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Phylogeny and divergence times of some racerunner lizards (Lacertidae: *Eremias*) inferred from mitochondrial 16S rRNA gene segments

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ABSTRACT

Eremias, or racerunners, is a widespread lacertid genus occurring in China, Mongolia, Korea, Central Asia, Southwest Asia and Southeast Europe. It has been through a series of taxonomic revisions, but the phylogenetic relationships among the species and subgenera remain unclear. In this study, a frequently studied region of the mitochondrial 16S rRNA was used to (i) reassess the phylogenetic relationships of some *Eremias* species, (ii) test if the viviparous species form a monophyletic group, and (iii) estimate divergence time among lineages using a Bayesian relaxed molecular-clock approach. The resulting phylogeny supports monophyly of *Eremias* sensu Szczerbak and a clade comprising *Eremias*, *Acanthodactylus* and *Latastia*. An earlier finding demonstrating monophyly of the subgenus *Pareremias* is corroborated, with *Eremias argus* being the sister taxon to *Eremias brenchleyi*. We present the first evidence that viviparous species form a monophyletic group. In addition, *Eremias przewalskii* is nested within *Eremias multiocellata*, suggesting that the latter is likely a paraphyletic species or a species complex. *Eremias acutirostris* and *Eremias persica* form a clade that is closely related to the subgenus *Pareremias*. However, the subgenera *Aspidorhinus*, *Scapteira*, and *Rhabderemias* seem not to be monophyletic, respectively. The Bayesian divergence-time estimation suggests that *Eremias* originated at about 9.9 million years ago (with the 95% confidence interval ranging from 7.6 to 12 Ma), and diversified from Late Miocene to Pleistocene. Specifically, the divergence time of the subgenus *Pareremias* was dated to about 6.3 million years ago (with the 95% confidence interval ranging from 5.3 to 8.5 Ma), which suggests that the diversification of this subgenus might be correlated with the evolution of an East Asian monsoon climate triggered by the rapid uplift of the Tibetan Plateau approximately 8 Ma.

1. Introduction

Over the last two centuries, the *Eremias* lizards have been one of the most difficult taxonomic groups within the family Lacertidae. *Eremias* was one of the "subgenera" into which Fitzinger (1834) divided the genus *Lacerta*. The taxon gained full generic status later by Fitzinger (1843), with the type species designated as *Eremias variabilis* [=*Eremias arguta* (Pallas, 1773) in the modern sense]. Today, the genus *Eremias* sensu stricto, is considered to comprise ~36 species, which inhabit sand, steppe, and desert regions from northern China, Mongolia, Korea, Central and Southwest Asia to Southeastern Europe (Fig. 1A; Guo et al., 2010 and references therein). The reproductive biology of *Eremias* (s. s.) is notable in that there exist two reproductive modes: viviparity and oviparity. Most are oviparous, whereas the *Eremias multiocellata* complex (comprising ~6 species or 8 subspecies; see Guo et al., 2010 and references therein), *Eremias buechneri*, and *Eremias przewalskii* are viviparous (Szczerbak, 1974, 2003).

Although the genus has been through a series of taxonomic revisions (e.g., Boulenger, 1918, 1921; Szczerbak, 1974; Eremchenko, 1999), the relationships among the species and subgenera are poorly understood (Orlov, 2008; Guo et al., 2010). Boulenger (1921) and FitzSimons (1943) assigned most of the species now placed in *Pedioplanis* to the subgenus *Mesalina* within the large genus *Eremias*. Szczerbak (1971) regarded *Eremias* sensu lato polyphyletic and considered *Eremias* (s. s.) endemic to Asia. Based on morphological characters and geographic distribution, Szczerbak (1974) subdivided the inclusive genus *Eremias* (s. l.) into two distinct genera: the genus *Mesalina* as a north African and

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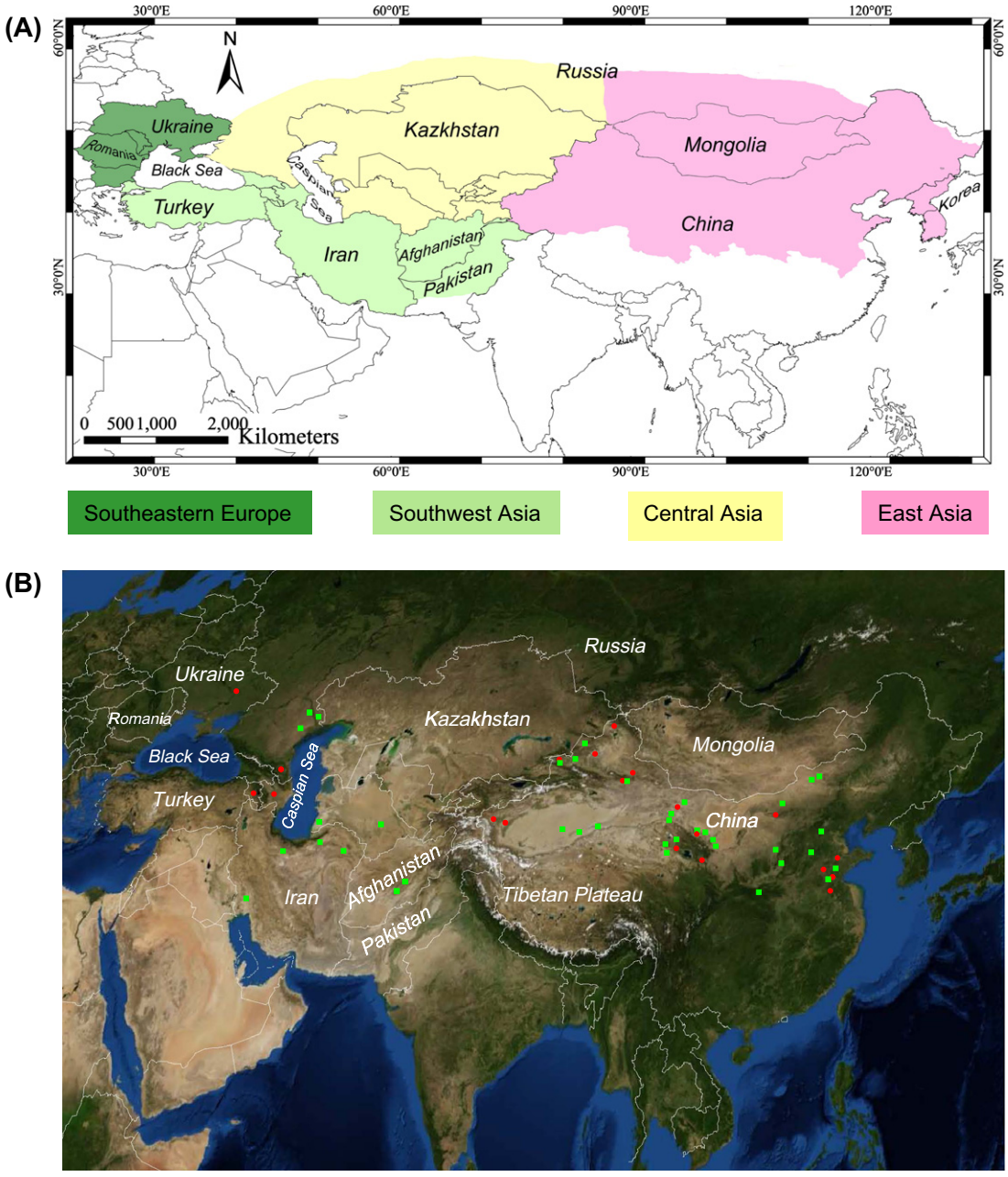

Fig. 1. (A) Schematic map showing the geographic distribution of *Eremias*. (B) Sampling localities for *Eremias* species (green squares), with red circles representing those retrieved from the GenBank.

lowland Southwest Asian clade, and the genus *Eremias* (s. s.), which is endemic to Asia. Furthermore, Szczerbak (1974) considered *Eremias velox* the type species of genus *Eremias*, and subdivided *Eremias* into five distinct subgenera: *Eremias* Fitzinger in Wiegmann, 1834 (group *E. velox*), *Rhabderemias* Lantz, 1928 (group *Eremias scripta–Eremias lineolata*), *Ommateremias* Lantz, 1928 (group *E. arguta*), *Scapteira* Fitzinger in Wiegmann, 1834 (group *Eremias grammica*), and *Pareremias* Szczerbak, 1973 (group *E. multiocellata*). The five subgenera were supported by Arnold (1986) on the basis of the hemipenial characters. However, the five subgenera were considered separate genera in some studies (e.g., Welch, 1983; Welch et al., 1990). As suggested by Barbanov (2009), *Aspidorhinus* Eichwald, 1841, should be regarded as the valid subgeneric name for a group of *E. velox*. Accordingly, as reviewed by Guo et al. (2010), the typical subgenus of *Eremias* is the group of *E. arguta* (with the synonym *Ommateremias*). Based on the species groups classified by Szczerbak (1971), Shine (1985) inferred that viviparity may have arisen twice within the genus *Eremias*. One of Szczerbak's species groups included the oviparous *Eremias argus*, the viviparous *E. multiocellata*, and *Eremias brenchleyi* for which the mode of reproduction was unknown at that time. Another species group contained the viviparous *E. przewalskii*, plus *E. buechneri*, *Eremias quadrifrons*, and *Eremias verimiculata* of unknown reproductive mode at that time.

In the past two decades, the mitochondrial 16S rRNA gene has been widely used to explore the phylogenetic relationships of lizards at varying taxonomic levels. An important aspect of rRNA genes is that they have conserved secondary structures that are moderately well conserved among distantly related taxa (Caetano-Anollés, 2002). Considering these secondary structural features, rRNA can be divided into paired (stem) and unpaired (loop) regions, and compensatory substitutions occur frequently

in the paired regions, a property that contradicts the assumption of independent mutations. Recently, likelihood-based methods have been successfully developed to account for compensatory substitutions in the rRNA genes (e.g., Savill et al., 2001; Xia et al., 2003a; Brown, 2005). Moreover, the use of partitioned Bayesian analyses has facilitated the exploration of partition-specific evolutionary models and should reduce systematic error, thus providing more precise posterior probability estimates (e.g., Nylander et al., 2004; Brandley et al., 2005; Guo and Wang, 2007).

Wan et al. (2007) made the first attempt to elucidate the phylogenetic relationships among the Chinese racerunner lizards on the basis of mitochondrial 16S rRNA data. Their results were preliminary because of incomplete taxonomic sampling and a lack of support values for the inferred relationships. 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). On the other hand, one potential shortcoming of the analyses of Wan et al. (2007) was that they did not incorporate secondary structural constraints of 16S rRNA into analyses.

Thus, as an extension of the molecular phylogenetics of the racerunner lizards, an effort has been made to collect some *Eremias* species from China, Turkmenistan, Russia, Iran and Afghanistan. We further tried to incorporate secondary structural constraints into analyses to improve the information content and performance of the mitochondrial 16S rRNA gene marker in the inference of *Eremias* relationships. This study specifically aims to: (i) reevaluate the phylogenetic relationships among some racerunner species, (ii) test if the viviparous species form a monophyletic group, (iii) estimate times of divergence within *Eremias* in an attempt to elucidate historical and ecological factors driving speciation in this group.

2. Materials and methods

2.1. Collection of samples

A total of 70 individuals of ~12 *Eremias* species were examined, including at least two individuals for most species whenever possible (see Table 1 and Fig. 1B). For most species, individuals from different localities were sampled to increase the reliability of the phylogenetic analyses. *Takydromus septentrionalis* and six other species were selected as outgroup taxa based on current understanding of the phylogenetic relationships among lacertid lizards (Mayer and Pavlicev, 2007). Voucher specimens are held in the Chengdu Institute of Biology, Chinese Academy of Sciences, the Department of Zoology, University of Guelph, and the Museum of Vertebrate Zoology, University of California. The details for all sequences used in this study are given in Table 1.

2.2. DNA extraction, amplification and sequencing protocols

The total genomic DNA was extracted from ethanol-preserved muscle or liver following the method of Aljanabi and Martinez (1997). The polymerase chain reaction (PCR) was used to amplify one segment approximately 540 bases from the 16S rRNA gene using universal primers 16Sar-L and 16Sbr-H (Palumbi, 1996). The PCR contained approximately 100 ng of template DNA, 2.5 μL of each primer (10 pmol/L), 5 μL of l0× reaction buffer, 2 μL dNTPs (each 2.5 mmol/L), and 2 units Taq DNA polymerase in total 50 μL volume. The PCR cycling conditions were 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 52–55 °C (adjusted according to the quality of template DNA) for 30 s and 72 °C for 1 min, and then a final elongation step at 72 °C for 8 min. The PCR amplification products were purified on a 1.0% agarose gel stained with ethidium bromide, using a commercial DNA purification kit following the manufacturer's protocol. Sequencing was performed using the same PCR primers with ABI Big Dye Terminator chemistry on an ABI 3730 automated sequencer. Sequences were then submitted for BLAST searching (Altschul et al., 1997) in GenBank to verify the data, with the accession numbers listed in Table 1.

2.3. Sequence alignment and analyses

A set of 16S rRNA sequences of *Eremias* and other related genera was also retrieved from GenBank, including 36 sequences representing nine *Eremias* species and 6 sequences representing 6 related genera (see Table 1). A total of 112 sequences were first aligned using Clustal X 1.83 (Thompson et al., 1997) with default gap penalties. The aligned matrix from this procedure was then checked by eye, and minor adjustments were made manually on the basis of secondary structure following the published model of *Darevskia caucasica* (Brown, 2005) with SeaView v.4.2.5 (Gouy et al., 2010). Nucleotide positions were designated as stem and loop regions. Some putative stem positions could not be confirmed since the complementary strands of some helices were not sequenced. The data matrices are available from the corresponding author.

Compositional heterogeneity was evaluated using chi-square (χ^2) tests implemented in PAUP* 4.0b10 (Swofford, 2002) and assessed using the software SeqVis v.1.5 (Ho et al., 2006) to visualize base composition and to conduct matched-pairs tests of symmetry of base substitution (Ababneh et al., 2006). Evidence of evolution under conditions more complex than that assumed by commonly applied models (i.e. stationary, reversible and homogeneous conditions) was inferred if the scatter of dots in the tetrahedral plots was widely dispersed and if a proportion X of the matched-pairs tests of symmetry rejected symmetry with *p*-values less than or equal to X. This procedure is consistent with that advocated by Jermiin et al. (2008, 2009). If the matched-pairs tests yield a larger than expected proportion of probabilities $\leqslant$0.05 (i.e., with >5% of the tests producing probabilities $\leqslant$0.05), then the conclusion is that the sequences have not evolved under stationary, reversible and homogeneous conditions. Substitutional saturation was tested by inspecting a new entropy-based index as implemented in DAMBE V.5.2.11 (Xia and Xie, 2001). For this approach, if I_{ss} (i.e., index of substitutional saturation) exceeds $I_{ss.c}$ (i.e., critical I_{ss}), then we can conclude that the sequences have experienced severe substitutional saturation (Xia et al., 2003b; Xia and Lemey, 2009).

2.4. Phylogenetic analyses

Phylogenetic hypotheses of *Eremias* were generated with 16S rRNA segments using two commonly applied phylogenetic methods: heuristic searches using equally weighted maximum parsimony (MP) analyses performed with the program PAUP* and Bayesian inference (BI) with the program MrBayes v.3.2 (Ronquist and Huelsenbeck, 2003). In both MP and BI analyses, each haplotype was treated as a taxon.

The MP analyses were performed with the following options implemented: heuristic search mode with ten random-addition-sequence replicates (RAS), tree bisection-reconnection branch swapping (TBR), MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered, and gaps were treated as missing data. Support for branches was assessed using 1000 non-parametric bootstrap replications (Felsenstein, 1985) with each bootstrap replicate performed as a heuristic search with ten RAS and TBR. *T. septentrionalis*, representing subfamily Lacertinae, was used to root the trees based on a recent publication (Mayer and Pavlicev, 2007).

Table 1
List of analyzed specimens, their geographical origin and the GenBank accession number.

Taxon and samples	Geographical origin	Voucher number[c]	Haplotype number	GenBank accession number	Reference
Subgenus *Aspidorhinus* Eichwald, 1841[a]					
E. velox	Tuokexun, Xinjiang, China	–	Hap1	DQ494814	Wan et al. (2007)
E. velox	Tuokexun, Xinjiang, China	–	Hap2	DQ494815	Wan et al. (2007)
E. velox	Daghestan, Russia	–	Hap3	AF206604	Fu (2000)
E. velox	Qitai, Xinjiang, China	–	Hap5	DQ494818	Wan et al. (2007)
E. velox	Buerjin/Karamay, Xinjiang, China	–	Hap6	DQ494817	Wan et al. (2007)
E. velox	Karamay /Qitai/Huocheng, Xinjiang, China	–	Hap7	DQ494816	Wan et al. (2007)
E. velox	Huocheng, Xinjiang, China	–	Hap8	DQ494819	Wan et al. (2007)
E. velox	Aydingkol, Xinjiang, China	W01494	Hap1	DQ658845	This study
E. velox	Aydingkol, Xinjiang, China	W01508	Hap2	DQ658844	This study
E. velox	Kalmykia, Russia	ZISP_TS175	Hap4	DQ658843	This study
E. velox	Astrakhan, Russia	ZISP_TS174	Hap4	DQ658842	This study
E. velox	Tacheng, Xinjiang, China	W9902	Hap9	DQ658841	This study
E. velox	Tacheng, Xinjiang, China	W9901	Hap7	DQ658840	This study
E. velox	Jinghe, Xinjiang, China	GUO523	Hap10	HQ615633	This study
E. velox	Jinghe, Xinjiang, China	GUO524	Hap10	HQ615634	This study
E. velox	Maryy Welayaty, Turkmenistan	MVZ233262	Hap11	HQ615639	This study
E. velox	Golestan, Iran	MVZ238535	Hap12	HQ615641	This study
E. velox	Maryy Welayaty, Turkmenistan	MVZ233263	Hap13	HQ615640	This study
E. persica	Delbar Field Station, Semnan, Iran	MVZ246012	Hap14	HQ615642	This study
E. persica	Delbar Field Station, Semnan, Iran	MVZ246013	Hap14	HQ615644	This study
E. persica	Sarhang, Khorasan, Iran	MVZ246010	Hap14	HQ615643	This study
E. persica	Izad Khast, Fars, Iran	MVZ234473	Hap15	HQ615645	This study
E. persica	Mobanakiye, Tehran, Afghanistan	MVZ234474	Hap16	HQ615646	This study
E. persica	Zamanabad, Seman, Iran	MVZ246014	Hap17	HQ615647	This study
E. persica	Takhteh Pol, Kandahar, Afghanistan	MVZ237049	Hap18	HQ615649	This study
Subgenus *Pareremias* Szczerbak, 1973					
E. brenchleyi	Suzhou, Anhui, China.	–	Hap20	EF490071	Rui et al. (2009)
E. brenchleyi	Xuzhou, Jiangsu, China	–	Hap21	DQ494826	Wan et al. (2007)
E. brenchleyi	Luquan, Shandong, China	–	Hap22	DQ494828	Wan et al. (2007)
E. brenchleyi	Taian/Luquan, Shandong, China	–	Hap23	DQ494827	Wan et al. (2007)
E. brenchleyi	Xuzhou, Jiangsu, China	W01525	Hap24	DQ658833	This study
E. brenchleyi	Laishui, Hebei, China	W01431	Hap25	DQ658832	This study
E. argus	Lanzhou, Gansu, China	–	Hap26	DQ494829	Wan et al. (2007)
E. argus	Zhouzhi, Shaanxi, China	W01527	Hap27	DQ658830	This study
E. argus	Yanchuan, Shaanxi, China	W01573	Hap28	DQ658831	This study
E. argus	Shahe, Hebei, China	W01433	Hap29	DQ658826	This study
E. argus	Zoucheng, Shandong, China	XSX1	Hap30	DQ658825	This study
E. argus	Shahe, Hebei, China	W01529	Hap30	DQ658827	This study
E. argus	Damao Qi, Inner Mongolia, China	W01278	Hap31	DQ658828	This study
E. argus	Abag Qi, Inner Mongolia, China	W01275	Hap32	DQ658829	This study
E. multiocellata	Guazhou, Gansu, China	W9904	Hap33	DQ658839	This study
E. multiocellata	Yulin, Shaanxi, China	W01549	Hap34	DQ658836	This study
E. multiocellata	Sonid Zuoqi, Inner Mongolia, China	W01273	Hap35	DQ658835	This study
E. multiocellata	Yulin, Shaanxi, China	W01277	Hap36	DQ658834	This study
E. multiocellata	Wuwei, Gansu, China	W01499	Hap37	DQ658838	This study
E. multiocellata	Sonid Youqi, Inner Mongolia, China	W01270	Hap38	DQ658837	This study
E. multiocellata	Golmud, Qinghai, China	GE0708016	Hap39	HQ615615	This study
E. multiocellata	Golmud, Qinghai, China	GE0708023	Hap39	HQ615616	This study
E. multiocellata	Golmud, Qinghai, China	GE0708017	Hap39	HQ615623	This study
E. multiocellata	Golmud, Qinghai, China	GE0708027	Hap39	HQ615617	This study
E. multiocellata	Akesai, Gansu, China	GE0708005	Hap39	HQ615618	This study
E. multiocellata	Golmud, Qinghai, China	GE0708026	Hap39	HQ615619	This study
E. multiocellata	Golmud, Qinghai, China	GE0708021	Hap39	HQ615620	This study
E. multiocellata	Golmud, Qinghai, China	GE0708022	Hap39	HQ615621	This study
E. multiocellata	Dacaidan, Qinghai, China	GE0708004	Hap39	HQ615622	This study
E. multiocellata	Dacaidan, Qinghai, China	GE0708003	Hap40	HQ615624	This study
E. multiocellata	Qitai, Xinjiang, China	–	Hap45	DQ494836	Wan et al. (2007)
E. multiocellata	Delingha, Qinghai, China	–	Hap46	DQ494833	Wan et al. (2007)
E. multiocellata	Yanchiwan, Gansu, China	–	Hap41	DQ494835	Wan et al. (2007)
E. multiocellata	Yanchiwan, Gansu, China	–	Hap42	DQ494834	Wan et al. (2007)
E. multiocellata	Sanchakou, Xinjiang, China	–	Hap47	DQ494839	Wan et al. (2007)
E. multiocellata	Sanchakou, Xinjiang, China	–	Hap49	DQ494838	Wan et al. (2007)
E. multiocellata	Yingisar, Xinjiang, China	–	Hap48	DQ494840	Wan et al. (2007)
E. multiocellata	Qitai, Xinjiang, China	–	Hap50	DQ494837	Wan et al. (2007)
E. multiocellata	Mazongshan, Gansu, China	–	Hap51	DQ494832	Wan et al. (2007)
E. multiocellata	Lanzhou, Gansu, China	–	Hap52	DQ494842	Wan et al. (2007)
E. multiocellata	Baotou/Lanzhou, China	–	Hap53	DQ494843	Wan et al. (2007)
E. multiocellata	Lanzhou, Gansu, China	–	Hap54	DQ494844	Wan et al. (2007)
E. multiocellata	Baotou, Inner Mongolia, China	–	Hap55	DQ494845	Wan et al. (2007)
E. multiocellata	Zhangye, Gansu, China	–	Hap56	DQ494841	Wan et al. (2007)
E. przewalskii	Zhangye, Gansu, China	–	Hap43	DQ494825	Wan et al. (2007)

(*continued on next page*)

Table 1 (*continued*)

Taxon and samples	Geographical origin	Voucher number[c]	Haplotype number	GenBank accession number	Reference
E. przewalskii	Minqin, Gansu, China	GUO403	Hap44	HQ615605	This study
E. przewalskii	Minqin, Gansu, China	GUO404	Hap44	HQ615608	This study
E. przewalskii	Minqin, Gansu, China	GUO405	Hap44	HQ615609	This study
E. przewalskii	Minqin, Gansu, China	GUO406	Hap44	HQ615610	This study
E. przewalskii	Minqin, Gansu, China	GUO450	Hap44	HQ615611	This study
E. przewalskii	Minqin, Gansu, China	GUO451	Hap44	HQ615612	This study
E. przewalskii	Minqin, Gansu, China	GUO454	Hap44	HQ615613	This study
E. przewalskii	Minqin, Gansu, China	GUO455	Hap44	HQ615614	This study
E. przewalskii	Minqin, Gansu, China	GUO459	Hap44	HQ615606	This study
E. przewalskii	Linze, Gansu, China	GUO449	Hap44	HQ615607	This study
Subgenus *Eremias* Fitzinger in Wiegmann, 1834[b]					
E. arguta	Yining, Xinjiang, China	–	Hap57	DQ494824	Wan et al. (2007)
E. arguta	Buerjin, Xinjiang, China	JF1314	Hap58	HQ615637	This study
E. arguta	Astrakhan, Russia	ZISP_TS579	Hap59	DQ658824	This study
E. arguta	Astrakhan, Russia	ZISP_TS173	Hap60	DQ658823	This study
E. arguta	Volgograd, Russia	ZISP_TS127	Hap59	DQ658822	This study
E. arguta	Volgograd, Russia	ZISP_TS130	Hap59	DQ658821	This study
E. arguta	Kalmykia, Russia	ZISP_TS559	Hap59	DQ658820	This study
E. arguta	Ukraine	–	Hap59	AY035837	Pavlicev and Mayer (2009)
E. arguta	Danizkanary, Azerbaijan	MVZ218770	Hap61	HQ615638	This study
Subgenus *Scapteira* Fitzinger in Wiegmann, 1834					
E. acutirostris	Takhteh Pol, Kandahar, Afghanistan	MVZ237044	Hap19	HQ615648	This study
E. grammica	Huocheng, Xinjiang, China	–	Hap63	DQ494820	Wan et al. (2007)
E. grammica	Huocheng, Xinjiang, China	–	Hap64	DQ494821	Wan et al. (2007)
E. grammica	Huocheng, Xinjiang, China	–	Hap65	DQ494822	Wan et al. (2007)
E. grammica	Huocheng, Xinjiang, China	GUO499	Hap66	HQ615635	This study
E. grammica	Huocheng, Xinjiang, China	GUO498	Hap66	HQ615636	This study
Subgenus *Rhabderemias* Lantz, 1928					
E. pleskei	Vedi, Armenia	–	Hap62	AY035838	Pavlicev and Mayer (2009)
E. scripta	Pir Zadeh, Afghanistan	MVZ237467	Hap67	HQ615650	This study
E. vermiculata	Dunhuang, Gansu, China	–	Hap68	DQ494831	Wan et al. (2007)
E. vermiculata	Dunhuang, Gansu, China	–	Hap68	DQ494830	Wan et al. (2007)
E. vermiculata	Linze, Gansu, China	GUO438	Hap70	HQ615625	This study
E. vermiculata	Linze, Gansu, China	GUO437	Hap70	HQ615626	This study
E. vermiculata	Linze, Gansu, China	GUO439	Hap70	HQ615627	This study
E. vermiculata	Linze, Gansu, China	GUO440	Hap70	HQ615628	This study
E. vermiculata	Tazhong, Xinjiang, China	WGXG08404	Hap69	HQ615629	This study
E. vermiculata	Tazhong, Xinjiang, China	WGXG08403	Hap69	HQ615630	This study
E. vermiculata	Ruoqiang, Xinjaing, China	WGXG08368	Hap69	HQ615631	This study
E. vermiculata	Qiemo, Xinjianag, China	WGXG08370	Hap69	HQ615632	This study
Outgroup					
Pedioplanis namaquensis	Cape Prov., South Africa	–	Hap75	AF206613	Fu (2000)
Adolfus jacksoni	Kabale District, Uganda	–	Hap76	AF206615	Fu (2000)
Latastia longicaudata	Rift Valley, Kenya	–	Hap72	AF206609	Fu (2000)
Acanthodactylus bedriagae	Taroudent, Morocco	–	Hap71	AY633438	Harris et al. (2004)
Mesalina guttulata	Harraat al Harrah, Egypt	–	Hap73	AY217969	Whiting et al. (2003)
Ophisops elegans	Chosrov, Armenia	–	Hap74	AF206605	Fu (2000)
Takydromus septentrionalis	Fuhai, Xinjiang, China	No Voucher	Hap77	DQ658847	This study

[a] Considering subgenus *Dimorphea* Eremchenko, 1999, as its synonym (Barabnov, 2009).
[b] Considering subgenus *Ommateremias* Lantz, 1928, as its synonym (Guo et al., 2010).
[c] The voucher numbers of the sequences retrieved from GenBank are not shown.

Prior to BI analyses, the best-fit models of evolution, TVM + I + G for the stems and GTR + G for loops, were selected using jModeltest v.0.1.1 (Posada, 2008) under the Akaike information criterion (AIC; Akaike, 1974), following recent recommendations of Posada and Buckley (2004). The aligned 16S rRNA data were partitioned by stem sites and loop sites. The rate parameters, base frequencies, and shape parameters of the gamma distributions used to model within-matrix heterogeneity were allowed to vary independently in each partition. We estimated posterior probability distributions by allowing four incrementally heated Markov chains (default heating values) to proceed for 20,000,000 generations, with samples taken every 1000 generations. Analyses were repeated beginning with different starting trees to ensure that our analyses were not restricted from the global optimum (Huelsenbeck et al., 2002). MCMC convergence was explored by examining the potential scale reduction factor (PSRF; Gelman and Rubin, 1992) convergence diagnostics for all parameters in the model (provided by the *sump* and *sumt* commands) and graphically using the program Tracer v.1.5.0 (Rambaut and Drummond, 2009). The first eight million generations, before this chain reached apparent stationarity, were discarded, and the remaining samples from the independent runs were pooled to obtain the final approximation of the posterior distribution of trees. To yield a single hypothesis of phylogeny, the posterior distribution was summarized as a 50% majority-rule consensus.

Additionally, as gap (or "indel") characters have been widely recognized as a valuable source of data for phylogenetic inference across the tree of life (e.g., Dessimoz and Gil, 2010), phylogenetic

information from indel events of 16S rRNA was also included in MP and BI analyses by coding indel events into a separate data matrix with the program SeqState (Müller, 2005) using the simple indel coding method (Simmons and Ochoterena, 2000). In the latter, all indels are scored as binary characters regardless of their length. In BI, a discrete model employing identical rates of forward and backward substitution (Lewis, 2001) was applied to the indel matrix. Gaps were also treated as a fifth state in MP analysis just to see if the phylogenetic resolution and nodal branch support increase.

2.5. Bayesian hypothesis testing

We used Bayes factors to compare our preferred Bayesian tree topology (see below) to Bayesian trees with constraints. The Bayes factor measures the amount by which one's opinion is changed after viewing the data. This can be interpreted as the change in odds in favor of a hypothesis and can be measured as the change in odds from the prior to the posterior (Lavine and Schervish, 1999) or as the relative success of two hypotheses at predicting the data (Kass and Raftery, 1995). Constraint analyses were conducted in MrBayes v.3.2 using the command *prset topologypr = constraint*. All analyses consisted of two simultaneous runs each with an abbreviated three MCMC chains run for ten million generations or more (as necessary). The Bayes factor was determined by calculating the marginal likelihood for both unconstrained and constraint analyses using Tracer v.1.5.0 (Rambaut and Drummond, 2009). The difference in these ln-transformed marginal likelihoods was compared to the table provided by Raftery (1996). Based on these tables, we consider a 2ln Bayes factor $\geqslant 10$ as significant evidence for choosing the favored hypothesis and rejecting alternatives (Kass and Raftery, 1995).

2.6. Divergence dating

A likelihood-ratio rest (Huelsenbeck and Crandall, 1997) revealed that these data do not evolve in a clock-like manner ($\chi^2 = 119.87514$, $df = 75$, $p < 0.001$). The relaxed Bayesian clock implemented in Estbranches and Multidivtime was used to generate an ultrametric tree (Kishino et al., 2001; Thorne and Kishino, 2002). In the Multidivtime analysis, parameters of the substitution model F84 + G were first estimated by the program Baseml in the PAML package v.4.3 (Yang, 1997). The output from Baseml was then used in the Multidistribute package to estimate the maximum likelihood of the branch lengths and a variance–covariance matrix, and to perform a MCMC Bayesian analysis for estimating the posterior distributions of substitution rates and divergence dates. The tree favored by Bayesian inference was used as the reference topology for molecular dating analysis. The northern grass lizard (*T. septentrionalis*) sequence served as the outgroup to root the tree relating the remaining 76 ingroup sequences. The priors for the mean and standard deviation of the ingroup root age (Lacertinae–Eremiadinae split, 16 Ma; Arnold et al., 2007), rttm and rttmsd were set to 16 million years and 4 million years, respectively (i.e., rttm = 1.6, rttmsd = 0.4). The mean and standard deviation of the prior distribution for the rate of molecular evolution at the ingroup root node (rtrate and rtratesd) were both set to 0.17. These values were based on the median of the substitution path lengths between the ingroup root and each terminal, divided by rttm (as suggested by the author). The prior mean and standard deviation for the Gamma distribution of the parameter controlling rate variation over time (i.e. brownmean and brownsd) were both set to 1.0. Markov chains in Multidivtime were run for 40,00,000 generations, sampling every 100th generation for a total of 40,000 trees, with a burn-in of 4000 trees before the first sampling of the Markov chain. To test whether the Markov chain was converging, four single runs were performed. Similar results from the four runs were observed.

The Multidivtime program allows for both minimum and maximum fossil constraints. Whereas minima are often based on earliest occurrences in the fossil record, maxima are intrinsically more difficult to estimate. So we used the estimated divergence time between Lacertinae and Eremiadinae (16 Ma) based on the results of Arnold et al. (2007) for the upper time of tip-root (Fig. 3). The stem and loop positions were treated as different partitions, with their heterogeneity being taken into account. The fossils of *Eremias* from Qinling Mountains in China (Li et al., 2004) and Builstyn Khudang (BUK-A) in Mongolia (Böhme, 2007) are recorded from the Pleistocene (493 ± 55 ka B.P.) and Late Miocene, respectively. Accordingly, estimates were calibrated using six time constraints (see Fig. 3). The C1 calibration point is a lower bound of 0.55 Ma based on the *Eremias* sp. fossil in Qinling Mountains (Li et al., 2004). C2 represents a lower bound of 5.3 Ma, derived from the *Eremias* sp. fossil in BUK-A (Böhme, 2007). C3 represents a lower bound of 11 Ma and an upper bound of 13 Ma for the divergence of the ancestor of *Eremias*, *Mesalina*, and *Ophisops* based on the results of Rastegar-Pouyani et al. (2010). C4 represents a lower bound of 12 Ma and an upper bound of 16 Ma for the divergence of the Eremiadinae based on the results of Arnold et al. (2007). Additionally, we specified what is the average rate of evolution of 16S rRNA gene sequences given the dates that we estimated.

3. Results

3.1. Base composition and nucleotide substitution patterns

One hundred and twelve sequences (including outgroup taxa) revealed 77 haplotypes (see Table 1). Of the 512 aligned characters, 211 were variable, with 158 parsimony-informative. The alignment of the ingroup required accommodation of 25–31 alignment gaps per sequence. Indels (insertion/deletion events) represented between 4.88% and 6.05% of the aligned sequence length. Most indels are 1 bp in length, and the maximum indel length is 6 bp. Table 2 shows the distribution of variable nucleotide positions in stem and loop regions. Average base composition is *A*: 30.51%, *C*: 25.1, *G*: 20.26%, *T*: 24.12%, when the outgroup taxa are combined. The *A* + *T* content is much higher than that of *G* + *C*. Average nucleotide compositions and transition-to-transversion (*Ts*/*Tv*) ratio for loop, stem, and all positions are given in Table 3. Loop regions exhibit a strong bias in favor of *A* (36.78%) and against *G* (14.88%), whereas stem regions are biased in favor of *G* (29.41%) and *C* (26.89%).

A base stationarity test shows insignificant differences among all taxa in base composition bias in the data: 16S rRNA, $\chi^2 = 26.12$, $df = 228$, $p = 1.00$; stem position, $\chi^2 = 18.57$, $df = 228$, $p = 1.00$; loop position, $\chi^2 = 35.85$, $df = 228$, $p = 1.00$. When all the sites are considered equal (i.e., all the sites placed in the same bin) and the tetrahedron is allowed to rotate, the 77 points are

Table 2
Sequence variations and phylogenetic information in 16S rRNA sequence data.

	Nucleotide sites	Variable sites	Variable sites (%)	Parsimony-informative sites	Parsimony-informative sites (%)
All positions	512	211	41.21	158	30.86
Stem regions	180	54	30	34	18.89
Loop regions	332	157	47.29	124	37.35

Table 3
Average nucleotide compositions and maximum likelihood *Ts*/*Tv* ratios for stem and loop regions of 16S rRNA sequence data.

	All positions	Stem regions	Loop regions
A %	30.51	19.87	36.78
C %	25.1	26.89	24.04
G %	20.26	29.41	14.88
T %	24.12	23.83	24.3
Ts/*Tv*	2.27	2.25	2.58

scattered tightly in an area where the proportion of *A* and *C* are >25% (Appendix 1A). The points are clearly spread within a confined area, implying that there may be more compositional heterogeneity in these data than the initial analysis suggested. To address this issue, we binned the nucleotides according to the structural information. The distributions of points differ for the stem and loop sites, with stem sites displaying a small amount of scatter (Appendix 1B), and loop sites displaying hardly any scatter (Appendix 1C). Rotating the two tetrahedral plots shows that the centroids differ for the stem/loop sites, thus suggesting that it would be necessary to apply two Markov models to these data to analyze them appropriately within a phylogenetic context. To corroborate whether this is the case, the matched-pairs test of symmetry is used in conjunction with 16S rRNA, stem sites and loop sites of the alignment. Table 4 summarizes the distribution of *p*-values for 16S rRNA, stem and loop sites. Of the 2926 pairwise tests conducted: (1) 95 tests (0.0325) for stem site reject symmetry with a probability $\leqslant$0.05, implying stem sites consistent with evolution under stationary, reversible, and homogeneous conditions; (2) 56 tests (0.019) for loop sites reject symmetry with a probability $\leqslant$ 0.05, implying loop sites consistent with evolution under stationary, reversible, and homogeneous conditions. Given the very small faction of asymmetrical results, a sensible approach to analyze these data phylogenetically would be to apply a time-reversible Markov model to the stem sites and another such model to the loop sites. With a proportion of invariant sites of 0.3544, the observed I_{ss} of 0.108 was significantly smaller than the $I_{ss.c}$ value of 0.664 for a symmetrical topology ($p < 0.0001$, two-tailed test) and the $I_{ss.c}$ value of 0.319 for an asymmetrical topology ($p < 0.0001$, two-tailed test), suggesting that the stems experienced little substitutional saturation. Similarly, as shown in Table 5, we inferred that the loops and 16S rRNA data as a whole experienced little substitutional saturation for a symmetrical tree.

3.2. Phylogenetic analyses

During the first replicate of an exploratory maximum-parsimony heuristic search more than one million equally parsimonious trees were obtained (786 steps long), and this computational demand compromised our ability to explore other islands of trees. The commands NCHUCK and CHUCKSCORE were therefore employed to perform a search from more than one starting point and to increase the probability of exploring other islands of trees. These options allow one to set a maximum number of trees (NCHUCK = 1000) of score greater than or equal to that specified by the CHUCKSCORE (=786 in our case) per random-addition-sequence replicate (Swofford, 2002). As 10 RAS were performed, a total of 7000 equally most parsimonious trees were found. These are presented as a majority-rule consensus tree where branches with bootstrap support lower than 50% are collapsed (see Appendix 2). The parsimony bootstrap support was also marked on the branches that receive such support in the Bayesian tree (see Fig. 2). Monophyly of genus *Eremias* was well supported (BP = 94%). The interrelationships of the earliest lineages are characterized by a basal polytomy (with most deep nodes support lower than 50%). Monophyly of the subgenus *Pareremias* is recovered albeit with a low support value (BP = 57%). However, monophyly of subgenera *Aspidorhinus*, *Scapteira*, and *Rhabdremias* is not supported individually. *Eremias acutirostris* and *Eremias persica* form a well supported clade (BP = 98%). *E. vermiculata* clusters with Hap57 of *E. arguta* (BP = 53%), making the latter species paraphyletic. In the subgenus *Pareremias*, the viviparous species form a monophyletic group (BP = 86%), whereas *E. multiocellata* and *E. przewalskii* are not reciprocally monophyletic. *E. argus* and *E. brenchleyi* form a clade (BP = 81%) that is the sister taxon to the viviparous species. When gaps are treated as a fifth state, the resultant consensus tree is similar to the MP consensus tree without gaps incorporated as phylogenetic characters, except for the placement of *E. arguta* (see Appendix 3). When gaps are coded as simple binary characters (i.e., simple indel coding), the MP analysis fails to recover subgenus *Pareremias* as monophyletic, placing these taxa in the polytomy with the rest of the genus (see Appendix 4).

For the BI analyses, the 50% majority consensus tree is shown in Fig. 2. The average PSRF was 1.000, implying convergence of runs. As with MP analyses, monophyly of the genus *Eremias* and the subgenus *Pareremias* is supported with strong posterior probabilities (PP = 0.99 and 0.98, respectively). Similarly, monophyly of the

Table 5
Tests for substitutional saturation using the index of substitutional saturation (I_{ss}). Substitutional saturation is indicated if I_{ss} exceeds critical value estimated for symmetrical and asymmetrical trees. Our data reject the hypothesis of substitutional saturation for a symmetrical tree, but reject this hypothesis only for stems in a highly asymmetrical tree.

			For a symmetrical tree		For an extreme asymmetrical (and generally very unlikely) tree	
	p-in[a]	I_{ss}	$I_{ss.c}$ sym.	*p*[b]	$I_{ss.c}$ asym.	*p*[b]
Stems	0.413	0.108	0.664	<0.0001**	0.319	<0.0001**
Loops	0.000	0.371	0.683	<0.0001**	0.348	0.7570
16S rRNA	0.140	0.309	0.704	<0.0001**	0.380	0.2152

[a] *p*-in is the proportion of invariable sites.
[b] Two tailed test.
** Little saturation.

Table 4
Matched-pairs tests for stationarity of base composition. Using 2926 tests for symmetry of base substitution, the number and proportion of tests rejecting the hypothesis of symmetry at the threshold level in column 1 are listed separately for tests using all 16S rRNA base sites, stem regions only, and loop regions only. Because the proportions of statistically asymmetrical results are approximately or below the threshold levels, we judge the data primarily with a model of base compositional stationarity.

Threshold[a]	16S rRNA		Stem regions		Loop regions	
	Number[b]	Proportion	Number[b]	Proportion	Number[b]	Proportion
0.05	76	0.026	95	0.032	56	0.019
0.01	3	0.001	39	0.013	3	0.001
0.005	0	0	23	0.008	0	0

[a] The smallest *p* value for stem regions is 0.0011. The smallest *p* value for loop regions is 0.0078. The smallest *p* value for 16S rRNA is 0.0064.
[b] The number of times that the matched-pairs test of symmetry resulted in a *p*-value below the threshold (number of test: 2926).

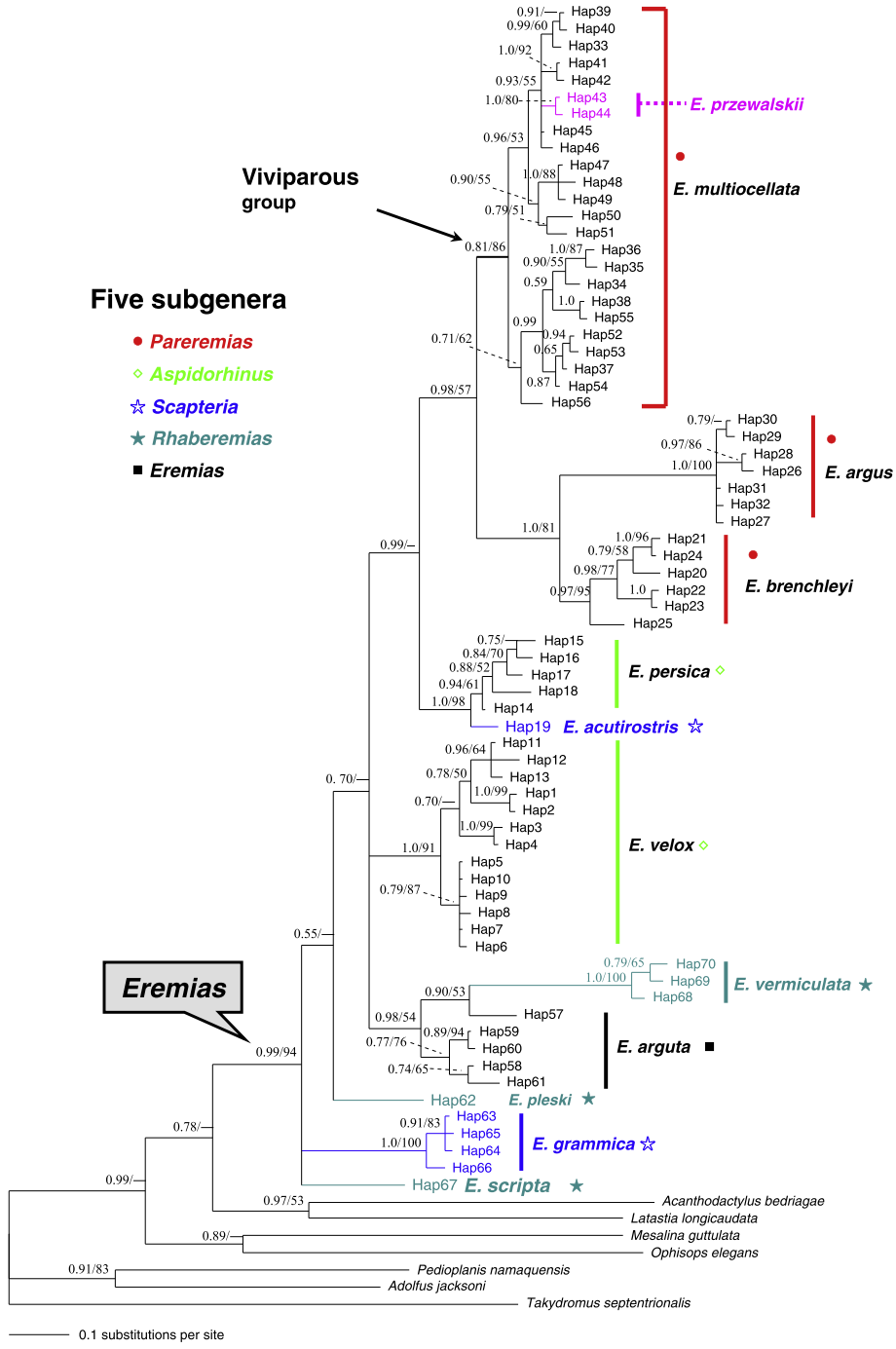

Fig. 2. 16S rRNA majority-rule consensus tree inferred from Bayesian inference by using MrBayes v.3.2, associations with less than 0.5 posterior probability were collapsed. Bayesian posterior probabilities, maximum parsimony bootstrap values are shown. Dashes represent nodes with non-parametric bootstrap support lower than 50% or represent nodes not existed. MP tree length = 786, CI = 0.4426, RI = 0.7564.

viviparous group is also supported, albeit with relatively low posterior probability (PP = 0.81). *E. argus* and *E. brenchleyi* form a clade (PP = 1.0) that is the sister taxon to the viviparous group. Monophyly of subgenera *Aspidorhinus*, *Scapteira*, and *Rhabdremias* is not supported individually. *E. vermiculata* clusters with Hap57 of *E. arguta* (PP = 0.90), making it paraphyletic. Contrary to MP trees, *E. persica* and *E. acutirostris* form a clade that is the sister taxon to the subgenus *Pareremias* (PP = 0.98). Genera *Eremias*, *Acanthodactylus* and *Latastia* form a clade, albeit with relatively low posterior probability (PP = 0.78), with the latter two genera forming a subclade that is the sister taxon to the genus *Eremias* (PP = 0.99). Gap incorporation within 16S rRNA changed neither topology nor support due to the conservative nature of this gene or short length of the segment (see Appendix 5).

3.3. Bayesian hypothesis testing

Bayes factor comparisons are summarized in Table 6. The analyses conducted reflect our primary interests of evaluating the alternative hypotheses of intrarelationships of genus *Eremais*. As mentioned above, analyses of the 16S rRNA data yield a monophyletic genus *Eremias* and viviparous species group. Bayes factor analyses of the 16S rRNA gene were conducted to compare topologies with constraints to the Bayesian tree topology. In most

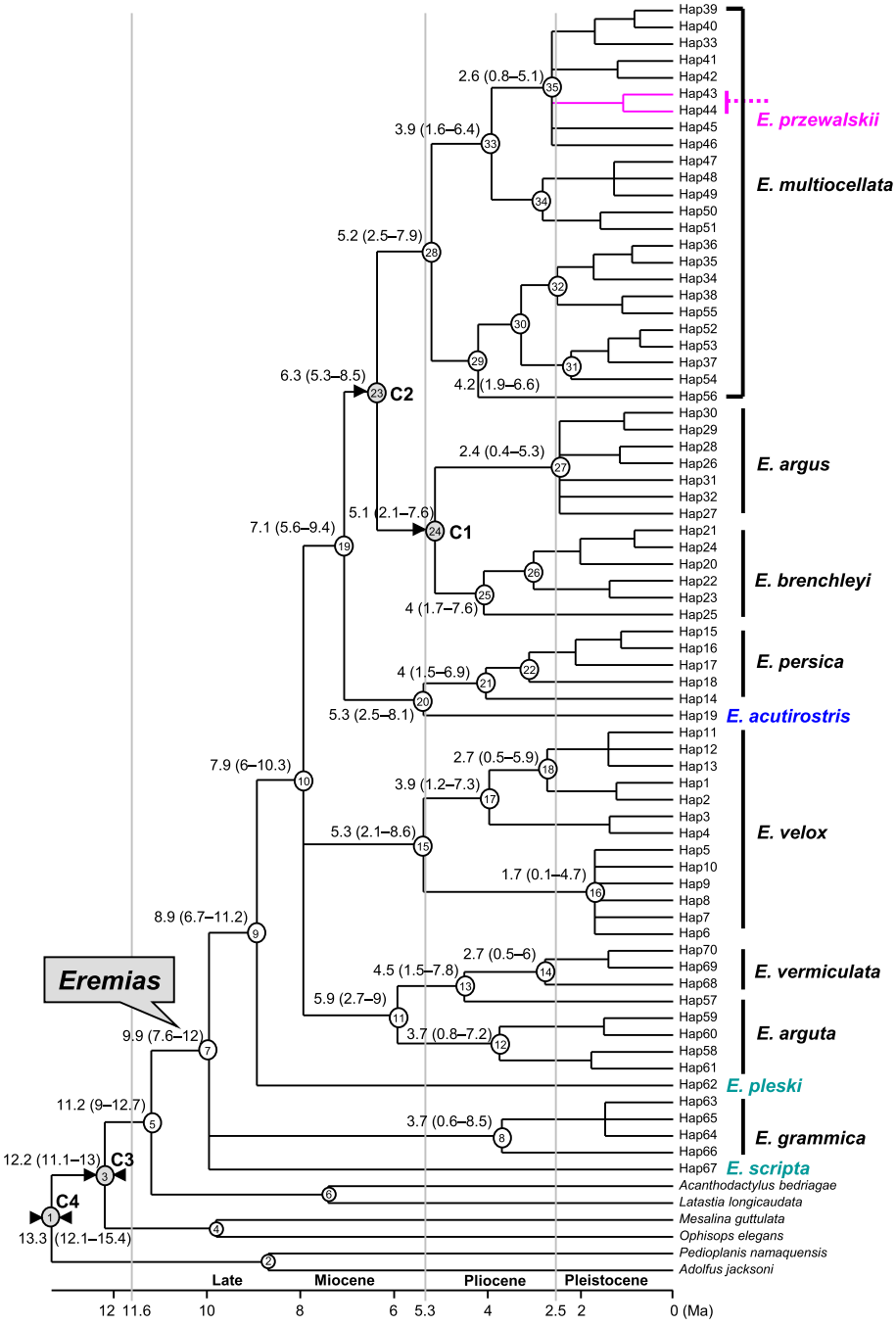

Fig. 3. Chronogram of *Eremias* species based on the 16S rRNA fragments under a Bayesian relaxed clock, and six time constraints. Branch lengths represent the mean values of the posterior distribution. The calibration points are indicated as shaded circles with left/right (minimum bound/maximum bound) pointing triangles beside them. Numbers and numbers in parentheses beside the nodes represent divergence time mean and 95% credibility intervals. More detailed time estimates are given in Table 7; node numbers in the table correlate with circled node numbers in the figure. Given the dates as presented above, the average substitution rate of 16S rRNA is estimated as ~0.19% per million years (with the 95% confidence interval ranging from 0.16% to 0.25%).

cases, there was very strong (2ln Bayes factor >10) evidence against the constrained topologies. Thus, several alternative phylogenetic hypotheses are significantly rejected, such as a monophyletic *E. multiocellata*, and monophyly for each subgenus *Aspidorhinus*, *Scapeteira*, and *Rhabderemias*.

3.4. Divergence dating

Bayesian dating methods allow comparison of results from prior (e.g., fossil constraints) and posterior distribution analyses to examine how prior specifications affect the final posterior distribution results. The prior distribution analysis ignores the information contained in the sequence data; hence, it is expected that there will be a larger amount of uncertainty in prior divergence-time estimates (Thorne and Kishino, 2002). Accordingly, we approximated the prior and posterior distributions of divergence times in the Bayesian dating analyses. The size of the 95% confidence interval (CI) of the prior distribution for node ages is considerably larger than the size of the 95% CI of the posterior distribution, and the means are also different in most cases (Table 7). These differences in means and the size of credibility intervals between the prior and posterior distributions indicate that the prior specification has

Table 6
Summary of 2ln Bayes factor comparisons of alternative phylogenetic hypotheses. Marginal likelihoods were calculated using the method of Newton and Raftery (1994) with modifications proposed by Suchard et al. (2001) using Tracer v.1.5.0 (Rambaut and Drummond, 2009). 2Ln Bayes factors ⩾10 are considered very strongly different (Kass and Raftery, 1995), indicating evidence against alternative hypotheses.

Alternative phylogenetic hypotheses	Ln marginal likelihood		2ln Bayes factor [$2\mathrm{Ln}(B_{10})$]
	Ln *L*: unconstrained	Ln *L*: constrained	
Monophyly of subgenus *Aspidorhinus*	−4431.950	−4443.870	23.840
Monophyly of subgenus *Scapteira*	−4431.950	−4557.329	250.758
Monophyly of subgenus *Rhabderemias*	−4431.950	−4442.206	20.512
Monophyly of *E. multiocellata*	−4431.950	−4466.610	69.320
Monophyly of *E. arguta*	−4431.950	−4436.121	8.342

Table 7
Bayesian estimates for divergence times with 95% credibility intervals.

Node[a]	Prior			Posterior		
	Date	SD	95% CI	Date	SD	95% CI
1	13.2	0.8	(12.1, 15.2)	13.3	0.9	(12.1, 15.4)
2	6.6	3.8	(0.3, 13.1)	8.7	3.1	(1.7, 13.6)
3	12.2	0.5	(11.1, 13)	12.2	0.5	(11.1, 13)
4	6.2	3.5	(0.3, 12)	9.8	2.4	(3, 12.5)
5	11.4	0.9	(9.3, 12.7)	11.2	0.9	(9, 12.7)
6	5.7	3.3	(0.3, 11.4)	7.4	2.9	(1, 11.7)
7	10.5	1.1	(8.2, 12.3)	9.9	1.2	(7.6, 12)
8	7.0	2.6	(1.6, 11.2)	3.7	2.1	(0.6, 8.6)
9	9.7	1.2	(7.2, 11.8)	8.9	1.2	(6.7, 11.2)
10	8.9	1.3	(6.5, 11.2)	7.9	1.1	(6, 10.3)
11	7.1	1.8	(3.3, 10.3)	5.9	1.6	(2.7, 9)
12	4.7	2.1	(1, 8.8)	3.7	1.7	(0.8, 7.2)
13	5.3	1.9	(1.7, 9.1)	4.5	1.6	(1.5, 7.8)
14	3.5	1.9	(0.6, 7.6)	2.7	1.5	(0.5, 6)
15	7.1	1.8	(3.3, 10.3)	5.3	1.7	(2.1, 8.6)
16	3.6	2.3	(0.2, 8.3)	1.7	1.2	(0.1, 4.7)
17	5.3	2.0	(1.6, 9.1)	3.9	1.6	(1.2, 7.3)
18	3.5	1.9	(0.6, 7.6)	2.7	1.4	(0.5, 5.9)
19	8.0	1.3	(5.9, 10.6)	7.1	1.0	(5.6, 9.4)
20	6.7	1.5	(3.6, 9.7)	5.3	1.4	(2.5, 8.1)
21	5.4	1.7	(2.2, 8.7)	4	1.4	(1.5, 6.9)
22	4.1	1.7	(1.1, 7.5)	3.1	1.3	(0.9, 5.9)
23	7.2	1.2	(5.4, 9.9)	6.3	0.9	(5.3, 8.5)
24	6.0	1.5	(3.2, 9)	5.1	1.2	(2.7, 7.6)
25	4.8	1.5	(1.9, 8)	4	1.3	(1.7, 6.6)
26	3.6	1.5	(1, 6.8)	3.0	1.2	(0.9, 5.5)
27	4.1	1.7	(0.9, 7.6)	2.4	1.3	(0.4, 5.3)
28	6.2	1.4	(3.6, 9.1)	5.2	1.1	(2.9, 7.5)
29	5.2	1.5	(2.4, 8.2)	4.2	1.2	(1.9, 6.6)
30	4.1	1.5	(1.5, 7.2)	3.3	1.2	(1.2, 5.7)
31	3.1	1.4	(0.8, 6.1)	2.2	1.0	(0.6, 4.5)
32	3.1	1.4	(0.8, 6.1)	2.5	1.1	(0.7, 4.8)
33	5.0	1.5	(2, 8.1)	3.9	1.2	(1.6, 6.4)
34	3.3	1.6	(0.7, 6.7)	2.8	1.2	(0.7, 5.4)
35	3.7	1.5	(1, 6.9)	2.6	1.1	(0.8, 5.1)

[a] Node numbers correspond to those in Fig. 3. The time unit is million years. The standard deviation (SD) is given for each node.

little influence on the posterior distribution and that most of the information about divergence time is retrieved from the sequence data.

The partitioned Bayesian approach estimated the divergence time for *Eremias* at 9.9 million years ago with the 95% CI of 7.6–12 Ma. Table 7 lists the divergence times obtained for key nodes within *Eremias* based on the phylogeny shown in Fig. 2. The divergence time for the tree root (divergence between the Lacertinae and the Eremiadinae) is at 13.3 Ma with the 95% CI of 12.1–15.4 Ma. The global clock approach also dated the divergence time for genus *Eremias* at about 10.3 ± 1.1 Ma (with the 95% CI ranging from 7.9 to 12.2 Ma), and for the tree root at around 13.3 ± 0.9 Ma (with the 95% CI ranging from 12.1 to 15.4 Ma), which is similar to those derived from the partitioned Bayesian approach. The divergence of the ancestor of *Eremias*, *Mesalina*, and *Ophisops* dated to about 12.2 ± 0.5 Ma. The viviparous group diverged from the oviparous species in subgenus *Pareremais* (node 23 in Fig. 3) approximately at 6.3 ± 0.9 Ma (95% CI, 5.3–8.5 Ma), while the MRCA of the viviparous species dated to 5.2 ± 1.1 Ma. Subsequently, rapid speciation events occurred in the viviparous group during the Pleistocene. The divergence time between *E. argus* and *E. brenchleyi* was dated to about 5.1 ± 1.2 Ma. And the divergence time between *E. persica* and *E. acutirostris* was estimated at about 5.3 ± 1.4 Ma. Fig. 3 and Table 7 demonstrate recurring cladogenesis occurring in the racerunner lizards since late Miocene. Thus, the racerunner lizards most probably originated in late Miocene and radiated into the Pleistocene. We tested the effect of varying the values of the priors by setting 0.5 (s.d. = 0.5) for the parameter (υ) that controls the degree of rate autocorrelation per 10 Myr along the descending branches of the tree. The results were very similar to the estimates given above, such as 10.2 ± 1.1 Ma for the genus *Eremias* divergence (with the 95% confidence interval ranging from 7.8 to 12.1 Ma), which further ensured that changing the priors does not have significant effect. Using the dates as presented in Figure 3 and Table 7, we estimated that the 16S rRNA gene segments in *Eremias* evolve at an average rate of approximately 0.19% per million years (with the 95% confidence interval ranging from 0.16% to 0.25%), meaning that any two racerunner lizards are diverging form each other at a rate of ~0.38% per million years (with the 95% confidence interval ranging from 0.32% to 0.5%).

4. Discussion

4.1. Phylogenetic relationships of racerunner lizards

On the one hand, phylogenetic relationships estimated using MP and BI were similar or identical for most part, with differences mostly due to resolution of some polytomies. All analyses provide strong support for monophyly of the genus *Eremias*. *E. argus* is the sister taxon to *E. brenchleyi*, which is congruent with the morphological character that both the species have two frontonasal shields, a synapomorphy that distinguishes them from other *Eremias*. On the other hand, almost all MP analyses revealed a basal polytomy with several unresolved deep nodes, whereas some deep nodes were resolved in the Bayesian trees (see Fig. 2 and Appendix 5). In addition, Bayesian support values in the BI trees were higher than bootstrap values for the clades in the MP trees, suggesting that Bayesian inference is the more precise phylogenetic analysis.

As discussed above, this study provided strong support for monophyly of racerunner lizards, consistent with the results of Arnold (1986) and Wan et al. (2007), and thus providing a firm basis for further testing the plausibility of the alternative biogeographic models. Similarly, the viviparous species form a monophyletic group, which is contrary to the speculation of Shine (1985). However, to date, the sister group of genus *Eremias* is still equivocal. Phylogenetically, *Eremias* is most closely related to a clade including *Acanthodactylus*, *Mesalina*, and *Ophisops-Cabrita* (Arnold, 1989). Mayer and Benyr (1994), on the basis of albumin-immunological studies, have proposed tentatively that *Eremias* is the sister taxon of *Mesalina* and that both of them belong to a larger clade also containing *Omanosanura* and *Ophisops*. They

proposed that *Eremias* was not closely related to *Acanthodactylus*. On the other hand, in a more recent study, the four genera *Acanthodactylus*, *Eremias*, *Mesalina* and *Ophisops* have been proposed to constitute a clade based on morphological characters (Arnold, 2004). In addition, both 4708 bp of mtDNA sequences and ~1600 bp nuclear DNA data (*rag1* and *c-mos* genes) suggested that the intergeneric relationships of Eremiadinae were not clear (Fu, 2000; Mayer and Pavlicev, 2007). Our result indicates that the genera *Acanthodactylus* and *Latastia* form a clade that is the sister taxon of genus *Eremias*. However, to resolve this discrepancy, it is necessary to include additional lacertid species and data to test these intergeneric relationships. As for the intrarelationships of genus *Eremias*, our molecular data further demonstrate that the subgenus *Pareremias* is monophyletic, in accordance to Arnold (1986). However, the subgenera *Aspidorhinus*, *Scapteira*, and *Rhabderemias* seem not to be individually monophyletic, as indicated by the results of Bayesian hypotheses testing.

4.2. E. multiocellata: a paraphyletic species or species complex?

The observation that *E. przewalskii* nests inside of *E. multiocellata* challenges the integrity of the latter species (Fig. 2). Support for this placement was statistically significant (BP = 80%; PP = 1.0). There are several potential explanations. First, *E. przewalskii* is not a valid species but part of *E. multiocellata*. This is unlikely because morphologically *E. przewalskii* is very distinct from *E. multiocellata*. The former has a large body size; its snout-to-vent length (SVL) ranges from 73.5 to 95 mm in males (n = 11) and 59 to 86 mm in females (n = 10), while the latter has a smaller SVL, ranging from 48 to 72 mm in males (n = 35) and 42–74 mm in females (n = 60) (Zhao, 1999). In addition, for *E. przewalskii*, the length of the lower side of the rostral shield is equal to or shorter than the length of the granular area before the first supraocular shield, whereas in *E. multiocellata*, the length of the lower side of the rostral shield exceeds the length of the granular area before the first supraocular shield. Furthermore, the dorsal pattern of *E. przewalskii* is net-like or crossbanded, whereas the dorsal pattern of *E. multiocellata* consists of eye-like spots, blotches, and stripes (Szczerbak, 2003). Specifically, all ten samples of *E. przewalskii* have brighter and widely transversal stripes on their back. Second, the *E. multiocellata* populations examined in this study are actually a species complex. According to the monophyly criterion for species diagnosis (Sites and Marshall, 2004) and several other criteria such as a large magnitude of divergence and sharp genetic discontinuity between the major clades, the populations appear to be at least three species. In fact, there is discrepancy on the taxonomy of *E. multiocellata*. One opinion is that *E. multiocellata* in China consists of five subspecies (Szczerbak, 2003): *E. m. multiocellata*, *E. m. yarkandensis*, *E. m. kozlowi*, *E. m. stummeri*, and *E. m. kokshaalensis*. An alternative hypothesis is that *E. multiocellata* in China contains only two subspecies (Eremchenko and Panfilov, 1999): *E. m. multiocellata* and *E. m. kozlowi*. Accordingly, the other three subspecies of *E. multiocellata* in China as Szczerbak (2003) interpreted them were elevated to specific status (Eremchenko and Panfilov, 1999; see Guo et al., 2010). The third possibility is that *E. multiocellata* is paraphyletic for mitochondrial haplotypes because of incomplete lineage sorting (Funk and Omland, 2003), which is widespread in recently diverged taxa that have speciated rapidly (Knowles and Carstens, 2007), and can obscure species relationships (Carstens and Knowles, 2007). With the current data, we cannot rule out the last two alternative hypotheses.

4.3. Racerunner lizards evolution timescale

Our results support a late Miocene to Pleistocene radiation of genus *Eremias*. This finding is similar to an albumin study by Mayer and Benyr (1994) who inferred that the MRCA of *Eremias* and *Mesalina* dated to about 12 Ma, but contrasts with the proposed scenario of Wan (2006), who suggested that the species divergence in Chinese *Eremias* began at about 18–9.1 Ma, with 5.5–3.4 Ma for subspecies divergence. In addition, our results echo Zhao et al. (2011) in that there is a late Miocene–Pliocene split between *E. argus* and *E. brenchleyi* (5.1 ± 1.2, wih 95% CI 2.1–7.6). Based on cyt *b* data, the divergence time of the two species was dated to about 4.1 ± 1.2 Ma (95% CI, 2.4–6.8 Ma) by using a Bayesian relaxed molecular-clock approach (Zhao et al., 2011).

In the Late Miocene (~10 Ma), faulting and uplifting of the Tibetan Plateau exceeded erosion, ending the continuous-plain topography. The period 12–7.6 Ma corresponds to the increased aridity of Central Asia and the uplift of the Tibetan Plateau (Harrison et al., 1992, 1995), which could have caused the split of the species of the genus *Eremias* in Central Asia, Southwest Asia, and the subgenus *Pareremias* species in East Asia (China, Mongolia and Korea). Subsequently, the nearly simultaneous environmental changes around 8 Ma, in and near Tibet, with tectonic events in the same area, have been interpreted as suggesting that the Tibetan Plateau grew rapidly at or just before *ca*. 8 Ma (see Molnar, 2005), and the rise of the Plateau affected regional climate changes, including a strengthening of the East Asian monsoon climate (Harrison et al., 1992, 1995; An et al., 2001). These events could have led to the evolutionary divergence of the remaining oviparous species and viviparous species in the subgenus *Pareremias*.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.06.022.

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