Volume 30 (January 2020), 1-11

Herpetological Journal

FULL PAPER

https://doi.org/10.33256/hj30.1.111

Published by the British Herpetological Society

Revised classification of the genus *Eryx* Daudin, 1803 (Serpentes: Erycidae) in Iran and neighboring areas, based on mtDNA sequences and morphological data

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Several attempts have recently been made to elucidate taxonomic status and phylogenetic relationships among the species and subspecies of sand boas of the genus *Eryx* throughout their distribution range, with no stable consensus about their taxonomy. Here the phylogenetic relationships among the populations of *Eryx* in Iran and adjacent areas are studied based on two mitochondrial markers (cyt*b* and 16S). Sixteen morphological characters were examined for evaluation of morphological differences among major populations. Ecological niche modeling was applied to demonstrate the potential distribution of the populations in Iran. ENMtools was also used to measure the degree of niche overlap among the major populations in Iran. Based on phylogenetic reconstruction and considering the genetic distances with specimens from type localities, *E. tataricus* is a junior synonym of *E. miliaris* and the subspecies rank for *E. m. nogaiorum* seems to be invalid. Considering the genetic distance of populations in western Iran and Iraq, and the habitat and morphological differences among the populations of *Eryx* in western Iran, Iraq and Egypt, the population of *Eryx* in western Iran is suggested as a different species from *E. jaculus*, named here as *Eryx* sp. and the ones from Iraq as *Eryx* cf. *jaculus*. Here, the evaluation and revision of taxonomic status, distribution ranges and descriptions of morphological characters of the studied species have been done.

Keywords: *Eryx*, taxonomy, phylogeny, mitochondrial markers, morphology, niche modeling, niche overlap

INTRODUCTION

Based on the most recent study, the family Erycidae comprises 13 named species: *Eryx borrii* Lanza & Nistri, 2005, *Eryx colubrinus* (Linnaeus, 1758), *Eryx conicus* (Schneider, 1801), *Eryx elegans* (Gray, 1849), *Eryx jaculus* (Linnaeus, 1758), *Eryx jayakari* Boulenger, 1888; *Eryx johnii* (Russell, 1801), *Eryx miliaris* (Pallas, 1773), *Eryx muelleri* (Boulenger, 1892), *Eryx somalicus* Scortecci, 1939, *Eryx tataricus* (Lichtenstein, 1823), *Eryx vittatus* Chernov, 1959 and *Eryx whitakeri* Das, 1991. These are distributed in north and east Africa, Europe, the Middle East, and south and central Asia (Pyron et al., 2014). During recent decades, several attempts have been made for the taxonomic modification among the members within this family, based on morphological traits and molecular data (Tokar, 1990; 1991; Kluge, 1993; Campbell, 1997; Eskandarzadeh et al., 2013; Pyron et al., 2013; 2014; Rastegar-Pouyani et al., 2014; Reynolds et al., 2014; Zarrintab et al., 2017). However, no consensus has been made about their taxonomic status.

It has been difficult to distinguish *E. miliaris* from *E. tataricus* either by morphological (Sorensen, 1988; Tokar, 1990; Eskandarzadeh et al., 2013; Zarrintab et al., 2017) or molecular evidence (Pyron et al., 2013; Rastegar-Pouyani et al., 2014; Reynolds et al., 2014). *Eryx miliaris* (type locality: north shore of the Caspian Sea between the Volga and Ural Rivers) is distributed across south-western Russia, east to China and Mongolia, south to Iran and Afghanistan (Wallach et al., 2014; Reynolds & Henderson, 2018). *Eryx tataricus* (type locality: Aral Sea region) is distributed from the eastern shore of the Caspian Sea south to Pakistan and Iran, and east to China and Mongolia (Wallach et al., 2014). The lack of a comperehensive study, especially on the type specimens of these two species and their subspecies, causes chaos in their taxonomic status. *Eryx miliaris* consists of two subspecies, the nominate form and *E. m. nogaiorum*

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(Nikolsky, 1910) (distribution range: western part of the distribution range of the species) (Ananjeva et al., 2006). Ross & Marzec (1990) elevated the taxonomic rank of the black sand boa, *E. m. nogaiorum*, to a distinct species, but without any references to document the change (Kluge, 1993). According to Harrison (2003), because of the trouble in defining *E. tataricus*, determining the distribution range of *E. tataricus* is confusing. He also announced that some authors have had problems with distinguishing *E. tataricus* from both *E. miliaris* and *E. jaculus*. According to Tokar (1990), *E. miliaris* and *E. tataricus* form a complex.

Differentiation of *E. elegans* from *E. jaculus* is also problematic in north-east Iran (Eskandarzadeh et al., 2013; Rastegar-Pouyani et al., 2014). *Eryx elegans* (type locality: Afghanistan) is distributed in the north of Iran and Afghanistan, and southern parts of Turkmenistan (Wallach et al., 2014; Reynolds & Henderson, 2018). *E. jaculus* (type locality: Egypt) distributed in south-eastern Europe, south-western Asia and north of Africa (Wallach et al., 2014). Boulenger (1893) described the holotype of *E. elegans* as follows: “Agrees in every respect with *E. jaculus*, except that the scales are larger, in 36 rows in the middle of the body”. The number of mid-body scales in the type specimen of *E. jaculus* from Egypt is 48 and in other studied specimens from Egypt varied from 43 to 51 (Anderson, 1898).

The number of dorsal scales at the mid-body is the most prominent and sometimes the only distinguishing character between *E. elegans* and *E. jaculus*. However, this number is uncertain in different works of literature (Table S1) (Boulenger, 1892; 1893; Tzarewsky, 1916; Terentyev & Chernov, 1965; Leviton & Anderson, 1970; Bannikov et al., 1977; Latifi, 1991; Zarrintab et al., 2017). In studied specimens of *E. jaculus* and *E. elegans* from north-eastern Iran, the minumum and maximum number of dorsal scales at mid-body were 36 and 46, respectively (36-41 for *E. elegans* and 42-46 for *E. jaculus*) (Eskandarzadeh et al., 2013).

Three subspecies have been described for *E. jaculus*: *E. j. jaculus* with three post-internasal scales, and *E. j. familiaris* (Eichwald, 1831) and *E. j. turcicus* (Oliver, 1801) each with two post-internasal scales. The subocular and supralabial scales are in contact in *E. j. familiaris* while these are separated by a row of scale in *E. j. turcicus* (Tzarewsky, 1916; Tokar, 1991; Kluge, 1993; Bannikov et al., 1977). Tokar (1991) studied 327 specimens of *E. jaculus* throughout its wide distribution range (Africa, Europe and Asia) and concluded that: “*Eryx jaculus* is a monotypic species, and the names *turcicus* and *familiaris* should be considered as its junior synonyms”. According to Tokar (1991), the eastern boundary of the distribution range of *E. jaculus* in central Iran is not known, yet he named the area between Shiraz and Kerman as the extreme south-eastern extent of the range.

Ecological niche modeling (ENM) makes it possible to reveal the potential distribution of a species based on environmental (physical) conditions even with limited locality data. Because of the presence of biogeographic component whitin the disciplines of systematics, the role of ENM in phylogenetic research is considerable (Phillips et al., 2006; Raxworthy et al., 2007). Some examples of such systematic applications include recognition of erroneous localities (Graham et al., 2004), discovery of new areas of endemism and new species (Raxworthy et al., 2003) and species delimitation (Wiens & Graham, 2005). One of the systematic utilities of ENM is in species delimitation and especially in recognition of cryptic species. One such example is the study of Raxworthy et al. (2007) about the genus *Phelsuma*, showing the utility of using ENM in species delimitation. Some studies have also been done using ENM for boid snakes (Di Cola et al., 2008; Eskandarzadeh et al., 2018a).

Here, we combine mitochondrial DNA and morphological data to evaluate the phylogenetic relationship and taxonomic position of populations within the genus *Eryx* throughout a wide range from Iran and adjacent areas, and apply the data of *E. tataricus* and *E. miliaris* from the type localities for the first time. We use univariate and multivariate analyses to explicate the main discriminator morphological characters among the main populations and investigate the separation of *Eryx* populations morphologically. We also apply ENM to reveal the potential distribution range of the populations in Iran and their habitat preferences. ENMtools is also used to calculate the degree of niche overlap between major populations in Iran.

MATERIAL AND METHODS

Phylogenetic analyses

A total of 182 sequences from two mitochondrial markers (cytochrome *b* and 16S rRNA) in 104 specimens were analysed, including 145 novel sequences produced in this study and 37 sequences retrieved from NCBI (details are presented in Fig. 1 and Table S2). Based on the reconstructed tree in the phylogenetic study of Pyron et al. (2013), *Calabaria reinhardtii* was chosen as outgroup (accession numbers are presented in Table S2). DNA extraction was done using proteinase K digestion followed by ammonium acetate extraction (Kapli et al., 2013). Amplification of the marker 16S rRNA (16S) was done using the primers 16SL (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SH (5′- CCGGTCTGAACTCAGATCACG -′) (Palumbi et al. 1991), and cytochrome *b* (cyt*b*) with the primers L14724 (5′-TGACTTGAAGAACCACCGTTG-3′) and H16064 (5′-CTTTGGTTTACAAGAACAATGCTTTA-3′) (Palumbi et al., 1991; Burbrink et al., 2000); and L14919 (5′-AACCACCGTTGTTATTCAACT-3′) and Ei700r (5′-GGGGTGAAAGGGGATTTTRTC-3′) (Burbrink et al., 2000; Rastegar-Pouyani et al., 2010). The amplified fragments were sequenced on an automated sequencer ABI 3730XL (Macrogen, Seoul, South Korea).

ClustalW multiple alignment implemented in the software Bioedit sequence alignment editor 7.0.9.0 (Hall, 1999) was used to align the sequences in addition to manual checking. Mega 7 (Kumar et al., 2016) was used for checking the stop codons and align reading frames in the protein coding cyt*b* gene as well as calculating the genetic distances (*p*-distance). To reconstruct the phylogenetic relationships, two phylogenetic approaches (Bayesian Inference and Maximum Likelihood) were used.

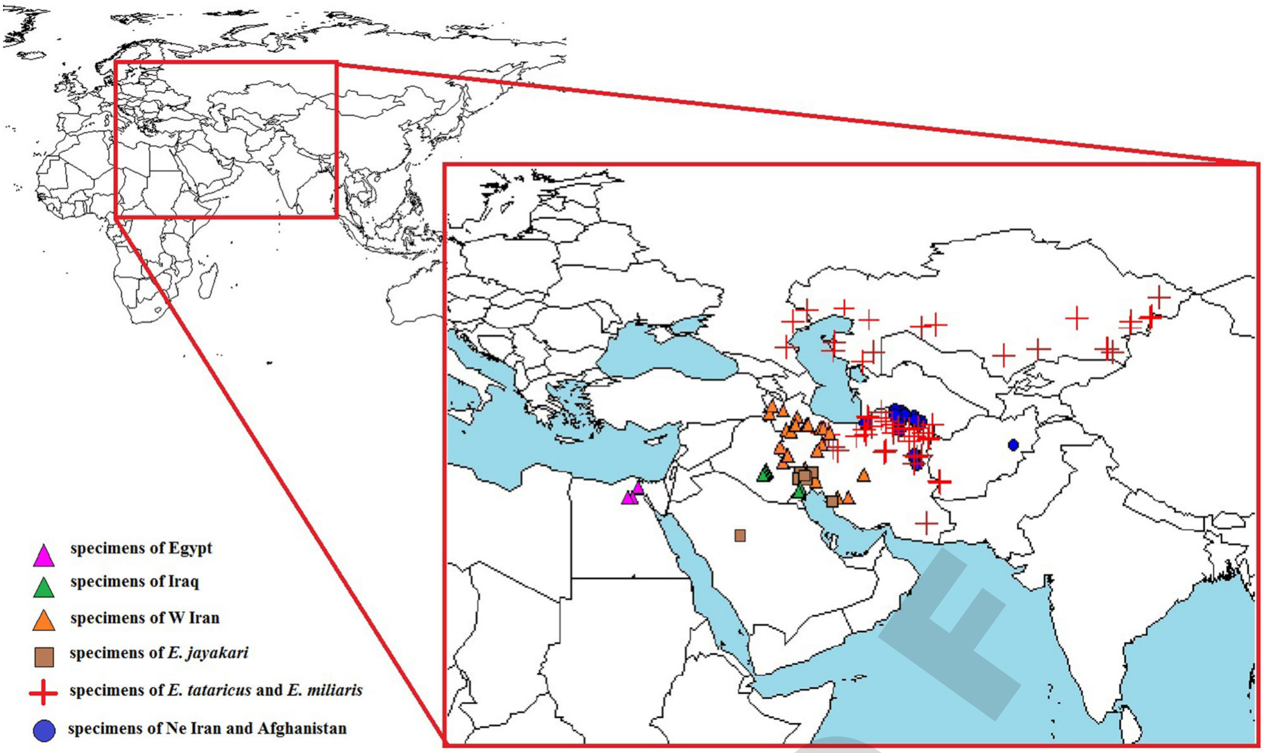

Figure 1. Localities of *Eryx* specimens applied in this study. Purple triangle: the specimens of Egypt (*E. jaculus*); green triangle: the specimens of Iraq (previously identified as *E. jaculus* and *E.* cf. *miliaris* (Rhadi et al., 2015)); orange triangle: the specimens of western Iran (morphologically identified as *E. jaculus*); brown square: the specimens of *E. jayakari* from Iran and Saudi Arabia; red cross: the specimens of *E. tataricus* and *E. miliaris*; blue circle: the specimens of north-eastern Iran and Afghanistan (morphologically identified as *E. elegans* and *E. jaculus*).

The software jModelTest 2.1.7 was used to determine appropriate models under the Akaike Information Criterion (Posada, 2008) which yielded in the model TIM2+G for 16S and TrN+G for cyt*b* as the best models, respectively. Bayesian inference (BI) was performed by Mr. BAYES 3.1.2 (Huelsenbeck & Ronquist, 2001) with two runs for 10 million generations, sampling every 1000 generations and discarding 25 % of the initial samples as burn-in. Maximum likelihood (ML) analysis was done with raxmlGUI v. 1.5. with 2000 replicates under the GTRGAMMA model (Silvestro & Michalak, 2012).

Morphological analyses

A total of 171 specimens were examined to determine the morphological divergence between major populations. Morphological data available from original descriptions and specimens from type localities were also included (Table S2). Seven meristic, two metric, and seven multistate characters were examined in the specimens (Table 1), considering the allometric variation the ratio of inter-ocular space width to distance between the posterior edge of the eye and the corner of the mouth was examined. For bilateral characters, only the right side was considered. Multistate characters were weighted numerically from 0 to 2 representing different status of these characters in the studied species. Nearest neighbor method using Minkowski implemented in SPSS 16 was used to perform cluster analysis. Based on previous studies performed on the *Eryx* spp., the most usual sexually dimorphic characters (number of subcaudal scales, length of tail, length of body and snout-vent length) (Shine, 1978; Tokar, 1991; Eskandarzadeh et al., 2018b) were omitted and both sexes were analysed together.

Homogeneity of variance was tested using Levene's test, and normality tests were used to evaluate the normal distribution of data. ANOVA with posthoc test, and Kruskal–Wallis test, were applied to parametric and non-parametric data, respectively, implemented in the statistical package SPSS 16, to reveal significantly different characters among groups. Two multivariate analyses; principal component analysis (PCA) and discriminative cluster analysis (DCA), were used to determine the form and pattern of variation among populations. The WI/DEM value was not available for all the Egyptian and some specimens from other clades, so this character was omitted from multivariate analyses.

Ecological niche modeling and ecological niche overlap

To reveal ecological requirements and the potential distribution for each major population in Iran, the altitude and 19 bioclimatic variables (in 30 arc-seconds resolution) were downloaded from DIVA-GIS (http://www.diva-gis.org) and the WorldClim database (http://www.worldclim.org/version1) (Hijmans et al., 2005), respectively. ENM was conducted only for the Iranian populations as data from other territories were sparse and were geographically limited (Fig.1). For each population the presence points (Table S2) and environmental layers were employed in Openmodeller v. 1.0.7. A total of 91

Table 1. Description of studied **(A)** meristic, morphometric and **(B)** multistate characters in populations of the genus *Eryx*.

(A)

Abbreviation	Character
RE	Number of scales around eye
LAB	Number of supralabial scales
BE	Number of scales between the eyes
PIN	Number of scales posterior to internasal
BEN	Number of scales between the eye and nasal
DS	Number of dorsal scales (in mid-body)
VS	Number of ventral scales
WI	Width of interocular space
DEM	Distance between posterior edge of the eye and the corner of the mouth

(B)

Character	No of states	Ch. State 0	Ch. State 1	Ch. State 2
Height of 2nd and 3rd upperlabial scales	3	2nd is higher	3rd is higher	2nd and 3rd are the same in height
The size of circum-orbital scales	2	Similar in size	One scales is bigger than the others	
Oblique line from eye to the corner of mouth	2	Present	Absent	
Connectional status of PIN with rostral scale	2	Connected	Not connected	
The status of eye position	3	Lateral	Somewhat upwarded	Upwarded
Keelness of scales	2	Scales not keeled	Scales somewhat keeled	
The shape of tail end	2	Pointed	Not pointed	

Table 2. Pairwise uncorrected genetic divergence (*p*-distance) among major clades of the genus *Eryx* from the mitochondrial markers for cyt*b* (upper-right in bold-italic) and 16S (lower-left in regular).

	Clade A	Clade B	Clade C	Clade D	*E. jayakari*
Clade A		***0.122***	***0.116***	***0.106***	***0.162***
Clade B	0.044		***0.059***	***0.111***	***0.154***
Clade C	0.034	0.023		***0.101***	***0.156***
Clade D	0.051	0.037	0.029		***0.155***
E. jayakari	0.062	0.064	0.058	0.063	

points were used. The Pearson correlation coefficient was calculated with SPSS 16 to determine the final layers for modeling each population and layers; correlations > 0.75 were omitted.

The selected layers and presence points were applied by MaxEnt 3.4.1 to produce the final model with maximum entropy method (Phillips et al., 2017). Ten percent of the data were used as test and 90 % as training. The convergence threshold and maximum number of iterations were 0.00001 and 500, respectively. The model was run with 10 replicates with cross validated replicate types. The area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the model performance. The niche overlap of concerned species was computed using Schoener's D metric implemented in ENMTools (Warren et al., 2010), ranging from 0 to 1, where the value 0 means that ecological niches do not overlap at all, and 1 means that the ecological niches are identical.

RESULTS

Phylogenetic analyses

Five major clades were revealed in both BI (Fig. 2) and ML (Fig. S1) phylogenetic trees. In addition to *E. jayakari*, four other major clades were revealed in the phylogenetic analyses. Clade A comprised the morphologically identified specimens of *E. elegans* and *E. jaculus* from north-east Iran (Eskandarzadeh et al., 2013; Rastegar-Pouyani et al., 2014). Clade B comprised the specimens from Iraq, previously identified as *E. jaculus* and *E.* cf. *miliaris* (Rhadi et al., 2015). Clade C comprised the specimens from western Iran morphologically identified as *E. jaculus*, and clade D comprised the specimens of *E. miliaris* and *E. tataricus* (from Chechnya, Kalmykia, Russia, Kazakhstan, Iran, and type specimens of *E. m. miliaris*, *E. t. tataricus*, and *E. m. nogaiorum*).

In both trees, the specimens from Iraq (clade B) and those from western Iran (clade C) were sister clades (with posterior probility 1 and bootstrap value 78). In the BI tree, clades B and C were clustered with clade D as their sister clade (with posterior probility 0.75), while in ML phylogram, clade D is clustered with clade A, but with a poor support value (with bootstrap value 36) (Fig. 2 and Fig. S1).

The calculated genetic divergence (*p*-distance) varied from 0 to 16.6 % in cyt*b* and from 0 to 8.4 % in 16S sequences. The minimum and maximum genetic distances of the two mitochondrial markers between clades were 5.9 % and 16.2 % for cyt*b* and 2.3 % and 6.4 % for 16S (Table 2). The genetic divergence (*p*-distance) within each clade is presented in Table 3. The genetic distance between *E. m. miliaris* and *E. t. tataricus* specimen from type localities was 2.2 % for cyt*b* and 0.6 % for 16S and that between *E. m. miliaris* and *E. m. nogaiorum* from the type locality was zero for both cyt*b* and 16S.

Morphological analyses

Univariate analyses revealed that all the studied characters were significantly different between major clades (P <0.05; Table 4). The results of LSD post hoc pair-wise test indicated that the largest number of significant

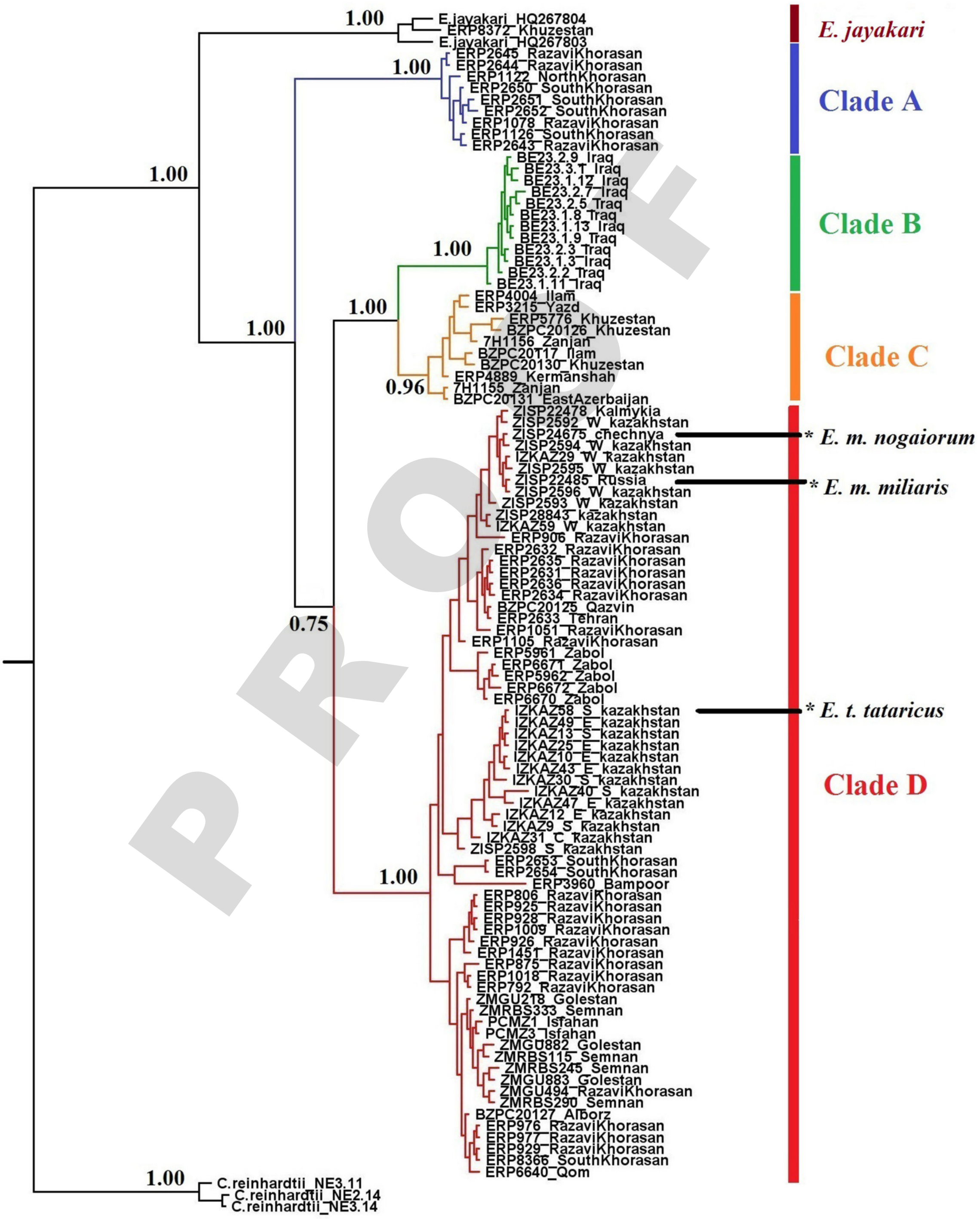

Figure 2. Bayesian inference phylogram based on 1263 base pairs of the concatenated cytochrome *b* and 16S sequence data set. The numbers next to the nodes indicate clade credibility in the Bayesian analysis. The specimens from the type localities are shown with an asterisk.

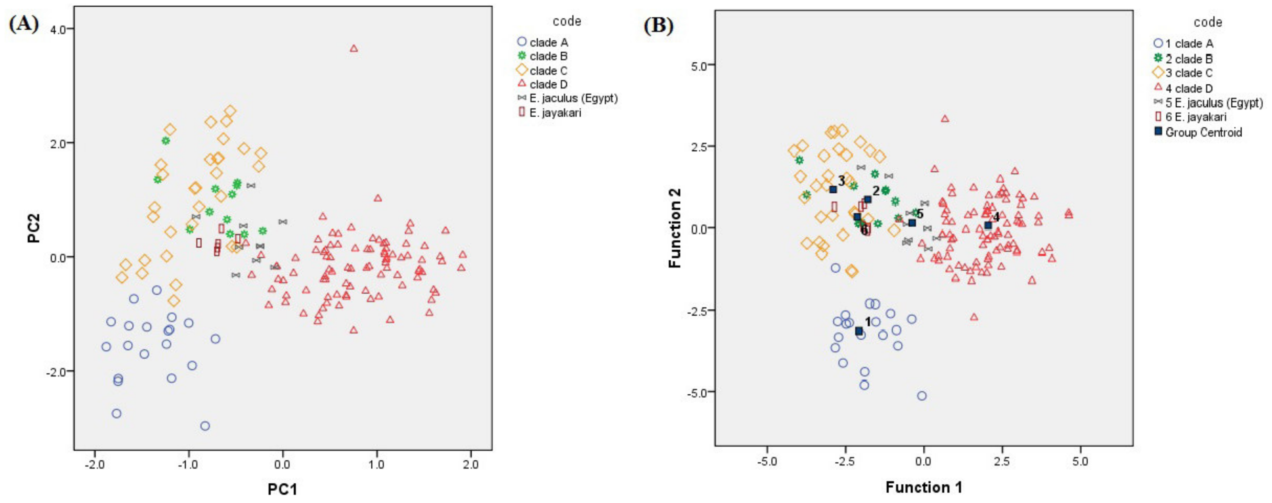

Figure 3. Plots of A: principal components and B: discriminant analyses of the populations of the genus *Eryx* for the seven studied characters (RE, LAB, BE, PIN, BEN, DS and VS).

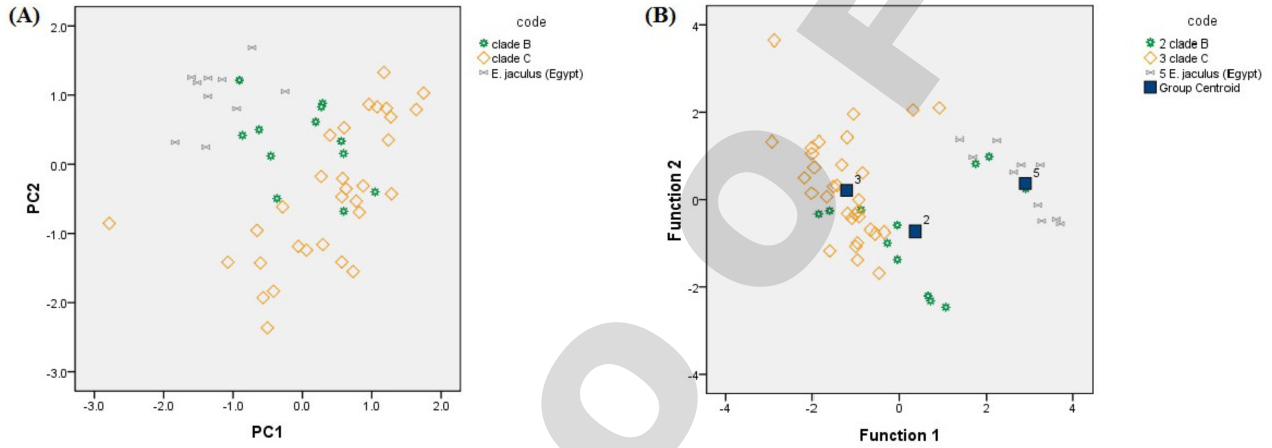

Figure 4. Additional A: principal components and B: discriminant analyses among the populations of clades B, C and *E. jaculus* from Egypt.

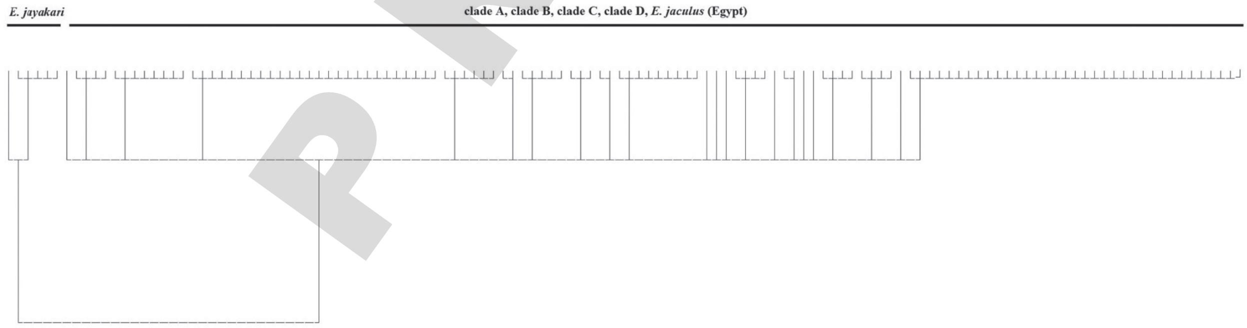

Figure 5. Dendrogram of cluster analysis showing morphological relationships in studied populations of the genus *Eryx*.

character differences are seen between the specimens of clades D and A and between clades D and B (Table S3).

In the PCA analysis, the first two components included 67.72 % of the total variance (Table S4, Fig. 3A). Based on classification results of DCA, 90.1 % of the original grouped cases were correctly classified. Five functions were computed for DCA and the first two functions explained 91.3 % of the total variance (Table S5, Fig. 3B). The plots of PCA and DCA indicate that the specimens of clades B, C and the populations of *E. jaculus* from Egypt and *E. jayakari* are not distinguishable from each other. The members of clades D and A are well separated from each other and also from other populations in both plots (Figs. 3A and 3B).

Additional multivariate analyses (PCA and DCA) were also performed on the populations of the clades B, C and *E. jaculus* from Egypt to check their separation in more details. In both analyses, the population of *E. jaculus*

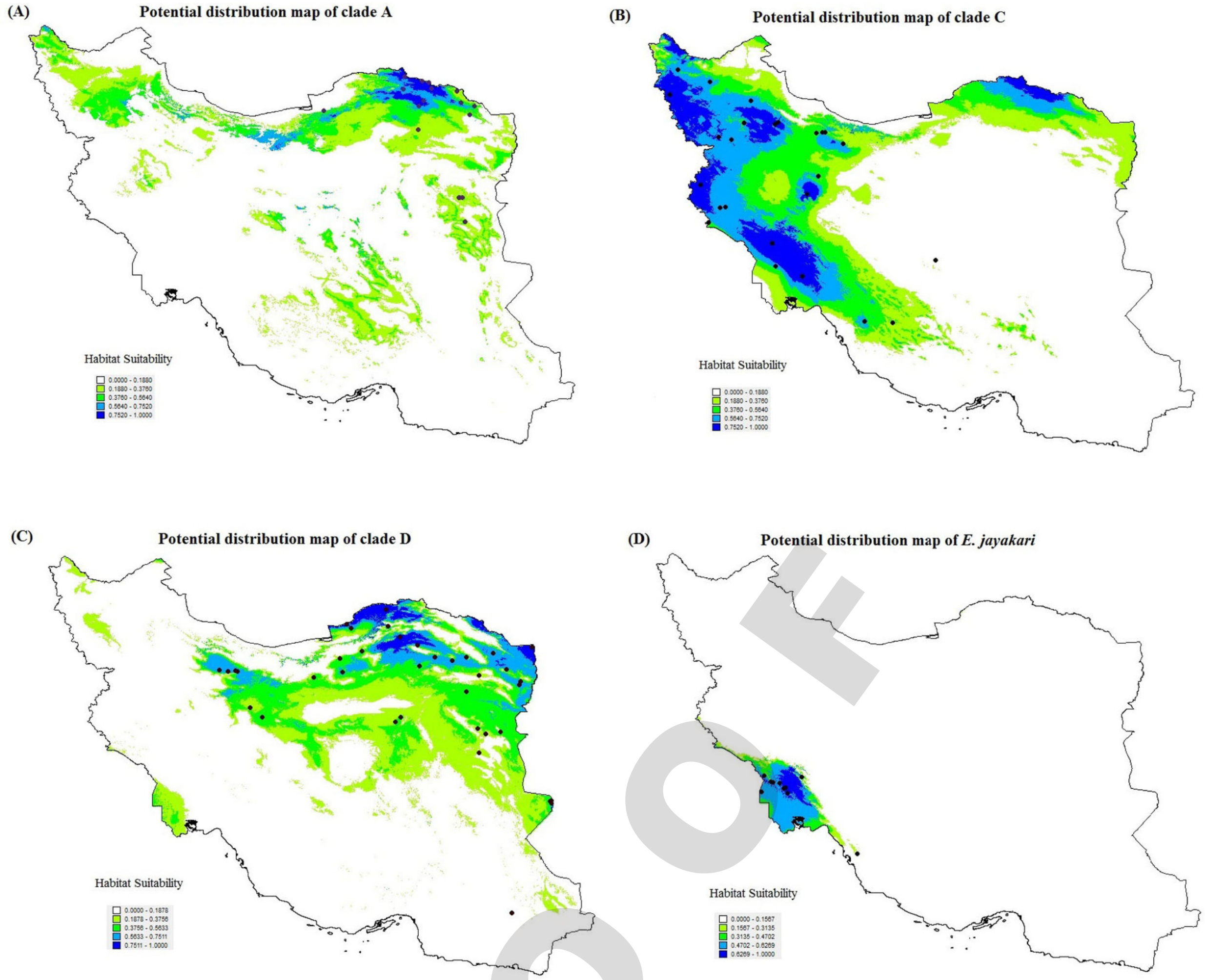

Figure 6. Potential distribution maps of Iranian clades of *Eryx*. A: the northeastern Iran clade (clade A; morphologically identified as *E. elegans* and *E. jaculus*); B: the western Iran clade (clade C; morphologically identified as *E. jaculus*); C: the *E. miliaris* and *E. tataricus* clade (clade D) and D: *E. jayakari*.

from Egypt was separated from the other two clades (B and C) (Figs. 4A and 4B). In the PCA, the first and second component respectively included 30.35 % and 24.38 % of total variance. BE, PIN and DS contribute most to the first component, and RE and VS contribute most to the second component. In the DCA, the first function with 92.9 % of total variance has the most correlation with PIN, BE, DS and RE; and the second function with 7.1 % of total variance has the most correlation with VS, BEN and LAB.

The results of the studied multistate characters are presented in Table S6. Based on the results of the cluster analysis (using multistate characters), only *E. jayakari* is well separated from other populations and no separation was detected between other clades (Fig. 5).

Ecological niche modeling and niche overlap

The AUC value, percent contribution, and permutation importance of the applied environmental factors are mentioned in Table S7. The niche modeling of clade A reveals that the northern parts of Iran, especially the Kopet Dagh and Alborz Mountains as the most suitable habitat for the presence of the members of this clade (Fig. 6A) with annual mean temperature as the most effective factor influencing their distribution (Table S7). The specimens of clade C distributed from north-west to south-west of Iran along the Zagros Mountains with one specimen from Yazd Province. The western part of Iran in addition to highland areas in NE Iran are revealed as the most suitable habitats for this clade (Fig. 6B). Annual precipitation is the most effective factor in the distribution of western clade (Table S7). The members of clade D distributed in the high land area and foothills of Alborz, Kopet Dagh and Eastern Mountains in addition to the Dasht-e Kavir and more interior area. The most suitable habitat for the members of clade D is located in the highland areas of NE and N Iran (Fig. 6C) and the distribution of *E. jayakari* specimens in Iran is limited to SW Iran (Fig. 6D). Altitude has the most effect on the distribution of members of both clades D and *E. jayakari* (Table S7). The Schoener's D metric value varied from 0.009 (between the populations of clades A and *E. jayakari*) to 0.431 (between the populations of clades A and C) (Table 5).

DISCUSSION

Clade A: the north-eastern clade: *E. elegans*

The clade A includes specimens with two post internasal scales belonging to *E. elegans* and those that have previously mistakenly been identified as *E. jaculus* (Eskandarzadeh et al., 2013; Rastegar-Pouyani et al., 2014). Members of this clade inhabit foothills and substrates covered with gravel and rocks. As is obvious from previous studies (Table S1), the reported maximum number of dorsal scales in mid-body for *E. elegans* was 43. It is notable that the number of dorsal scales in mid-body overlaps in all the other studied species (Table 4). This number in the studied specimens from north-east of Iran and Afghanistan (including the holotype and three syntypes ZISP 8711, ZISP 8473 and ZISP 8462 of *E. elegans*) varied from 35 to 46. The minimum number of dorsal scales in the mid-body in *E. jaculus* specimens from Egypt and in the members of clade D were 43 and 39, respectively. Considering the overlap in the number of dorsal scales in the mid-body among the studied populations, this character cannot be an informative, distinctive character for separation of different erycid species.

Table 3. Calculated genetic divergence (*p*-distance) whitin each clades of the genus *Eryx* for the two mitochondrial markers cyt*b* and 16S with estandard error. One cyt*b* sequence of *E. jayakari* included in this study.

	cyt*b*	**16S**
Clade A	0.572%±0.16	0.086%±0.082
Clade B	0.189%±0.079	0.038%±0.036
Clade C	1.546%±0.305	0.332%±0.139
Clade D	1.854%±0.297	0.619%±0.156
E. jayakari	-	0.605%±0.298

Clades B and C: the Iraqi and western Iran clades

In spite of the overlapping in multivariate analyses (Figs. 3A, 3B, 4A, and 4B), two characters, BE and WI/DEM, were significantly different between these two clades (P < 0.05) (with overlapped limits) (Tables 4 and S3). The genetic distance between these two clades was considerable (Table 2). The *Eryx* population of western Iran inhabit mostly in foothills, among and under rocks and shrubs and in some regions (e.g. in Khuzistan plain in SW Iran) in desert areas with gravel substrate. The specimens of Iraq were collected from agriculture and pasture lands with a softer substrate. The number of post internasal scales in these two clades was two or three.

The post hoc analysis of morphological data of the Egyptian population (type locality of *E. jaculus*) showed that this is significantly different from populations of both Iraq and western Iran (P <0.05) (Table S3). Additional multivariate analyses showed that the Egyptian population was separated from Iraqi and western Iran populations (Figs. 4A and 4B). *Eryx jaculus* in north Africa generally occupies different kinds of habitats: including sandy coastal areas, and sometimes sparse forests and mountains with sparse tree cover and clayey and marly soil, as well as in the steppes and the desert limits (Ouni Ridha pers. comm.).

Considering the morphological and habitat differences between the populations of *Eryx* in western Iran and Egypt, and the considerable genetic distance between the populations from western Iran and Iraq, we suggest that the population of *Eryx* in western Iran can be considered as a distinct species from *E. jaculus*. Considering the results and lack of the tissue sample of *E. jaculus* from the type locality, here we named the population of *Eryx* in western Iran as *Eryx* sp. and those from Iraq as *Eryx* cf. *jaculus*.

Table 4. Descriptive statistics and univariate analysis of studied morphological (meristic and metric) characters among *Eryx* populations. See Table 1 for descriptions of character abbreviations.

		RE	**LAB**	**BE**	**PIN**	**BEN**	**DS**	**VS**	**WI/DEM**
Clade D	Mean ± SE	11.54±0.09	11.88±0.09	7.63±0.07	3.31±0.06	3.75±0.05	47.1±0.28	182.59±0.89	0.88±0.02
N=92	Range	10-13	10-14	6-9	2-4	3-4	39-54	167-235	0.6-1.25
Clade A	Mean ± SE	8.89±0.20	9.40±0.13	6.63±0.11	2.00±0.00	2.94±0.06	39.75±0.61	167.95±1.64	1.31±0.04
N=20	Range	7-11	8-10	6-7	2-2	2-3	35-46	156-184	1.2-1.7
Clade B	Mean ± SE	9.58±0.19	10.75±0.18	6.42±0.29	2.25±0.13	3.00±0.00	49.33±0.64	190.92±1.33	1.08±0.04
N=12	Range	9-11	10-12	5-8	2-3	3-3	45-53	185-198	0.90-1.37
Clade C	Mean ± SE	9.31±0.23	10.53±0.16	5.70±0.11	2.07±0.05	2.96±0.04	51.07±0.59	185.34±2.00	1.41±0.06
N=31	Range	7-11	9-12	5-7	2-3	2-3	44-57	166-203	1.1-1.95
E. jaculus (Egypt)	Mean ± SE	10.00±0.21	10.50±0.17	7.22±0.15	3.00±0.00	2.90±0.10	47.00±0.76	191.10±1.97	-
N=10	Range	9-11	10-11	7-8	3-3	2-3	43-51	181-200	-
E. jayakari	Mean ± SE	10.17±0.17	10.83±0.17	5.00±0.00	3.00±0.00	3.00±0.00	47.00±1.05	176.00±1.96	0.43±0.10
N=6	Range	10-11	10-11	5-5	3-3	3-3	45-50	172-181	0.25-0.6
p value		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table 5. Calculated Schoener's D metric between major clades of *Eryx* in Iran reveals the degree of ecological niches overlap.

	Clade A	Clade C	Clade D
Clade C	0.431		
Clade D	0.414	0.359	
E. jayakari	0.009	0.116	0.083

Clade D: *E. miliaris*

The genetic variations among the members of clade D, including specimens of *E. t. tataricus*, *E. m. miliaris* and *E. m. nogaiorum* from Russia, Chechnya, Kalmykia, Kazakhstan and Iran, is low (Table 3). The genetic distance between the specimens of *E. t. tataricus* and *E. m. miliaris* from their type localities reveal that these two taxa should be attributed to the same species and considering the principle of priority, *E. tataricus* is a junior synonym of *E. miliaris*. Also considering the genetic distance between *E. m. miliaris* and *E. m. nogaiorum*, the subspecies rank for *E. m. nogaiorum* seems to be invalid. The members of this clade dwell in various types of habitats, including agriculture fields, sandy deserts, semi-desert and desert areas with a hard substrate, foothills, among and under rocks and shrubs. In spite of the diversity in morphological characters and habitat, the genetic diversity among populations of this major clade is low. Maximum intraspecies genetic distance (except for a specimen from Bampoor in south-eastern Iran) for cyt*b* is 3.4 %. The maximum calculated intraspecies genetic distance for the specimen from Bampoor is 5.9 % for cyt*b*. Based on the potential distribution map of the clade D (Fig. 6C), the locality of this specimen in south-eastern Iran is out of the predicted suitable habitat. So, more specimens from the area (south-eastern Iran) are needed to shed more light on its taxonomic status. Based on the molecular and morphological results the members of this clade are well separated from other studied *Eryx* spp. The position of eyes in the members of this clade is dorsolateral, upward and/or lateral with 2, 3 or 4 post internasal scales. The morphology of this species has been well discussed in previous studies (Eskandarzadeh et al., 2013; Rastegar-Pouyani et al., 2014; Zarrintab et al., 2017).

E. jayakari:

Eryx jayakari is the sister taxon to the other studied species which were considered here, and well distinguished from other species in the morphological cluster analysis. The bent scale on the tail tip and absence of the oblique line from the eye to the corner of mouth are the prominent characteristics of this species in comparison to other studied *Eryx* spp. This species inhabits sand dunes of south-western Iran. Based on the findings of niche overlap, the most overlap is between *E. elegans* and *Eryx* sp. (43 %) and the least overlap is between *E. elegans* and *E. jayakari* (0.9 %). The most amount of niche segregation among the studied population of *Eryx* spp. in Iran is seen between *E. jayakari* and the other species (Table 5). As mentioned above, *E. jayakari* is a well-adapted species for a burrowing lifestyle and is highly depended on sand dunes. The niche similarities among *E. elegans*, *E. miliaris* and *Eryx* sp. are the highest (Table 5).

CONCLUSION

Based on the results, we confront cryptic diversity in studied erycids, which is not uncommon among serpentes (Feldman & Spicer, 2002; Rawlings & Donnellan, 2003; Marin et al., 2013; Avcı et al., 2015; Fathinia et al., 2017; Torki, 2017; Portillo et al., 2018; Ruane et al., 2018; Wüster et al., 2018). In spite of the high morphological similarities in the examined groups of erycids, the results reveal considerable genetic divergence. *Eryx jayakari* is the only species that can be morphologically distinguished by key characters from the other studied erycids in the region. The descriptions of morphological characters of four species (*E. elegans*, *E. miliaris*, *Eryx* sp. and *E. jayakari*) are revised here. More specimens, especially the molecular data of *E. jaculus* from Egypt, are needed to clarify the exact taxonomic status of the Iraqi population (*Eryx* cf. *jaculus*).

ACKNOWLEDGEMENTS

We wish to thank the authorities of Razi University, Hakim Sabzevari University and Razi vaccine & serum research institute of Karaj for their support and kind collaboration in the lab tasks. We also would like to thank Konstantin D. Milto, Tatjana Dujsebayeva, Reza Babaie Savasari, Morteza Moadab, Manouchehr Roudini, Bahman Zangi and Behzad Fathinia for helping us in providing the specimens; Reza Nikfalak and Omid Rahimi for their kind help during the field work and Seyyed Saeed Hosseinian Yousefkhani for his kind help in the molecular process in laboratory.

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Accepted: 12 August 2019

Please note that the Supplementary Materials are available via the Herpetological Journal website: https://thebhs.org/publications/the-herpetological-journal/volume-30-number1-january-2020