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A new species of the genus *Eremias* Fitzinger, 1834 (Squamata: Lacertidae) from Central Iran, supported by mtDNA sequences and morphology

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Abstract

A new species of the lacertid genus *Eremias* Fitzinger, 1834 is described from northwest of Isfahan province, Central Iran. Two mitochondrial genes (cyt *b* and 12S DNA) were sequenced and analyzed as reliable molecular markers for the separation of this newly discovered species from closely related species within the genus *Eremias*: *E. velox, E. persica, E. papenfussi, E. lalezharica, E. montana, E. strauchi, E. kopetdaghica* and *E. suphani*. Genetic distances (K2-p) between any of these species with the newly described species are relatively high (27.5–32.8% for cyt *b* and 5.2–10.4% for 12S DNA). Phylogenetic analyses (MP, ML and BI) generated trees with very similar topologies. According to molecular and morphological data, *Eremias isfahanica* **sp. nov.** belongs to the subgenus *Aspidorhinus*, and is closely related to *E. papenfussi*. Because several new *Eremias* species have recently been described from the Iranian Plateau, we additionally provide an updated identification key.

Key words: Lacertidae, mtDNA, Iranian Plateau, *Eremias isfahanica* **sp. nov.**

Introduction

Lizards of the family Lacertidae have a widespread distributional range in Eurasia and all of Africa (Fu, 1998). The genus *Eremias* Fitzinger, 1834 is one of the members of the tribe Eremiadini (and within it assigned to the Saharo-Eurasian clade; Arnold, 1989; Mayer & Pavlicev, 2007; Kapli *et al.,* 2011; Pyron *et al.,* 2013) that inhabits mostly arid and xeric habitats in the Saharo-Euroasian region (Sindaco & Jeremčenko, 2008).

The genus *Eremias* comprises about 35 species (Uetz & Hošek, 2015) that inhabit steppe, sand and desert regions from northern China, Mongolia, Central and southwest Asia, and Korea to southeastern Europe (Sindaco & Jeremčenko, 2008). Among them, 18 species have been reported from Iran territory in all parts of the country except regions of high elevation (Anderson, 1999; Rastegar-Pouyani *et al.,* 2010; Rastegar-Pouyani & Nilson, 2011; Šmid *et al*., 2014). According to Szczerbak (1974). Based on morphological characters (Ananjeva *et al.,* 1998; Chirikova, 2004; Guo *et al.*, 2010), hemipenial features (Arnold, 1986), and revisions (Barabnov, 2009; Gou et al., 2011), five subgenera were classified within *Eremias* (though the assignment of some species is still unclear): *Eremias* Fitzinger, 1834 (*E. velox* group); *Rhabderemias* Lantz, 1928 (*E. scripta–E. lineolata* group); *Aspidorhinus* Eichwald, 1841 (*E. montana* group); *Scapteira* Fitzinger, 1834 (*E. grammica* group); *Pareremias* Szczerbak, 1973 (*E. multiocellata* group).

Eremias montana, from the subgenus *Aspidorhinus*, was described from Siyah Darreh village in northeastern of Kermanshah, and is endemic to Iran (Rastegar-Pouyani & Rastegar-Pouyani, 2001). After its description, several

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individuals of this species have been collected from populations near the Zagros and Elburz Mountains in southwestern and northern Iran (Fig. 1). The substantial morphological variation among these populations led to the classification of *E. montana* as a species complex (Rastegar-Pouyani & Rastegar-Pouyani, 2001). Recently, Mozaffari *et al.* (2011) described *Eremias papenfussi* from central Elburz Mts. as a member of this complex.

During our expedition to Central Iran, five specimens tentatively assigned to the *E. montana* complex were collected from the Ghamishloo National Park in the Central Zagros Mts. Preliminary examination of the collected specimens, using morphological keys (Minton *et al*., 1992; Anderson, 1999), indicated that this population is similar to species within the subgenera *Aspidorhinus* and *Eremias*; however, it could not be attributed to any of the known species of the genus in Iran. In this study, we present the description of this population from Isfahan Province, Central Zagros, as a new species of the genus *Eremias* in Iran. We used two mitochondrial markers (cyt *b* and 12S DNA) to estimate the phylogenetic structure and genetic distances among the new population and those of the morphologically similar Iranian species of the subgenera *Aspidorhinus* and *Eremias*. In addition, we provide a new identification key for *Eremias* species in Iran.

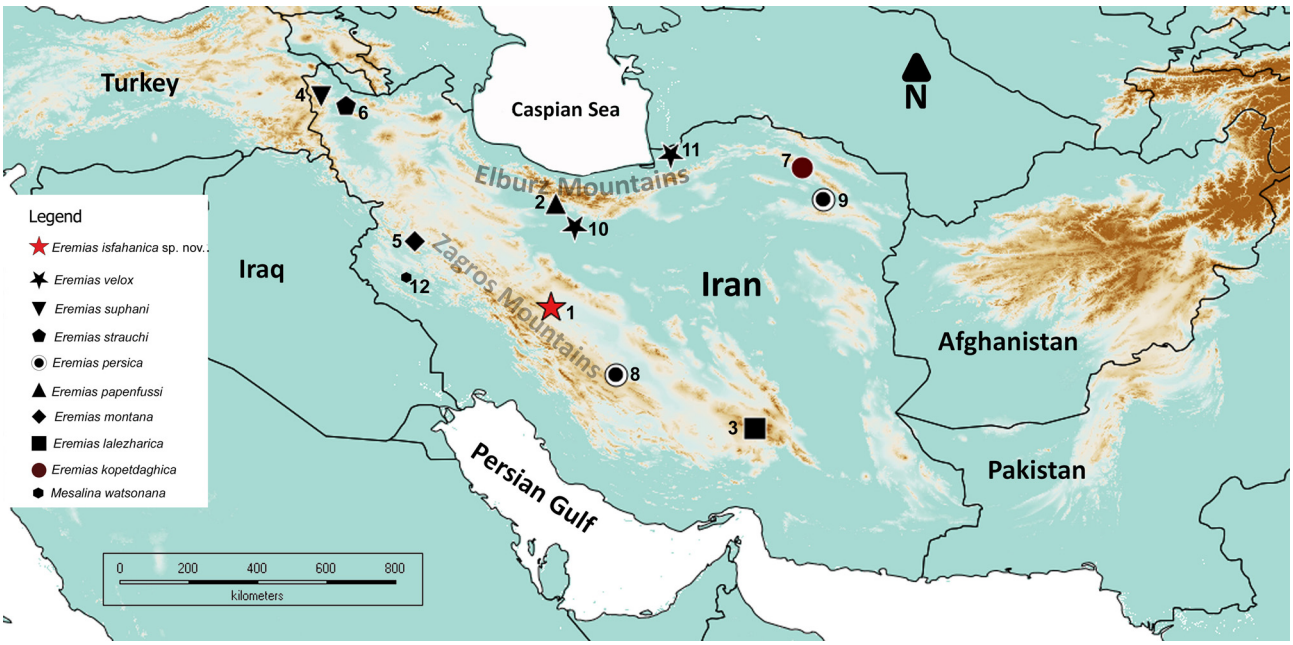

FIGURE 1. Map of Iran with sampling locations included in this study. Locality codes correspond to localities presented in Table 1. Taxon names correspond to changes proposed in this paper.

Materials and methods

The five *Eremias* specimens of the new population from Isfahan Province, Central Zagros, and their tissue samples have been deposited in Sabzevar University Herpetological Collection (SUHC) with related museum numbers. For the taxonomic and phylogenetic analyses, we examined and compared the new population with the following eight morphologically related Iranian species from the subgenera *Eremias* and *Aspidorhinus*: *E. kopetdaghica, E. lalezharica, E. montana, E. papenfussi, E. persica, E. strauchi, E. suphani* and *E. velox* (see Table 1 for specimen codes and localities).

Molecular analysis. The five individuals of the newly recognized population and 30 sequences of closely related species of *Eremias* in Iran were included in the molecular study (25 sequences were retrieved from GenBank; Table 1). A distribution map with the type locality of the new species, and the localities for the other species included in this study, is presented in Figure 1. Localities and GenBank accession numbers are presented in Table 1. To root the phylogenetic tree, *Mesalina watsonana* was selected as the outgroup taxon (Harris *et al.,* 1998; Fu, 2000; Kapli *et al.,* 2011; Pyron *et al.,* 2013).

Genomic DNA was extracted from tissue samples using the salt-based method (Kabir *et al.,* 2012). Two

mitochondrial genes, cytochrome *b* (cyt *b*) and 12S ribosomal DNA (12S DNA) were selected for this study. Partial fragments of the two genes were amplified using the following primers: For cyt *b*, Mtanew 5′-CTCCCAGCCCCATCCAAC ATCTCAGGATGATGAAAC-3′ and Mtfsh 5′-TAGTTGGCCAATGATGATGAAT GGGTG TTCTACTGG-3′ (Rastegar-Pouyani *et al.,* 2013); For 12S (forward strand solely), 12Sa 5′-AAACTG GGATTAGATACCCCACTAT-3′ and 12Sb 5′-GAGGGT GAC GGG CGG TGT GT-3′ (Kocher *et al.,* 1989).

We used Clustal W, as implemented in BioEdit sequence alignment editor v.7.0 (Hall, 1999), to align sequences, with default parameters. Sequences of cyt *b* were analysed with Mega v.6.0 (Tamura *et al.,* 2013) and no stop codons were observed. Genetic distances (K2-p distances) were calculated using Mega v.6.0 for each marker independently. Phylogenetic analyses were performed using Maximum Parsimony (MP), Bayesian Inference (BI) and Maximum Likelihood (ML). The appropriate model of nucleotide substitution of each gene was selected using the program JModeltest v.2.1.3 (Posada, 2008) according to the Akaike Information Criterion (AIC; Akaike, 1974), the GTR+I+G for Cyt *b* and GTR+I for 12S.. PAUP* v.4.0 (Swofford, 2003) was employed for the MP and ML analyses, with reliability assessed using nonparametric bootstrapping with 2000 replicates. Bayesian analysis was performed using MrBayes v.3.2.2 (Ronquist *et al.,* 2012). For the BI analysis, four simultaneous Markov chains were run for 10 million generations with a sampling frequency of every 100 generations, and burn-in was performed discarding 100,000 trees of each run. Convergence of runs was examined by plots of ln *L* scores and low standard deviation of split frequencies as implemented in MrBayes v.3.2.2. Two independent Bayesian runs were performed as an additional check that the chains mixed well and converged. A majority rule consensus tree was calculated from the posterior distribution of trees, and the posterior probabilities were calculated as the percentage of samples recovering any particular clade, where probabilities greater than 95% indicate significant support.

Morphological analyses. Material for the morphological examinations included 35 alcohol-preserved specimens from SUHC, five of the newly described species and 30 specimens of the eight closely-related species in Iran (list of species is present in Table 1). Characters for the morphological analyses were selected based on previous taxonomic studies of the genus *Eremias* (Szczerbak, 1971) (Table 3), and on personal observations. Nineteen metric and eight meristic characters were examined in all specimens of the newly recognized population, in comparison with other closely related species of the genus *Eremias* (Supplementary Table S1). Mensural characters were taken by the second author on the right side of each specimen using a digital caliper with accuracy to the nearest 0.01 mm. According to the previous identification key for *Eremias* (Anderson, 1999), informative characters were employed to upgrade the identification key of the genus in Iran.

Results

Molecular analyses. The concatenated alignment included 692 nucleotide (nt) of cyt *b* (316 nt variable; 292 nt parsimony informative; 360 nt conserved) and 372 nt of 12S DNA (109 nt variable; 86 nt parsimony informative; 262 nt conserved). The MP, ML and BI phylogenetic trees produced almost identical topologies with high support values (Fig. 2).

All species of the genus *Eremias* included in the genetic analysis as well as the new species described herein constitute monophyletic lineages (Fig. 2). The new species and *E. papenfussi* are sister species, though this relationship is weakly supported in the ML analysis (72%). The close phylogenetic relationship to *E. papenfussi* is also supported by morphological data (see below). According to the phylogenetic tree (Fig. 2), the lineage composed of the new species and *E. papenfussi* is sister to the clade of the remaining species: *E. suphani, E. persica*, *E. lalezharica, E. montana, E. strauchi, E. kopetdaghica* and *E. velox*. These remaining species form a distinct clade with high support values. Within this clade, *E. persica* diverged first, followed by *E. suphani*. The five remaining species are divided into two monophyletic lineages, one composed of the sister species *E. kopetdaghica* and *E. velox,* and the other including *E. lalezharica* and the sister species *E. montana* and *E. strauchi*. The species assigned within the sugenus *Aspidorhinus* subgenus divided, as *E. montana* is nested among species classified within the subgenus *Eremias*, whereas *E. papenfussi* is phylogenetically positioned within a different clade with the new species. The genetic diversity among all species is relatively high (Table 2) suggesting that each species, including the newly described species, is a unique and differentiated entity.

TABLE 1. List of material examined in this study with the museum numbers and GenBank accession numbers. Locality codes correspond to localities presented in Fig. 1. Taxon names correspond to changes proposed in this paper.

Species	Museum number	Locality/Code	Coordinates	GenBank accession number		Reference
				cyt *b*	12S DNA	
Eremias isfahanica sp. nov.	SUHC 3009	45 km NW Isfahan/1	32°52' 12.2" N; 51°06' 41.2" E	KP317958	KP317970	This study
Eremias isfahanica sp. nov.	SUHC 3011	45 km NW Isfahan/1	32°52' 12.2" N; 51°06' 41.2" E	KP317959	KP317971	This study
Eremias isfahanica sp. nov.	SUHC 3012	45 km NW Isfahan/1	32°52' 12.2" N; 51°06' 41.2" E	KP317957	KP317969	This study
Eremias isfahanica sp. nov.	SUHC 3014	45 km NW Isfahan/1	32°52' 12.2" N; 51°06' 41.2" E	KP317961	KP317973	This study
Eremias isfahanica sp. nov.	SUHC 3017	45 km NW Isfahan/1	32°52' 12.2" N; 51°06' 41.2" E	KP317960	KP317972	This study
Eremias papenfussi	SUHC 1127	North of Tehran/2	35° 47' 44.9" N; 51° 14' 20.2" E	KP317962	KP317974	This study
Eremias papenfussi	SUHC 1128	North of Tehran/2	35° 47' 44.9" N; 51° 14' 20.2" E	KP317963	KP317975	This study
Eremias lalezharica	SUHC 151	Lalehzar mountain/3	29°29' 27.9" N; 56°48' 58.3" E	KJ468077	KJ468089	Rastegar-Pouyani *et al*. (2015)
Eremias lalezharica	SUHC 153	Lalehzar mountain/3	29°29' 27.9" N; 56°48' 58.3" E	KJ468078	KJ468090	Rastegar-Pouyani *et al*. (2015)
Eremias lalezharica	SUHC 158	Lalehzar mountain/3	29°29' 27.9" N; 56°48' 58.3" E	KJ468079	KJ468091	Rastegar-Pouyani *et al*. (2015)
Eremias lalezharica	SUHC 159	Lalehzar mountain/3	29°29' 27.9" N; 56°48' 58.3" E	KJ468080	KJ468092	Rastegar-Pouyani *et al*. (2015)
Eremias suphani	SUHC 301	Chaldoran, NW Iran/4	38°42' 58.2" N; 44°37' 55.6" E	KP317965	KP317977	This study
Eremias suphani	SUHC 302	Chaldoran, NW Iran/4	38°42' 58.2" N; 44°37' 55.6" E	KP317964	KP317976	This study
Eremias suphani	SUHC 310	Chaldoran, NW Iran/4	38°49' 08.6" N; 44° 34' 48.5" E	KF797806	KP317967*	Rastegar-Pouyani *et al*. (2013)
Eremias suphani	SUHC 311	Chaldoran, NW Iran/4	38°49' 08.6" N; 44° 34' 48.5" E	KF797807	KP317968*	Rastegar-Pouyani *et al*. (2013)
Eremias montana	SUHC 216	NE of Kermanshah/5	34°30' 08.5" N; 47°21' 12.3" E	FJ 416293	FJ445366	Rastegar-Pouyani *et al*. (2010)
Eremias montana	SUHC 217	NE of Kermanshah/5	34°30' 08.5" N; 47°21' 12.3" E	FJ 416294	FJ445367	Rastegar-Pouyani *et al*. (2010)
Eremias montana	SUHC 218	NE of Kermanshah/5	34°30' 08.5" N; 47°21' 12.3" E	FJ 416295	FJ445368	Rastegar-Pouyani *et al*. (2010)
Eremias montana	SUHC 219	NE of Kermanshah/5	34°30' 08.5" N; 47°21' 12.3" E	FJ 416296	FJ445369	Rastegar-Pouyani *et al*. (2010)
Eremias strauchi	SUHC 315	44 km NW Marand/6	38°33' 59.3" N; 45°18' 37.2" E	KJ468070	KJ468082	Rastegar-Pouyani *et al*. (2015)
Eremias strauchi	SUHC 317	44 km NW Marand/6	38°33' 59.3" N; 45°18' 37.2" E	JQ690099	JQ690168	Rastegar-Pouyani *et al*. (2012)
Eremias strauchi	SUHC 318	44 km NW Marand/6	38°33' 59.3" N; 45°18' 37.2" E	KJ468072	KJ468084	Rastegar-Pouyani *et al*. (2015)
Eremias strauchi	SUHC 316	44 km NW Marand/6	38°33' 59.3" N; 45°18' 37.2" E	KP317966	KP317978	This study
Eremias kopetdaghica	SUHC 351	Yengeje, Neyshabur, Khorasan/7	36°46' 10.2" N; 57°31' 0.2" E	KJ468076	KJ468088	Rastegar-Pouyani *et al*. (2015)
Eremias kopetdaghica	SUHC 352	Yengeje, Neyshabur, Khorasan/7	36°46' 10.2" N; 57°31' 0.2" E	KJ468075	KJ468087	Rastegar-Pouyani *et al*. (2015)
Eremias kopetdaghica	SUHC 353	Yengeje, Neyshabur, Khorasan/7	36°46' 10.2" N; 57°31' 0.2" E	KJ468073	KJ468085	Rastegar-Pouyani *et al*. (2015)
Eremias kopetdaghica	SUHC 354	Yengeje, Neyshabur, Khorasan/7	36°46' 10.2" N; 57°31' 0.2" E	KJ468074	KJ468086	Rastegar-Pouyani *et al*. (2015)
Eremias persica	SUHC 172	Near Abadeh, north Shiraz/8	30°56' 37.7" N; 52°55' 58.2" E	FJ416246	FJ445324	Rastegar-Pouyani *et al*. (2010)
Eremias persica	SUHC 173	Near Abadeh, north Shiraz/8	30°56' 37.7" N; 52°55' 58.2" E	FJ416250	FJ445325	Rastegar-Pouyani *et al*. (2010)
Eremias persica	SUHC 174	Near Abadeh, north Shiraz/8	30°56' 37.7" N; 52°55' 58.2" E	FJ416252	FJ445326	Rastegar-Pouyani *et al*. (2010)
Eremias persica	SUHC 196	South of Neyshaboor, Khorasan/9	36°06' 15.6" N; 59°03' 45.1" E	FJ416243	FJ445320	Rastegar-Pouyani *et al*. (2010)
Eremias persica	SUHC 197	South of Neyshaboor, Khorasan/9	36°06' 15.6" N; 59°03' 45.1" E	FJ416245	FJ445321	Rastegar-Pouyani *et al*. (2010)
Eremias velox	SUHC 268	South of Tehran/10	35°04' 06.9" N; 51°46' 57.5" E	JQ690195	JQ690127	Rastegar-Pouyani *et al*. (2012)
Eremias velox	SUHC 269	South of Tehran/10	35°04' 06.9" N; 51°46' 57.5" E	JQ690196	JQ690128	Rastegar-Pouyani *et al*. (2012)
Eremias velox	SUHC 359	Around Gorgan, north of Iran/11	36°48' 25.8"N; 54°29' 58.7" E	JQ690194	JQ690126	Rastegar-Pouyani *et al*. (2012)
Mesalina watsonana	1b	Ilam, Western Iran/12	33°15' 12.9" N; 48°04' 14.8" E	FJ416173	FJ445251	Rastegar-Pouyani *et al*. (2010)

Morphological characters. According to the 27 morphological characters, some variation is present between the new species and the other species in head scale counts (i,e., the gular, infralabial, supralabial, collar scales and submaxillary shields), and in the dorsal and ventral scale counts (it is presented in the diagnosis section). The new species has two diagnostic characters (i.e., the gular and collar scale counts) from its closest species, *E. papenfussi*. Computational morphological analyses were not performed, due to insufficient samples sizes. Morphological characters were used for the new species description and diagnosis with respect to its closely related species.

TABLE 2. Genetic distances (K2-p distance) between nine species of the genus *Eremias* included in this study inferred from Cyt *b* (below diagonal) and 12S (above diagonal) genes.

	E. isfahanica **sp. nov.**	*E. papenfussi*	*E. persica*	*E. montana*	*E. strauchi*	*E. kopetdaghica*	*E. l alezharica*	*E. suphani*	*E. velox*
E. isfahanica **sp. nov.**		5.4%	10.4%	5.2%	8.3%	8.5%	9.4%	8.2%	9.7%
E. papenfussi	30.1%		9.2%	8.2%	8.1%	7.6%	7.9%	5.8%	8.5%
E. persica	29.1%	27.1%		10.7%	7.9%	9.8%	11.3%	8.7%	9.8%
E. montana	30.4%	28.2%	18.5%		7.1%	8.0%	9.8%	8.0%	10.9%
E. strauchi	28.6%	26.7%	17.4%	16.6%		6.7%	9.1%	7.3%	8.9%
E. kopetdaghica	27.5%	28.3%	18.9%	19.5%	17.8%		9.3%	7.1%	7.8%
E. lalezharica	27.9%	26.6%	19.2%	17.3%	17.3%	18.3%		9.6%	9.4%
E. suphani	28.7%	27.7%	16.4%	17.2%	17.4%	16.2%	16.5%		8.8%
E. velox	32.8%	28.2%	21.1%	20.1%	18.5%	21.0%	21.6%	21.2%	

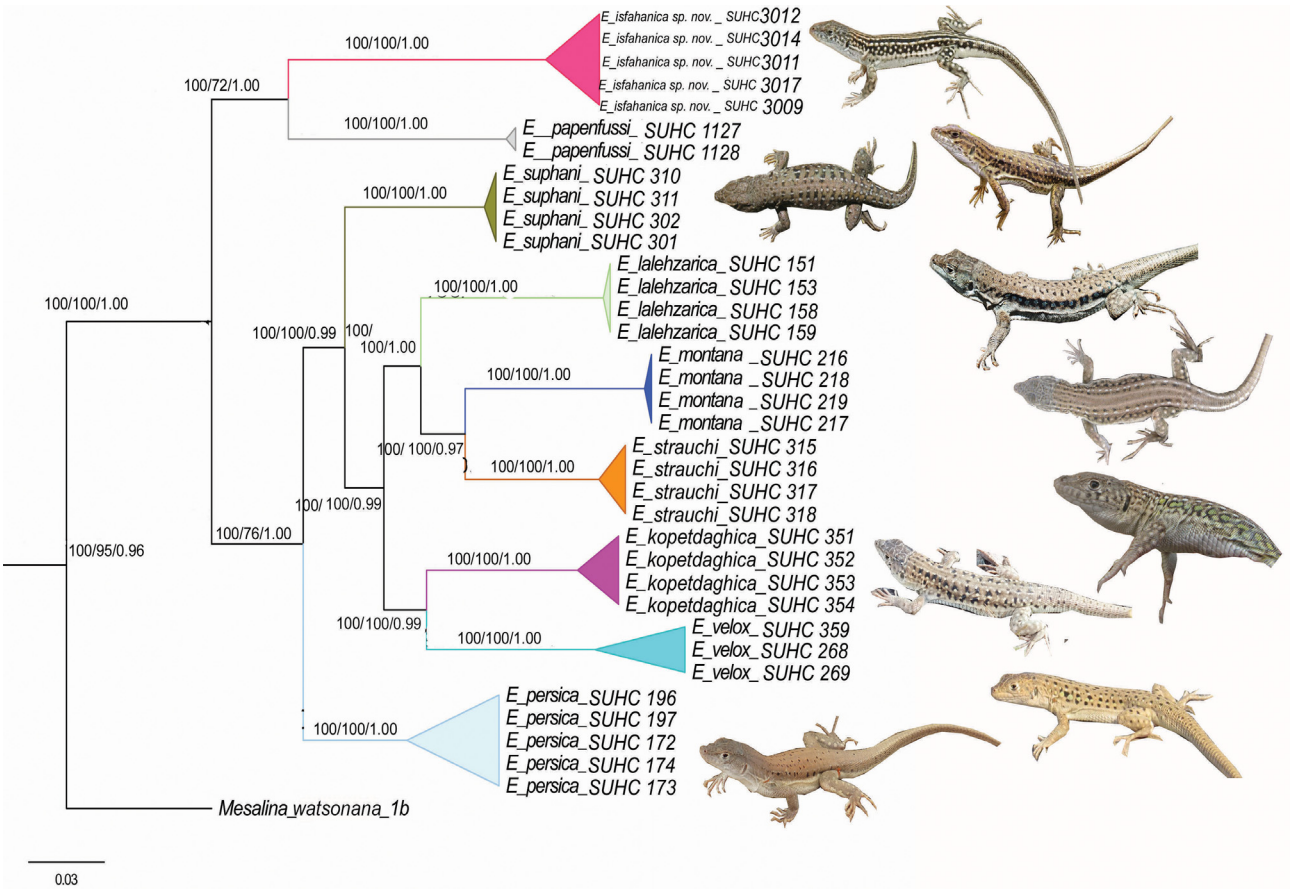

FIGURE 2. Bayesian Inference phylogenetic tree of nine species of the genus *Eremias* in Iran inferred using 12S DNA and cyt *b* mitochondrial gene fragments. *Mesalina watsonana* was used as the outgroup. Numbers near the nodes present the MP and ML bootstrap values and BI posterior probabilities, respectively (MP/ML/BI). Information on the samples included is given in Table 1. Taxon names correspond to changes proposed in this paper.

TABLE 3. list of morphological characters (19 metric and 8 meristic) and their abbreviations that used in this study.

characters	abbreviation
Snout-vent length (from tip of snout to anterior edge of cloaca)	SVL
Tail length (from posterior edge of cloaca to tip of tail)	TL
Trunk length (distance between hindlimb and forlimb)	LHF
Head length (from tip of snout to the posterior edge of tympanum)	HL
Head height (maximum distance between upper head and lower jaw)	HH
Head width (distance between posterior eye corners)	HW
Length of forelimb (from top of shoulder joint to tip of 4th finger)	LFL
Length of hindlimb (from hip joint to tip of 4th toe)	LHL
Length of femur (from hip joint to top of knee)	LFO
Length of tibia (from top of knee to beneath wrist)	LA
Eye Diameter (distance from anterior corner to posterior corner to its posterior)	EL
Rostral length (from tip of nostril to anterior corner of eye)	RED
Distance between posterior edge of eye and tympanum	EED
Length of neck (distance between posterior edge of tympanum and shoulder joint)	NL
Tympanum diameter (largest size)	TD
Interorbital distance (largest size)	IOR
Length of cloaca crevice (largest size)	LV
Length of widest part of tail base	LBT
Length of widest part of belly	LWB
Number of labial scales anterior to the center of eye on the right side of head	NSL
Number of scales on the right lower labial region	NIL
Number of gular scales in a straight median series	NGS
Number of collar scales	NCS
Number of transverse series of ventral scales counted in straight median series between collar and the row of scales separating the series of femoral pores	NVS
Number of dorsal scales across midbody	NDS
Number of subdigital lamellae along underside of 4th toe (defined by their width, the one touching the claw included), counted bilaterally	SDLT
Number of femoral pores	NFP

Systematics

The genetic distinctiveness of the *Eremias* population from the central Zagros Mountains from other *Eremias* species as seen in two mitochondrial gene fragments (cytb *b* and 12S DNA), in addition to several morphological differentiations (see results above) presented in this study, led us to describe this population as a new species:

Eremias isfahanica sp. nov.

(Figs. 3 and 4)

Holotype. The holotype is a male, SUHC 3012, collected 54 km northwest of Isfahan city, near Hassanije village within the Ghomishloo National Park (32.84 N, 51.10 E; 1200 m asl.); collected during August 2009 by S. Rafiei.

Paratypes. Four males (SUHC 3008, 3009, 3013, 3014) and two females (SUHC 3016, 3017) were collected from the same locality as the holotype.

Etymology. The epithet *isfahanica* was attained from the species locality (Isfahan Province, Central Iran).

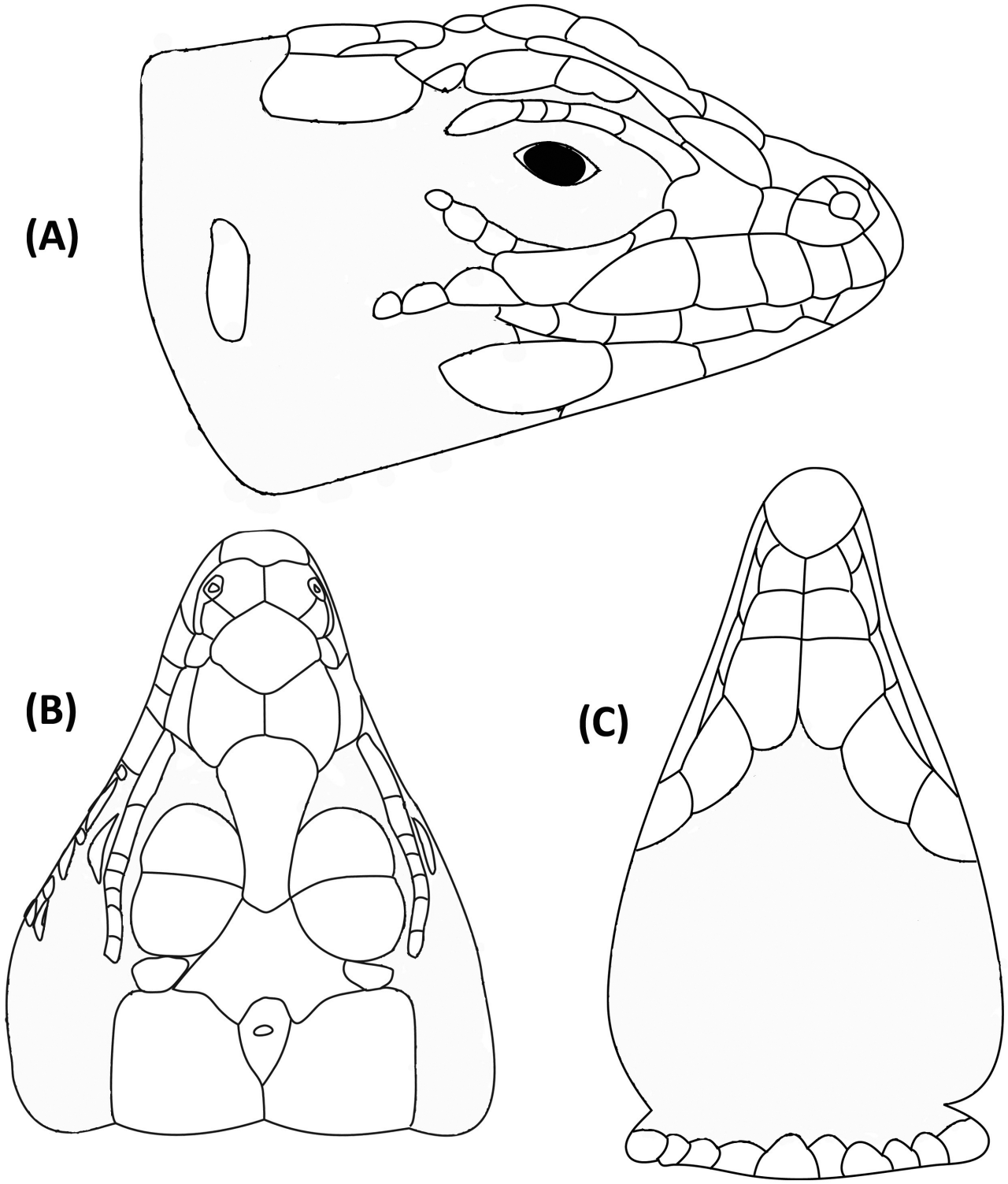

FIGURE 3. Schematic drawings of the holotype of *Eremias isfahanica* **sp. nov.** (SUHC 3012). (A) Lateral view of the head; (B) Dorsal view of the head(C) Ventral view of the head.

Description of the Holotype (SUHC 3012). Size: SVL of the holotype is 67.6 mm and tail length is 114.1 mm.

Scalation (Figs. 3 and 4): A subocular scale borders the mouth and five supralabial scales in front of it; three scales in contact with the nostril (first three supralabials); two lorial scales before the eye; seven supraciliars; three supraoculars, with the third smaller than the first two; occipital scale absent; five inframaxillary scales et each side, the first three pairs in contact with each other;33 gular scales; eight infralabials; two series of 23 femoral pores et each side, separated by three scales,; 25 subdigital lamella under 4th toe; Five submaxillary shields, first three pairs

in contact; collar includes 14 scales; 59 longitudinal scales in mid-dorsum; 31 transversal rows of ventral scales; scales in the temporal region are unkeeled.

Coloration in life (Fig. 4): the dorsal color is dark brown with longitudinal cream dashed strips between them. In adult specimens, two median dark stripes are joined and create a light brown stripe in the mid-dorsum region. Two dark brown stripes are present on both sides of the mid-dorsum stripe. In total, there are two dark brown, one light brown and three cream dashed stripes in dorsum of adults. Two lateral stripes extend onto the tail and consists of erratic light spots. Two light dorsal stripes between the lateral and dark brown stripes extend to the tail and reach together on the tail. The ventral surface of the body and tail are cream. The dorsal view of the limbs has many light spots.

Diagnosis. *Eremias isfahanica* **sp. nov.** is different from most morphologically similar species within the subgenera *Eremias* and *Aspidorhinus* as follows: It can be distinguished from *E. suphani* by lacking the extension of gular scales in contact with the second inframaxillary scales (Fig. 3C). It can be distinguished from *E. lalezharica* by having fewer gular scales (NGS, 27–33 versus 33–40), additional inframaxillaries (five versus four), and higher number of femoral pores (NFP, 18–23 versus 16–18). It can be distinguished from *E. papenfussi* by having higher number of gular scales (NGS, 27–33 versus 24–28) and collar scales (NCS, 12–15 versus 10–12). It can be distinguished from *E. montana* by having fewer mid-dorsum scales (NDS, 55–63 versus 63–67), additional transverse rows of ventral plates (NVS, 30–31 versus 27–28), and gulars (NGS, 27–33 versus 23–25). It can be distinguished from *E. velox* by having higher number of mid-dorsum scales (NDS, 55–63 versus 46–56) and the absence of a lateral dark-margined blue ocellus.

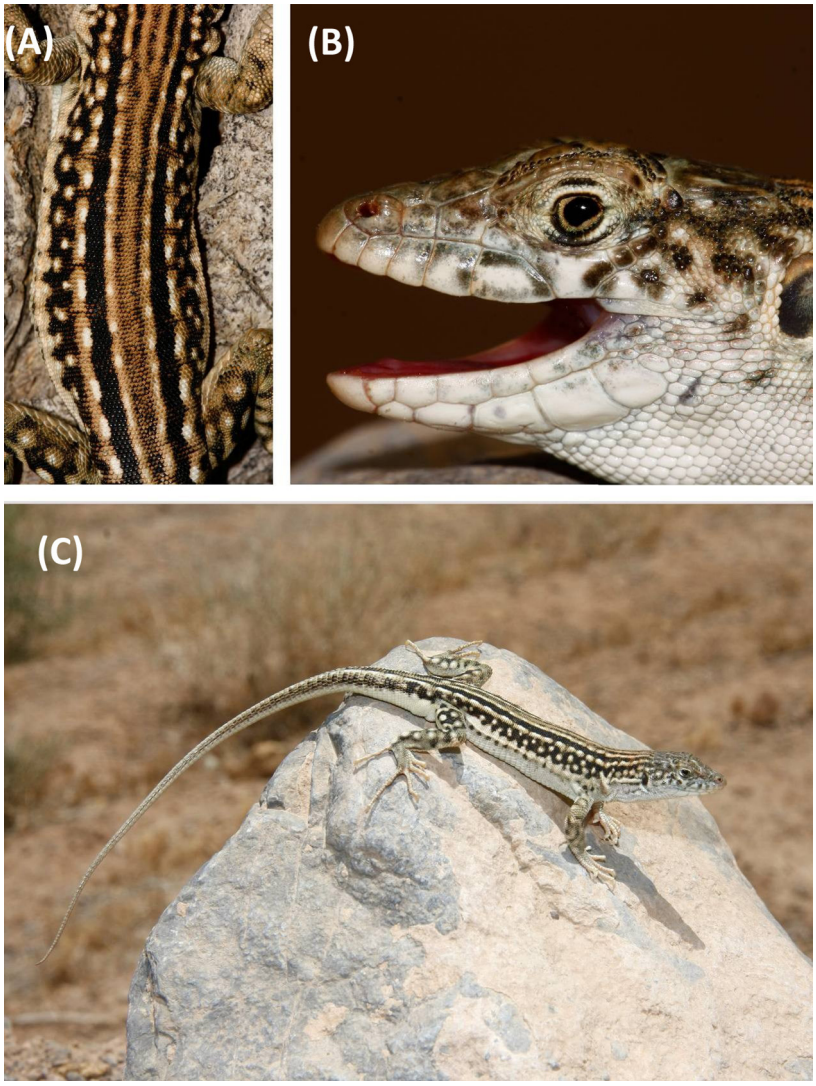

FIGURE 4. Photos of the holotype of *Eremias isfahanica* **sp. nov.** (SUHC 3012). (A) Dorsal patterns and line stripes along the body; (B) Lateral view of the head; (C) General view of the species in a natural setting. Photos by F. Heidary.

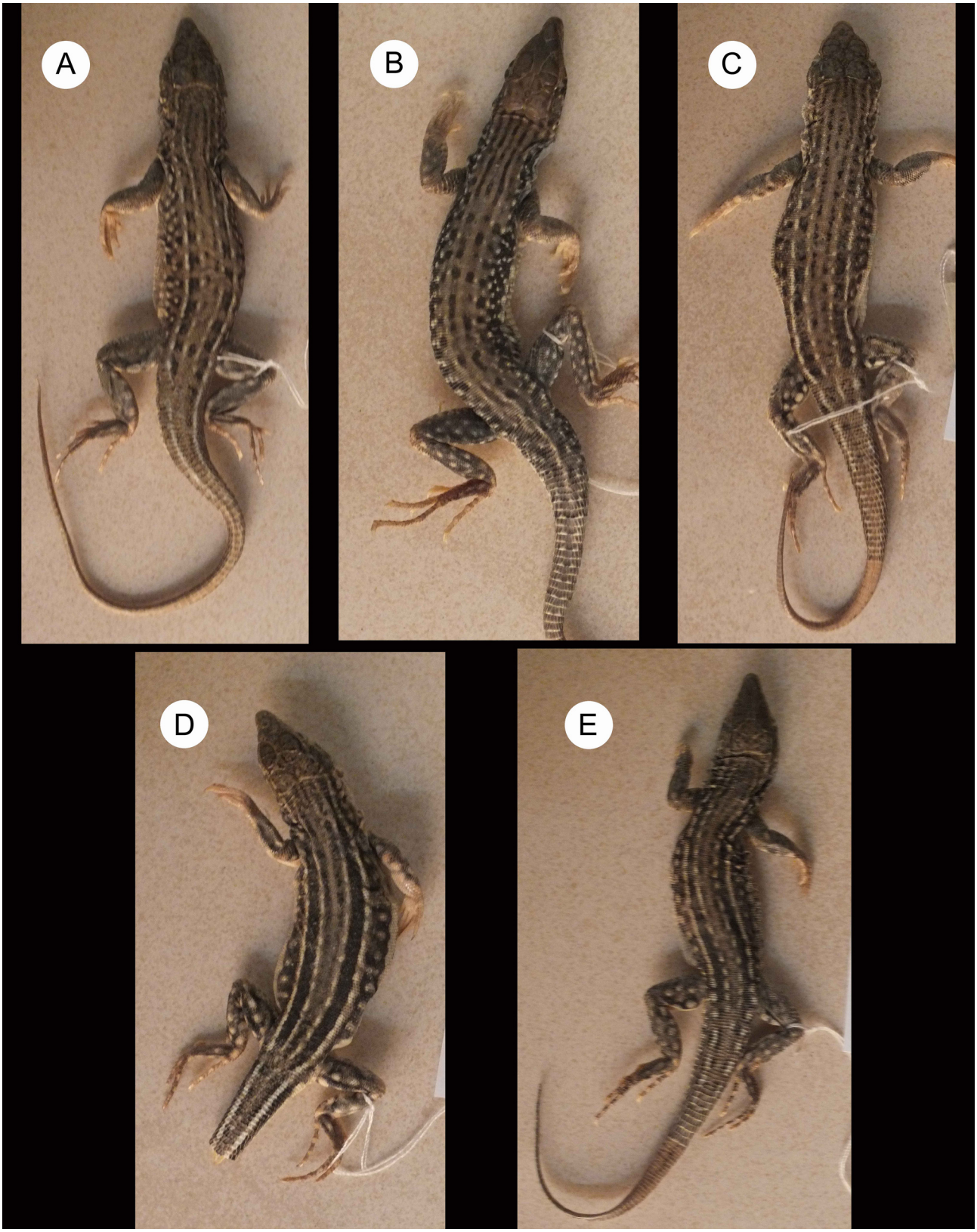

FIGURE 5. Dorsal color pattern of five paratypes. (A) SUHC 3008, Female; (B) SUHC 3009, Female; (C) SUHC 3013, Female; (D) SUHC 3014, Female; (E) SUHC 3016, Male.

Range and variation among paratypes. Snout-vent length 57.2–63.6 mm; five inframaxillary shields; 17–20 femoral pores; 27–30 gular scales; 12–15 collar scales; number of dorsal scales varies from 55–68; supralabials 5–6; number of infralabials 6–8; 30–33 ventral plates in transversal series; 22–26 subdugital lamella under 4th toe.

Paratypes show different color patterns from sharp stripes in the back to those in which the vertebral stripe is not present (Fig. 5).

Habitat. The type locality is a wide desert area that consists of small scattered shrubs, and the habitat is a sub-mountainous region extending to the mountains. Overgrazing is the most important destructive factor in the area because the region has low plant diversity (Fig. 6).

FIGURE 6. Type locality of *Eremias isfahanica* **sp. nov.**,54 km northwest of Isfahan city.

Discussion

The new species, *Eremias isfahanica* **sp. nov.**, is morphologically distinct and well supported by mitochondrial molecular data. Five species within the subgenus *Eremias* can be divided into two groups, according to the tree topology (Fig. 2). The first group includes the foothill and mountain dwelling species *E. montana, E. strauchi, E. lalezharica.* The second group includes two desert-dwelling species, *E. velox* and *E. kopetdaghica. Eremias velox* and *E. persica* have a wide range distribution on the Iranian Plateau, whereas other species such as *E. lalezharica, E. montana, E. papenfussi, E. suphani, E. strauchi* and *E. kopetdaghica* have limited ranges (especially the recently described species *E. papenfussi* and *E. montana*; Rastegar-Pouyani *et al.,* 2010; Mozaffari *et al.,* 2011; Rastegar-Pouyani *et al.,* 2012; Smid *et al.,* 2014). *Eremias isfahanica* **sp. nov.** has a limited distribution range similar to other species from the *E. montana* complex (Smid *et al.,* 2014). To date, it is known only from the type locality.

Our phylogenetic results suggest that *E. isfahanica* **sp. nov.** and *E. papenfussi* are quite distinct from each other genetically (K2p-distance: 30.1% for cyt *b* and 5.4% for 12S DNA; Table 2). K2p-distance values among the species within the *Eremias* subgenus show high genetic divergence between any pair of species included in the study. *Eremias velox* and *E. persica* have the greatest genetic distances from *E. isfahanica* **sp. nov.** for cyt *b* (32.8%) and 12S DNA (10.4%), respectively. The high values of genetic divergence suggest that *E. isfahanica* **sp.**

nov. has been genetically isolated from the other species of the genus for a long period of time. This has probably occurred due to their limited distribution range.

An interesting result from the molecular analysis concerns the assignment of species to subgenera, in contrast to their known systematic division (Fig. 2). *Eremias montana* and *E. papenfussi* are classified within the subgenus *Aspidorhinus*, though they are not closely related in the phylogenetic tree. *Eremias montana* is in fact nested among the species assigned to the subgenus *Eremias*. A proper revision of the subgenera in Iran is necessary to resolve this systematic perplexity.

The Iranian Plateau is a region composed of a high number of endemic taxa (Smid *et al.,* 2014) Environmental changes in this region, such as the climate oscillations during the Miocene to Pleistocene, the tectonic collusion between the Eurasian-Arabian plates and uplift of mountain ridges, as well as a series of local faults, have had a key role in the habitat fragmentation of several taxa in the region (e.g., Macey et al., 1998, 2000; Veith *et al.,* 2003; Ahmadzadeh *et al.,* 2013). It may be hypothesized that these environmental events have promoted vicariance and may have facilitated speciation within the genus *Eremias* on the Iranian Plateau, as was suggested for other species within the genus in central and eastern Asia (Gue *et al.,* 2011).

Although several species within the genus *Eremias* have been recently described, the diagnostic key to the genus has not been updated. Below we present an updated key for the genus *Eremias* in Iran based on diagnostic morphological characters.

Key to the genus *Eremias* Fitzinger, 1834

1a.	Subocular bordering mouth	2
1b.	Subocular not bordering mouth	15
2a.	A complete row of lateral scales of the 4th toe forming a distinct fringe or comb on its entire length	3
2b.	Lateral scales of 4th toe not forming distinct fringe	4
3a.	Row of femoral pores reaches well short of knee; 4th toe with 2 complete of subdigital scales, i.e., a total of 4 scales counted around toe (except that an extra scale may be present at a joint); supracaudal scales keeled, but not pointed behind; broad dark dorsolateral stripe from nostril through eye, along body and side of tail, one or two additional narrower dark stripes medial to these on each side, the remainder of the dark dorsal stripes interrupted and form reticulate pattern	*Eremias scripta*
3b.	Row of femoral pores reaches knee; 4th toe with single row of subdigital scales, i.e., a total of 3 scales counted around toe (except an extra scale may be present at a joint); supracaudal scales strongly keeled and acuminute; dorsal pattern of 7 dark stripes, outer dorsolateral stripe broadest	*Eremias lineolata*
4a.	The 2 series of femoral pores broadly separated, space between series at least 1/3 length of each	*Eremias pleskei*
4b.	The 2 series of femoral pores meeting, or separated by space not greater than 1/3 length of each	5
5a.	Back with 5–11 dark stripes, broader than interspaces, none of the stripes containing light ocelli or spots; stripes persistent in adults, but sometimes indistinct so that back appears almost uniform sandy; usually only single median collar scale distinctly larger than adjacent gulars	6
5b.	Light ocelli or spots on upper flanks (rare exception), dark stripes of juvenile breaking up in adults to form spots or broken lines; usually several collar scales distinctly larger than adjacent gulars	7
6a.	Frontal and supraocular scales separated by complete row of granules; 4th toe with 2 complete rows of subdigital scales and a complete row of sharply pointed lateral scales, i.e., total of 4 scales counted around penultimate phalanx	*Eremias fasciata*
6b.	Frontal and supraocular scales not separated by complete row of granules; 4th toe lacking complete row of distinctly pointed lateral scales; i.e., total of 3 scales counted around penultimate phalax	*Eremias andersoni*
7a.	Four submaxillary shield, smaller shields lateral and posterior to 4th submaxillary	*Eremias lalezharica*
7b.	Five submaxillary shields	8
8a.	Adults with dark interrupted dorsolateral black stripe forming ocelli with white spots, this dorsolateral pattern not contrasting strongly with interrupted dark stripes and spots of dorsum; juveniles with 3 dark stripes on dorsum between white-spotted dorsolateral stripes, vertebral stripe black, bifurcated on nape (dark stripes breaking up into several irregular rows of dark spots with age); ventral surface of tail carmine red in juveniles (in life)	*Eremias velox velox*
8b.	Adults usually with black dorsolateral stripe more or less continuous for at least major portion of the length; Black dorsolateral stripe containing white spots, black stripe contrasting strongly with dorsal color pattern; less than 40 gular scales; juvenile with 4 dark stripes on dorsum between dorsolateral white-spotted stripes, vertebral stripe light colored (dark stripes breaking up into 4 more or less regular rows of dark spots with age); ventral surface of tail not red in juveniles	9
9a.	Adults with more or less distinct rows of dark spots on dorsum between dorsolateral dark stripes, the latter usually with white spots in a single row within each stripe; distal portion of tail bluish in juveniles (in life)	*Eremias persica*
9b.	Juveniles with 3 distinct dark stripes on dorsum; adults have two white spotted rows upper dorsolateral strip	10
10a.	9 collar scales; 23–24 gulars; 63–68 middorsum scales; 31–33 ventral in transvers series; ventral region dirty white, suffused by bluish-brown; 18–19 femoral pores	*Eremias montana*
10b.	more than 9 collars; less than 63 middorsum scales; more than 30 transverse rows of ventral plates; more than 24 gulars	11

11a. Adults with two distinct segregated dark strips in dorsolateral region; lateral dark strips with white ocelli; lacking the colored ocelli on flanks 12
11b. Adults usually without dark stripes or spots on middorsum; dorsolateral region with alternate light and dark spots, often fusing longitudinally, forming 2–4 longitudinal stripes, often broken, the impression being 3–4 rows of light spots on flanks; ventral surface of tail yellow in juveniles (in life) 13
12a. collar scales less than 13; 24–28 gular scales; 19–26 femoral pores; Ventral surface in juveniles reddish; normally SVL less than 60mm; Central Elburz mountains in Iran . . . . . *Eremias papenfussi*
12b. 14–15 collars; 17–23 femoral pores; 27–33 middorsum scales; Snout-Vent length more than 62mm; two lateral strips with several small white spots; three first pairs of submaxillary shields in contact; 27–33 gulars . . . . . *Eremias isfahanica*
13a. 34–38 gulars; third pair of chin shields separated; no enlarged gular scales bordering to the third chin shields; presence of large numbers of white spots in ventolateral row . . . . . *Eremias suphani*
13b. less than 30 gulars; third pair of chin shields contacted; enlarged gular scales bordering to the third chin shields; presence of green ocelli in ventrolateral row 14
14a. 23–33 gulars; 56–68 dorsals; 24–35 scales in 9–10th caudal annulus; west and east Azarbaijan in Iran . . . . . *Eremias strauchi*
14b. 19–28 gulars; 48–59 dorsals; 20–26 scales in 9–10th caudal annulus; eastern Mazandaran, northern Khorasan . . . . . *Eremias kopetdaghica*
15a. 4th toe with distinct fringe on both lateral and medial sides, formed by complete row of sharply pointed lateral scales and complete row of similar medial scales; ungual lamellae of fingers and toes with prominent flat lateral expansions 16
15b. 4th toe without distinct fringe; ungual lamellae without prominent lateral expansion 18
16a. Scales of flanks not larger than those of back; broad plates on lower surface of tibia more than twice as broad as adjacent scales . . . . . *Eremias acutirostris*
16b. Scales of flanks larger than those of back; broad plates on lower surface of tibia not twice as broad as adjacent scales 18
17a. 18–19 subdigital lamella; enlarge tibial scales; dorsal background is yellowish-brown and broken by grey transverse bars; just known from Maranjab desert, central Iran . . . . . *Eremias kavirensis*
17b. 20–22 subdigital lamella; small tibial scales; dorsal pattern is completely reticulum of dark-brown spots; northeastern Iran . . . . . *Eremias grammica*
18a. 5th toe with 2 complete rwos of subdigital scales and incomplete row of small lateral scales; 2nd supraocular (1st of 2 large undivided supraoculars) as long as or shorter than its distance from 2nd loreal …….……. *Eremias arguta*
18b. 5th toe with single complete row of subdigital scales and a few scattered lateral scales not forming complete row; 2nd supraocular (1st of 2 large undivided supraoculars) longer than its distance from 2nd loreal 19
19a. 4th toe with single row of subdigital scales . . . . . *Eremias intermedia*
19b. 4th toe with 2 rows of subdigital scales, internal much the larger . . . . . *Eremias nigrocellata*

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