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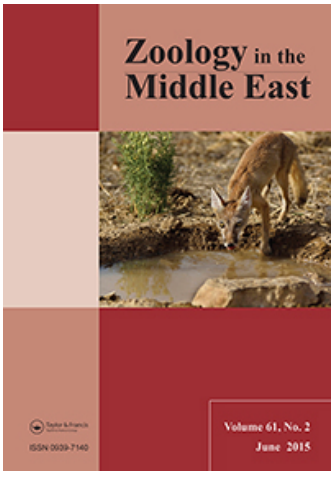

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Zoology in the Middle East
Publication details, including instructions for authors and subscription information:
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Taxonomic reevaluation of Eremias strauchi strauchi Kessler, 1878 and Eremias strauchi kopetdaghica Szczerbak, 1972, based on nuclear and mitochondrial DNA sequences (Reptilia: Lacertidae)

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Published online: 11 Mar 2015.

To cite this article: Eskandar Rastegar-Pouyani, Seyyed Saeed Hosseinian Yousefkhani & Michael Wink (2015) Taxonomic reevaluation of Eremias strauchi strauchi Kessler, 1878 and Eremias strauchi kopetdaghica Szczerbak, 1972, based on nuclear and mitochondrial DNA sequences (Reptilia: Lacertidae), Zoology in the Middle East, 61:2, 118-124, DOI: 10.1080/09397140.2015.1020615

To link to this article: http://dx.doi.org/10.1080/09397140.2015.1020615

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Zoology in the Middle East, 2015
Vol. 61, No. 2, 118–124, http://dx.doi.org/10.1080/09397140.2015.1020615

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Taxonomic reevaluation of *Eremias strauchi strauchi* Kessler, 1878 and *Eremias strauchi kopetdaghica* Szczerbak, 1972, based on nuclear and mitochondrial DNA sequences (Reptilia: Lacertidae)

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(Received 9 September 2014; accepted 2 February 2015; first published online 12 March 2015)

Strauch's Racerunner, *Eremias strauchi*, is represented by two subspecies, *Eremias strauchi strauchi* Kessler, 1878 and *Eremias strauchi kopetdaghica* Szczerbak, 1972, occurring in opposite margins on the northern Iranian Plateau. We sequenced 3926 base pairs of nuclear and mitochondrial DNA from 16 samples of *Eremias strauchi*, *Eremias lalezharica* and *Eremias velox* collected from northeastern, northwestern and southern Iran. Phylogenetic analyses revealed that *Eremias lalezharica* is sister to *Eremias strauchi kopetdaghica* and caused the currently recognised species *Eremias strauchi* to be paraphyletic. According to the estimated genetic distances in the mitochondrial fragments among the lineages, *E. s. strauchi* diverged from *E. s. kopetdaghica* and *E. lalezharica* with a mean genetic distance of 14.0% and 13.9% respectively. Our data indicate enough molecular divergence between the two currently recognised subspecies of *E. strauchi* and justify upgrading them to full species level as *Eremias strauchi* (for the north-western clade) and *Eremias kopetdaghica* (for the north-eastern clade).

Keywords: Cytochrome *b;* RAG1; *Eremias strauchi*; Iranian Plateau; taxonomy

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Introduction

The Eurasian lacertid genus *Eremias* consists worldwide of 35 species most of which occur on the Iranian Plateau, Central Asia and the Lesser Caucasus. 17 of them have been recorded from Iran (Uetz & Hošek, 2013). *Eremias strauchi* is one of ten species of the subgenus *Dimorphea* Eremchenko, 1999, which Barabnov (2009) considers to be a synonym of *Aspidorhinus* Eichwald, 1841.

Eremias strauchi Kessler, 1878 is found in Iran and the countries around the Caspian Sea in two geographically distinct subspecies (Anderson, 1999): *Eremias strauchi strauchi* inhabits the northwestern part of Iran where it extends into the Caucasus region and the eastern part of the Anatolian plateau (Ahmadzadeh, Kami, Hojjati, & Rezazadeh, 2009). *Eremias strauchi kopetdaghica* is restricted to the Kopet Dagh region on the northeastern margin of the Iranian Plateau (Anderson, 1999; Hosseinian Yousefkhani, Yousefi, Rastegar Pouyani, & Khani, 2013). Zoogeographic evidence suggests that the two subspecies in Iran became isolated in the Kopet Dagh and Caucasus regions by uplifting of the Elburz mountain range (Rastegar-Pouyani, Kazemi Noureini, Rastegar-Pouyani, Joger, & Wink, 2012). This geological event changed the areas suitable for

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this species and increased high elevation mountains in the Elburz region and deep valleys. It is thought that geological events and climatic factors (such as temperature and precipitation) are limiting gene flow and leading to two separated subspecies in the northeast and northwest portion of Iran along with some relict and isolated populations of the *Eremias* species complex in the highlands and foothills of the Elburz Mountains (Rastegar-Pouyani, 2009; Mozaffari, Ahmadzadeh, & Parham, 2011; Rastegar-Pouyani et al., 2012).

To further understand the taxonomy and evolution of *Eremias strauchi* populations in the region, we compared the two established subspecies of *Eremias strauchi* using one nuclear and mitochondrial molecular marker for population and species level discrimination (RAG1, Cyt *b* and 12S rDNA). These markers can help to understand whether geological events and disjunction have affected the population differentiation of *E. strauchi*. We included samples of the related *Eremias lalezharica* Moravec, 1994 and *E. velox* (Pallas, 1771) in the analyses, to clarify the taxonomic status and relationships among this subclade of the genus *Eremias*.

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Material and Methods

Sampling. 16 specimens were collected from four distinct areas: four *Eremias strauchi* from Marand, East Azarbaijan province, four *Eremias kopetdaghica* from Yengeje village between Sabzevar and Neyshabour, Khorasan Razavi province, four *Eremias velox* from the Jajarm area in Northern Khorasan province, and four *Eremias lalezharica* from the type locality, Lalezar Mountains, Kerman province, southeastern Iran (Figure 1). The voucher DNA material is kept in the tissue and DNA collection of the Institute of Pharmacy and Molecular Biotechnology (IMPB) at Heidelberg University, Germany. All tissue samples of these specimens were obtained from liver, muscle or tail tip. A single specimen of *Ophisops elegans* was used as an outgroup taxon in the phylogenetic analyses. The complete lists of specimens as well as accession number of sequences used in this study are presented in Appendix 1.

DNA extraction and PCR. Total genomic DNA was extracted from tissues using a high salt method (Aljanabi & Martinez, 1997). We amplified a partial sequence of nuclear gene RAG1 and mitochondrial 12S ribosomal DNA and a complete fragment of mitochondrial cytochrome *b* gene. Appropriate primers, PCR condition and programs to amplify these DNA fragments of Cyt *b* and 12S have been described elsewhere (Rastegar-Pouyani et al., 2012). Fragments of RAG1 were amplified using primers E-R1f (5′- CAAAGTGAGATCATTTAGCAA-3′) and E-R2838r (5′-TGCCTGGCATTCATTTTTCGAA-3′) under the PCR condition as follows: first denaturation 95°C for 5 min, amplifying stage with 37 cycles and precycle 95°C for 50 seconds, 50°C for 40 seconds, 72°C for 3 min and the final extension stage with 72°C for 10 min. Following the manufacture protocols, the amplified fragments were sequenced using the automated sequencer ABI prism 3100, Applied Biosystems.

Phylogenetic analyses. Sequences obtained from the sequencer were aligned using Clustal W as implemented in the program BioEdit Sequence Alignment Editor ver.7.0 (Hall, 1999) and checked visually. Prior to the final analyses, sequences of cyt *b* gene were examined to avoid the presence of nuclear pseudogenes by checking the absence of premature stop codons and GC content of the L-strand. Translation of cyt *b* DNA to amino acid sequences and calculation of the genetic distances (uncorrected sequence divergence) were carried out using MEGA 5.0 (Tamura et al., 2011).

Phylogenetic trees were reconstructed with different methods of phylogetic analysis. Bayesian Inference (BI) was employed using the program Mr. Bayes 3.2.2 (Ronquist & Huelsenbeck, 2003). Maximum Parsimony (MP) and Maximum Likelihood (ML) were performed, using the program PAUP* 4.0b10 (Swofford, 2001). In order to reconstruct ML and BI trees, the program Modeltest 3.7 (Posada & Crandall, 1998) was used to choose the best fit model of evolution for the individual and combined datasets. Nodal support for the MP and ML trees was assessed by nonparametric bootstrap analysis with 5000 and 2000 replicates respectively (Felsenstein, 1985).

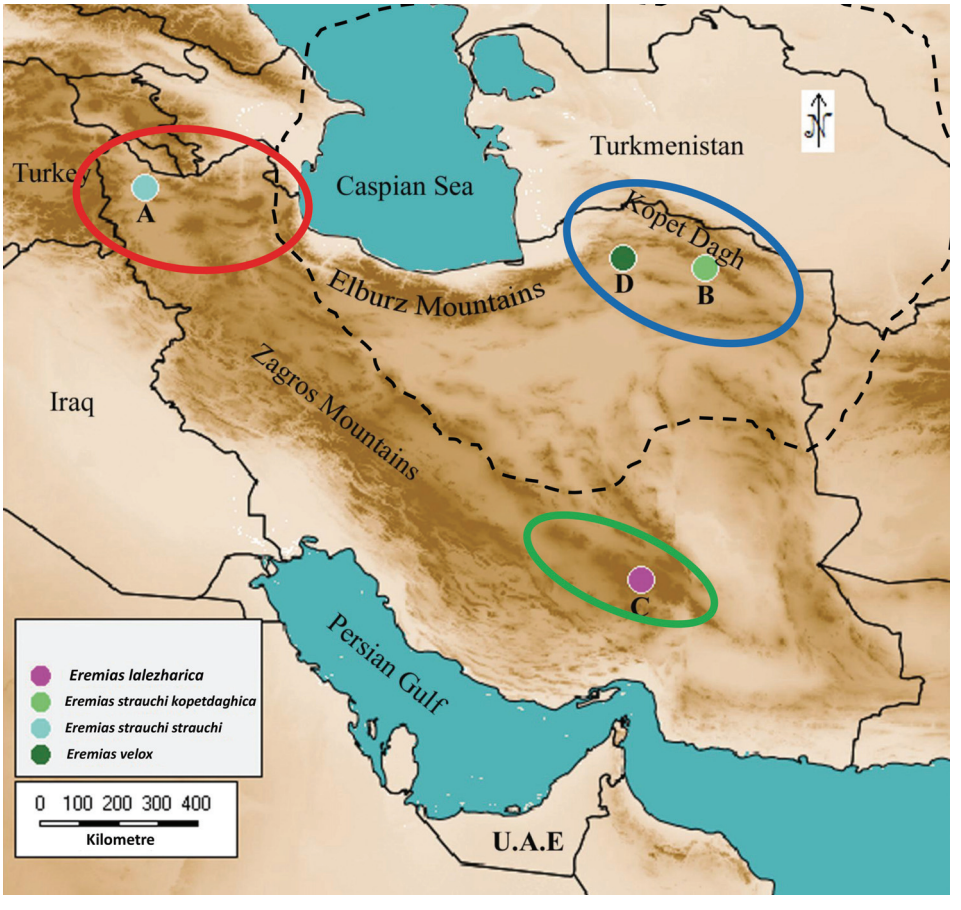

Figure 1. Map of Iran and sampling localities where the specimens used in this study were collected. (A: *Eremias strauchi,* B: *Eremias kopetdaghica,* C: *Eremias lalezharica* and D: *Eremias velox*). Overall distribution of each taxon is as follow: Green circle: *E. lalezharica*; Red circle: *E.strauchi*; Dark blue circle: *E.kopetdaghica*; and Dotted line: *E. velox.*

Results

In total, 3926 nucleotides of nuclear (2414 bp) and mitochondrial DNA (1512 bp) were unambiguously aligned (RAG1 with 2414 bp, cyt *b* with 1143 bp and 12S rDNA with 369 bp). In this dataset 718 sites were variable and 507 were parsimony informative. In addition, out of 1512 bp of mitochondrial DNA used in this study 518 positions were variable and 393 parsimony informative. MP analysis produced the tree with a length of 1042, Consistency Index CI = 0.8177, Homoplasy Index HI = 0.1823 and Retention Index RI = 0.9114. The best fit model of nucleotide substitutions for the combined dataset based on the Akaike information criterion was the GTR+G+I model for the combined dataset. ML, BI and MP analyses of the individual datasets (results not shown), combined 12S rDNA and cyt *b* (results not shown) and concatenated mitochondrial and nuclear datasets (Figure 2) produced the same tree topology regarding the major clades. Since the posterior probabilities of nodes in the Bayesion tree were almost identical with bootstrap values of ML, only the second ones are indicated in Figure 2

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Table 1. Uncorrected genetic distances (p-distance) among the species used in this study based on 1479 bp the mitochondrial cyt *b* and 12S.

	O.elegans	*E.velox*	*E.lalezharica*	*E.s.strauchi*	*E.s.kopetdaghica*
O.elegans					
E.velox	0.224				
E.lalezharica	0.219	0.179			
E.s.strauchi	0.225	0.153	0.139		
E.s.kopetdaghica	0.219	0.166	0.147	0.140	

Table 2. Uncorrected genetic distances (p-distance) among the species used in this study based on 2414 bp the nuclear RAG1.

	O.elegans	*E.velox*	*E.s.strauchi*	*E.s.kopetdaghica*	*E.lalezharica*
O.elegans					
E.velox	0.050				
E.s.strauchi	0.052	0.026			
E.s.kopetdaghica	0.050	0.025	0.015		
E.lalezharica	0.051	0.026	0.022	0.020	

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along with the bootstrap values of the MP tree. In all analyses the populations of *E. strauchi* exhibit two distinct clades that were always paraphyletic with *E. lalezharica* with high bootstrap support (100%). According to these results, *E. s. kopetdaghica* is closer to *E. lalezharica* and *E. s. strauchi* than to *E. velox*. Uncorrected sequences divergence of the mitochondrial fragments between the two subspecies, *E. s. strauchi* and *E. s. kopetdaghica* is higher than that between *E. s strauchi* and *E. lalezharica.* The average genetic distances among the species included in this study are summarised in Table 1. As shown in Figure 2, *Eremias lalezharica* is situated between the two subspecies of *Eremias strauchi* and the genetic divergence among them is an important argument for upgrading the two subspecies to species level.

Discussion

The phylogenetic analysis of nuclear and mitochondrial DNA indicated that the two subspecies of *E. strauchi* are genetically far from each other, so that the *E. lalezharica* population is placed between them. According to both genetic distances (Table 1–2) among species with Cyt *b* and 12S, the distance between of *E. s. strauchi* and *E. s. kopetdaghica* is more than that between *E. s. strauchi* and *E. lalezharica* and this result shows a deep divergence between *E. s. strauchi* and *E. s. kopetdaghica.* Here we used molecular markers to determine relationship between two subspecies and the uncorrected p-distances of up to 14% in mitochondrial cytochrome *b* gene indicated the threshold for setting the species delimitation in lacertid lizards (Harris, 2002; Arribas & Carranza, 2004). Considering the amounts of genetic distance between these two subspecies and the substantial periods of isolation time related to certain genetic distance in the family Lacertidae (Carranza et al., 2004; Arnold, Arribas, & Carranza, 2007; Mayer & Palvicev, 2007; Rastegar-Pouyani et al., 2010), it is assumed that these two subspecies have been isolated at least since the Late Miocene, about 7 million years ago. We therefore suggest promoting the two subspecies of *E. strauchi* to full species status, i.e. *E. strauchi* (Kessler, 1878) and *E. kopetdaghica* (Szczerbak, 1972). The morphology congruences together with the high genetic distance between *E. s. strauchi* and *E. s. kopetdaghica* (Table 1) support the taxonomic change. This includes the lateral spots that are

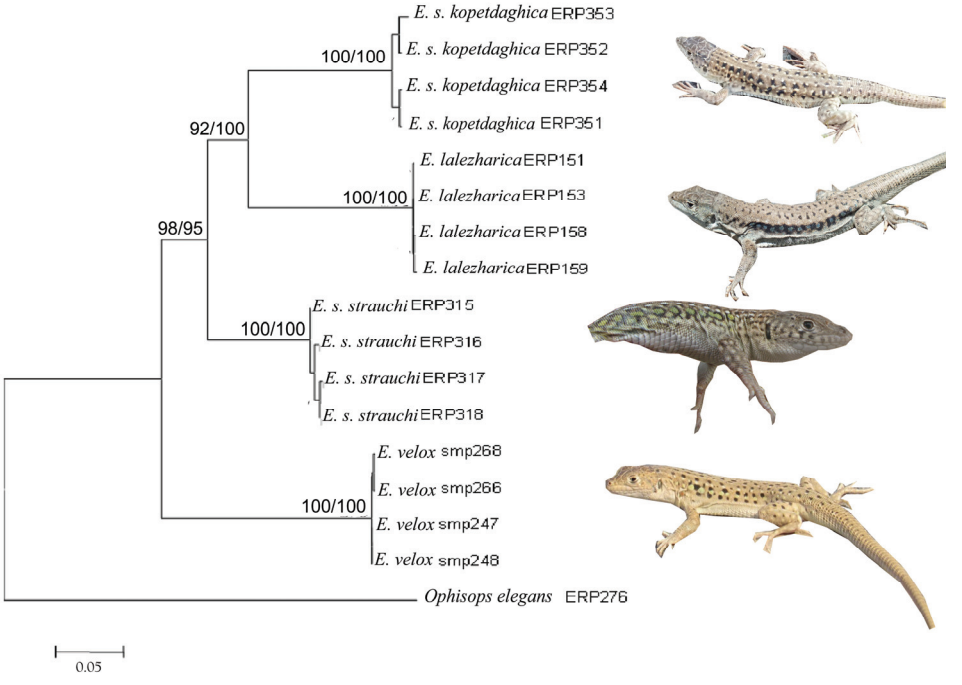

Figure 2. Phylogenetic relationships among the populations of *Eremias* included in the analysis using the combined dataset, based on the Maximum Likelihood method. *Ophisops elegans* was used as the outgroup taxon. Numbers next to the nodes indicate the bootstrap values of ML (2000 replicates) followed by MP (5000 replicates). All photos are from male specimens.

sharply expressed in green and blue in both *E. strauchi strauchi* and *E. lalezharica,* but absent in *E. strauchi kopetdaghica* (Moravec, 1994). Instead, the latter taxon has ambiguous spots and a dorsal pattern. In general, *E. s. strauchi* has significantly more scales in the gular and dorsal regions than *E. s. kopetdaghica* (Anderson, 1999). Collectively these observations support the recognition of the two distinct subspecies as distinct species. On the other hand, the high genetic divergence between *E. lalezharica* and two other species of the genus *Eremias* (*E. velox* and *E. kopetdaghica*) indicated that we cannot consider it as a subspecies or population of *E. kopetdaghica* (Table 1, 2).

The current distribution pattern for this *Eremias* complex apparently represents some relict populations. It is likely that several other populations of the genus *Eremias* became restricted and differentiated from the ancestral form and are now probably limited in the Zagros and Elburz mountain systems. The molecular phylogeny (Figure 2) suggests that *E. kopetdaghica* is placed as the sister taxon of the clade comprising *E. strauchi* and *E. lalezharica.*

Supplementary Material

The Annex is available as supplementary information via the "Supplementary" tab on the articles online page (http://dx.doi.org/10.1080/09397140.2015.1020615).

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Acknowledgements

We wish to thank Prof. Richard Wassersug from British Columbia University, USA, for improving the English of this manuscript. Our special thanks go to Majid Sabeti for his valuable assistance during the field work in Iran.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Iranian National Science Foundation (INSF) under the project number of 89001493.

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