



## Alternative methods of phylogenetic inference for the Patagonian lizard group *Liolaemus elongatus-kriegi* (Iguania: Liolaemini) based on mitochondrial and nuclear markers

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### ABSTRACT

We present different approaches to a multi-locus phylogeny for the *Liolaemus elongatus-kriegi* group, including almost all species and recognized lineages. We sequenced two mitochondrial and five nuclear gene regions for 123 individuals from 35 taxa, and compared relationships resolved from concatenated and species tree methods. The *L. elongatus-kriegi* group was inferred as monophyletic in three of the five analyses (concatenated mitochondrial, concatenated mitochondrial + nuclear gene trees, and SVD quartet species tree). The mitochondrial gene tree resolved four haploclades, three corresponding to the previously recognized complexes: *L. elongatus*, *L. kriegi* and *L. petrophilus* complexes, and the *L. punmahuida* group. The BEAST species tree approach included the *L. punmahuida* group within the *L. kriegi* complex, but the SVD quartet method placed it as sister to the *L. elongatus-kriegi* group. BEAST inferred species of the *L. elongatus* and *L. petrophilus* complexes as one clade, while SVDquartet inferred these two complexes as monophyletic (although with no statistical support for the *L. petrophilus* complex). The species tree approach also included the *L. punmahuida* group as part of the *L. elongatus-kriegi* group. Our study provides detailed multilocus phylogenetic hypotheses for the *L. elongatus-kriegi* group, and we discuss possible reasons for differences in the concatenation and species tree methods.

### 1. Introduction

Biologists interested in conservation and macroecology have shown concern about the “taxonomic inflation” that has been evidenced in many systematic studies (Isaac et al., 2004). In these reports, new species are usually diagnosed based solely on a single or a few molecular markers of the matrilineal mitochondrial locus. Consistent evidence indicates that, in some cases, the exclusive use of the mitochondrial locus for phylogenetic reconstructions could lead to erroneous species tree hypotheses (Brito and Edwards, 2009). Several processes can lead to discordance between gene and species trees, but the most common are hybridization and incomplete lineage sorting (Funk and Omland, 2003). These two processes can leave a similar phylogenetic signal that might be difficult to distinguish without independent lines of evidence (Hird and Sullivan, 2009; Joly et al., 2009; Maddison, 1997). Given the limitations of the mitochondrial genome to

infer species trees, and the processes mentioned above, there has been an increase in the use of multiple nuclear markers in phylogenetic/phylogeographic studies of many types of organisms, including lizards (e.g. Avila et al., 2012; Camargo et al., 2012; Hackett et al., 2008; Stöck et al., 2008). However, these multi-locus studies should take into account the gene tree heterogeneity that can result from incomplete lineage sorting, interspecific gene flow, estimation error, or mutational stochasticity (Avice, 1989; Maddison, 1997; Pamilo and Nei, 1988).

Dayrat (2005) proposed considering species as hypotheses, and this conceptualization is at the core of the general lineage species (GLC) concept, which defines species as a population or metapopulation of lineages that have diverged separately from other similar lineages (de Queiroz, 2005). Under the GLC, a species is the only biological category above organism, and speciation events are stochastic processes of lineage separation. However, different characters/attributes are not expected to differ in a predictable manner in most cases. Consequently,

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this view of species as evolutionary lineages isolated by stochastic processes suggests that researchers will often find inconsistencies between different lines of evidence, due to the nature of the evolution of different types of characters, i.e., molecular, phenotypic, proteomic (Adams et al., 2009).

With the development of DNA sequencing, a rapid increase on the use of molecular data alone to define species boundaries has been popularized among some taxonomists (e.g. Blaxter, 2003; Hebert et al., 2003). More recently, others proposed a more comprehensive and integrative taxonomic approach that includes several independent lines of evidence in addition to the molecular characters, e.g., niche envelopes (but see Meik et al., 2015), and several classes of morphological characters (Aguilar et al., 2017; Dayrat, 2005; Padial and De la Riva, 2007; Padial and De La Riva, 2009; Will et al., 2005). In spite of this, among closely related species/populations, incongruent lines of evidence have become interesting in themselves, as they can provide evidence of evolutionary processes that contribute to the origin and maintenance of biodiversity (Knowles and Carstens, 2007; Moritz, 1994; Moritz et al., 1995; Templeton, 2001). Well-supported hypotheses of species boundaries require multiple lines of evidence, ideally combined with statistical tests that this concordance among data sets is not due to stochastic processes (Padial and De la Riva, 2007, 2009).

*Liolaemus* is the largest genus of vertebrates endemic to southern South America, and it is the world's second-richest genus (in species numbers) of extant amniotes (Pincheira-Donoso et al., 2013). Within *Liolaemus* two subgenera are recognized, *Eulaemus* and *Liolaemus* (*sensu stricto* [SS]) (Etheridge, 1995; Laurent, 1983; Morando et al., 2003; Schulte II et al., 2000). Within the subgenus *Liolaemus* (SS), also known as the *L. chiliensis* group, the *L. elongatus-kriegi* group was initially defined by Cei (1979) based on morphological characters, and it has a long taxonomic history which we describe in extensive detail (see Appendix A). In the first molecular phylogenetic/phylogeographic study of this group (from mtDNA sequence data), Morando et al. (2003) differentiated three complexes: *L. elongatus*, *L. kriegi* and *L. petrophilus*. These complexes have been supported by morphological data in some recent studies (Avila et al., 2012), whereas others (Lobo et al., 2010) have recognized modified *L. kriegi* and *L. elongatus* groups (= *L. elongatus* + *L. petrophilus sensu Morando et al. (2003)* complexes). However, concatenated and species tree methods based on presumably unlinked nuclear gene regions, have not been carried out on this group.

In the last 30 years, several authors (Lobo, 2001, 2005; Lobo et al., 2010; Nuñez et al., 1991; Videla and Cei, 1996) have suggested further species additions to the *L. elongatus-kriegi* group, such as the *L. cristiani* clade (Esquerré et al., 2013). Most of its component species were described based on morphological characters (Abdala et al., 2010; Cei, 1974; Donoso Barros and Cei, 1971; Espinoza and Lobo, 2003; Espinoza et al., 2000; Esquerré et al., 2013; Hulse, 1979; Koslowsky, 1896; Müller and Hellmich, 1939; Nuñez et al., 1991; Pincheira-Donoso and Scolaro, 2007; Quinteros et al., 2008; Werner, 1907), and a few of these have been corroborated by molecular (mostly mtDNA) characters (Avila et al., 2015, 2004, 2010, 2012; Escobar Huerta et al., 2015; Torres-Pérez et al., 2009; Troncoso-Palacios et al., 2015).

One of the three main subgroups of the *L. elongatus-kriegi* is the *L. kriegi* complex (squares in Fig. 1). This species' complex extends from 37°S to 42°S (Medina et al., 2014; Morando et al., 2003; Pincheira-Donoso and Nuñez, 2005), and includes four nominal species: *L. buergeri*, *L. kriegi*, *L. ceii* and *L. zabalai* (Cei, 1986; Medina et al., 2013, 2014; Troncoso-Palacios et al., 2015), and possibly a fifth, *L. tregenzai* (Medina et al., 2014). Three candidate species are also identified in this complex based on mitochondrial markers: *L. sp. A*, *L. sp. B*, and *L. sp. C*; samples from one locality of *L. sp. A* were recently described as *L. zabalai* (Troncoso-Palacios et al., 2015).

The other main subgroup is the *L. elongatus* complex (Fig. 1, set of circles) that is distributed from 35° S to 45° S (Morando et al., 2003), and includes eight nominal species: *L. elongatus*, *L. shitan*, *L. antumalguen*, *L. chillanensis*, *L. carlosgarini*, *L. burmeisteri*, *L. smaug*, *L.*

*crandalli*, and three candidate species: *L. sp. 5*, *L. sp. 6* and *L. sp. 7* (Morando et al., 2003). However, the taxonomy might be more complex as evidenced by Medina et al. (2017). In this phylogeographic study, the authors clearly resolved seven described species, but individuals within the *L. elongatus* haploclade were interdigitated with individuals identified as *L. shitan*. Likewise, the *L. carlosgarini* haploclade also included individuals of *L. sp. 1*, and the *L. antumalguen* haploclade included individuals of *L. sp. 7* identified by Morando et al. (2003). Medina et al. (2017) also identified three candidate species: *L. sp. 2*, *L. sp. 3*, and *L. sp. 6*.

Finally, the *L. petrophilus* complex, which extends from northern Tucumán Province in the north to southern Chubut Province, Argentina (Fig. 1, set of triangles), includes ten described species; *L. austromendocinus*, *L. capillitas*, *L. dicktracyi*, *L. gununakuna*, *L. heliodermis*, *L. parvus*, *L. petrophilus*, *L. talampaya*, *L. tulkas*, and *L. umbrifer*. *Liolaemus* sp. B (mentioned above as part of the *L. kriegi* complex) is inferred nested in this complex with nuclear data.

As summarized above, it is clear that the current taxonomic knowledge of the *Liolaemus elongatus-kriegi* group is still limited. Here, we provide, to our knowledge, the first multilocus phylogenetic hypothesis for this group, which is based on the most complete taxon sampling to date. The main goal of this paper is to provide the first comprehensive molecular phylogenetic hypothesis including all described taxa known from the three complexes: *L. kriegi*, *L. elongatus* and *L. petrophilus* (*sensu Morando et al., 2003*). We present novel phylogenetic hypotheses, further discuss the taxonomic and systematic implications for the *Liolaemus elongatus-kriegi* group, and at a more inclusive level, for the entire genus.

## 2. Materials and methods

### 2.1. Taxon sampling

We included individuals from type localities of the six described species of the *L. kriegi* complex, nine of the *L. elongatus* complex, and from the ten described species of the *L. petrophilus* complex. In addition, we included samples from nine localities of eight previously proposed candidate species of the *L. elongatus* and *L. kriegi* complexes. Jointly, sampled localities represent the known geographic ranges for these three complexes (Fig. 1). We sampled species from several other clades of *Liolaemus* that have been considered closely related to the *Liolaemus elongatus-kriegi* group, including *L. thermarum*, *L. coeruleus*, *L. neuquensis*, *L. punmahuida*, *L. flavipiceus*, *L. chiliensis*, *L. bibronii*, *L. pictus*, *L. septentrionalis*, and three representatives of main clades of the *Eulaemus* subgenus, *L. archeformis*, *L. vallecurensis*, and *L. rothi*, that we use to root the trees. Specimen vouchers and tissues are catalogued in Centro Nacional Patagónico Herpetological Collection (LJAMM-CNP) in Puerto Madryn, Argentina (<http://www.cenpat.edu.ar/nuevo/coleccion03.html>). Appendix B, and Table 1 summarize locality data and GenBank accession numbers for the 142 specimens used in this study.

### 2.2. Gene sampling

We sequenced two mitochondrial regions, cytochrome *b* (709 bp,  $n = 123$ , Kocher et al., 1989) and 12S (832 bp,  $n = 117$ , Wiens and Penkrot, 2002); and five nuclear fragments, including three protein coding loci [NPCL]: EXPH5 (841 bp,  $n = 115$ ), KIF24 (417 bp,  $n = 119$ ), MXRA5 (849 bp,  $n = 114$ ) (Portik et al., 2011), one intron [NIL]: BA3 (270 bp,  $n = 108$ , Waltari and Eduards, 2002), and one anonymous locus [ANL]: LPB4G (627 bp,  $n = 104$ , Olave et al., 2011). Of the total 797 sequences used in this study, 402 [50.5%] were newly generated sequences, 311 [39%] were downloaded from Genbank, and 84 [10.5%] (*L. petrophilus* complex) were taken from an unpublished PhD thesis (Feltrin, 2013) (Genbank accession numbers: MG659992-MG660442 & MG674076-MG674081, Table 1). For some individuals for which we could not obtain all sequences, we used other individuals

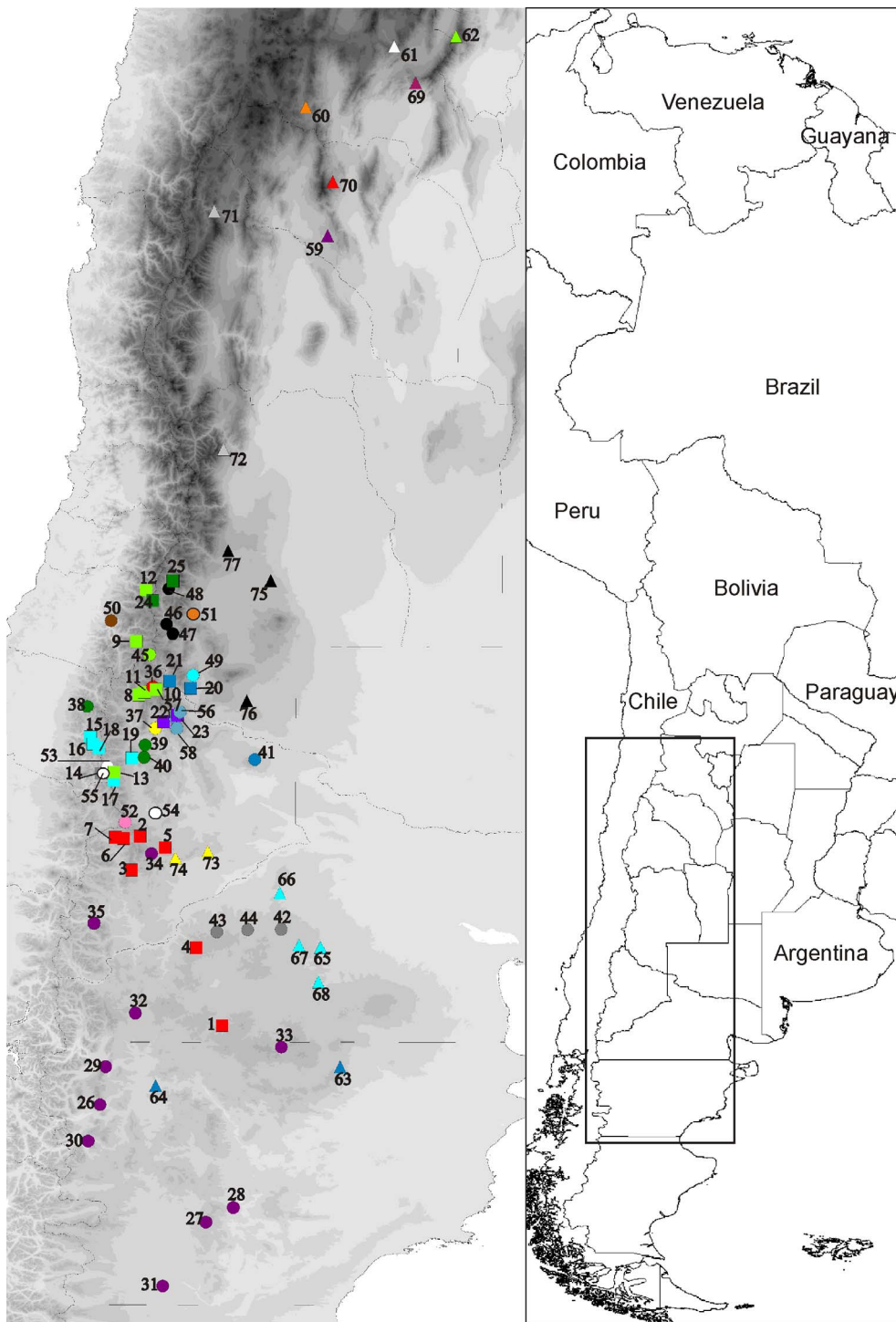


Fig. 1. Sampled localities of the *Liolaemus elongatus-kriegi* group. Species of the *L. elongatus* complex marked with a circle, *L. kriegi* complex with a square, and *L. petrophilus* complex with a triangle. *Liolaemus kriegi* + *L. ceii* (1–7); *L. buergeri* (8–13); *L. tregenzai* (14); *L. zabalai* (15–19); *L. sp. B* (20 and 21); *L. sp. C* (22 and 23); *L. sp. D* (24 and 25); *L. elongatus* (26–35); *L. antumalguen* (36); *L. burmeisteri* (37); *L. chillanensis* (38–40); *L. crandalli* (41); *L. shitan* (42–44); *L. carlosgarini* (45); *L. smaug* (46–48); *L. choique* (49); *L. sp. 1* (50); *L. sp. 2* (51); *L. sp. 3* (52); *L. sp. 6* (53–55); *L. sp. 7* (56–58); *L. talampaya* (59); *L. tulkas* (60); *L. umbrifer* (61); *L. heliodermis* (62); *L. petrophilus* (63–68); *L. capillitas* (69); *L. dicktracy* (70); *L. parvus* (71–72); *L. gunnakuna* (73–74) and *L. austromendocinus* (75–77).

from the same locality (Appendix B).

### 2.3. DNA extraction, amplification and sequencing

Genomic DNA was extracted using Qiagen® DNeasy® 96 kit for animal tissue following the manufacturer's protocol. Mitochondrial and nuclear gene PCR and sequencing protocols followed Morando et al. (2003) and Noonan and Yoder (2009), respectively. All sequences were edited with Sequencher v4.8 (™Gene Codes Corporation Inc., 2007), and NPCL were translated to amino acids to verify that there were no stop codons. Alignments were straightforward (no algorithms were needed), and in all cases, missing data were coded as "?". We selected

the best-fitting molecular evolution models for all regions with JModelTest v0.1.1 (Guindon and Gascuel, 2003; Posada, 2008), using a corrected Akaike (AICc, Table 2) information criterion. Recombination was tested for nuclear genes using RDP: Recombination Detection Program v3.44 (Heath et al., 2006; Martin and Rybicki, 2000). Before running concatenated analyses, we evaluated different codon partitions for the *cyt-b* fragment through the Bayesian factor analysis in MrBayes v3.2 (Ronquist and Huelsenbeck, 2003). We tested an unpartitioned model and then a codon-partitioned model, and for both we ran  $10^7$  generations with their respective molecular evolution models. We followed the same scheme for the nuclear coding genes. Based on these results, we used a concatenated matrix with partitioned *cyt-b* and

**Table 1**

Detail of individuals sequenced by gene, organized by complex or group. In LJAMM-CNP column voucher numbers and in the following columns GenBank accession numbers for the individual sequences, alternative voucher number (with its own GenBank accession number) if other individual from the same locality was use or a question mark if we were not able to obtain a sequence for that gene. New generated sequence have GenBank accession numbers in italics.

Specie	LJAMM-CNP	Cytochrome-b	12S	BA3	EXPH5	KIF24	LPB4G	MXRA5
<i>L. kriegi</i> complex								
<i>L. kriegi</i> + <i>L. ceii</i>	3565	KJ494150.1	MG660025	MG674076	MG660069	MG660143	MG660202	MG660272
	2733	KJ494124.1	MG660026	MG674077	MG660070	?	MG660203	MG660273
	5383	KJ494184.1	MG660027	MG674078	MG660071	MG660144	MG660204	MG660274
	5562	KJ494190.1	KJ493993.1	MG674079	KP121288.1	KP121233.1	KP121264.1	KP789610.1
	5393	KJ494186.1	MG660028	?	MG660072	MG660145	MG660205	MG660275
	13870	KJ494246.1	KJ493997.1	KJ493947.1	KP121281.1	KJ493962.1	KP121249.1	MG660276
	2613	KJ494186.1	KP121213.1	KJ493951.1	KP121284.1	KP121227.1	KP121258.1	KP789602.1
<i>L. buergeri</i>	5313	KJ494173.1	KJ493987.1	MG660360	MG660073	14119	MG660206	MG660277
	14096	KJ494068.1	KJ494003.1	MG660361	KP121283.1	KJ493968.1	KP121253.1	MG660278
	6413	KJ494201.1	KJ493990.1	KJ493959.1	KP121291.1	KP121237.1	KP121267.1	KP789612.1
	6439	KJ494208.1	KJ493989.1	MG674080	KP121292.1	KP121238.1	KP121268.1	KP789613.1
	5294	KJ494165.1	KJ493986.1	KJ493956.1	KP121287.1	KP121232.1	KP121263.1	KP789608.1
<i>L. tregenzai</i>	13908	KJ494036.1	KJ493999.1	KJ493949.1	KP789559.1	KJ493964.1	KP789591.1	?
	13918	KJ494039.1	KJ494000.1	KJ493950.1	KP789560.1	KJ493965.1	KP789592.1	MG660279
<i>L. zabalai</i>	3433	KJ494142.1	KP789548.1	KJ493955.1	KP789566.1	KJ493972.1	KJ493958.1	KP789606.1
	13991	KJ494060.1	KJ494001.1	?	?	KJ493966.1	KP789593.1	MG660347
	13907	KJ494035.1	KJ493998.1	KJ493948.1	KP789558.1	KJ493963.1	KP789590.1	MG660348
	14152	KJ494074.1	KJ494006.1	?	?	MG660194	MG660207	2532
	5339	KJ494180.1	KJ493992.1	KJ493957.1	?	KJ493974.1	KP789599.1	KP789609.1
<i>L. sp. B</i>	5756	KJ494193.1	KJ493991.1	?	KP789569.1	KJ493976.1	?	?
	2667	KJ494120.1	KJ494008.1	KJ493953.1	KP789564.1	KJ493970.1	KP789595.1	KP789616.1
	12174	KJ494240.1	KJ493996.1	?	MG660074	MG660195	MG660208	MG660349
<i>L. sp. C</i>	12148	KJ494020.1	KJ493995.1	KJ493946.1	KP789556.1	KJ493961.1	KP789589.1	KP789601.1
	2616	AY173822.1	KJ493982.1	MG674081	MG660075	MG660196	MG660209	MG660350
<i>L. sp. D</i>	5797	KJ494195.1	KP789549.1	KJ493958.1	KP789570.1	KJ493977.1	KP789600.1	KP789611.1
	2758	KJ494130.1	KJ493984.1	KJ493954.1	KP789565.1	KJ493971.1	KP789597.1	KP789605.1
<i>L. elongatus</i> complex								
<i>L. elongatus</i>	2128	KY127782	AY173872.1	?	?	MG660146	MG660210	MG660280
	9060	KP789554.1	MG660029	?	KP789575.1	MG660147	MG660211	KP789618.1
	9100	KY128157	MG660030	?	MG660076	MG660148	MG660212	MG660281
	3675	KY127900	KY127486	MG660363	MG660077	KY127589	MG660213	MG660282
	3578	KY127888	KY127485	MG660364	3575	KY127588	KP121261.1	MG660283
	3047	KY127838	KY127480	MG660365	3046	KY127583	MG660214	3715/KP789607.1
	3492	KY127855	KY127482	MG660366	MG660078	KY127585	MG660215	MG660284
	10975	KY127635	KY127455	MG660367	MG660079	KY127560	KP121244.1	MG660285
	8078	KY128117	KY127508	MG660368	MG660080	MG660149	MG660216	MG660286
	12987	KY127690	KY127460	MG660369	MG660081	KY127565	MG660217	MG660287
<i>L. antumalguen</i>	6155	KY128016	KY127494	MG660370	?	KY127599	MG660218	MG660288
	6167	KY128018	KY127495	MG660371	KP121290.1	KY127600	KP121266.1	MG660289
<i>L. burmeisteri</i>	7637	KY128070	KY127500	MG660372	?	KY127605	KP121270.1	MG660290
	7644	KY128077	KY127501	MG660373	MG660082	MG660150	KP121271.1	MG660291
<i>L. chillanensis</i>	14041	KY127734	KY127466	MG660374	5327	KY127571	MG660219	5327
	14042	KY127735	KY127467	11305	11305	KY127572	11305	11305
<i>L. crandalli</i>	12220	KY127666	KY127458	MG660375	KP121277.1	KY127563	KP121247.1	MG660292
	12225	KY127671	KY127459	MG660376	KP121280.1	KY127564	KP121248.1	MG660293
<i>L. shitan</i>	6853	KY128060	KY127498	MG660377	MG660083	KY127603	MG660220	MG660294
	1915	AY173575.1	MG660031	MG660378	MG660084	MG660151	MG660221	MG660295
	5532	KY127990	KY127492	MG660379	MG660085	KY127597	MG660222	MG660296
	5537	MG659992	MG660032	MG660380	MG660086	MG660152	MG660223	MG660297
	13498	MG659993	MG660033	MG660381	MG660087	MG660153	MG660224	MG660298
	13527	KY127710	KY127461	?	MG660088	KY127566	MG660225	?
<i>L. carlosgarini</i>	14061	KY127736	MG660034	MG660382	MG660089	MG660154	MG660226	MG660341
	14064	KY127739	MG660035	?	MG660090	MG660155	MG660227	MG660299
	3435	KY127850	KY127481	MG660383	MG660091	KY127584	MG660228	MG660300
<i>L. smaug</i>	2679	KY127823	KY127477	MG660384	KP121286.1	KY127477	KP121260.1	MG660301
	7916	KY128105	KY127506	MG660385	KP121294.1	KY127611	KP121274.1	MG660302
	2764	KY127836	KY127479	MG660386	MG660092	KY127582	MG660229	MG660303
<i>L. choique</i>	7767	KY128092	KY127505	MG660387	MG660135	KY127610	?	MG660304
	7768	KY128093	MG660036	MG660388	MG660136	MG660156	?	MG660305
	7771	KY128096	MG660037	MG660389	MG660137	MG660157	?	MG660306
	7772	KY128097	?	?	MG660138	MG660158	?	MG660307
	7770	KY128095	MG660038	MG660390	7769	KP121240.1	KP121272.1	MG660308
<i>L. sp. 1</i>	14075	KY127744	KY127470	MG660391	MG660093	KY127574	MG660230	MG660309
	14076	KY127745	KY127471	MG660392	MG660094	14073/KY127573	MG660231	MG660310
<i>L. sp. 2</i>	7994	KY128108	MG660039	MG660393	MG660095	MG660197	8000	8000
	7995	KY128109	MG660040	MG660394	MG660096	MG660198	MG660232	MG660351
<i>L. sp. 3</i>	13887	KY127715	?	MG660395	MG660097	KY127567	MG660233	MG660352
	MIC1642	KY128168	KY127512	MG660396	MG660098	KY127615	MG660234	MG660353
<i>L. sp. 6</i>	13899	KY127717	KY127463	13902	13902	KY127568	MG660235	13902
	4446	MG659994	KY127488	MG660397	MG660099	MG660199	MG660236	MG660354
	2522	KY127802	KY127476	MG660398	MG660100	KY127578	MG660237	MG660355

(continued on next page)

Table 1 (continued)

Specie	LJAMM-CNP	Cytochrome-b	12S	BA3	EXPH5	KIF24	LPB4G	MXRA5
<i>L. sp. 7</i>	5225 2602 2693 10442	KY127930 KY127811 KY127826 KY127620	MG660041 AY173888.1 KY127478 KY127454	MG660399 MG660400 MG660401 MG660402	MG660101 MG660102 MG660103 MG660104	KY127592 KY127579 KY127581 KY127559	MG660238 MG660239 MG660240 MG660241	MG660342 MG660311 MG660312 MG660313
<i>L. petrophilus</i> complex								
<i>L. talampaya</i>	1980 [FML 13045] 2737 [MLP.S 2401]	MG659995 AY173552.1	MG660042 MG660043	MG660403 MG660404	MG660105 MG660106	MG660159 MG660160	MG660242 MG660243	MG660314 MG660315
<i>L. tulkas</i>	4219 4227	MG659997 MG659998	MG660044 MG660045	MG660405 MG660406	MG660107 MG660108	MG660200 MG660201	MG660244 MG660245	MG660356 MG660357
<i>L. umbriifer</i>	5031 5032	MG659999 MG660000	MG660046 MG660047	MG660407 MG660408	MG660109 MG660110	MG660161 MG660162	MG660246 MG660247	MG660316 MG660343
<i>L. heliodermis</i>	8569	MG660001	MG660048	MG660409	MG660111	MG660163	?	MG660317
<i>L. petrophilus S</i>	5481 8861	JN846994.1 JN847063.1	MG660049 ?	MG660410 ?	MG660112 MG660139	MG660164 ?	MG660248 MG660249	MG660318 MG660319
<i>L. petrophilus N</i>	6982 11224 11355	KP121326.1 JN847096.1 KP789552.1	KP121216.1 MG660050 KP121211.1	KP789551.1 ? KP789550.1	KP789572.1 MG660113 KP789555.1	KP789577.1 MG660165 MG660166	KP121269.1 MG660250 KP121246.1	KP789617.1 11122/KF968003.1 KP789615.1
<i>L. capillitas</i>	2786 [BYU 47100] 2788	AY367811.1 AY173555.1	AY367841.1 MG660051	MG660411 2789	MG660114 MG660115	MG660167 MG660168	MG660251 MG660252	2789 MG660320
<i>L. dicktracy</i>	5816	MG660002	MG660052	MG660412	MG660116	MG660169	MG660253	MG660321
<i>L. parvus</i>	2706 [FML 13059] 2711 [BYU 47106]	AY173611.1 AY367809.1	2705 AY173906.1	MG660413 MG660414	2762 MG660117	MG660170 MG660171	MG660254 MG660255	2762 MG660322
<i>L. gununakuna</i>	10403 2690 4443	MG660003 AY173545.1 MG660004	MG660053 AY173903.1 MG660054	MG660415 MG660416 MG660417	MG660118 MG660119 MG660120	MG660172 MG660173 MG660174	MG660256 MG660257 MG660258	MG660323 MG660324 MG660344
<i>L. austromendocinus</i>	2716 5147 10574	AY173838.1 AY367815.1 MG660005	AY173907.1 AY367843.1 MG660055	MG660418 MG660419 MG660420	?	MG660175 MG660176 MG660177	MG660259 MG660260 MG660261	MG660325 MG660326 MG660327
<i>L. punmahuida</i> group								
<i>L. flavipiceus</i>	7906 7907	KP121330.1 MG660006	MG660056 MG660057	MG660421 MG660422	KP121293.1 MG660140	KP121241.1 MG660178	KP121273.1 ?	MG660328 MG660329
<i>L. punmahuida</i>	2626 2649	MG660007 KP121336.1	MG660058 KP121214.1	MG660423 MG660424	MG660122 KP121285.1	MG660179 KP121228.1	MG660262 KP121259.1	? MG660339
Sister groups								
<i>L. chiliensis</i>	14360 14361	MG660024 MG660008	942 ?	MG660425 MG660426	MG660123 MG660141	? ?	? ?	MG660330 ?
<i>L. bibronii</i>	8212 9896	MG660009 MG660010	MG660059 KF968265.1	MG660427 MG660428	MG660359 KF968265.1	MG660180 KF968073.1	8211 9897/ JN410479.1	MG660358 8211
<i>L. pictus</i>	14359 14343	MG660011 MG660012	EU649350.1 EU649352.1	MG660429 MG660430	MG660142 MG660124	MG660181 MG660182	MG660263 MG660264	MG660331 MG660340
<i>L. septentrionalis</i>	14099 14100	MG660013 MG660014	MG660060 MG660061	MG660431 MG660432	MG660125 MG660126	MG660183 MG660184	MG660265 MG660266	MG660332 MG660333
<i>L. coeruleus</i>	14206 978 979	MG660015 MG660016 MG660017	? MG660062 ?	MG660433 MG660434 MG660435	MG660127 MG660128 MG660129	MG660185 MG660186 MG660187	MG660267 ? ?	MG660334 MG660335 ?
<i>L. neuquensis</i>	980 985	MG660018 MG660019	MG660063 MG660064	MG660436 ?	MG660130 ?	MG660188 ?	? ?	? ?
<i>L. thermanum</i>	14130 14170	MG660020 MG660021	MG660065 MG660066	MG660437 MG660438	? MG660271	MG660189 MG660190	MG660268 MG660269	MG660345 MG660336
Outgroups								
Section <i>L. lineomaculatus</i>								
<i>L. archeforus</i>	9238 9240	KF968825.1 JF272765.1	KF969003.1 KF969004.1	MG660439 MG660440	MG660131 MG660132	KF968068.1 KF968069.1	? ?	MG660337 MG660346
Group								
<i>L. montanus</i>								
<i>L. vallecurensis</i>	2713 2714	MG660022 MG660023	MG660067 MG660068	MG660441 MG660442	MG660133 MG660134	MG660191 MG660192	? ?	MG660338 ?
Group								
<i>L. boulengeri</i>								
<i>L. rothi</i>	3091 3092	KF968906.1 KF968907.1	KF969097.1 KF969098.1	? ?	KF968338.1 KF968339.1	MG660193 KF968157.1	? ?	KF968007.1 KF968008.1

unpartitioned 12S and nuclear genes.

#### 2.4. Phylogenetic analyses

**Gene tree analyses.**—Bayesian Inference (IB) was performed with MrBayes v3.2 (Ronquist and Huelsenbeck, 2003), all analyses were run for  $10^7$  generations and evaluated for convergence using Tracer v1.5.0. Maximum Likelihood (ML) analyses were conducted using RAxML

v7.0.4 based on 1000 rapid bootstrap analyses, and the GTRGAMMA evolution model. We also obtained bootstrap support values with RAxML v7.0.4 (Stamatakis, 2006) based on 1000 quick replicates and a GTRGAMMA model for all genes. Both analyses, IB and ML, were performed for each of the seven genes.

**Concatenated gene tree analyses.**—We explored a wide range of scenarios via analyses for three different data partitions: 1 – concatenated mitochondrial markers, 2 – concatenated nuclear markers, and 3 – all

**Table 2**

Summary of each gene fragment, nature, best-fitting evolutionary model selected with JModelTest (under AICc), rate of change of the gamma distribution across sites, recombination test results performed with RDP for nuclear genes (R) and number of polymorphic sites (S).

Gen	Nature	Evolutionary model	Base	Nst	Rates	R	S
Cytochrome-b	Mitochondrial coding	HKY + I + G	0.3518 0.3194 0.0885 0.2403	2	Gamma	–	207
12S	Mitochondrial ribosomal	GTR + I + G	0.2058 0.1853 0.2500 0.3590	6	Gamma	–	198
BA3	Nuclear Intron	JC	Equal	1	Equal	NO	40
MXRA5	Nuclear coding	HKY + G	0.3294 0.1822 0.2073 0.2811	2	Gamma	NO	48
LPB4G	Nuclear anonymous	HKY + G	Equal	6	Gamma	NO	17
EXPH5	Nuclear coding	GTR + G	0.2513 0.1783 0.2052 0.3652	6	Gamma	NO	72
KIF24	Nuclear coding	TrNef + G	Equal	6	Gamma	NO	65

genes concatenated, except the mitochondrial fragment of *L. sp. B*, for which we have evidence of mitochondrial introgression (Medina et al., 2014, 2015). Bayesian analyses were performed using MrBayes v3.2 (Ronquist and Huelsenbeck, 2003), and equilibrium samples (assessed with Tracer v1.5.0) were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) were considered significant when  $\geq 0.95$  (Huelsenbeck and Ronquist, 2001). Likelihood analyses were conducted using RAxML v7.0.4 based on 1000 rapid bootstrap analyses and the GTRGAMMA evolution model. We also obtained bootstrap support values with RAxML v7.0.4 (Stamatakis, 2006) based on 1000 quick replicates and a GTRGAMMA model for all genes.

**Species tree and chronogram.**—We estimated a time-calibrated species tree using a Bayesian approach with an ultrametric ML phylogeny as a guide tree. The age of the nodes and their confidence intervals were determined using Bayesian MCMC analysis of molecular sequences (BEAST; Drummond and Rambaut, 2007a), and summarized in the following steps. A user-specified starting ultrametric tree was estimated using the best ML topology under a penalized likelihood rate smoothing (PLRS) approach, with r8s v 1.7 (Sanderson, 2002). The nodal age for calibration of the PLRS guide chronogram was derived from Fontanella et al. (2012), which included the species from the *Liolaemus elongatus* and *kriegi* complexes, as well as member of the subgenus *chiliensis*. For the r8s chronogram, we used the following nodal ages: (1) the divergence between *L. archeoforus* (group *L. lineomaculatus*) and *L. rothi* (group *L. boulengeri*) constrained between 13.08 and 23.08 millions of years ago (MYA); and (2) the divergence between *L. vallecurensis* (group *L. montanus*) and *L. rothi* (group *L. boulengeri*) constrained between 8.98 and 17.17 millions of years ago (MYA). This chronogram provided the guide tree for the BEAST species tree subsequent estimations. The *Liolaemus* species tree chronogram was estimated using a lognormal relaxed clock (uncorrelated) model approach (\*BEAST) implemented as in BEAST v. 1.5.3 (Drummond and Rambaut, 2007a).

This approach can use multiple genes by unlinking partition trees, site and clock models. Genes on the same locus (cytochrome *b* and 12S) were linked for the clock model. We provided user-defined sets of priors that included: the molecular model for each gene, the user-specified starting r8s ultrametric tree, a two node-age constraints for divergence between *L. lineomaculatus* and *L. boulengeri* groups at  $18.08 \pm 2.17$  MYA, and between *L. montanus* and *L. boulengeri* groups at  $12.7 \pm 2.6$  MYA; an exponential  $\mu$  of 10 for its standard deviation; and a species tree prior (Yule process) with population size model (piecewise linear and constant root). Ploidy levels were assigned as mitochondrial (cytochrome *b* and 12S) or autosomal nuclear, and an exponential hyperprior (initial value 1, mean 10 and offset 0) was used for the ucl.d.mean parameter. The suggested modifications for default MCMC operators were determined after two runs of 2 million generations each, with a sampling rate every 1000 generations. The species tree chronogram estimation included the suggested MCMC operator calibrations, and four runs of 100 million generations sampled every 5000 generations. The convergence of the runs and the optimal burnin were determined using Tracer v 1.4 (Rambaut and Drummond, 2007a). The tree files were combined using LogCombiner (Drummond and Rambaut, 2007b), and  $\sim 20,000$  initial trees were discarded as burnin.

The maximum clade credibility summary tree was determined with the retained trees using TreeAnnotator (Rambaut and Drummond, 2007b). The *Liolaemus* chronogram was determined from a summary tree using FigTree v 1.2.3 (Rambaut, 2009).

We also generated a species tree using the SVDquartet algorithm as implemented in PAUP 4a149 ([http://people.sc.fsu.edu/~dswofford/paup\\_test/](http://people.sc.fsu.edu/~dswofford/paup_test/)); data were scored at the level of the individual, with species set as the taxon partitions. We used default settings except for the species tree option and ran 1000 bootstrap replicates in all analyses.

### 3. Results

#### 3.1. Phylogenetic analyses

Results of concatenated analyses for all genes are depicted in Fig. 2, Fig. 3 shows the inferred BEAST species tree, and Fig. 4 the SVDquartet species tree. Individual gene tree analyses are included in Appendix C, and Bayesian Inference for the concatenated mitochondrial markers and concatenated nuclear markers are depicted in Appendixes D and E, respectively. The concatenated mitochondrial gene tree (Appendix D) inferred as well-supported monophyletic groups (IB = 1/ML = 98) the three main haploclades corresponding to the three complexes of the *L. elongatus-kriegi* group: *Liolaemus kriegi* (IB = 1/ML = 100), *L. elongatus* (IB = 1/ML = -) and *L. petrophilus* (IB = 1/ML = 93), and a sister relationship between the first two (IB = 1/ML = 100). However, two individuals of *L. shitan* were inferred within the *L. elongatus* complex, another two were placed outside of this group. Several species within the *L. elongatus* complex are not inferred as monophyletic, but a recent phylogeographic study addresses these issues in detail (Medina et al., 2017). The *L. punmahuida* group (IB = 1/ML = 100) was inferred as sister of the *L. elongatus-kriegi* group (IB = 1/ML = 99). *Liolaemus thermanum* was not inferred within the *L. elongatus-kriegi* group but more closely related to other species of the *L. chiliensis* subgenus, with (*L. septentrionalis* + (*L. pictus* + *L. chiliensis*)). *Liolaemus trengzai* is inferred as part of the *L. kriegi* complex, and *L. parvus* is included in the *L. petrophilus* complex.

Although the concatenated nuclear gene tree (Appendix E) differs in several relationships relative to the mitochondrial gene tree (Appendix D), most of these differences do not have statistical support. The *L. elongatus* and *L. petrophilus* complexes were not inferred as monophyletic and the *L. kriegi* complex had high support only with ML bootstrap (95%) (IB = 0.87). Candidate species *L. sp. B* was nested within the *L. petrophilus* complex and not in the *L. kriegi* complex, as it was in the mitochondrial tree (Appendix D). *Liolaemus trengzai* was inferred as closely related to *L. flavipiceus* (0.98/99) in the *L. punmahuida* group (and not within the *L. kriegi* complex as it was in the mitochondrial gene tree, Appendix D). In agreement with the mitochondrial gene tree, *L. thermanum* was not inferred as part of the *L. elongatus-kriegi* group.

The concatenated analyses with all genes inferred a single tree that include one main clade with high statistical support (Fig. 2, IB = 1/ML = 99); this clade includes the three complexes *L. kriegi*, *L. elongatus*, and *L. petrophilus*, but only the latter with high support (1/98); the *L.*

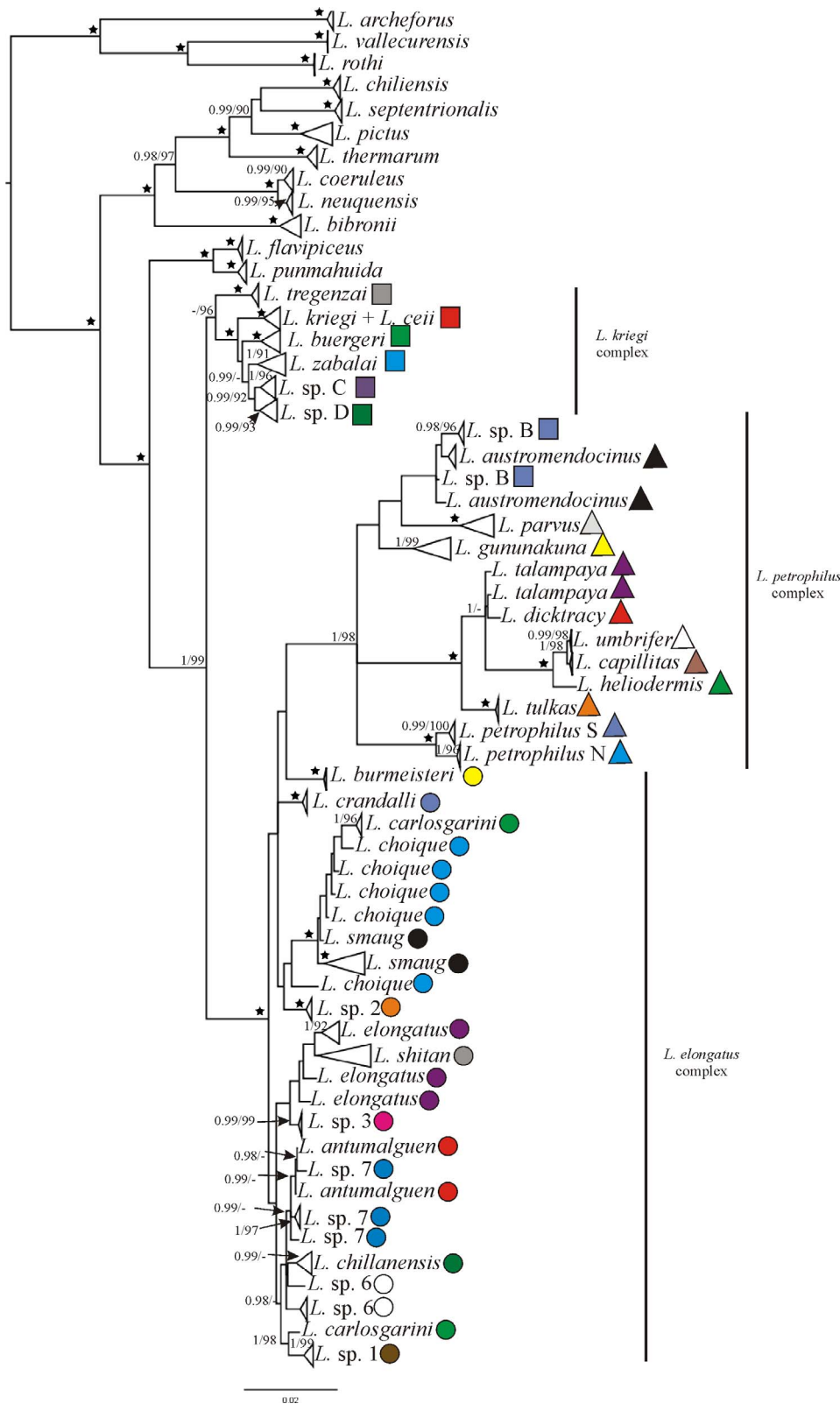


Fig. 2. Concatenated Bayesian mitochondrial and nuclear tree. Stars in nodes represent Bayesian inference posterior probability = 1 and ML bootstrap = 100%. Icons on the side of species names correspond to those in Fig. 1.

*kriegi* complex only had support with ML-bootstrap (96%, IB = 0.81). The *L. punmahuida* group was inferred as sister to the *L. elongatus-kriegi* group with high support (IB = 1, ML = 100%), and *L. tregenzai* and *L. parvus* were inferred within the *L. kriegi* and *L. petrophilus* complexes respectively, similar to the mitochondrial tree (Appendix D). Species of the *L. elongatus* and *L. petrophilus* complexes were resolved as a single large clade with high support (IB = 1/ML = 100); this topology

contrasts with the mitochondrial tree, which places the *L. elongatus* complex as sister to the *L. kriegi* complex (Appendix D). Within the *L. kriegi* complex each of the described or candidates species was inferred as monophyletic. Species of the *L. petrophilus* complex that were not resolved as monophyletic included *L. austromendocinus*, *L. sp. B*, and *L. talampaya*. In contrast, within the *L. elongatus* complex, *L. burmeisteri*, *L. chillanensis*, *L. crandalli*, *L. shitan*, *L. sp. 1*, *L. sp. 2* and *L. sp. 3* were

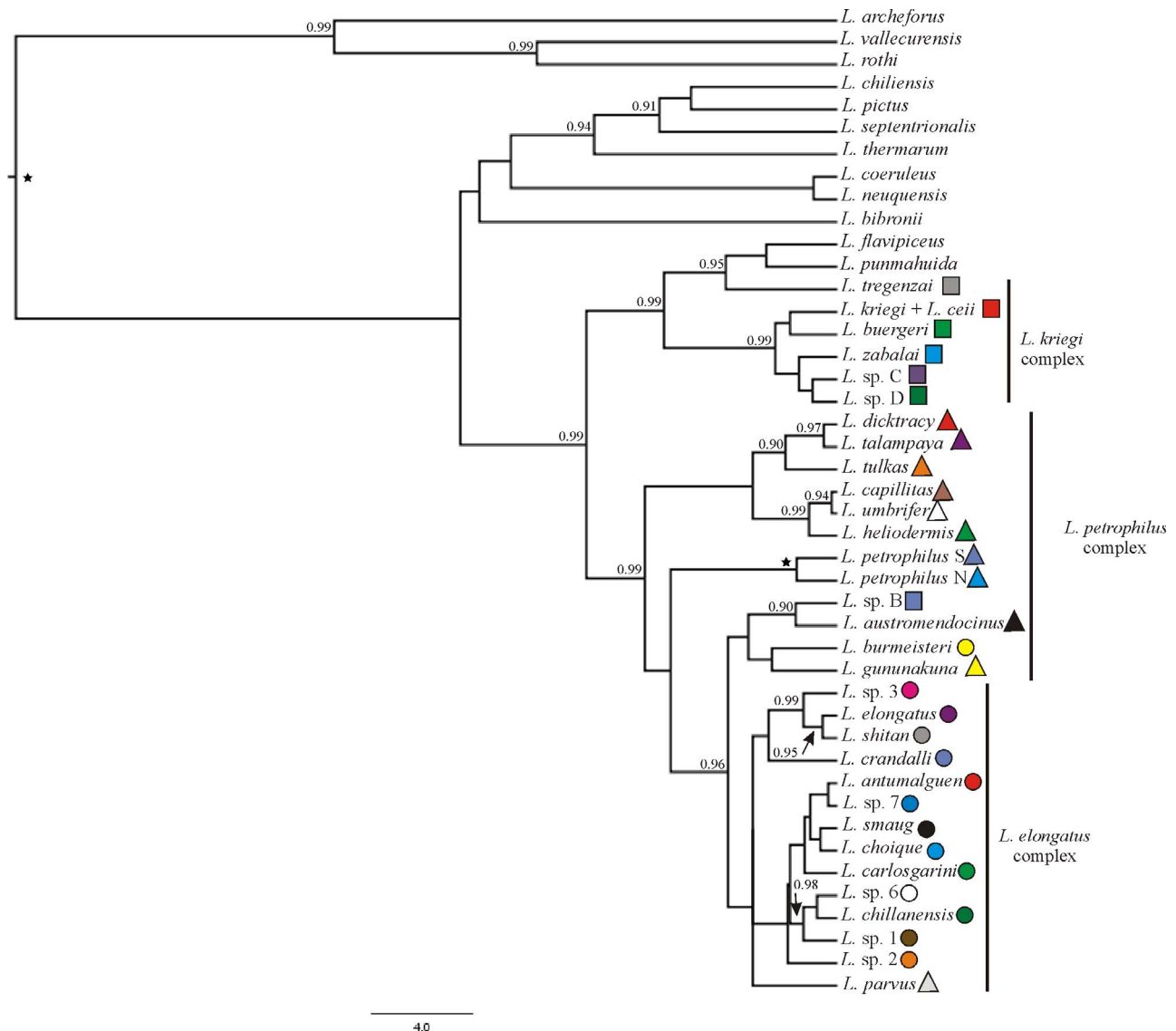


Fig. 3. Species tree inferred with BEAST (mitochondrial and nuclear genes) with posterior probability values on nodes.

inferred as monophyletic.

In the BEAST species tree approach (Fig. 3), the *L. elongatus* and *L. petrophilus* complexes were not resolved as monophyletic, but in combination their species were inferred as a single clade ( $P = 0.99$ ), and this topology was also resolved in the concatenated analyses (Fig. 2). The *L. kriegi* complex ( $P = 0.99$ ) was inferred as sister to the *L. punmahuida* group ( $P = 0.95$ ), including *L. flavipiceus* and *L. tregenzai* ( $P = 0.99$ ), and this clade is the sister group to the (*L. elongatus* + *L. petrophilus*) complexes ( $P = 0.99$ ). *Liolaemus thermarum* was inferred as closely related to (*L. chillanensis* + *L. pictus* + *L. septentrionalis*) with high support ( $P = 0.94$ ); while *L. tregenzai* was nested within the *L. punmahuida* group, similar to the concatenated nuclear tree (Appendix E). The northernmost species of the *L. petrophilus* complex (*L. dicktracyi*, *L. talampaya*, *L. tulkas*, *L. capillitas*, *L. umbrifer*, and *L. heliodermis*) were inferred as a clade but with no statistical support. The SVDquartet species tree (Fig. 4), presents some contrasting results with either the concatenated or the BEAST analyses: 1-the *L. punmahuida* group (*L. flavipiceus* + *L. punmahuida*) is inferred as sister to the *L. elongatus-kriegi* group (bootstrap value [BV] = 98), and *L. tregenzai* as part of the *L. kriegi* complex (BV = 100). The *L. elongatus* complex is inferred to be monophyletic (BV = 96), and although the *L. petrophilus* complex is inferred as a clade, it does not have statistical support. These two

complexes are resolved as sister taxa, although without statistical support.

#### 4. Discussion

The aim of this work is to present the first comprehensive *L. elongatus-kriegi* group multilocus phylogeny, including all recognized lineages and implementing concatenated methods using Bayesian inference and maximum likelihood algorithms, as well as species tree approaches.

As expected given the stochasticity associated to evolutionary processes on each gene, we found different topologies between gene trees (Appendix C); the variance in genealogical histories can be the result of various biological processes (reviewed in Maddison, 1997; Degnan and Rosenberg, 2009); but in general, we found high concordance between IB and ML topologies and level of support. Mitochondrial gene trees (cytochrome-b and 12S) inferred species of the *L. elongatus* and *L. kriegi* complexes closer to each other than to species of the *L. petrophilus* complex. In contrast, nuclear gene trees did not show clear differentiation between species of these two complexes. This result is a yet another clear example of why it is important to move *Liolaemus* studies to an integrative taxonomic (IT) approach (Aguilar et al., 2017) and

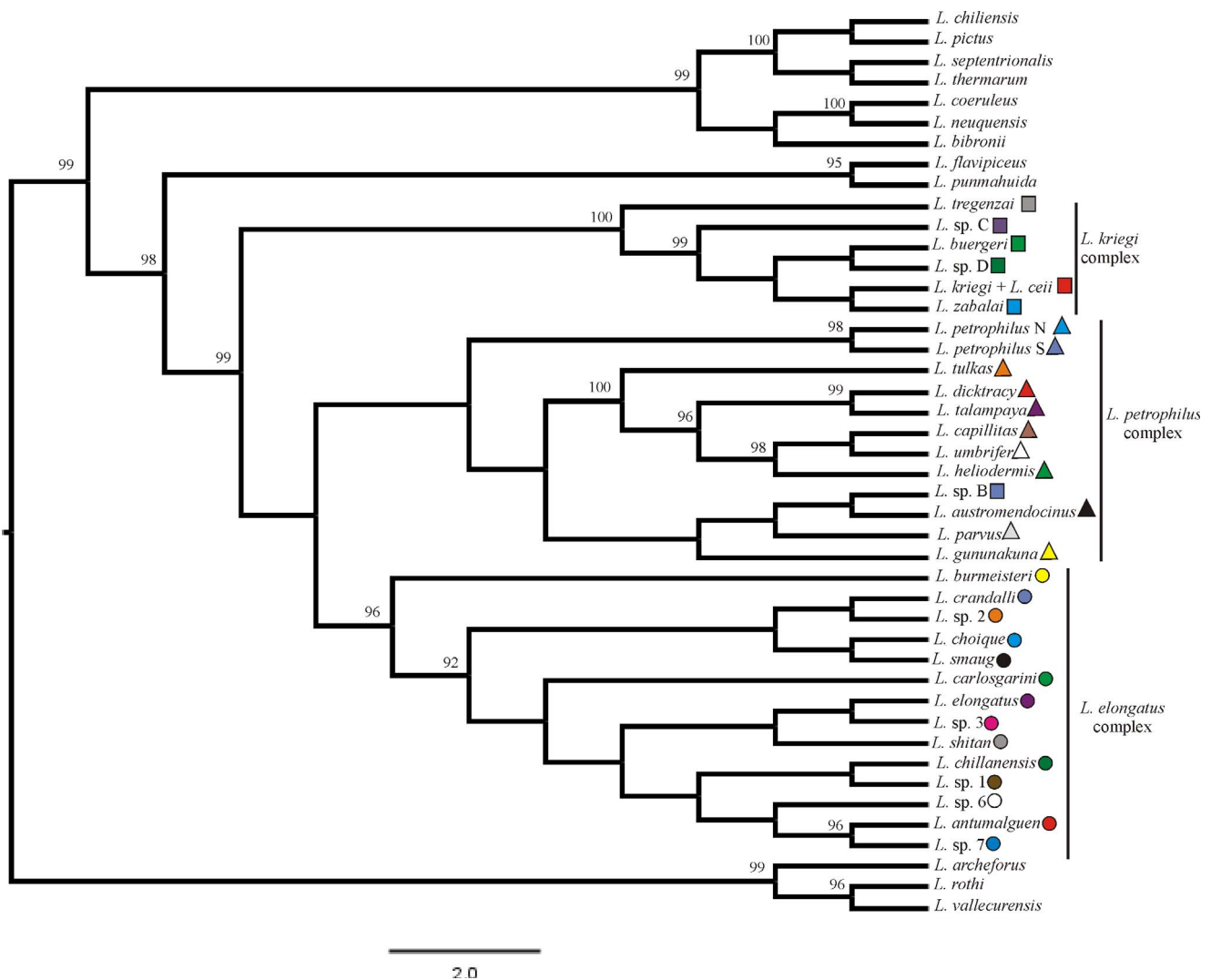


Fig. 4. Species tree inferred with SVDquartet. Values on nodes represent bootstrap inferences.

avoid misleading phylogenetic inferences based on a single type of marker. Even IT approaches, however, will sometimes find incongruence that is real, and reveals interesting evolutionary and/or demographic processes such as speciation accompanied by introgression/hybridization. These processes have now been documented in various animal taxa, including several groups of *Liolaemus* (i.e. Good et al., 2003; Kubatko, 2009; McGuire et al., 2007; Morando et al., 2004; Olave et al., 2011; Renoult et al., 2009). However, as our focus here is on phylogenetic relationships, we continue with discussion of the different combined analyses, and highlight some differences with partial datasets.

The *Liolaemus elongatus-kriegi* group, including the *L. elongatus*, *L. kriegi* and *L. petrophilus* complexes (26 described species and nine candidate species) was inferred as monophyletic with high support in the mitochondrial tree (Appendix D), in the all-data concatenated tree (Fig. 2), and in the SVDquartet species tree (Fig. 4). With the BI concatenated analyses, the species included in the *L. elongatus* and *L. petrophilus* complexes were inferred as a single clade with strong support (1/100), with the *L. kriegi* complex (including *L. tregenzai*) as its sister clade (1/99), and then the *L. punmahuida* group as sister to these two (1/100) (Fig. 2). The BEAST species tree analysis also inferred the *L. elongatus* and *L. petrophilus* complexes as a clade with strong support (99), but the sister group was the *L. kriegi* complex + *L. punmahuida* group (PP = .99, Fig. 3). Finally, the SVDquartet species tree method (Fig. 4) inferred both complexes as sister clades, although without

statistical support. Our results are largely congruent with Lobo (2001, morphological characters) and Lobo et al. (2010, bibliographical review) that inferred the *L. kriegi* complex as sister to the *L. elongatus* plus *L. petrophilus* complexes, but Lobo et al., 2010 included the *L. punmahuida* complex as part of the *L. elongatus* group (although these authors had reduced taxonomic sampling). In addition, (Esquerré et al., 2014) hypothesized that the *L. leopardinus* clade (*L. leopardinus*, *L. ramonensis*, *L. valdesianus*, *L. ubaghsi* and *L. frassinetti*) could be part of the *L. elongatus-kriegi* group, but provided no empirical support for this topology.

The *L. elongatus* and *L. petrophilus* complexes were inferred as strongly supported clades in the mitochondrial gene tree (Appendix D) and in the SVDquartet species tree (Fig. 4), and the *L. kriegi* complex was inferred as monophyletic in all of our analyses, in agreement with other authors (Lobo, 2001, 2005; Lobo et al., 2010; Pyron et al., 2013; Schulte II et al., 2000). Support for a monophyletic *L. kriegi* complex was high to moderate with the four approaches we used (Appendix D, E and Figs. 2 and 3), and included four described (*L. kriegi*, *L. ceii*, *L. buergeri*, *L. zabalai*) and two candidate species (*L. sp. C*, *L. sp. D*). Two other species were not consistently inferred in this clade: *L. tregenzai* is included within this complex in the mitochondrial gene tree (Appendix D), the concatenated analyses (Fig. 2) and in the SVDquartet species tree (Fig. 4), but the concatenated nuclear data (Appendix E) and the BEAST species tree approaches (Fig. 3) placed this species as the sister taxon to the *L. punmahuida* group. In addition *L. sp. B* is included within

this complex in the mitochondrial gene tree (Appendix D), while the nuclear data (Appendix E), concatenated analyses (Fig. 2), and the two species tree approaches (Figs. 3 and 4), placed this taxon in the *L. petrophilus* complex.

These strongly supported but mutually exclusive results between mitochondrial trees and concatenated analyses versus nuclear tree and species tree approaches for *L. tregenzai*, suggest that either of two processes could have produced this pattern: 1 - mitochondrial gene tree stochasticity, and/or 2 - historical or ongoing interspecific hybridization (Avice, 1989; Maddison, 1997; Pamilo and Nei, 1988). *Liolaemus tregenzai* is only known from its type locality (Fig. 1, #14), an area that also harbours species of the *L. kriegi* complex, and in geographic proximity towards the north, the two species included in the *L. punmahuida* group. This geographic proximity suggests the possibility of historical and/or ongoing hybridization. Dense sampling in this area and inclusion of a larger number of nuclear markers will be necessary to further test these hypotheses.

For the case of *L. sp. B*, contrasting results between types of markers are consistent with previous studies (Feltrin, 2013; Medina et al., 2014; Morando et al., 2003); and the same hypotheses suggested for *L. tregenzai* are also valid in this case, as discussed in Medina et al. (2014). At a more general level, introgression between *Liolaemus* species on different time scales has been well-documented in other *Liolaemus* species complexes (e.g. Camargo et al., 2012; Grummer, 2017; Olave et al., 2017; Olave et al., 2011). Relationships among species within the *L. kriegi* complex are mostly congruent with Medina et al. (2014) and with Schulte II et al. (2000), but not with Pyron et al. (2013). These last two studies only included three species of this complex, but the selected species differed between the two.

The *L. elongatus* complex was inferred as monophyletic in the mitochondrial gene tree (Appendix D), and in the SVDquartet species tree (Fig. 4), while in the concatenated tree (Fig. 2) it was paraphyletic with respect to the *L. petrophilus* complex (Fig. 2). *Liolaemus shitan* is characterized by divergent mitochondrial haplotypes that are placed in different topological regions of the *L. elongatus* complex topology, and also external to it (Appendix D). However, the nuclear tree inferred all of these individuals as one clade nested within *L. elongatus* (Appendix E).

Similarly, several other species of this complex were also not inferred as monophyletic in one or more analyses, including *L. elongatus* (only if *L. shitan* is considered valid), *L. antumalguen*, *L. choique*, *L. smaug*, *L. sp. 6*, and *L. sp. 7*, and results of a detailed phylogeographic study suggested possible gene flow and/or incomplete lineage sorting between these and other species of the complex (Medina et al., 2017). Both of these processes violate basic assumptions of phylogenetic methods and likely explain our results (Funk and Omland, 2003; Harrison, 1991; Sullivan et al., 2002). This complex seems to be a very recent diversification (1.2 myr, Medina et al., 2017), thus other classes of markers (SNPs) will be needed to delimit species and infer relationships among them; this approach has been proven to be very useful for other lizard complexes (e.g. West African forest geckos *Hemidactylus fasciatus* complex, Leaché et al., 2014).

The *L. petrophilus* complex was inferred as monophyletic in the mitochondrial (Appendix D), the concatenated trees (Fig. 2), and in the SVDquartet species tree (although with no statistical support, Fig. 4), and includes three main clades for which species relationships reflect geographic concordance. The northernmost of these, the *capillitas* group (Lobo, 2010), is inferred with strong support and includes *L. talampaya*, *L. dicktracy*, *L. tulkas*, *L. umbrifer*, *L. capillitas* and *L. heliodermis* (Figs. 1 and 2). *Liolaemus talampaya* was not inferred as monophyletic but it is closely related to *L. dicktracy* (Appendixes C, D, Figs. 2 and 4). The three species with a relative central distribution between the northern and the southernmost taxa within this complex (*L. austromendocinus*, *L. gununakuna*, *L. parvus*), and the two lineages of the southernmost distributed *L. petrophilus*, are inferred as independent lineages (Figs. 2 and 3).

More than a decade ago, Morando et al. (2003) sampled 207 mitochondrial sequences (198 individuals and 49 localities) representing the above three complexes, and proposed: (1) three candidate species for *L. petrophilus* complex; (2) three species and four candidate species for *L. kriegi* complex; and (3) one species and three candidate species for *L. elongatus* complex. Since then, all candidate species from *L. petrophilus* have been described, as well as two from the *L. kriegi* complex and one for *L. elongatus* complex, and we anticipate discovery of additional new species within these complexes and from other groups of *Liolaemus*.

The results of this study provide detailed multilocus phylogenetic hypotheses for the *L. elongatus-kriegi* group, based on samples from almost all of the species and candidate species recognized in this group, coupled with extensive geographic sampling. We found alternative topologies with different methodological approaches and/or groups of data (Figs. 2–4 and Appendixes C, D, E), these incongruences highlight several issues that, although previously raised in the literature in general (i.e. Rokas et al., 2003), we consider necessary to summarize here as important points for future study, for researchers interested in this lizard family. First, unlinked genes will have different genealogical histories influenced by multiple biological factors, i.e. incomplete lineage sorting, hybridization, natural selection, and/or stochasticity of the evolutionary process (reviewed in Degnan and Rosenberg, 2009; Maddison, 1997). Second, from a taxonomic perspective, discordance among gene trees reinforces the importance of an integrative taxonomy approach (Dayrat 2005, Padial and De la Riva, 2009) to test and delimit species boundaries as hypotheses supported by multiple lines of evidence; this should reduce “taxonomic inflation” (Isaac et al., 2004; Padial and De la Riva, 2007). By way of example, this study is an important contribution to the taxonomic knowledge of this group, but it leaves several issues unresolved. Future studies will need to include: (1) additional field work to fill in gaps in coverage and to increase sample sizes in some regions, specifically west of the Andes (2) a larger number of high-resolution molecular markers (SNPs) suitable for newer species tree methods; and (3) newer morphological analyses for delimiting and describing species (Aguilar et al., 2013, 2017).

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## Appendix A. A, B, C, D and E. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ymp.2017.11.017>.

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