Zootaxa 3860 (4): 379–395
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3860.4.6
http://zoobank.org/urn:lsid:zoobank.org:pub:7F7EF005-6D3E-4680-8EE6-3772DB04C337

Molecular phylogeny and biogeography of the genus *Acanthodactylus* Fitzinger, 1834 (Reptilia: Lacertidae) in Iran, inferred from mtDNA Sequences

NASTARAN HEIDARI[1,2], ESKANDAR RASTEGAR-POUYANI[3], NASRULLAH RASTEGAR-POUYANI[1] & HIVA FAIZI[1]

[1]*Department of Biology, Faculty of Science, Razi University, 6714967346 Kermanshah, Iran,*
[2] *Department of Animal Biology, Faculty of Biological Science, Kharazmi University. Tehran, Iran*
[3]*Department of Biology, Hakim Sabzevari University, Sabzevar, Iran*

Abstract

Phylogenetic relationships of Iranian *Acanthodactylus* species were investigated using 1407 bp of mitochondrial DNA including 606 bp of cytochrome b and 801 bp of NADH dehydrogenase subunit 4 (ND4). Analyses done with maximum parsimony, maximum-likelihood, and Bayesian inference included 67 specimens from 27 geographically distinct localities in Iran. Our molecular results proposed three clear and geographically isolated clades by their phylogenetic positions and genetic differences. These three major clades are: (1) *A. micropholis*+ *A. grandis*+ *A. khamirensis;* (2) *A. blanfordi*+ *A. schmidti*+ *Acanthodactylus sp*$_1$, (3) *A. nilsoni*+ *A. boskianus* + *Acanthodactylus sp*$_2$. The phylogenetic analyses of the genus did not group *A. grandis* with the remaining species of the *A. boskianus* group and clustered it along with *A. khamirensis* within the *A. micropholis* group. In addition, phylogenetic results revealed a monophyletic status for *A. schmidti* and *A. micropholis* groups. Molecular clock approach indicated that the most recent divergence event splits *A. micropholis* from *A. khamirensis* about 2 MYA and results of dispersal-vicariance analyses showed that this diversification occurred by dispersal event rather than vicariance. Results of Reconstruct Ancestral State in Phylogenies (RASP) showed that Most Recent Common Ancestor (MRCA) of *A. micropholis*, *A. blanfordi* and *A. sp*$_1$ originated in eastern Iran. The first diversification of the genus in Iran most likely occurred between 8.5–9 MYA corresponding with the hypothesis that the genus has entered Iran long after the complete uplifting of the Zagros Mts. (10–12 MYA) which limited its dispersal only to the Persian Gulf shores and western slopes of the Zagros Mts.

Key words: Lacertidae *Acanthodactylus*, Mitochondrial genes, Cytochrome b, ND4, Phylogeny, Biogeography, Iran

Introduction

Nine genera and 41 species of lacertid lizards occur in Iran (Rastegar Pouyani *et al.* 2008; Ahmadzadeh *et al.* 2012; Heidari *et al.* 2013). One of these genera is *Acanthodactylus*, which is Saharo-Sindian in its distribution (Anderson, 1999). Of these, so far, seven species have been documented from Iran: *Acanthodactylus blanfordi* Boulenger, 1918, *A. boskianus* (Daudin, 1802), *A. grandis* Boulenger, 1909, *A. micropholis* Blanford, 1874; *A. nilsoni* Rastegar-Pouyani, 1998, *A. schmidti* Haas, 1957, and recently, *A. khamirensis* Heydari, Rastegar-Pouyani, Rastegar-Pouyani and Rajabizadeh, 2013. Current distribution pattern of *Acanthodactylus* species in Iran shows a great potential role of the Zagros Mountains in forming and diversifying its species to a great extent, especially along western slopes of the mountain chain. This mountain chain has played a pivotal role in modeling distribution patterns of various taxa from its emergence to present day (Macey *et al.* 1998, 2000; Rastegar-Pouyani, 1999a, b, c; Rastegar-Pouyani & Nilson 2002; Rastegar-Pouyani *et al.* 2009). The effect of the Zagros Mountains in modeling the evolutionary history of taxa distributed in its western and eastern slopes is a prominent biogeographic question that should be addressed by studying distributional patterns of different species in the area.

The Zagros mountainous ecosystem can be a major physical barrier to the distribution of species that are not rock dwellers and thus unable to live in this type of habitat. Fringed-toed lizard genus, *Acanthodactylus,* is an interesting case to address the influence of this mountainous ecosystem on the phylogeography and taxonomy of its

Accepted by S. Carranza: 4 Aug. 2014; published: 8 Sept. 2014

species. The members of this genus are fully discordant with stony and rocky habitats, which act as barriers to their dispersal. The gradual uplifting of the Zagros mountain chain due to the collision of the Arabian tectonic plate with Eurasia occurred around 35–20 MYA (Dercourt *et al.*, 1986; Mouthereau, 2011), only to stabilize around 10–12.4 MYA (Sborshchikov *et al.* 1981; Mouthereau, 2011). This process has generated extensive vicariance events on different species of reptiles during its history (Macey *et al.* 1998, 2000; Rastegar-Pouyani, 1999a, b, c; Rastegar-Pouyani & Nilson 2002). The ancestors of the genus *Acanthodactylus* originated in south-west Asia around 17-19 MYA (Harris & Arnold, 2000; Mayer & Pavlicev, 2007), followed by two emigrations, westwards to Africa and eastwards to Pakistan and India (Harris & Arnold, 2000).

The genus *Acanthodactylus* forms a monophyletic group composed of 42 species within the family Lacertidae (Uetz, 2013). Complex relationships either inter- or intraspecific, make this genus an excellent group for understanding evolutionary patterns and elucidating phylogenetic relationships among its taxa. The first traditional studies on the genus were carried out using morphological and anatomical data only by Boulenger (1918, 1921), Salvador (1982) and Arnold (1983). Salvador (1982) and Arnold (1983) have extensively defined species groups within the genus and determined species boundaries with few differing arguments. Salvador (1982) defined nine species groups with *A. grandis* as a single species group. However, Arnold (1983) defined eight species groups and defined *A. grandis* as a species complex that is close to the *A. boskianus* group. After these first reviews, molecular data have been used to elucidate the phylogenetic relationships of different species groups of the genus (Harris & Arnold, 2000; Harris *et al.*, 2004; Fonseca *et al.*, 2008, 2009; Carretero *et al.*, 2011). Due to the large number of species and the wide distribution of the genus, a molecular and phylogenetic study of the whole genus is hard to acquire. *Acanthodactylus* is a taxonomically difficult genus, due in part to its species being apparently similar, but also extremely variable in some details (Salvador, 1982; Arnold, 1983). However, Fonsesca et al. (2008) have studied some of species groups in the genus such as the *A. pardalis* and *A. erythrurus* groups. In their study, *A. mechriguensis* was synonymized with *A. maculatus*. Crochet et al. (2003) examined the *A. scutellatus* group using multivariate analysis and they revalidated *A. senegalensis*, which had been considered a synonym of *A. dumerili* by previous authors. In another extensive study by Harris &Arnold (2000), 15 species of the genus were examined phylogenetically by a combination of 12S and 16S mitochondrial genes and morphologically examined over 30 species of the genus. They determined three main clades including a western clade, an eastern clade and a *scutellatus* clade. Based on the definition by Harris & Arnold (2000), *Acanthodactylus* species in Iran all belong to the eastern clade. On the basis of environmental factors (topography and climate), most distribution areas of the genus in Iran occur in the Khalij-o-Omani ecological zone. This zone extends throughout southern parts of Sistan and Baluchistan, Hormozgan, Bushehr, Khuzistan provinces along coastlines of the Persian Gulf and Oman Sea dominated by a sub-equatorial climate zone (Heshmati, 2007). In addition, a significant part of its distribution extends to the Zagros ecological zone in Ilam and Kermanshah Provinces (see Figure 1).

The lowland coastal, arid and desert zones of southern, southeastern and southwestern Iran towards the western corners of Iran (Khalij-o-Omani ecological zone) represent similar and unique habitats with sandy soils in accordance with preferable habitats of the genus *Acanthodactylus*. The genus occurs in these types of habitats across Iberia, North Africa and southwestern Asia, from southeastern Turkey to southern Arabia and from the Mediterranean and the Red Sea to Pakistan and northwestern India (Salvador, 1982; Anderson, 1999; Harris & Arnold, 2000; Sindaco & Jeremčenko, 2008). *Acanthodactylus* was previously thought to be closely related to *Eremias* and *Mesalina* (Arnold, 1981) but currently its sister taxa have proved to be *Mesalina* and *Ophisops* (Mayer & Benyr, 1994; Fu 2000; Harris & Arnold, 2000; Pyron *et al.*, 2013).

In this study, we have used information from two mitochondrial genes, ND4 and Cyt b to infer the phylogenetic relationships among *Acanthodactylus* taxa in Iran. Cyt b has enough variability for considering population level studies, and conservatively enough, for revealing unclear phylogenetic relationships. These features make Cytochrome b one of the most commonly used mitochondrial genes in phylogenetic studies, served to clarify phylogenetic relationships at long divergence times (Graybeal, 1994).

n this study, we tried to discover: (i) the phylogentic relationships of the extant species of the genus in Iran. This by examining representative taxa from four out of the nine species groups, including the *A. schmidti*, *A. boskianus*, *A. micropholis*, and *A. grandis* groups. We use this data to assess previous morphological and molecular phylogenetic studies (Harris & Arnold, 2000); (ii) evaluate the divergence time of extant lineages using molecular clock approaches; (iii) inferring historical biogeography of the genus in Iran using dispersal vicariance analyses.

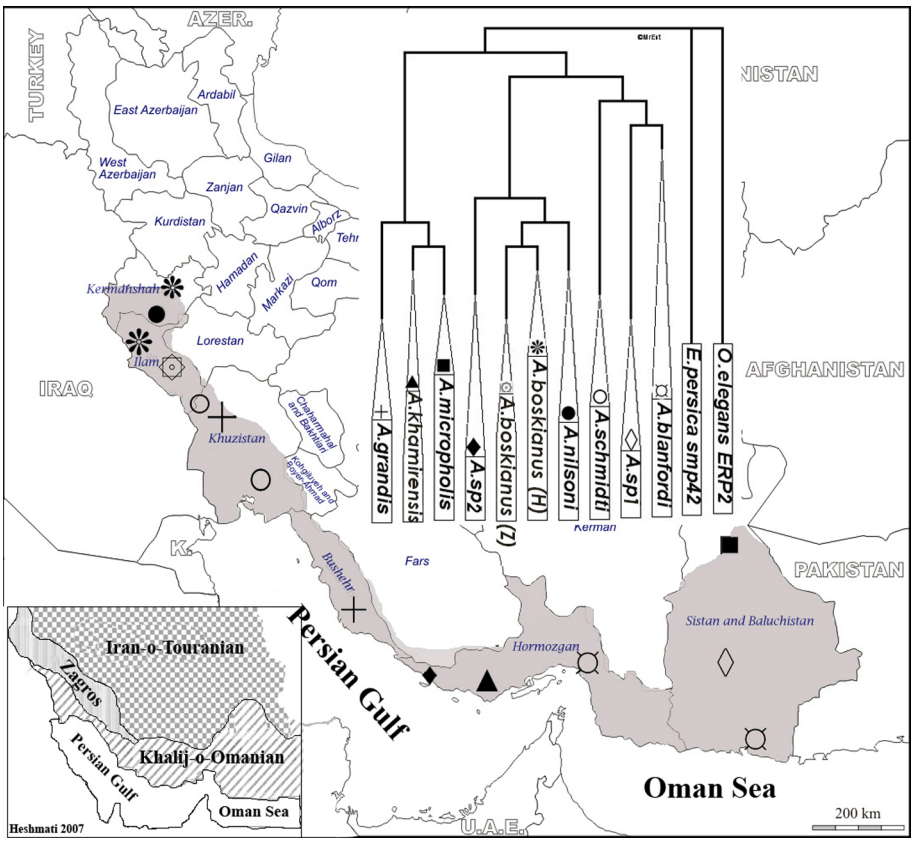

FIGURE 1. Distribution Map of genus *Acanthodactylus* in Iran (gray shaded area) and approximate sampling points for each of the species used in this study. Distinct sign is used for each species. Any of these signs is included in the phylogenetic tree in the map. Precise positioning with GPS locations for all samples used in this study are presented in Appendix A. Khalij-o-Omani and the Zagros ecological zones in a separate small map (Heshmati 2007) at the bottom.

Materials and methods

Study area and sample collection: A total of 67 specimens belonging to six known species of *Acanthodactylus* and some unknown populations of the genus from 27 geographically distinct localities were collected and examined morphologically and molecularly. Field work was conducted in Kermanshah, Ilam, Khuzistan, Bushehr, Hormozgan, Sistan and Baluchistan provinces, covering the whole geographic distribution of the genus in Iran. Sampling localities for specimens used in this study are shown in Figure 1. Specimens were collected during 2011–2012 at the localities presented in Table 1. Current studies on Lacertid phylogenetic relationships propose *Ophisops* and *Eremias* as close and distant relatives of the genus, respectively (Harris, *et al.,* 1998; Harris & Arnold, 2000; Mayer & Pavlicev, 2007). Consequently, we chose *Eremias persica* and *Ophisops elegans* to root the phylogenetic tree. Original DNA and tissue samples deposited in the Department of Biology, Hakim Sabzevari University, Iran. All specimens have been preserved in 95% alcohol and deposited in the Razi University Zoological Museum (RUZM) collection.

DNA extraction, amplification and sequencing

DNA was extracted from preserved tissue samples (thigh muscles) using non-organic DNA Extraction Procedure (Proteinase K and Salting out). MT-ND4 (NADH dehydrogenase subunit 4; from 1 to 642, ND4; from 643 to 801, tRNA His [complete], tRNA Ser [complete] and tRNA Leu [Partial]), and Cytochrome b genes were amplified by the polymerase chain reaction (PCR) procedure using primers presented in Table 2. PCR reactions performed in 30µl with the following conditions: Initial denaturation stage of 95° C (04:00) followed by the 35 cycles with denaturation at 95°C (00:40), annealing at 49° C (00:40) and extension at 72° C (01:20) then single extension cycle at 72° C (10:00). The amplified genes were sequenced with an automatic DNA sequencer by relevant protocols of BIONEER Company.

TABLE 1. Specimens used in this study, collection numbers, GenBank accession numbers, collecting localities and their exact coordination. RUZM (Razi University Zoological Museum); ERP (Eskandar Rastegar Pouyani); SMP (Sabzevar- Mashhad Project).

Species	Collection number	GenBank accession numbers		Coordinates	Locality
		Cyt b	ND4 (tRNAs His+Ser+Leu)		
Ophisops elegans	ERP 276	KJ652769	KJ652838	36 ° 08′ 27.4″ N; 57º 40′ 47.1″ E	5km western Eshtehard
Eremias persica	SMP 42	KJ652770	KJ652839	35º 44′ 70.3″ N; 50º 14′ 10.5″ E	50 km SE Jajrom
A.khamirensis	RUZM 148	KJ652702	KJ652796	26º 30′ 47.4″ N; 55º 58′ 44.2″ E	7 km East of Khamir Port
A.khamirensis	RUZM 146	KJ652703	KJ652795	26º 30′ 47.4″ N; 55º 58′ 44.2″ E	7 km East of Khamir Port
A.khamirensis	RUZM 37	KJ652704	KJ652794	26º 30′ 47.4″ N; 55º 58′ 44.2″ E	7 km East of Khamir Port
A.khamirensis	RUZM 38	KJ652705	KJ652793	27º 02′ 58.9″ N; 53º 17′ 02.3″ E	7 km East of Khamir Port
A.khamirensis	RUZM 40	KJ652706	KJ652792	27º 02′ 58.9″ N; 53º 17′ 02.3″ E	7 km East of Khamir Port
A.khamirensis	ERP 863	KJ652707	KJ652790	26º 30′ 47.4″ N; 55º 58′ 44.2″ E	Hormozgan, Parsian
A.khamirensis	RUZM 149	KJ652708	KJ652797	26º 30′ 47.4″ N; 55º 58′ 44.2″ E	7 km East of Khamir Port
A.khamirensis	ERP 860	KJ652709	KJ652791	26º 30′ 47.4″ N; 55º 58′ 44.2″ E	Hormozgan, Parsian
A.blanfordi	ERP 851	KJ652710	KJ652809	29° 40′ 20.8″ N; 60° 50′ 01.9″ E	Qeshm Island
A.blanfordi	ERP 850	KJ652711	KJ652810	29° 40′ 20.8″ N; 60° 50′ 01.9″ E	Qeshm Island
A.blanfordi	ERP 848	KJ652712	KJ652811	29° 40′ 20.8″ N; 60° 50′ 01.9″ E	Qeshm Island
A.blanfordi	RUZM 47	KJ652713	KJ652812	29° 40′ 20.8″ N; 60° 50′ 01.9″ E	Qeshm Island
A.blanfordi	RUZM 101	KJ652714	KJ652813	29° 40′ 20.8″ N; 60° 50′ 01.9″ E	Chabahar-Beriis
A.blanfordi	RUZM 12	KJ652715	KJ652814	25° 15′ 07.9″ N; 60° 49′ 13.1″ E	Chabahar-Tiskoopan
A.blanfordi	RUZM 44	KJ652716	KJ652815	25° 15′ 07.9″ N; 60° 49′ 13.1″ E	Hormozgan-Minab
A.blanfordi	RUZM 45	KJ652717	KJ652816	25° 15′ 07.9″ N; 60° 49′ 13.1″ E	Hormozgan-Minab
A.blanfordi	RUZM 41	KJ652718	KJ652817	25° 15′ 07.9″ N; 60° 49′ 13.1″ E	Hormozgan-Minab
A.blanfordi	RUZM 43	KJ652719	KJ652818	25° 15′ 07.9″ N; 60° 49′ 13.1″ E	Hormozgan-Minab
A.blanfordi	RUZM 10	KJ652720	KJ652819	25° 15′ 07.9″ N; 60° 49′ 13.1″ E	Chabahar-Tiskoopan
A.blanfordi	RUZM 17	KJ652721	KJ652820	28º 56′ 56.19″ N; 51º 05′ 29.4″E	Chabahar-Govater
A.blanfordi	RUZM 15	KJ652722	KJ652821	28º 56′ 56.19″ N; 51º 05′ 29.4″E	Chabahar-Govater
A.blanfordi	RUZM 4	KJ652723	KJ652822	26° 56′ 23.37″N; 56° 2′ 46.23″E	Bampoor
A.blanfordi	RUZM 113	KJ652724	KJ652823	26° 56′ 23.37″N; 56° 2′ 46.23″E	Chabahar-Beriis
A.blanfordi	RUZM 102	KJ652725	KJ652824	26° 56′ 23.37″N; 56° 2′ 46.23″E	Chabahar-Beriis
A.blanfordi	RUZM 104	KJ652726	KJ652825	26° 56′ 23.37″N; 56° 2′ 46.23″E	Chabahar-Lipar
Acanthodactylus sp$_1$	RUZM 8	KJ652727	KJ652789	25° 15′ 07.9″ N; 60° 49′ 13.1″ E	Bampoor to Bazman road
Acanthodactylus sp$_1$	RUZM 5	KJ652728	KJ652788	27° 13′ 34.9″ N; 56° 23′ 22.3″ E	Bampoor to Bazman road
Acanthodactylus sp$_1$	RUZM 7	KJ652729	KJ652785	27° 13′ 34.9″ N; 56° 23′ 22.3″ E	Bampoor to Bazman road
Acanthodactylus sp$_1$	RUZM 97	KJ652730	KJ652787	25° 07′ 11.1″ N; 61° 16′ 33.1″ E	Bampoor to Bazman road
Acanthodactylus sp$_1$	RUZM 9	KJ652731	KJ652786	25° 15′ 07.9″ N; 60° 49′ 13.1″ E	Bampoor to Bazman road
A.nilsoni	RUZM 28	KJ652732	KJ652826	27° 13′ 34.9″ N; 56° 23′ 22.3″ E	Qasr-e-Shirin
A.nilsoni	RUZM 22	KJ652733	KJ652827	27° 13′ 34.9″ N; 56° 23′ 22.3″ E	Qasr-e-Shirin
A.nilsoni	RUZM 25	KJ652734	KJ652828	25° 10′ 12.7″ N; 61° 28′ 31.1″ E	Qasr-e-Shirin
A.nilsoni	RUZM 21	KJ652735	KJ652829	25° 10′ 12.7″ N; 61° 28′ 31.1″ E	Qasr-e-Shirin
A.nilsoni	RUZM 19	KJ652736	KJ652830	25° 07′ 11.1″ N; 61° 16′ 33.1″ E	Qasr-e-Shirin

.....continued on the next page

TABLE 1. (Continued)

Species	Collection number	GenBank accession numbers		Coordinates	Locality
		Cyt b	ND4 (tRNAs His+Ser+Leu)		
A.micropholis	RUZM 91	KJ652737	KJ652771	27° 12′18.6″ N; 60° 26′16.52″ E	Zahedan-Mianbazar
A.micropholis	RUZM 85	KJ652738	KJ652772	25° 07′ 11.1″ N; 61° 16′ 33.1″ E	Zahedan-Mianbazar
A.micropholis	RUZM 14	KJ652739	KJ652773	25° 06′ 58.5″ N; 61° 25′ 49.1″ E	Chabahar-Tiskoopan
A.micropholis	RUZM 131	KJ652740	KJ652774	27° 11′10.43″ N; 60° 22′46.2″ E	Chabahar-Tiskoopan
A.micropholis	RUZM 130	KJ652741	KJ652775	27° 11′10.43″ N; 60° 22′46.2″ E	Chabahar-Tiskoopan
A.micropholis	RUZM 129	KJ652742	KJ652776	27° 11′10.43″ N; 60° 22′46.2″ E	Chabahar-Tiskoopan
A.micropholis	RUZM 132	KJ652743	KJ652777	27° 11′10.43″ N; 60° 22′46.2″ E	Chabahar-Tiskoopan
A.micropholis	RUZM 1	KJ652744	KJ652778	27° 11′10.43″ N; 60° 22′46.2″ E	Chabahar-Tiskoopan
A.micropholis	RUZM 94	KJ652745	KJ652779	31° 30′ 38.7″ N; 48° 37′ 41.9″ E	Zahedan-Mianbazar
A.micropholis	RUZM 82	KJ652746	KJ652780	31° 30′ 38.7″ N; 48° 37′ 41.9″ E	Zahedan-Mianbazar
A.micropholis	RUZM 89	KJ652747	KJ652781	31° 30′ 38.7″ N; 48° 37′ 41.9″ E	Zahedan-Mianbazar
Acanthodactylus sp_2	RUZM 143	KJ652748	KJ652782	32° 14′ 56.2″ N; 47° 43′ 54.5″ E	Parsian
Acanthodactylus sp_2	RUZM 144	KJ652749	KJ652783	32° 14′ 56.2″ N; 47° 43′ 54.5″ E	Parsian
Acanthodactylus sp_2	RUZM 145	KJ652750	KJ652784	34 °32′10.95″N; 45° 38′47.10″E	Parsian
A.grandis	RUZM 150	KJ652751	KJ652837	34 °32′10.95″N; 45° 38′47.10″E	Bushehr- Alichangii
A.grandis	RUZM 157	KJ652752	KJ652836	34 °32′10.95″N; 45° 38′47.10″E	Bushehr- Alichangii
A.schmidti	RUZM 48	KJ652753	KJ652831	34 °32′10.95″N; 45° 38′47.10″E	30km N Ahwaz, Albaji
A.schmidti	RUZM 52	KJ652754	KJ652833	34 °32′10.95″N; 45° 38′47.10″E	30km N Ahwaz, Albaji
A.schmidti	RUZM 50	KJ652755	KJ652832	33°06′42.10″ N; 47°21′ 28.21″E	30km N Ahwaz, Albaji
A.schmidti	RUZM 67	KJ652756	KJ652834	33°06′42.10″ N; 47°21′ 28.21″E	Southern Ilam
A.schmidti	RUZM 65	KJ652757	KJ652835	34°16′19.25″N; 47°31′ 02.20″ E	Southern Ilam
A.boskianus (Z)	RUZM 135	KJ652758	KJ652798	34°16′19.25″N; 47°31′ 02.20″ E	Dehloran-Zarrinabad
A.boskianus (Z)	RUZM 134	KJ652759	KJ652799	34°16′19.25″N; 47°31′ 02.20″ E	Dehloran-Zarrinabad
A.boskianus (H)	RUZM 60	KJ652760	KJ652800	33° 22′ 56.7″ N; 46° 14′ 58.8 ″E	Dehloran-Golan
A.boskianus (H)	RUZM 63	KJ652761	KJ652801	33° 22′ 56.7″ N; 46° 14′ 58.8 ″E	Dehloran-Golan
A.boskianus (H)	RUZM 61	KJ652762	KJ652802	33° 22′ 56.7″ N; 46° 14′ 58.8 ″E	Dehloran-Golan
A.boskianus (H)	RUZM 59	KJ652763	KJ652803	33° 22′ 56.7″ N; 46° 14′ 58.8 ″E	Dehloran-Golan
A.boskianus (H)	RUZM 35	KJ652764	KJ652804	33° 03′ 56.4″N; 46° 54′ 04.47″E	Kermanshah, Harsin
A.boskianus (H)	RUZM 34	KJ652765	KJ652805	33° 03′ 56.4″N; 46° 54′ 04.47″E	Kermanshah, Harsin
A.boskianus (H)	RUZM 32	KJ652766	KJ652806	26°52′57.90″ N; 53° 35′37.12″E	Kermanshah, Harsin
A.boskianus (H)	RUZM 127	KJ652767	KJ652807	26°52′57.90″ N; 53° 35′37.12″E	Ilam, Darre Shahr
A.boskianus (H)	RUZM 126	KJ652768	KJ652808	26°52′57.90″ N; 53° 35′37.12″E	Ilam, Darre Shahr

Data analyses

DNA alignment: Sequences were aligned using ClustalW (Thompson, 1994) implemented in Bioedit version 7.0.0 (Hall, 1999). Before any analysis, all aligned sequences were translated into amino acids using vertebrate mitochondrial translation code implemented in the program MEGA V.5.0 (Kumar et al., 2008) to check if there were any inspected stop codons and to ensure that all the sequences were protein coding and functional instead of pseudogenes. Because substitution saturation would decrease, phylogenetic information contained in sequences (Xia & Lemey, 2009) saturation of our dataset was assessed with the program DAMBE V.4.2.7 (Xia & Xie, 2001).

TABLE 2. Primers used in this study and the sequence of each primer. F=Forward, R=Reverse primers. ND4 also include (tRNAs His+Ser+Leu).

Gene	Primer Name	Orientation	Primer Sequence	Reference(s)
Cytb	Lglulk	F	5′—AACCGCCTGTTGTCTTCAACTA–3′	Podnar *et al*. (2007), Schulte *et al*. (2012)
	Mtfsh	R	5'—TAGTTGGCCAATGATGATGAATGGGTGTTCTACTGG–3'	Dietzen *et al.* (2003)
	Ei700r	R	5′—GGGGTGAAAGGGGATTTT(AG)TC–3′	Rastegar Pouyani *et al.* (2009)
ND4	ND4F	F	5′—CACCTATGACTACCAAAAGCTCATGTAGAAGC–3′	Thaung *et al.*, 2009
	LeuR	R	5′—CATTACTTTTACTTGGATTTGCACCA–3′	Arevalo *et. al*. (1994)

Model determination and phylogeny reconstruction: To perform maximum likelihood and Bayesian analyses, the best-fitting evolutionary model was chosen for our dataset using jModelTest 2.1.1 (Posada, 2008), under Corrected Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC) criteria.

The software MEGA5 was used to infer the *p*-distance among taxa and the percentage of variable sites and parsimony informative sites in the data. Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic analyses were performed with the combined data set of the two genes using the heuristic search algorithm using PAUP 4.0b10 (Swofford, 2003) and MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003), respectively. All MP and ML tree reconstructions were performed with tree-bisection-reconnection (TBR) branch swapping (Swofford *et al.,* 1996), with 1000 bootstrap pseudoreplicates (Felsenstein, 1985) and random-additions of taxa for 1000 replications (in MP) and 100 replications (in ML). In running Bayesian analyses, default-heating values were used for four Markov chains and the same set of branch length for each partition. Each analysis was run for 10 million generations and started with randomly generating trees with sampling every 1000 generations. The first 25% of all saved trees were discarded as relative burning for diagnostics. Convergence of two runs was supported by average standard deviation of split frequencies lower than 0.01. The resulted trees were visualized and edited using MrEnt V.2.4 (Zuccon & Zuccon, 2013).

Estimation of divergence time and molecular clock. The likelihood ratio test did not reject the null hypothesis of a homogeneous clock-like rate of the data set. Comparison of the chronogram and phylogram by this test did not yield significant differences in the likelihood of these trees, suggesting that the error introduced by rate heterogeneity is not great. The log-likelihood value of the ML tree (ln= 9709.49799) was compared with that of the same tree constructed under the molecular-clock assumption (ln= 9720.75654); therefore, the likelihood ratio test statistic is LR = 22, $P < 0.05$. This suggests that we can probably use the genetic distance between populations inhabiting different geographical regions, in conjunction with geological information about the age of the events that are responsible for the separation of the regions, in order to estimate a local rate of evolution for *Acanthodactylus*.

We used the estimates of the age of the split and separation between two groups of lineages at the deeper nodes of lacertid lizards including Eremiadinae—Lacertinae as the calibration point Mayer & Pavlicev (2007) assumed this separation process and it assumes contact of Africa and Eurasia 17–19 million years ago, as a well dated geological event (Rögl 1999) and colonization of Africa by Lacertids in the early Neogenic. Based on this calibration point, we calibrated the molecular clock within our ML phylogeny.

The phenomenon of the Zagros Mountains uplift acted as a strong physical barrier against distribution of this genus toward the central Iranian plateau around 10–12.4 MYA (Sborshchikov *et al.*, 1981; Mouthereau, 2011), but this estimation cannot be correctly used as a calibration point, because it cannot express correctly the evolutionary history of lineages' speciation in the studied species.

Biogeography Analyses

The RASP (Reconstruct Ancestral State in Phylogenies software) V.2.1 (Yu *et al.*, 2011) was used to infer historical biogeography of the genus in Iran based on the ML estimate of phylogeny. This software was applied to Iranian species of the genus *Acanthodactylus* to find the possible ancestral ranges of lineages leading to terminal nodes and to identify the possible factors of dispersal and vicariance events responsible for the current distributional pattern

of the species. The genus is distributed in arid zones along the coastal line of the Persian Gulf and Oman Sea from western Iran to the border of Pakistan in southeastern Iran. Two geographic regions were chosen: 1—Western and south-western Iran represented by "A" and 2—South and south-eastern Iran represented by "B". These regions were defined based on the habitat characteristics, current distribution of the genus, geographic isolation and available distance and barriers for reconstruction of the historical distribution of lineages and distributional relationships in the biogeographic analyses. Default setting in RASP used for dispersal vicariance analyses (DIVA) includes: number of chains = 10, frequency of sample = 100, discard samples = 100, temperature = 0.1, maximum number of areas = 2, state frequencies = fixed (Jukes–Cantor) and an across-site rate variation= equal.

Results

The dataset included 606bp of Cytb, (265 positions [43.7%] variable and 234 positions [38.6%] parsimony informative), and 646bp of ND4 (327 positions [50.6%] variable and 288 positions [44.6%] parsimony informative). ND4 was followed by tRNAs, from 647 to 801, includes tRNA His (complete) + tRNA Ser (complete) + tRNA Leu (Partial). The combined dataset included 1407bp, of which 636 positions (45.2%) were variable and 564 positions (40.1%) were parsimony informative. Base composition average was 27.7% A, 13.6% G, 30.3% C and 28.4% T. A+T proportion (56.1%) is much higher than the C+G (43.9%) proportion. Statistical test for substitution saturation analyses (Xia *et al.,* 2003) showed no significant saturation in the dataset. The estimated Transition/Transversion bias (R) is 3.21. Uncorrected *p*-distance for the studied genes was between 4%–18% as genetic divergence among studied taxa (Tables 3 and 4), and 2.5–2.7% as intraspecific genetic divergence among *A. blanfordi* individuals as the only species with considerable genetic variability. Here, the 4% refers to the divergence between two populations of *A. boskianus* (Z) belongs to Zarrinabad (Ilam Province) and *A. boskianus* (H) belongs to Harsin and Mehran (Kermanshah and Ilam provinces). The divergence between different species is very high and starts at 7% to 18%, which is comparable to the reported genetic distances for Lacertid species (Carranza *et al.*, 2004; Rastegar-Pouyani *et al.*, 2009; Smid & Frynta, 2012).

TABLE 3. Pairwise uncorrected genetic divergence (*p*-distance) within *Acanthodactylus spp.,* derived from the mitochondrial gene Cytb.

R	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	*O.elegans*												
2	*E.persica*	0.20											
3	*A khamirensis*	0.21	0.21										
4	*A.blanfordi*	0.22	0.20	0.15									
5	*A.sp*$_1$	0.20	0.20	0.17	0.12								
6	*A.nilsoni*	0.21	0.23	0.17	0.16	0.17							
7	*A.micropholis*	0.21	0.20	0.06	0.15	0.16	0.17						
8	*A.sp*$_2$	0.21	0.22	0.17	0.16	0.15	0.16	0.17					
9	*A.grandis*	0.23	0.22	0.15	0.18	0.19	0.18	0.15	0.18				
10	*A.schmidti*	0.19	0.19	0.16	0.14	0.14	0.17	0.16	0.16	0.19			
11	*A.boskianus* (H)	0.20	0.21	0.16	0.17	0.19	0.13	0.17	0.16	0.19	0.19		
12	*A. boskianus* (Z) *(H)*	0.19	0.21	0.15	0.16	0.18	0.12	0.14	0.16	0.18	0.18	0.05	

Selected evolutionary model by jModelTest 2.1.1 for the combined dataset using AICc and BIC criteria was TrN+I+G as the best-fitting model. The proportion of invariable sites (I) = 0.4450, for among-site rate variation, its Gamma distribution shape parameter (a) = 0.8900. Each dataset of Cytb and ND4 sequences yielded strongly corresponding tree topologies with the combined data set for MP, ML and Bayesian analyses (relevant tree topologies not shown). So, only the topology based on the combination of both mitochondrial DNA genes (Cytb+ND4) is shown. Finally, only the tree inferred from Bayesian analysis as the single optimal tree is presented due to similar topologies resulting from ML, MP and Bayesian analysis.

TABLE 4. Pairwise uncorrected genetic divergence (*p*-distance) within *Acanthodactylus spp.,* derived from the mitochondrial gene ND4.

R	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	*O. elegans*												
2	*E. persica*	0.22											
3	*A. micropholis*	0.24	0.25										
4	*A. blanfordi*	0.26	0.27	0.17									
5	*A. sp$_1$*	0.25	0.26	0.17	0.11								
6	*A. boskianus* (H)	0.25	0.25	0.16	0.17	0.16							
7	*A. boskianus* (Z)	0.25	0.26	0.18	0.17	0.17	0.04						
8	*A. khamirensis*	0.25	0.24	0.07	0.17	0.18	0.18	0.19					
9	*A .nilsoni*	0.26	0.26	0.16	0.16	0.17	0.10	0.12	0.17				
10	*A. sp$_2$*	0.24	0.24	0.16	0.16	0.15	0.14	0.15	0.17	0.16			
11	*A. schmidti*	0.26	0.26	0.17	0.12	0.13	0.16	0.17	0.17	0.16	0.16		
12	*A. grandis*	0.25	0.25	0.15	0.16	0.16	0.17	0.18	0.15	0.17	0.17	0.17	

The phylogenetic analysis revealed three clades. One main clade (Clade A) includes *A. grandis, A. micropholis* and *A. khamirensis,* with weak support values for the divergence within (55.8% posterior probabilities and 73.5% [in ML] and 77.2% [in MP] bootstrap values). The next, clade B, is divided into two not supported subclades, B_1 and B_2. The results showed relatively strong support values for the radiation within subclade B_2 (the *A. schmidti* group), but not the same for subclade B_1 (the *A. boskianus* group) and clade A (the *A. micropholis* and *A. grandis* groups). Subclade B_2 includes *A. blanfordi*, *Acanthodactylus sp$_1$* and *A. schmidti* with high supporting values for the divergence within (1 posterior probabilities and 100% [in ML] and 94.1% [in MP] bootstrap values). Subclade B_1 contains *A. nilsoni, A. boskianus* complex and *Acanthodactylus sp$_2$* with low support values for the radiation within (posterior probabilities of 62% and bootstrap values of 50.3% [in ML] and 63% [in MP]).

The phylogenetic analysis shows *A. grandis* is close to the *A. micropholis* group (*A. micropholis* and *A. khamirensis*; Figure 2). In addition, the phylogenetic results revealed a monophyletic status for *A. schmidti* and *A. micropholis* groups in Iran.

Based on our examined materials, *Acanthodactylus sp$_1$* and *Acanthodactylus sp$_2$* are well-differentiated species, uniform in their distinctive features. The most similar species of the genus in the neighboring area of Iran with *Acanthodactylus sp$_2$* is *Acanthodactylus hardyi*. Three specimens of this species were also compared phylogenetically with *Acanthodactylus sp$_2$* using Cytochrome b gene and resulted in about 10% genetic differences and have a well supported and separated phylogenetic position with this species (Heidari, N. 2014, PhD thesis; Heidari et al 2014; works in progress on the description of the species).

Based on the molecular clock results (Figure 3), the ancestor of the existing species of the genus had undergone a diversification process around 8.5–9 MYA that led to the evolution of two lineages. One lineage includes *A. blanfordi, Acanthodactylus sp$_1$* and *A. schmidti*, and another includes *A. nilsoni, A. boskianus,* and *Acanthodactylus sp$_2$* with *A. micropholis, A. khamirensis* and *A. grandis*. Based on molecular clock analysis and dated tree, it seems that divergence time between *A. nilsoni* and *A. boskianus* is coincident with the divergence time between *A. blanfordi* and *Acanthodactylus sp$_1$* at around 3.5–4 MYA. The split of *A. schmidti* from *A. blanfordi* and *Acanthodactylus sp$_1$*, at about 5–5.5 MYA, is concurrent with the splitting time of *A. grandis* from *A. micropholis* and *A. khamirensis*. The discrepancy between lineage topology of *A. nilsoni*+ *A. boskianus*+ *Acanthodactylus sp$_2$* in the phylogram and the chronogram trees (Figures 2 and 3) may be due to the low support values. This subclade may find a fixed position by adding more samples of the studied taxa and closely related species as well as alternative mitochondrial alleles and the inclusion of nuclear genes,

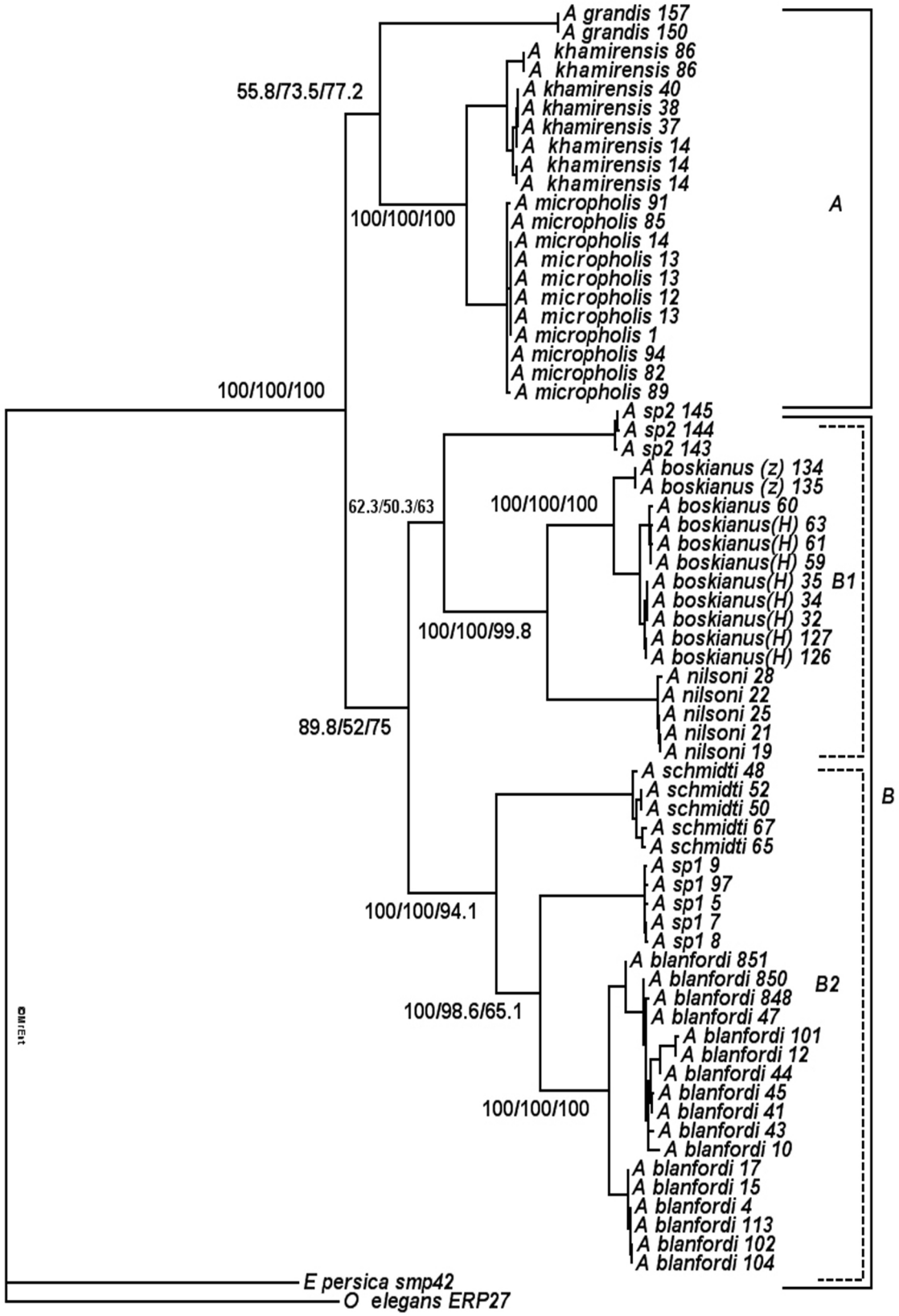

FIGURE 2. 50% majority rule consensus tree of the Bayesian analysis (under TrN+I+G evolutionary model) based on combined data set of 1407 bp of Cyt b and ND4 (tRNAs His+Ser+Leu). Numbers next to the nodes are posterior probabilities followed by ML/MP bootstrap support values. Significant values are usually over that 90% for BI and 80% for MP a ML.

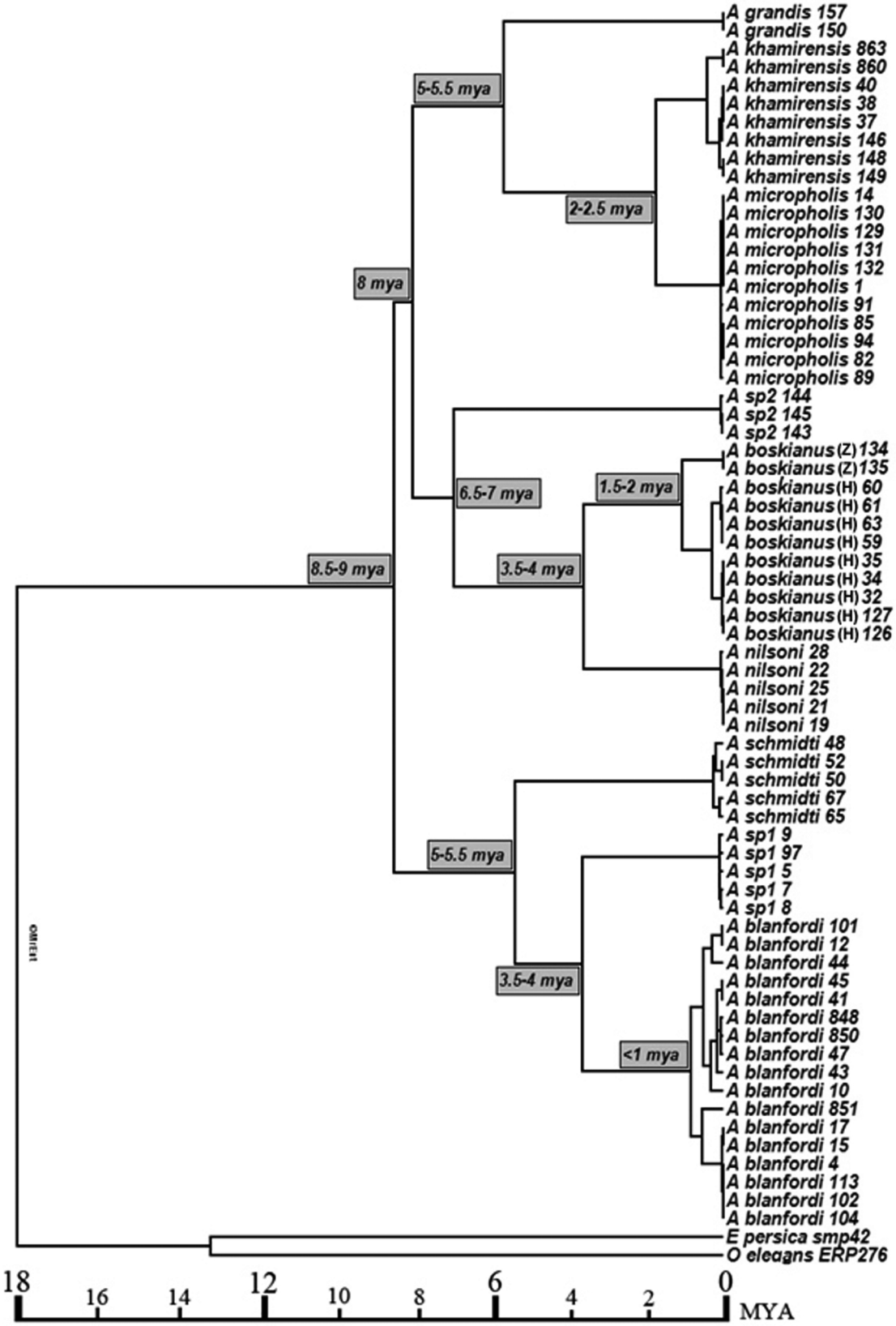

FIGURE 3. The Maximum Likelihood (ML) chronogram based on the Cyt b and ND4 fragments (ND4 was followed by tRNAs His+Ser+Leu) showing phylogenetic relationships and estimated divergence times of *Acanthodactylus* lineages in Iran. Numbers in gray rectangles beside the nodes represent divergence time of taxa.

Results of biogeography analysis

Lagrange results of vicariance analysis suggests an early vicariance event at node 137 and this event separated *Ohisops elegance* and *Eremias persica* (outgroup lineages) from remaining lineages of *Acanthodactylus* in Iran (Figure 4). This may have happened around 8.5–9 MYA according to the resulting chronogram at Figure 3. This node is also supported by 100% marginal probability and Bayesian support value for this node is 1.00. The *Acanthodactylus* lineage (node 136, see following) remained in western Iran and then underwent more diversification in the western slopes of the Zagros Mts. followed by subsequent dispersals towards southeastern, southern and then eastern Iran.

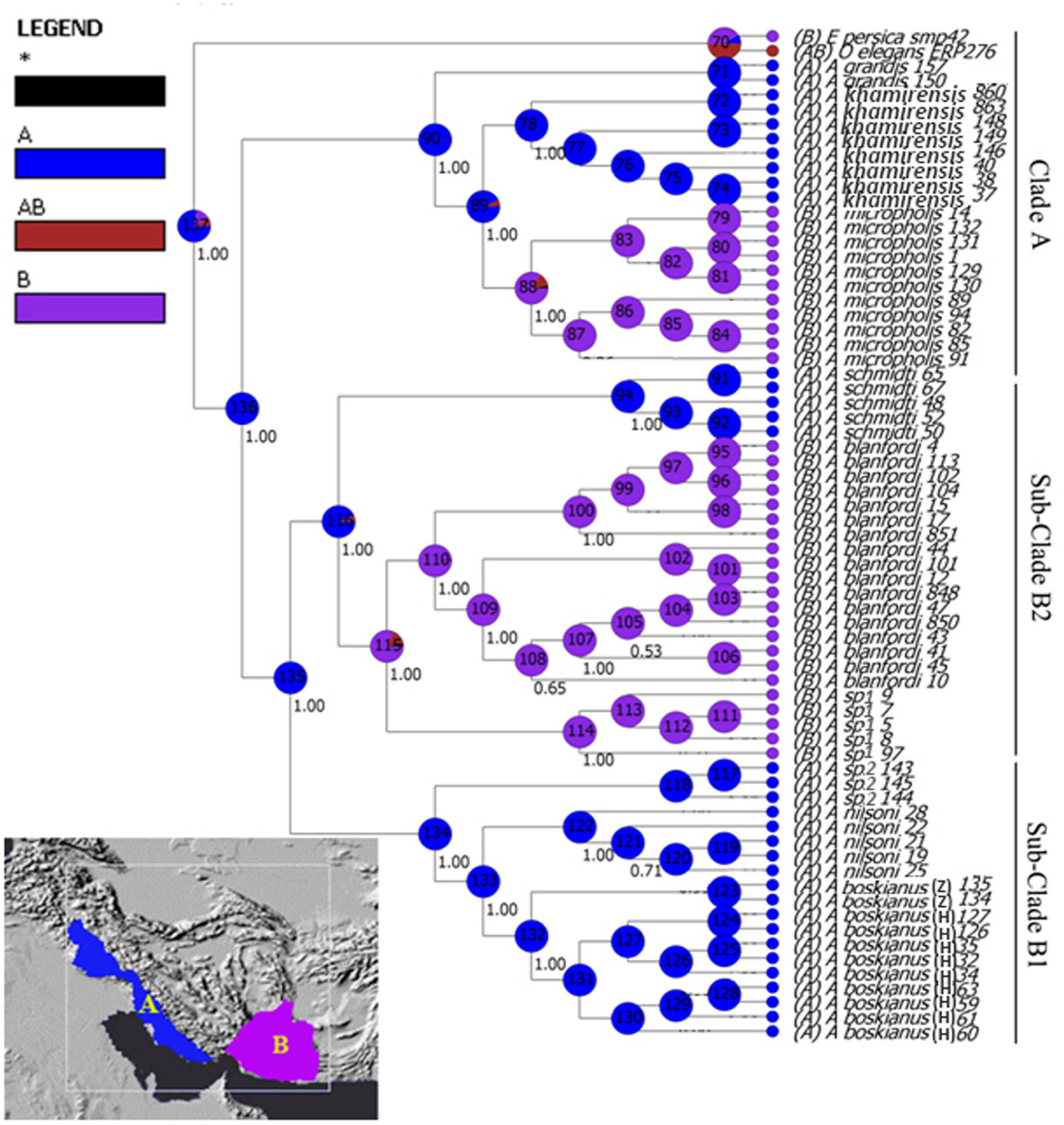

FIGURE 4. Graphical output of BBM analyses exported from RASP for *Acanthodactylus* in Iran. Single color means the node has a specific and single ancestral range, and multicolor mean; the node has multiple possible ancestral ranges. (A) Defined geographic range as western and southwestern of Iran. (B) Defined geographic range as south and southeastern Iran. (*) The black color means unknown origin or geographic range. Numbers inside the pies are node numbers and numbers beside pies or next to the nodes are Bayesian credibility values.

S-DIVA analysis suggests that one possible ancestral range for node 136 includes A (occurrence of this range is 100%). This node (node 136), proposes an early dispersal to eastern and southern Iran and the ancestors of taxa originated in region B (includes *A. micropholis, A. blanfordi* and *Acanthodactylus sp$_1$*) have one main source of distribution (A region), and have dispersed from region A to region B.

The ancestors of node 89 originated in A, B, and AB regions with 93.32% marginal probability (for A region), 0.69% (for B region) and 6% (for AB region) (Figure 4). In addition, the Bayesian support value for this node is 1.00. The same dispersal events of node 136 happened for node 89 and the ancestors of A (with most marginal probability, 98%) have dispersals to B region, creating *A. micropholis* and its relatives.

Taxa belonging to subclade B_2 (i.e., *A. boskianus, A. nilsoni* and *Acanthodactylus sp$_2$*) have ancestors from A and B regions, but taxa belonging to subclade B_1 (i.e., *A. blanfordi, A. schmidti* and *Acanthodactylus sp$_1$*) have ancestors from A region solely. The ancestor of both sub-clade B_1 and B_2, at node 135, originated in western Iran (region A) with 98.5% marginal probability and bayesian support value of 100.

The ancestral area reconstruction at node 116 proposes regions A and B as ancestral areas with 100% support values and 93.9% marginal probability for A and 5.5% marginal probability for AB. This points to a dispersal route eastward, with A region as a source area of this dispersal event to eastern Iran as a sink area. Results of dispersal vicariance analysis have clarified two separate ancestral sources for *A. micropholis* and *A. blanfordi* with *Acanthodactylus sp$_1$*. This means that *A. micropholis* originated during a separate phenomenon from an ancestral range for node 89 and had a recent common ancestor with *A. khamirensis*. However, the lineages leading to *A. blanfordi* and *Acanthodactylus sp$_1$* originated from a separate phenomenon (but both through dispersal, because no vicariance event has happened after originating node 137; data not shown). This node (115) has the most recent common ancestor with *A. schmidti*.

Discussion

Phylogenetic relationships among clades, species groups and systematic accounts.

We have produced the first detailed and well-supported molecular phylogeny for the Iranian species of the genus *Acanthodactylus*. Based on this study, the most genetic, ecologic and morphologic diversity amongst the Iranian species of *Acanthodactylus* is found in the western and southwestern slopes of the Zagros Mountains . Existing 7–18% genetic divergence of Cyt b among different species of the genus in the study area is very high compared to the calculated genetic difference of the Cyt b gene among other species of the genus (9.4–12.3% in the *A. erythrurus* group, Fonseca *et al.,* 2009). Genetic divergence using cytochrome b, between two lizard lacertid species of *Psammodromus hispanicus* and *Psammodromus edwardsianus* was estimated at 13% (Carranza *et al.,* 2006). High level of intraspecific genetic divergence in both *A. blanfordi* (2.5–2.7%, from 13 distinct localities over most of the species range in Iran) and *A. boskianus* populations (2%) indicates that these may be signs of a species complex or existence of different taxonomic units at the subspecific level and these taxa need more revision and sampling from more localities. Analyses of geographic variation in four population of *A.blanfordi* in S and SE Iran revealed some intraspecific variations based on morphological characters (Rastegar-Pouyani *et al.,* 2011).

In this study, two definite and species-rich sister clades (Clades A and B), were discovered within *Acanthodactylus* in Iran. A basal, not well supported, dichotomy in clade A separates *A. grandis* (lives in soft sandy habitats) from *A. khamirensis* and *A. micropholis*, which inhabit hard substrates and drier habitats. Clade B is divided into two distinct subclades (B_1 and B_2) that are fairly correlated with their habitat characteristics in nature. There is a highly variable and polymorphic unit in each of these subclades including *A. blanfordi* in subclade B_2 and *A. boskianus* in subclade B_1. In subclade B_2, a basal dichotomy separates *Acanthodactylus sp$_2$* (lives in soft sandy habitats) from *A. nilsoni* and *A. boskianus*. The two latter species inhabit hard substrates and drier habitats. This new species also forms a monophyletic group exhibiting a 15–16% genetic distance from its closest relatives. There is an incompatibility in position of subclade B_2 in the tree. Reconstructing phylogenetic trees using more mtDNA or nuclear DNA would certainly improve the posterior probability and bootstrap values and would fix the unstable position of these subclades in the resulting trees.

Based on the classifications presented by Arnold (1983) and Salvador (1982) and our phylogenetic analysis, four species groups within *Acanthodactylus* have been recognized in Iran. These include the A. *micropholis* group

with *A. micropholis* and *A. khamirensis*; The monotypic *A. grandis* group; *A. boskianus* group with *A. boskianus*, *A. nilsoni* and *Acanthodactylus sp*$_{2,}$ and *A. schmidti* group, with *A. blanfordi, A. schmidti* and *Acanthodactylus sp*$_{1}$. Our analysis confirmed the validity of *A. nilsoni* (Rastegar-Pouyani, 1998) as a distinct species in Iran and as a member of the *A. boskianus* species group, as there is no published data on this species after its description. Moreover, *A. khamirensis* is branched within the *A. micropholis* group that is coincident with their habitat characterization and other morphological characters including three series of scales on the fingers (Heidari *et al*., 2013). One population (n=6; Specimens 1, 14, 129–132 in Figure 1) of *A. micropholis* from Chabahar, Tisskopan shows a 1.5% genetic difference from another population of *A. micropholis* from Mianbazar, northern Zahedan, about 300km away (Specimens 82, 85, 89, 91, 94 in Figure 1). This divergence is fully supported by differences in habitat characterizations between northern and southern groups of *A. micropholis* (Personal observation). Two additional suspected *Acanthodactylus* species in Iran are revealed in this study. *Acanthodactylus sp*$_{1}$ is located within the *A. schmidti* group, with most ecological characters similar to other members of this group in Iran, as *A. blanfordi* (Heidari et al., unpublished). *Acanthodactylus sp*$_{2}$ is located within or close to the *A. boskianus* group (Heidari et al., unpublished). Each of the mentioned new species exhibited distinct genetic distance with other species. This implies that these levels of genetic divergence are in the range of interspecific genetic divergence among former recognized species of the genus and suggests that these forms should be assigned to the species level.

The close relationships between *A. schmidti* and *A. blanfordi* in our analysis are congruent with the results of Harris and Arnold (2000). Our study shows a different position for *A. grandis* as close to *A. khamirensis* and *A. micropholis* rather than to *A. boskianus* as presented by Arnold (1983) and Harris and Arnold (2000) using molecular and morphological studies. The split between *A. grandis* and the members of the *A. micropholis* group, however, is not supported.

Acanthodactylus boskianus, the most widespread species of the genus, ranging from North Africa to Iran, has already been divided into five subspecies (Salvador, 1982; Arnold, 1983; Trape *et al.,* 2012). The populations of *A. boskianus* in Iran are highly diverse; two individuals from southern Ilam (*A.boskianus* [Z]), showed 4% genetic differences (and 5% genetic differences based on Cyt b) from other *boskianus* populations from Kermanshah and Ilam (*A.boskianus* [H]) (Subclade B1 in Figure 2; Table 3). Morphological differences in *A. boskianus* (Z) compared to the formerly known populations of *A. boskianus* (H) in Iran includes larger dorsal scales, extending large ventral scales to laterals, more juxtaposed in ventral scales and larger temporal scales in *A. boskianus* (Z) compare to *A. boskianus* (H) (Heidari et al., work in progress).

Biogeography of the genus *Acanthodactylus* in Iran

The whole of African Lacertid lineages including *Acanthodactylus* split from Palearctic clades at about 24 MYA (Bohme & Corti, 1993). This splitting may coincide with the collision of the Arabian tectonic plate with the Eurasian landmass, which happened around 20–35 MYA (Dercourt *et al.*, 1986; Mouthereau, 2011; Smid & Frynta, 2012). A consequent event is the uplifting of the Zagros Mountains in western and southwestern Iran, dated around 10–12.4 Mya (Sborshchikov *et al.*, 1981; Mouthereau, 2011). This event has certainly acted as a strong biogeographical barrier to *Acanthodactylus*, affecting its systematics and distribution, as it did for many other lacertid lizards. The isolation of *Mesalina watsonana* from the remaining species of the genus, as well as the evolutionary history of *Eremias montanus* from a lineage of *Eremias persica* (Rastegar-Pouyani *et al.,* 2009) exemplify the important role of the Zagros Mts. uplift during the Miocene (Smid & Frynta, 2012),. Within *Acanthodactylus* this geological process has influenced clade B (Figure 2) more than the other clades since the origin and diversification of three taxa in clade B has happened in this area. In addition, *A. khamirensis* in clade A experienced diversification because of this process. The Zagros Mountains run from northwest to southeast of Iran which is situated at the direct route of distribution of *Acanthodactylus* lineages (from west to east) and serves as a barrier for dispersal from the western parts of Iran to the central Iranian plateau. This hypothesis is supported by the observation that the same favorite habitats for the genus (sandy soils) are found in the central Iranian plateau but with no occurrence of the genus there (Arnold, 1983; Leviton *et al.*, 1992; Anderson, 1999). All the evidence on current distribution patterns of the genus in Iran, herpetological studies, and our fieldwork studies support this hypothesis. This uplifting as a barrier could play a key role in driving *Acanthodactylus* species along western and southern margins of the Zagros Mountains towards eastern Iran and along sandy, arid and shoreline habitats of the Persian Gulf and Oman Sea and then towards Afghanistan and Pakistan. Therefore, the invasion of the ancestors of

the genus *Acanthodactylus* from Africa and the Middle East towards Iran would have happened after the uplifting of the Zagros Mountains. With this hypothesis, we can date back the entrance of the genus into Iran during the Late Miocene, around 10–12.4 MYA. Similar dispersal events have occurred during the late Miocene (9–10 MYA) in accordance with the geological event of the uplifted Zagros Mts. The agamid *Paralaudakia erythrogastra* was formed and originated from the main lineage of *P. caucasia* (Macey *et al.*, 1998, 2000), and in the *Eremias persica* complex, the uplifting of the Zagros Mts. has created two eastern and western clades along the sides of the mountains around 10–11 MYA (Rastegar-Poyani *et al.*, 2008). Specialized animals have less ability to disperse and this may explain why *Acanthodactylus* dispersed across the western and southern margins of the Zagros Mountains along shorelines of the Persian Gulf and did not disperse towards the central Iranian Plateau (through the Zagros Mts.), We suggest that the eastern taxa of the genus *Acanthodactylus* in Iran have originated from western Iran.
The most suitable scenario for the distribution and evolution of the Iranian species of the genus *Acanthodactylus* is by dispersal after uplifting of the Zagros Mts. Other alternative scenarios regarding the occurrence of this genus in Iran cannot be supported here because the species of the genus are absent in the opposite slope of the Zagros Mts. (the eastern slopes), and the high diversity of species in the western and southwestern parts of Iran and the southern slopes of the Zagros Mts. Since dispersal to new and restricted ecological niches in western and southern Iran, the genus has radiated into several species (currently there are about seven defined species in the western towards southern parts of Iran). These processes may have been forced by ecological divergence, competition for acquiring habitats among populations and then by acquiring reproductive isolations (Rundell & Price, 2009). Diverse ecological environments and existing different habitats (sandy hills with soft and hard substrates; plains at low and high elevations) in these regions have facilitated the speciation process of the genus in Iran.

Combined molecular and morphological analyses by Harris and Arnold (2000) suggested that the genus *Acanthodactylus* originated in southwestern Asia and Middle East during the mid-Miocene connection between Asia and Africa and later invaded North Africa on several occasions. Accordingly, it can be suggested that eastward invasion from southwestern Asia occurred during the lower Pliocene. Therefore, the extant species of the genus in Iran and along the shores of Persian Gulf and Oman Sea, towards Pakistan and northwestern India (*A. cantoris*), may be descendants of these lineages, which then led to the establishment of *A. micropholis*, *A. blanfordi*, *A. khamirensis, Acanthodactylus sp$_1$, Acanthodactylus sp$_2$*, and *A. nilsoni*. The current interspecific and intraspecific genetic and morphological variation and divergence of *Acanthodactylus* lineages in Iran may be due to the long time from the initial dispersal of the main lineages from western Iran towards the east to Pakistan, Afghanistan and northwestern India.

Among these 10 out of 42 species of *Acanthodactylus*, it seems that *A. nilsoni* (western Iran), *Acanthodactylus sp$_1$* (Southeastern Iran), *A. khamirensis* (southern Iran) and *Acanthodactylus sp$_2$* (southern Iran) are endemic species for different parts of Iran.

Acknowledgments

We wish to thank Razi University authorities for their help and support during fieldwork and collecting specimens. We also thank the honorable authorities of Hakim Sabzewari University for help in doing lab works. The authors wish to thank Professor Göran Nilson (from the Gothenburg Natural History Museum, Sweden) for loan of *Acanthodactylus* specimens from his Collection, Prof. Steven C. Anderson for reading the manuscript and providing helpful improvements, and Dr. Yu Yan for invaluable technical support during analyzing and interpreting the RASP analyses. We would also like to thank the following persons contributing greatly in different parts of this study, either during field work or during lab work; Behzad Fathinia, Rasoul Karamiani, Azar Khosrawani, Hamze Orei and Saeed Hosseinian Yousefkhani. The authors thank Mr. Parviz Heidari for his cooperation and great effort in collecting specimens and his financial support during field work.

Literature cited

Ahmadzadeh, F., Carretero, M.A., Harris, D.J., Perera, A. & Bohme, W. (2012) A molecular phylogeny of the eastern group of ocellated lizard genus *Timon* (Sauria: Lacertidae) based on mitochondrial and nuclear DNA sequences. *Amphibia-Reptilia*, 33, 1–10.

http://dx.doi.org/10.1163/156853811X619718
Anderson, S.C. (1999) *The Lizards of Iran.* Society for the Study of Amphibians and Reptiles. Oxford, Ohio, 442 pp.
Arevalo, E., Davis, S. & Sites, J. (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology*, 43, 387–418.
http://dx.doi.org/10.1093/sysbio/43.3.387
Arnold, E.N. (1981) Estimating phylogenies at low taxonomic levels. *Journal of Zoological Systematics and Evolutionary Research*, 19, 1–35.
http://dx.doi.org/10.1111/j.1439-0469.1981.tb00230.x
Arnold, E.N. (1983) Osteology, genitalia and the relationships of *Acanthodactylus* (Reptilia: Lacertidae). *Bulletin of the British Museum* (*Natural History*). *Zoology Series*, 44 (5), 1–86.
Boulenger, G.A. (1918) Sur les lezards du genre *Acanthodactylus* Wieg. *Bulletin de la Société zoologique de France*, 43, 143–155.
Boulenger, G.A. (1921) *A monograph of the Lacertidae*. *Vol. 2.* British Museum (Natural History), London, viii + 451 pp.
Böhme, W. & Corti, C. (1993) Zoogeography of the lacertid lizards of the western Mediterranean basin. *Lacertids of the Mediterranean region,* pp. 17–33
Carretero, M.A., Fonseca, M., García-Muñoz, E., Brito, J. & Harris, D.J. (2011) Adding *Acanthodactylus beershebensis* to the mtDNA phylogeny of the *Acanthodactylus pardalis* group. *North-Western Journal of Zoology*, 7 (1), 138–142.
Carranza, S., Arnold, E.N. & Amat, F. (2004) DNA phylogeny of *Lacerta* (*Iberolacerta*) and other lacertine lizards (Reptilia: Lacertidae): did competition cause long term mountain restriction? *Systematic Biodiversity*, 2, 57–77.
http://dx.doi.org/10.1017/S1477200004001355
Crochet, P.A., Geniez, P. & Ineich, I. (2003) A multivariate analysis of the fringe-toed lizards of the *Acanthodactylus scutellatus* group (Squamata: Lacertidae): systematic and biogeographical implications. *Zoological Journal of the Linnaean Society*, 137, 117–155. [with 21 figures]
Dercourt, J., Zonenshain, L.P., Ricou, L.E., Le Pichon, X., Knipper, A.L., Grandjaquet, C., Sbortshikov, I.M., Geussant, J., Lepvrier, C., Pechersku, D.H., Boulin, J., Bazhenov, M.L., Lauer, J.P. & Biju-Duval, B. (1986) Geological evolution of the Tethys belt from the Atlantic to the Pamirs since the Lias. *Tectonophysics,* 123, 241–315.
http://dx.doi.org/10.1016/0040-1951(86)90199-X
Dietzen, C., Witt, H.H. & Wink, M. (2003) The phylogeographic differentiation of the European robin *Erithacus rubecula* on the Canary Islands revealed by mitochondrial DNA sequence data and morphometrics: evidence for a new robin taxon on Gran Canaria? *Avian Science,* 3, 115–131.
Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution,* 39, 783–791.
http://dx.doi.org/10.2307/2408678
Fonseca, M.M., Brito, J.C., Kaliontzopoulou, A., Rebelo, H., Kalboussi, M., Larbes, S., Carretero, M.A. & Harris, D.J. (2008) Genetic variation in the *Acanthodactylus pardalis* group in North Africa. *African Zoololgy*, 43, 8–15.
http://dx.doi.org/10.3377/1562-7020(2008)43[8:GVASLI]2.0.CO;2
Fonseca, M.M., José, C.B., Octávio, S.P., Miguel, A.C. & Harris, D.J. (2009) Systematic and phylogeographical assessment of the *Acanthodactylus erythrurus* group (Reptilia: Lacertidae) based on phylogenetic analyses of mitochondrial and nuclear DNA. *Molecular Phylogenetics and Evolution*, 51, 131–142.
http://dx.doi.org/10.1016/j.ympev.2008.11.021
Graybeal, A. (1994) Evaluating the phylogenetic utility of genes: a search for genes informative about deep divergences among vertebrates. *Systematic Biology*, 43, 174–193.
http://dx.doi.org/10.1093/sysbio/43.2.174
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.
Harris, D.J., Arnold, E.N. & Thomas, R.H. (1998) Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proceedings of the Royal Society B: Biological Sciences,* 265, 1939–1948.
http://dx.doi.org/10.1098/rspb.1998.0524
Harris, D.J. & Arnold, N. (2000) Elucidation of the relationships of spiny-footed lizards, *Acanthodactylus spp.* (Reptilia: Lacertidae) using mitochondrial DNA sequence, with comments on their biogeography and evolution. *Journal of Zoology London*, 252, 351–362.
http://dx.doi.org/10.1111/j.1469-7998.2000.tb00630.x
Harris, D.J., Batista, V. & Carretero M.A. (2004) Assessment of genetic diversity within Acanthodactylus erythrurus (Reptilla: Lacertidae) in Morocco and the Iberian Peninsula using mitochondrial DNA sequence data. *Amphibia-Reptilia,* 25 (2), 227–232.
http://dx.doi.org/10.1163/1568538041231229
Heshmati, G.A. (2007) Vegetation characteristics of four ecological zones of Iran. *International Journal of Plant Production,* 1 (2), 215–224.
Heidari, N. (2014) *Systematics and phylogeny of lizard's genus Acanthodactylus Fitzinger, 1843 in Iran based on molecular and morphological characters.* PhD thesis, Razi University, 255 pp.

Heidari, N., Rastegar Pouyani, N., Rastegar-Pouyani, E. & Rajabizadeh, M. (2013) A new species of *Acanthodactylus* Fitzinger 1834 (Sauria: Lacertidae) from southern Iran. *Zootaxa,* 3722 (3), 333–346.
http://dx.doi.org/10.11646/zootaxa.3722.3.3

Fu, J. (2000) Toward the phylogeny of the family Lacertidae, Why 4708 base pairs of mtDNA sequences cannot draw the picture. *Biological Journal of the Linnean Society*, 71, 203–217. [with two figures]
http://dx.doi.org/10.1111/j.1095-8312.2000.tb01254.x

Kumar, S., Dudley, J., Nei, M. & Tamura, K. (2008) MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics*, 9, 299–306.
http://dx.doi.org/10.1093/bib/bbn017

Leviton, A.E., Anderson, S.C., Adler, K. & Minton, S.A. (1992) *Handbook to Middle East Amphibians and Reptiles.* SSAR, Oxford, Ohio, 252 pp. [Contr. to Herpetol. No. 8]

Macey, J., Robert, J.A., Schulte, J.A., Ananjeva, N.B., Larson, A., Rastegar-Pouyani, N., Sakhat, M.S. & Papenfuss, T.J. (1998) Phylogenetic Relationships among Agamid Lizards of the *Laudakia caucasia* Species Group: Testing Hypotheses of Biogeographic Fragmentation and an Area Cladogram for the Iranian Plateau. *Molecular Phylogenetics and Evolution*, 10 (1), 118–131.
http://dx.doi.org/10.1006/mpev.1997.0478

Macey, J.R., Schulte, J.A., Kami, H.G., Ananjeva, N.B., Larson, A. & Papenfuss, T.J. (2000) Testing hypotheses for vicariant separation in the agamid lizard *Laudakia caucasia* from mountain ranges of the Northern Iranian plateau. *Molecular Phylogenetics and Evolution,* 14 (3), 479–483.
http://dx.doi.org/10.1006/mpev.1999.0722

Mayer, W. & Benyr, G. (1994) Albumin-Evolution und Phylogenese in der Familie Lacertidae (Reptilia: Sauria). *Annuals of Natural History Museum,* 96B, 621–648.

Mayer, W. & Pavličev, M. (2007) The phylogeny of the family Lacertidae (Reptilia) based on nuclear DNA sequences: convergent adaptations to arid habitats within the subfamily Eremiainae. *Molecular Phylogenetics and Evolution,* 44 (11), 55–63.

Mouthereau, F. (2011) Timing of uplift in the Zagros belt/Iranian plateau and accommodation of late Cenozoic Arabia-Eurasia convergence. *Geological Magazine,* 148 (5–6), 726–738.
http://dx.doi.org/10.1017/S0016756811000306

Podnar, M., Pinsker, W. & Haring, E. (2007) Unusual origin of a nuclear pseudogene in the Italian wall lizard: intergenomic and interspecific transfer of a large section of the mitochondrial genome in the genus *Podarcis* (Lacertidae). *Journal of Molecular Evolution*, 64, 308–320.
http://dx.doi.org/10.1007/s00239-005-0259-0

Posada, D. (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution,* 25, 1253–1256.
http://dx.doi.org/10.1093/molbev/msn083

Pyron, R.A., Frank, T.B. & John, J.W. (2013) A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology*, 13, 1–93.

Rastegar-Pouyani, N. (1998) A new species of *Acanthodactylus* (Sauria: Lacertidae) from Qasr-e-Shirin, Kermanshah Province, Western Iran. *California Academy of Science*, 50 (9), 257–265.

Rastegar-Pouyani, N. (1999a) *Systematics and Biogeography of Iranian Plateau Agamids (Reptilia: Agamidae).* PhD Thesis. Gothenburg University, Gothenburg, v + 182 pp. [Sweden]

Rastegar-Pouyani, N. (1999b) Analysis of Geographic Variation in *Trapelus agilis* Complex (Sauria: Agamidae). *Zoology in the Middle East*, 18, 75–99.
http://dx.doi.org/10.1080/09397140.1999.10637798

Rastegar-Pouyani, N. (1999c) Two New Subspecies of *Trapelus agilis* Complex (Sauria: Agamidae) from Lowland Southwestern Iran and Southeastern Pakistan. *Asiatic Herpetological Research,* 8, 90–101.

Rastegar-Pouyani, N. & Nilson, G. (2002) Taxonomy and biogeography of the Iranian species of *Laudakia* (Sauria: Agamidae). *Zoology in the Middle East,* 26, 93–122.
http://dx.doi.org/10.1080/09397140.2002.10637926

Rastegar Pouyani, E., Rastegar Pouyani, N., Kazemi Noureini, S., Joger, U. & Wink, M. (2009) Molecular phylogeny of the *Eremias persica* complex of the Iranian plateau (Reptilia: Lacertidae), based on mtDNA sequences. *Zoological Journal of the Linnean Society,* 158 (3), 641–660.
http://dx.doi.org/10.1111/j.1096-3642.2009.00553.x

Rastegar-Pouyani, N., Heydari, N. & Faizi, H. (2011) Analysis of Geographic Variation in Four Populations of *Acanthodactylus blanfordi* Boulenger, 1918 (Sauria: Lacertidae) from Southern and Southeastern Iran. *Russian Journal of Herpetology*, 18 (3), 84–93.

Rastegar-Pouyani, N., Kami, H.G., Rajabizadeh, M., Anderson, S.C. & Shafiei, S. (2008) Annotated Checklist of Amphibians and Reptiles of Iran. *Iranian Journal of Animal Biosystematics*, 4 (1), 43–66.

Rögl, F. (1999) Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short overview). *Geologica Carpathica,* 50, 339–349.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics,* 19, 1572–1574.

http://dx.doi.org/10.1093/bioinformatics/btg180
Rundell, R.J. & Price, T.D. (2009) Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology and Evolution*, 24 (7), 394–399.
http://dx.doi.org/10.1016/j.tree.2009.02.007
Salvador, A. (1982) A review of the lizards of the genus *Acanthodactylus* (Sauria: Lacertidae). *Bonner Zoologische Monographien*, 16, 1–167.
Sborshchikov, I.M., Savostin, L.A. & Zonenshan, L.P. (1981) Present plate tectonics between Turkey and Tibet. *Tectonophysics,* 79, 45–73.
http://dx.doi.org/10.1016/0040-1951(81)90232-8
Schulte, U. & Lotters, A. (2012) Cryptic niche conservatism among evolutionary lineages of an invasive lizard. *Global Ecology and Biogeography*, 21, 198–211.
http://dx.doi.org/10.1111/j.1466-8238.2011.00665.x
Sindaco, R. & Jeremcenko, V.K. (2008) *The reptiles of the Western Palearctic*. Edizioni Belvedere, Latina (Italy), 579 pp.
Smid, J. & Frynta, A. (2012) Molecular variability of *Mesalina watsonana* (Reptilia: Lacertidae) on the Iranian plateau and its phylogenetic and biogeographic affinities inferred from mtDNA sequences. *Acta Herpetologica*, 7 (1), 139–153.
Swofford, D.L. (2003) PAUP*. Phylogenetic Analysis Using Parsimony (and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
Swofford, D.L., Olsen, G.J., Waddell, P.J. & Hillis, D.M. (1996) Phylogenetic inference. *In*: Hillis, D.M., Moritz, C. & Mable, B.K. (Eds.), *Molecular Systematics*. Sinauer Associates, Sunderland, Massachusetts, pp. 407–514.
Thaung, H., Willoughby, T., Pradya, S., Duong S., Setha, T., Sein, M., Chang, M. & Catherine, W. (2009) Mitochondrial pseudogenes in the nuclear genome of *Aedes aegypti* mosquitoes: implications for past and future population genetic studies. *BMC Genetics,* 10, 11.
http://dx.doi.org/10.1186/1471-2156-10-11
Thompson, J.D. (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.
http://dx.doi.org/10.1093/nar/22.22.4673
Trapé, J.F., Trapé, S. & Chirio, L. (2012) *Lézards, crocodiles et tortues d'Afrique occidentale et du Sahara*. IRD Orstom, Montpellier, 503 pp.
Uetz, P. (2013) The Reptile database. Available from: http://wwwreptile-databaseorg/ (accessed 13 August 2014)
Xia, X. & Xie, Z. (2001) DAMBE: Software Package for Data analysis in molecular biology and evolution. *Journal of Heredity,* 92 (4), 371–373.
http://dx.doi.org/10.1093/jhered/92.4.371
Xia, X. & Lemey, P. (2009) *Assessing substitution saturation with DAMBE*. *In*: Lemey, P., Salemi, M. & Anne-Mieke Vandamme, A.-M. (Eds.), *The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny. 2nd Edition*. United States of America by Cambridge University Press, New York, pp. 615–630.
Xia, Y., Yang, P. & Sun, Y. (2003) "One-Dimensional Nanostructures: Synthesis, Characterization, and Applications". *Advanced Materials,* 15 (5), 353–389.
http://dx.doi.org/10.1002/adma.200390087
Yu, Y., Harris, A.J. & He, X.J. (2011) *RASP (Reconstruct Ancestral State in Phylogenies) 2.0* beta. Available from: http://mnh.scu.edu.cn/soft/blog/RASP (accessed 13 August 2014)
Zuccon, A. & Zuccon, D. (2013) *MrEnt V.2.4.* The drawing tool for phylogenetic trees. Available from: http://www.mrent.org (accessed 13 August 2014)