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Molecular Phylogeny of the *Liolaemus kriegi* Complex (Iguania, Liolaemini)

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Abstract: We provide a well-supported phylogenetic hypothesis for all recognized lineages of the *Liolaemus kriegi* complex based on a multilocus dataset. We used 29 individuals from the eight taxa included in this complex for which we sequenced eight gene regions (two mitochondrial and six nuclear). We implemented maximum likelihood and Bayesian inference methods for the mitochondrial, nuclear, and concatenated sequences and employed BEAST to estimate the species tree. The all genes concatenated analyses and the species trees recovered the *L. kriegi* complex as monophyletic with high support, including three described species (*L. kriegi*, *Liolaemus ceii*, and *Liolaemus buergeri*) and three previously identified candidate species (*Liolaemus* sp. A, *Liolaemus* sp. C, and *Liolaemus* sp. D), with *Liolaemus tregenzai* as a closely related taxon. Another previously proposed candidate species (*L.* sp. B) has a labile topological position that varies depending on the type of markers and analytical methods used. In the mitochondrial gene tree, *L.* sp. B is recovered within the *L. kriegi* complex whereas in the "all genes concatenated" analyses and in the nuclear species tree analyses, it is recovered outside of this complex as sister to *Liolaemus petrophilus* (a representative of the *L. petrophilus* group). Morphologically, *L.* sp. B is indistinguishable from *L. austromendocinus* (also included in the *L. petrophilus* group); thus, we do not consider *L.* sp. B as part of the *L. kriegi* complex. We estimated divergence times for the major clades of the complex based on the species tree hypothesis, and all were inferred to have a Pleistocene origin.

Key words: Concatenated gene tree; Divergence times; Lizard; Patagonia; Species tree

Systematists have a long history of using mitochondrial DNA (mtDNA) for reconstructing phylogenies, but the exclusive analysis of mitochondrial genomes could provide misleading depictions of the species tree (Brito and Edwards 2009). A variety of processes can be responsible for the discordance among gene trees and the species tree, but hybridization or incomplete lineage sorting (or both) are considered to be the most common (Funk and Omland 2003). These two processes can leave similar phylogenetic signals that might be difficult to distinguish without independent lines of evidence (Maddison 1997; Hird and Sullivan 2009; Joly et al. 2009). Hybridization is more widespread than previously considered, and recently separated, closely related species are most likely to hybridize (Mallet 2007). Several cases of hybridization have been reported in lizards (Leaché and McGuire 2006; McGuire et al. 2007; Leaché 2009), and Olave et al. (2011) found evidence of hybridization between two species of the highly diverse South American lizard genus *Liolaemus*. Incomplete lineage sorting is expected in species having rapid divergence or large effective population sizes (or both) and has also been reported in several groups of lizards (Godinho et al. 2005; McGuire et al. 2007) including species of *Liolaemus* (Morando et al. 2004; Avila et al. 2006). Follow-up studies that include nuclear loci and other types of data (e.g., morphological, ecological niche envelopes, etc.), analyzed in a precise geographical context, usually help to distinguish between these two processes (McGuire et al. 2007; Olave et al. 2011).

Given the limitations of mitochondrial genomes to recover phylogenetic relationships between species, there is an increasing use of multiple nuclear markers in studies of the evolutionary history of many types of organisms (Hackett et al. 2008; Stöck et al. 2008; Camargo et al. 2012). These multilocus studies avoid biases associated with mitochondrial loci and can accommodate nuclear gene tree heterogeneity that might result from incomplete lineage sorting, interspecific gene flow, estimation error, or mutational stochasticity (Pamilo and Nei 1988; Avise 1989; Maddison 1997). This is now a preferred approach for reconstructing the evolutionary history of closely related populations or species (Markolf et al. 2011). Traditionally, multilocus datasets have been analyzed using concatenated sequences with optimality criteria such as maximum parsimony, maximum likelihood (ML), and Bayesian inference (BI), but these methods do not take into account the between-locus stochasticity that is characteristic of species trees. Kubatko and Degnan (2007) recently showed that under some conditions, multilocus concatenation can lead to poor phylogenetic estimates. Among the conditions affecting phylogenetic reconstructions, the most important ones are coalescent assumptions, incomplete lineage sorting, and sampling a single individual per species (Kubatko and Degnan 2007).

Recognition of the limitations of concatenation analyses has led to a paradigm shift in systematic biology (Edwards et al. 2007; Edwards and Bensch 2009). This shift has been accompanied by the rapid development of algorithms using multiple gene trees to estimate a species tree (Liu and Pearl 2007; Kubatko et al. 2009; Heled and Drummond 2010). In these analyses, each gene tree is independently estimated (based on its estimated substitution rate and molecular clock), and the collection of gene trees is then analyzed in a coalescent framework to estimate the species tree. The structure of a species tree is determined by the processes of speciation, extinction, and in some cases hybridization, whereas the structure of the gene trees reflect not only the proliferation and loss of populations but also processes of mutation and coalescence between lineages (Knowles and Kubatko 2010).

The genus *Liolaemus* includes over 257 currently described species in temperate South America (Abdala and Quinteros 2014). The genus is distributed over a wide geographic area and occupies latitudes from 14°S–52°S, altitudes from 0 m to almost 5000 m, and a variety of climatic

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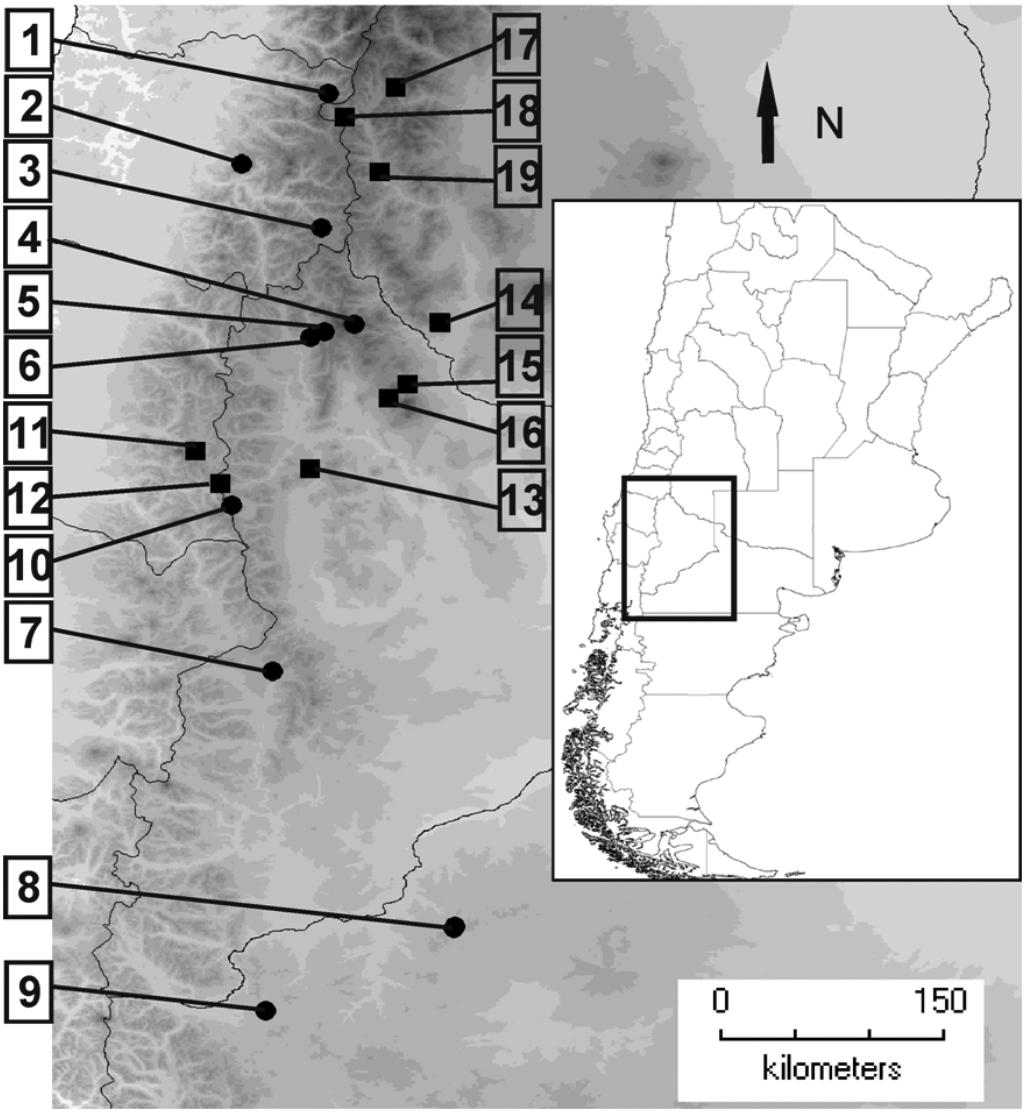

FIG. 1.—Map showing sampling localities of the *Liolaemus kriegi* complex and two related taxa. Circles and squares correspond to localities for described and candidate species, respectively. *Liolaemus buergeri* (1–6); *L. ceii* (7), *L. kriegi* (8, 9), and *L. tregenzai* (10); *L.* sp. A (11–13), *L.* sp. B (14); *L.* sp. C (15, 16), and *L.* sp. D (17–19). Locality 14 includes two sampled sites in close geographic proximity that are distinct (see Appendix).

regions ranging from the world's driest desert to the humid *Nothofagus* forests (Donoso-Barros 1966; Cei 1986, 1993; Lobo et al. 2010). *Liolaemus* includes two major subgenera, *Liolaemus* and *Eulaemus* (Laurent 1983; Etheridge 1995; Schulte et al. 2000; Pincheira-Donoso et al. 2008; Lobo et al. 2010). Within the *Liolaemus* clade, several species complexes have been described, one of which is the *L. kriegi* complex (Cei 1972). This group was defined as the *L. elongatus–kriegi* complex (Cei 1974), on the basis of several diagnostic morphological characters, and later redefined again as the *L. kriegi* complex (Cei 1986). More recently, different taxonomic groupings have been proposed for this complex (Morando et al. 2003; Avila et al. 2004; Lobo et al. 2010).

The *L. kriegi* complex can be considered as a natural set of closely related forms that extends latitudinally from 37°S (near El Planchón habitats typical of *L. buergeri* in Region VII in Chile) to its southern distributional limit at the northern edge of Chubut province at 42°S (Morando et al. 2003; Pincheira-Donoso and Núñez 2005). Until recently, the *L. kriegi* complex included three morphologically described species, *Liolaemus buergeri*, *Liolaemus kriegi*, and *Liolaemus ceii* (Fig. 1). In a recent taxonomic review of the genus, based mainly on morphological data, Lobo et al. (2010) also included *Liolaemus cristiani* within the *L. kriegi* complex. In an earlier mtDNA-based study, Morando et al. (2003) proposed three candidate species within this complex: *Liolaemus* sp. A, *Liolaemus* sp. B, and *Liolaemus* sp. C; they also proposed *Liolaemus* sp. 8 as a closely related taxon possibly nested within this species group. Based on morphology, specimens of *L.* sp. B seem to be conspecific with specimens of *L. austromendocinus* (*L. petrophilus* group), and there is no evidence that these two taxa are different species (Feltrin 2013). Based on mitochondrial markers, Medina et al. (2014) recovered *L.* sp. B within the *L. kriegi* complex, consistent with results from Morando et al. (2003), and hypothesized either ancient introgression or hybrid origin for this taxon. *Liolaemus* sp. 8 has been described as *L. tregenzai* (Pincheira-Donoso and Scolaro 2007). Using traditional morphological characters, Lobo

et al. (2010) included this species in the *elongatus* group based on characters listed in the original species description (F. Lobo, personal communication), but a recently published phylogeographic study, recovered *L. tregenzai* (*L.* sp. 8) as part of the *L. kriegi* complex (Medina et al. 2014). Nonetheless, we believe that further evidence is needed in order to test its phylogenetic position. In a recently published morphological study that included specimens sampled by Morando et al. (2003) and those from the type locality of *L. buergeri*, Medina et al. (2013) showed that these specimens represent morphologically distinct lineages and, therefore, recognized a new candidate species within the *L. kriegi* complex called *Liolaemus* sp. D. The phylogeographic study of Medina et al. (2014), based on two mitochondrial and two nuclear genes, found that the *L. kriegi* complex includes: *L. buergeri*, *L. kriegi* + *L. ceii*, *L.* sp. A, *L.* sp. B, *L.* sp. C, and *L.* sp. D and might also include *L. tregenzai*.

Taxonomic knowledge of the *Liolaemus kriegi* complex is still limited, with species limits unclear and no inclusive phylogenetic hypothesis available. The main objective of our study was to provide a well-supported phylogenetic hypothesis including all recognized lineages of the *Liolaemus kriegi* complex, based on a multilocus data set (six nuclear and two mitochondrial genes), using traditional concatenated approaches and a multispecies coalescent method. We included individuals from all lineages thought to be included in this complex: four described species (including *L. tregenzai*) and three candidate species, plus the closely related taxon (*L.* sp. B).

MATERIALS AND METHODS

Taxon Sampling

We sampled three of the four described species from their type localities and, because the type locality of *L. kriegi* is not precise and describes only a general region, we sampled a population located 27 km northwest from its most-probable type locality. Candidate species A–D were obtained from the sites at which these lineages were originally collected (Morando et al. 2003; Medina et al. 2013); collectively these localities represent the known geographic range of the complex (Fig. 1). We also included individuals of two related species of the *L. kriegi* complex representing the *L. elongatus* complex (*L. elongatus*) and *L. petrophilus* group (*L. petrophilus*); these three groups comprise the *L. elongatus–kriegi* complex of the subgenus *Liolaemus* (sensu Cei 1975), and we used as an outgroup *Liolaemus bibronii* from another clade within the subgenus. Voucher specimens and tissues were catalogued in the herpetological collection Centro Nacional Patagónico in Puerto Madryn (LJAMM-CNP), Argentina (http://www.cenpat.edu.ar/nuevo/colecciones03.html). We used a total of 29 specimens (see Appendix for detail on examined material).

Gene Sampling

We collected complete sequence data for most individuals. The two mitochondrial fragments amplified were cytochrome *b* (cyt-*b*; 712 base pairs [bp], $n = 24$; Kocher et al. 1989) and 12S (868 bp, $n = 29$; Wiens et al. 2010). The six nuclear fragments included three protein-coding loci (NPCL): EXPH5 (841 bp, $n = 24$), KIF24 (489 bp, $n = 22$), MXRA5 (848 bp, $n = 20$; Portik et al. 2011); one intron: BA3 (265 bp, $n = 17$; Waltari and Eduards 2002); and two anonymous loci (ANL): LPB4G (656 bp, $n = 24$; Olave et al. 2011), LDA1B (517 bp, $n = 23$; Camargo et al. 2012). Some sequences we used were taken from Medina et al. (2014) and Avila et al. (2015); new sequences generated unique to this paper were deposited in GenBank (accession numbers KP789547–KP789618). Two additional cyt-*b* sequences were used from Morando et al. (2003), one each representing *L. ceii* and *L.* sp. D (GenBank AY367810.1 and AY173631.1).

Molecular Data

Genomic DNA was extracted using the Qiagen® DNeasy® 96 Tissue Kit (Qiagen) for animal tissues following the protocol provided by the manufacturer. Protocols for PCR and sequencing for the mitochondrial genes follow Morando et al. (2003), while protocols for nuclear loci are according to Noonan and Yoder (2009). All sequences (ANL, NPCL, intron, and mitochondrial) were edited using Sequencher™ v4.8 (2007 Gene Codes Corporation, Inc.), and NPCL were translated to amino acids to check for stop codons, while the other loci were aligned by eye to maximize blocks of sequence identity. We did not use alignment software and, in all cases, missing data were coded as "?" For each gene, we selected the best-fitting evolutionary model in JModelTest v0.1.1 (Table 1; Guindon and Gascuel 2003; Posada 2008). Recombination was tested and excluded in nuclear genes using RDP: Recombination Detection Program v3.44 (Martin and Rybicki 2000; Heath et al. 2006). Before we ran the concatenated analyses, we evaluated different codon partitions for the cyt-*b* fragment through Bayesian factor analysis (Kass and Raftery 1995) on MrBayes v3.2 (Ronquist and Huelsenbeck 2003). The first model we tested was an unpartitioned model and the second one was partitioned by codon. For both models we ran 10 million generations with their respective selected molecular evolution models. We followed the same scheme for the nuclear coding genes. Based on these results, we used a combined matrix with partitioned cyt-*b* and unpartitioned 12S and nuclear gene.

Phylogenetic Analyses

Separate gene trees analyses.—We used BI as implemented in MrBayes v3.2 (Ronquist and Huelsenbeck 2003) for each of the eight genes; we used Tracer v1.5.0 (Rambaut and Drummond 2007) to assess convergence. Because Bayesian posterior probabilities are often quite different from ML bootstrap values, we also conducted ML analyses with the program RAxML v7.0.4 (Stamatakis 2006) to obtain bootstrap values based on 1000 rapid replicates and the GTRGAMMA evolution model for all genes.

Combined gene trees analyses.—In order to explore a wider range of scenarios, we also ran concatenated analyses for two different data combinations: (1) the combined mtDNA markers, and (2) all gene regions except for the mitochondrial genes of *L.* sp. B (for which an ancient mitochondrial introgression or hybridization was hypothesized). In both combinations, we again implemented BI and ML methods. Bayesian analyses were conducted using MrBayes v3.2, and equilibrium samples (assessed with Tracer v1.5.0) were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) were considered

TABLE 1.—Summary of each gene sampled from representatives of the *Liolaemus kriegi* complex, with details of the function and the best-fitting models of molecular evolution (selected with JModelTest) implemented in BEAST and in MrBayes. For all genes used in the RAxML analyses, we used the GTR-GAMMA model. Nst = Nucleotide substitution type.

Gene	Function	JModelTest	BEAST	MrBayes
Cytochrome *b* 1st position	Mitochondrial coding	K80+G	HKY+G	Nst = 2, rates = gamma
Cytochrome *b* 2nd position	Mitochondrial coding	HKY	HKY	Nst = 2, rates = equal
Cytochrome *b* 3rd position	Mitochondrial coding	TIM2	GTR	Nst = 6, rates = equal
12S	Mitochondrial ribosomal	TIM3+I+G	GTR+G	Nst = 6, rates = gamma
BA3	Nuclear intron	JC	HKY	Nst = 1, rates = equal
MXRA5	Nuclear coding	TPM2μf	HKY	Nst = 2, rates = equal
LDAB1D	Nuclear anonymous	F81	HKY	Nst = 1, rates = equal
LPB4G	Nuclear anonymous	TPM3μf+G	HKY+G	Nst = 2, rates = gamma
EXPH5	Nuclear coding	TPM3uf	GTR	Nst = 6, rates = equal
KIF24	Nuclear coding	HKY+G	HKY+G	Nst = 2, rates = gamma

significant when ≥0.95 (Huelsenbeck and Ronquist 2001). Likelihood bootstrap analyses were conducted using RAxML v7.0.4 based on 1000 rapid bootstrap analyses and the GTRGAMMA evolution model.

Species tree approach.—We ran analyses for two different data combinations: (1) the all nuclear genes combined, and (2) all gene regions except for the mitochondrial genes of *L.* sp. B (for the reason given above). To reconstruct the species trees incorporating the multispecies coalescent approach, we ran two independent analyses for each data combination with BEAST v1.6.0 (Drummond and Rambaut 2007), which is also a Bayesian approach, for 300 million generations, sampled every 1000 generations, and assuming a Yule tree prior. To ensure that convergence was reached before default program burn-in values, we evaluated convergence by examining likelihood and parameter estimates over time in Tracer v1.5.0. All parameters had effective sample sizes greater than 200, a good indication that the analyses adequately sampled the posterior distributions. We combined the parameters of the trees from the two runs in LogCombiner v1.6.0 and then summarized those trees with TreeAnotator v1.6.0 to produce a maximum clade credibility tree and median node heights (this option rescales the node heights to reflect the posterior median node heights for the clades contained in the target tree). For this analysis, individuals were aggregated (identified) into species on the basis of a published phylogeographic study (Medina et al. 2014) in combination with their geographic distributions (sampling localities).

Divergence Time Analysis

We estimated divergence times between the main clades of the *L. kriegi* complex based on the species tree. We did not include mitochondrial genes of *L.* sp. B because of its possible hybrid origin (detailed below). We used the all genes combined dataset for these analyses and performed a likelihood ratio test (LRT) using JModeltest v0.1.1 (Guindon and Gascuel 2003; Posada 2008) to evaluate deviation from a strict molecular clock for each gene. Because there is no fossil from the subgenus *Liolaemus* to calibrate the tree, we used the following rates of evolution: cyt-*b* (2.23^{-2}, 95% HPD 1.43^{-2}–3.14^{-2}), 12S (5.76^{-3}, 95% HPD 3.92^{-3}–7.82^{-3}) and MXRA5 (6.56^{-4}, 95% HPD 4.32^{-4}–9.05^{-4}), taken from Fontanella et al. (2012) based on those authors' estimates of a *Eulaemus* fossil. We used BEAST v1.6.0 to estimate divergence times based on a species tree method, with a relaxed molecular clock model under the uncorrelated relaxed clock distribution for all genes (Table 2; Drummond and Rambaut 2007). Two independent analyses were performed for 100 million generations, sampled every 1000 generations, and assumed a Yule tree prior as above. Parameter convergence was checked using Tracer v1.5.

RESULTS

Phylogenetic Analyses

We illustrate main phylogenetic results with Bayesian concatenated mitochondrial and all genes trees, with ML bootstrap support values (Fig. 2a,c), and two species trees (nuclear only and all genes except mitochondrial genes for *L.* sp. B; Fig. 2b,d). Separated gene tree results are provided in the Supplementary Material. The mitochondrial gene tree recovered the *L. kriegi* complex, including four described species (*L. kriegi*, *L. ceii*, *L. buergeri*, *L. tregenzai*) and four candidate species (*L.* sp. A, *L.* sp. C, and *L.* sp. D, *L.* sp. B), with high support (BI = 0.99; ML = 95; Fig. 2a). The majority of the species were recovered as clades with high support, with the exception of *L. ceii*. Relationships between

TABLE 2.—Results of a likelihood-ratio test (LRT) for a molecular clock based on samples from representatives of the *Liolaemus kriegi* complex. The likelihood values (expressed as the negative natural logarithm [−ln L]) are given for an enforced (E) or nonenforced (NE) molecular clock along with the LRT and *P* values.

Gene	−ln L (E)	−ln L (NE)	LRT	*P*-value
Cytochrome *b*	1936.564	1927.651	17.823	<0.0001
12S	1810.255	1796.440	27.629	<0.0001
BA3	463.471	458.270	10.400	0.0013
MXRA5	1269.670	1266.682	05.977	0.0145
LDAB1D	838.904	836.112	05.581	0.0181
LPB4G	1139.983	1130.145	19.677	<0.0001
EXPH5	1453.554	1449.711	07.686	0.0056
KIF24	944.589	937.658	13.862	0.0002

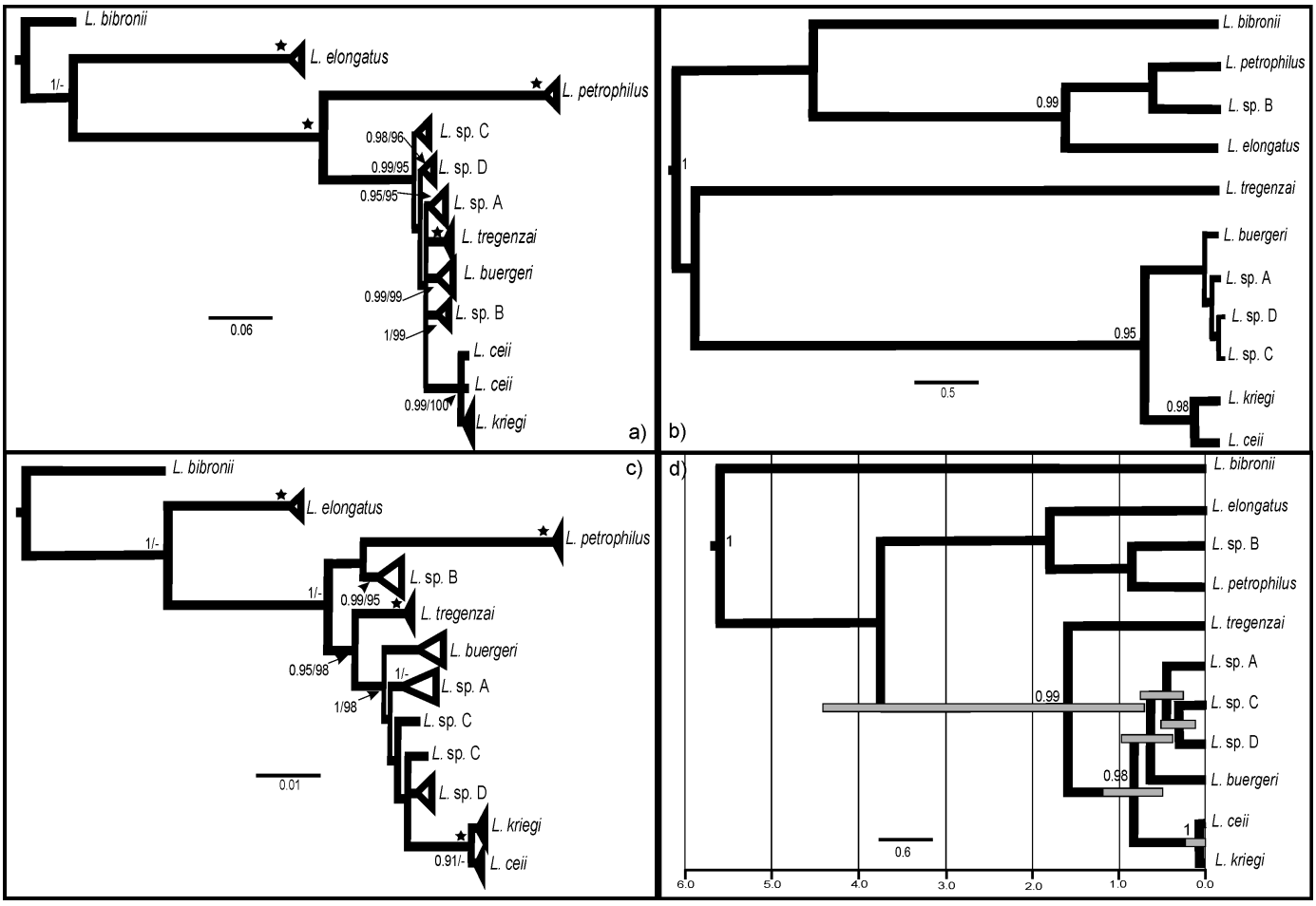

FIG. 2.—Different phylogenies for the *Liolaemus kriegi* complex and related taxa: (a) Bayesian concatenated mitochondrial tree; (b) BEAST (v1.6.1) species tree based on nuclear genes with posterior probability values; (c) Bayesian concatenated tree that includes all genes except the mitochondrial genes of *L.* sp. B; and (d) BEAST species tree without *L.* sp. B mitochondrial genes, with posterior probability values. Where given at each node in (a) and (c), Bayesian posterior probability values (BI) are shown to the left of the slash and maximum likelihood (ML) bootstrap values are to the right (the "-" indicates no significant support); stars on nodes represent BI = 1.0 and ML = 100%. Estimated divergence times in (d) are marked in light grey; units on the abscissa are expressed in millions of years ago.

these clades did not have statistical support and internodes were very short in all cases; however, all terminals corresponding to *L. ceii* and *L. kriegi* were recovered as a strongly supported clade (BI = 0.99; ML = 100).

The all genes concatenated analyses recovered a highly supported clade (BI = 0.95; ML = 98; Fig. 2c) that included four described species (*L. kriegi*, *L. ceii*, *L. buergeri*, *L. tregenzai*) and three candidate species (*L.* sp. A, *L.* sp. C, *L.* sp. D). *Liolaemus tregenzai* was sister to the rest of the species of the *L. kriegi* complex, with high support (BI = 1.0; ML = 98).

The nuclear species tree reconstruction recovered *Liolaemus* sp. B external to the *L. kriegi* complex, and nested within the clade (*L. petrophilus* + *L. elongatus*), with high statistical support (PP = 0.99; Fig. 2b). Similarly, all separate nuclear gene trees recovered *L.* sp. B outside of the *L. kriegi* complex (Supplementary Material), and *L. tregenzai* was recovered as the sister taxon of the rest of the species of the *L. kriegi* complex which formed a distinct clade (PP = 0.95). *Liolaemus kriegi* and *L. ceii* were recovered as sister taxa with high support (PP = 0.98).

In agreement with the all genes concatenated analyses, the species tree approach for which the mitochondrial genes of *L.* sp. B were excluded (Fig. 2d) recovered *L.* sp. B outside the *L. kriegi* complex and nested within the *L. elongatus* and *L. petrophilus* group representatives, although there is no statistical support for this relationship. *Liolaemus tregenzai* was recovered with high support (PP = 0.99) as the sister taxon of the rest of the species of the *L. kriegi* complex (PP = 0.98) and with *L. ceii* as sister to *L. kriegi* (PP = 1.0).

Divergence Time Estimation

The divergence of *L. tregenzai* from the rest of the *L. kriegi* complex was estimated to have occurred 3.7 million years ago (Mya; 95% HPD = 5.3–2.6 Mya; Fig. 2d). The split within this clade of the ancestral taxon from the rest of the species in the complex occurred an average of 1.6 Mya (95% HPD = 0.704–4.409). Divergences among lineages within this last clade occurred entirely within the Pleistocene (2.6–0.001 Mya).

DISCUSSION

Phylogenetic Analyses

We have presented the first comprehensive multilocus phylogeny of the *Liolaemus kriegi* complex, including all the

recognized lineages, and by implementing traditional concatenated methods and species tree approaches (Liu and Pearl 2007). Almost all analyses found strong support for the monophyly of the *L. kriegi* complex, including the three described species (*L. kriegi*, *L. ceii*, *L. buergeri*) and three of the candidate species (*L.* sp. A, *L.* sp. C, *L.* sp. D). The mitochondrial tree included *L. tregenzai* and *L.* sp. B within the *L. kriegi* complex, whereas the nuclear species tree approach did not include *L.* sp. B within the complex. Both species trees and the all genes concatenated tree consistently recovered *L. tregenzai* as the sister taxon of the rest of the *L. kriegi* complex. Thus, the inclusion of *L. tregenzai* as part of this complex is questionable, and detailed analyses based on wider taxonomic sampling, including other members of the *L. petrophilus* group, are needed to assess the phylogenetic affiliation of *L. tregenzai*.

In the concatenated mitochondrial tree, most of the taxa included in the *L. kriegi* complex were recovered as clades with high support, with the exception of *L. ceii*. Given that this is a single locus analysis, the inclusion of *L.* sp. B within the *L. kriegi* complex is worth noting and is in agreement with previous cyt-*b* results (Morando et al. 2003; Medina et al. 2014). This result contrasts, however, with the nuclear species tree analyses. Morando et al. (2003) called attention to the fact that, although the mitochondrial gene tree recovered *L.* sp. B within the *L. kriegi* complex, the specimens used in that study were phenotypically almost identical to *L. austromendocinus*, a species belonging to the *L. petrophilus* group. A recent study did not report statistically supported differences in morphology between *L.* sp. B and *L. austromendocinus* and, based on 16 nuclear genes, *L.* sp. B was recovered within the *L. petrophilus* group (Feltrin 2013). The morphological similarity and the unresolved phylogenetic position of *L.* sp. B (mitochondrial vs. nuclear genes) led Medina et al. (2014) to suggest that *L.* sp. B might have experienced mitochondrial introgression in the past or perhaps have a hybrid origin. The results presented here are in agreement with both of these hypotheses, and detailed analyses, based on more-extensive population and gene sampling, are needed in order to fully evaluate these alternatives.

Although coalescent phylogenetic reconstructions might present lower posterior probabilities compared to those recovered by concatenation methods, this likely reflects the conflicting genealogies of unlinked loci used in a multispecies coalescent framework, an issue that is not accounted for by the concatenation method (Avise 1994; Wollenberg and Avise 1998; Edwards et al. 2007; Liu and Pearl 2007). As in many other empirical studies, we found fewer nodes with strong statistical support in the species tree results than in the all genes concatenated analyses (cf. Fig. 2b,d), but the same three nodes were recovered with high support using both approaches for relationships among the focal taxa. We feel it likely that the stochastic history of each marker, and the relatively recent origin of the species of the *L. kriegi* complex, is responsible for a low number of nodes with high statistical support. Despite poor resolution at some nodes, we advocate the use of multispecies coalescent methods because they generate clear evolutionary hypotheses that can be tested with both phylogenetic and phylogeographic methods. The inclusion of more markers and individuals per taxon will allow refinement of these hypotheses in future studies of the *L. kriegi* complex and the evaluation of *L.* sp. B.

Evolutionary History and Divergence Times

All the estimated divergence times among clades of the *Liolaemus kriegi* complex occurred within the last 1.5 Mya, placing the radiation of this group well within the Pleistocene. After the initial divergence of this clade, the Great Patagonian Glaciations took place between 1.168 and 1.016 Mya, and these were followed by 14–16 glacial geoclimatic events separated by warm interglacial periods. These glacial–interglacial cycles were characterized by temperature shifts of up to 7°C (Rabassa et al. 2005), and some ice sheets that formed during glacial advances reached areas of Neuquén Province now inhabited by the *L. kriegi* complex. The orogenic history of the Neuquén Province produced a complex landscape; the westernmost region is strictly Andean and the northwestern region includes at least five high mountain peaks. In contrast, the west-central portion of the mountain range is more acute but of lower elevation while the easternmost area is characterized by low isolated hills. This topographic complexity, along with the glacial cycles, probably shifted the geographic distribution of these lineages on multiple occasions. Some populations likely persisted in isolated pockets of suitable environments while others almost certainly shifted their distributions either altitudinally (on mountain peaks), latitudinally, or both. Collectively, these events could have promoted both the divergence of closely related lineages and (possibly) secondary contact and introgression on a very recent geological time scale.

Taxonomic Implications

The phylogenetic analyses based on the concatenated multilocus approach recovered with strong support the species of the *Liolaemus kriegi* complex with *L. tregenzai* as its sister taxon (previously included in the *L. elongatus* group by Lobo et al. 2010 but without a formal phylogenetic analysis). Detailed morphological analyses that compare *L. tregenzai* with members of the *L. elongatus* and *L. kriegi* complexes are needed in order to provide further support for the taxonomic affiliation of this taxon. A recent morphological comparison among *L. buergeri* and the candidate species *L.* sp. A, *L.* sp. C, and *L.* sp. D revealed several differences, including the degree of sexual dimorphism (Medina et al. 2013). In the species tree reported here, *L.* sp. C is the sister group to *L.* sp. D; these taxa are morphologically similar, but their distributional ranges are separated by the Colorado River, which serves as a barrier for gene flow for other lizard species (Morando et al. 2007; Feltrin 2013). Similarly, the Colorado River could have recently isolated *L.* sp. C from *L.* sp. D; if population sizes have remained relatively large throughout this isolation history, then many loci would show incomplete lineage sorting.

Liolaemus ceii and *L. kriegi* were recovered as reciprocally monophyletic sister taxa in almost all analyses except the mtDNA. The geographic ranges of these species overlap extensively, and Morando et al. (2003) suggested that they might represent one lineage. A recent phylogeographic study of these clades found a similar pattern, with almost complete geographic overlap and no molecular differences (Medina et al. 2014). Present evidence supports the hypothesis that

these two clades are conspecific, but additional classes of morphological data (Aguilar et al. 2013) and rapidly evolving molecular markers are needed to distinguish between the alternatives of conspecific versus incipient species.

We have shown that the *L. kriegi* group is a relatively young species complex that includes three described and three candidate species, with different levels of support for their taxonomic status. The evidence indicates that most of the divergence of these taxa occurred during the last 500,000 yr. If further support is found for the distinct nature of these taxa, most of them would represent microendemics whose evolution might have been favored by the recent glacial cycles extending over the topological landscape of Neuquén Province.

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SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found online at http://dx.doi.org/10.1655/HERPETOLOGICA-D-13-00083.S1.

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APPENDIX
Specimens Examined

List of specimens from representatives of the *Liolaemus kreigi* complex that were sequenced for this study; included are attribution of the taxon, sampling localities, and geographic coordinates (GPS datum = WGS84). The numbers in Locality correspond to values depicted in Fig. 1. LJAMM-CNP = Centro Nacional Patagónico in Puerto Madryn.

Species	Descriptor (year)	LJAMM-CNP	Locality	Latitude	Longitude
L. buergeri	Werner (1907)	14119	(1) Chile; VII Región; Curicó; Road to "El Planchon," 6.3 km junction road to Pichuante-Paso Vergara	−35.13556	−70.51617
		14090	(2) Chile; VII Región; Talca; "El Peine" Hill	−35.59944	−71.04455
		14096	(3) Chile; VII Región; Talca; Laguna del Maule	−36.01694	−70.56208
		6413	(4) Argentina; Neuquén; Minas; Paso Malo, Arroyo Domuyo	−36.64622	−70.36124
		6439	(5) Argentina; Neuquén; Minas; Arroyo Covunco, near Puente de Carrizo	−36.68926	−70.5407
		5294	(6) Argentina; Neuquén; Minas; 14 km S Aguas Calientes	−36.72819	−70.62517
L. ceii	Donoso-Barros (1971)	2613, 13870	(7) Argentina; Neuquén; Picunches; Pampa de Lonco Luan	−38.90402	−70.85525
L. kriegi	Müller and Hellmich (1939)	5562	(8) Argentina; Río Negro; El Cuy; 20 km S Mencue	−40.56794	−69.74980
		14301	(9) Argentina; Río Negro; Pilcaniyeu; Dina Huapi	−41.11947	−70.89741
L. tregenzai	Pincheira-Donoso and Scolaro (2007)	13908, 13918	(10) Argentina; Neuquén; Ñorquín; W Termas de Copahue	−37.81983	−71.10108
L. sp. A		3433, 13991	(11) Chile; VIII Región; Bío Bío; Laguna de la Laja	−37.47213	−71.32000
		13907	(12) Argentina; Neuquén; Ñorquín; W Termas de Copahue, 1 km from the exit	−37.81983	−71.10108
		5339	(13) Argentina; Neuquén; Ñorquín; 20 km S El Cholar	−37.58513	−70.62688
L. sp. B		2667	(14) Argentina; Mendoza; Malargüe; 5 km N Ranquil Norte	−36.63250	−69.83722
		5756	(14) Argentina; Mendoza; Malargüe; 3.2 km N Ranquil Norte	−36.64013	−69.83205
L. sp. C		2615	(15) Argentina; Neuquén; Chos Malal; 15 km N Los Barros	−37.03472	−70.03527
		12148	(16) Argentina; Neuquén; Chos Malal; Entrance "Área Natural Protegida Tromen," Laguna Los Barros	−37.12991	−70.14502
L. sp. D		2758	(17) Argentina; Mendoza; Malargüe; 7 km N Las Leñas	−35.09888	−70.10861
		5797	(18) Argentina; Mendoza; Malargüe; 11.4 km S Termas del Azufre	−35.29727	−70.41355
		2744	(19) Argentina; Mendoza; Malargüe; Mallines Colgados	−35.65083	−70.20222
L. petrophilus	Donoso-Barros and Cei (1971)	6982	Argentina; Río Negro; El Cuy; Cerro Policia	−39.73380	−68.47905
		11355	Argentina; Río Negro; 9 de Julio; 9.7 km N Sierra Colorada	−40.56138	−67.85991
L. elongatus	Koslowsky (1896)	3715	Argentina; Chubut; Paso de Indios; 110 km S Paso de Indios	−44.51736	−69.19052
		9060	Argentina; Chubut; Sarmiento; 87.8 km SE junction Provincial Road 20, between Los Flamencos and La Blanca ranches	−44.73952	−69.60811
		8852	Argentina; Chubut; Cushamen; 9.1 km E Embarcadero La Cancha, road to Gualjaina	−42.79561	−70.85225
L. bibronii	Bell (1843)	8211	Argentina; Río Negro; Valcheta; Aguada del Toro, Meseta de Somuncurá	−41.28447	−66.47363