JOURNAL OF ZOOLOGICAL SYSTEMATICS AND EVOLUTIONARY RESEARCH

Accepted on 12 April 2012
J Zool Syst Evol Res doi: 10.1111/j.1439-0469.2012.00662.x

[1]*Department of Biology, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran;* [2]*Department of Biology, Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Heidelberg, Germany;* [3]*Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran;* [4]*State Natural History Museum, Braunschweig, Germany*

Molecular phylogeny and intraspecific differentiation of the *Eremias velox* complex of the Iranian Plateau and Central Asia (Sauria, Lacertidae)

Eskandar R. Pouyani[1,2], Sakineh K. Noureini[1], Nasrullah R. Pouyani[3], Ulrich Joger[4] and Michael Wink[2]

Abstract

The Central Asian racerunner, *Eremias velox*, is a widely distributed lizard of the Eurasian lacertid genus *Eremias*. Nucleotide sequences of mitochondrial genes, cyt *b* and 12*S* rDNA from 13 geographically distant localities in Iran and Central Asia, were analysed. Phylogenetic analyses of the sequence data unambiguously recovered five major clades within the *E. velox* complex with a high level of genetic divergence, indicating long periods of isolation. The basal position of the Iranian clades in the phylogenetic trees suggests that the *E. velox* clade originated on the Iranian plateau in the Middle Miocene. According to our calibrations, the northern Iranian clade diverged first some 10–11 Ma and that the Central Asian lineages split from the northeastern Iranian lineage approximately 6 Ma, most likely as a result of uplifting of the Kopet-Dagh Mountains in the northern margin of the Iranian plateau. Topology of the phylogenetic trees, combined with the degree of the genetic distances among the independent lineages recovered in this study, provide a solid foundation for a fundamental revision of the taxonomic status of the major clades within this species complex.

Key words: Mitochondrial DNA – *Eremias velox* complex – Lacertidae – phylogeny

Introduction

The Central Asian racerunner, *Eremias velox* (Pallas 1771), is one of the most widely distributed lizards within the Eurasian lacertid genus, *Eremias*. Its distribution range covers a large territory in Daghestan (Russia), the northeastern Caucasus and Transcaucasus, Lower Volga, Kazakhstan, and all of the middle Asian republics, as far east as northern Afghanistan and western China. In the south, it occurs through the Iranian plateau, on the southern coast of the Caspian Sea, the valleys of Kopet-Dagh, and scattered localities on the northern and western margin of the Central Plateau (Fig. 1) (Szczerbak 1974; Zhao and Adler 1993; Anderson 1999). Ecologically, *E. velox* inhabits a wide variety of surfaces and habitats. In the west, north, and east of its range, the preferred habitats are desert sands and loose soils with poor vegetation. To the south of its range, it occurs on foothills and in river valleys with scattered vegetation and mountains up to 1900 m above sea level (Eremchenko and Panfilov 1999). Morphologically, *E. velox* is a medium-sized lacertid of nearly 20 cm in length, including a tail of up to 12 cm. The juveniles differ radically from the adults in color pattern. Sexual dimorphism is also frequently observed in many populations (Szczerbak 1974; Leviton et al. 1992; Anderson 1999).

Within the large geographical range, several isolated populations have been reported, representing well-distinguished morphotypes (possibly subspecies). Larger regions of the Central Asian range are occupied by the nominate form. The territory to the west is inhabited by the Caspian subspecies, *E. velox caucasia* (Lantz 1928). In western China, eastern Uzbekistan, and eastern Kazakhstan another subspecies, *E. velox roborowski* (Bedriaga 1912), has been reported (Szczerbak 1974). The adults of this subspecies have a dorsal pattern of irregular dark spots and laterally rows of bright blue-edged spots (Szczerbak 2003). Recently, Eremchenko and Panfilov (1999) reported a fourth subspecies, *E. velox borkini* from the highlands of the Issik-kul lake depression of the Tien-Shan Mountains in Kyrgyzstan. It differs from all other subspecies in lacking sexual dimorphism and in having higher numbers of dorsal and gular scales. *Eremias velox* from Iran is poorly known, and large gaps in collections from various parts of the plateau exist. However, based on some limited collections, all of the Iranian populations have been allocated to the nominate subspecies, *E.velox velox* (Anderson 1999). Despite several morphological and ecological studies, particularly on the Central and northern Asian populations, the systematic status and subspecies boundaries within the *E. velox* clade are still in debate (Eremchenko and Panfilov 1999; Szczerbak 2003). To date, no attempt has been made to reveal the intraspecific phylogeny and phylogeography of the species based on molecular genetic data. Origin, distribution, fragmentation, diversification, and subsequent evolution of *E. velox* in this area are still, to a great extent, unknown. A phylogenetic study of the Chinese species of *Eremias* using sequences of 16S rDNA resulted in an unclear picture of relationships within the *E. velox* clade (Lixia et al. 2007).

While herpetologists have traditionally relied upon morphological data for making phylogenetic decisions; contemporary techniques facilitate the discovery of distinct genetic lineages. Indeed, once genetically different populations are identified, and their geographic distributions determined, analyses focused on morphological differentiation can be initiated. Molecular markers are of great value to study intraspecific variation and geographic association and to infer the evolutionary history of a species, especially in cases of little or mostly clinal phenotypic variation (Moritz and Hillis 1996; Cruzan and Templeton 2000). We therefore addressed the

Corresponding author: Eskandar R. Pouyani (rastegarpouyani45@gmail.com; e.rastegar@sttu.ac.ir)
Contributing authors: Sakineh K. Noureini (sakine80@yahoo.com), Nasrullah R. Pouyani (nasrullah.r@gmail.com), Ulrich J. (ulrich.joger@snhm.niedersachsen.de), Michael Wink (wink@uni-hd.de)

Fig. 1. Entire distribution range of *Eremias velox* (gray background) and 13 localities, from which the specimens included in the present study were collected

question of intraspecific differentiation in the Central Asian racerunner by inferring a molecular phylogeny using mitochondrial cytochrome *b* (cyt *b*) and 12S rDNA sequences. These markers have proven very useful in various investigations of molecular phylogeography and systematics in reptiles (e.g., Wink et al. 2001; Guicking et al. 2002a,b; Nagy et al. 2002; Carranza et al. 2004; Carranza and Arnold 2006).

Intraspecific differentiation of a species is a consequence of geographical, demographical, and ecological factors that have operated throughout the evolutionary history (Walker and Avise 1998). It should be particularly apparent in taxa that show only limited mobility, such as reptiles. As several recent studies have shown (e.g., *Nerodia erythrogaster* in the eastern United States), intraspecific variability in these vertebrates provides evidence for the existence of distinct lineages or morphotypes that can be well correlated with geographic regions (Lenk et al. 1999, 2001; Guicking et al. 2002b; Fritz et al. 2005a,b).

In this communication, we report on the molecular phylogeny of the *E. velox* complex inferred from mitochondrial cyt *b* and 12S rDNA nucleotide sequences. The main goals were to reveal (1) whether distinct evolutionary lineages exist in this morphologically heterogeneous species complex, (2) how these correlate with geographic regions, and (3) which inferences can be drawn from these data about the evolutionary history of the species.

Material and Methods

Samples

Nearly 70 specimens of the *E. velox* complex collected between 2002 and 2005 from 13 geographically distant localities, covering large parts of the species' distribution range, were available for phylogenetic analyses (Fig. 1 and Table 1). Based on present knowledge of the phylogenetic relationships within the genus using morphological and molecular data (Szczerbak 1974; Rastegar Pouyani 2007), *Eremias strauchi* and *Ophisops elegans* were included as outgroup taxa. Details on the sample localities, reference numbers of aliquots, and GenBank accession numbers for the cyt *b* and *12S* rDNA sequences are listed in Table 1. The voucher specimens were deposited at the State Natural History Museum, Braunschweig, Germany, and the voucher DNA samples were deposited at the Institute of Pharmacy and Molecular Biotechnology (IPMB), Heidelberg University, Germany.

Laboratory protocols

Isolation of total genomic DNA from preserved liver or muscle tissues followed standard protocols involving phenol–chloroform, chloroform–isoamylalcohol extraction and ethanol washing (Sambrook and Russell 2001). Complete sequences of the mitochondrial gene encoding cyt *b* and partial sequences of the 12S rDNA gene were amplified and sequenced following standard PCR conditions and methods described elsewhere (Rastegar Pouyani et al. 2010). Primers used in both amplification and sequencing were Lgluk 5′ –AACCGCTGTTGTC TTCAACTA-3′, NtheH 5′-GGTTTACAAGACCAGTTGCTTT-3, Mt-E600f 5′ CCATAATTCACCTTCTTTTCC-3′, Ei700r 5′-GGGT GAAAGGGGATTTT (AG)TC-3 for the *cyt b* gene and 12sA 5′-AAACTGGGATTAGATACCCCACTAT-3′ and 12sB 5′-GAG GGT GAC GGG CGG TGT GT-3′ (Kocher et al. 1989) for the12S rDNA gene.

Sequence alignment

Using ClustalW as implemented in the program package Bioedit sequence alignment editor (Hall 1999) with default parameters, nucleotide sequences of cyt *b* (1143 bp) and 12S rDNA (371 bp) were aligned. No gaps were necessary to align the cyt *b,* but when including outgroup taxa, three gaps appeared in all ingroup samples. 12S rDNA sequences. Prior to analysis, cyt *b* sequences were translated into amino acids, using the program package Mega 5 (Tamura et al. 2011) to check for stop codons, suggesting that they were functional sequences. To check for sequencing errors, the sequences were compared with closely related species.

Phylogenetic reconstructions

A test for substitution saturation effect was performed using the program DAMBE (Xia and Xie 2001). ILD test, also known as partition homogeneity test, (Farris et al. 1995; Swofford 2001) was applied to the data for the combination of cyt *b* and 12S rDNA sequence partitions. Calculation of the genetic divergence within and among populations was performed using MEGA version 4 (Tamura et al., 2007). Result of the ILD test (ILD, $p = 0.387$) clearly showed that the two gene fragments are congruent, and subsequently, they were combined in a total-evidence analysis. However, because this test has received much criticism recently (Dolphin et al. 2000; Barker and Lutzoni 2002), we examined congruence of tree topologies derived from the individual data sets with that derived from the combined data set (Nagy et al. 2004; Guicking et al. 2006).

Three different methods of phylogenetic analysis were employed and the results compared: maximum likelihood (ML) and maximum parsimony (MP), using the program PAUP* 4.0b10 (Swofford 2001), and Bayesian inference using the program MR.BAYES 3.1.2 (Huelsenbeck and Ronquist 2001). The MP analysis was performed with all sites weighted equally, because saturation effects were negligible in our data set. For ML and BI analyses, MODELTEST 3.07 (Posada and Crandall 1998) was used to select the most appropriate model of sequence evolution for the genetic regions, both independently and in combination. The selected models for cyt *b* and 12S, under Akaike information criterion, were GTR + I + G and HKY + I + G, respectively. For the combined data set, this was the GTR + I + G model with the following parameter settings: base frequencies: A = 0.2866, C = 0.3096, G = 0.1356, T = 0.2681, six substitution types: A–C = 5.4756, A–G = 23.2875, A–T = 4.9936, C–G = 3.6434, C–T = 29.4806, G–T = 1.0000, proportion of invariable sites I = 0.4419, among site rate variation followed a gamma distribution with shape parameter = 0.8849. The MP trees were estimated using the heuristic search algorithm with tree-bisection–reconnection (TBR) branch swapping and 100 random addition replicates, and all changes had the same weight. The robustness of the branches of the shortest MP and ML trees were assessed using nonparametric bootstrapping (Felsenstein 1985) performed with 1000 replicates.

doi: 10.1111/j.1439-0469.2012.00662.x

Table 1. List of the materials used in this study with the localities and reference numbers. Numbers of the localities correspond to those defined in Fig. 1

Field number	Species	Locality number	IPMB number	Locality	Accession number	
					Cyt b	12S
02-74	*E. velox*	1	40605	North Tehran[1]	JQ690170	JQ690101
02-75	*E. velox*	1	40606	North Tehran[1]	JQ690169	JQ690100
ERP263	*E. velox*	2	41048	SE Tehran, the Central Desert of Iran	JQ690197	JQ690129
ERP266	*E. velox*	2	41049	SE Tehran, the Central Desert of Iran	JQ690172	JQ690131
ERP267	*E. velox*	2	41050	SE Tehran, the Central Desert of Iran	JQ690198	JQ690130
ERP268	*E. velox*	2	41051	SE Tehran, the Central Desert of Iran	JQ690195	JQ690127
ERP269	*E. velox*	2	41052	SE Tehran, the Central Desert of Iran	JQ690196	JQ690128
Smp188	*E. velox*	3	40725	SE Golestan National park[2]	JQ690181	JQ690113
Smp189	*E. velox*	3	40726	SE Golestan National park[2]	Q690182	JQ690114
Smp206	*E. velox*	3	40729	SE Golestan National park[2]	JQ690176	JQ690108
Smp207	*E. velox*	3	40730	SE Golestan National park[2]	FJ416174	JQ690107
Smp208	*E. velox*	3	40731	SE Golestan National park[2]	JQ690180	JQ690112
Smp210	*E. velox*	3	40733	SE Golestan National park[2]	JQ690179	JQ690111
Smp211	*E. velox*	3	40734	SE Golestan National park[2]	JQ690178	JQ690110
Smp221	*E. velox*	3	40735	SE Golestan National park[2]	JQ690175	JQ690106
Smp230	*E. velox*	3	40736	SE Golestan National park[2]	JQ690177	JQ690109
ERP359	*E. velox*	3	41011	SE Golestan National park[2]	JQ690194	Q690126
ERP250	*E. velox*	4	41041	10 km S Jajarm town[3]	FJ416175	JQ690132
ERP251	*E. velox*	4	41042	10 km S Jajarm town[3]	JQ690201	JQ690134
ERP252	*E. velox*	4	41043	10 km S Jajarm town[3]	JQ690202	JQ690135
ERP253	*E. velox*	4	41044	10 km S Jajarm town[3]	JQ690200	JQ690133
ERP255	*E. velox*	4	41046	10 km S Jajarm town[3]	JQ690203	JQ690136
Smp247	*E. velox*	4	40739	10 km S Jajarm town[3]	JQ690173	JQ690104
Smp267	*E. velox*	4	40742	10 km S Jajarm town[3]	JQ690174	JQ690105
Smp268	*E. velox*	4	40734	10 km S Jajarm town[3]	JQ690171	JQ690102
Smp266	*E. velox*	4	40741	10 km S Jajarm town[3]	JQ690199	JQ690103
Smp15	*E. velox*	5	40718	15 km SW Sabzevar[4]	JQ690183	JQ690115
Smp16	*E. velox*	5	40719	15 km SW Sabzevar[4]	JQ690186	JQ690118
Smp17	*E. velox*	5	40720	15 km SW Sabzevar[4]	JQ690185	JQ690117
Smp18	*E. velox*	5	40721	15 km SW Sabzevar[4]	JQ690184	JQ690116
ERP188	*E. velox*	6	40840	S Naishaboor, N Khorasan, Iran	JQ690187	JQ690119
ERP199	*E. velox*	6	40841	S Naishaboor, N Khorasan, Iran	JQ690192	JQ690124
ERP190	*E. velox*	6	40842	S Naishaboor, N Khorasan, Iran	JQ690188	JQ690120
ERP191	*E. velox*	6	40843	S Naishaboor, N Khorasan, Iran	JQ690189	JQ690121
ERP192	*E. velox*	6	40844	S Naishaboor, N Khorasan, Iran	JQ690190	JQ690122
ERP198	*E. velox*	6	40845	S Naishaboor, N Khorasan, Iran	JQ690191	JQ690123
ERP201	*E. velox*	6	40847	S Naishaboor, N Khorasan, Iran	JQ690193	JQ690125
Ev1	*E. velox*	7	40568	S Aral Sea, Uzbekistan	JQ690218	JQ690151
Ev2	*E. velox*	7	40569	S Aral Sea, Uzbekistan	JQ690216	JQ690149
Ev3	*E. velox*	7	40570	S Aral Sea, Uzbekistan	JQ690217	JQ690150
Ev4	*E. velox*	7	40571	S Aral Sea, Uzbekistan	JQ690219	JQ690152
Ev5	*E. velox*	7	40572	S Aral Sea, Uzbekistan	JQ690220	JQ690153
Ev6	*E. velox*	7	40573	S Aral Sea, Uzbekistan	JQ690221	JQ690154
Ev7	*E. velox*	7	40574	S Aral Sea, Uzbekistan	JQ690222	JQ690155
Ev8	*E. velox*	7	40575	S Aral Sea, Uzbekistan	JQ690223	JQ690156
Ev9	*E. velox*	7	40576	S Aral Sea, Uzbekistan	JQ690224	JQ690157
Ev11	*E. velox*	7	40578	S Aral Sea, Uzbekistan	JQ690225	JQ690158
Ev12	*E. velox*	7	40579	S Aral Sea, Uzbekistan	JQ690226	JQ690159
Ev13	*E. velox*	7	40580	S Aral Sea, Uzbekistan	JQ690227	JQ690160
M90	*E. velox*	8	40926	W Aral Sea, Kazakhstan	JQ690212	JQ690145
D4	*E. velox*	9	41056	Extreme W Kazakhstan	JQ690233	JQ690166
D10	*E. velox*	10	41058	SW Balkhash Lake, Kazakhstan	JQ690228	JQ690161
D9	*E. velox*	10	41057	SW Balkhash Lake, Kazakhstan	JQ690229	JQ690162
RL112	*E. velox*	10	41065	SW Balkhash Lake, Kazakhstan	JQ690234	JQ690167
RL26	*E. velox*	10	41053	SW Balkhash Lake, Kazakhstan	JQ690230	JQ690163
RL28	*E. velox*	10	41054	SW Balkhash Lake, Kazakhstan	JQ690231	JQ690164
RL65	*E. velox*	10	41055	SW Balkhash Lake, Kazakhstan	JQ690232	JQ690165
K04-6	*E. velox*	10	40931	SW Balkhash Lake, Kazakhstan	JQ690215	JQ690148
04-8	*E. velox*	11	40933	NE Aral Sea, Kazakhstan	JQ690213	JQ690146
K04-12	*E. velox*	11	40934	NE Aral Sea, Kazakhstan	JQ690214	JQ690147
T105	*E. velox*	12	40930	Turkistan, S Kazakhstan	JQ690206	JQ690139
M6	*E. velox*	13	40929	E Kazakhstan	JQ690204	JQ690137
U16	*E. velox*	13	40928	E Kazakhstan	JQ690205	JQ690138
A11	*E. velox*	13	40924	E Kazakhstan	JQ690208	JQ690141
A1	*E. velox*	13	40923	E Kazakhstan	JQ690209	JQ690142

doi: 10.1111/j.1439-0469.2012.00662.x

Table 1. (Continued)

Field number	Species	Locality number	IPMB number	Locality	Accession number	
					Cyt b	12S
Pp	*E. velox*	13	40925	E Kazakhstan	JQ690207	JQ690140
U12	*E. velox*	13	40927	E Kazakhstan	JQ690210	JQ690143
M41	*E. velox*	13	40920	E Kazakhstan	JQ690211	JQ690144
ERP 276	*O. elegans*	Outgroup	40627	Tehran province, Iran	FJ416172.1	FJ445250
ERP317	*E. strauchi*	Outgroup	40997	NW Iran	JQ690099	JQ690168

[1]Ninety kilometre, north Tehran, northern Iran.
[2]Golestan province, Iran.
[3]NW Khorasn, Iran.
[4]W Khorasan, Iran.

A partitioned Bayesian analysis was performed in four chains and two independent runs for four million generations with model parameters for each gene partition (GTR + I + G for cyt *b* and HKY + I + G for 12S rDNA) being independently estimated as part of the analysis. The analyses were started with randomly generated trees and every 100th tree was sampled. The log-likelihood of the 40 000 trees in each analysis was plotted against the generation time. After verifying that saturation had been reached, both in the term of likelihood scores and parameter estimation, the first 8000 trees were discarded in both runs, and a majority-rule consensus tree was generated from the remaining 32 000 (postburnin) trees. The frequency of any particular clade among the individual trees contributing to the consensus tree represents the posterior probability of that clade (Huelsenbeck and Ronquist 2001).

Rough molecular clocks may give some idea of the absolute date of colonization, and this can be used to distinguish different kinds of natural colonization (Carranza and Arnold 2003). To apply the concept of the molecular clock, it is necessary to first check the data set for constancy of the substitution rate. For this purpose, relative rate tests were performed either on pairwise comparisons of individual taxa (following the procedure of Tajima (1993), as implemented in MEGA vers 4 (Tamura et al., 2007) or on group wise comparisons as implemented in PHYLTEST vers. 2.0 (Kumar 1996). In addition, a log-likelihood ratio test was used to examine the clock-like evolution of sequences of the ingroup in the combined data set by calculating a x^2 statistic (Likelihood Ratio Test, LRT) based on ML values with and without rate constancy enforced (x^2 = 2_[(_lnLclock)_(_lnLunconstrained)], df = number of terminal nodes-2) (Felsenstein 1981). As calibration points, we used the previously estimated maximum age of divergence time in the subfamily Eremiadini (*Ophisops, Eremias* in our study), which was setting up at 16 Ma (Arnold et al. 2007) or 13.6 Ma (Kyriazi et al. 2008) and estimated divergence time between the Iranian and Central Asian caldes of *Eremias velox* at approximately 6 Ma (Rastegar Pouyani 2007) caused by uplifting the Elburz and Lesser Caucasus Mountains in late Miocene (Abdrakhmatov et al. 1996).

Results

Phylogenetic analysis

A total of 1514 characters were aligned unambiguously in the 70 specimens included in this study (including 68 ingroup and two outgroup taxa). Of these, 599 characters were variable and 404 were parsimony informative. The stop codon in cyt *b* was usually TAA but it was TAG in specimens from north Iran and northwest Khorasan (localities 1 and 4). A distinct bias against G in the base composition of the light strand, and the absence of unexpected stop codons in the cyt *b* protein, indicated that the aligned sequences represent functional mitochondrial genes and not paralogous copies (Macey et al. 1997; Zhang and Hewitt 1999; Nagy et al. 2004; Guicking et al. 2006). In the sequence data transitions exceeded transversions at low levels of divergence. This agrees with previous studies of animal mtDNA that have reported an initially high (> 50%) transition bias gradually decreasing over time (Brown et al. 1982; Hedges et al. 1991; Fuller et al. 1998; Honda et al. 2000a,b). The scatter plots did not exhibit a distinct transition plateau (not shown), at which multiple substitutions occur at the same site (Thomas et al. 1989; Hedges et al. 1991). In addition, statistical tests of saturation demonstrated that the data sets (12S and cyt *b*), either individually or in combination, exhibit no substitution saturation (the observed I_{ss} values (0.136) are significantly lower than $I_{ss.c}$ (0.834).

The partition homogeneity test (ILD test) of the two mitochondrial genes, tested simultaneously, revealed no significant heterogeneity between cyt *b* and 12S rDNA data set, p = 0.387, suggesting that the level of congruence in the phylogenetic signal among these genes is similar and could be subsequently combined for phylogenetic analysis.

To resolve intraspecific differentiation based on cyt *b* and 12S rDNA sequences, phylogenetic trees were calculated under MP and ML criteria, as well as, Bayesian inference. All phylogenetic analyses produced trees of the same overall (general large-scale structure) topology (Fig. 2). MP analysis yielded 548 equally parsimonious cladograms of 1285 steps, consistency index (CI) = 0.51315, homoplasy index (HI) = 0.4070, and retention index (RI) = 0.9020. The large number of equally parsimonious solutions was largely owing to small differences in terminal branches, particularly among specimens originating from the same or geographically proximal populations. ML analyses of the combined data set under the same model of evolution (GTR + G + I) resulted in a tree of lnL = −8431.70391, which was identical to the BI tree. Bayesian inference under the GTR + I + G model for cyt *b* and the HKY + I + G model for 12S, resulted in a topology with mean lnL = −8538.12. Posterior probability values from the BI were highly congruent with ML bootstrap support. Similarity in lnL values and nodal supports (see Fig. 2) suggest that two methods successfully converged on the same tree space.

The phylogenetic trees provided strong support for monophyly of geographically distinct populations of the *E. velox* clade with highly resolved intraspecific relationships among the major lineages (Fig. 2). Five major clades could be identified in all reconstructions with considerable bootstrap and Bayesian supports, which generally correspond to geographical regions within the distribution range. In addition, the single specimen from west Kazakhstan is well differentiated from all other Central Asian populations, forming a distinct lineage. These clades are designated A, B, C, D and E in Fig. 2. The most basal dichotomy in the tree separated the northern

doi: 10.1111/j.1439-0469.2012.00662.x

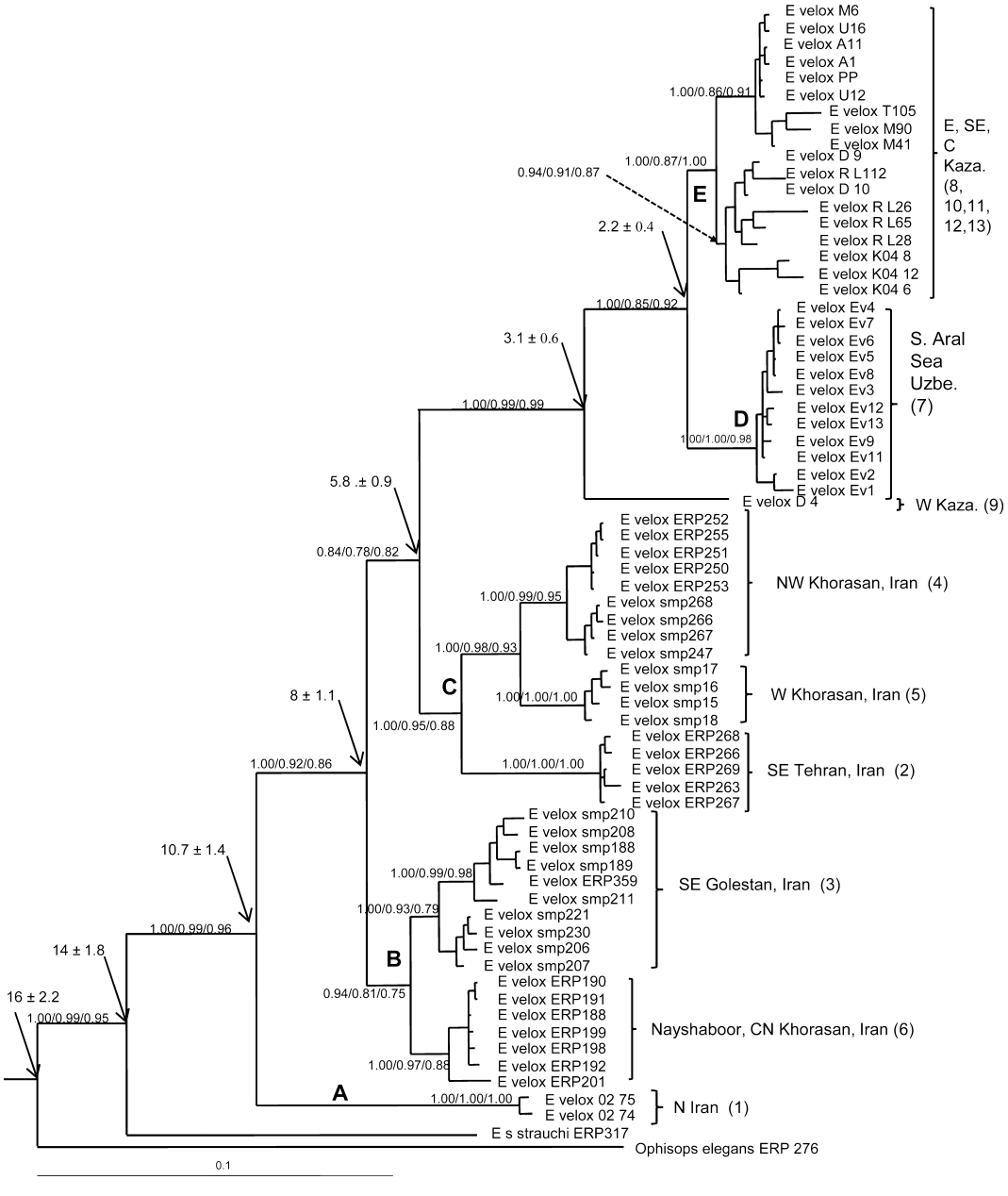

Fig. 2. Phylogenetic relationships among the *Eremias velox* populations included in the analysis. Individuals of *Ophisops elegans* and *Eremias strauchi* were used as outgroup taxa. Phylogenetic analyses of maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) produced trees with the same topology with regard to the major lineages. Only the BI tree is presented. Numbers close to the branches are posterior probabilities of BI followed by MP and ML bootstrap supports (1000 replicates). A, B, C, D and E indicate the major clades. The arrows indicate the estimated time of divergences of the major clades (in Ma) along with the related standard deviations (see the text for details). Numbers within the pranthesis indicate the localities of the related clades correspond to those indicated in Fig. 1 and Table 1

Iranian specimens, collected from the deep valleys of Elburz Mountains (clade A, locality 1), with very statistical high supports (100% bootstrap and posterior probability values). The second divergence divided the remaining into two major units leading to the clades B and C, D, E which are genetically very well differentiated (Table 2). In addition, they both received high bootstrap and posterior probability supports. Clade B consists of two discernible subunits from the southeast region of the Golestan province (locality 3) and the specimens from Nayshaboor in northern-Central Khorasan (locality 6). Clade C was also subdivided into three well-distinguished assemblages corresponding to the localities 2, 4 and 5. All reconstructions clearly suggested a sister relationship between the lowland-inhabiting specimens from the northeastern portion of the Iranian plateau (clade C) and all the Central Asian clades, in which the single specimen of west Kazakhstan forms the sister taxon of the rest. It should be noted that only one sample from west Kazakhstan (locality 9) was available for this study. Therefore, any interpretations regarding the actual position of this population should be made cautiously. The remaining materials from the Central Asian localities were fragmented into two major units forming clades D and E, which refer to the specimens examined from Uzbekistan (locality 7) and several localities in Kazakhstan (localities 8,

doi: 10.1111/j.1439-0469.2012.00662.x

Table 2. Genetic distances (p-distance) between 13 populations of the *Eremias velox* complex included in this study

	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	0.094												
3	0.076	0.065											
4	0.085	0.056	0.058										
5	0.086	0.060	0.064	0.034									
6	0.075	0.062	0.029	0.050	0.056								
7	0.113	0.098	0.097	0.093	0.090	0.087							
8	0.108	0.092	0.092	0.089	0.088	0.089	0.033						
9	0.100	0.095	0.089	0.086	0.088	0.085	0.068	0.055					
10	0.103	0.088	0.090	0.088	0.085	0.086	0.033	0.020	0.056				
11	0.108	0.084	0.087	0.084	0.080	0.079	0.036	0.024	0.067	0.030			
12	0.104	0.083	0.084	0.085	0.081	0.076	0.037	0.033	0.062	0.029	0.013		
13	0.106	0.080	0.085	0.084	0.080	0.079	0.039	0.031	0.067	0.021	0.013	0.011	

Numbers of the populations refers to those in Fig. 1 and Table 1.

Table 3. Genetic diversity (p-distance) for major clades of the *Eremias velox* complex and the outgroup taxa

	Oe	Es	A	B	C	D	E	WK
[Oe]								
[Es]	0.202							
[A]	0.194	0.135						
[B]	0.198	0.132	0.082					
[C]	0.193	0.139	0.088	0.059				
[D]	0.194	0.138	0.113	0.093	0.094			
[E]	0.190	0.132	0.105	0.086	0.085	0.036		
[WK]	0.199	0.148	0.100	0.087	0.089	0.068	0.061	

Oe = *Ophisops elegans,* Es = *Eremias strauchi,* WK = West Kazakhstan sample and the letters A–D correspond to the major clades indicated in Fig. 2

10, 11, 12, 13). All the major clades within the *E. velox* complex suggested by the present study are generally highly supported by bootstrap and posterior probabilities as well as the considerable level of genetic divergence (Fig. 2 and Table 3). Comparison of the chronogram and phylogram by a likelihood ratio test did not yield significant differences in the likelihoods of these trees, suggesting that the error introduced by rate heterogeneity was not very great (–lnChronogram = 8452.4854 –lnPhylogram = 8431.70391, LR = 41.56291, df = 68, $p > 0.05$). The relative rate test performed with PHYLTEST (Kumar 1996) indicated that consistency of mutation rate at the 5% level is not rejected by our data. (Z value of 7.564×10^{-4}). Consequently, the major divergence times derived from a ML tree with a calibrated time bar (not shown) are indicated close to relative nodes in Fig. 2. Using the calibration points presented here (see above) an evolutionary rate of 0.65% per lineage per million years was calculated for the combination of the two mitochondrial gene fragments. This amount corresponds to the amounts calculated in other lacertid lizards, derived from exactly the same genetic markers (Maca-Meyer et al. 2003; Carranza et al. 2004; Arnold et al. 2007; Pavlichev and Mayer 2009).

Genetic diversity among populations and the major clades

Genetic divergence data among 13 populations of *E. velox* included in this study are shown in Table 2. The highest genetic diversity (p-distance) between populations was 11.3%, between the northern Iran population (locality 1) and specimens from the southern Aral Sea in Uzbekistan (locality 7). The lowest value was 1.1%, between southern and eastern Kazakhstan populations (localities 12 and 13, respectively). Genetic diversity (not shown) within populations was generally low; the highest value was 1.6%, which scored within the Southeastern Golestan population (locality 3).

Pairwise genetic distances based on cyt *b* and 12S rDNA sequences are summarized as interclade comparisons in Table 3. Distances of 3.6% to 11.3% separated the five major clades from each other. Between the Iranian samples from locality 1 and the rest of the Iranian clades (clades B and C), genetic distances were in the range of 5.6–8.8%. Intraclade distances were highest in clade C (3.3%). Lower interclade genetic distances among the Central Asian units than those scored among the Iranian clades may indicate less diversity. Considerably lower within- than among-clade distances (Table 3) and generally shallow branch topologies among the crown nodes (Fig. 2), might suggest that differentiation into extant haplotypes occurred comparatively recently.

Discussion

The present study clearly indicates the occurrence of independent lineages in the *E. velox* complex, which experienced long periods of isolation as suggested by previous studies based on morphology and ecology (Szczerbak 1974, 2003; Eremchenko and Panfilov 1999). This information may serve to elucidate the evolutionary history of the species and to provide guidelines for the revision of taxonomic status of the major lineages.

According to the results presented here, with special reference to the observed levels of genetic divergence, at least five major clades can be distinguished within the *E. velox* complex. These clades correlate well with geographic regions of Central Asia and the Iranian Plateau. However, evidence for the Western Kazakhstan population is based on a single specimen and therefore cannot reliably represent a clade and an evolutionary lineage. Further sample material from this region could be informative to verify the persistence of an independent genetic lineage in western Kazakhstan. Note, however, that the locality of this single specimen is located

doi: 10.1111/j.1439-0469.2012.00662.x

within the range of one of the well-established subspecies of the *E. velox* complex, *E. velox caucasia*, with several diagnostic morphological features (Szczerbak 1974, 2003). Unfortunately, no morphological data were available from this specimen to clarify its subspecies affiliation. However, the only partial sequence of the cyt *b* belonging to *E. velox*, available in GenBank (AF 206549; Fu 2000), is identical to the cyt *b* sequences of our single specimen of Western Kazakhstan in the available fragment (660 bp). As the locality of this sample, Dagestan (southwestern Russia), is close to the type locality of *E. velox caucasia*, most likely, both specimens might be attributed to *E. velox caucasia*. The remaining three Iranian and two Central Asian lineages are well-differentiated from one another by considerable genetic distance (Table 3). Position of the north Iranian population at the base of the phylogenetic tree with high genetic distances from all other clades indicates a long period of isolation. This separation is also supported by ecological and morphological evidence. This population is the only record of *E. velox* in Iran inhabiting the valleys of Central Elburz at an elevation of over 2000 m above sea level. They also differ morphologically from other Iranian populations of *E. velox* by having several diagnostic features (unpublished data). According to our mitochondrial markers, they are indeed a factual outgroup for all remaining populations of the *E. velox* clade. Many morphological traits of this population are closer to *E. strauchi* than to *E. velox*. However, the genetic distance of 13.5% between them (Table 3) makes it very unlikely that it is attributable to *E. strauchi (sensu stricto*). The remaining population of the Iranian clades B and C correlate with two geographically distinct habitats in the general area of the northeastern Iranian Plateau. Populations of clade B inhabit the foothills and generally rugged areas of the eastern Elburz Mountains. During several long-term expeditions in the area, they have never been observed at altitudes below 1800 m. In contrast, the specimens of clade C were always observed on open plains and desert habitats of the south and southeastern Elburz Mountains in Tehran, Semnan and Khorasan provinces of northeastern Iran. Clade C is genetically highly heterogeneous, with 3.3% intraclade diversity. Homogeneity in the habitat notwithstanding, a geographical distance of at least 500 km separates these populations from each other. Indeed, they may be reproductively isolated because of geographical distance, although no apparent barrier can be defined between them. In addition, divergence of the west Khorasan specimens (locality 5), from northwest Khorasan clade, might be a result of introgression between this population of *E. velox* and the specimens of its closest related taxon, *Eremias persica,* in the deserts and steppes of the northeastern Iran, where they could frequently be seen sympatrically. This of course can be addressed with including specimens of *E. persica* of the area in the analysis, if this hypothesis is true (that the Khorasan specimens have in fact mtDNA from *E. persica*) it would mean that *E. persica* would make *E. velox* paraphyletic. Even mor informative solution could be using a nuclear marker that most likely it can reveal gene flow and introgresion between these taxa(the work that is in progress using nuclear gene PRLR).

Genetic divergence between the Iranian and Central Asian clades was at least 8.6% (p-distance) (Table 3). Furthermore, the branching pattern of the phylogenetic tree supports the monophyly of the Central Asian populations, which are well separated from the Iranian specimens. In the eastern parts of Kazakhstan and northwestern China, *E. velox roborowskii* (Bedriagai, 1912) has been reported (Szczerbak 1974). Clade E in the present study seems to represent this subspecies. Although the specimens of clade E were collected from several geographically distant localities, they are well defined as belonging to the same clade with very low interpopulation and intraclade genetic distances (Tables 2 and 3). It is also noteworthy that the populations of the northeast Aral Sea (locality 11) are definitely out of the range for *E. velox roborowskii*, however, they are grouped within clade E. This might also reflect a degree of gene flow in the boundaries between the nominate subspecies and *E. velox roborowskii*.

Biogeography

A particular advantage of the use of molecular techniques is the possibility to estimate divergence times of evolutionary lineages (molecular clock). Knowledge of the evolutionary rate allows dating divergence times from sequence data. This is of course notble that besides other problems with any molecular clock, the problems with caliberation of a molecular clock is usually inevitable. In the best situation, caliberations are only an approximate of time divergence, particularly among deep and basal nodes. According to our calibrated molecular clock, the first splitting event within the *E. velox* complex isolated the North Iranian unit, some 10.7 Ma. This was most likely caused by first uplifting of the Elburz Mountains in the late Miocene some 12–10 Ma (Abdrakhmatov et al. 1996; Macey et al. 1998, 2000a,b). This geological event apparently caused fragmentation of the *E. velox* clade into two well isolated groups, the western subunit inhabiting deep valleys of the Central Elburz and the eastern subunit distributed in foothills and open plains of the eastern and southeastern Elburz Mountains. Further expansion of the western clade should have been prevented by very deep valleys of the Central Elburz rimmed by a series of highly elevated rocks, so that they are now found in a few scattered localities in this area in the form of relict populations. In contrast, the eastern clade seems to have undergone several successive and relatively rapid speciation events during the late Miocene to mid-Pliocene. The first split within the eastern clade separated the highland-inhabiting populations of southeast Golestan and northwest Khorasan (clade B) from the desert dwelling lineages of northwest and west Khorasan and the Iranian Central Desert population (clade C) at approximately 8 Ma. The split between the Iranian and Central Asian clades was estimated to have occurred around 5.7 Ma. Genetic distance between the northeast Iranian unit (clade C) and the Central Asian clades reaches 9.4%. Considering paleogeographical evidence, we hypothesize that the genetic distance between Central Asian *E. velox* and their closest relatives in the Iranian plateau suggests independent evolutionary history since the uplifting of the Kopet-Dagh Mountains, some 6 Ma (Abdrakhmatov et al. 1996; Macey et al. 1998, 2000a, b). This phenomenon presumably formed a geographic barrier preventing gene flow between the Iranian and Central Asian populations. This event can also help to calibrate a molecular clock within the *E. velox* complex of about 1.25% sequence divergence in the mitochondrial genome among lineages per one million years. By taking into account evolutionary rate estimates for the same marker in other members of the genus *Eremias*, as well as in other scleroglossan lizards (Maca-Meyer et al. 2003; Carranza et al. 2004), the estimated evolutionary rate for the *E. velox* complex lies within the range of the previous evolutionary rate estimates.

doi: 10.1111/j.1439-0469.2012.00662.x

Genetic distances between clades and within populations were generally greater within the Iranian clades than in the non-Iranian taxa (Table 2 and 3). In addition, the inferred phylogeny clearly assigned basal position to the Iranian populations, suggesting that the rapid fringe-toed lizard probably originated on the Iranian plateau and invaded Central Asia by the late Miocene. Considering the maximum genetic distance among the Iranian clades on one hand and those between the Iranian and Central Asian clades on the other, it seems that the *E. velox* clade started to diverge in the late Miocene relatively rapidly. Possibly one or two independent lineages invaded Central Asia via the land bridge between the Caspian and the high plateaus of Badkyz and Pamir, the region which had evidently become arid as a result of the retreat of the Caspian Sea in the mid-Miocene (Abdrakhmatov et al. 1996). In the late Miocene, the invaders seem to have dispersed into Central Asia relatively rapidly owing to unoccupied niches and lack of ecological competitors. Rapid cladogenesis resulted in producing several morphologically distinct morphotypes which could not be resolved by genetic markers. This is possibly responsible for lower genetic divergence and relatively less resolved relationships among the Central Asian clades, in spite of their vast distribution range. The pattern is not restricted to *E. velox*, it has also been shown in a phylogenetic study of the agamid lizard *Trapelus agilis*, which is one of the most widely distributed lizards in the area (Macey and Ananjeva 2004). Low resolution of phylogenetic relationships among the Central Asian clades of *E. velox* complex is even more pronounced in a phylogenetic tree reconstructed using the ISSR data set (Rastegar Pouyani et al. 2009). This is, of course, most likely due to slower lineage sorting in nuclear than mitochondrial DNA (Moore 1995; Moritz and Hillis 1996). Furthermore, the Central Asian lineages are mainly isolated by distance alone, whereas between the Iranian lineages geographical barriers can be defined.

Taxonomic implications

The data presented here provide strong evidence for intraspecific subdivision of the *E. velox* complex. Six distinct lineages can be recognized within the species complex. These have most likely evolved independently over the last 6–11 Ma. To account for this high intraspecific diversity and long divergence times, it seems desirable to assign species or at least subspecies rank to the genealogical lineages. Good congruence between the present mitochondrial and recently published nuclear data (Rastegar Pouyani et al. 2009) may justify the designation of subspecies even when distinguishing these subspecies from phenotype would probably not be possible.

The data provide strong evidence for the north Iranian lineage being a distinct taxonomic entity at the species level. This unit is highly differentiated from all other lineages genetically, morphologically, and ecologically. The other two Iranian lineages (clades B and C) are also well-distinguished genetically and ecologically, but less pronounced, morphologically. A distinctive color pattern discriminates them from each other but the scalation supports their divergence relatively weakly (unpublished data). Nonetheless, considering their high degree of genetic distance and well-differentiated ecological features, we recommend recognizing these lineages as distinct species. However, increased sampling covering the entire distribution range and extension of molecular and morphological investigations seems desirable to define geographic boundaries between these evolutionary lineages more accurately. The fact that several genetic units are discernible within the clade C should not be used as grounds to partitioning them into several distinct subspecies. Morphologically, the group is well defined as a whole. The populations at localities 2, 3, and 5 are easily recognized as belonging to the same clade, but this is not strictly true of the subclades, which are difficult to define morphologically.

The Central Asian lineages, as a whole, represent a clearly distinguishable unit, genetically, geographically, and, to a great extent, morphologically, such that they can be easily distinguished from each other. Hence, the Central Asian clade should also be recognized as a distinct species. The data presented here provide good evidence to identify three independent lineages within the Central Asian clade corresponding, to a great extent, to the previously recognized morphotypes (subspecies) in the area. These can also be related to the geographic expansion of the recorded subspecies. The eastern and southern Kazakhstan populations, grouped within clade E, correspond to the traditional *E. velox roborowskii*, subspecies, as do likewise the clade D and single specimen of west Kazakhstan in re-identification of *E. v. velox* and *E. v. caucasia*, respectively.

Eremchenko and Panfilov (1999) reported a new subspecies, *E. velox borkini* from the highlands of Kyrgyzstan. Unfortunately, no material from this area was available for the present study; therefore, the taxonomic status of this new morphotype remains uninvestigated. As the type locality of *Eremias velox* is located in Central Asia, all the Central Asian clades should be assigned to *E. velox,* and the Iranian lineages should await new scientific names.

To conclude, the present study provides good arguments for a fundamental revision of the taxonomic status of the *E. velox* complex. Subdividing the clade into four distinct species is recommended: (1) The former *E. velox* should be applied to the whole Central Asian clade, within which three subunits (subspecies) can be identified. (2) The reconstructed phylogeny and amount of the genetic distances strongly suggest that the northern Iranian lineage represents a distinct species. (3) Populations of the northeastern Iranian Plateau (clades B and C) represent the third and fourth species, respectively. However, although the molecular basis to split these clades into two distinct species is provided by this study, a more comprehensive ecological and morphological investigation seems to be necessary to define the species boundaries and figure out the diagnostic morphological features.

Acknowledgements

We would like to thank Göran Nilson, Claes Andren, Daniela Guicking, Marina Chirikova, Hilde Enting, Yurij Chikin, and Tatjana Dujsebayeva for their generous assistance in providing some of the Iranian and Central Asian tissue specimens. We are thankful to authorities at Hakim Sabzevari University, Iran, for providing the field work facilities in Iran. Special thanks go to Majid Sabeti, Alireza Hashemi, and Mohammad Talebi for their unforgettable help in collecting samples in the hot and dry deserts of the Iranian plateau. The work in Iran was partly financed by National Geography Society and in Central Asia by a grant from the German Research Association (DFG Jo-9).

doi: 10.1111/j.1439-0469.2012.00662.x

Zusammenfassung

Molecular phylogeny and intraspecific differentiation of the Eremias velox *complex of the Iranian Plateau and Central Asia (Sauria, Lacertidae)*

Eremias velox (Gattung *Eremias*) aus Zentralasien gehört zu den weit verbreiteten Eidechsen Eurasiens. Nucleotidsequenzen mitochondrialer DNA (Cytochrom b und 12 S rDNA) von 13 geographisch unterschiedlichen Standorten aus dem Iran und Zentralasien wurden genauer analysiert. Die phylogenetische Auswertung fand 5 genetische Linien innerhalb des *E. velox* – Komplexes, die sich durch große genetische Distanzen unterscheiden und auf lange Zeiten der Isolation hinweisen. Innerhalb der Gesamtphylogenie liegen die iranischen Kladen basal. Dies deutet darauf hin, dass der *E. velox* Komplex im mittleren Miozän auf dem iranischen Plateau entstand. Aufgrund von Kalkulationen über eine molekulare Uhr wird angenommen, dass die nordiranischen Linien von 10–11 Millionen Jahren entstanden, während sich die zentralasiatischen Taxa vor 6 Ma abzweigten. Dies wurde vermutlich durch die Entstehung der Kopet-Dagh-Berge am Nordrand des iranischen Plateaus ausgelöst. Die Baumtopologie und die genetischen Distanzen bieten eine gute Grundlage dafür, den taxonomischen Status der hier gefundenen Hauptlinien des *E. velox* – Komplexes neu zu bewerten.

References

Abdrakhmatov K, Aldazhanove Y, Hager SA, Hamburger B, Herring MW, Kalabaev TA, Makarov KB, Molnar VI, Panasyuk P, Prilepin SV, Reilinger MT, Sadybakasove RE, Souter IS, Trapeznikov BJ, Tsurkov YA, Zubovich AV (1996) Relatively recent construction of the Tien Shan inferred from GPS measurements of present day crustal deformation rates. Nature **384:**450–453.

Anderson SC (1999) The Lizards of Iran. Society for the Study of Amphibians and Reptiles, Ithaca, NY, pp 442.

Arnold EN, Arribas O, Carranza S (2007) Systematics of the palaearctic and oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera. Zootaxa **1430:**1–86.

Barker FK, Lutzoni FM (2002) The utility of the incongruence length difference test. Syst Biol **51:**625–637.

Brown WM, Prager EM, Wang A, Wilson AC (1982) Mitochondial DNA sequences of primates: tempo and mode of evolution. J Mol Evol **18:**225–239.

Carranza S, Arnold EN (2003) Investigating the origin of transoceanic distributions: mtDNA shows *Mabuya* lizards (Reptilia, Scincidae) crossed the Atlantic twice. Syst Biodivers **1:**275–282.

Carranza S, Arnold EN (2006) Systematics, biogeography and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. Mol Phylogenet Evol **38:**531–545.

Carranza S, Arnold EN, Amat F (2004) DNA phylogeny of *Lacerta* (*Iberolacerta*) and other lacertinae lizards (Reptilia: Lacertidae): did competition cause long-term mountain restriction? Syst Biodivers **2:**57–77.

Cruzan MB, Templeton AR (2000) Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record. Trends Ecol Evol **15:**491–496.

Dolphin K, Belshaw R, Orme CDL, Quicke DLJ (2000) Noise and incongruence: interpreting results of the incongruence length difference test. Mol Phylogenet Evol **17:**401–406.

Eremchenko V, Panfilov A (1999) Taxonomic position and geographic relations of a lacertid lizard *Eremias velox* from the Issyk-Kul lake depression, Tien Shan mountains, Kyrgyzstan. Science and New Technology **99:**119–125.

Farris JS, Kallersjo M, Kluge AG, Bul C (1995) Constructing a significance test for incongruence. Syst Biol **44:**570–572.

Felsenstein J (1981) Evolutionary trees from DNA-sequences—a maximum likelihood approach. J Mol Evol **17:**368–376.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**783–791.

Fritz U, Siroky P, Kami H, Wink M (2005a) Environmentally caused dwarfism or a valid species-Is *Testudo weissingeri* Bour, 1996 a distinct evolutionary lineage? New evidence from mitochondrial and nuclear genomic markers. Mol Phylogenet Evol **37:**389–401.

Fritz U, Fattizzo T, Guicking D, Tripepi S, Grazia Pennisi M, Lenk P, Joger U, Wink M (2005b) A new cryptic species of pond turtle from southern Italy, the hottest spot in the range of the genus *Emys* (Reptilia,Testudines, Emydidae). Zool Scr **34:**351–371.

Fu J (2000) Toward the phylogeny of the family Lacertidae - why 4708 base pairs of mtDNA sequences cannot draw the picture. Biol J Linn Soc Lond **71:**203–217.

Fuller S, Baverstock PB, King D (1998) Biogeographic origins of goannas (Varanidae): a molecular perspective. Mol Phylogenet Evol **9:**294–307.

Guicking D, Fritz U, Wink M, Lehr E (2002a) New data on the diversity of the Southeast Asian leaf turtle genus *Cyclemys* Bell, 1834. Molecular results (Reptilia: Testudines: Geoemydinae). Faunistische Abhandlungen Staatliches Museum für Tierkunde Dresden **23:**75–86.

Guicking D, Joger U, Wink M (2002b) Molecular phylogeography of the viperine snake (*Natrix maura*) and the Dice snake (*Natrix tessellata*): first results. Biota **3:**49–59.

Guicking D, Lawson R, Joger U, Wink M (2006) Evolution and phylogeny of the genus *Natrix* (Serpentes: Colubridae). Biol J Linn Soc **87:**127–143.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser **41:**95–98.

Hedges SB, Nussbaum RA, Maxon LR (1991) Caecilian phylogeny and biogeography inferred from mitochondrial DNA sequences of the 12S rRNA and 16S rRNA genes (Amphibia: Gymnophiona). Herpetological Monographs **7:**64–76.

Honda M, Ota H, Kobayashi M, Nabhitabhata J, Yong HS, Hikida T (2000a) Phylogenetic relationships, character evolution and biogeography of the subfamily Lygosominae (Reptilia: Scincidae) inferred from mitochondrial DNA sequences. Mol Phylogenet Evol **15:**452–461.

Honda M, Ota H, Kobayashi M, Nabhitabhata J, Yong HS, Sengoku S, Hikida T (2000b) Phylogenetic relationships of the family Agamidae (Reptilia: Iguania) inferred from mitochondrial DNA sequences. Zool Sci **17:**527–537.

Huelsenbeck JP, Ronquist F (2001) MR. BAYES: Bayesian inference of phylogeny. Bioinformatics **17:**754–755.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablance FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A **86:**6196–6200.

Kumar S (1996) Phyltest: A Program for Phylogenetic Hypothesis Testing. Institute of Molecular Evolutionary Genetics and Department of Biology, The Pennsylvania State-University, University Park, PA.

Kyriazi PN, Poulakakis A, Parmakelis A, Crochete A, Moravec J, Rastegar-Pouyani N, Tsigenopoulos S, Magoulas A, Mylonas M, Lymberakis P (2008) Mitochondrial DNA reveals the genealogical history of the snake-eyed lizards (*Ophisops elegans* and *O. occidentalis*) (Sauria: Lacertidae). Mol Phylogenet Evol **49:**795–805.

Lenk P, Fritz U, Joger U, Wink M (1999) Mitochondrial phylogeography of the European pond turtle *Emys orbicularis* (Linnaeus 1758). Mol Ecol **8:**1911–1922.

Lenk P, Joger U, Wink M (2001) Phylogenetic relationship among European ratsnakes of the genus *Elaphe* Fitzinger based on mitochondrial DNA sequence comparisons. Amphibia-Reptilia **22:**329–339.

Leviton AE, Anderson SC, Adler KK, Minton SA (1992) Handbook to Middle East Amphibian and Reptiles. Contribution to Herpetology No: 8. Society for study of Amphibians and Reptiles. Oxford, OH.

Lixia W, Shihong S, Yuanting J, Jongfeng Y, Naifa L (2007) Molecular Phylogeography of the Chinese lacertids of the genus *Eremias* (Lacertidae) based on 16S rRNA mitochondrial sequences. Amphibia-Reptilia **28:**33–41.

Maca-Meyer N, Carranza S, Rando JC, Arnold EN, Cabrera V (2003) Status and relationships of the extinct giant Canary Island lizard *Gallotia goliath* (Reptilia: Lacertidae), assessed using ancient

doi: 10.1111/j.1439-0469.2012.00662.x

mitochondrial DNA from its mummified remains. Biol J Linn Soc **80:**659–670.
Macey JR, Ananjeva NB (2004) Genetic variation among agamid lizards of the *Trapelus agilis* complex in the Caspian-Aral Basin. Asia Herpet Res **10:**1–7.
Macey JR, Larson A, Ananjeva NB, Papenfuss TJ (1997) Evolutionary shifts in three major structural features of the mitochondrial genome among iguanian lizards. J Mol Evol **44:**660–674.
Macey JR, Shulte JA, Ananjeva NB, Larson A, Rastegar Pouyani N, Shamakov SM, Papenfuss TJ (1998) Phylogenetic relationship among agamid lizards of the *Laudakiacaucasia* species group: testing the hypotheses of biogeographic fragmentation and an area cladogram for the Iranian plateau. Mol Phylogenet Evol **10:**118–131.
Macey JR, Shulte JA, Larson A, Ananjeva NB, Wang Y, Petiyagoda R, Rastegar-Pouyani N, Papenfuss TJ (2000a) Evaluating trans Tethys migration: an example using acrodont lizards phylogenetics. Syst Biol **49:**233–256.
Macey JR, Shulte JA, Kami HG, Ananjeva NB, Larson A, Papenfuss TJ (2000b) Testing hypothesis of vicariance in the agamid lizard *Laudakia caucasia* from the mountain ranges on the Northern Iranian Plateau. Mol Phylogenet Evol **14:**479–483.
Moore WS (1995) Inferring phylogenies from mtDNA variation-mitochondrial-gene trees versus nuclear-gene trees. Evolution **49:**718–726.
Moritz C, Hillis DM (1996) Molecular systematics: context and controversies. In: Hillis DM, Moritz C, Mable BK (eds), Molecular Systematics. Sinauer Associates, Sunderland, MA, pp 1–113.
Nagy ZT, Joger U, Guicking D, Wink M (2002) Phylogeography of the European Whip snake *Coluber* (*Hierophis*) *viridiflavus* as inferred from nucleotide sequences of the mitochondrial cyt *b* gene and ISSR genomic fingerprinting. Biota **3:**109–118.
Nagy ZT, Lawson R, Joger U, Wink M (2004) Molecular phylogeny and systematics of racers, whipsnakes and relatives (Reptilia: Colubridae) using mitochondrial and nuclear markers. J Zool Syst Evol Res **42:**223–233.
Pavlichev M, Mayer M (2009) Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact? Mol Phylogenet Evol **52:**727–734.
Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics **14:**817–818.
Rastegar Pouyani E (2007) Molecular phylogeography and evolution of the lacertid genus *Eremias* of the Iranian Plateau and Central Asia (Reptilia, Lacertidae). PhD thesis, Faculty of Life Science, Heidelberg University, Heidelberg.
Rastegar Pouyani E, Kazemi Noureini S, Joger U, Wink M (2009) A Phylogeny of the *Eremias velox* complex of the Iranian Plateau and Central Asia (Reptilia, Lacertidae): Molecular evidence from ISSR-PCR fingerprints. Iranian Journal of Animal Biosystematics **5:** 15–25.
Rastegar Pouyani E, Kazemi Noureini S, Rastegar Pouyani N, Joger U, Wink M (2010) Molecular phylogeny and biogeography of the *Eremias persica* complex of the Iranian plateau (Reptilia: Lacertidae) based on sequences of the mtDNA. Zool J Linn Soc **158:**641–660.
Sambrook J, Russell DW (2001) Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.
Swofford DL (2001) PAUP*. Phylogenetic Analysis Using Parsimony (*and other Methods), Version 4.0b10. Sinauer Associates, Sunderland.
Szczerbak NN (1974) The Palaearctic Desert Lizards. Akadeimya Nauk Ukrienskoi SSR Institut Zoologii, Naokova Dumka, Kiev.
Szczerbak NN (2003) Guides to the Reptiles of the Eastern Palaearctic. Krieger Publishing Company, Malabar, FL.
Tajima F (1993) Simple methods for testing molecular clock hypothesis. Genetics **135:**599–607.
Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol **24:**1596–1599.
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA 5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol **28:**2731–2739.
Thomas RH, Schaffner W, Wilson AC, Pääbo S (1989) DNA phylogeny of the extinct marsupial wolf. Nature **340:**465–467.
Walker D, Avise JC (1998) Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. Annu Rev Ecol Sys **29:**23–58.
Wink M, Guicking D, Fritz U (2001) Molecular evidence for hybrid origin of *Mauremys iversoni* Pritchard et McCord, 1991, and *Mauremys pritchardi* McCord, 1997 (Reptilia: Testudines: Bataguridae). Zoologische Abhandlungen Staatliches Museum für Tierkunde Dresden **51:**41–50.
Xia X, Xie Z (2001) DAMBE: Software package for data analysis in molecular biology and evolution. J Hered **92:**371–373.
Zhang DX, Hewitt GM (1999) Nuclear integration: challenges for mitochondrial DNA markers. Trends Ecol Evol **11:**247–251.
Zhao E, Adler KK (1993) Herpetology of China. Society for the Study of Amphibians and Reptiles, Oxford, OH.

doi: 10.1111/j.1439-0469.2012.00662.x