



Deep Mitochondrial and Morphological Differentiation of *Hemidactylus persicus* Anderson, 1872 (Squamata: Gekkonidae) in Iran

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With currently 149 species, *Hemidactylus* Oken, 1817 is one of the most species-rich genera of the family Gekkonidae. In this study, 50 *Hemidactylus persicus* and *H. romeshkanicus* from southern Iran and three specimens of the newly described species *H. kurdicus* from north-eastern Iraq were screened using sequences of the mitochondrial 12S rRNA gene (approximately 400 bp) with two *H. hajarensis* as outgroups. In addition, 58 specimens were analysed morphologically using 25 mensural and six meristic characters. The genetic data recovered six well supported clades of *H. persicus* and *H. romeshkanicus* in southern Iran, which also showed morphological differentiation with the exception of specimens from Khuzestan and Fars provinces. Principal Coordinates Analysis (PCoA) and haplotype networks are compatible with our phylogenetic tree and morphological analyses. These findings highlight deep mitochondrial and morphological variation of *H. persicus* from Iran. Interestingly, our phylogenetic inference revealed that *H. romeshkanicus* should be regarded as a valid species, whereas *H. kurdicus* is not a distinct evolutionary lineage and synonymous with *H. romeshkanicus*.

Key words: Gekkonidae; Iranian plateau; Phylogeny; Radiation; Species complex

INTRODUCTION

The diverse herpetofauna of the Iranian plateau has been of interest to herpetologists, particularly with respect to ecology and zoogeography (e.g., Anderson, 1968; Hosseinzadeh et al., 2014a). Topographically, the Iranian plateau consists of a complex of mountain chains enclosing two main mountain ranges: the Elburz, which extends from north-west to north-east, and the Zagros, which extends from north-west to south-eastern Iran (Fisher, 1968). Descriptions of species using molecular tools resulted in the detection of cryptic taxa, and the raising of geographically isolated subspecies to the rank of species (Ahmadzadeh et al., 2013; Ficetola et al., 2013). However, further molecular and integrative studies are necessary in order to gain a more complete understanding of the Iranian herpetofauna.

With 149 recognised species (Uetz et al., 2018), the genus *Hemidactylus* Oken, 1817 is one of the most species-rich genera of the family Gekkonidae. It is globally distributed in tropical and subtropical regions. Four species of *Hemidactylus* have been reported from Iran: *Hemidactylus persicus* Anderson, 1872, *H. robustus* Heyden, 1827, *H. flaviviridis* Rüppell, 1840, and *H. romeshkanicus* Torki 2011 (Anderson, 1999; Bauer et al., 2006; Torki et al., 2011; Šmíd et al., 2014; Hosseinzadeh

et al., 2014b). *Hemidactylus persicus* is distributed in the northern Arabian Peninsula, southern Iran, Iraq, Kuwait, Pakistan and India (Sindaco & Jeremčenko, 2008; Carranza & Arnold, 2012; Khan, 2013; Castilla et al., 2013; Šmíd et al., 2014). Molecular studies of Iranian *H. persicus* have shown a high level of genetic differentiation (Carranza & Arnold, 2012; Šmíd et al., 2013). Recently, a new species, *H. kurdicus*, has been reported from the oak woodlands of Zagros forest steppe of Qara Dag Mountains, Sulaimani, north-eastern Iraq (Safaei-Mahroo et al., 2017).

The occupation of Iran by *H. persicus* in different climates and habitats along with deep intraspecific variation suggests that it might comprise a species complex. According to Torki et al. (2011), *H. romeshkanicus* is endemic to Iran, inhabiting western slopes of the Zagros Mountains and southern Lorestan. According to Šmíd et al. (2014), the species probably belongs to the arid clade together with its sister taxa *H. persicus*, *H. robustus* and *H. turcicus*. Here, we study the genetic variability of *H. persicus* across its entire range in the Iranian Plateau using 12S rRNA mtDNA sequences, together with multivariate analyses of mensural and meristic characters. Further, we evaluate the validity of *H. romeshkanicus* using these methods.

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METHODS

Fifty-three *Hemidactylus* were included in the phylogenetic analyses which were procured from collections (Collection of The Biology Department of Shiraz University (CBSU), Zoological Museum of University of Tehran (ZUTC), Department of The Environment of Hormozgan Zoological Collection (DHZC), Farhang Torki Herpetology Museum (FTHM), Collection of The California Academy of Sciences: (CAS), Centre for Ecological Sciences, Bangalore, India (CES), Museum of Vertebrate Zoology, Berkeley (MVZ), National Museum, Prague (NMP)). Morphological characters of specimens collected from the type locality of *H. romeshkanicus* were compared with the holotype from Zoologisches Museum of Berlin (ZMB). Other samples were obtained from recent expeditions and have been deposited in the Sabzevar University Herpetological Collection (SUHC), the Zoological Museum of Ferdowsi University of Mashhad (ZMFUM) and the Zoological Museum of University of Birjand (ZMUB) with appropriate sampling permission from the Department of Environment of Iran (see Table 1, Fig. 2). Specimens were euthanised with chloroform and tissues extracted and fixed in 75% ethanol. In total, 42 *H. persicus* were sequenced for this study. A further eight and three sequences of *H. persicus* and *H. kurdicus*, respectively, and two of *H. hajarensis* (used as outgroup) were downloaded from GenBank. We used 12Sa and 12Sb primers (Kocher et al., 1989) to amplify a section (approximately 400 bp) of the mitochondrial 12S ribosomal RNA gene. Sequences were imported to BioEdit, version 7.0.9.0 (Hall, 1999), aligned using ClustalW multiple alignment, and adjusted by hand. A distance matrix using uncorrected p-distances was calculated with MEGA, version 5 (Tamura et al., 2011). Two phylogenetic analyses were performed: Maximum Likelihood (ML) and Bayesian Inference (BI). We choose GTR+I+G as the best-fitting model of nucleotide substitution based on the Akaike Information Criterion as implemented in ModelTest, version 3.7 (Posada & Crandall, 1998). The maximum likelihood (ML) tree was produced using RAxML v 7.0.3 (Stamatakis, 2006). To test the robustness of the nodes we ran 1000 bootstrap pseudo-replications under ML (Templeton et al., 1992). Bayesian analyses were performed in MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). Four Markov Chain Monte Carlo analyses (MCMC) were run simultaneously for 10 million generations and the first 1,000,000 trees (as a conservative 'burn-in') were discarded. Posterior probabilities for nodes were calculated from the remaining trees using a majority-rule consensus analysis. Clades are regarded as strongly supported if they have bootstrap values higher than 70% in ML, or posterior probabilities (pp) of 95% or above in the Bayesian analysis (Hillis et al., 1993). To visualise the number of specimens sharing certain haplotypes, haplotype networks of the 12S were constructed using the TCS software package (Clement et al., 2000). This program estimates the number of mutational steps by which pairwise haplotypes differ and computes the probability of parsimony for pairwise differences until the probability exceeds 0.95 (Templeton et al., 1992). To

further evaluate relationships among populations of *H. persicus* we performed a Principal Coordinate Analysis (PCoA) using GenAlEx v.6.5 (Peakall & Smouse, 2006). We used the same software to perform a Mantel test to examine the correlation between geographic and genetic distances based on point localities in the populations of *H. persicus* (Jensen et al., 2005). An analysis of molecular variance (AMOVA) was performed to evaluate the population structure and mutational differences between the loci in different populations using GenAlEx v.6.5 (Peakall & Smouse, 2006). Additionally, to calculate the genetic differentiation fixation index, the partitioning of among-group genetic variation (PhiPT) values were calculated in order to examine the distribution of differences within and between populations using GenAlEx v.6.5 (Peakall & Smouse, 2006).

Fifty-eight specimens of *H. persicus* were examined morphologically, including 29 males and 29 females. All specimens were studied for 25 mensural and six meristic characters following Kluge (1969), Vences et al. (2004), Busais & Joger (2011), and Carranza & Arnold (2012, see supplementary section; Table 2).

Statistical analysis was performed with SPSS 16.0 and PAST v. 2.17c (Hammer et al., 2001). The multivariate canonical variate analysis (CVA) was conducted on the transformed matrix to determine if individuals would be assigned to the correct population group based on morphological measurements.

RESULTS AND DISCUSSION

A total of 399 characters of the 12S rRNA gene were used in the phylogenetic analyses, of which there were 44 parsimony-informative characters (224 invariant or monomorphic sites and 44 variable or polymorphic sites). The proportion of invariable sites, $I = 0$, for among-site rate variation followed a gamma distribution, with the shape parameter $\alpha = 0.2402$. The frequencies of nucleotides were: freq A = 0.3285, freq C = 0.3047, freq G = 0.1913, freq T = 0.1755. Both methods (ML and BI) gave very similar results and showed only minor differences, at the base of the trees, where relationships had little support (Fig. 1). The phylogeny recovered six well-supported clades comprising the following populations: Clade A from south-west Iran (Behbahan city, East of Khuzestan Province); south-east Iran (Lipar, Jod Village and Bazman, Sistan and Baluchistan), extreme south-west Iran (Mahshar, Khuzestan Province); Clade B₁ from south Iran (south of Lorestan, Romeshkan, Pole-e-Dokhtar); north-east Iraq (western border of the Zagros forest steppe in south-western Sulaimani, Kurdistan region); Clade B₂ from south-west Iran (northern and central Khuzestan; western Ilam); Clade C₁ from south Iran (Bushehr and southern Fars Province); Clade C₂ from central Iran (Kerman and northern Fars Province); and Clade C₃ from south Iran (central and eastern Fars Province; south-eastern Khuzestan, Fig. 1). Uncorrected genetic distances ranged between 0.000 and 0.008 and between 0.026 and 0.097 within and between clades of *H. persicus*, respectively (Table 3). There is no genetic distance between *H. kurdicus* and clade B₁

Table 1. Details of studied specimens of *H. persicus* and *H. romeshkanicus*. The abbreviations refer to: Collection of The Biology Department of Shiraz University (CBSU), Zoological Museum of University of Tehran (ZUTC), Department of The Environment of Hormozgan Zoological Collection (DHZC), Farhang Torki Herpetology Museum (FTHM), Collection of The California Academy of Sciences (CAS), Centre for Ecological Sciences, Bangalore, India (CES), Museum of Vertebrate Zoology, Berkeley (MVZ), National Museum, Prague (NMP), Sabzevar University Herpetological Collection (SUHC), Zoological Museum of Ferdowsi University of Mashhad (ZMFUM) and Zoological Museum of University of Birjand (ZMUB). M. = Morphological study, G. = Genetic study.

Species	Voucher Code	Locality; number in Figure S ₁	GenBank Accession No	Source	Type of Study
<i>H. romeshkanicus</i>	SUHC 1153	40 Km east of Haftgel, Iran;1	MG744524	This study	M., G.
<i>H. romeshkanicus</i>	SUHC 1154	40 Km east of Haftgel, Iran;1	MG744525	This study	M., G.
<i>H. romeshkanicus</i>	SUHC 1155	40 Km east of Haftgel, Iran;1	MG744526	This study	M., G.
<i>H. romeshkanicus</i>	SUHC 1156	40 Km east of Haftgel, Iran;1	MG744527	This study	M., G.
<i>H. persicus</i>	SUHC 1222	5Km west of Dayyer, Iran;2	MG744529	This study	M., G.
<i>H. persicus</i>	SUHC 1223	5Km west of Dayyer, Iran;2	MG744530	This study	M., G.
<i>H. persicus</i>	SUHC 1414	Nourabad, Iran;3	MG744531	This study	G.
<i>H. persicus</i>	SUHC 1415	Nourabad, Iran;3	MG744532	This study	G.
<i>H. persicus</i>	SUHC 1425	Nourabad, Iran;3	MG744535	This study	M., G.
<i>H. persicus</i>	SUHC 1433	Nourabad, Iran;3	-	This study	M.
<i>H. romeshkanicus</i>	SUHC 3622	Masjedsolyeman, Iran;4	MG744539	This study	G.
<i>H. romeshkanicus</i>	SUHC 3623	Masjedsolyeman, Iran;4	MG744540	This study	M., G.
<i>H. romeshkanicus</i>	SUHC 3624	Masjedsolyeman, Iran;4	MG744541	This study	M., G.
<i>H. romeshkanicus</i>	SUHC 3625	Masjedsolyeman, Iran;4	MG744542	This study	G.
<i>H. persicus</i>	SUHC 3643	Ahram mountain, Iran;5	-	This study	M.
<i>H. persicus</i>	SUHC 3644	Ahram mountain, Iran;5	-	This study	M.
<i>H. persicus</i>	SUHC 3645	Ahram mountain, Iran;5	-	This study	M.
<i>H. persicus</i>	SUHC 3693	Khahr national park, Iran;6	MG744544	This study	M., G.
<i>H. persicus</i>	SUHC 3694	Khahr national park, Iran;6	MG744551	This study	M., G.
<i>H. persicus</i>	SUHC 3696	Khahr national park, Iran;6	MG744545	This study	M., G.
<i>H. persicus</i>	SUHC 3691	Ahram mountain, Iran;5	MG744543	This study	G.
<i>H. persicus</i>	ZMFUM 10005	Gakal Cave, Gachsaran,Iran;7	MG744548	This study	M., G.
<i>H. romeshkanicus</i>	ZMFUM10001	Izeh,Iran;8	MG744515	This study	M., G.
<i>H. romeshkanicus</i>	ZMFUM10002	Izeh,Iran;8	MG744522	This study	M., G.
<i>H. romeshkanicus</i>	ZMFUM10003	Izeh,Iran;8	MG744523	This study	M., G.
<i>H. persicus</i>	CBSU R082	25km NW of Lamerd,Iran;9	-	This study	M.
<i>H. persicus</i>	CBSU R083	25km NW of Lamerd,Iran;9	-	This study	M.
<i>H. persicus</i>	ZMFUM10007	Varavi, Iran;10	MG744547	This study	M., G.
<i>H. persicus</i>	ZMFUM10008	Varavi, Iran;10	MG744549	This study	M., G.
<i>H. persicus</i>	ZMFUM10009	Varavi, Iran;10	MG744550	This study	M., G.
<i>H. persicus</i>	ZMFUM10010	Behbahan,Iran;11	-	This study	M.
<i>H. persicus</i>	ZMFUM10011	Behbahan,Iran;11	MG744546	This study	M., G.
<i>H. persicus</i>	CBSU 8071	GoohGorm Jahrum,Iran;12	-	This study	M.
<i>H. persicus</i>	CBSU 8068	GoohGorm Jahrum,Iran;12	-	This study	M.
<i>H. persicus</i>	CBSU 8091	GoohGorm Jahrum,Iran;12	-	This study	M.
<i>H. persicus</i>	CBSU 8083	GoohGorm Jahrum,Iran;12	-	This study	M.
<i>H. persicus</i>	CBSU 4217	Jahrum,Iran;13	-	This study	M.
<i>H. persicus</i>	CBSU 8055	Kazeran,Iran;14	-	This study	M.
<i>H. persicus</i>	CBSU 8056	Shiraz, Iran;15	-	This study	M.
<i>H. persicus</i>	CBSU 5395	Shiraz, Iran;15	-	This study	M.
<i>H. persicus</i>	CBSU R111	Gachsaran,Iran;16	-	This study	M.
<i>H. romeshkanicus</i>	SUHC 3788	Masjed soleman,Iran;17	MG744553	This study	M., G.
<i>H. romeshkanicus</i>	SUHC 3784	Pole-e-dokhtar,Iran;18	MG744555	This study	M., G.
<i>H. romeshkanicus</i>	SUHC 3786	Pole-e-dokhtar,Iran;18	MG744554	This study	M., G.
<i>H. romeshkanicus</i>	SUHC 3789	Pole-e-dokhtar,Iran;18	-	This study	M.
<i>H. persicus</i>	SUHC 3785	Mehran,Iran;19	-	This study	M.
<i>H. persicus</i>	SUHC 2097	Bazman,Iran;20	MG744520	This study	G.
<i>H. romeshkanicus</i>	ZMFUM 10024	Romeshkan, Lorestan, Iran;21	MG744556	This study	M., G.
<i>H. romeshkanicus</i>	ZMB 75020	Romeshkan, Lorestan, Iran;21	-	Torki et al., 2011	M.
<i>H. persicus</i>	SUHC 1558	Jahrom,Iran;22	MG744536	This study	M., G.
<i>H. persicus</i>	SUHC 1974	Marvdasht,Iran;23	MG744538	This study	M., G.
<i>H. persicus</i>	DHZCH132	Qeshm island, Iran;24	-	This study	M.
<i>H. persicus</i>	ZUTC R.1256	Bibi Hakemieh, KohgiluyehvaBoyerahmad, Iran;25	-	This study	M.
<i>H. persicus</i>	ZUTC R.1222	Bibi Hakemieh, KohgiluyehvaBoyerahmad, Iran;25	-	This study	M.
<i>H. persicus</i>	ZUTC R.1234	Bibi Hakemieh, KohgiluyehvaBoyerahmad, Iran;25	-	This study	M.
<i>H. persicus</i>	ZUTC R.1476	Jod Village, Sistan and Baluchistan, Iran;26	MG744552	This study	M., G.
<i>H. persicus</i>	SUHC 451	10 Km East of Evaz,Iran;27	-	This study	M.
<i>H. persicus</i>	SUHC 1787	10 Km East of Evaz,Iran;27	-	This study	M.
<i>H. persicus</i>	SUHC 1416	Parishan region,Iran;28	MG744533	This study	G.
<i>H. persicus</i>	SUHC 1421	Parishan region,Iran;28	MG744534	This study	G.
<i>H. persicus</i>	SUHC 1837	Darab,Iran;29	MG744537	This study	G.
<i>H. persicus</i>	SUHC 1211	Bushehr,Iran;30	MG744528	This study	G.
<i>H. persicus</i>	CBSU R004	Kazeran,Iran;31	-	This study	M.
<i>H. persicus</i>	CBSU B636	Kazeran,Iran;31	-	This study	M.
<i>H. persicus</i>	ZMUB 41	Behbahan,Iran;11	MG744516	This study	M., G.
<i>H. persicus</i>	ZMUB 42	Behbahan,Iran;11	MG744517	This study	M., G.
<i>H. romeshkanicus</i>	ZMUB 43	Mehran,Iran;19	MG744518	This study	M., G.
<i>H. romeshkanicus</i>	ZMUB 44	Mehran,Iran;19	MG744519	This study	M., G.
<i>H. persicus</i>	ZMFUM10004	Farur Island,Iran;36	MG744521	This study	G.
<i>H. persicus</i>	MVZHERP234385	Lipar Village, Sistan and Baluchistan, Iran;32	JQ957077	Šmid et al.,2013	G.
<i>H. persicus</i>	FTHM005000	Mahshahr,Iran;33	JQ957074	Šmid et al.2013	G.
<i>H. persicus</i>	FTHM005001	Mahshahr,Iran;33	JQ957075	Šmid et al.2013	G.
<i>H. romeshkanicus</i>	FTHM005100	Bushehr,Iran;34	JQ957076	Šmid et al.2013	G.
<i>H. persicus</i>	NMP6V74807/1	Booreki,Iran;35	KC818691	Šmid et al.,2013	G.
<i>H. persicus</i>	NMP6V74807/2	Booreki,Iran;35	KC818690	Šmid et al.,2013	G.
<i>H. kurdicus</i>	CAS 262258	Kurdistan Region, Iraq;37	MG549189	Safaei-Mahroo et al.,2017	G.
<i>H. kurdicus</i>	CAS 262259	Kurdistan Region, Iraq;37	MG549190	Safaei-Mahroo et al.,2017	G.
<i>H. kurdicus</i>	CAS 262260	Kurdistan Region, Iraq;37	MG549191	Safaei-Mahroo et al.,2017	G.
<i>H. persicus</i>	CES 08027	Nabhdongar, Rajasthan, India;38	KC735107	Bansal and Karanth, 2013	G.
<i>H. persicus</i>	CES 1_08027	Nabhdongar, Rajasthan, India;38	HM595701	Bansal and Karanth, 2010	G.
<i>H. hajarensis</i>	CAS 227612	Tanuf,Oman;39	DQ120337	Carranza and Arnold,2006	G.
<i>H. hajarensis</i>	CAS 227614	Tanuf,Oman;39	DQ120338	Carranza and Arnold,2006	G.

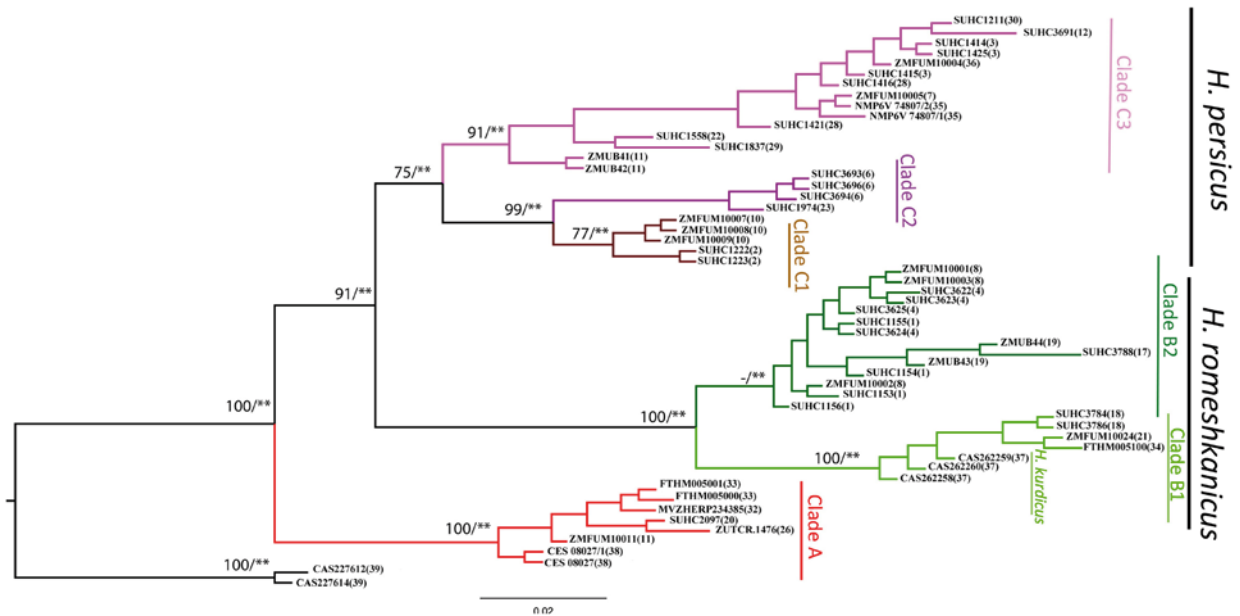


Figure 1. Bayesian 12S rRNA tree. Posterior probability and ML bootstrap values are indicated in star symbol (>99% (**), >95% (*)) and number on each branch of phylogenetic tree, respectively. Number in parenthesis showed locality of the specimens according to figure 2.

(*H. romeshkanicus*) (0.000). Apart from this case, the lowest genetic distance was found between clades B₁ (*H. romeshkanicus*) and B₂ (0.026). The highest genetic distance was found between clades B₁ (*H. romeshkanicus*) and A as well as *H. kurdicus* and A (0.097). The most genetically divergent group was clade A, being sister to all other *H. persicus* clades. Haplotype network analyses revealed 18 haplotypes including five haplotype networks and three unique haplotypes recovered by TCS. Specimens of *H. kurdicus* with *H. romeshkanicus* formed the same haplotype (Fig. 3).

Principal Coordinate Analysis (PCoA) distinguished six groups of individuals along discriminate axes 1 and 2, which accounted for 61.91 % and 25.26 % of the genetic variation, respectively (Fig. 4). Along the first axis, clade A, separated from clades *H. romeshkanicus*, B₂ and C₁, while the second axis resolved the other clades, but clade C₂ and C₃ are very close to each other. Genetic distances were positively correlated with geographic distances among six population (Rxy=0.003). The AMOVA analyses revealed that more genetic variation within populations (60%) was observed than among the six populations of *H. persicus* (40%). The largest PhiPT value was between clades A and C₁ (0.666), with the smallest value between clades B₁ and B₂ (-0.044). Clade B₁ represents *H. romeshkanicus*. The PhiPT distances between clades B₁ (*H. romeshkanicus*) and B₂ (-0.044), and between clades C₁ and C₂ (0.138) were not statistically significant.

There was no significant sexual dimorphism in *H. persicus*, excluding the number of preanal pores, which are only present in males. Morphology divided the individuals of *H. persicus* and *H. romeshkanicus* into six groups according to the clades in the 12S rRNA topology. Morphological character summaries are shown in Table 4. CVA analyses of meristic and morphometric characters showed that clades A and *H. romeshkanicus*

are fully differentiated from other groups, clades C₁ and C₂ are distinct from other groups, and clades C₃ and B₂ overlap with each other (Table 3, Fig. 5). The holotype of *H. romeshkanicus* falls within clade B₁, hence forth the *H. romeshkanicus* clade. Of thirty-one studied variables, SED/SVL, IO2/SVL and EEd/SVL had the highest CV1 and CV2 loadings (Table 5).

According to Vasconcelos & Carranza (2014), uncorrected genetic distances of up to 5.7% in 12S rRNA are considered to reflect high levels of genetic differentiation between different populations of *Hemidactylus* species. Interestingly, *H. kurdicus* shares the same haplotype with specimens of clade B₁ (*H. romeshkanicus*) (without genetic distance, 0.000). There is also little genetic differentiation between clade B₂ from Khuzestan and Ilam provinces and clade B₁ (*H. romeshkanicus*) from Lorstan province and Sulaimani, north-eastern Iraq, suggesting that these clades represent the same species at however high mitochondrial level of variation. Generally, *H. kurdicus* is not a distinct evolutionary lineage and synonymous with *H. romeshkanicus*, which has been described first by Torki et al. (2011). Taken together, five clades with significant genetic distances and eighteen different haplotypes are found within *H. persicus* of Iran, with *H. romeshkanicus* forming a distinct clade with a unique haplotype. However, unique haplotype networks according to defined clades probably imply the presence of isolated populations without gene flow. In addition, six haplotypes occur in clade C₃ that include all individuals from Fars Province, with the exception of specimens from mountainous areas in the north, which are included in clade C₂, and lowland regions in southern Fars which are assigned to clade C₁. With respect to different geographical conditions, three clades of *H. persicus* exist in Fars Province that show high genetic variation and most likely long-term

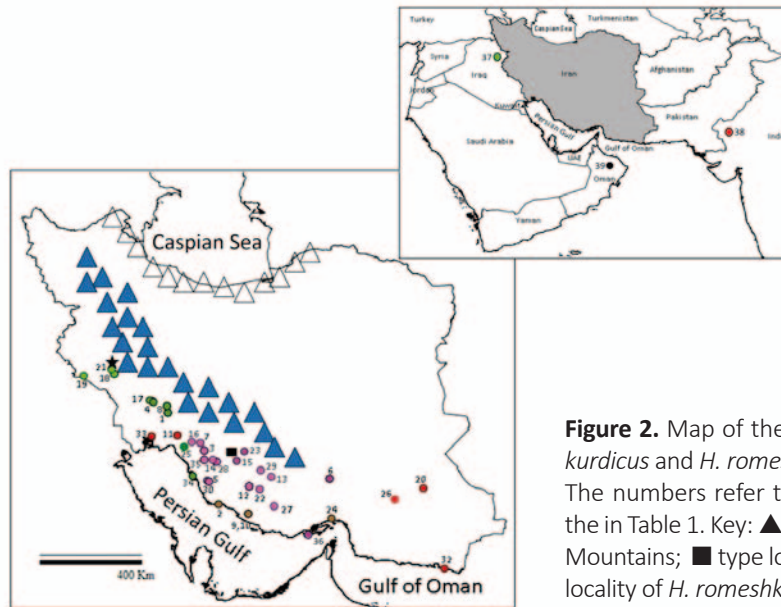


Figure 2. Map of the samples of *H. persicus*, *H. kurdicus* and *H. romeshkanicus* used in this study. The numbers refer to the samples indicated in the in Table 1. Key: ▲ Zagros Mountains; △ Elburz Mountains; ■ type locality of *H. persicus*; ★ type locality of *H. romeshkanicus*.

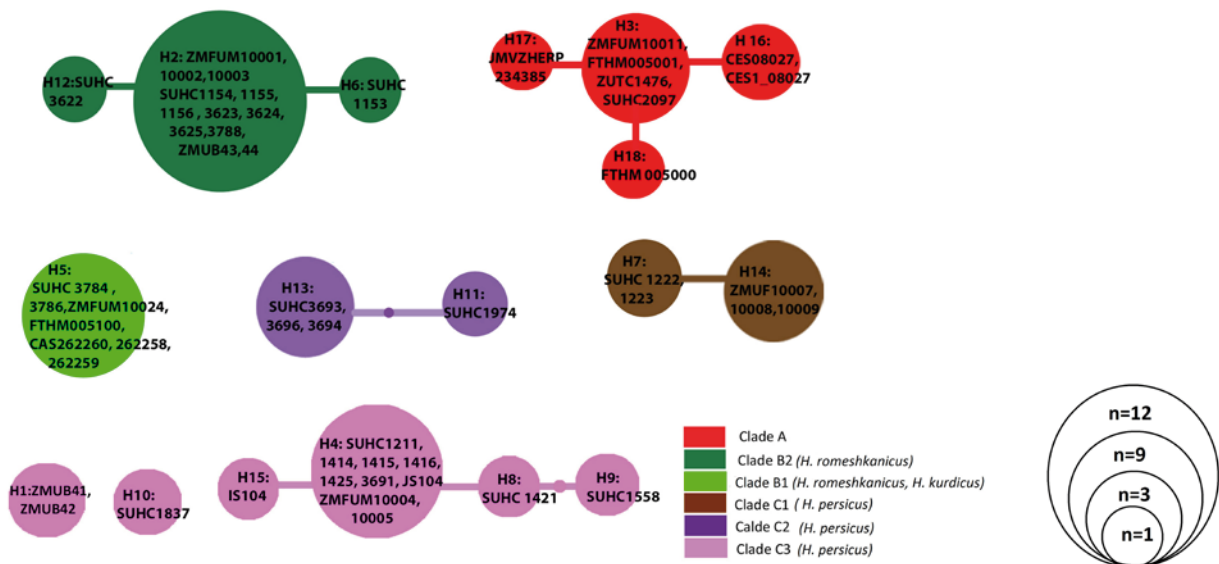


Figure 3. Haplotype networks constructed with statistical parsimony based on 399 bp of the mitochondrial 12S ribosomal RNA gene of *H. persicus*, *H. kurdicus* and *H. romeshkanicus* (50 individuals). Each circle represents one haplotype; size of circles is proportional to haplotype frequency.

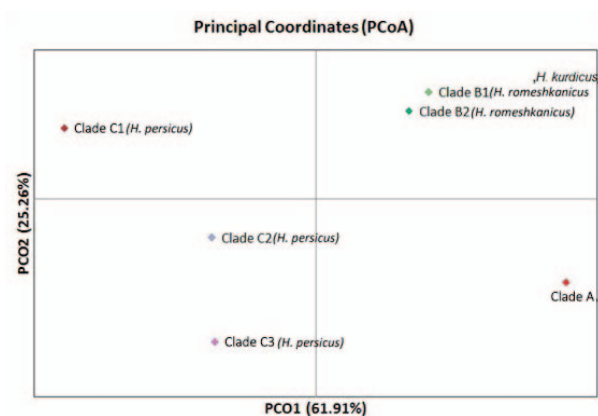


Figure 4. Principal coordinates analysis of five populations of *H. persicus*, *H. kurdicus* and *H. romeshkanicus*.

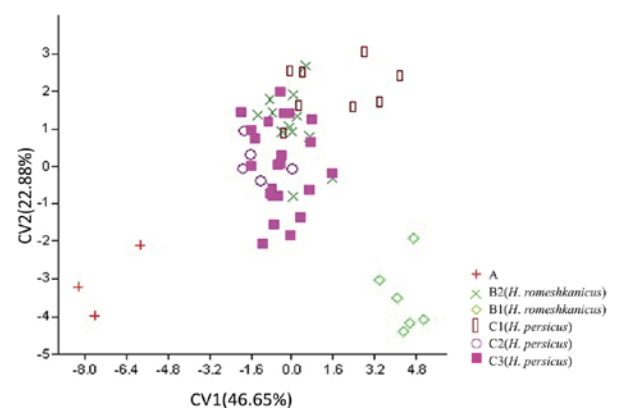


Figure 5. Multivariate morphological analyses of the five populations of *H. persicus* and *H. romeshkanicus*.

isolation. Morphologically, many external features of *Hemidactylus* species appear quite plastic, often varying within and between species (Carranza & Arnold, 2006). As a result, morphological characters may not be able to differentiate populations. Genetic distances suggest that clade C is characterised by deep interspecific variation between three main local populations from southern Iran (Bushehr and southern Fars Province; Clade C₁), central Iran (Kerman and northern Fars Province; Clade C₂), and southern Iran (central and eastern Fars Province; south-eastern Khuzestan; Clade C₃). Research is currently ongoing to clarify the phylogenetic relationships of *H. persicus* complex with more mitochondrial and nuclear genes.

The results derived from PCoA are compatible with our phylogenetic tree and morphological analyses. The individuals of clades C₂ and C₃ are closer in PCoA, PhiPT and morphological analyses, whereas in the phylogenetic tree clades C₃ and C₁ and clades C₁ and C₂ are closer than clades C₂ and C₃. The inconsistent results of clades C₂ and C₃ might be related to short geographic distances between the two clades which influence the PCoA, PhiPT and morphological analyses. The Mantel test showed a significant correlation with geographic and genetic distance, indicating that populations of *H. persicus* show a pattern of isolation by distance, which is usually explained by gene flow (Rousset, 1997). According to Šmíd et al. (2013), the long presence of *H. persicus* in Iran has resulted in high levels of intraspecific differentiation within the Iranian populations. Iran has two main mountain ranges that have played a significant role in the distribution, isolation and separation of reptile species; the Elburz Mountains that run from north-west to north-east and the Zagros Mountains that range from north-western to south-eastern Iran (Fisher, 1968; Macey et al., 1998). The formation of the Zagros Mountains began by the collision of the Arabian lithospheric plate moving in a north-easterly direction with the Eurasian landmass, which took place from the Oligocene to the Miocene 35 – 20 million years ago (Ma) (Mouthereau, 2011). According to Šmíd et al. (2013), the oldest reported dispersal of *Hemidactylus* from Arabia onto the Iranian Plateau occurred 13.1 Ma when the ancestor of *H. persicus* colonised Iran. The closest relatives of the Iranian *H. persicus* are found in UAE and northern Oman including *H. luqueorum* and *H. hajarensis* which are sister taxa of *H. persicus*. Dispersal therefore occurred most probably via the Gomphotherium land bridge connecting the Arabian and Anatolian plates approximately 18 Ma (Gardner 2009; Šmíd et al., 2013).

Geological events have led to the formation of different habitats and climatic conditions, separating the mountain regions from the Mesopotamian lowland populations and undoubtedly influencing the radiation, isolation, and differentiation of the Iranian herpetofauna (Wischuf & Fritz, 1996; Hrbek & Meyer, 2003; Feldman & Parham 2004; Rastegar-Pouyani et al., 2010). It seems likely that the ancestor of *H. persicus* penetrated the Iranian plateau from the south-west (basic dichotomy on the tree; clade A) and then dispersed to the more eastern parts (Gardner, 2009; Šmíd et al., 2013). Two

Table 2. The mensural and meristic characters used in this study.

Characters	Definition
SVL	Maximum snout to vent length (from tip of snout to cloacal aperture)
HW	Head width (at the widest point of head)
HH	Head height (from occiput to underside of jaws)
HL	Head length (from tip of snout to the retroarticular process of jaw)
CL	caudal length (from posterior edge of cloaca to tip of tail)
IO1	anterior interorbital distance (distance between left and right supraciliary scale rows at anteriormost point of eyes)
IO2	posterior interorbital distance (distance between left and right supraciliary scale rows at posterior-most point of eyes)
SL	supralabial scales (right)
IL	Infralabial scales (right)
4th SC	Scansors under 4th toe (Counts the sub digital lamellae in a single row of scales from the base of toe to the tip of the 4th toe)
1st SC	Scansors under 1st toe (Counts the sub digital lamellae in a single row of scales from the base of toe to the tip of the 1st toe)
OD	Orbital diameter (from greatest diameter of orbit)
EED	Eye to ear distance (from anterior edge of ear opening to posterior corner of eye)
SED	Snout to eye distance (from anterior point of eye to tip of snout)
DS	No. of dorsal scales (Counts the mid-way scales between the fore and hind limbs)
VS	No. of ventral scales (Counts the transverse row across the belly that includes the greatest number)
HLS	HL/SVL
HWS	HW/SVL
HHS	HH/SVL
OS	OD/SVL
O1S	IO1/SVL
O2S	IO2/SVL
ES	EED/SVL
SS	SED/SVL
HWH	HW/HL
HHH	HH/HL
HWHH	HW/HH
OH	OD/HL
EH	EED/HL
SH	SED/HL
O1H	IO1/HL

samples from India grouped with individuals of clade A, suggesting an eastward distribution from south-western Iran to India. The seven samples from Šmíd et al. (2013) are dispersed in our phylogenetic tree, including three samples from Brooki (Fars Province, Iran) that are located in clade C₃; one sample from Bushehr that is located in the *H. romeshkanicus* clade; three samples including one from Lipar village (Sistan and Baluchistan Province, Iran) and two others from Mahshar (extreme south-western Iran) are placed in clade A. The latter three samples

Table 3. Average uncorrected genetic distances (p-distance) between and within individual clades of *H. persicus*, *H. kurdicus* and *H. romeshkanicus* from the Iranian plateau based on 399 bp fragment of 12SrRNA.

Population	<i>H. kurdicus</i>	Clade B ₂	Clade C ₃	Clade A	Clade C ₁	Clade C ₂	Clade B ₁	Within clades
<i>H. kurdicus</i>								0.000
Clade B ₂	0.026							0.001
Clade C ₃	0.088	0.085						0.008
Clade A	0.097	0.094	0.065					0.003
Clade C ₁	0.076	0.080	0.044	0.054				0.002
Clade C ₂	0.090	0.086	0.054	0.076	0.041			0.004
Clade B ₁	0.000	0.026	0.088	0.097	0.076	0.090	0.000	0.000

Table 4. Descriptive parameters of 25 metric and six meristic characters including maximum, minimum, mean, and standard error in the studied clades of *H. persicus* and *H. romeshkanicus*.

Population	Clade A (n=3)		Clade B ₂ (n=13)		Clade B ₁ (n=6)	
Characters	Mean ± std. Error	Range	Mean ± std. Error	Range	Mean ± std. Error	Range
SVL	59.02±7.21	51.81-66.23	60.97±2.93	42.31-72.73	64.48±2.81	55.65-73.06
HW	12.23±1.54	10.69-13.78	11.96±0.64	7.72-14.06	13.01±0.44	12.05-14.49
HH	6.55±1.04	5.51-7.59	5.88±0.34	3.88-7.43	6.94±0.63	4.83-9.38
HL	15.93±0.73	15.20-16.66	17.8±0.80	12.95-20.73	19.63±0.89	16.42-22.47
CL	-	-	69.22±4.84	53.07-81.05	86.00	86.00-86.00
IO1	4.97±0.26	4.71-5.23	4.49±0.30	2.68-5.65	4.81±0.23	3.89-5.53
IO2	6.68±0.82	5.86-7.51	6.69±0.49	3.21-8.30	7.75±0.34	6.83-8.73
SL	10	10-10.00	11.09±0.16	10.00-12.00	12.66±0.66	11.00-15.00
IL	8.5±0.5	8.00-9.00	8.54±0.2	8.00-10.00	9.66±0.49	8.00-11.00
OD	3.55±0.62	2.93-4.18	4.04±0.31	2.54-6.49	4.51±0.23	3.68-5.36
EED	4.92±0.73	4.19-5.65	4.72±0.23	3.00-5.48	4.82±0.29	3.97-6.00
SED	6.57±0.83	5.74-7.40	6.72±0.33	5.14-8.52	6.98±0.51	4.98-8.66
DS	43±1	42.00-44.00	43.1±2.01	32.00-50.00	45.60±4.54	32.00-60.00
VS	43.5±7.5	36.00-51.00	43.3±2.28	27.00-53.00	40.00±1.84	34.00-46.00
1st SC	6.5±1.5	5.00-8.00	8.63±0.36	6.00-10.00	9.66±0.33	9.00-11.00
4th SC	12±2	10.00-14.00	12.45±0.15	12.00-13.00	13.00±0.25	12.00-14.00
CL/SVL	-	-	1.16±0.03	0.97-1.25	1.2113±0	1.21-1.21
HL/SVL	0.27±0.02	0.25-0.29	0.29±0.004	0.27-0.32	0.30±0.003	0.30-0.32
HW/SVL	0.20±0.0008	0.21-0.21	0.19±0.006	0.14-0.21	0.20±0.006	0.18-0.22
HH/SVL	0.11±0.004	0.11-0.11	0.09±0.003	0.07-0.11	0.10±0.008	0.07-0.13
OD/SVL	0.05±0.003	0.06-0.06	0.06±0.005	0.06-0.12	0.06±0.001	0.07-0.07
IO1/SVL	0.08±0.005	0.08-0.09	0.07±0.002	0.06-0.09	0.07±0.003	0.07-0.09
IO2/SVL	0.11±0.0001	0.11-0.11	0.1±0.004	0.08-0.13	0.12±0.002	0.11-0.13
EED/SVL	0.08±0.002	0.08-0.09	0.07±0.001	0.07-0.09	0.07±0.003	0.06-0.09
SED/SVL	0.11±0.0004	0.11-0.11	0.11±0.005	0.09-0.16	0.10±0.008	0.07-0.13
HW/HL	0.76±0.061	0.70-0.83	0.67±0.01	0.58-0.76	0.66±0.01	0.61-0.74
HH/HL	0.40±0.046	0.36-0.46	0.33±0.01	0.27-0.41	0.35±0.02	0.24-0.43
HW/HH	1.87±0.06	1.82-1.94	2.04±0.06	1.80-2.60	1.93±0.13	1.54-2.49
OD/HL	0.22±0.02	0.19-0.25	0.22±0.01	0.20-0.41	0.22±0.005	0.21-0.25
EED/HL	0.30±0.03	0.28-0.34	0.26±0.006	0.23-0.30	0.24±0.01	0.21-0.28
SED/HL	0.41±0.03	0.38-0.44	0.37±0.01	0.34-0.54	0.35±0.02	0.22-0.41
IO1/HL	0.31±0.002	0.31-0.31	0.25±0.01	0.21-0.31	0.24±0.01	0.21-0.30
IO2/HL	0.41±0.03	0.39-0.45	0.37±0.02	0.25-0.50	0.39±0.01	0.37-0.45

Population	Clade C ₁ (n=8)		Clade C ₂ (n=5)		Clade C ₃ (n=23)	
Characters	Mean ± std. Error	Range	Mean ± std. Error	Range	Mean ± std. Error	Range
SVL	54.63±1.37	50.01-61.06	49.38±4.95	35.72-62.35	59.67±1.15	51.40-68.63
HW	10.63±0.22	9.70-11.68	9.45±0.94	6.78-11.80	11.72±0.25	9.75-14.27
HH	4.96±0.21	4.09-5.70	4.10±0.51	2.65-5.53	5.79±0.20	4.17-7.35
HL	16.15±0.41	14.56-17.76	14.95±1.07	11.77-17.63	17.33±0.34	14.00-19.90
CL	57.74±4.68	44.87-65.75	48.5±5.29	43.21-53.80	71.25±4.05	46.73-90.14
IO1	4.22±0.14	3.72-5.01	3.92±0.33	3.15-5.03	4.36±0.09	3.76-5.15
IO2	6.03±0.23	5.13-6.98	5.53±0.82	3.20-7.90	6.33±0.19	4.79-8.05
SL	11.50±0.32	10.00-13.00	11.40±0.50	10.00-13.00	11.57±0.28	9.00-15.00
IL	9.00±0.32	8.00-11.00	9.0±0.00	9.00-9.00	8.95±0.17	8.00-11.00
OD	3.39±0.14	3.01-4.08	3.37±0.18	2.77-3.75	3.76±0.11	2.60-4.72
EED	4.20±0.14	3.70-5.07	3.65±0.37	2.55-4.58	4.45±0.12	3.58-6.03
SED	5.81±0.12	5.25-6.42	5.64±0.43	4.25-6.82	6.38±0.13	5.41-7.63
DS	41.87±2.07	33.00-48.00	38.2±2.47	30.00-45.00	46.41±2.26	35.00-78.00
VS	42.87±0.91	40.00-47.00	40.40±1.69	36.00-46.00	43.40±1.17	31.00-53.00
1st SC	8.75±0.45	7.00-11.00	9.20±0.20	9.00-10.00	8.19±0.11	7.00-9.00
4th SC	13.25±0.25	12.00-14.00	12.6±0.50	11.00-14.00	11.90±0.15	11.00-13.00
CL/SVL	1.06±0.07	0.86-1.18	1.27±0.06	1.21-1.33	1.159±0.05	0.83-1.35
HL/SVL	0.29±0.003	0.28-0.31	0.30±0.009	0.28-0.33	0.29±0.003	0.27-0.33
HW/SVL	0.19±0.002	0.18-0.20	0.20±0.011	0.18-0.24	0.19±0.004	0.12-0.21
HH/SVL	0.09±0.003	0.08-0.10	0.08±0.006	0.07-0.11	0.09±0.003	0.08-0.12
OD/SVL	0.06±0.001	0.06-0.07	0.06±0.003	0.06-0.08	0.06±0.001	0.05-0.07
IO1/SVL	0.07±0.003	0.07-0.10	0.08±0.003	0.07-0.09	0.07±0.001	0.06-0.09
IO2/SVL	0.11±0.003	0.09-0.12	0.11±0.009	0.08-0.13	0.10±0.002	0.08-0.13
EED/SVL	0.07±0.002	0.07-0.09	0.07±0.0007	0.07-0.08	0.07±0.001	0.06-0.09
SED/SVL	0.10±0.001	0.10-0.11	0.11±0.003	0.11-0.13	0.10±0.004	0.10-0.12
HW/HL	0.65±0.01	0.60-0.69	0.62±0.02	0.58-0.69	0.67±0.009	0.57-0.77
HH/HL	0.30±0.009	0.26-0.33	0.27±0.02	0.23-0.35	0.33±0.01	0.25-0.41
HW/HH	2.16±0.08	1.89-2.58	2.34±0.11	1.96-2.56	2.05±0.06	1.63-2.61
OD/HL	0.20±0.005	0.19-0.24	0.22±0.004	0.21-0.24	0.21±0.003	0.18-0.24
EED/HL	0.26±0.007	0.23-0.29	0.24±0.007	0.22-0.26	0.25±0.006	0.20-0.31
SED/HL	0.36±0.006	0.33-0.39	0.37±0.006	0.36-0.40	0.35±0.01	0.03-0.41
IO1/HL	0.26±0.01	0.23-0.32	0.26±0.009	0.23-0.29	0.25±0.005	0.21-0.29
IO2/HL	0.37±0.01	0.31-0.40	0.36±0.03	0.24-0.45	0.36±0.009	0.29-0.47

Table 5. Factor loadings of canonical variate analysis (CVA) of 31 morphological characters for the 58 specimens of *H. persicus* and *H. romeshkanicus*.

Characters	CV ₁	CV ₂
SVL	39.705	26.865
HW	29.252	18.666
HH	41.437	28.425
HL	45.242	30.248
IO1	41.148	44.035
IO2	41.194	16.873
OD	54.858	11.709
EED	43.834	40.644
SED	32.829	19.96
HL/SVL	-772.13	-107.82
HW/SVL	-5.962	-7.9426
HH/SVL	-308.6	-118.62
OD/SVL	-931.89	-1818.7
IO1/SVL	-279.09	1072.1
IO2/SVL	1699.7	18.347
EED/SVL	-1404.8	701.88
SED/SVL	2778.4	579.07
HW/HL	251.45	206.15
HH/HL	93.773	-82.777
HW/HH	34.986	9.873
OD/HL	96.726	811.84
EED/HL	387.34	-409.82
SED/HL	-654.89	-34.759
IO1/HL	104.97	-578.45
IO2/HL	-459.2	183.4
SL	18.386	-4.128
IL	-12.804	-4.4937
DS	1.2861	7.2884
VS	7.6616	-0.19007
1st SC	14.834	-2.5593
4th SC	38.408	21.415
Eigenvalue	5.853	2.871
Accumulated percentage of variability	46.65	22.88

of *H. persicus* have a basal position in the phylogenetic tree relative to the other samples. The topology and phylogenetic positions of the Persian gecko of Šmíd et al., (2013) is consistent with their position in our data.

The results support the validity of *H. romeshkanicus* using morphological and molecular data. Interestingly, our phylogenetic inference revealed that *H. kurdicus* shares haplotypes with *H. romeshkanicus*. The new reported species is not representing a distinct evolutionary lineage and is synonymous with *H. romeshkanicus*. Hence, *H. romeshkanicus* is no longer endemic to Iran, expanding the distribution from the type locality (Iran, south of Lorestan, Romeshkan, Pole-e-Dokhtar) and the Khuzestan and Ilam provinces (all locations of specimens from clade B₂) to Iraq (south-western Sulaimani, Kurdistan region). Furthermore, the habitat of the two species is identical, representing by in oak woodlands of the Zagros forest steppe on western slopes separated only by the political border (Torki et al., 2011; Safaei-Mahroo et al., 2017). Hence, distribution of the species might be extended to central Iraq. Probably, the species is synonymous with previous described species of Iraq, *Hemidactylus bornmuelleri* Werner, 1895 that has been considered a synonym of *H. persicus* by Smith 1935. However, there is a need to collect specimens of Persian gecko from different regions of Iraq for final conclusion. These findings highlighted deep mitochondrial and morphological

variations between different populations of the Persian gecko in Iran. Eventually, three definite species based on the molecular clades could be recognised: clade C corresponds to type locality of *H. persicus* and we therefore use the name *H. persicus* for this clade; clade B with the name *H. romeshkanicus*; and clade A, which might further represent a new cryptic species. Describing new species and studying variation in all populations is ongoing with additional loci for shedding more light on the clades of *H. persicus* in a further study.

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REFERENCES

- Ahmadzadeh, F., Flecks, M., Carretero, M. A., Mozaffari, O., Bohme, W., Harris, D. J., Freitas, S. & Rodder, D. (2013). Cryptic speciation patterns in Iranian rock lizards uncovered by integrative taxonomy. *PLOS ONE* 8, 12.
- Anderson, S.C. (1968). Zoogeographic analysis of the lizard fauna. Pages 305–371 in W. B. Fisher, ed., *The Land of Iran*. In A.J. Arberry, (ed), *The Cambridge History of Iran*, Vol. 1. Cambridge: Cambridge University Press.
- Anderson, S.C. (1999). *The lizards of Iran*. Contributions to Herpetology Volume 15. Ohio: Society for the Study of Amphibians and Reptiles.
- Bansal, R. & Karanth, K. P. (2010). Molecular phylogeny of *Hemidactylus* geckos (Squamata: Gekkonidae) of the Indian subcontinent reveals a unique Indian radiation and an Indian origin of Asian house geckos. *Molecular Phylogenetics and Evolution* 57, 459–465.
- Bansal, R. & Karanth, K. P. (2013). Phylogenetic analysis and molecular dating suggest that *Hemidactylus anamallensis* is not a member of the *Hemidactylus* radiation and has an ancient late Cretaceous origin. *PLoS one* 8(5), p.e60615:1-8
- Bauer, A.M., Jackman, T., Greenbaum, E. & Papenfuss, T.J. (2006). Confirmation of the occurrence of *Hemidactylus robustus* Heyden, 1827 (Reptilia: Gekkonidae) in Iran and Pakistan. *Zoology in the Middle East* 39, 59–62.
- Busais, S.M. & Joger, U. (2011). Three new species and one new subspecies of *Hemidactylus* Oken, 1817 from Yemen (Squamata, Gekkonidae). *Vertebrate Zoology* 61, 267–280.
- Carranza, S. & Arnold, E.N. (2006). Systematics, biogeography,

- and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 38, 531–545.
- Carranza, S. & Arnold, E.N. (2012). A review of the geckos of the genus *Hemidactylus* (Squamata: Gekkonidae) from Oman based on morphology, mitochondrial and nuclear data, with descriptions of eight new species. *Zootaxa* 3378, 1–95.
- Clement, X., Posada, D. & Crandall, K. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology* 9, 1657–1659.
- Feldman, C.R. & Parham, J.F. (2004). Molecular systematics of Old world stripe-necked turtles (Testudines: Mauremys). *Asiatic Herpetological Research* 10, 28–37.
- Ficetola, G.F., Bonardi, A., Sindaco, R. & Padoa-Schioppa, E. (2013). Estimating patterns of reptile biodiversity in remote regions. *Journal of Biogeography* 40, 1202–1211.
- Fisher, W. B. (1968). *The land of Iran, Vol. 1. The Cambridge history of Iran*. Cambridge, England: Cambridge University Press.
- Gardner, A. S. (2009). Two new gecko species records for the United Arab Emirates: *Pristurus carteri* (Gray, 1863) and *Hemidactylus persicus* Anderson, 1872. *Tribulus* 18, 18–23.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41, 95–98.
- Hammer, Q., Harper, D.A.T. & Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4, 1–9.
- Hillis, D. M., Ammerman, L. K., Dixon, M. T. & de Sa, R. O. (1993). Ribosomal DNA and the phylogeny of frogs. *Herpetological Monographs* 7, 118–131.
- Hosseinzadeh, M. S., Aliabadian, M., Rastegar-Pouyani, E. & Rastegar-Pouyani N. (2014a). The roles of environmental factors on reptile richness in Iran. *Amphibia-Reptilia* 35, 215–225.
- Hosseinzadeh, M.S., Aliabadian, M., Rastegar-Pouyani, E., Rastegar-Pouyani, N. (2014b). Morphological study of *Hemidactylus* geckos (Squamata:Gekkonidae) from Iran. *Iranian Journal of Animal Biosystematics (IJAB)* 10,175–184.
- Hrbek, T. & Meyer, A. (2003). Closing of the Tethys sea and the phylogeny of Eurasian killifishes (Cyprinodontiformes: Cyprinodontidae). *Journal of Evolution and Biology* 16, 17–36.
- Jensen, J.L., Bohonak, A.J. & Kelley, S.T. (2005). Isolation by distance, web service. *BMC Genetics* v. 3.16.
- Khan, M.S. (2013). An Addition to Geckos of the Genus *Hemidactylus* Oken 1817 in Pakistan, with Notes on Morphology, Ecology and Distribution of Species. *Chicago Herpetological Society* 48, 130–134.
- Kluge, A.G. (1969). The evolution and geographic origin of the New World *Hemidactylus mabouia-brookii* complex (Gekkonidae, Sauria). *Miscellaneous publications Museum of zoology, University of Michigan* 138, 1–78.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X. & Wilson, A.C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America* 86, 6196–6200.
- Macey, J.R., Shulte, J.A., Ananjeva, N.B., Larson, A., Rastegar-Pouyani, N., Shamakove, S.M. & Papenfuss, T.J. (1998). Phylogenetic relationshi among agamid lizards of the *Laudakia caucasia* species group: testing the hypotheses of biogeographic fragmentation and an area caldogram for the Iranian Plateau. *Molecular Phylogenetics and Evolution* 10, 118–131.
- Peakall, R. & Smouse, P.E. (2006). Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288–295.
- Posada, D. & Crandall, K. A. (1998). Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Rastegar-Pouyani, E., Rastegar Pouyani, N., Kazemi Nouredini, S., Joger, U. & Wink, M. (2010). Molecular phylogeny of the *Eremias persica* complex of the Iranian plateau (Reptilia: Lacertidae), based on mtDNA sequences. *Zoological Journal of the Linnean Society* 158, 641–660.
- Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145,1219–1228.
- Safaei-Mahroo, B., Ghaffari, H., Ghafoor, A., & Amini, S. (2017). A new species of *Hemidactylus* (Squamata: Gekkota: Gekkonidae) from Qara Dag Mountain, Kurdistan Region, with a key to the genus in Iraq. *Zootaxa* 4363(3), 377–392.
- Sindaco, R. & Jeremčenko, V.K. (2008). The reptiles of the western Palearctic. Latina, Italy: 1. Annotated checklist and distributional atlas of the turtles, crocodiles, amphisbaenians and lizards of Europe, North Africa, Middle-East and Central Asia.
- Šmíd, J., Carranza, S., Kratochvíl, L., Gvoždík, V., Nasher, A.K. & Moravec J. (2013). out of Arabia: A Complex Biogeographic History of Multiple Vicariance and Dispersal Events in the Gecko Genus *Hemidactylus* (Reptilia: Gekkonidae). *PLOS ONE* 8, e64018.
- Šmíd, J., Moravec, J., Kodym, P., Kratochvíl, L., Hosseinian Yousefkhani, S.S., Rastegar-Pouyani, E. & Frynta, D. (2014). Annotated checklist and distribution of the lizards of Iran. *Zootaxa*, 3855.
- Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688– 2690.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28, 2731–2739.
- Templeton, A.R., Crandall, K.A. & Sing, C.F. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132, 619–633.
- Torki, F., Manthey, U. & Barts, M. (2011). Ein neuer *Hemidactylus* Gray, 1825 aus der Provinz Lorestan, West-Iran, mit Anmerkungen zu *Hemidactylus robustus* Heyden, 1827 (Reptilia: Squamata: Gekkonidae). *Sauria* 33, 47–56.
- Uetz, P., Freed, P. & Hošek, J. (2018). The Reptile Database. Zoological Museum, Hamburg. Available from: <http://www.reptiledatabase.org>. Last accessed on 19 Jun 2018.
- Vences, M., Wanke, S., Vieites, D.R., Branch, B. & Glaw, F. (2004). Natural colonisation or introduction? High genetic divergences and phylogeographic relationships of house geckos (*Hemidactylus*) from Madagascar. *Biological Journal of Linnean Society* 83, 115–130.
- Wischnuf, T. & Fritz, U. (1996). Eine neue Unterart der Bachschildkröte (*Mauremys caspica ventrimaculata* subsp. nov.) aus dem Iranischen Hochland. *Salamandra* 32, 113–122.

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