethanol-preserved tissue samples from five geographically distant populations of *Teratoscincus bedriagai* in Iran (Table 1 & Fig. 1). Voucher specimens were deposited in the Herpetological collections of Hakim Sabzevari University, Sabzevar (ERP) and Shahrekord University, Shahrekord (HAC).

We performed DNA extraction using Aron-Gene Kit (Aron-Gene, Isfahan, Iran) following the instructions provided by the manufacturer. A fragment of the mitochondrial cytochrome C oxidase subunit I (COI) was amplified using combinations of primers L 1498 (Folmer *et al.*, 1994), RepCOI F and RepCOI R (Nagy *et al.*, 2012). Standard PCR reaction was done as described by Khosravani *et al.* (2017). The amplified DNA was then visualized and assessed on a 1.5% agarose gel. Positive PCR products were sent for sequencing to Macrogen Inc. (Seoul, South Korea, <http://www.macrogen.com>). All newly obtained sequences were submitted in GenBank under accession numbers MH476256 - MH476270 (Table 1). Published sequences of *Teratoscincus bedriagai* (MF573787- MF573790) were downloaded from NCBI (<http://www.ncbi.nlm.nih.gov>) and included in the final dataset. For outgroups, we used *T. keyserlingii* (MF573791 and MF573792) and *T. microlepis* (MF573800 and MF573798) which are closely related to *T. bedriagai* (Nazarov *et al.*, 2017).

**TABLE 1.** Information on the *Teratoscincus bedriagai* specimens used in the study, with locality and Genbank accession numbers.

NO	Voucher specimens	Locality	Haplotype Number	Latitude /longitude	Genbank Number
1	ERP 5470	Semnan Province, Kaal-e Shour- 60 Km E of Biarjomand	H1	35.88, 56.33	MH476256
	ERP 5471	Semnan Province, Kaal-e Shour- 60 Km E of Biarjomand	H2	35.88, 56.33	MH476257
	ERP 5472	Semnan Province, Kaal-e Shour- 60 Km E of Biarjomand	H3	35.88, 56.33	MH476258
2	ERP 3868	Razavi Khorasan province, on the road of Niaz-Abad to Moussa-Abad	H9	34.1818, 60.31088	MH476264
	ERP 3869	Razavi Khorasan province, on the road of Niaz-Abad to Moussa-Abad	H10	34.1818, 60.31088	MH476265
3	ERP 6042	Razavi Khorasan province, Around Niaz-Abad	H14	---	MH476270
	ERP 5974	South Khorasan Province- On the road of Nehbandan to Deh-Salm- 45 km E of Deh-Salm	H11	31.4582, 59.6094	MH476266
	ERP 5975	South Khorasan Province- On the road of Nehbandan to Deh-Salm- 45 km E of Deh-Salm	H12	31.4582, 59.6094	MH476268
	ERP 5977	South Khorasan Province- On the road of Nehbandan to Deh-Salm- 45 km E of Deh-Salm	H11	31.4582, 59.6094	MH476267
	HAC 172	Sistan and Baluchistan Province, NW Zahak, near the Khamak village	H4	30.915453, 61.631615	MH476259
4	HAC 173	Sistan and Baluchistan Province, NW Zahak, near the Khamak village	H5	30.915453, 61.631615	MH476260
	HAC 174	Sistan and Baluchistan Province, NW Zahak, near the Khamak village	H6	30.915453, 61.631615	MH476261
	HAC 175	Sistan and Baluchistan Province, NW Zahak, near the Khamak village	H7	30.915453, 61.631615	MH476262
	HAC 176	Sistan and Baluchistan Province, NW Zahak, near the Khamak village	H8	30.915453, 61.631615	MH476263
5	ERP 831	Indefinite locality from Razavi Khorasan province	H13	----	MH476269

Genetic distances within and between populations were calculated using Mega 7.0 (Kumar *et al.*, 2016). The Median-joining algorithm (Bandelt *et al.*, 1999) implemented in Network 5.0.0.3 ([www.fluxus-engineering.com](http://www.fluxus-engineering.com)) was used to depict divergence among haplotypes. The DNA sequences were divided initially into three partitions, the first, second and third codon positions of COI. A greedy search with PartitionFinder2 (Lanfear *et al.*, 2016) was conducted to select optimal partitioning schemes and the best-fit evolutionary models based on the Akaike information criterion (AIC) allowing for separate estimation of branch lengths for each partition. The suggestion of PartitionFinder2 analysis is using the predefined partitions except for the second codon positions which should be combined in a single partition with first codon positions. Bayesian inference (BI) of

phylogeny was performed using MrBayes v.3.2.5 (Ronquist *et al.*, 2012). Two simultaneous and independent runs consisting of eight MCMC chains in MrBayes were run for 10,000,000 generations with default priors, trees sampled every 1,000 generations and separate estimation of parameters for individual partitions under default heating using best-fit models as suggested by PartitionFinder2 (HKY+I for first and second codon positions as one subset and GTR for third codon positions). We assessed convergence of the runs by discarding the 25% of the initial samples as burn-in and a majority rule consensus tree was generated from the remaining post-burn-in trees. Maximum Likelihood (ML) analysis was performed using RaxML GUI v. 0.95 (Silvestro & Michalak, 2012) with ‘GTR’ option and 1000 bootstrap replicates (partition set by codon and 10 independent runs).

## RESULTS

The dataset used for the phylogenetic analyses consisted of 608 nucleotides, there were 435 invariable sites and 173 polymorphic sites, of which 141 sites were phylogenetically informative and 32 were singletons. Our dataset could be translated into amino acid sequences with the vertebrate mtDNA genetic code. Nucleotide composition analyses revealed 17 haplotypes, haplotype diversity of 0.9883 and the average of 0.496 for GC content for *Teratoscincus bedriagai*.

Uncorrected genetic *p*-distances between five populations of *T. bedriagai* and outgroup taxa are shown in Table 2. The minimum genetic distance between pairs of populations referred to Gonabad and Biarjomand was 0.009 while the maximum divergence between Deh-Salm and Niaz-Abad was 0.024 (Table 2 and Fig.1). The genetic distance between *T. bedriagai* with *T. microlepis* and *T. keyserlingii* was almost the same (0.171 and 0.166 respectively). The tree inferred from the Bayesian analysis was similar to those constructed using Maximum Likelihood (not shown) generally (Fig. 2). In both trees, *T. bedriagai* recovered as a monophyletic group relative to the outgroup taxa, *T. microlepis* and *T. keyserlingii*, with high statistical supports, PP (1) and ML (100%) (Fig. 2). Included populations of *T. bedriagai* show relatively high genetic homogeneity. Nevertheless, a clade including individuals from Sistan, Deh-Salm and one sample from Niaz-Abad (Fig. 1) was discovered with strong support (100%) in ML tree, albeit not found in Bayesian framework. Some degrees of polytomous relationships among *T. bedriagai* individuals revealed in both analyses. Median-joining haplotype network of 17 haplotypes is illustrated in Figure 3.

**TABLE 2.** Uncorrected genetic *p*-distances among five populations *Teratoscincus bedriagai* and outgroup taxa. Since one individual (ERP 831) comes from an indefinite locality in Razavi Khorasan province, it was not included.

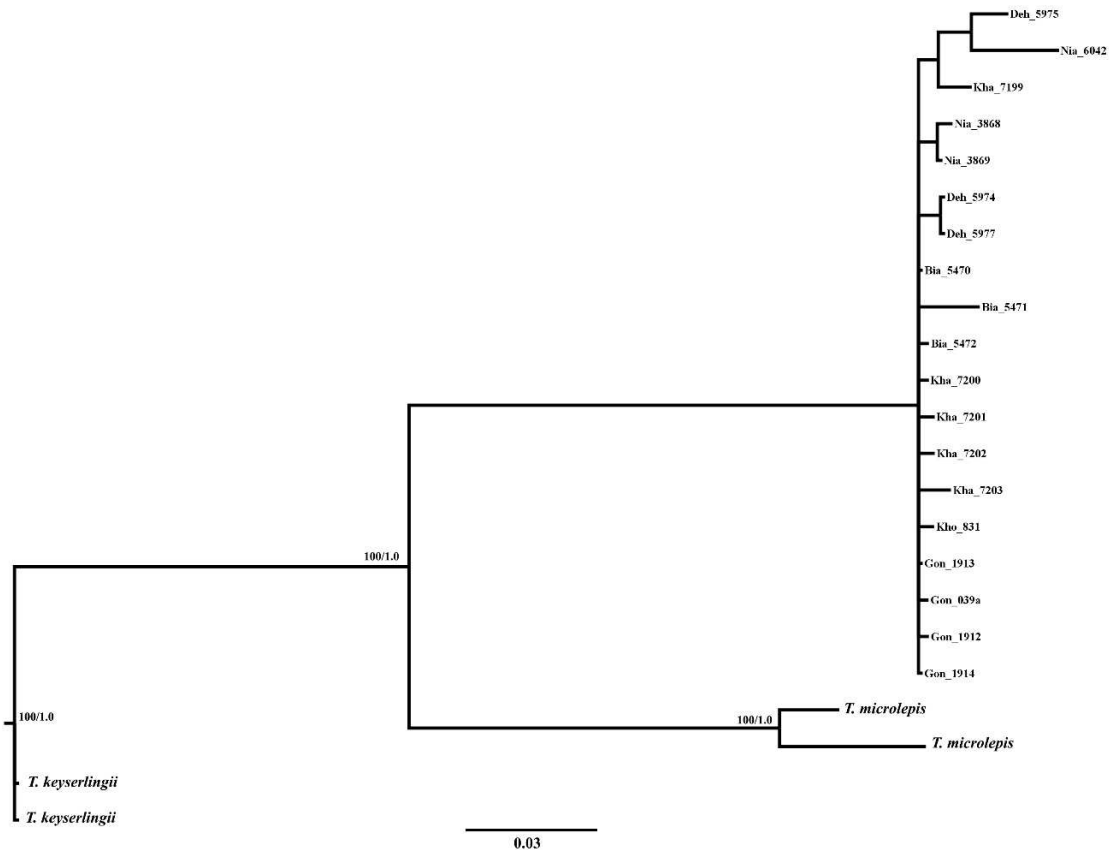
	1	2	3	4	5	6
1 <i>T. keyserlingii</i>						
2 <i>T. microlepis</i>	0.146					
3 <i>T. bedriagai</i> (Biarjomand)	0.167	0.173				
4 <i>T. bedriagai</i> (Niaz-Abad)	0.171	0.174	0.019			
5 <i>T. bedriagai</i> (Deh-Salm)	0.167	0.173	0.021	0.024		
6 <i>T. bedriagai</i> (Khamak)	0.166	0.173	0.017	0.021	0.015	
7 <i>T. bedriagai</i> (Gonabad)	0.160	0.166	0.009	0.016	0.015	0.012

## DISCUSSION

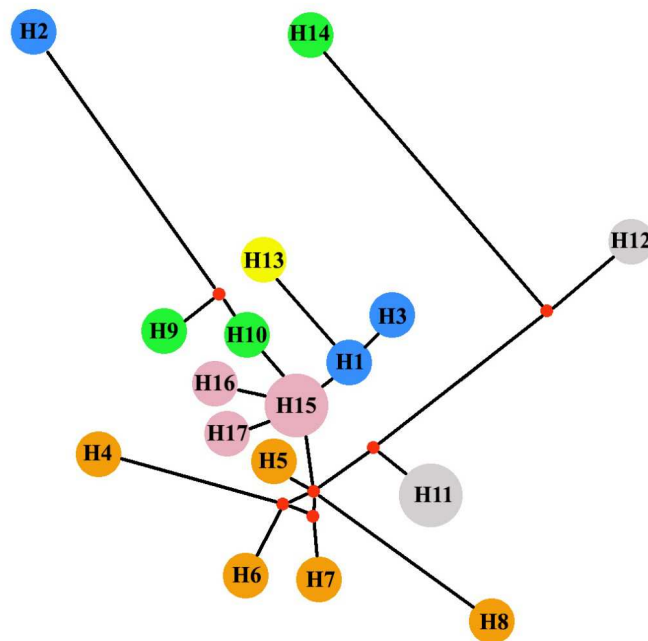
The genetic variability between five populations of *Teratoscincus bedriagai* was analyzed using mtDNA COI partial sequences. Although *T. bedriagai* as a monophyletic group was recovered in our analysis. However, our data added nothing more to the previous finding on its phylogenetic position among other species of the Genus *Teratoscincus* (Nazarov *et al.*, 2017). Inferring phylogenetic relationships

based on gene trees is one of the most reason for current obstacle, which most likely might be improved and shed new light on the issue by taking advantages of multilocus phylogeny (e.g. Martens *et al.*, 2008; Liu *et al.*, 2017).

Our findings are compatible with the current taxonomy of *T. bedriagai* as a valid species with numerous populations in Iran (Uetz & Hošek, 2017). This conclusion is derived from little genetic differences between *T. bedriagai* populations in Iran (Table 2), together with their phylogenetic relationships with other species of the Genus *Teratoscincus* (Fig. 2). According to Star-like topology of Median-joining network (Fig.3) and Gonabad's geographic position (H15 likely ancestral haplotype) located in the center of the rest of populations, a recent demographic expansion could be a plausible scenario. However, the spatial distribution of the haplotypes (Fig. 3) could be the consequences of multiple colonization and extinction events recently. It seems that current distribution pattern of *T. bedriagai* is shaped by a huge influx of climate changes and glaciations in the Quaternary (Taberlet *et al.*, 1998; Hewitt, 2004; Popov *et al.*, 2006), but additional analyses of more populations of *T. bedriagai* are necessary to testify this hypothesis.



**FIGURE 2.** The Phylogenetic tree resulting from a Bayesian framework reconstruction based on 608 base pairs of the COI sequence. Bootstrap support values (left) from maximum likelihood analysis and posterior probabilities (right) from Bayesian inference are indicated at the nodes. The tree was rooted by *Teratoscincus keyserlingii*.



**FIGURE 3.** A Median-joining network connecting 17 haplotypes found in the COI sequence data set. Median vector shown with orange nodes, the branches length is indicated the numbers of mutational steps joining the haplotypes and the size of each circle is proportional to the number of haplotypes represented. Biarjomand (H1: ERP 5470, H2: ERP 5471, H3: ERP 5472); Khamak village (H4: HAC 172, H5: HAC 173, H6: HAC 174, H7: HAC 175, H8: HAC 176); Niaz-Abad (H9: ERP 3868, H10: ERP 3869, H14: ERP 6042); Deh-Salm (H11: ERP 5974 and ERP 5977, H12: ERP 5975); Gonabad (H15: 1913 and 1914, H16: 039a, H17: 1912) and H13: ERP 831. For more information, refer to Table 1.

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