

SHORT COMMUNICATION

Phylogenetic position of two species of the *Liolaemus elongatus-kriegi* Complex and a new northern limit for *L. buergeri* (Squamata: Liolaemidae)

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Palavras-chave: Chile, citocromo b, lagartos, *Liolaemus scorialis*, *Liolaemus zabalai*.

Liolaemus is an extraordinarily diverse lizard genus containing 257 species (Abdala and Quinteros 2014). The genus has been split into several groups (e.g. Lobo *et al.* 2010) of which the *L. elongatus-kriegi* Complex (Ceí 1979) is one of the most characteristic in the Patagonian region of Chile and Argentina (Esquerré *et al.* 2013). This complex is composed of four clades: *L. elongatus* Koslowsky, 1896, *L. kriegi* Müller and Hellmich, 1939, *L. petrophilus* Donoso-Barros and Ceí, 1971, and *L. punmahuida* Avila, Perez, and Morando, 2003 (Avila *et al.* 2012). Troncoso-Palacios *et al.* (2015) described two species in this complex based in

morphological features; both—*L. scorialis* Troncoso-Palacios, Díaz, Esquerré, and Urra, 2015 and *L. zabalai* Troncoso-Palacios, Díaz, Esquerré, and Urra, 2015—are from the surroundings of the Laja Lagoon in the region of Biobío, Chile. The authors proposed that *L. scorialis* might belong to either the *L. elongatus* or *L. kriegi* clade, but the lack of molecular data did not allow them to determine its phylogenetic position. Besides, Troncoso-Palacios *et al.* (2015) description of *L. zabalai* was based on a population previously assigned to *L. kriegi* by Donoso-Barros (1974). They suggested that this population also corresponds to the candidate species *Liolaemus* sp. A of the *L. kriegi* Clade, proposed by Morando *et al.* (2003), with a distribution that includes the type locality of *L. zabalai*; however, in the absence of molecular data of the type specimens of *L. zabalai*,

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Troncoso-Palacios *et al.* (2015) could not confirm this supposition. In a phylogenetic study based on cytochrome b (cyt-b) obtained from two paratypes of *L. scorialis*, Troncoso-Palacios *et al.* (2018) found that *L. scorialis* belonged to the *L. elongatus* Clade; however, the relationship of *L. zabalai* remains unknown.

Here, we provide for first time cyt-b data for the holotype of *L. zabalai* and discuss its phylogenetic relationships. We also provide comments about *L. buergeri* Werner, 1907 and *L. scorialis*, with the latter being based on cyt-b data of two paratypes.

Laboratory procedures for DNA extraction, amplification, and sequencing are the same as those of Troncoso-Palacios *et al.* (2016). We generated cyt-b data for the holotype of *L. zabalai* and two specimens identified as *L. buergeri* from Los Humos (34°50' S, 70°26' W). Sequences of *L. elongatus* from Lonquimay Volcano were provided by G. Escobar-Huerta, and other sequences were taken from GenBank. The *L. kriegi* Clade was represented by sequences for *L. buergeri*, *L. kriegi*, *L. tregenzai* Pincheira-Donoso and Scolaro, 2007, and the candidate species *Liolaemus* sp. A, *Liolaemus* sp. B, *Liolaemus* sp. C (Morando *et al.* 2003) and *Liolaemus* sp. D (Medina *et al.* 2014). The sequences for *L. buergeri* from GenBank include data for lizards from the type locality of El Planchón, Chile (generated by Medina *et al.* 2014). The *L. elongatus* Clade was represented by the following taxa: *L. antonietae* Troncoso-Palacios, Esquerré, Urra, Díaz, Castro-Pastene, and Ruiz, 2018; *L. antumalguen* Avila, Morando, Perez, and Sites, 2010; *L. burmeisteri* Avila, Perez, Medina, Sites, and Morando, 2012; *L. carlosgarini* Esquerré, Núñez, and Scolaro, 2013; *L. crandalli* Avila, Medina, Perez, Sites, and Morando, 2015; *L. curis* Núñez and Labra, 1985; *L. elongatus*; *L. janequeoae* Troncoso-Palacios, Díaz, Puas, Riveros-Riffo, and Elorza, 2016; *L. leopardinus* Müller and Hellmich, 1932; *L. scorialis*; *L. smaug* Abdala, Quinteros, Scrochii, and Stazzonelli, 2010; and the candidate species *Liolaemus* sp.1, *Liolaemus* sp.2,

Liolaemus sp.3, *Liolaemus* sp. 6A, *Liolaemus* sp. 6B (Medina *et al.* 2017) and *Liolaemus* sp. “Lircay” (Troncoso-Palacios *et al.* 2018). We consider *Liolaemus* sp.7 (Morando *et al.* 2003) to be conspecific with *L. antumalguen* following Troncoso-Palacios *et al.* (2018). Accession numbers of the cyt-b mitochondrial sequences generated in this study and the sequences obtained from GenBank are in Appendix I. Sequences were aligned using the Software MUSCLE (Edgar 2004) included in MEGA v.6. For each terminal (described or candidate species) we identified the haplotypes using DnaSP v5.10 (Librado and Rozas 2009), keeping only one sequence of each haplotype in the Bayesian inference (BI) analyses. We used JModelTest v2.1.7 (Guignon and Gascuel 2003, Darriba *et al.* 2012) to select a substitution model (HKY + G + I) and performed a BI analyses with MrBayes v3.2.6 (Ronquist *et al.* 2012) with two independent analyses, each consisting of two groups of four chains that ran independently. These were run for 15×10^6 generations, sample frequency = 1000. *Phymaturus vociferator* Pincheira-Donoso, 2004 was the outgroup. The initial 25% of the samples were discarded as burn-in when calculating the convergence diagnostic, assessed by examining values of average standard deviation of the Potential Scale Reduction Factor (PSRF = 1.000 for all parameters; Gelman and Rubin 1992) and the minimum and average Estimated Sample Size (ESS > 5000 for all parameters).

Our results (Figure 1) are highly congruent with previous cyt-b phylogenies (Avila *et al.* 2012, Troncoso-Palacios *et al.* 2018) and multilocus phylogenies (Avila *et al.* 2015, Medina *et al.* 2017). *Liolaemus buergeri* was recovered as monophyletic (posterior probability = 1) and our samples (Colección de la Pontificia Universidad Católica de Chile, SSUC Re 776 and 777; Figure 2) expand its northern distributional limit to Los Humos, in the region of O'Higgins, Chile, more than 33 km (airline) from the current northern limit in its type locality El Planchón in the region of Maule, Chile

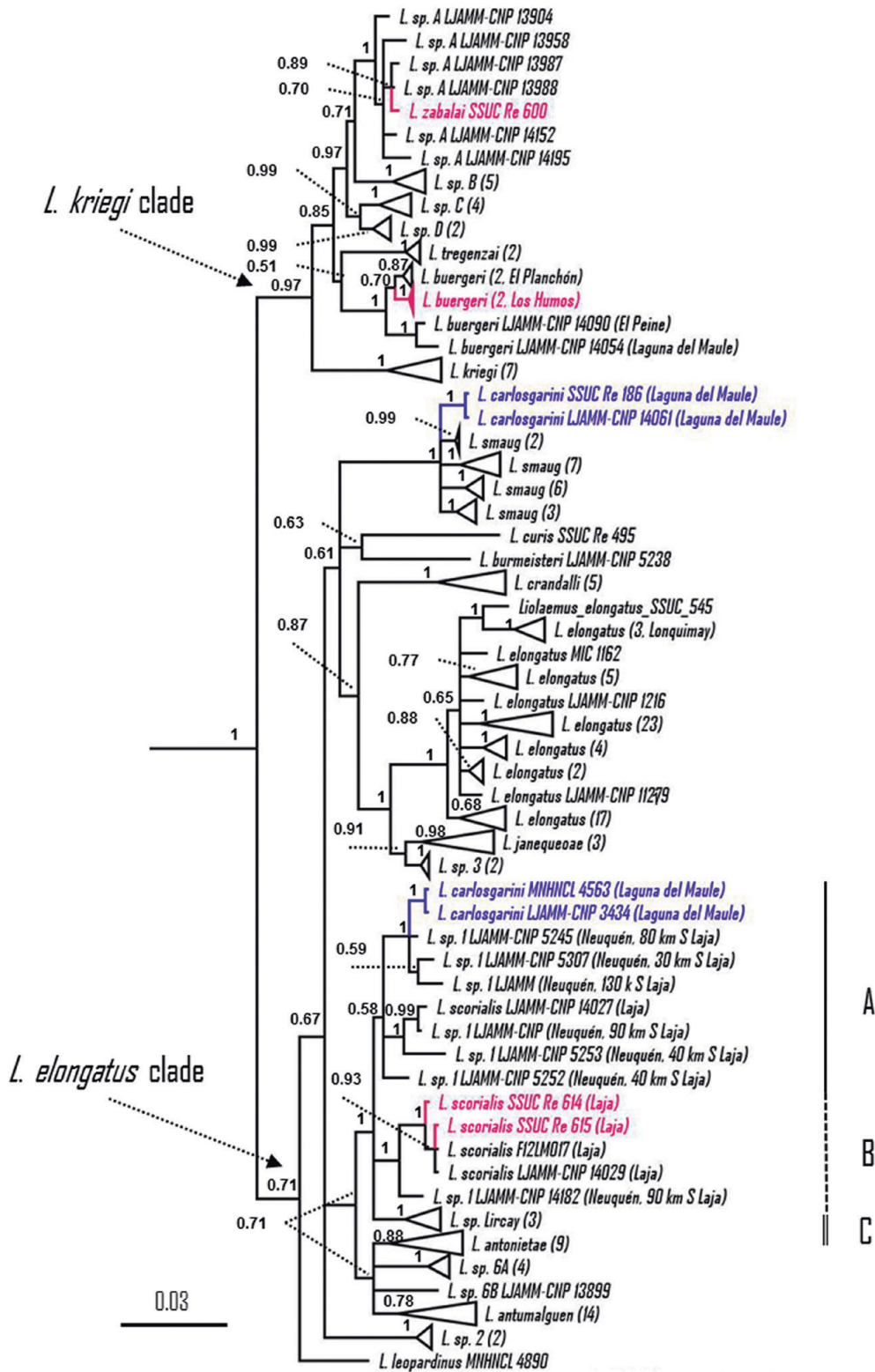


Figure 1. Phylogenetic relationships using Bayesian inference (50% consensus tree) based on *cyt-b* (HKY + G + I). In red: new northern limit for *Liolaemus buergeri*, *L. zabalai* holotype and two *L. scorialis* paratypes. In blue: samples of *L. carlosgarini*. Posterior probabilities are indicated. Scale shows the number of changes per site. Numbers between parentheses indicate the number of sequences collapsed of each node. Outgroups (*P. vociferator*, *L. petrophilus* clade, and *L. punmahuida* clade) are not shown.

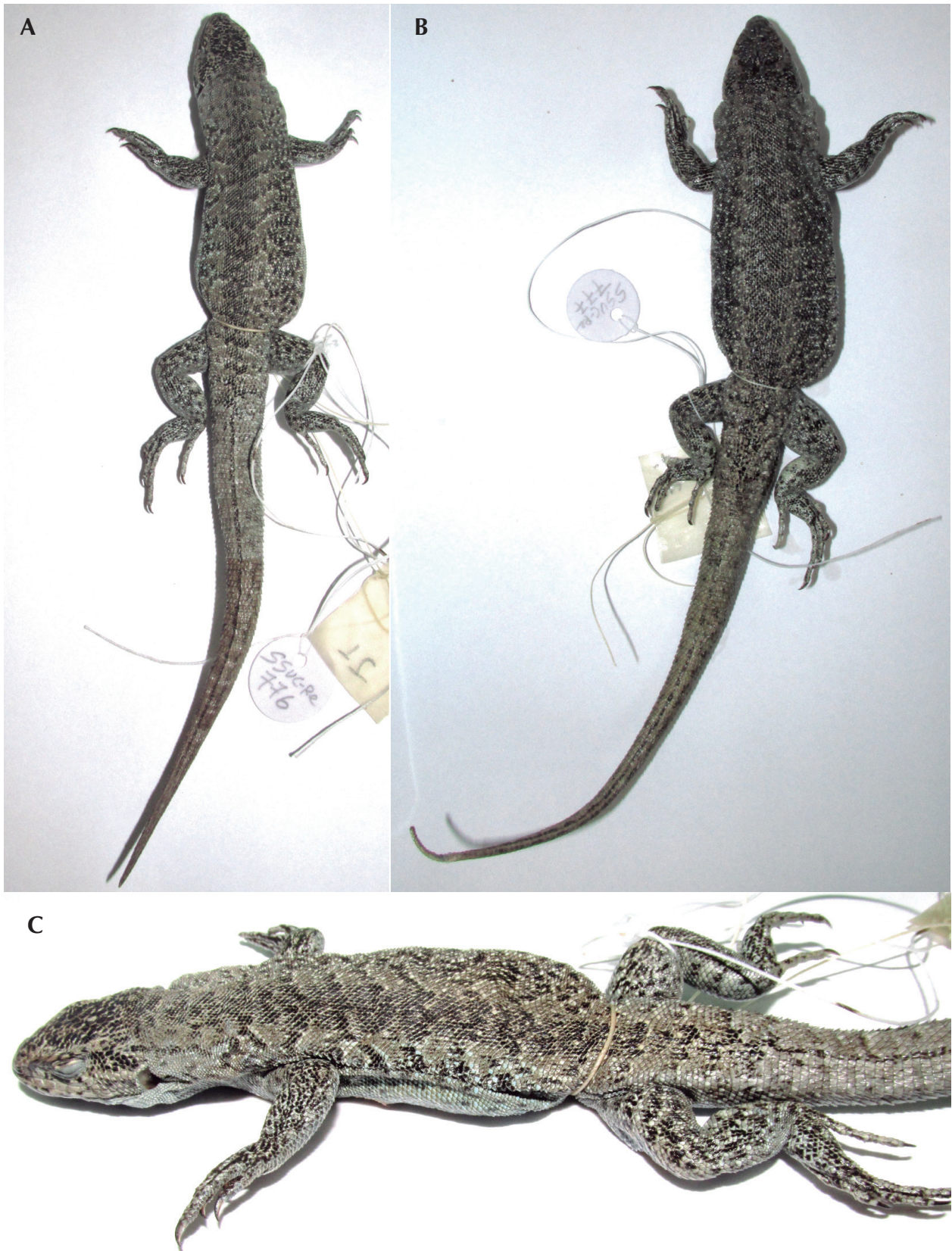



Figure 2. Specimens of *Liolaemus buergeri* from Los Humos (new record). (A) Dorsal view of SSUC Re 776. (B) Dorsal view of SSUC Re 777. (C) Side view of SSUC Re 776.

(35°08' S; 70°31' W; Werner 1907). This new record was suggested by Troncoso-Palacios *et al.* (2011) in an unreviewed naturalist publication, but was not substantiated by evidence.

The holotype of *Liolaemus zabalai* and all sequences of *Liolaemus* sp. A form a strongly supported node (posterior probability = 1), thereby confirming the conspecific status of these specimens. In addition, the placement of *L. zabalai* in the *L. kriegi* Clade, as was proposed by Troncoso-Palacios *et al.* (2016), is confirmed.

The taxonomic relationships of *Liolaemus scorialis* are complex. In our phylogeny, *L. scorialis* is related to *L. carlosgarini*. However, the latter was paraphyletic in a cyt-b phylogeny (Troncoso-Palacios *et al.* 2016) and in a concatenated mtDNA + nuclear DNA phylogeny (Medina *et al.* 2018; p. 164). We found that *L. carlosgarini* was represented in two disparate clades (Figure 1); one is closely allied with *L. smaug*, whereas the other is closely related to *Liolaemus* sp.1. In contrast, in our phylogeny, *L. scorialis* is nested in a node with strong support (posterior probability = 1) which has three main clades (Figure 1). Clade A (posterior probability = 0.58) is formed by part of *L. scorialis* + part of *L. carlosgarini* + part of *Liolaemus* sp.1. Clade B (posterior probability = 1) is composed of part of *L. scorialis* (including the paratypes) + part of *Liolaemus* sp.1. Clade C (posterior probability = 1) contains sequences of *Liolaemus* sp. “Lircay”. The description of *L. carlosgarini* (Esquerré *et al.* 2013) was based on a morphological cladistic analysis which did not include *L. smaug*, but did include data from the taxon’s description (Abdala *et al.* 2010) in the diagnosis. Additionally, Esquerré *et al.* (2013; p. 433) pointed out that *L. carlosgarini* and *L. smaug* have “striking exomorphological similarity” and that “[*L. smaug*] is found relatively close to the type locality of *L. carlosgarini*.” We propose two hypotheses that require further research. In the first hypothesis, *L. scorialis*, *L. carlosgarini*, and *Liolaemus* sp. “Lircay” are full species, but *L. carlosgarini* show evidence of introgression from *L. scorialis* and from *L. smaug*. This should be explored with a

multilocus DNA study in conjunction with morphological comparisons based on the examination of vouchers of both species to diagnose *L. carlosgarini* from *L. smaug*, and then *Liolaemus* sp. “Lircay” should be described. In the second hypothesis, *L. scorialis* and *Liolaemus* sp. “Lircay” are conspecific, and *L. carlosgarini* is a hybrid generated from *L. scorialis* and *L. smaug*. These issues need to be clarified because if *L. carlosgarini* is based on hybrid specimens, then it is an unavailable name (ICZN 1999, Art. 1.3), but if *L. carlosgarini* is a taxon of hybrid origin, then it is an available name (ICZN 1999, Art. 17.2). Although our phylogeny is based on a single molecular marker, the fact that the same paraphyletic result for *L. carlosgarini* was found in a multilocus phylogeny (Medina *et al.* 2018) strongly suggests that this pattern is not related to the lack of informative characters in our phylogeny. Although it is not possible reach a conclusion at the moment on the validity of either of these hypotheses, we hope that additional research will resolve this issue.

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Appendix I. Sequences used in the phylogeny.

Sequences generated in this study: *Liolaemus buergeri* SSUC Re 776 (MK838610), SSUC Re 777 (MK838611), *L. zabalai* SSUC Re 600 (MK838612).

Sequences obtained from GenBank. For each terminal we used only non-redundant haplotypes (representative haplotypes are in bold): *L. kriegi* clade. *L. buergeri*: **KJ494070**, KJ494071, KJ494072, **KJ494079**, **KJ494066**, **KJ494062**, KJ494069. *L. kriegi*: **AY173802**, AY173814, KJ494012, **KJ494027**, KJ494028, **KJ494049**, **KJ494076**, KJ494150, KJ494155, **KJ494186**, KJ494188, KJ494190, KJ494191, **KJ494223**, **KJ494235**. *L. tregenzai*: **KJ494230**, KJ494037, KJ494038, KJ494039, KJ494040, **KJ494036**. *Liolaemus* sp.A: **KJ494032**, **KJ494052**, **KJ494056**, **KJ494057**, KJ494058, KJ494060, KJ494061, KJ494073, KJ494084, **KJ494074**, KJ494087, **KJ494085**. *Liolaemus* sp. B: **KJ494192**, KJ494220, **KJ494193**, **KJ494213**, **KJ494224**, KJ494225, **KJ494236**, KJ494237, KJ494238, KJ494239, KJ494240, KJ494241, KJ494242, KJ494243. *Liolaemus* sp. C: **KJ494009**, KJ494010, **KJ494019**, **KJ494022**, **KJ494212**. *Liolaemus* sp. D: KJ494122, **KJ494194**, KJ494196, KJ494214, **KJ494215**. *L. elongatus* clade. *L. antonietae*: **AY529901**, AY730669, AY730673, AY850621, **KY127643**, KY127644, **KY127645**, KY127648, KY127649, KY127650, **KY127651**, KY127652, **KY127730**, KY127731, KY127963, KY127964, **KY127965**, KY127969, KY127970, **MH178605**, **MH178606**, **MH178607**. *L. antumalguen*: **KY128092**, KP121325, KP121335, **KY128015**, KY128017, KY128020. AY173580, **KY127620**, KY127622, **KY127623**, KY127624, KY127625, KY127627, KY127628, KY127629, **KY127630**, KY127654, **KY127658**, KY127795, **KY127796**, KY127797, KY127807, **KY127808**, **KY127809**, KY127810, KY127811, KY127813, **KY127814**, **KY127815**, AY173823, KY127827, KY127923, **KY127924**, KY127925, KY127926, KY127927, KY127928, **KY127929**, KY127931, KY127934, KY127936, KY127937, KY128082, KY128083, KY128084, KY128085, **KY128088**, KY128091. *L. burmeisteri*: KP121327, KP121328, **KY127939**, KY127941. *L. carlosgarini*: **KY127736**, KY127737, KY127738, KY127739, KY127740, **KY127849**, KY127850, **MH178578**, MH178579, **MH178580**, MH178581. *L. crandalli*: KY127616, KY127617, KY127631, KY127632, KY127633, KY127634, KY127660, KY127661, **KY127662**, KY127664, **KY127665**, KY127668, KY127669, KY127670, **KY127672**, KY127675, KY127676, KY127677, KY127700, KY127702, KY127703, KY127704, KY127761, KY127762, **KY127764**, **KY127765**, KY127766, KY127678. *L. curis*: MH178584, MH178585, MH178586, **MH178587**. *L. elongatus*: AY173557, **AY173564**, AY173568, AY173571, **AY173585**, AY173704, AY173798, **AY173803**, AY173806, AY173809, **AY173815**, AY173818, AY173826, **AY173827**, AY173840, AY173848, AY173853, AY173854, AY173855, **KY127635**, KY127637, **KY127638**, KY127639, KY127640, KY127641, **KY127642**, **KY127657**, KY127679, KY127681, KY127684, **KY127687**, **KY127688**, **KY127689**, KY127693, **KY127695**, KY127696, KY127697, KY127706, KY127708, **KY127759**, KY127768, **KY127769**, KY127770, KY127771, **KY127774**, KY127775, KY127776, KY127777, KY127778, **KY127780**, KY127781, **KY127782**, KY127783, KY127787, **KY127790**, KY127803, **KY127805**, **KY127820**, KY127828, KY127837, **KY127839**, KY127841, KY127842, KY127851, KY127852, **KY127853**, KY127854, KY127857, KY127858, KY127859, **KY127860**, KY127862, KY127866, **KY127869**, KY127874, **KY127875**, **KY127878**, KY127880, KY127884, KY127887, KY127891, KY127892, KY127894, KY127895, **KY127897**, KY127905, KY127908, KY127909, KY127910, KY127912, **KY127914**, KY127916, KY127919, KY128027, KY128053, **KY128054**, KY128056, **KY127974**, **KY127975**, KY127976, **KY127977**, **KY127978**, KY127979, KY127980, **KY127981**, **KY127982**, **KY127985**, KY127986, **KY127988**, **KY127990**, **KY127993**, KY127994, **KY127995**, KY127996, KY127997, KY127998, **KY127999**, **KY128000**, **KY128001**, KY128024, KY128025, **KY128039**, KY128040, **KY128051**, KY128062, KY128064, **KY128068**, KY128115, KY128116, KY128117, **KY128118**, KY128119, KY128120, **KY128121**, KY128123, **KY128124**, KY128125, KY128128, KY128130, KY128131, KY128132, KY128134, KY128137, KY128138, KY128139, KY128147, KY128150, KY128151, **KY128156**, **KY128163**, **KY128164**, **KY128169**, KY128170, **MH178588**, MH178589. *L. janequeoae*: **MH178590**, **MH178591**, **MH178592**. *L. leopardinus*: **MH178596**. *L. scorialis*: **EU649244**, **KY127725**, **KY127727**, **MH178603**, **MH178604**. *L. smaug*: AY173598, AY173599, **AY173600**, **AY173601**, AY173603, AY173691, AY367806, AY173830, **KP121329**, KY127758, KY127773, **KY127821**, **KY127823**, **KY127824**, KY127825, **KY127834**, **KY127835**, **KY128002**, **KY128003**, KY128006, KY128008, **KY128011**, KY128012, **KY128093**, KY128098, **KY128100**, **KY128104**, KY128105, KY128106, **KY128165**, KY128166. *Liolaemus* sp.1: KY127663, KY127741, KY127744, KY127748, KY127749, KY127751, **KY127753**, KY127754, **KY127946**, **KY127948**, **KY127949**, KY127950, KY127951, KY127954, KY127955, KY127957, **KY127958**, **KY127961**, KY127966, KY127967, KY127968, KY127972, KY127973, KY128030, KY128032, KY128034, KY128036, KY128041, **KY128043**, KY128167. *Liolaemus* sp.2: **KY128107**, KY128108, **KY128110**. *Liolaemus* sp.3: **KY127711**, **KY127713**, KY127714. *L. sp.6* (A+B): AY173595, AY173719, AY173816, **KY127717**, KY127718, KY127756, **KY127791**, KY127792, KY127794, KY127798, **KY127799**, **KY127800**, KY127844, **KY127917**, KY127971. *Liolaemus* sp. Lircay: **KY127741**, **KY127744**, **MH178608**. *L. petrophilus* clade. *L. austromendocinus*. **AY173547**, **AY173550**, **AY173551**, **AY367815**, **AY173838**, **AY173839**. *L. capillitas*. **AY173844**. *L. dicktracy*. **AY367816**. *L. gununakuna*. **AY173859**. *L. parvus*. **AY173836**. *L. petrophilus*. **JN847103**, **JN847211**, **AY173796**. *L. talampaya*. **AY173797**. *L. tulkas*. **AY367813**. *L. umbrifer*. **AY367814**. *L. punmahuida* clade. *L. flavipiceus*: **MG660006**. *L. punmahuida*: **KP121336**. *Phymaturus vociferator*: **JX969016**.