Molecular Assessment and Taxonomic Status of the Rapid Racerunner (*Eremias velox* complex) with Particular Attention to the Populations in Northwestern China

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Abstract   The rapid racerunner, *Eremias velox*, is a widely distributed lizard from the northern Caucasus across entire Central Asia eastward to China. It is increasingly common to accept *E. velox* as a species complex in its entire range. To date, published morphological and molecular systematic hypotheses of this complex are only partially congruent, and its taxonomic status and evolutionary history are still far from clear. The mitochondrial cytochrome *b* gene and 12S rRNA sequences were used to evaluate the taxonomy of this complex, with particular attention to the phylogenetic placement of populations in northwestern China. Examination of the phylogenetic analyses recovers seven distinct, biogeographically discrete, and well-supported clades, revealing genetically identifiable populations corresponding to some previously morphology-defined subspecies. Chinese *E. v. roborowskii* appears to have split from other Central Asian rapid racerunner lizards well before differentiation occurred among the latter taxa. Specifically, we corroborate that there are two subspecies occurring in China, i.e., *E. v. velox* and *E. v. roborowskii*. We recommend a novel subspecific status for the phenotypically and genetically distinct populations in southern Aral Sea region of Uzbekistan previously assigned to *E. v. velox*. Finally, each of the three independently evolving lineages from Iranian Plateau should be recognized as three species new to science under the general lineage concept.

Keywords   mtDNA, subspecies, *Eremias velox* complex, taxonomy, phylogeography

1. Introduction

Racerunner lizards of the genus *Eremias* (family Lacertidae) are the dominant reptiles in the deserts and steppes of Central Asia. The taxonomy of this genus has a long complex history, and the delimitation of species/subspecies of *Eremias* is known for being notoriously difficult. This is, in part, caused by the great geographic variation in most morphological characters in many of the species (e.g., Szczerbak, 1974, 1975; Ananjeva *et al.*, 1998; Chirikova, 2004). Currently, over 36 species have

been assigned to the genus, and much of their phylogeny and taxonomy is controversial (e.g., Szczerbak, 1974; Guo *et al.*, 2011). Among the many controversies, the taxonomy of the *Eremias velox* complex is perhaps one of the most confusing. The distribution of this group ranges from the northern Caucasus across entire Central Asia eastward to China (Figure 1). Traditionally, the rapid racerunner was considered as a single species with three or four described subspecies (Szczerbak, 1974, 1975; Eremchenko and Panfilov, 1999). However, as noted by Rastegar-Pouyani (2009), it is increasingly common to accept *E. velox* as a clade in its entire range representing a species complex. The nominate form occurs in the larger parts of the distribution range. The Caspian subspecies *E. v. caucasia* occurs at the western part of the range. The

subspecies *E. v. roborowskii* is endemic in Turpan-Hami Depression and Dunhuang Basin in northwestern China. Recently Eremchenko and Panfilov (1999) described the fourth subspecies, *E. v. borkini* from the highlands of Issyk-Kul lake depression in Kyrgyzstan.

Many recent taxonomic treatments reveal varied opinions as to the number of species. Some herpetologists recognized a single widespread species (e.g., Szczerbak, 2003; Ananjeva *et al.*, 2006; Sindaco and Jeremcenko, 2008), whereas others (Rastegar-Pouyani, 2009; Rastegar-Pouyani *et al.*, 2012) hypothesized the existence of at least four distinct species and three well-supported subspecies (Figure 2). Some of the differences of opinion result from the accumulation of mitochondrial DNA sequence information (Rastegar-Pouyani *et al.*, 2012). At present it seems reasonable that mtDNA sequence information supports the recognition of the Iranian populations of *E. velox* as three distinct species based on differences in distribution, morphology, habitat preference, and mtDNA sequence divergence (Figure 2).

The subspecific status of *E. v. roborowskii* is still controversial, although Szczerbak (1975) confirmed its status according to color, pattern and characters of pholidosis. Boulenger (1921) suggested that the morphological variation in the populations of *E. v. roborowskii* is not sufficient to support its subspecies status. The validity of this subspecies has not been recognized for a long time (Mertens and Wermuth, 1960). More recently, on the basis of multivariate morphological

analyses, Wang *et al.* (2014) argued that there is no subspecies differentiation between populations from Turpan-Hami Depression and from Dzungarian Basin and Ily Valley in China. Thus, Wang *et al.* (2014) implied that *E. v. roborowskii* may be a synonym of nominate subspecies.

Establishing correct species/subspecies limits depends on adequate sampling of populations throughout the range. Given the previous studies, it might appear that taxonomic limits were clear. Rastegar-Pouyani (2009) made the first attempt to elucidate the phylogenetic relationships among the rapid racerunner in Iran and Central Asia on the basis of ISSR-PCR fingerprints data. The results were tentative due in part to limited taxonomic sampling. As shown by simulations and empirical studies, more taxa and data do affect the phylogenetic reconstruction (e.g., Pollock *et al.*, 2002; Zwickl and Hillis, 2002). Rastegar-Pouyani *et al.* (2012) studied patterns of mitochondrial DNA cytochrome *b* (cyt *b*) and 12S rRNA variation in 70 specimens from 13 geographically distant localities in Iran and Central Asia. Their results contradicted previous systematic hypotheses; revealed surprising relationships between Iranian and Central Asiaian lineages and uncovered novel, cryptic evolutionary lineages (i.e. new putative species). Moreover, they argued that the eastern and southern Kazakhstan populations correspond to the traditional *E. v. roborowskii*. However, Rastegar-Pouyani *et al.* (2012), who used most samples already analyzed by Rastegar-
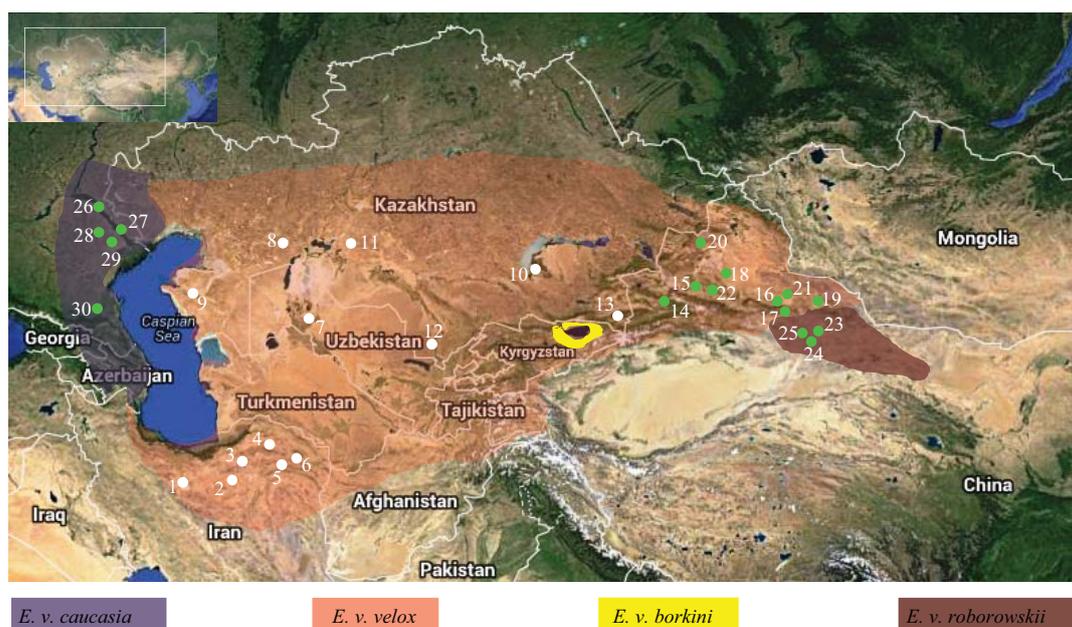

Figure 1 Entire distribution range of *Eremias velox* complex showing traditional subspecies' range with different background color, and the sampling localities for different populations (numerical labels 14–30 with green dots), white dots with numbers 1–13 representing those from Rastegar-Pouyani *et al.*(2012). The locality number corresponds with that in Appendix A.
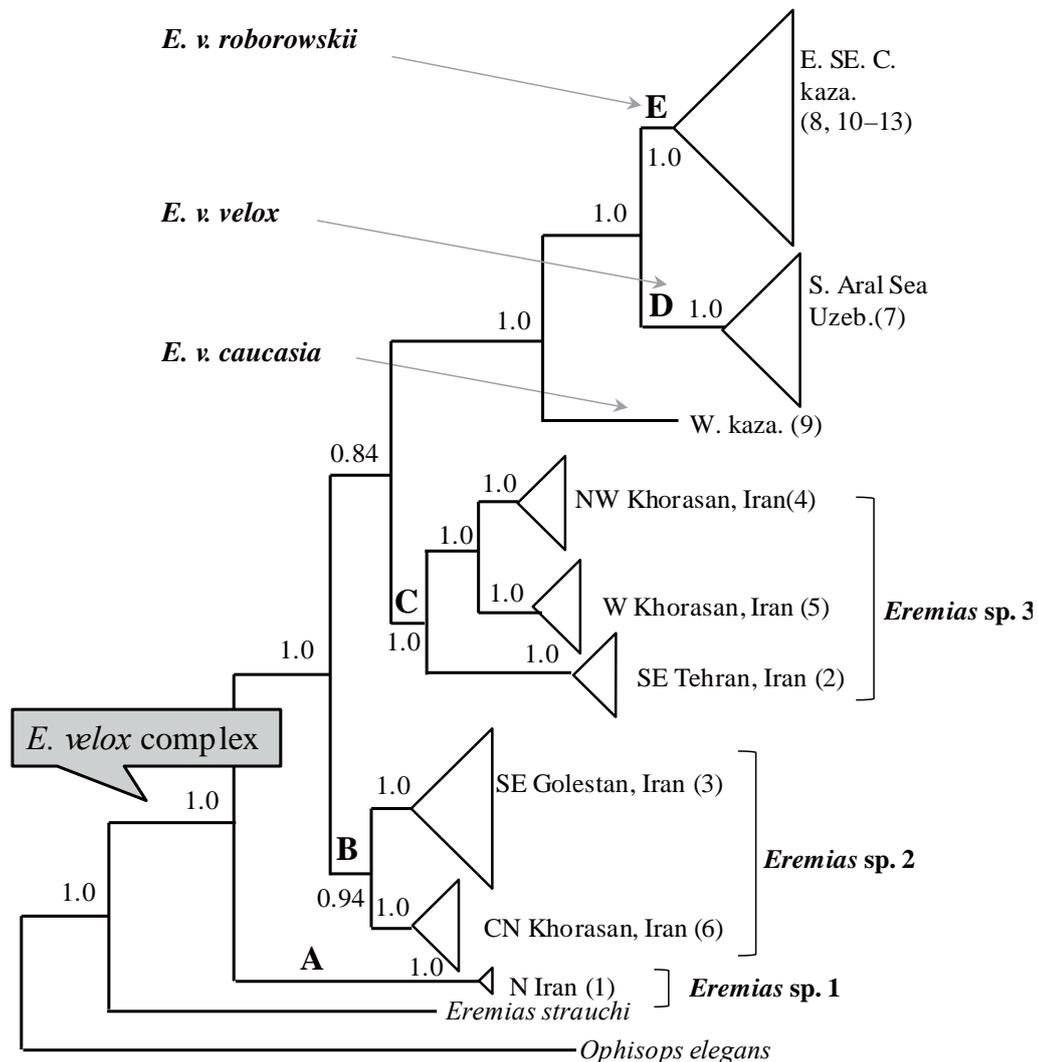
Figure 2 Rastegar-Pouyani *et al.*'s (2012) phylogenetic hypothesis for populations of the *Eremias velox* complex from Iranian Plateau and Central Asia using partitioned Bayesian analyses of mtDNA data. A, B, C, D and E indicate the major clades. Values beside the node represent Bayesian posterior probabilities.

Pouyani (2009), were unable to sample adequately the eastern part of the taxon's range. There was no sample from China included in their work. In addition, Rastegar-Pouyani *et al.* (2012) speculated that *E. v roborowskii* occurs in western China, eastern Kazakhstan and Eastern Uzbekistan. This inappropriate interpretation may give confusing clues about the identity of this subspecies, resulting in incorrect inference of its phylogenetic placement. It appears likely that the inclusion of more rapid racerunner samples may get a better illustration of their phylogeographic structure, and may also provide valuable information for their taxonomy.

Thus, as an extension of the molecular phylogenetics of the rapid racerunner, an effort has been made to collect some specimens from northwestern China and Russia. We further determined the complete cyt *b* gene and 12S

rRNA segment to gain a more comprehensive view on phylogeographic patterns in *E. velox* complex. This study specifically aims to: (1) re-evaluate the phylogeographic structure of *E. velox* complex, (2) test the phylogenetic affinities of the rapid racerunner populations in China, and (3) test the validity of the subspecific status of *E. v. roborowskii*. The hypothesis that *E. v. roborowskii* is valid particularly predicts that it will correspond with distinct, well-supported, and moderately divergent mtDNA haplotype clade.

2. Materials and Methods

2.1 Taxon sampling We collected a total of 87 samples representing natural populations at 17 localities. These included 57 samples from 12 localities in China, 30

samples from five localities in Russia, covering a large territory occupied by *E. velox* in the two regions (Appendix A, Figure 1). To ensure accuracy more than four representatives of each population were collected. However, only a single individual was collected from six sites due in part to sampling difficulties. Populations were assigned subspecific status based on location and phenotype. Due to logistical and legal obstacles to filed work, no sample of *E. velox borkini* from the highlands of Kyrgyzstan was available. Based on present knowledge of phylogenetic relationships among *Eremias* lizards, *E. argus* and *E. persica* were chosen as outgroup taxa (Guo *et al.*, 2011). All voucher specimens were deposited in the herpetological collections of Chengdu Institute of Biology, Chinese Academy of Sciences or the Department of Zoology, University of Guelph. Detailed information about all specimens and sequences used in this study is listed in Appendix A.

2.2 DNA extraction, amplification, and sequencing Genomic DNA was extracted from liver or tail muscle tissues stored in 95% ethanol following the protocol of Aljanabi and Martinez (1997). Complete sequence of the mitochondrial DNA cyt *b* gene was amplified using the primers L14919 and H16064 from Burbrink *et al.* (2000). A ~371bp fragment of 12S rRNA was amplified using the primers L1091 and H1478 from Kocher *et al.* (1989). Each of our 50 μl PCR reactions contained 25 μl of 2 × EasyTaq SuperMix, 0.4 μM of each primer, and 1–2 μl genomic DNA. The PCR protocol involved initial denaturation at 94°C for 180 s followed by 30 cycles of 94°C for 30 s, 43°C for 30 s (50–52°C for 12S rRNA), and elongation at 72°C for 90 s (60 s for 12S rRNA); and final extension at 72°C for 8 min. Negative controls were run for all amplifications. Amplified products were visualized on 0.8% agarose gels and purified using the DNA gel extraction kit (Omega Bio-Tek). The purified products were sequenced directly by Majorbio or Sangon (Shanghai) Co., Ltd., using the same primers as PCR for 12S rRNA, and L14919 from Burbrink *et al.* (2000) in combination with L14761 as well as H14892 from Zhao *et al.* (2011) for cyt *b*. All novel sequences are deposited in GenBank with accession numbers KF999318–KF999491.

2.3 Sequence alignment and analyses We have retrieved sequences of cyt *b* and 12S rRNA of 39 individuals from GenBank, which included sequences of 37 individuals published in Rastegar-Pouyani *et al.* (2012) and sequences of two individuals used as outgroup in this study (Appendix A). Sequences of cyt *b* were translated to amino acid sequences using MEGA5 (Tamura *et al.*, 2011) to verify the data. A total of 126 sequences of cyt *b* and 12S rRNA were first aligned using Clustal X 2.0 (Larkin *et al.*, 2007), respectively, with default gap penalties. Then the aligned matrice were re-checked visually.

Phylogenetic congruence of cyt *b* and 12S rRNA data sets were tested by the partition homogeneity test of Farris *et al.* (1995) with PAUP* 4.0b10 (Swofford, 2003). The partition homogeneity test supported the combination of the cyt *b* and 12S rRNA data sets ($P = 0.1$). Homogeneity of base frequencies was evaluated using Chi-square (χ^2) tests implemented in PAUP*. Base composition at the third position exhibited no heterogeneity: the combined data, $\chi^2 = 16.66$, $df = 237$, $P = 1.00$; first position, $\chi^2 = 7.18$, $df = 237$, $P = 1.00$; second position, $\chi^2 = 2.02$, $df = 237$, $P = 1.00$; third position, $\chi^2 = 48.99$, $df = 237$, $P = 1.00$; and 12S rRNA, $\chi^2 = 12.11$, $df = 237$, $P = 1.00$. Given these results, a sensible approach to analyze these data phylogenetically would be to apply a time-reversible Markov model.

2.4 Phylogenetic analyses To estimate phylogenetic relationships, Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) were implemented for the combined data. Each haplotype was treated as a taxon in the analyses. MP analyses with PAUPRat (Sikes and Lewis, 2001), based on Parsimony Ratchet (Nixon, 1999), were conducted using 1000 ratchets with 10 iterations per replicate. The shortest equally most parsimonious trees were combined to produce a strict consensus tree. Consistency (CI), retention (RI), and rescaled consistency (RC) indices were used to evaluate homoplasy in character data and overall support for optimal trees. Support for individual branches was evaluated using non-parametric bootstrapping (Felsenstein, 1985) in PAUP* with 1000 pseudoreplicates, TBR branch swapping, simple taxon addition with one tree held at each step, and a maximum of 100 trees saved per replicate in order to decrease the time needed to run large bootstrap replicates.

To infer relationships under BI we used MrBayes v3.2.2 (Ronquist *et al.*, 2012). We optimized several parameters prior to conducting our final analyses using the Markov Chain Monte Carlo (MCMC) method implemented in MrBayes, including partitioning strategy, models of molecular evolution, length of MCMC analyses. We assessed two alternative partitioning strategies: (1) a single partition and (2) a four-partition strategy that applied a single partition to each of the three codon positions of cyt *b* and a fourth partition for 12S

rRNA sequence.

The Akaike Information Criterion (AIC; Akaike, 1974), as implemented in MrModeltest v2.3 (Nylander, 2008), was used to select the appropriate model of sequence evolution for each partition. To compare the two alternative partitioning strategies, we conducted two independent analyses of each strategy in MrBayes. Each analysis consisted of three heated chains and one cold chain, with an incremental heating temperature of 0.02, and an exponential distribution with a rate parameter of 25 as the prior on branch lengths (Marshall, 2010). All analyses were run for 20 million generations with parameters and topologies sampled every 1000 generations. To ensure MCMC convergence, a burn-in period for all analyses was diagnosed in two ways: (1) by the average standard deviation of split frequencies (ASDSF; Lakner *et al.*, 2008) between two MCMC analyses run independently, with levels below 0.01 being considered indicative of convergence, and (2) by the potential scale reduction factor (PSRF; Gelman and Rubin, 1992) comparing the variance within and between runs, with levels approaching 1.0 as runs converge. To compare performance of the two partitioning strategies we calculated Bayes factors from the marginal likelihood scores estimated by the stepping-stone method (Xie *et al.*, 2011) in MrBayes 3.2.2. Comparison of alternative partitioning strategies strongly favored four partitions over one partitioning scheme (2Ln Bayes factor > 600). After selecting the optimal partitioning strategy, we conducted two analyses with different starting trees using the optimized parameters. We then used the *sumt* command in MrBayes to generate a consensus topology and associated Bayesian posterior probabilities (BPP) from a pooled post-burnin sampled from two analyses.

Partitioned maximum likelihood (ML) analyses were conducted in RAxMLHPC v7.0 (Stamatakis, 2006) on the combined dataset with the same partitioning strategy as for Bayesian inference. The more complex model (GTR + I + Γ) was used for all subsets, and 100 replicate ML inferences were performed for each analysis. Each inference was initiated with a random starting tree and nodal support was assessed with 1000 bootstrap pseudoreplicates (Stamatakis *et al.*, 2008).

2.5 Bayesian tests of topology hypotheses Bayes factors were used to compare the unconstrained Bayesian tree topology to Bayesian trees with 'hard' constraints. Here we tested the following taxonomy and geography-based hypotheses to evaluate the taxonomic status within *E. velox* complex: (1) Are populations within the historically accepted distributions in northwestern China monophyletic? (2) Are the Iranian populations monophyletic? (3) Do Iranian populations belong to nominate subspecies? (4) Are populations of *E. v. roborowskii* and nominate subspecies monophyletic? We constrained the topology for each of the above putative groupings and performed a stepping stone run of 10 million generations (50 steps with convergence being reached in each step) from which we obtained a mean marginal likelihood estimate. Convergence was examined through diagnostic plots of standard deviation of the split frequencies and screening similarity in the two independent marginal likelihood estimates (Ronquist *et al.*, 2011). Each of these estimates were compared using Bayes factors (Kass and Raftery, 1995) to the marginal likelihood estimate obtained from the unconstrained MrBayes run of the same length. The stepping stone function (Xie *et al.*, 2011) implemented in MrBayes v3.2.2, offers an improved estimation of marginal likelihood over harmonic mean estimation.

3. Results

3.1 Sequence characteristics Two mitochondrial genes (cyt *b* and 12S rRNA) were obtained, yielding alignments of 1143 and 372 bp, respectively. 126 sequences (including two outgroup taxa) revealed 80 haplotypes. Alignment was unambiguous and 1 indel was inferred in 12S rRNA between outgroup and ingroup taxa. No premature stop codons were observed in cyt *b*, indicating that the obtained sequences were mitochondrial in origin and not nuclear pseudocopies. Average base composition is *A*: 27.55%, *C*: 30.69, *G*: 14.72%, *T*: 27.04%, when the outgroup taxa are combined. Nucleotide composition showed an anti-*G* bias and *A*+*T*-richness (54.59%), which is a characteristic of the mitochondrial genome. Variable and parsimony-informative characters are: 441 and 319 of 1143 (cyt *b*); 84 and 57 of 371 (12S rRNA).

A χ^2 test at the 5% level of significance for differences in base frequencies showed that there was no base compositional heterogeneity among sequences, which is known to adversely affect phylogenetic inference (Jermiin *et al.*, 2004).

3.2 Phylogenetic analyses The heuristic search of the combined data resulted in 914 equally parsimonious trees of 1136 steps, with moderate CI (0.579) and high RI (0.887). These are presented as a majority-rule consensus tree where branches with bootstrap support lower than 50% are collapsed (data not shown). AIC analyses conducted with the aid of MrModeltest identified the

HKY + *I* + *Γ* model as the most appropriate model for partition of cyt *b* 1st codon position, and the GTR + *I* + *Γ* model for partitions of cyt *b* 2nd codon position, cyt *b* 3rd codon position, 12S rRNA or combined data. Bayesian analyses were conducted for 20 million generations, with a conservative burn-in of six million generations that provided dense sampling of the posterior distribution. The MP, ML and Bayesian trees differed in support values, but not in the compositions of the major lineages. The bootstrap support was also marked on the branches that receive such support in the Bayesian tree (Figure 3). The phylogenetic trees recovered the monophyly of the *E. velox* complex with high support values. As shown in Figure 3, seven major clades connected deep in the complex phylogenetic history were identified in all reconstructions with strong posterior probabilities and bootstrap values, which generally correspond well to geographical regions within the distribution range. These clades were designated I, II, III, IV, V, VI and VII in Figure 3. The subdivision and phylogenetic positions of the Iran populations (I, II, III) are completely identical with the clades A, B, C of Rastegar-Pouyani *et al.* (2012), respectively. The basal split in the complex is between the northern Iranian population (clade I) and a highly supported clade of the other populations from Iran, Central Asia, China and Russia. However, clade IV, corresponding to *E. v. roborowskii*, covers the populations from Turpan-Hami Depression, and forms the sister taxon to the other populations excluding those from Iran. The clade V, corresponding to *E. v. caucasia*, covers populations from North-eastern Russian Caucasus and Western Kazakhstan. The clade VI is composed of populations from Uzbekistan, conventionally assigned to nominate subspecies. The clade VII covers populations from Eastern Kazakhstan and Dzungarian Basin as well as Ily Valley in China, which corresponds to nominate subspecies. Overall, the notable differences between Rastegar-Pouyani *et al.* (2012) and our results presented here lie in the phylogenetic placement of *E. v. roborowskii.*

3.3 Bayesian tests of topology hypotheses We compared the marginal likelihood estimates of topologically constrained and unconstrained run of MrBayes for possible monophyletic group within *E. velox* complex (Table 1). In all cases, there was very strong (Bayes factor > 100) evidence against the constrained topologies. Thus, four alternative phylogenetic hypotheses were significantly rejected, such as a monophyletic group of Chinese rapid racerunner populations, monophyly of Iranian populations, assignment of Iranian populations to nominate subspecies, and assignment of *E. v. roborowskii* to nominate subspecies.

4. Discussion

This study provided strong support for the monophyly of *E. velox*, consistent with the results of Guo *et al.* (2011) and Rastegar-Pouyani *et al.* (2012), and thus providing a firm basis for future testing of alternative biogeographic models. The Iranian populations were recovered here as a paraphyletic assemblage, representing three distinct, biogeographically discrete, well-supported clades that are not each other's closet relatives. This is in accord with the results of Rastegar-Pouyani *et al.* (2012).

The morphological differences (Rastegar-Pouyani *et al.*, 2012), allopatric ranges and substantial genetic divergence in the mtDNA bear evidence of long standing isolation between several populations. The haplotype clades associated with each lineage on Iranian Plateau are differentiated by mean uncorrected levels of sequence divergence that often exceed 10% (Table 2). This degree of divergence suggests that evolutionary separation of the three clades occurred millions of years ago, likely sometime during the Miocene based on a molecular clock calibration of 1.25% pairwise divergence per million years for mtDNA within the *E. velox* complex (Rastegar-Pouyani *et al.*, 2012). Concordance between strongly differentiated mtDNA haplotype clades and phenotypic

Table 1 Bayes factors of stepping-stone-based estimates of marginal likelihood for comparisons of alternative phylogenetic hypotheses. 2ln Bayes factors ≥10 are considered very strongly different (Kass and Raftery, 1995), indicating evidence against alternative hypotheses.

Alternative phylogenetic hypotheses		Description of constraint	Ln Marginal likelihood		2Ln Bayes factor
			Ln*L*: unconstrained	Ln*L*: constrained	
H1	Monophyly of rapid racerunner populations in China	All samples from China	–8022.39	–8121.34	197.9
H2	Monophyly of rapid racerunner populations in Iran	All samples from Iran: clade I, II, III	–8022.39	–8076.55	108.32
H3	Nominate subspecies affinity of Iranian rapid racerunner populations	clade I, II, III, VI, VII	–8022.39	–8082.49	120.2
H4	Monophyly of *E. v. velox* and *E. v. roborowskii*	clade IV, VI, VII	–8022.39	–8090.19	135.6
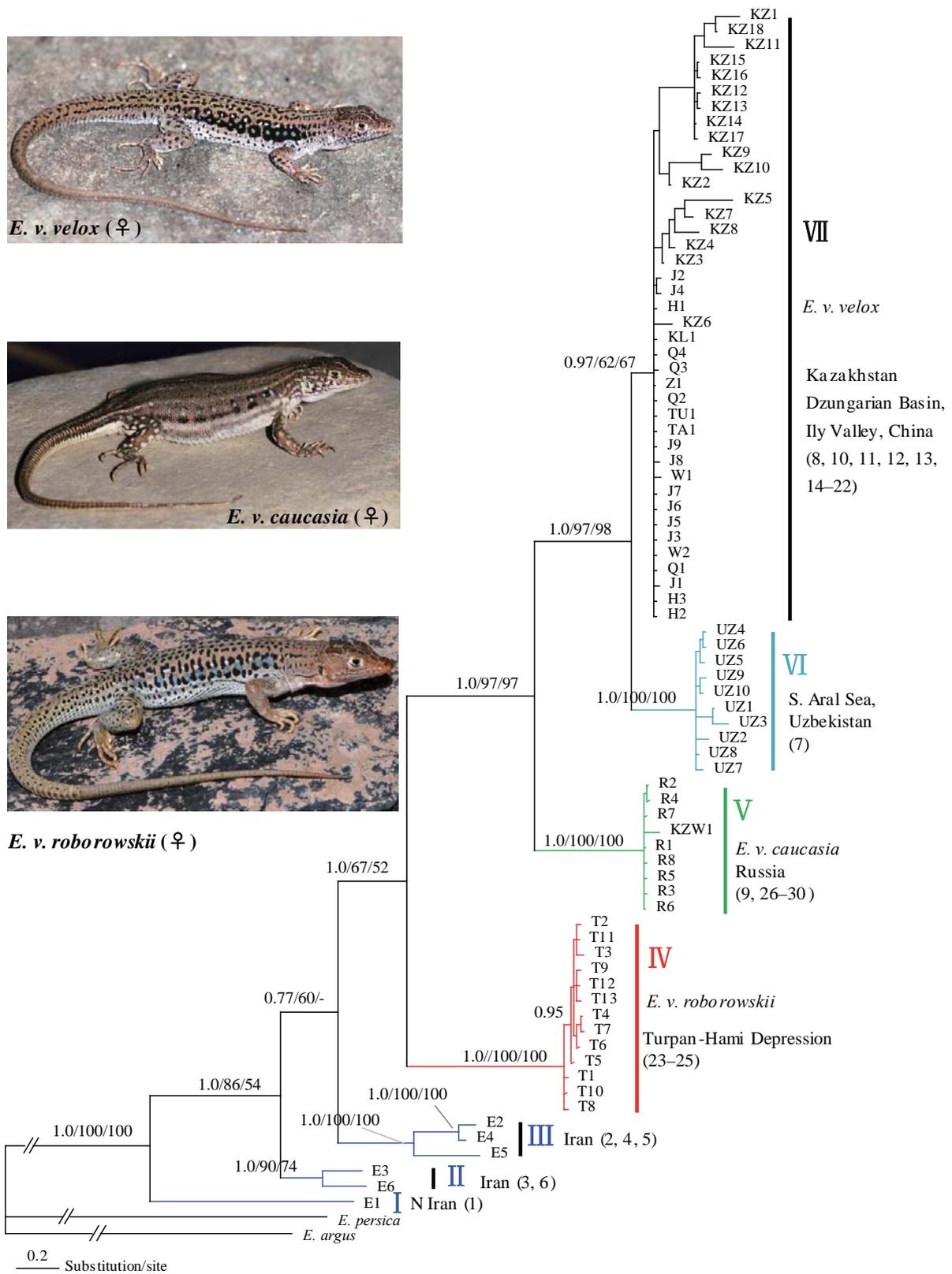
Figure 3 Hypothesized relationships of *Eremias velox* complex included in this study, illustrated by the majority-rule consensus tree resulting from partitioned Bayesian analyses. Bayesian posterior probabilities, maximum parsimony and likelihood bootstrap values are shown. Nodes supported by ≥ 0.95 Bayesian PP and ≥ 70% BP were considered highly supported. Terminals are labeled with haplotypes. Roman alphabet labels correspond to clades referred to the Results and Discussion. Representative photographs of female adults show the dorsal and lateral differentiation among three subspecies.

Table 2 Genetic distances within and between outgroup species and *E. velox* complex included in this study. Mean uncorrected (*p*) distances are below diagonal; distances calculated using MEGA's Maximum Composite Likelihood model with rate variation among sites defined by the gamma shape parameter (0.2366) are above the diagonal. Mean maximum likelihood divergences within each species or subspecies are in the diagonal with bold text.

		1	2	3	4	5	6	7	8	9
1	*E.argus*	**NA**	0.419	0.483	0.442	0.443	0.454	0.453	0.471	0.433
2	*E. persica*	0.161	**NA**	0.416	0.442	0.489	0.462	0.441	0.433	0.401
3	clade I	0.172	0.158	**NA**	0.173	0.251	0.267	0.272	0.325	0.299
4	clade II	0.164	0.159	0.099	**0.03**	0.092	0.145	0.152	0.171	0.164
5	clade III	0.165	0.17	0.122	0.066	**0.033**	0.14	0.167	0.186	0.17
6	clade IV	0.17	0.165	0.128	0.092	0.089	**0.003**	0.152	0.17	0.153
7	clade V	0.168	0.161	0.129	0.095	0.101	0.094	**0.002**	0.111	0.093
8	clade VI	0.166	0.154	0.14	0.099	0.105	0.098	0.075	**0.006**	0.04
9	clade VII	0.162	0.152	0.135	0.097	0.1	0.093	0.067	0.033	**0.012**

variation (Rastegar-Pouyani *et al.*, 2012) supports the hypothesis that each of the three independently evolving lineages from Iranian Plateau deserves recognition at the species-level under the general lineage concept of species (de Queiroz, 1999; 2007) and associated operational species-delimitation criteria (Wiens and Penkrot, 2002).

Rastegar-Pouyani *et al.* (2012) demonstrated the basal positions of the Iranian clades for the first time and considered them three distinct species. However, their inference of the phylogenetic placement of *E. v. roborowskii* was misleading. In support of Rastegar-Pouyani *et al.* (2012), our results confirm the basal position of the Iranian clades and statistically reject the placement of Iranian clades into the nominate subspecies from Central Asia. Surprisingly, we find that *E. v. roborowskii* is the sister taxon to *E. velox caucasia* plus *E. v. velox*, i.e., other populations from Central Asia. Our results clearly confirm that all of the major clades inferred from mitochondrial haplotypes are concordant with geographical regions. Iranian clades at the basal position of the phylogenetic tree may imply that *E. velox* originated from the Iranian Plateau and invaded Central Asia as Rastegar-Pouyani *et al.* (2012) suggested. If we take this scenario as a reliable explanation for the origin and evolution of *E. velox*, we consider the clade IV (*E. v. roborowskii*) as first split from entire Central Asian populations, resulting from the barrier effect of Tianshan Mountains with their dramatic uplift since Tertiary (Macey *et al.*, 1999; Guo and Wang, 2007; Zhang *et al.*, 2008; Melville *et al.*, 2009; Solovyeva, 2013). Geographically, with the uplift of Tianshan Mountains, Turpan-Hami Depression and Dzungarian Basin were blocked from each other, resulting in a historical vicariant pattern and allopatric distribution between *E. v. roborowskii* and *E. v. velox* as well as *E. v. caucasia*. Wang *et al.* (2014) failed to recognize the validity of *E. v. roborowskii* due to a

lack of understanding the discrete characters between *E. v. velox* and *E. v. roborowskii*, including dorsal pattern and lateral color pattern. In general, the adults of *E. v. roborowskii* have a dorsal pattern of irregular dark spots, with rows of bright blue-edged, dark eye-like spots on the lateral sides (Zhao, 1999; Szczerbak, 2003).

In addition, clade V, corresponding to traditional *E. velox caucasia*, forms the sister taxon to Central Asian clades, in accord with the results of Dolotovskaya *et al.*(2007) inferred from ~ 1800 bp mtDNA of cyt *b*, COI and 16S rRNA . Rastegar-Pouyani *et al.* (2012) speculated that a single specimen from Western Kazakhstan belongs to *E. velox caucasia*. The affinity of that single specimen was corroborated in this study for there to be strong support for its nesting inside clade V. The Central Asian clades, corresponding to conventional nominate subspecies, comprise clades VI and VII. The patterns of mtDNA variation support a sister relationship between clade VI and clade VII. Clade VI comprises samples from Uzbekistan, whereas clade VII consists of populations from Kazakhstan and Dzungarian Basin and Ily River Valley, Xinjiang, China. Judging from the type locality of the nominate form of *E. velox*, Inderskija mountain in Kazakhstan, we consider clade VII as nominate subspecies. Szczerbak (1975) examined the relationships of *E. v. roborowskii* from Ssatschsheu (Dunhuang Basin, Gansu, China) and Lukuchun (Shanshan, Xinjiang, China) with a population from Dzungarian Basin. He found that the samples from Dzungarian Basin stood an intermediate position between the subspecies *E. v. roborowskii* and nominative form. On the one hand, judging from pholidosis, the population from Dzungarian Basin is closer to *E. v. roborowskii* than to the populations from Balkhash in Kazakhstan. On the other hand, the dorsal and lateral pattern of the population from Dzungarian Basin is closer to that of the latter, i.e. nominate form.

Interestingly, Szczerbak (1975) referred to the population from Dzungarian Basin as nominative form. Our results statistically support that the rapid racerunner populations in Dzungarian Basin cluster with those from Kazakhstan. In fact, biogeographic connections to the Dzungarian Basin are to the northwest, with a number of passes leading to Kazakhstan. The Dzungarian Gate is the main pass through the western ranges, leading to Lake Alaköl and Lake Balkhash in Kazakhstan. There are a number of taxa that have similar distribution pattern, including *Phrynocephalus melanurus* (Melville *et al.*, 2009), *P. heliocopus* (Solovyeva *et al.*, 2011), and *Eremias arguta* (Szczerbak, 2003).

Traditionally, the subspecies category is used to diagnose geographically distinct populations that were thought to be in the early stages of speciation (Mayr, 1963; Mayr and Ashlock, 1991). Despite some criticisms surrounding the concept of subspecies (e.g., Burbrink *et al.*, 2000; Patten and Unitt, 2002; Zink, 2004), recent studies in which researchers used multiple criteria (e.g., morphological, behavioral, and genetic characters) have confirmed that many subspecies are evolutionarily definable entities (e.g., Gavin *et al.*, 1999; Phillimore and Owens, 2006; Guo *et al.*, 2012). In Central Asia, the clinal and discrete variation among *E. velox* populations from different regions have been documented (Szczerbak, 1975; Chirikova, 2004). As noted by Szczerbak (1975), the most deviating populations of *E. velox* were found in Fergana Valley and neighborhood of Badkhyz desert, reaching subspecies level. Chirikova (2004) studied geographic variations of *E. velox* populations from Kazakhstan and Uzbekistan. She found some variations in phenotypical traits between populations from the two regions. In contrast with the nominate form in Kazakhstan, populations from the southern Aral Sea region in Uzbekistan have very light elongated spots on the middle of the back in adults. Even some individuals from the southern Aral Sea region lack this kind of dorsal pattern completely. Our results demonstrate that the populations in southern Aral Sea region of Uzbekistan correspond with distinct, well-supported, and moderately divergent mtDNA haplotype clade (clade VI) . The mean uncorrected level of sequence divergence between clades VI and VII falls within 3.3%. In support of Chirikova (2004), we recommend that the populations of rapid racerunner from southern Aral Sea region of Uzbekistan be assigned to a novel subspecies, which will facilitate an effective short-cut for estimating patterns of intraspecific genetic diversity of *E. velox*.

5. Conclusions

The mtDNA data confirms the subspecific status for three of the four morphology-defined subspecies: *E. v. roborowskii*, *E. v. caucasia*, *E. v. velox* (sensu stricto). Specifically, we corroborate that there are two subspecies occurring in China, i.e., *E. v. velox* and *E. v. roborowskii*. We recommend a novel subspecies for the phenotypically and genetically distinct populations in Uzbekistan previously referred to as *E. v. velox*. We also support species-level recognition for each of the three independently evolving lineages from Iranian Plateau traditionally assigned to *E. v. velox*. We do so recognizing that more comprehensive geographic sampling and investigation of additional molecular markers are required to clarify hypothesized species/subspecies boundaries, taxonomic status and evolutionary history of the *E. velox* complex.

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Appendix A List of analyzed specimens, their geographical origin and the GenBank accession number.

Taxon	Locality No.	Geographical origin	Latitude, longitude	Sample No.	Haplotype type	cyt *b*	12S rRNA	Reference
						Accession number		
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	Guo475	H1	KF999318	KF999405	This study
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	Guo476	Z1	KF999319	KF999406	This study
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	Guo478	Z1	KF999320	KF999407	This study
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	Guo479	Z1	KF999321	KF999408	This study
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	Guo481	Z1	KF999321	KF999409	This study
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	Guo483	H2	KF999323	KF999410	This study
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	Guo484	H3	KF999324	KF999411	This study
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	Guo485	Z1	KF999325	KF999412	This study
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	QY1015	Z1	KF999396	KF999483	This study
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	QY1016	TU1	KF999397	KF999484	This study
E. v. velox	14	Huocheng, Xinjiang, China	44.02°N, 80.81°E	QY1017	Z1	KF999398	KF999485	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo520	Z1	KF999326	KF999413	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo521	Z1	KF999327	KF999414	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo522	J1	KF999328	KF999415	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo523	Z1	KF999329	KF999416	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo524	Z1	KF999360	KF999447	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo525	J2	KF999361	KF999448	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo526	J3	KF999362	KF999449	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo527	J4	KF999363	KF999450	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo528	J5	KF999364	KF999451	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo529	Z1	KF999365	KF999452	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo530	Z1	KF999366	KF999453	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo531	J6	KF999367	KF999454	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo532	Z1	KF999368	KF999455	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo533	Z1	KF999369	KF999456	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo534	J7	KF999370	KF999457	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo535	Z1	KF999371	KF999458	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo536	J8	KF999388	KF999475	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo537	J9	KF999389	KF999476	This study
E. v. velox	15	Jinghe, Xinjiang, China	44.55°N, 82.59°E	Guo538	Z1	KF999390	KF999477	This study
E. v. velox	16	Fukang, Xinjiang, China	44.41°N, 87.86°E	WGXG08103	Z1	KF999330	KF999417	This study
E. v. velox	17	Dabancheng, Xinjiang, China	43.34°N, 88.22°E	WGXG08525	Z1	KF999384	KF999471	This study
E. v. velox	17	Dabancheng, Xinjiang, China	43.34°N, 88.22°E	WGXG08526	W1	KF999385	KF999472	This study
E. v. velox	17	Dabancheng, Xinjiang, China	43.37°N, 88.14°E	WGXG08536	Z1	KF999386	KF999473	This study
E. v. velox	17	Dabancheng, Xinjiang, China	43.37°N, 88.14°E	WGXG08537	Z1	KF999387	KF999474	This study
E. v. velox	17	Dabancheng, Xinjiang, China	43.37°N, 88.14°E	WGXG08538	W2	KF999359	KF999446	This study
E. v. velox	17	Dabancheng, Xinjiang, China	43.37°N, 88.14°E	WGXG08539	W2	KF999358	KF999445	This study
E. v. velox	18	Karamay, Xinjiang, China	45.27°N, 85.03°E	GP07080433	KL1	KF999404	KF999491	This study
E. v. velox	19	Qitai , Xinjiang, China	44.29°N, 90.11°E	WGXG08116	Q1	KF999331	KF999418	This study
E. v. velox	19	Qitai , Xinjiang, China	44.29°N, 90.11°E	WGXG08118	Q2	KF999399	KF999486	This study
E. v. velox	19	Qitai , Xinjiang, China	44.29°N, 90.11°E	WGXG08119	Z1	KF999400	KF999487	This study
E. v. velox	19	Qitai , Xinjiang, China	44.29°N, 90.11°E	WGXG08120	Z1	KF999401	KF999488	This study
E. v. velox	19	Qitai , Xinjiang, China	44.29°N, 90.11°E	WGXG08121	Q3	KF999402	KF999489	This study
E. v. velox	19	Qitai , Xinjiang, China	44.29°N, 90.11°E	WGXG08122	Q4	KF999403	KF999490	This study

(Continued Appendix A)

Taxon	Locality No.	Geographical origin	Latitude, longitude	Sample No.	Haplotype type	Accession number cyt *b*	12S rRNA	Reference
E. v. velox	20	Tacheng, Xinjiang, China	46.64°N, 82.95°E	JF1293	TA1	KF999395	KF999482	This study
E. v. velox	21	Fuyun, Xinjiang, China	44.42°N, 88.81°E	JF1383	Z1	KF999393	KF999480	This study
E. v. velox	22	Gaoquan, Xinjiang, China	44.48°N, 84.17°E	JF1230	Z1	KF999394	KF999481	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	WGXG08202	T1	KF999332	KF999419	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	WGXG08203	T2	KF999333	KF999420	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	WGXG08204	T1	KF999334	KF999421	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	WGXG08205	T3	KF999335	KF999422	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	WGXG08206	T1	KF999336	KF999423	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	WGXG08207	T4	KF999337	KF999424	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	LJE0707006	T5	KF999372	KF999459	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	LJE0707005	T6	KF999373	KF999460	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	LJE0707004	T7	KF999374	KF999461	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	LJE0707003	T8	KF999375	KF999462	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	LJE0707002	T9	KF999376	KF999463	This study
E. v. roborowskii	23	Turpan, Xinjiang, China	42.86°N, 89.19°E	LJE0707001	T10	KF999377	KF999464	This study
E. v. roborowskii	24	Jiaohe Ruins, Xinjiang, China	42.95°N, 89.07°E	WGXG08208	T11	KF999338	KF999425	This study
E. v. roborowskii	25	Toksun, Xinjiang, China	42.99°N, 88.75°E	WGXG08258	T12	KF999339	KF999426	This study
E. v. roborowskii	25	Toksun, Xinjiang, China	42.99°N, 88.75°E	WGXG08259	T12	KF999379	KF999465	This study
E. v. roborowskii	25	Toksun, Xinjiang, China	42.99°N, 88.75°E	WGXG08260	T12	KF999379	KF999466	This study
E. v. roborowskii	25	Toksun, Xinjiang, China	42.99°N, 88.75°E	WGXG08261	T12	KF999380	KF999467	This study
E. v. roborowskii	25	Toksun, Xinjiang, China	42.99°N, 88.75°E	WGXG08262	T13	KF999381	KF999468	This study
E. v. roborowskii	25	Toksun, Xinjiang, China	42.99°N, 88.75°E	WGXG08263	T12	KF999382	KF999469	This study
E. v. roborowskii	25	Toksun, Xinjiang, China	42.99°N, 88.75°E	WGXG08264	T12	KF999383	KF999470	This study
E. v. caucasia	26	Baskunchak, Astrakhan, Russia	46.98°N, 48.21°E	Guo1266	R1	KF999340	KF999427	This study
E. v. caucasia	27	E Volga River, Astrakhan, Russia	47.91°N, 46.57°E	Guo1268	R1	KF999341	KF999428	This study
E. v. caucasia	27	E Volga River, Astrakhan, Russia	47.91°N, 46.57°E	Guo1269	R1	KF999342	KF999429	This study
E. v. caucasia	27	E Volga River, Astrakhan, Russia	47.91°N, 46.57°E	Guo1270	R2	KF999343	KF999430	This study
E. v. caucasia	27	E Volga River, Astrakhan, Russia	47.91°N, 46.57°E	Guo1271	R1	KF999344	KF999431	This study
E. v. caucasia	27	E Volga River, Astrakhan, Russia	47.91°N, 46.57°E	Guo1272	R1	KF999345	KF999432	This study
E. v. caucasia	27	E Volga River, Astrakhan, Russia	47.91°N, 46.57°E	Guo1273	R1	KF999346	KF999433	This study
E. v. caucasia	27	E Volga River, Astrakhan, Russia	47.91°N, 46.57°E	Guo1274	R3	KF999347	KF999434	This study
E. v. caucasia	28	W Volga River, Astrakhan, Russia	46.59°N, 47.88°E	Guo1285	R3	KF999350	KF999437	This study
E. v. caucasia	28	W Volga River, Astrakhan, Russia	46.59°N, 47.88°E	Guo1286	R6	KF999349	KF999436	This study
E. v. caucasia	28	W Volga River, Astrakhan, Russia	46.59°N, 47.88°E	Guo1287	R7	KF999348	KF999435	This study
E. v. caucasia	28	W Volga River, Astrakhan, Russia	46.59°N, 47.88°E	Guo1289	R1	KF999357	KF999444	This study
E. v. caucasia	28	W Volga River, Astrakhan, Russia	46.59°N, 47.88°E	Guo1290	R1	KF999356	KF999443	This study
E. v. caucasia	29	Kalmykia, Russia	46.67°N, 46.29°E	Guo1276	R4	KF999355	KF999442	This study

(Continued Appendix A)

Taxon	Locality No.	Geographical origin	Latitude, longitude	Sample No.	Haplotype type	Accession number cyt *b*	12S rRNA	Reference
E. v. caucasia	29	Kalmykia, Russia	46.67°N, 46.29°E	Guo1277	R1	KF999354	KF999441	This study
E. v. caucasia	29	Kalmykia, Russia	46.67°N, 46.29°E	Guo1278	R5	KF999353	KF999440	This study
E. v. caucasia	29	Kalmykia, Russia	46.67°N, 46.29°E	Guo1279	R1	KF999352	KF999439	This study
E. v. caucasia	29	Kalmykia, Russia	46.67°N, 46.29°E	Guo1284	R1	KF999351	KF999438	This study
E. v. caucasia	30	Dagestan, Russia	43.61°N, 46.34°E	Guo1711	R8	KF999391	KF999478	This study
E. v. caucasia	30	Dagestan, Russia	43.61°N, 46.34°E	Guo1712	R1	KF999392	KF999479	This study
Eremias sp. 1	1	North Tehran, Iran	–	02-74	E1	JQ690169	JQ690100	*
Eremias sp. 2	3	SE Golestan National park, Iran	–	Smp221	E3	JQ690175	JQ690106	*
Eremias sp. 2	6	S Naishaboor, N Khorasan, Iran	–	EMP188	E6	JQ690187	JQ690119	*
Eremias sp. 3	2	SE Tehran, Iran	–	ERP266	E2	JQ690172	JQ690131	*
Eremias sp. 3	4	10 km S Jajarm town, Iran	–	Smp268	E4	JQ690171	JQ690102	*
Eremias sp. 3	5	15 km SW Sabzevar, Iran	–	Smp15	E5	JQ690183	JQ690115	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev2	UZ1	JQ690216	JQ690149	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev3	UZ2	JQ690217	JQ690150	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev1	UZ3	JQ690218	JQ690151	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev4	UZ4	JQ690219	JQ690152	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev6	UZ4	JQ690221	JQ690154	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev7	UZ5	JQ690222	JQ690155	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev8	UZ6	JQ690223	JQ690156	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev5	UZ6	JQ690220	JQ690153	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev9	UZ7	JQ690224	JQ690157	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev11	UZ8	JQ690225	JQ690158	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev12	UZ9	JQ690226	JQ690159	*
E. velox	7	S Aral Sea, Uzbekistan	–	Ev13	UZ10	JQ690227	JQ690160	*
E. v. caucasia	9	Extreme W Kazakhstan	–	D4	KZW1	JQ690233	JQ690166	*
E. v. velox	8	W Aral Sea, Kazakhstan	–	M90	KZ1	JQ690212	JQ690145	*
E. v. velox	10	SW Balkhash Lake, Kazakhstan	–	K04-6	KZ2	JQ690215	JQ690148	*
E. v. velox	10	SW Balkhash Lake, Kazakhstan	–	D10	KZ3	JQ690228	JQ690161	*
E. v. velox	10	SW Balkhash Lake, Kazakhstan	–	D9	KZ4	JQ690229	JQ690162	*
E. v. velox	10	SW Balkhash Lake, Kazakhstan	–	RL26	KZ5	JQ690230	JQ690163	*
E. v. velox	10	SW Balkhash Lake, Kazakhstan	–	RL28	KZ6	JQ690231	JQ690164	*
E. v. velox	10	SW Balkhash Lake, Kazakhstan	–	RL65	KZ7	JQ690232	JQ690165	*
E. v. velox	10	SW Balkhash Lake, Kazakhstan	–	RL112	KZ8	JQ690234	JQ690167	*
E. v. velox	11	NE Aral Sea, Kazakhstan	–	04-8	KZ9	JQ690213	JQ690146	*
E. v. velox	11	NE Aral Sea, Kazakhstan	–	K04-12	KZ10	JQ690214	JQ690147	*
E. v. velox	12	Turkistan, S Kazakhstan	–	T105	KZ11	JQ690206	JQ690139	*
E. v. velox	13	E Kazakhstan	–	M6	KZ12	JQ690204	JQ690137	*
E. v. velox	13	E Kazakhstan	–	U16	KZ13	JQ690205	JQ690138	*
E. v. velox	13	E Kazakhstan	–	Pp	KZ14	JQ690207	JQ690140	*
E. v. velox	13	E Kazakhstan	–	A11	KZ15	JQ690208	JQ690141	*
E. v. velox	13	E Kazakhstan	–	A1	KZ16	JQ690209	JQ690142	*
E. v. velox	13	E Kazakhstan	–	U12	KZ17	JQ690210	JQ690143	*
E. v. velox	13	E Kazakhstan	–	M14	KZ18	JQ690211	JQ690144	*
E. pesica	–	Iran	–	ERP164	–	FJ416285	FJ445362	*
E. argus	–	South Korea	–	LEGO-F415	–	JQ086345	JQ086345	#

* Rastegar-Pouyani *et al*. (2012); # Kim *et al*. (Unpublished data)