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The Stummer's Racerunner (*Eremias stummeri* Wettstein, 1940) does occur in Northwest China

Jinlong Liu[1], Tatjana N. Dujsebayeva[2], Marina A. Chirikova[2], Qi Song[1], Xiong Gong[1], Xianguang Guo[1,*]

Abstract. The status of *Eremias stummeri* in China has been a matter of controversy for over two decades, although it is well acknowledged that this species occurs in Northeast Kyrgyzstan and Southeast Kazakhstan. To date, whether its occurrence extends to the adjacent Chinese region (possibly in the Ily River Valley) has not been confirmed yet. To overcome constraints on field surveys imposed by the region's remoteness and rugged terrain, we targeted areas of further field inventories by estimating the potential distribution of the species. Ecological niche modeling (ENM) enabled us to predict a continuous distribution area from Kazakhstan to the Chinese part of the Ily River Valley in Zhaosu county for the species' occurrence. We conducted extensive field surveys in the predicted area. In 2017, for the first time, the occurrence of *E. stummeri* in China was confirmed with a single find in Zhaosu county. Further morphological and multilocus phylogenetic analyses congruently supported the taxonomic status of the recently discovered population as *E. stummeri*. In contrast to its distribution continuity between Kazakhstan and China, the ENM indicates distribution discontinuity between Kazakhstan and Kyrgyzstan. This geographic distribution pattern of *E. stummeri* is supported by morphological and molecular evidence, which highlights that individuals from China and Kazakhstan are more closely related to each other than to those from Kyrgyzstan. The mismatch between mitochondrial and nuclear phylogeographic pattern implies that historical mitochondrial introgression occurred from *E. stummeri* individuals from Kyrgyzstan to those from Kazakhstan.

Keywords: distribution range, *Eremias multiocellata* complex, lizard, population discovery, Tianshan Mountains.

Introduction

The Stummer's Racerunner, *Eremias stummeri* Wettstein, 1940, is one of the species in *Eremias multiocellata* complex. Its terra typica lies near Karakol (Issyk-Kul Basin) in Northeast Kyrgyzstan, and initially this species was assigned to an oviparous species, i.e. a subspecies of *Eremias velox* (Wettstein, 1940). Szczerbak (1974) revised its status to the viviparous multiocellated racerunner, but taxonomically classified it as a synonymy of *Eremias multiocellata yarkandensis*. However, based on thorough morphological studies on *E. multiocellata* complex in Northeast Kyrgyzstan, the Stummer's Racerunner was proposed as a new subspecies of multiocellated racerunner, i.e. *E. m. stummeri* (Eremchenko et al., 1992). The authors suggested for the first time that this subspecies in Northeast Kyrgyzstan should possess geographic range connecting to that of "*E. multiocellata*" in the Narinkol River Valley (Paraskiv, 1956) in extreme Southeast Kazakhstan (ESK in this paper) and further extending eastward well into the Chinese part of the Ily River Valley (CPIRV in this paper). A few years later, the subspecies status of Stummer's Racerunner was elevated as an independent species through long-term hybridization experiments (Eremchenko and Panfilov, 1999). The population of *E. multiocellata* complex was rediscovered in Central Tianshan Mountains in ESK (Dujsebayeva et al., 2009), but was not allocated as *E. stummeri* because of its morphological peculiarities. Orlova et al. (2016) confirmed that the Kazakhstan records are indeed conspecific with *E. stummeri* through comparative morphological analysis.

In this way, *E. stummeri* is a typical high mountain dwelling lizard with elevation no less

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1 - Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China
2 - Institute of Zoology of Republic of Kazakhstan, Almaty 050060, Kazakhstan
*Corresponding author; e-mail: guoxg@cib.ac.cn

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than 1600 m above sea level (a.s.l.) (Eremchenko et al., 1992; Orlova et al., 2016). Its narrow distribution range is restricted in Central Tianshan Mountains along the Issyk-Kul Basin to the Ily River Valley, and its habitats in these areas are characterized with diverse alpine meadow with gravel or soil clay (Dujsebayeva et al., 2009; Orlova et al., 2016). Given the significant similarities of the habitats among the areas in CPIRV and the occurrence localities of *E. stummeri* in Kazakhstan and Kyrgyzstan, the discovery of *E. stummeri* in ESK has raised the possibility of occurrence of this species in the adjacent CPIRV.

The status of *E. stummeri* in China has been a matter of controversy for over two decades, although it is known that this species occurs in Kyrgyzstan and Kazakhstan. On the one hand, this species has not been listed in China's herpetofauna until recently (Guo et al., 2010; Cai et al., 2015). Sindaco and Jeremčenko (2008) reported it from the Ily River Valley in immediately adjacent China. Neither Zhao and Adler (1993) or Zhao et al. (1999) nor Dujsebayeva et al. (2009) referred to any records from China. This is part of a very complex group, and it is possible that Chinese records refer to a related species. Guo et al. (2010) reviewed the systematics of the genus *Eremias*, and argued that *E. stummeri* should occur in China by following the perspective of Eremchenko and Panfilov (1999). Subsequently, Cai et al. (2015) agreed with Guo et al. (2010) regarding the systematics of *Eremias* in the updated checklist of reptiles of China. On the other hand, until recently, the field collection of *E. stummeri* in China has been lacking yet. This is in part due to remoteness and rugged terrain of the geographic setting in CPIRV. As summarized by Song et al. (2014), the Ily Basin is a Mesozoic-Cenozoic fault-bounded depression surrounded by the Tianshan orogenic belt with an average elevation exceeding 3500 m a.s.l. The Ily River originates on the northern slope of Khan Tengri Peak (6995 m a.s.l.), flows through the Ily Basin into Kapchagay Reservoir and finally westwards into Balkhash Lake.

Recently developed ecological niche modeling (ENM) approaches have been successfully employed to model the potential geographic distribution of diverse taxa (e.g. Joyner et al., 2010; Nazeri et al., 2014; Bobrowski et al., 2017). Of these, presence-only methods (e.g. Maxent and GARP) requiring a set of known occurrence data and correlated environmental variables at those locations make them in a relatively easy and concise way describe and delimit geographic ranges of species distributions. Many studies have employed these predictions to conduct field surveys to locate new populations, especially for those species with restricted distribution and sparse populations (e.g. Bourg et al., 2005; Siqueira et al., 2009; Menon et al., 2010). These efforts can have great contributions to biodiversity conservation management and evaluation (e.g. Zhang and Zhang, 2014), especially for rare, endangered and poorly known species (Menon et al., 2010). Moreover, ENMs themselves can be considered as independent evidence to evaluate or develop phylogeographic hypotheses about the processes generating patterns of diversification in disparate taxa (Alvarado-Serrano and Knowles, 2014).

In this study, we utilized ecological niche modeling to explore the potential distribution of *E. stummeri*. Then, we conducted extensive field surveys in the predicted areas in CPIRV to verify previous speculation on the occurrence of *E. stummeri* in China. Subsequently, morphological and molecular data were used to confirm the lizards collected to be *E. stummeri*. Finally, this evidence was used to understand the diversification of this species in its distribution range.

Material and methods

Ecological niche modeling

We conducted ENM analysis using MAXENT v3.4.1 (Phillips et al., 2006; Phillips and Dudík, 2008). Nineteen bioclimate variables representing present period at a resolution of 30 arc-seconds were retrieved from the WorldClim database, version 1.4 (http://www.worldclim.org; Hijmans et al., 2005). Because strong colinearity between environmental variables could inflate model accuracy (Synes and Osborne, 2011), we excluded those variables with Pearson correlation coefficients $r > 0.85$ based on pairwise comparison of raster files in SDMtoolbox v1.1c (Brown, 2014). Nine variables were retained for subsequent analysis: Bio1 (Annual Mean Temperature), Bio3 (Isothermality), Bio7 (temperature Annual Range), Bio8 (Mean Temperature of Wettest Quarter), Bio9 (Mean Temperature of Driest Quarter), Bio12 (Annual Precipitation), Bio15 (Precipitation of Seasonality), Bio17 (Precipitation of Driest Quarter), and Bio18 (Precipitation of Warmest Quarter). Georeferenced occurrence records were obtained from our field surveys in Kazakhstan and Kyrgyzstan as well as recent literature (Jablonski and Koleska, 2017), and the coordinates of old records (Eremchenko et al., 1992; Eremchenko and Panfilov, 1999) were restored using topographic maps of scale 1:200 000 (http://sasgis.ru/sasplaneta). To ensure that input occurrence data are spatially independent and to reduce sampling bias, all sampling localities were also rarefied at a spatial distance of 10 km using SDMtoolbox in ArcGIS v9.1 (Esri, Redlands, CA, USA). The rarefying procedure was implemented three times independently. Thus, 35 occurrence points were retained for the following modeling analysis (fig. 1A, supplementary table S1).

To account for sampling bias, we followed the methods used in Mizsei et al. (2016). We delimited the background areas for model training according to the known occurrences of *E. stummeri* in three provinces in Kyrgyzstan (including Chuy, Naryn and Issyk-Kul) and Raiymbekskiy district in Kazakhstan (fig. 1A). Ecological niche models were first built in the areas of Central Tianshan Mountains in Kyrgyzstan and Kazakhstan (i.e. the background areas); then projected to southwestern CPIRV in China (including three counties of Zhaosu, Tekes and Gongliu; fig. 1A). We conducted Maxent analyses using the default settings except employing 75% of the species records for training and 25% for testing the model, 100 bootstrap replicates and the fade by clamping function. We compiled information on two measures of model performance, including the area under the curve (AUC) of the receiving operator characteristics (ROC) for the test data and the true skill statistic (TSS; Allouche et al., 2006) for each algorithm. The AUC value is the probability that the model would rank a randomly chosen presence observation higher than the randomly chosen absence observation. Swets (1988) classified values of AUC as follows: those > 0.9 indicated high accuracy (excellent), from 0.7 to 0.9 indicated good accuracy, and those < 0.7 indicated low accuracy. The TSS, a threshold-dependent statistics, is presented as an improved measure of model accuracy that, unlike the common kappa statistics (Allouche et al., 2006), is not dependent on species prevalence (i.e. proportion of occurrence points for which the species is present). Acceptable models are those with at least a TSS of 0.5, and excellent those with TSS around 0.7. The TSS was calculated with four possible prediction results of the model: true positive (TP), false positive (FP); true negative (TN) and false negative (FN), where TP and TN were related to the threshold that discriminates presence and absence. The average of logistic thresholds of ten percent training presence, which was generated in the Maxent output, was used to define the minimum probability of suitable habitat.

Sampling and morphological analyses

With extensive fieldwork conducted during 2017 in potential distribution areas in the Ily River Valley, Xinjiang, China where suggested by our ENM results, individuals from a single population in Zhaosu county belonging to *E. multiocellata* complex have been discovered. Further morphological examination indicated that the characters of these individuals were consistent with the diagnostic descriptions of *E. stummeri*.

Moreover, in order to investigate the potential differentiations among the populations of *E. stummeri* from three different countries (i.e. China, Kazakhstan, Kyrgyzstan), the morphological measurement has been conducted on 15 individuals (11 ♂♂, 4 ♀♀) from the population in China, which were preserved as voucher specimens and deposited in the herpetological collection of the Chengdu Institute of Biology. Through international collaborations, we also conducted morphological measurement on 4 (1 ♂, 3 ♀♀) and 8 (4 ♂♂, 4 ♀♀) individuals of *E. stummeri* from Kyrgyzstan and Kazakhstan, respectively (see table 1).

Animals were euthanized with an overdose of sodium pentobarbital via intraperitoneal injection, and liver tissues were extracted and preserved in 95% ethanol following animal use protocols approved by Chengdu Institute of Biology, Chinese Academy of Sciences. Morphological analyses were conducted on 11 metric and 15 meristic characters (supplementary table S2). One female specimen from the population in China was considered as juvenile because of its snout-vent length (SVL) less than 40 mm. Metric measurements were conducted using digital caliper, and recorded to two decimal places. All one side measurements were performed on the left side body of individuals.

Multivariate morphometric methods were employed using correlation based principal component analysis (PCA) on our data matrices. Metric and meristic traits were analyzed in separate PCA due to the lack of correlation between these two trait types. Sexual dimorphism was tested separately for individuals from populations in China and Kazakhstan, except Kyrgyzstan due to only a single male individual. An analysis of variance (ANOVA) was conducted on both the metric and meristic dataset to test sexual dimorphism. Only two metric and one meristic traits ($p < 0.05$; supplementary table S2) in individuals from Kazakhstan exhibited sexual dimorphism, which is consistent with the results in Dujsebayeva et al. (2009) and Orlova et al. (2016). However, most metric and only three meristic traits ($p < 0.05$; supplementary table S2) in individuals from China were observed with sexual dimorphism. As a result, metric data collected from juvenile specimens and the single male from Kyrgyzstan were not included in the PCA, and data collected from male and female specimens were analyzed in separate PCA. Moreover, all individuals including one juvenile were included into a single PCA analysis in the meristic dataset. All morphometric analyses were performed with SPSS 19 (SPSS Inc., Chicago, USA).

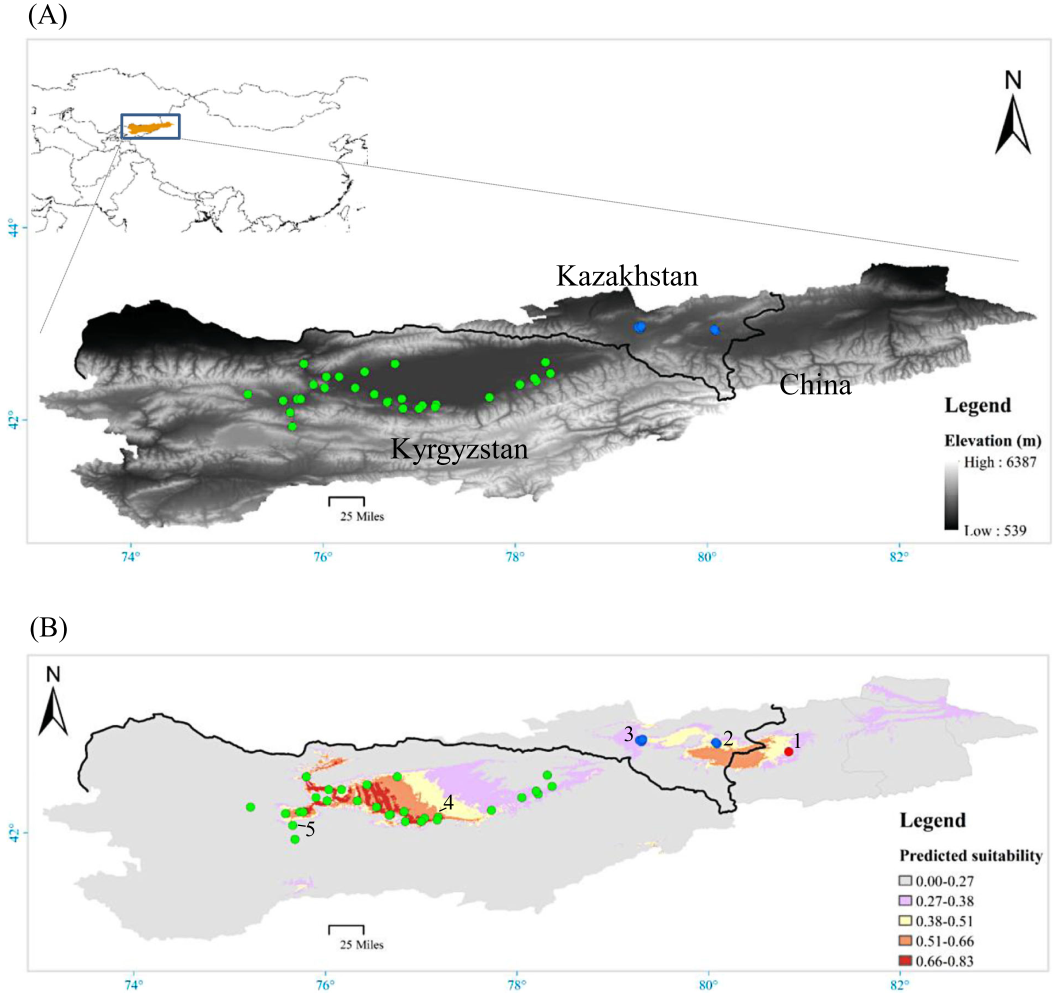

Figure 1. The map inserted in the upper left corner indicates our studying area with goldish color; the magnified areas and the presence points in Kyrgyzstan and Kazakhstan were used for model training and the magnified area in China for model projection; green and blue circles represent presence points in Kyrgyzstan and Kazakhstan, respectively (A). Modeled geographic distribution of *E. stummeri* in Kyrgyzstan, Kazakhstan and China; red regions indicate higher environmental suitability; the average value of ten percentile training presence logistic threshold is 0.2722; red circle indicates our field record of *E. stummeri* in Zhaosu county, Xinjiang, China; sites in this study are numbered as in table 1 (B).

Molecular data collection and phylogenetic analyses

Genomic DNA was extracted from liver tissues stored in 95% ethanol using the popular high-salt protocols (Aljanabi and Martinez, 1997). We amplified one mitochondrial gene (cytochrome c oxidase 1 [*COI*]) and one nuclear gene (microtubule associated protein 1A [*MAP1A*]) fragments. The primers were designed according to Ward et al. (2005) and Portik et al. (2012) with minor modifications (supplementary table S3). Each of our 25 μl PCR reactions contained 12.5 μl of 2 × EasyTaq SuperMix (Tsingke BiolTech, Chengdu, China), 0.2 μM of each primer, and 1-2 μl genomic DNA. The PCR protocol involved initial denaturation at 94°C for 4 min followed by 35 cycles of 94°C for 30 s, 52°C for 30 s (55°C for *MAP1A*), and elongation at 72°C for 50 s (105 s for *MAP1A*); and final extension at 72°C for 10 min. All PCR products were commercially purified and sequenced for double strands with the primers used for amplification. All novel sequences are deposited in GenBank with accession numbers MN524184-MN524221 (see table 1).

Fifteen individuals from China, eight from Kazakhstan and four from Kyrgyzstan were sequenced for *COI* gene segments. To verify and complement the mitochondrial data, at least one individual from each population was sequenced for *MAP1A* gene (table 1). Both protein-coding genes were translated to amino acids using MEGA v7.0.26 (Kumar et al., 2016) and no stop codons were observed.

Table 1. List of analyzed specimens, sex, geographic origin and GenBank accession number.

Voucher No.	Sex	Site No.	Locality	Longitude	Latitude	Accession number	
						COI	*MAP1A*
Guo6384	♀	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524196	-
Guo6503	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524197	-
Guo6504	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524198	-
Guo6505	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524199	-
Guo6506	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524200	-
Guo6507	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524201	-
Guo6508	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524202	-
Guo6509	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524203	-
Guo6510	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524204	-
Guo6511	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524205	-
Guo7251	♀	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524206	-
Guo7477	♀	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524207	MN524215
Guo7478	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524208	-
Guo7481	♂	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524209	MN524216
Guo7484	♀	1	Zhaosu, Xinjiang, China	80.84	42.84	MN524210	
KZG080	♂	2	Tekes River Valley, Kazakhstan	80.09	42.93	MN524195	MN524221
KZ5	♂	3	Kegen River Valley, Kazakhstan	79.28	42.96	MN524188	MN524217
KZ6	♀	3	Kegen River Valley, Kazakhstan	79.28	42.96	MN524189	MN524220
KZ51	♂	3	Kegen River Valley, Kazakhstan	79.28	42.96	MN524190	MN524218
KZ52	♂	3	Kegen River Valley, Kazakhstan	79.28	42.96	MN524191	MN524219
KZ53	♀	3	Kegen River Valley, Kazakhstan	79.28	42.96	MN524192	-
KZ54	♀	3	Kegen River Valley, Kazakhstan	79.28	42.96	MN524193	-
KZ55	♀	3	Kegen River Valley, Kazakhstan	79.28	42.96	MN524194	-
Guo4721	♂	4	Kaji-Say, Kyrgyzstan	77.17	42.16	MN524184	MN524211
Guo4723	♀	5	Kok-Dzhar, Kyrgyzstan	75.66	42.08	MN524185	MN524212
Guo4724	♀	5	Kok-Dzhar, Kyrgyzstan	75.66	42.08	MN524186	MN524213
Guo4725	♀	5	Kok-Dzhar, Kyrgyzstan	75.66	42.08	MN524187	MN524214

All sequences of *COI* gene fragment used in Orlova et al. (2017) were retrieved from GenBank. Two loci were aligned separately using Clustal X v2.0 (Larkin et al., 2007). No indel was found in both gene matrices. The final dataset contained 651 bp *COI* and 1428 bp *MAP1A* gene fragments. For *MAP1A*, we phased it using the PHASE algorithm (Stephens et al., 2001) in DnaSP v5.0 (Librado and Rozas, 2009), and retained haplotypes with posterior probabilities greater than 0.9. Identical sequences for *COI* were collapsed into a single haplotype using DnaSP.

PartitionFinder v1.1.1 (Lanfear et al., 2012) was employed to select the best partitioning strategy and the optimal nucleotide substitution model for mitochondrial dataset which first was separated into three codon positions using the Bayesian information criterion (BIC) (Schwarz, 1978). Three partitions and their respective model of DNA evolution have been proposed: 1st codon with $K80 + G + I$, 2nd with F81, 3rd with $GTR + G$. Bayesian inference analysis was conducted with MrBayes v3.2.2 (Ronquist et al., 2012). Two independent runs were carried out with four Monte Carlo Markov chains (MCMCs) for two million generations with parameters and topologies sampled every 200 generations. Each run consisted of three heated chains and one cold chain, with an incremental heating temperature of 0.01. Convergence of the runs was assessed by average split frequency standard deviation lower than 0.01 and the effective sample size (ESS) $\geqslant 200$ evaluated with Tracer v1.7.1 (Rambaut et al., 2018). A 50% majority-rule consensus tree and posterior probability (PP) of clades were assessed by combining the sampled trees from the two independent runs after a 25% burn-in phase.

The median-joining network (MJN) reconstruction method (Bandelt et al., 1999) was conducted in Network v5.0.1.1 (Fluxus Technology, www.fluxus-engineering.com) with all the *COI* and *MAP1A* sequences of *E. stummeri* to evaluate the genealogies of the haplotypes and their relatedness with their geographic origin, respectively. Geographic subareas were defined according to the three different countries where individuals inhabited.

Results

Ecological niche modeling

Our distribution model indicated a better than random prediction with high AUC and TSS values, 0.95 and 0.81 respectively. The predicted distribution of *E. stummeri* covered the most part of the areas near the shores around the Issyk lake in Kyrgyzstan and ESK in the Ily River

Valley (fig. 1B), which fits well with the known occurrences. However, the predicted areas in Kyrgyzstan and Kazakhstan were separated at Kungey and Terskey intermountain depression crossed by two larger rivers – Tyup and Karkara near the border of these two countries. More importantly, our models suggested the continuous distribution from Kazakhstan into the large part of the areas of Zhaosu county and the separate distribution around the edge of the Tianshan Mountains in Tekes and Gongliu counties in CPIRV (fig. 1B).

Morphological description

The preliminary qualitative present/absent traits and certain stable meristic traits including the scalations and dorsal patterns (see fig. 2A) have been used as diagnostic traits to identify the individuals from the population in Zhaosu county as *E. stummeri*. Furthermore, these traits exhibited no sexual dimorphism. As a result, we gave a general description of these traits irrespective of sex in supplementary table S4.

Morphometric analyses

Our original morphometric data were given in supplementary table S5. The results of PCA of the metric dataset showed that both of the first two principal components account for 95.27% and 87.13% of the observed variation in respective males and females (supplementary table S6). Based on the factor loadings, PC1 represented a measure of body size (all metric traits positively correlated with high loads) for both males and females, whereas PC2 represented a measure of body length (positively correlated with moderate loads) and tail length (negatively correlated with moderate loads) and distance between femoral pore rows (Dist.P.fm.; positively correlated with extremely high loads) for female and male, respectively. The plots of PC1 and PC2 scores for male and female individuals revealed quite different scenarios (fig. 3A, B). In males, individuals from China and Kazakhstan showed apparent morphological differentiation along the PC2 scores, which indicated that the differentiation between individuals from China and Kazakhstan is most relevant to the character of Dist.P.fm (distance between femoral pore rows), as individuals from Kazakhstan possessed wider distance between femoral pore rows. However, in females, individuals from China and Kazakhstan appeared little differentiation, whereas individuals from Kyrgyzstan showed significant differentiation from individuals from China and Kazakhstan along the PC1 scores, which indicated that the individuals from Kyrgyzstan usually have smaller body size.

Five principal components explained 70.35% of the total variation in the PCA of meristic dataset (supplementary table S7). The plots of first three principal components showed a similar geographic pattern that the overlaps in morphology decreased in individuals from China to Kazakhstan, then to Kyrgyzstan. Most apparent differentiation among the individuals from the three different countries was exhibited along the PC2 scores, which was positively associated with higher loads for number of scales between femoral pore rows (Sc.p.fm.) and modest loads for number of infralabials (Infralab.) and negatively associated for modest loads for number of collar scales (Coll.) (fig. 3C, supplementary fig. S1).

Phylogenetic relationships

Our Bayesian phylogenetic tree is highly congruent with that in Orlova et al. (2017) regarding the relationships in *E. multiocellata-E. przewalskii* species complex. As shown in fig. 4, three major clades (I-III) were recovered. In accord with Orlova et al. (2017), the relationships among the three clades were still unresolved. *E. stummeri* was recovered as the sister species to *E. szczerbaki* albeit with moderate support (PP = 0.81). More importantly, the specimens examined in this study were nested within *E. stummeri*, which further supported their taxonomic status identified by morpholo-

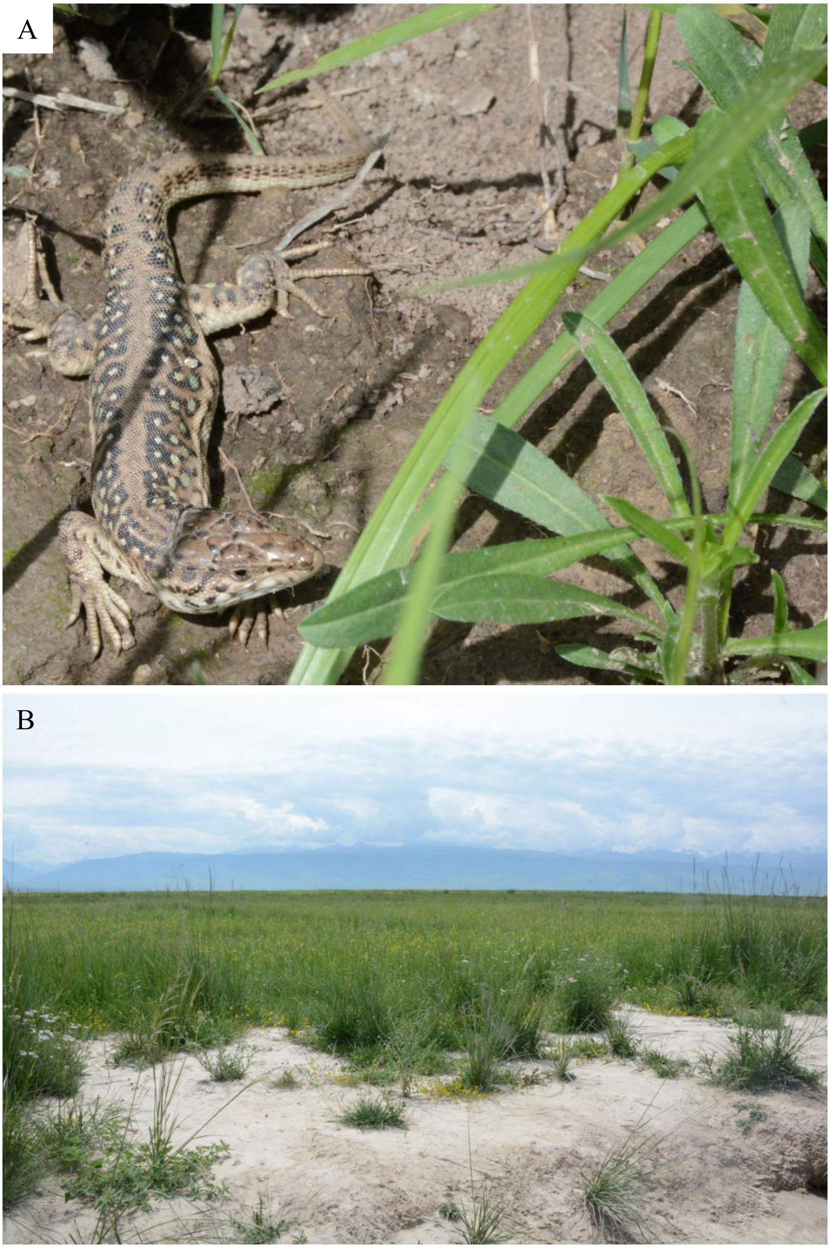

Figure 2. Specimen of *E. stummeri* in life (A) and biotope (B) of our field record in Zhaosu county, Xinjiang, China.

gical characters. However, there was apparent differentiation associated with the formation of three subclades within *E. stummeri* (fig. 4). Subclade A included all individuals from China, and subclade B represented most individuals from populations in Kazakhstan, whereas subclade C consisted of individuals from Kyrgyzstan and some from Kazakhstan. The relationships among the three subclades were unresolved with low support (PP = 0.65).

Moreover, MJN analysis showed that all of the *COI* sequences from specimens in Zhaosu

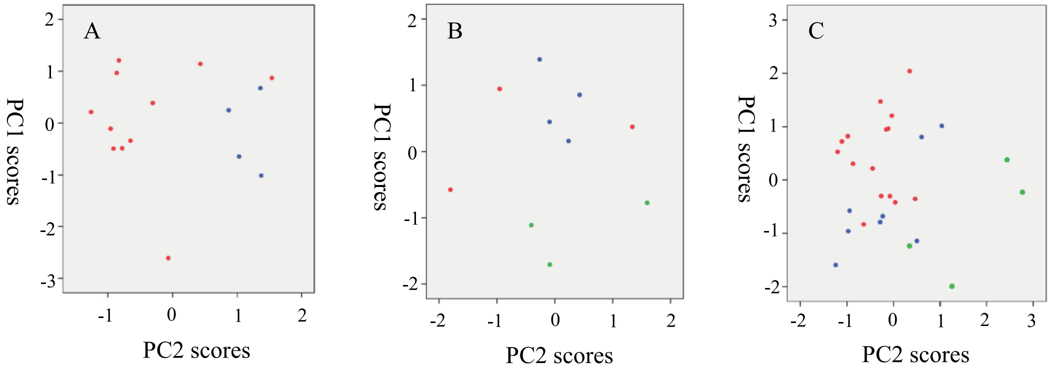

Figure 3. Morphometric analyses of the 11 metric (A and B) and 15 meristic (C) traits. Colored circles correspond to individuals from Kyrgyzstan (green), Kazakhstan (blue) and China (red). Projection of PC1 and PC2 scores from PCA analysis. A: all male specimens; B: all female specimens; C: all specimens.

county, China shared one unique haplotype (subclade A), which was closely related to subclade B. The group comprising individuals from China and most from Kazakhstan was at least ten substitutions from subclade C (fig. 5A).

Consistent with the mtDNA results, the MJN based on nuclear dataset indicated that individuals from China shared a haplotype with some individuals from Kazakhstan. All the genotypes from Kyrgyzstan formed a separate cluster, which was at least seven substitutions from the group comprising the genotypes from China and Kazakhstan (fig. 5B).

Discussion

Eremias stummeri has been classified by IUCN as "Least Concern" (Orlova et al., 2017) on the basis that this species is found in a moderately wide area of desert where no major threats exist; and at least in part of its range, available data suggests that the population has been stable for more than 50 years. Recent taxonomic work has led to a range extension into Kazakhstan (Orlova et al., 2016), suggesting that this species might be more widespread than presently recognized. Indeed, *E. stummeri* has been expected a possible distribution in CPIRV for nearly two decades (Eremchenko et al., 1992; Sindaco and Jeremčenko, 2008). This speculation was confirmed by a sophisticated treatment that incorporated ecological niche modeling with morphological and molecular data under the rubric of integrative taxonomy in this study.

The results of our ENM guided us to survey the predicted area in Zhaosu county, and led to the discovery of a new population of *E. stummeri*. While this work was being prepared for publication, Dujsebayeva et al. (2019) reported ENM of *E. stummeri* using ArcGIS with expanded set of the relief and climatic variables. Their niche-based GIS modeling pointed to the Tekes Valley in China as still relatively suitable habitats for *E. stummeri* but located in its easternmost ecological limits. As mentioned in Dujsebayeva et al. (2019), their suggestion was verified by the recent find of *E. stummeri* in the rangelands with pasture and *Achnatherum splenderns* in Zhaosu county. Overall, the potential distribution range of *E. stummeri* suggested by Dujsebayeva et al. (2019) was consistent with that in our ENM developed by MAXENT with key bioclimatic variables.

Only a single population of *E. stummeri* has been discovered in Zhaosu county in CPIRV up to present, although our ENM analysis suggested a relatively wider potential distribution range of this species in Zhaosu county in this region. The habitat of this population is characterized by soft clay soils with dense vegetation of

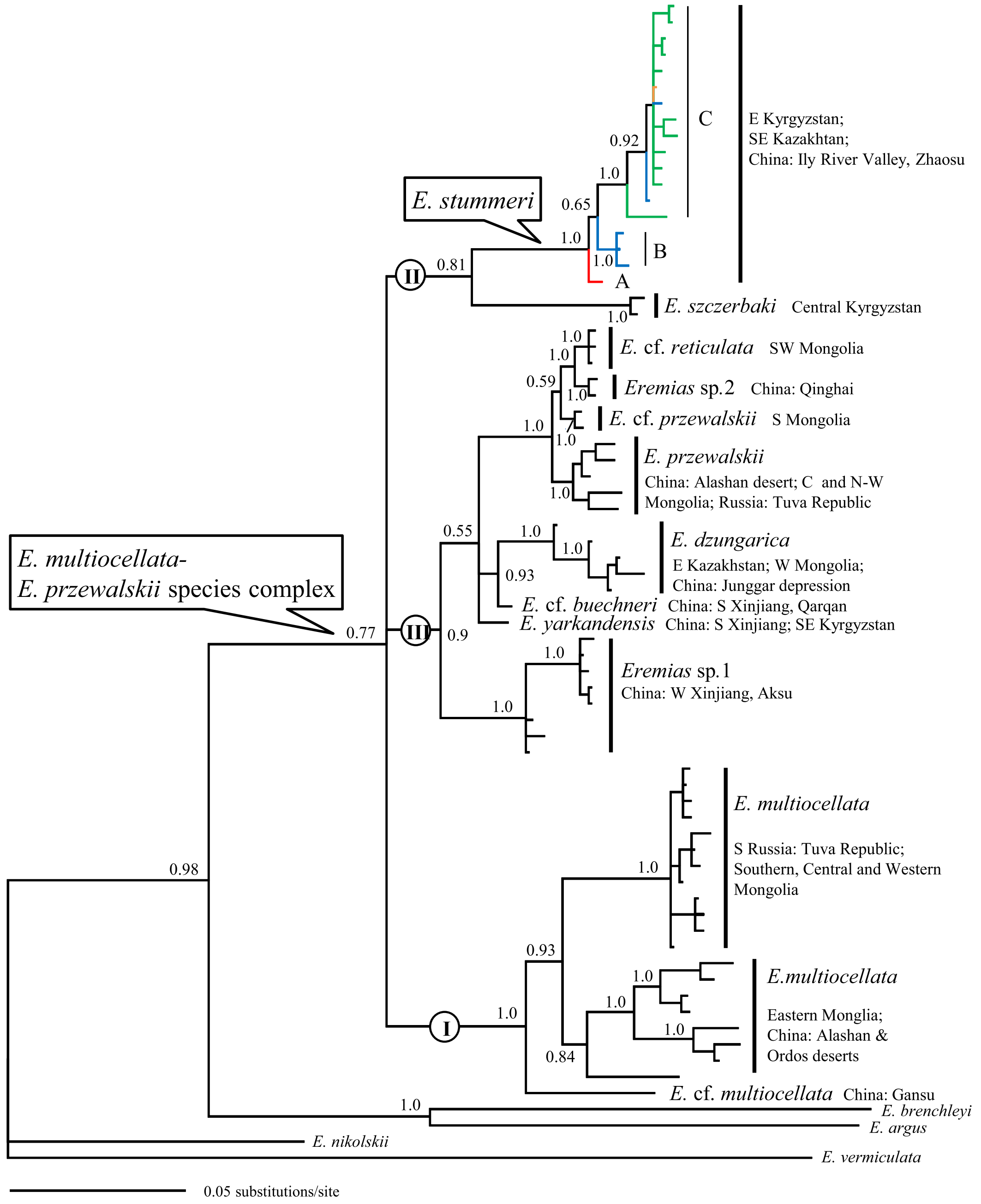

Figure 4. The 50% majority-rule consensus tree of *E. multiocellata-E. przewalskii* complex resulting from partitioned Bayesian analyses based on mitochondrial *COI* haplotypes. Numbers on branches indicate posterior probabilities (PP). Three major clades (I-III) in the tree correspond to those in Orlova et al. (2017). Colored branches within *E. stummeri* correspond to individuals from Kyrgyzstan (green), Kazakhstan (blue), China (red) and Kyrgyzstan and Kazakhstan (orange).

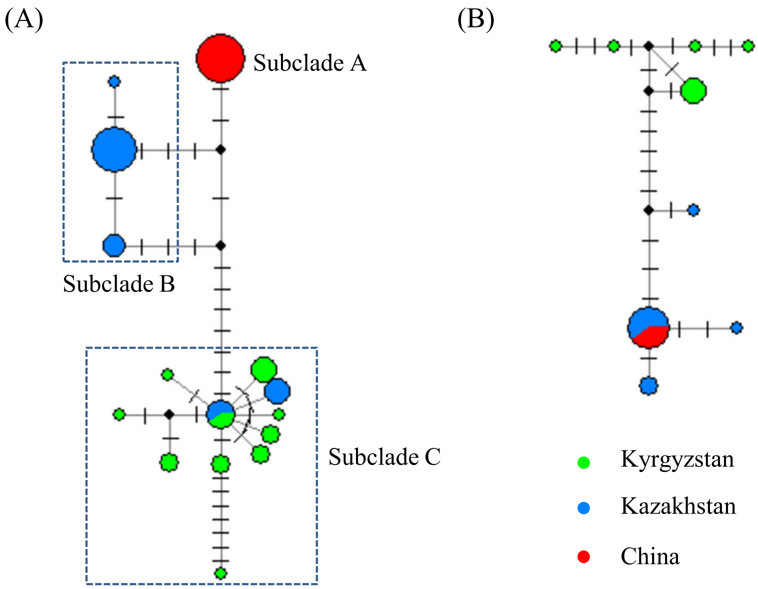

Figure 5. Median-joining network of *E. stummeri* based on mitochondrial (A) and nuclear (B) haplotypes. Short bars crossing network branches indicate mutation steps; small dark circles indicate median vectors inferred by NETWORK software. Circle size corresponds to relative numbers of individuals sharing a particular haplotype. Different filled colors represent the corresponding geographic origin, as given below the network.

Festuca-Achnatherum steppes (fig. 2B), which is different from those records from stony and gravel desert with sparse bushes in Kazakhstan and Kyrgyzstan (Dujsebayeva et al., 2009). During our field investigation around these areas, we found that most of the areas had been transformed to farmland and pastureland. These situations have led us to presume that habitat fragmentation and losses may have caused the extensive decline of populations of *E. stummeri* in these areas. It is necessary to further evaluate the genetic diversity and demography of this species, with particular attention to the population in CPIRV. Whatever the result of future research, our data suggests that it is urgent for our relevant Chinese authorities to pay attention to and protect the existence of this species in China.

The predicted distribution range of *E. stummeri* indicated a continuous distribution from Kazakhstan to China, whereas a discontinuous distribution from Kyrgyzstan to Kazakhstan. Dujsebayeva et al. (2009) once suggested that the connection between the populations from Kazakhstan and Issyk-Kul area in Kyrgyzstan was impossible, because the unsuitable marshy habitats in the Kungey-Terskey intermountain depression separated the two areas. As expected, the distribution separation between Kazakhstan and Kyrgyzstan was also supported by our morphological and molecular data, so was the continuity between China and Kazakhstan. Our morphological analyses suggested that the individuals from Kazakhstan and China had little differentiation, but were more differentiated from individuals from Kyrgyzstan. On the one hand, the results of PCA of metric traits may be biased, as all of females from Kyrgyzstan are apparently smaller (SVL less than 50 mm) than those from Kazakhstan and China, which may lead values of metric traits correlated with SVL to be relatively low either. Eremchenko et al. (1992) documented larger and wider values of SVL in females from Kyrgyzstan (47 specimens, mean SVL ± SE, 53.1 ± 0.54 mm). On the other hand, Orlova et al. (2016) noted the statistically significant differences between populations from Kazakhstan and Kyrgyzstan by signs of body length and the number of femoral pores (P.fm.). Accordingly, this evidence points out that the differentiation between individuals from Kyrgyzstan and China and Kazakhstan may be related to the size of the body. Although the sample size of populations from Kyrgyzstan is quite small (totally four individuals), the results of PCA of meristic traits still indicated that individuals from Kyrgyzstan apparently diverged from individuals from China and Kazakhstan. Most of apparent differentiations are associated with loading scores on PC2, which indicated the differentiation of individuals from Kyrgyzstan may be related to larger number of scales between femoral pore rows and number of infralabials and lesser number of collar scales.

In mtDNA dataset, the results from MJN of *E. stummeri* suggested that individuals from China were more differentiated from individuals from Kyrgyzstan. This evidence coincided well with the results from the uncorrected pairwise distance (*p*-distance) among the populations from our three focused countries, with the lowest differentiation (*p*-distance of 1.3%) between the populations from China and Kazakhstan, moderate differentiation (*p*-distance of 1.5%) between those from Kazakhstan and Kyrgyzstan, and the highest differentiation (*p*-distance of 2.0%) between those from China and Kyrgyzstan. Moreover, in the nuclear dataset, the relationships among the genotypes from the three different countries explicitly indicated that individuals from Kyrgyzstan were differentiated from those from China and Kazakhstan (see fig. 5B). On the one hand, the fact that the mitochondrial haplotypes in some individuals from Kazakhstan are clustered and even shared with those individuals from Kyrgyzstan to form subclade C (see figs 4 and 5A) seems to contradict above morphological and molecular suggestions. On the other hand, the mismatch between mitochondrial and nuclear phylogeographic pattern prompts us to speculate that the haplotypes from Kazakhstan more closely related to those from Kyrgyzstan may be possessed by historical mitochondrial introgression from individuals from Kyrgyzstan to those from Kazakhstan. However, incomplete lineage sorting may explain the retained polymorphisms in the diverged populations; this is probably not valid in our case in consideration of the apparent divergence between individuals from Kazakhstan and Kyrgyzstan in nuclear data and the more rapidly evolving mitochondrial gene that did not coalescence at the level of divergence between them. Future work for *E. stummeri* may require more extensive sampling, especially in Kyrgyzstan, and obtain more refined molecular data, such as microsatellite or population genomic data to address the questions related to introgression, speciation, and other topics.

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Ethics statement. This study is conducted with appropriate permissions (letter voucher: CIBCAS [2017] 005) for field survey and specimens' collecting, and guidelines from the responsible authority, the Forest Department, Ministry of Forest and Environment, the People's Republic of China. Collections of tissue samples were carried out in strict accordance with "Regulation for the Collection of Genetic Resources (HJ 628–2011)", and all practical efforts were made to ameliorate specimens suffering throughout this study. This study was approved by the Animal Ethics Committee of Chengdu Institute of Biology (Chinese Academy of

Sciences) and Institute of Zoology (Committee of Scientific Ministry of Education and Science, Kazakhstan), and animal experiments were carried out in line with the institutional guidelines.

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