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The mitochondrial phylogeography and intraspecific taxonomy of the Steppe Racerunner, *Eremias arguta* (Pallas) (Lacertidae: Sauria, Reptilia), reflects biogeographic patterns in Middle Asia

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Abstract

Steppe racerunner, *Eremias* (*Eremias*) *arguta*, is one of the most widespread species of the Asian racerunners (genus *Eremias*). Several subspecies were traditionally recognized however, morphological variability is so high that delimitation of these subspecies was always problematic. Here we present a phylogenetic hypothesis for this species based on cytochrome *b* sequences (55 samples from 35 populations, 900 bp partial sequences), infer it biogeography and the revise its subspecific structure. Six major phylogenetic lineages were revealed. The southernmost populations (*E. a. uzbekistanica*) from Uzbekistan form a clade together with the Issyk-Kul Lake subspecies (*E. a. darevskii*) based on both molecular and morphological evidence. Within more northern populations, there is a split between populations from Northern Caucasus, Europe and Western Kazakhstan (*E. a. deserti*) and Central and Eastern Kazakhstan populations (*E. a. arguta*). Transcaucasian (*E. a. transcaucasica*) steppe racerunners are grouped with Middle Asian populations. Finally, the easternmost samples, assigned to "*E. a. potanini*" are nested within the *E. a. arguta* clade. Populations from the Ili River Valley form a separate lineage sister to the clade joining all other *E. arguta* lineages and might represent a yet undescribed taxon. Species distribution in relation to historical biogeography of Middle Asia is discussed.

Key words: Biogeography, *cyt b*, Kazakhstan, Turan, Central Asia, *Eremias*

Introduction

The arid realm of Middle and Central Asia is one of World's largest and oldest desert regions (West 1983). Tracing its history to early Cenozoic Era (Fedorovich 1946; Kramarenko 1974), which expanded after the Indian collision, this arid area not only separated Oriental and Western regions but was also a place of origin for unique biotic complexes. Whereas the refugial hypothesis has been widely used in studies of phylogenetic history of European Biota (see Hewitt 2000), the arid areas of the Middle East and Middle Asia have not yet been sufficiently studied (Dolotovskaya *et al*. 2007; Graham *et al.* 2012; Guo *et al.* 2011; Melville *et al.* 2009; Nazarov & Poyarkov 2013; Orlova *et al.* 2007; Solovyeva *et al.* 2011; 2012; 2014).

Racerunners, or *Eremias,* are an Asian genus of the lizard family Lacertidae. The genus *Eremias* belongs to the lacertid subfamily Lacertinae, tribe Eremiadini of sub-Saharan origin (Arnold 1989; Arnold *et al.* 2007; Harris *et al.* 1998; Fu 1998, 2000; Mayer & Pavlicev 2007), and is the only lizard genus in this sub-family found exclusively in Asia. Taxonomy of this group is quite complicated and insufficiently studied (Sczcerbak 1974). Today the genus consists of ca. 35-40 recognized species found in Middle and Central Asia. The steppe racerunner, *Eremias arguta* (Pallas)*,* belongs to the subgenus *Eremias*, which includes several species found exclusively in southern and central parts of Middle Asia (*E. aria* Anderson & Leviton, *E. intermedia* (Strauch), *E. nigrocellata* Nikolsky), whereas *E. arguta* has the largest range among other species and is found in the Black Sea region from Romania to Ciscaucasia, Eastern Transcaucasia and Middle Asia to the east as far as Chinese Dzhungaria and Dzhungar Gobi in Western Mongolia.

The steppe racerunner has a vast range and inhabits various climatic and landscape zones (Chernov 1934; Sczcerbak 1974, 1993; Brushko 1995). Some of the isolated populations of this species are found in remote mountain valleys (e.g. Issyk-Kul depression, Kyrgystan; Sevan depression, Armenia). The forms inhabiting plain areas often demonstrate zones of clinal variation and intergradations with morphological characters and transition zones between the subspecies (Sczcerbak 1970, 1974, 1993). Based on complex analysis of morphological characters, pholidosis and coloration pattern, six subspecies were recognized in the last taxonomic revision by Sczcerbak (1993):

The nominate form *E. a. arguta* (Pallas 1773) inhabits the central part of the species range from Volga – Ural watershed in the west to Balkhash Lake area in the east.

The western form *E. a. deserti* (Gmelin 1789) inhabits the northwestern part of the species range from eastern Romania in the west along the Black Sea coast to southern Ukraine, the Crimean peninsula and Ciscaucasia including the northwest coast of the Caspian Sea to the Ural River in the east.

The Transcaucasian form *E. a. transcaucasica* Darevskii 1953 was described from an isolated population in the Sevan Lake depression in Armenia. The taxonomic status of the rest of Transcaucasian populations remained somewhat vague; they were considered to be intermediate between *E. a. deserti* and *E. a. transcaucasica*.

The eastern form *E. arguta potanini* (Bedriaga 1912) was assumed to be different from the nominate form mostly in coloration pattern and is found in Chinese Dzhungaria (the northern region of Xinjiang province of the Peoples Republic of China) and Dzhungar Gov in Mongolia.

The southern form *E. a. uzbekistanica* Chernov 1934 is known from the southernmost limit of the species range in the semi-desert areas of the Uzbekistan foothills.

The isolated population of the Issyk-Kul Lake valley in Kyrgyzstan was described as a separate subspecies: *E. a. darevskii* Tsaruk 1986.

Sczcerbak (1993) proposed that, based on geographical variation of morphological characters, the area of probable formation of this species should be considered to be somewhere in the steppes of central Kazakhstan. Since morphological characters in many areas demonstrate clinal variation and are dependant from local environment, the delimitation of the subspecies is a difficult task within the taxonomy of *Eremias* racerunners (Chernov 1934; Okulova 1973; Sczcerbak 1993; Orlova & Terbish 1997; Chirikova & Kolbinzev 2003). Thus, to test the level of molecular differentiation between the presently recognized subspecies is a relevant task for a better understanding of the taxonomy of this group.

However, until now no molecular genetic research has been applied to this species and phylogeographic data for the lizards of the genus *Eremias* have been sparse and exists primarily for species within this genus in China (Xin *et al.* 2004; Wan *et al.* 2007; Orlova 2008). Recently Guo *et al.* (2011) provided a preliminary account on the phylogeny and divergence times of some *Eremias* lizards inferred from 16S rRNA partial sequences. The resultant phylogeny, covering approximately 12 species, is however poorly resolved within the majority of internal nodes. And while this confirms the monophyly of *Eremias*, it nonetheless questions the validity of the subgeneric clades within the genus *Eremias*. Moreover, Guo *et al*. (2011) actually found *E. arguta* to be paraphyletic in relation to *E. vermiculata* Blanford with haplotype 57 grouping with *E. vermiculata* (though with poor node support). This fact is not discussed in the text of the article but questions the monophyly of *E. arguta* or the validity of the phylogenetic hypothesis presented. Since morphologically *E. vermiculata* and *E. arguta* appear to be quite different (Sczcerbak 1974), we consider that this fact could be the result of misidentification or improper nuclear pseudogene sequencing. The purpose of this study is to provide the first molecular mtDNA data concerning the taxonomic structure of the steppe racerunner *E. arguta*, to analyze the biogeographical patterns in line with data on historical biogeography of Middle Asia, and to elaborate a preliminary phylogeographic hypothesis for this species as a model representative of the herpetofauna of semi-desert areas of Middle-Asia.

Material and methods

Sample collection. The sampling strategy covers the entire range of *E. arguta*; 60 samples from 35 populations were analyzed (see Table 1 and Figure 1). Tissue samples were stored in absolute ethanol. In the most cases voucher specimens were collected, preserved, and deposited in the herpetological collection of the Zoological Museum of Moscow University (ZMMU), Russia. Outgroup included: *Darevskia praticola* (Eversmann) (locality

of origin—Goriachiy kluch, NW Caucasus, Russia), and two congeneric taxa—*E. velox* (Pallas), representative of the distantly related subgenus *Aspidorhinus* (formerly *Dimorphea*, see Barabanov 2009; Guo *et al.* 2011), and assumingly a closer relative, *E. intermedia* (subgenus *Eremias* sensu stricto).

TABLE 1. *E. arguta* populations sampled in this study. Names of localities and countries of origin are given; numbers appointed to *E. arguta* populations correspond to those in Fig. 1. N is the sample size per population. ZMMU ID corresponds to the numbers of the voucher specimens kept in the herpetological collection of the Zoological Museum of Mscow University, Russia. GenBank accession numbers are given.

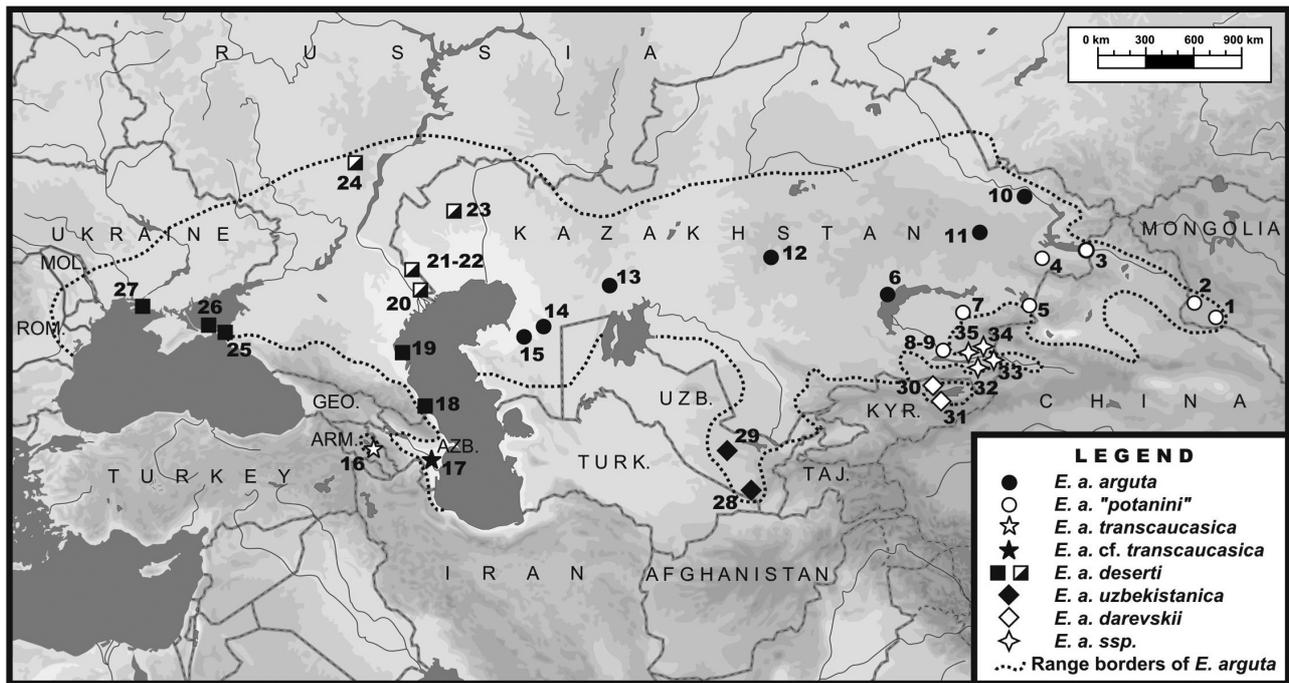| Subspecies	Country	Locality		N	ZMMU ID	GenBank A.N.
E. a. potanini	Mongolia	1	Bulgan Somon (1)	2	R-12604	KM977908–977909
-	Mongolia	2	Bulgan Somon (2)	1	R-12459	KM977910
-	Kazakhstan	3	Aigyrkum sands	2	R-12417	KM977911–977912
-	Kazakhstan	4	Oichilik	2	R-12420	KM977913–977914
-	Kazakhstan	5	Zhalanashkol Lake	1	R-12415	KM977915
-	Kazakhstan	6	W coast of Balkhash	3	R-12488	KM977916–977918
E. a. cf. *potanini*	Kazakhstan	7	Ainabulak	2	R-12489	KM977919, KM977960
-	Kazakhstan	8	Kerbulak	1	R-12414	KM977920
E. a. arguta	Kazakhstan	9	Shingeldy	1	R-12419	KM977921
-	Kazakhstan	10	Chernyi Irtysh	1	R-12416	KM977970
-	Kazakhstan	11	Kopa	1	R-12412	KM977922
-	Kazakhstan	12	Dzhezkazgan	2	–	KM977923–977924
-	Kazakhstan	13	Baikodam	2	R-12424	KM977925–977926
-	Kazakhstan	14	N Ustyurt, Ozektyk	2	R-12409	KM977927–977928
-	Uzbekistan	15	NW Ustyurt	1	R-11981	KM977969
E. a. transcaucasica	Armenia	16	Noraduz, Sevan lake	1	R-12237	KM977929
E. a. cf. *transcaucasica*	Azerbaijan	17	E Azerbaijan	1	R-12473	KM977930
E. a. deserti	Russia	18	Papas	1	R-12455	KM977931
-	Russia	19	Chechen Island	2	R-12072	KM977932–977933
-	Russia	20	Akhtuba river	2	–	KM977936–977937
-	Russia	21	Bogdo mt.	2	R-12433	KM977940–977941
-	Russia	22	Baskunchak lake	2	–	KM977938–977939
-	Kazakhstan	23	Manat	2	R-12224	KM977942–977943
-	Russia	24	Dikovka village	2	–	KM977934–977935
-	Russia	25	Taman	3	–	KM977944–977946
-	Ukraine	26	Kerch	3	–	KM977947–977949
-	Ukraine	27	Golaya Pristan	2	R-12256	KM977950–977951
E.a. uzbekistanica	Uzbekistan	28	Kyzylcha	1	R-12249	KM977953
-	Uzbekistan	29	Aidarkul Lake	1	R-11698	KM977952
E.a. darevskii	Kyrgyzstan	30	Turu-Aigyr	2	R-12490	KM977954–977955
-	Kyrgyzstan	31	Ak-Olen	4	R-12943	KM977956–977959
E. arguta ssp.	Kazakhstan	32	Kokpek environs	2	R-12410	KM977964–977965
-	Kazakhstan	33	Kainar	1	R-12425	KM977963
-	Kazakhstan	34	Konyrolen	1	R-12426	KM977961
-	Kazakhstan	35	Konyrolen environs	1	Kn-1	KM977962

FIGURE 1. Distribution of *Eremias arguta* subspecies (following Sczcerbak et al., 1993, modified) and localities studied. Populations of *E. arguta* used in this study are shown, numbers correspond to those in Table 1. Icons represent the main lineages of *Eremias arguta* (see Fig. 2).

DNA isolation and sequencing. Total genomic DNA was extracted from a relatively small amount of tissue (tail muscles, voucher material remains) using the standard phenol-chloroform method (see Sambrook *et al.* 1989) and the Diatom DNA Prep 100 Tissue Kit (IsoGene) following the manufacturer instructions. The obtained DNA was purified, visualized in UV-light electrophoresis and the resultant concentration was measured. The final concentration of DNA was adjusted to approximately 100 ng/µL using NanoDrop 2000 (Thermo Scientific). One mitochondrial protein-coding gene—cytochrome *b* (cyt *b*) fragment was amplified by polymerase chain reaction (PCR): the partial cyt *b* sequence comprising 780-790 bp. Four primers were used in this study (see Table 2 for details regarding the primers); in most cases the primer combination mt-A-new and mt-FS-H worked well. Additional primers were used in sequencing PCRs.

TABLE 2. Primers used for PCR amplification and sequencing. Origins of primers and references: a—Kalyabina-Hauf & Ananjeva (2004), b—Kocher *et al.* (1989).

Primer name	Primer sequence
mt-A-new[a] (L-14991[b])	(5'-CTC CCA GCC CCA TCC AAC ATC TCA GCA TGA TGA AAC-3')
mt-FS-H[a] (H-16060[b])	(5'-TAG TTG GCC AAT GAT GAT GAA TGG GTG TTC TAC TGG TT-3')
L-14841[b]	(5'-AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA-3')
H-15149[b]	(5'-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A-3')

PCR was conducted using the GenePack Core (IsoGene) in a total volume of 20 ml, which contained from 1.0 to 5.0 ml of DNA extraction, 1.0 ml of forward and reverse primers (10 mM), 0.2 ml of *Taq* polymerase (5 U/ml), 1 ml dNTP mix, and PCR buffer containing 15 mM MgCl$_2$. PCR conditions were as follows: initial denaturation for 5 min at 94° C, 31-35 cycles composed of the 30 seconds denaturation at 94° C, 45 seconds of annealing at 50-57° C, and 1 min extension at 72° C. PCR products were purified with the Wizard SV Gel and PCR Clean-up System (Promega). Cycle sequencing was done commercially through VGNKI RAMS, Moscow.

Phylogenetic analysis. The sequence data were translated into amino acids to confirm the absence of premature stop codons. The forward and reverse sequences were checked visually in Chromas 2.0 and a consensus sequence was compiled with BioEdit 5.0.9 (Hall 1999). Sequences were also submitted to a BLAST search in GenBank to confirm that the required sequences had been amplified. Nucleotide sequences were initially aligned using ClustalX 1.81 (Thompson *et al.* 1997) with default parameters, and then checked for presence of deletions, stop-codons and optimized manually in BioEdit 7.0.5.2 (Hall 1999) and MEGA 5.0 (Tamura *et al.* 2011). Mean uncorrected genetic distances (*p*-distances) between sequences were determined with MEGA 5.0 (Tamura *et al.* 2011).

MODELTEST v.3.06 (Posada & Crandall 1998) was used to estimate the optimal evolutionary model to be used for the data set analysis. The preferred model was (GTR + I + G), as suggested by the Akaike information criterion (AIC).

Phylogenetic trees were inferred using three different methods, Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian analyses (BA). The MP analyses were conducted with the program PAUP version 4.0b4a (Swofford 1998), using a heuristic search and the closest step-wise sequence addition algorithm. Most-parsimonious trees were generated with 100 random-addition sequences and the tree-bisection-reconnection (TBR) algorithm, for branch swapping. Transitions and transversions were equally weighted. The Maximum Parsimony tree was reconstructed with random addition, 20 replicates with CNI (Close Neighbor Interchange) value = 3. The ML analyses were conducted using Treefinder (Jobb *et al.* 2004). Transitions and transversions were equally weighted, and gaps were treated as missing data. The BA analyses were carried out with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) by implementing the model of sequence evolution. The analysis was run with four chains for 10 million generations and trees sampled in every 1000 generations, producing an output of 10000 trees. To confirm that the chains had achieved stationary, we evaluated "burn-in" plots by plotting log-likelihood scores and tree lengths against generation number using the software Tracer v.1.4 (Drummond & Rambaut 2007). After assessing for apparent convergence, the first 2500 trees of each run were discarded as "burn-in". A majority rule consensus tree (BI tree) was calculated from the posterior distribution of remaining trees.

Confidence in tree topology was tested by non-parametric bootstrap analysis (Felsenstein 1985) with 1000 replicates for the MP and ML analyses, and by posterior probability (pp) for BA in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Branches with bootstrap values 70% or greater and pp values over 0.95 were regarded as strongly supported (Huelsenbeck & Hillis 1993).

Results

Sequence data. Among the 60 *E. arguta* sequences we identified, 49 haplotypes of cyt *b* fragment and all outgroup samples represented unique haplotypes (see Table 1; GenBank Accession numbers: KM977908–KM977970). The final alignment used for phylogenetic analysis comprised 780 bp of 60 *Eremias arguta* specimens and sequences of *Eremias velox* and *Eremias intermedia*; sequence of *Darevskia praticola* was used as an outgroup. No length variation, deletions or insertions were observed in *Eremias* sequences. Of the 780 aligned sites, 538 were found to be conserved, 242 variable and 173 parsimony-informative; the transition–transversion bias was estimated at 3.767 (all data given for ingroups only). The nucleotide frequencies were 26.60% (A), 29.55% (T), 13.88% (C), and 29.98% (G).

Geographic distribution of mtDNA haplotypes. All studied samples of *E. arguta* form a monophyletic group, although this was not unexpected given the low number of outgroups included. The revealed haplotypes of *E. arguta* mitochondrial DNA are clearly divided into six clades, most of them well structured and comprising several subclades (see Figure 2). These six clades were named from A to F, whereas subclades, if their variation was pronounced and had some geographical pattern, were marked as A1, A2 etc. The distribution of the lineages obtained shows clear geographical structure (see Figures 1 and 2 for details).

The first clade to branch off is clade D, which includes the narrow-ranged populations from the Ili River Valley in east Kazakhstan, which forms a sister clade to all other *E. arguta* lineages. The monophyly of this clade is well-supported (100/100/1.0; hereafter the node support values are given for ML/MP/BA analyses respectively); two well-supported subclades are revealed for the populations from the right (D1; 88/93/0.93) and the left (D2; 100/100/1.0) banks of the Ili River.
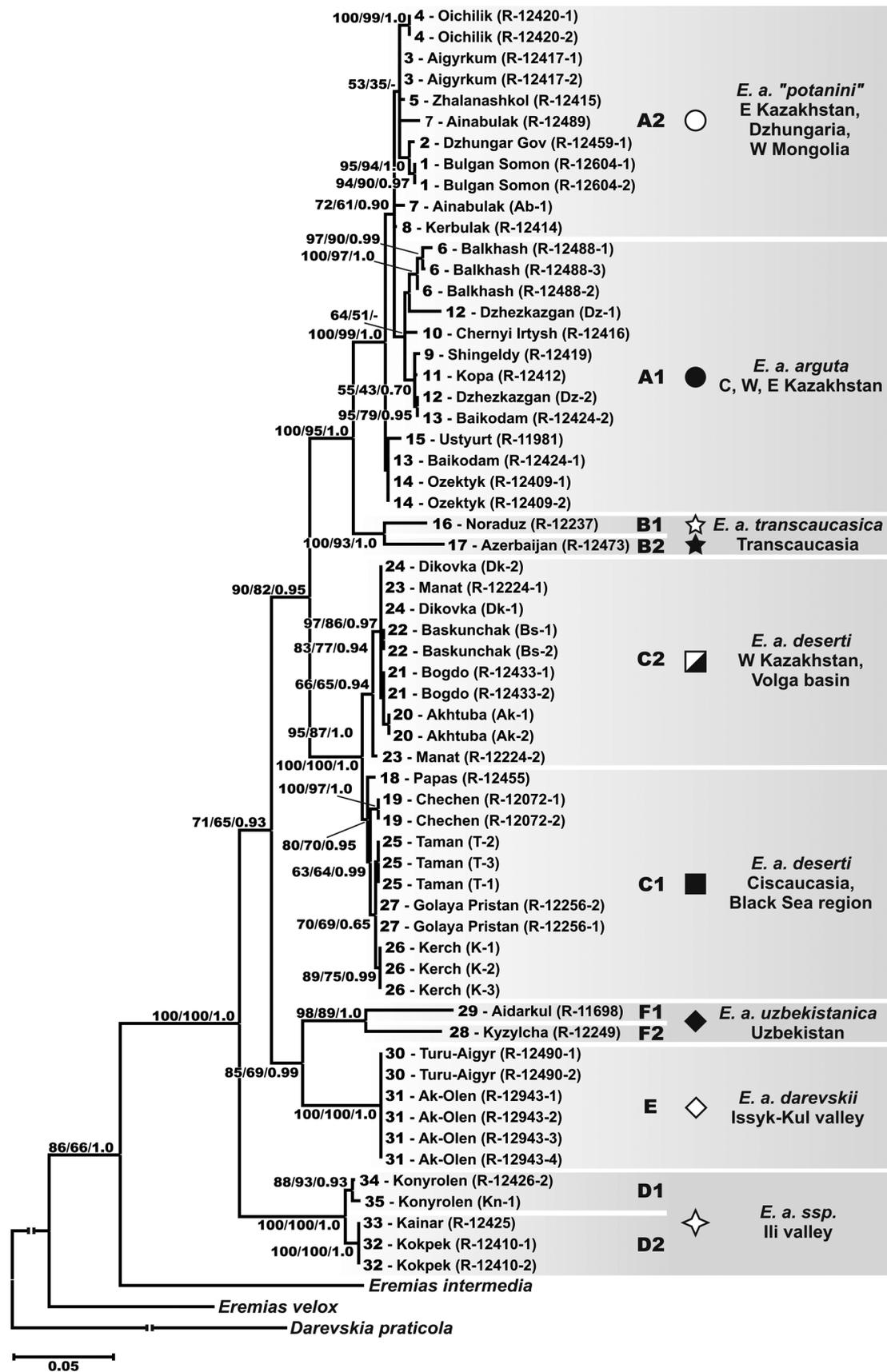
FIGURE 2. The tree resulting from the Maximum Likelihood phylogenetic analysis of the studied samples of *Eremias arguta*. For the major cladogenetic events ML/MP/BA bootstrap and posterior probabilities values are given. The geographic information is given for all major clades identified. Icons representing main *Eremias arguta* lineages correspond to those in Fig. 1. Cyt b sequences of *Darevskia praticola*, *Eremias velox* and *Eremias intermedia* are used as outgroups.

The monophyly of the clade joining all other lineages of *E. arguta* has moderate support values (71/65/0.93); it is further divided into the two subclades: one encompassing populations from the southern part (E + F; 85/69/0.99), and the other one from the northern part of the species range (A + B + C; 90/82/0.95) (see Figure 2).

Within the southern subclade, two samples from the southernmost limit of the species range from Uzbekistan, which correspond to the subspecies *E. a. uzbekistanica*, form lineage F (98/89/1.0) and consist of two highly divergent haplotypes (F1 and F2). Lineage F is sister to the populations from the Issyk-Kul Lake depression in the Tien Shan Mountains of Kyrgyzstan (clade E; 100/100/1.0), which were described as a separate subspecies *E. a. darevskii*.

Within the northern group of *E. arguta*, the populations from the northwest region of the species range—northern Caspian region, Ciscaucasia, and Black Sea region, including Crimean peninsula are combined into clade C (100/100/1.0) and correspond to subspecies *E. a. deserti*. There is a notable structuring within clade C, with populations from the western part of the subspecies range in the Black Sea region to Ciscaucasia (as far east as Dagestan Republic of Russia on Caspian coast) forming subclade C1 (80/70/0.95), and the eastern populations from the Volga basin and western Kazakhstan grouped into subclade C2 (66/65/0.94).

The two populations from Transcaucasia (eastern Azerbaijan and Sevan Lake in Armenia) corresponding to *E. a. transcaucasica* form clade B (100/93/1.0), joining two highly divergent haplotypes (B1 and B2). The Transcaucasian clade B is surprisingly distantly related to the Ciscaucasian populations (clade C), and forms a well-supported monophyletic group (100/95/1.0) with the populations from central and Eastern Kazakhstan (clade A). Clade A (100/99/1.0) joins the populations which belong to the nominate form (*E. a. arguta* - populations from Central to Eastern Kazakhstan, Aral Sea region and Ustyurt Plateau; group A1) and the form known as *E. a. potanini* (from the Balkhash Lake area in the west to Xinjiang province of China and Mongolian Dzhungaria (Khovd Aimak) of Mongolia in the east; group A2). These groups of populations do not form monophyletic groups on the phylogenetic tree (see Figure 2) and the relationships within clade A remain essentially unresolved. Populations from the western part of the subspecies range (Ustyurt Plateau) tend to form a basal lineage within clade A, though with moderate support values (72/61/0.90).

Intra- and interspecific differentiation of mtDNA haplotypes. Mean uncorrected genetic *p*-distances (%) within *E. arguta* and between *E. arguta* and two outgroup *Eremias* species—*E. intermedia* and *E. velox* are shown in Table 3. All studied samples were grouped according to their arrangement in the phylogenetic trees and current taxonomy (see Figure 2); eleven units were allocated (A1, A2, B1, B2, C1, C2, D1, D2, E, F1 and F2); they correspond to major geographical populations. Genetic distances between the groups (hereafter given in percentages; $p = 1.28–15.29$ %) are generally higher then the distances between geographically and morphologically closely related local populations, belonging to one unit ($p < 1.32\%$).

Mean genetic distances between phylogenetic lineages of *Eremias arguta* vary from 1.28% (between left and right bank populations of the unnamed clade D from the Ili River Valley), 1.50% (between eastern and western clades of *E. a. deserti*) and 1.77% (between *E. arguta potanini* and *E. a. arguta*) to 15.77–15.90% (between *E. a. uzbekistanica* and the unnamed clade D from the Ili River Valley) (see Table 3). Within the phylogenetic lineages, genetic differentiation varies from 0.00 for the populations of *E. a. darevskii* and the left bank populations from the Ili River valley to 0.33%–1.32% (for other lineages) (see Table 3).

Clade F (*E. a. uzbekistanica*) shows remarkable genetic differentiation between the two samples from Uzbekistan (F1 and F2) estimated as $p = 7.82\%$. Quite deep intrasubspecific differentiation is also revealed in clade B (*E. a. transcaucasica*), with $p = 5.51\%$ for the distance between Armenian (B1) and Azerbaijani (B2) populations (see Table 3 for details). Both clades are poorly sampled in our study and the distant localities are most probably isolated from each other. Within the unnamed clade D from the Ili River valley a certain differentiation ($p = 1.28\%$) is found between the populations from the left and right banks of the Ili River, respectively. The southern part of *Eremias arguta* species range shows presumably quite an old differentiation between local populations. The differentiation within clade B can be explained through presumably old isolation of geographic populations and possibly due to an incomplete knowledge of the taxonomy of this group, since the taxonomic position of *E. arguta* from Azerbaijan requires further clarification.

TABLE 3. Mean uncorrected genetic *p*-distances distances (percentage) between (below diagonal) and within (diagonal, bold font) the different lineages revealed within *Eremias arguta*. The standard error (500 bootstrap replicates) is given above diagonal. Not all lineages are equally sampled. Codes used for *Eremias arguta* lineages: 1—A1 – Arg – *E. a. arguta*; 2—A2 – Pot – *E. a. "potanini"*; 3—B1 – Trans – *E. a. transcaucasica* (Armenia); 4—B2 – cf. Trans – *E. a. cf. transcaucasica* (Azerbaijan); 5—C1 – W Des – *E. a. deserti*, western populations; 6—C2 – E Des – *E. a. deserti*, eastern populations; 7—D1 – R Ili – the unnamed clade from the Ili River Valley, right bank populations; 8—D2 – L Ili – the unnamed clade from the Ili River Valley, left bank populations; 9—E – Dar – *E. a. darevskii*; 10—F1 – Uzb – *E. a. uzbekistanica*; 11—F2 – Uzb – *E. a. uzbekistanica*; 12—*E. intermedia*; 13—*E. v. velox*; 14—*Darevskia praticola*.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1—A1 - Arg	**1.04**	0.30	0.79	1.02	1.07	1.11	1.27	1.22	1.11	1.28	1.05	1.73	1.89	2.01
2—A2 - Pot	1.77	**1.32**	0.87	1.01	1.01	1.05	1.28	1.25	1.11	1.28	1.09	1.76	1.89	2.02
3—B1 - Trans	5.60	6.10	-	0.88	1.09	0.99	1.37	1.31	1.15	1.40	1.21	1.77	1.95	1.98
4—B2 - cf. Trans	6.86	6.93	5.51	-	1.19	1.05	1.45	1.39	1.20	1.46	1.29	1.87	1.89	2.08
5—C1 - W Des	7.37	7.24	7.63	8.33	**0.50**	0.38	1.39	1.37	1.17	1.42	1.23	1.78	1.82	1.98
6—C2 - E Des	7.38	7.24	6.81	7.50	1.50	**0.33**	1.35	1.33	1.12	1.43	1.25	1.77	1.79	2.00
7—D1 - R Ili	10.41	10.41	11.67	11.41	11.01	11.04	**0.51**	0.39	1.33	1.57	1.33	1.67	1.83	2.05
8—D2 - L Ili	10.68	10.75	11.67	11.28	11.21	11.15	1.28	**0.00**	1.31	1.53	1.30	1.69	1.84	1.98
9—E - Dar	9.78	9.66	10.26	10.13	8.35	7.94	10.51	10.64	**0.00**	1.33	1.09	1.73	1.68	1.98
10—F1 - Uzb	14.08	14.25	14.74	15.26	13.80	13.96	15.77	15.90	12.44	-	1.01	1.74	1.75	1.89
11—F2 - Uzb	9.23	9.60	10.38	10.26	9.06	9.35	11.28	11.41	7.95	7.82	-	1.72	1.82	1.97
12—*E. intermedia*	17.87	18.03	17.31	18.46	16.85	16.85	18.91	19.10	18.08	20.00	18.59	-	1.69	1.90
13—*E. velox*	18.04	18.26	17.82	17.31	17.09	16.77	18.01	18.08	16.28	18.08	16.28	17.95	-	1.85
14—*Darevskia*	23.73	23.97	23.21	24.36	22.88	23.10	23.91	24.10	23.59	24.23	24.23	23.97	22.44	-

Discussion

Geographic structuring of haplotypes within phylogenetic lineages and biogeographic patterns in Middle Asia. The formation of the arid fauna in Middle and Central Asia has a long history (Geptner 1938; Korovin 1961). The most significant aridification process and formation of the continental climate started in the late Oligocene (29–24 MYA) in the territory of Central Asia and neighboring areas of Kazakhstan, whereas in such regions as Xinjiang, Tibet, and the eastern regions of Middle Asia, the desertification process started approximately 24–22 MYA (Kramarenko 1974). According to Sinitsyn (1962) in late Oligocene–early Miocene (24–18 MYA) the arid belt spread from eastern Anatolia to Iranian land, southern Middle Asia and western Central Asia (Xinjiang, Tibet). This process favored formation of autochthonous arid herpetofauna, combining African and Asian faunal elements (Geptner 1938, Chernov 1949, Rustamov & Szczerbak 1985). Central and Middle Asia appear to have had a shared herpetofauna during the Miocene but these were separated since the early to mid-Pliocene (5.6–3.5 MYA) following the orogenetic processes in the Tien Shan region (Chkhikvadze *et al.* 1983).

The first known remains of the genus *Eremias* appear in middle–late Miocene deposits of Middle Asia (8.5–7.0 MYA) (Bakradze & Chkhikvadze 1988). This time period corresponds with reduction of the Sarmatian Paratethys Basin and uplift of the Turan Plate (Popov *et al.* 2004) which followed with a significant aridification of the Middle Asian climate (Alisov 1969); it is likely that the common ancestor of Asian Racerunners already existed 8 MYA before present. This assumption agrees with the preliminary divergence time estimate for the basal split of *Eremias* as calculated by Guo *et al.* (2011): ca. 9.9 MYA (range: 7.6–12.0 MYA).

E. arguta and other species of the subgenus *Eremias* belong to the Middle Asian autochthonous herpetofaunal center (Bogdanov 1965). The sister taxon of *E. arguta* according to Guo *et al.* (2011) is reconstructed as *E. vermiculata* from western China (Xinjiang province), however this clade is poorly supported (MP BS = 54; BPP = 0.98). We assume that the area of *E. arguta* origin is likely to be situated in the northern part of the Irano-Turanian region. This assumption agrees with the maximum species diversity for the subgenus *Eremias* in the area–*E. nigrocellata* and *E. intermedia* are found in Turan, whereas *E. aria* is restricted to southeastern slopes of Hindukush in Afghanistan (Anderson 1968; Szczerbak 1974). Guo *et al.* (2011) dated *E. vermiculata*–*E. arguta* split at the Miocene—Pliocene transition, ca. 5.9 MYA (range 2.7–9.0 MYA). We assume that a common ancestor of *E. arguta* might have occupied Turanian area around middle—late Pliocene. Guo *et al.* (2011) estimate basal split with *E. arguta* as 3.7 (0.8–7.2) MYA in the middle Pliocene, however this estimate actually corresponds to the split between clades A (*E. a. arguta*) and B+C (*E. a. deserti + E. a. transcaucasica*) (see Fig. 2).

The geographic pattern of the haplotype distribution in *Eremias arguta* is quite peculiar and likely has some biogeographic background. The highest genetic diversity is observed in the south and southeast of the species range, which is likely to be the area of the autochthonous origin of *E. arguta*. Further to the steppe areas in the north and west of *E. arguta*'s range, genetic distances among local populations tend to get lower, which probably indicates relatively more recent expansion events. Differentiation within the phylogenetic lineages and its value for the taxonomy of the group requires more detailed discussion, so we will examine each one of them.

Clade A. Studied *E. arguta* populations from central and eastern Kazakhstan do not show high differentiation levels (Table 3) and there is no clear geographic structuring within the clade. Two main color patterns were reported from this territory (see Sczcerbak 1974, 1993): blotched morph *E. arguta arguta* (see Figure 3B), corresponding to the populations from western Kazakhstan (Ustyurt plateau) to Aral Sea area, Balkhash Lake and the northern part of eastern Kazakhstan; and a banded form *E. arguta potanini* (see Figure 3A), which is found in populations from the east of the species range: western Mongolia, east Kazakhstan and the Balkhash Lake area. *E. a. potanini* was described by Bedriaga (1912) basing primarily on differences in dorsal pattern from the easternmost part of the species range in Chinese Dzhungaria. Later, Chernov (1934) identified large collections from Semirechye region (eastern Kazakhstan) and assumed that they might be intermediate between *E. a. arguta* and *E. a. uzbekistanica*. Despite material from Dzhungaria (westernmost China), which was sparse, Chernov included this part of the species range within the range of the subspecies *E. a. uzbekistanica*. Yakovleva (1964) studied populations from environs of Almaty, Kaskelen, Balkhash Lake area, Zailiyskiy and Dzhungariskiy Alatau Mountains in Kazakhstan and argued that morphologically these populations are closely related to *E. a. uzbekistanica*. Later Sczcerbak (1974) included populations found east from the Balkhash Lake in the subspecies *potanini*; in the Balkhash Lake area a wide transition zone between *E. a. potanini* and *E. a. arguta* was assumed, since many morphological characters, including coloration pattern demonstrate clinal variation from west to east

(Sczcerbak 1993). Sczcerbak (1993) also considered that the Ili River Valley population is a form "transitional" between *E. a. potanini* and *E. a. arguta*. According to Orlova (1989) Mongolian populations, traditionally regarded as the subspecies *potanini*, correspond well to the original description of this form by Bedriaga (1912), but are quite different from the description given by Sczcerbak (1993) and in major diagnostic characters they are similar to the nominative subspecies (Orlova & Terbish 1997). Racerunners from eastern Kazakhstan also show many characters typical of the nominative form and thus represent marginal populations of *E. a. arguta* (Chirikova 2001, 2006; Chirikova & Kolbinzev 2003; Orlova 2008). The two easternmost of all studied populations in our analysis (localities 1 and 2 in Bulgan Somon, Dzhungar Gov; Mongolia) are close to the type locality of the subspecies *E. arguta potanini* and they do not form separate lineages nor do they group together with other populations from east Kazakhstan and the Balkhash Lake area.

Populations morphologically identified as *E. a. arguta* do not form a monophyletic group. Thus, populations from Balkhash area, Ainabulak and Kerbulak in eastern Kazakhstan, Almaty Province, show some morphological characters typical of *E. a. arguta*, however genetically are close to typical *E. a. potanini* populations (see Figure 2). Some diagnostic characters in *E. arguta* subspecies, such as coloration pattern, might depend on ecological features, such as soil type (Orlova 2008). In the vicinity of Shingeldy and Kerbulak settlements in Almaty Province (pp. 8 and 9, see Fig. 1) two types of haplotypes, belonging to groups A1 and A2 were found in close vicinity from each other.

A west-east clinal pattern in coloration from the blotched "*arguta*"-type to banded "*potanini*"-type was reported (Orlova *et al.* 2012), which generally corresponds to the observed variation of mtDNA haplotypes. Generally, the unclear geographic structuring within clade A, the clinal pattern in variation of the main diagnostic character (coloration pattern), and the low genetic distances between geographic populations ($p \leq 1.77\%$) does not allow us to recognize *E. a. potanini* as a valid subspecies, but rather as a color morph.

Clade B. The *E. arguta* populations from the Transcaucasia region form a monophyletic group, which is the sister group of clade A (*E. a. arguta*–*E. a. potanini*), but not with clade C found in Northern Caucasus. This can be explained by the assumption that the Steppe racerunner likely could have colonized the Caucasian isthmus several times independently (Chernov 1954; Darevsky 1957; Sczcerbak 1974).

Clade B is restricted to a relatively small part of the species range in Transcaucasia—from a mountainous area of eastern Armenia and western Azerbaijan to the Caspian Sea coast in central Azerbaijan. Morphological specificity of Transcaucasian *E. arguta* was first noticed by Chernov (1934), and later the population from the isolated Sevan Lake Valley was described as *E. arguta transcaucasica* by Darevsky (1953). The Armenian population is morphologically significantly different from all other *E. arguta* populations and is isolated from the rest of the species range by mountainous ridges of the Lesser Caucasus and Karabakh. Sczcerbak (1974) noticed the specificity of Georgian and Azerbaijani populations from eastern Transcaucasia and their differences from the Sevan form and considered them to be an intermediate form between *E. a. deserti* and *E. a. transcaucasica* without subspecific designation.

Our data suggest a monophyly of the Transcaucasian clade, but indicate a quite deep divergence between the Armenian (terra typical of *E. a. transcaucasica*) and the Azerbaijan populations ($p = 5.51\%$). Observed genetic distances between them indicate pronounced geographical isolation of the Sevan Lake population and an old differentiation in the Transcaucasian lineage of *Eremias arguta*. It is noteworthy, that there was an unsuccessful attempt to describe a subspecies from southeastern Azerbaijan: the name "*Eremias arguta lenkorana*" is regarded as a *nomen nudum* according to Sczcerbak (1974). Tentatively, we consider Armenian and Azerbaijan populations as one subspecies: *E. a. transcaucasica*, however, further sampling in central Azerbaijan and eastern Georgia is essential for a more detailed study of Transcaucasian evolutionary lineage of *E. arguta*; the status of the Azerbaijani population requires clarification.

Eremias arguta's range in eastern Transcaucasia encompasses the Apsheron Peninsula, middle and lower parts of the Kura River Valley penetrating to the west as far as the easternmost Georgia (Darevsky 1959). In this area the steppe racerunner inhabits stony and semidesert biotopes around 300–400 m a. s. l. The population of the southern and southeastern coast of the Sevan Lake (originally described as *E. a. transcaucasica*) occupies mountain steppe at ca. 2000 m a. s. l. This population is completely isolated from the main area of the species range possessing forest slopes within the Lesser Caucasus Mountains and Sevan Mountain ridge (ca. 3000 m a. s. l.). Assumingly, the species could colonize the Sevan Valley from the Kura River Valley since the Sevan Mountain ridge was higher than 1500 m a. s. l. having been formed during the Pleistocene (Varadanianz 1948; Darevsky 1959).

FIGURE 3. Major coloration patterns in *Eremias arguta*. A—banded form *Eremias arguta* "*potanini*" from Tokty river, Alakol valley, East Kazakhstan (photo by O. Belyalov); B—spotted form *Eremias arguta arguta* from Dzhezkazgan, central Kazakhstan (photo by A. Kornelyuk); C—ocellated form *Eremias arguta ssp.* from Konyrolen, Ili valley, East Kazakhstan (photo by O. Belyalov); D—ocellated form *Eremias arguta ssp.* from Kaindytau, Ili valley, East Kazakhstan, showing bluish ocelli (photo by O. Belyalov).

Clade C. Clade C is a sister-clade to the group joining clades A and B. These three clades together form a group of subspecies surrounding the Caspian Sea basin and their initial radiation is likely to be related to the Parathethys transgressions at the end of the pre-Pleistocene epoch (Rustamov & Szczerbak 1985; Anajeva & Tuniyev 1992; Popov *et al.* 2004).

Clade C occupies the northwestern part of the species range and is found in an area of the Steppes from southern Ukraine to west Kazakhstan as far as Dagestan to the south and corresponds well to the distribution of the subspecies *E. a. deserti*. This lineage is divided in two subclades. Clade C1 is found in the west – southern Ukraine and Crimean Peninsula to Northern Caucasus (Ciscaucasia) from Krasnodar territory to Dagestan. Clade C2 is distributed in the lower part of Volga Basin (from Saratov to Astrakhan Province) and western Kazakhstan. The revealed genetic diversity is in concordance with morphological variation (Sczcerbak 1993). Within the *E. a. deserti* range an east-west variation of several morphological (meristic and scaling) characters was reported (Sczcerbak 1966). Morphologically, the most diverse populations inhabit the Volga – Ural watershed area and Kalmykia (Okulova 1973; Kireyev 1977, Sczcerbak *et al.* 1993); some specific characters were revealed for the populations from Saratov province (Tabachishin & Zavyalov 1998). The easternmost locality of this lineage—Manat—is situated in the Ural River Basin, where the presence of the contact zone between *E. a. arguta* and *E. a. deserti* was assumed (Sczcerbak 1993).

The known distribution of *E. arguta* in the Caucasian Isthmus shows the break between *E. a. deserti* (C) and *E. a. transcaucasica* (B) from the Apsheron Peninsula in northern Azerbaijan to the Samur River delta in Dagestan, Russia. The same zoogeographic pattern is known for other reptile species, such as *Eremias velox, Phrynocephalus helioscopus* (Pallas) etc. (Darevsky 1957), in addition to several mammal species as well (Geptner 1938). This break corresponds to the foothills of the Great Caucasus and to the riverbed of Samur River, which might form a barrier for dispersal.

Clade D. This clade is sister to a monophyletic group joining all other *E. arguta* lineages and likely represents the most basal split within *E. arguta*. Clade D is restricted to the small territory of the Ili River Valley between Tien Shan and the Dzhungar Alatau Mountains from the Altyn-Emel Mountains in the west presumably to the Boro-Khoro Mountains (China) in the east (the extent of this lineage in China has to be confirmed by further studies).

This narrow area hosts quite a noticeable genetic diversity assuming an old isolation and stable demographic conditions of this population. Two well-supported subclades are present—the D1 occupies the areas on the right bank of the Ili River, whereas the D2 is found on the left bank of the river. Such differentiation is not surprising since the Ili riverbed is surrounded by swampy and wet areas with numerous tugai forests. Due to regular floods the riverbed often changes with many new streams and islands being formed every year (Korelov 1948). Moreover, along the river on both sides there are areas with loose sands, which are unsuitable for steppe racerunners, whose preferences are clay and stone deserts. Together these habitats might form a barrier for *Eremias arguta* dispersal. The specificity of the Ili River populations was also shown for other lizards in the region, such as toad-headed agamas *Phrynocephalus helioscopus cameranoi* (see Solovyeva *et al.* 2011, 2012) and *Ph. alpherakii* (Dunayev *et al.* 2009) (Agamidae), which are endemic to the Ili River valley. Climatic conditions in the Ili River Valley remained relatively stable during the Pleistocene (Korovin 1961; Markov *et al.* 1965; Alisov 1969), thus the valley likely served as a refugium of desert herpetofauna, which caused the observed endemism.

The population of *E. arguta* from the Ili River Valley in east Kazakhstan was previously recognized as *E. a. uzbekistanica* by Chernov (1934), or as an intermediate form between *E. a. uzbekistanica* and the nominative subspecies by Yakovleva (1964), or as *E. a. potanini* in the latest revision by Sczcerbak (1993). However, our data indicate that this population represents a separate mtDNA lineage, corresponding to the basal split within *E. arguta*. Morphological differences of this population are also significant (Orlova *et al.* 2012); dorsal pattern in this form is characterized by presence of bluish or green ocelli in breeding males (Dujsebayeva *et al.* 2007; see Figure 3C, D), a feature not observed in any other *E. arguta* populations. Currently, the taxonomic status of the D lineage has not been established, though both genetically and morphologically it represents a unique phylogenetic unit.

Clade E. This clade corresponds to the subspecies *E. a. darevskii*, morphologically closely related to *E. a. uzbekistanica*. Our mtDNA data confirm this assumption and indicate a sister relationships of clade E with the subspecies from Uzbekistan.

Clade E is restricted to the valley of the Issyk-Kul Lake in Kyrgyz Tien Shan Mountains. We examined two populations from the valley, which show no differences in mtDNA sequences. It is assumed that *E. arguta* populations of the Issyk-Kul valley are related to the populations from the Chu River valley, an important

colonization route for reptiles (Shnitnikov 1928; Zimina 1959; Sczcerbak 1993; Eremchenko & Panfilov 1998). However, Yakovleva (1964), based on morphological analysis of external morphological characters, argued that steppe racerunners most likely colonized the Issyk-Kul Valley from the southwest—through the Talass Valley and Fergana region in Uzbekistan. Morphologically, Ili Valley racerunners show some similarities with Issyk-Kul Valley subspecies (Yakovleva 1964; Orlova 1989, 2008); ecologically, these forms are also quite similar by preferring mountain semi-deserts at 1000-2000 m a. s. l. According to Yakovleva (1964) these similarities confirm Tarasov's (1958) opinion that faunas of Ili River and Issyk-kul Valleys were connected in the past and contradict Shnitnikov (1928) hypothesis on the colonization of Issyk-Kul Valley through the Chu River Valley along the Boom Gorge. Our data do not support the hypothesis of a common origin for Issyk-Kul and Ili steppe racerunners but indicate their basal position in the phylogenetic tree.

Clade F. This clade corresponds to the subspecies *E. a. uzbekistanica*. It is found in eastern Turan region—the southernmost part of the species range—from southern Uzbekistan along the foothills of Zeravshan and Nuratau mountain ridges to southern Kazakhstan in the north. This lineage shows the highest value of within-group genetic differentiation (p = 7.82%; which is higher than between-group distances for lineages A, B, C, see Table 3). High level of genetic differentiation between local populations is an indicative of the long history of the *E. arguta* in the Turan area. The territory of southern Kazakhstan and Turan is a probable area of autochthonous origin of the species. *Eremias a. uzbekistanica* and *E. a. darevskii* are morphologically quite distant from all other subspecies of the steppe racerunner (Sczcerbak 1974; Sczcerbak *et al.* 1993; Orlova *et al.* 2012).

In the present paper we analyzed just two populations of *E. a. uzbekistanica*—one (F2 lineage) from the extreme southern range of the species (Kyzylcha, Kashkadarya Province of Uzbekistan) on the southern side of Nuratau Mountains, and the second population (F1 lineage, Aidarkul Lake) north to this mountain range. The mountains of Nuratau and Zeravshan might be the barrier separating subclades F1 and F2; however, additional sampling from Uzbekistan is essential for further clarification of differentiation within the clade F.

Taxonomic implications of the mtDNA data. Our preliminary mtDNA data indicate comparatively deep divergence within the *E. arguta* species complex. Divergence among the revealed *E. arguta* clades is comparable or even higher to those in other genera of Lacertidae (see Fu 1998; Kalyabina-Hauf & Ananjeva 2004; Harris *et al.* 2002; Carranza *et al*. 2004). Our preliminary results have the following taxonomic implications:

1) The Uzbekistan subspecies, *E. a. uzbekistanica*, is the most distinct taxon within *E. arguta*.
2) Validity of *E. a. darevskii* is supported by mtDNA sequence data.
3) Forms, possibly related with its origin to the Caspian Basin area (*E. a. arguta, E. a. deserti, E. a. transcaucasica*), form a monophyletic group.
4) Transcaucasian populations form a clade tentatively joined under the name *E. a. transcaucasica*.
5) Deep differentiation within *E. a. uzbekistanica* and *E. a. transcaucasica* clades should not be considered as a basis for any taxonomical conclusions prior to a detailed study of morphological and molecular geographical differentiation of these forms at a population level.
6) From the mtDNA perspective, there are no reasons for subspecific status of the Dzhungarian form, *E. a. potanini.* Molecular variation does not coincide with morphological variation and shows clinal pattern and assumes relatively recent dispersal from central Kazakhstan to the east (Dzhungaria). Thus, populations of *E. arguta* from western China and Mongolia should be regarded as the nominate form, *E. a. arguta.*
7) A population from the Ili River Valley represents an unnamed taxon, which status is supported by both morphological specificity and molecular methods. Its taxonomic status will be discussed in detail elsewhere.

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