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The True Identity of the New World Iguanid Lizard *Liolaemus chillanensis* Müller and Hellmich 1932 (Iguania: Liolaemidae) and Description of a New Species in the *Liolaemus elongatus* Group

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Jaime Troncoso-Palacios, Damien Esquerré, Félix A. Urra, Hugo A. Díaz, Carlos Castro-Pastene, and María Soledad Ruiz (2018) *Liolaemus* is a particularly species-rich radiation of New World iguanid lizards from southern South America. Thanks to intense systematic and taxonomic research, the knowledge on its species-level diversity has increased rapidly over the past several years. The *L. elongatus* species-complex has received considerable attention and a remarkable case is *Liolaemus chillanensis*, a name that has been used for two different species that are sympatric in Termas de Chillán, central Chile. Since the holotype is lost, we propose that the first step to identify the true *L. chillanensis* is through the analysis of the original description. Then we provide a morphological and molecular characterization of *L. chillanensis* based on topotypes and a description of the taxon previously confused with it.

Key words: Chile, Cytochrome *b*, *Liolaemus monticola*, Phylogeny, Principal component analysis.

BACKGROUND

Liolaemus Wiegmann, 1834 is the second most diverse genera of lizards, currently with 257 species (Abdala and Quinteros 2014) and new species described almost every year, especially in the Andean and Patagonian regions of Chile and Argentina (*e.g.* Avila et al. 2015; Esquerré et al. 2013; Troncoso-Palacios et al. 2015). Both morphological and molecular data support two main clades that are considered subgenera, roughly separated by the Andes: the *Liolaemus* (*sensu stricto*) subgenus or "Chilean" group and the *Eulaemus* subgenus or "Argentinean" group

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(Espinoza et al. 2004; Etheridge 1995; Laurent 1985; Schulte et al. 2000).

There is a group of Andean and Patagonian viviparous and saxicolous lizards within the *Liolaemus* (*sensu stricto*) subgenus known as the *L. elongatus* clade, which is part of a complex of clades known as the *L. elongatus-kriegi* complex (Cei 1975 1979). Research on this complex has supported four main clades: *punmahuida*, *petrophilus*, *kriegi* and *elongatus* (Avila et al. 2004 2012; Morando et al. 2003). The *L. elongatus* clade comprises species almost exclusively confined to rocky environments along the eastern slope of the Andes and south of the Mendoza River basin (Argentina), extending to the volcanic hills of Patagonia in Chubut Province, Argentina, and the Araucanía Region in Chile (Avila et al. 2015; Escobar-Huerta et al. 2015b; Minoli et al. 2013; Morando et al. 2003). Members of the *L. elongatus* clade are medium to large sized *Liolaemus* (maximum snout vent length = 107.8), long-tailed, with absent or reduced sexual dichromatism, viviparous, insectivorous, and almost exclusively saxicolous, with a high amount of midbody, ventral and dorsal scales (Abdala et al. 2010; Avila et al. 2015). Currently, this clade is comprised of nine species: *L. antumalguen* Avila et al. 2010, *L. burmeisteri* Avila et al. 2012, *L. choique* Abdala et al. 2010, *L. crandalli* Avila et al. 2015, *L. elongatus* Koslowsky 1896, *L. janequeoae* Troncoso-Palacios et al. 2016, *L. lonquimayensis* Escobar-Huerta et al. 2015b, *L. shitan* Abdala et al. 2010, *L. smaug* Abdala et al. 2010; and possibly *L. carlosgarini* Esquerré et al. 2013 (*fide* Esquerré et al. 2013) and *L. cristiani* Núñez et al. 1991 (*fide* Medina et al. 2014); but recently *L. lonquimayensis* and *L. shitan* were suggested as junior synonyms of *L. elongatus* in Avila et al. (2015) and Troncoso-Palacios et al. (2016), respectively.

One of the most confusing issues in the *Liolaemus elongatus* clade is the identity of the true *L. chillanensis* Müller & Hellmich 1932, a name that has been used indistinctly for two different species of *Liolaemus* that inhabit Termas de Chillán, Biobío Region, Chile. This species has been both included (Avila et al. 2010 2012 2015; Escobar-Huerta et al. 2015b; Medina et al. 2017; Torres-Pérez et al. 2009) and excluded from the *L. elongatus* clade (Lobo et al. 2010; Troncoso-Palacios et al. 2015). One major problem is that the holotype of *L. chillanensis* is lost (Franzen and Glaw 2007). Here, we analyze the original description (Müller and Hellmich 1932), review several vouchers used by Torres-Pérez et al. (2009) and several specimens from different collections, and provide an identification for the species that we conclude is the true *L. chillanensis*. We use both a molecular (our data and data from GenBank) and a morphological characterization. We also provide a description for the taxon previously confused with *L. chillanensis*.

MATERIALS AND METHODS

Morphological data and analyses

We collected specimens in the field by hand or noose. Specimens were dissected to extract a sample of liver/muscle for DNA extraction, and fixed in 99% ethanol. These and all examined specimens (n = 80) are indicated in Appendix 1. Morphological characters were examined according to Etheridge (1995), Lobo (2005) and Avila et al. (2010 2012 2015). Body measurements were taken using a digital Vernier calliper (0.02 mm precision). Body measurements are provided as mean ± standard deviation. The stomach and intestinal contents of the individuals were observed under a binocular microscope for a preliminary description of the species' diet. Data for: *L. choique* and *L. smaug* were taken from Abdala et al. (2010); *L. antumalguen* were mainly taken from Avila et al. (2010), plus some specimens reviewed by us; *L. burmeisteri* were taken from Avila et al. (2012); *L. crandalli* were taken from Avila et al. (2015); *L. lonquimayensis* were taken from Escobar-Huerta et al. (2015b); and *L. cristiani* were taken from Núñez et al. (1991) and two specimens that we reviewed.

As a visualization and exploratory analysis, we performed a Principal Component Analysis (PCA) on the morphometric and meristic variables with the R package *FactoMineR* (Lê et al. 2008). These analyses were performed in the R statistical environment version 3.2.3 (RDCT 2016). We used the residuals of a regression between the snout-vent length (SVL) and the following six variables: head length (distance between anterior edge of auditory meatus and tip of the snout), head width, head height, axilla-groin distance, arm length and foot length. Missing data from some measurements of some individuals were imputed using the *imputePCA* function from the *MissMDA* R package (Josse and Husson 2012).

We performed a nonparametric Kruskal-Wallis test on the meristic variables with a Dunn test for *post hoc* comparisons (Dunn 1964) of

the following: midbody scales, dorsal scales (counted between the occiput and the level of the anterior border of the hind limbs), ventral scales, supralabial scales, infralabial scales and fourth toe lamellae; using data from the following species: *L. antumalguen*, *L. carlosgarini*, *L. chillanensis*, *L. curis*, *L. elongatus*, *L. janequeoae*, *L. scorialis*, the candidate species *L.* sp. Chillán and *L.* sp. 6.

We excluded species without a published dataset from the PCA and Kruskal-Wallis test, but we performed a diagnosis based on scale count and SVL ranges following the diagnosis previously published for the description of *Liolaemus* included in this work (Abdala et al. 2010; Avila et al. 2010 2012 2015; Escobar-Huerta et al. 2015b; Esquerré et al. 2013). Color pattern features were used as qualitative features of diagnosis for all species.

Molecular laboratory procedures

Genomic DNA was extracted using a salt-extraction protocol (slightly modified from Miller et al. 1988). We amplified the mitochondrial gene cytochrome *b* (*Cyt-b*) with Polymerase Chain Reaction (PCR) using the IguaCytob_F2 and IguaCytob_R2 primers (Corl et al. 2010), with the following thermocycle: denaturation at 94°C for 5 minutes, then cycle 35 times at 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 1.5 minutes, and then a final hold at 72°C for 5 minutes. Sequencing reactions were done using a Big Dye Kit (Applied Biosystems, Foster City, CA). Sequencing was run on an ABI 3130xl Genetic Analyzer. Sequences were edited on Geneious 9.0.4 (Biomatters, Auckland, New Zealand, 2015).

Phylogenetic reconstruction

Accession numbers of the *Cyt-b* mitochondrial loci sequences generated in this study and the sequences obtained from GenBank are indicated in Appendix 2. Sequences of the *L. lonquimayensis* type specimens (n = 4) were provided by G. Escobar-Huerta. The 164 nucleotide sequences involved in the analysis were aligned using MUSCLE (Edgar 2004). We used JModelTest v2.1.7 (Darriba et al. 2012; Guidon and Gascuel 2003) to select a substitution model (HKY + G), considering both BIC and AIC information criteria. We identified the non-redundant haplotypes of each terminal taxa using DnaSP v5.10 (Librado and Rozas 2009) and performed a Bayesian inference (BI) analyses with MrBayes v3.2.6 (Ronquist et al. 2012). Two independent analyses,

each consisting of two groups of four chains that run independently, were run for 10 × 10⁶ generations, sample frequency = 1,000. Priors were left by default. *Phymaturus vociferator* Pincheira-Donoso, 2004 was selected as out group because *Phymaturus* is the sister genus of *Liolaemus* (Schulte et al. 2000). 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; Gelmar and Rubin 1992) and the minimum and average Estimated Sample Size (ESS > 5000 for all parameters). Then, to estimate speciation, we used Bayesian implementation of the Poisson Tree Processes (bPTP, Zhang et al. 2013) for species delimitation using the non-ultrametric consensus tree obtained through the BI analyses described above, in the online server (http://species.h-its.org/) run with 50000 MCMC generations (all other parameters by default). Additionally, we generated a maximum clade credibility tree (MCC) using TreeAnnotator v1.8 (Drummond et al. 2012), from the posterior distribution of an MCMC Bayesian phylogenetic inference with BEASTv1.81 (Drummond and Rambaut 2007), which was run for 100 × 10⁶ generations. Relative divergence times (branch lengths) were calculated using the logNormal relaxed clock in BEAST. We then used this ultrametric tree to run the Generalized Mixed Yule Coalescent model (GMYC, Pons et al. 2006). The two sequences of *L.* aff. *chillanensis* from Avila et al. (2015) were not included in the GMYC analysis.

RESULTS

Identification of the true *Liolaemus chillanensis*

Müller and Hellmich (1932) described *L. monticola* and included three subspecies, all these with allopatric distributions: *L. monticola monticola*, *L. monticola chillanensis* and *L. monticola villaricensis*. In the same publication, they referenced an additional *L. monticola* ssp. sympatric with *L. monticola chillanensis*, but did not describe it. Currently, these three described taxa are accepted at the species level (Abdala and Quinteros 2014; Lobo et al. 2010).

The identification of *Liolaemus chillanensis* is problematic because this species name has been used for two different species of *Liolaemus* that occur sympatrically in Termas de Chillán and

share two features: a fragmented vertebral line and a black lateral band. However, one of these *Liolaemus* species has an olive or bluish dorsal and ventral color, lacks precloacal pores and does not belong to the *L. elongatus* clade. The other does not have an olive or bluish dorsal and ventral color, its males have precloacal pores and it belongs to the *L. elongatus* clade. Unfortunately, the holotype of *L. chillanensis*, collected in Termas de Chillán at 1700 m, is lost (Franzen and Glaw 2007) and was not illustrated in the original description by Müller and Hellmich (1932). However, the authors did provide a black and white dorsal picture of one male paratype. Müller and Hellmich (1932) indicated that the color pattern of the holotype's dorsum is olive-brown ("*Färbung der Oberfläche ein dunkles*, *leicht ins Oliv gehendes Rehbraun*", p. 184) and the ventral color is olive ("*Gesamte Unterseite olivgrün*", p. 185). They do not describe the coloration of the male paratypes, but stated that the female paratypes have green or blue shades ("*dunkel-bis hellblaugrün*", Müller and Hellmich 1932:186). Later, Hellmich (1950 1952) stated that this species has a dark "gray-blue" dorsal and ventral color.

At this point it is clear that the true *L. chillanensis* is the lizard from Chillán with olive or bluish dorsal and ventral color. However, Müller and Hellmich (1932) point out that the holotype of *L. chillanensis* has four precloacal pores, although these are difficult to see ("*schwer sichtbar*", p. 184), and according to Hellmich (1950) the precloacal pores in *L. chillanensis* are extraordinarily difficult to see ("*auBerordentlich schwer sichtbar*", p. 147). Note that in the time of *L. chillanensis* description Müller and Hellmich (1932), the absence of precloacal pores in male *Liolaemus* species was unknown, being first reported by Cei and Scolaro (1982), so it is very likely that the mention of precloacal pores in the holotype of *L. chillanensis* was a mistake or that precloacal pores in *L. chillanensis* males are present at an extremely low frequency (we failed to find a topotype with them). Based on the original description (Müller and Hellmich 1932) and the posterior publications of one of the species authorities (Hellmich 1950 1952), we conclude that the true *L. chillanensis* is the lizard that occurs in Termas de Chillán (Chile) and that it can be differentiated by its olive or bluish dorsal and ventral coloration (Fig. 1; see more comparative features in Table 1). This has already been noted by other authors (Donoso-Barros 1966; Pincheira-Donoso and Núñez 2005). In fact, a photograph of the first record of *L.*

chillanensis from Argentina (Avila et al. 2013:228) clearly displays the olive coloration.

In a recent phylogenetic work based on *Cyt-b*, Torres-Pérez et al. (2009) included both "*monticola*" taxa from Chillán: *L. monticola chillanensis* (currently *L. chillanensis*) and the taxon that they identified as the undescribed *L. monticola* ssp. However, we recently reviewed part of the vouchers used as *L. chillanensis* (MZUC 28249 and 28251, Fig. 2) and determined that these do not belong to *L. chillanensis* because they lack olive or bluish dorsal and ventral color and the male has precloacal pores. Thus, we conclude that these belong to an undescribed species. We also reviewed the vouchers MZUC 28257-59 and 28263, used by Torres-Pérez et al. (2009) as *L. monticola* ssp., and noted that they have olive or bluish dorsal and ventral color and the males lack precloacal pores (Fig. 3). To confirm the identification of these two taxa in Torres-Pérez et al. (2009), we sequenced *Cyt-b* for the two species from our own samples, the undescribed *Liolaemus* sp. and *L. chillanensis*. In our *Cyt-b* phylogeny, our sample of *L. chillanensis* is nested with the *L. monticola* ssp. from Torres-Pérez et al. (2009) and our undescribed species is nested with *L. monticola chillanensis* from Torres-Pérez et al. (2009), so the obvious conclusion is that Torres-Pérez et al. (2009) used the name "*L. chillanensis*" for the undescribed species from Termas de Chillán (= *L.* sp. Chillán) and used "*L. monticola* ssp." for *L. chillanensis*.

Phylogenetic relationships

Our Bayesian inference found that neither *Liolaemus chillanensis* nor the undescribed taxon from "Termas de Chillán" (*L.* sp. Chillán) are closely related to *L. monticola* (50% consensus tree) (Fig. 4); this was also found by Torres-Pérez et al. (2009). The inference also concluded that *L. chillanensis* is not part of the *L. elongatus* clade, but is instead sister to *L. cristiani* - a species that also features olive dorsal color and lacks precloacal pores - with strong support. We propose that the name "*L. chillanensis* clade" refer to this group in the future. *Liolaemus* sp. Chillán is recovered as part of the *L. elongatus* clade, with strong support. This clade is also comprised of *L. antumalguen*, *L. burmeisteri*, *L. carlosgarini*, *L. choique*, *L. curis*, *L. leopardinus*, *L. elongatus*, *L. scorialis*, *L. shitan*, *L. smaug*, two candidate species (*Liolaemus* sp. 6 and *Liolaemus* sp. 7) proposed by Morando et al. (2003) and *Liolaemus* sp. from Lircay, this last

referred as *L. carlosgarini* by Escobar-Huerta et al. (2015a). *Liolaemus* sp. 7 and *L. antumalguen* were not found to be reciprocally monophyletic (see below). The same occurs with *L. shitan* and *L. elongatus*, which raises doubts about the validity of the first (see Avila et al. 2015). *Liolaemus* sp. Chillán is closely related to *L. antumalguen*, *L. sp. 6* and *L. sp. 7* with strong support. On the other hand, *L.* aff. *chillanensis* by Avila et al. (2015), listed by Medina et al. (2017) as *L.* sp. 1, is closely related to *L. scorialis*, a species with type locality in Laja, 5 km NW from where the samples of *L.* aff. *chillanensis* were collected (Fig. 4).

The species delimitation through GMYC shows the presence of several species in the data set (likelihood ratio = 12.08; *P* = 0.002). *Liolaemus chillanensis* was recovered as a full species, while eleven lineages were recognized in the *L. elongatus* clade (Fig. 5), *L.* sp. Chillán among them. In the MCC tree, *L.* sp. Chillán is sister to the lineage comprised of *L. antumalguen* + *L.* sp. 7, with *L.* sp. 6 basal to this clade. The GMYC model did not discriminate several species and candidate species as independent lineages: 1) *L. antumalguen* + *L.* sp. 7, therefore considered here as conspecifics, in fact Medina et al. (2017) were also unable to differentiate these two taxa at the genetic level, even with a larger data set. 2) *L. elongatus* + *L. shitan* + *L. lonquimayensis*, previously thought to be junior synonyms of *L.*

Fig. 1. Variation in the true *Liolaemus chillanensis*. (A-B) Male from Termas de Chillán (SSUC Re 708). (C) Male from near Aguas Calientes (SSUC Re 710). (D) Male from Termas de Chillán (SSUC Re 707). (E-F) Female from Termas de Chillán (SSUC Re 709).
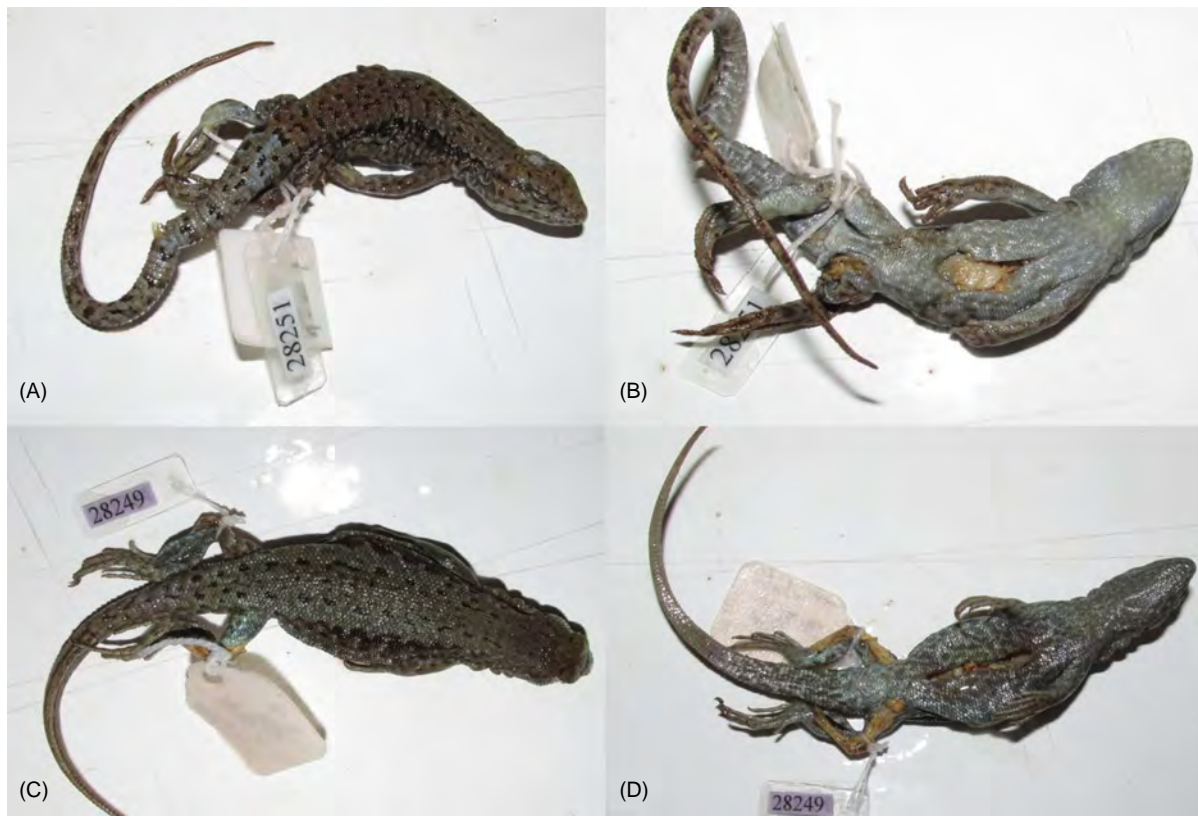
Fig. 2. Some specimens previously misidentified as *L. chillanensis* in Torres-Pérez et al. (2009): (A-B) MZUC 28251 and (C-D) MZUC 28249. Here identified as *L.* sp. Chillán.

Table 1. Characteristics of *Liolaemus antonietae* sp. nov. and the species of the *L. elongatus* clade that occur near it, plus *L. chillanensis.* Juvenile specimens examined are excluded. M = males; F = females

	L. antonietae sp. nov. (M = 6, F = 2)	*L. antumalguen*	*L. burmeisteri*	*L. carlosgarini* (M = 6, F = 11)	*L. chillanensis* (M = 9, F = 4)	*L. elongatus* (M = 3, F = 3)	*L. scorialis* (M = 8, F = 3)	*L. smaug*
Max SVL (mm)	77.6	107.8	85.2	68.8	85.8	73.7	69.9	71.3
Midbody scales range	86-98	72-82	70-81	80-95	82-92	76-88	76-90	73-80
Ventral scales	118-131	105-118	99-110	112-124	113-127	119-129	115-131	119-131
Dorsal scales	71-78	70-78	76-85	68-82	75-88	67-73	72-81	69-83
Dorsal pattern	Fragmented vertebral line, dark paravertebral spots and lateral dark bands	Variable, from patternless to two dorsolateral series of black ocelli sometimes fused longitudinally	Light brown speckled with white spots, flanked by band of dark brown between axilla and groin, with few white spots	Marked or inconspicuous dark occipital band and lateral dark bands	Fragmented vertebral line, dark paravertebral spots and lateral dark bands	Vertebral and lateral bands	Marked dark occipital band and lateral dark bands	Dark occipital band with white dots in males, and lateral dark bands
Ventral melanism	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent
Head color	Light brown	Variable, from completely black to light-tan or ochre	Ochre	Light brown	Brown	Dark brown	Brown/Light brown	Gray to brown
Body color	Light brown	Light gray to ochre	Light brown/kaki	Yellowish brown or light brown	Olive-brown/ bluish brown	Ochre to almost black	Brown/Gray	Yellowish brown (males) or brown (females)
Tail rings	Marked/Weak	Absent	Weak	Marked/Weak	Absent/Weak	Marked/Weak	Marked	Absent
Precloacal pores in males	3-5	3-4	0-5	0-3	0	4-5	3-4	3-4
Behavior	Arboreal-saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Arenicolous

elongatus in Avila et al. (2015) and Troncoso-Palacios et al. (2016) and therefore considered here as part of the *L. elongatus* variation. 3) *L. carlosgarini* was not found to be monophyletic and instead forms part of two not closely related clades: *L. smaug* + *L. choique* + *L. carlosgarini* and *L. carlosgarini* + *L. scorialis*. Our sample of *L. carlosgarini* consists of the type specimens and several topotypes, among which it is not possible to differentiate two morphotypes and should all be assigned to *L. carlosgarini* according to the features provided by Esquerré et al. (2013). These results suggest hybridization or introgression in *L. carlosgarini*, as have been recorded for other *Liolaemus* (Morando et al. 2004 2007). 4) *L. choique* was not found to be an independent lineage (forming part of the *L. smaug* + *L. choique* + *L. carlosgarini* clade); therefore, its taxonomic status should be evaluated in the future. Besides, our bPTP species delimitation analysis recognized thirteen different lineages in the *L. elongatus* clade, among them *L.* sp. Chillán as the fourth with the highest posterior probability (Fig. 6). Results only differ from GMYC in that part of *L. carlosgarini* was recovered as a full species, sister to *L. scorialis*; and part of *L.* aff. *chillanensis* was also recovered as a full species.

Morphologic analyses results

The first three Principal Components (PCs) cumulatively account for 76.86% of the total variation (Table 2). PC1 is mainly explained by variation in head length, head height and head width (Fig. 7). PC2 mostly represents variation in arm length, foot length and AGD (Fig. 7). PC3

Fig. 3. Some misidentified specimens of *Liolaemus chillanensis*. Part of the vouchers used as "*Liolaemus monticola* ssp." in Torres-Pérez et al. (2009) from Termas de Chillán: (A-B) MZUC 28257, (C-D) MZUC 28259. Part of the specimens of "*L. cristiani*" from Termas de Chillán deposited in the MZUC collection (E-F).

mostly represents variation in AGD, head width and foot length (Fig. 7).

The PCA plots (Fig. 7) have 95% confidence ellipses around the centroid of the species and illustrate the morphometric differences between the species included in the analysis. The PC1 vs PC2 graphic shows a partial overlap between *Liolaemus* sp. Chillán, *L. chillanensis*, *L. carlosgarini*, *L. scorialis* and *L. elongatus*, but there is no overlap between *Liolaemus* sp. Chillán and *L. chillanensis* in the PC1 vs PC3 graphic. Although *Liolaemus* sp. Chillán partially overlaps with *L. carlosgarini* in the PC1 vs PC3 graphic, the ellipses have different orientation. *Liolaemus* sp. Chillán, *L. scorialis* and

L. elongatus partially overlap in all PC analyses, but they are not sister species in our phylogenetic reconstruction. According to the mDNA phylogeny, *Liolaemus* sp. Chillán, *L. antumalguen* and *L.* sp. 6 are closely related, but show no overlap in the PC1 vs PC2 graphic; in the PC1 vs PC3 graphic only *L. antumalguen* and *L.* sp. 6 show partial overlap. Besides, *Liolaemus* sp. Chillán and *L. janequeoae* show no overlap in any of the PCs graphics.

A Kruskal-Wallis test on the meristic variables revealed significant differences in three of the six variables analysed: scales around midbody, dorsal scales and ventral scales (Table 3). *Liolaemus* sp. Chillán differs from *L. antumalguen* (closely
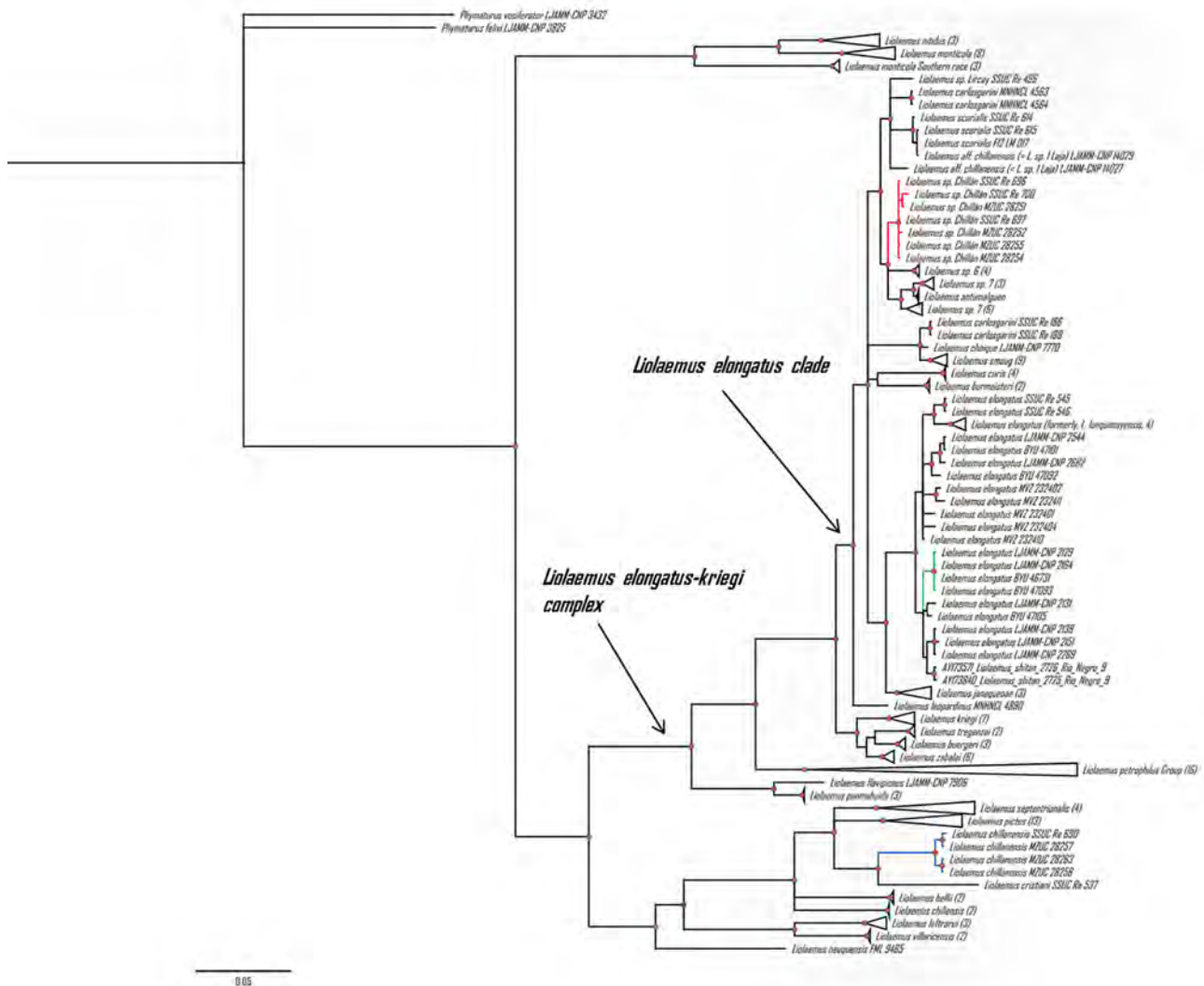

Fig. 4. Phylogenetic relationships using Bayesian inference (50% consensus tree) based on *Cyt-b* (HKY + G). In color: *Liolaemus* sp. Chillán (red, our data from Shangrila, Termas de Chillán and GenBank data from Termas de Chillán), *L. chillanensis* (blue, our data from Termas de Chillán and data from GenBank) and *L. elongatus* from Chubut, type locality (green). Red circle on the node denotes posterior probabilities > 0.95. Scale shows the number of changes per site. Numbers between parentheses indicate the number of sequences of the collapsed nodes.
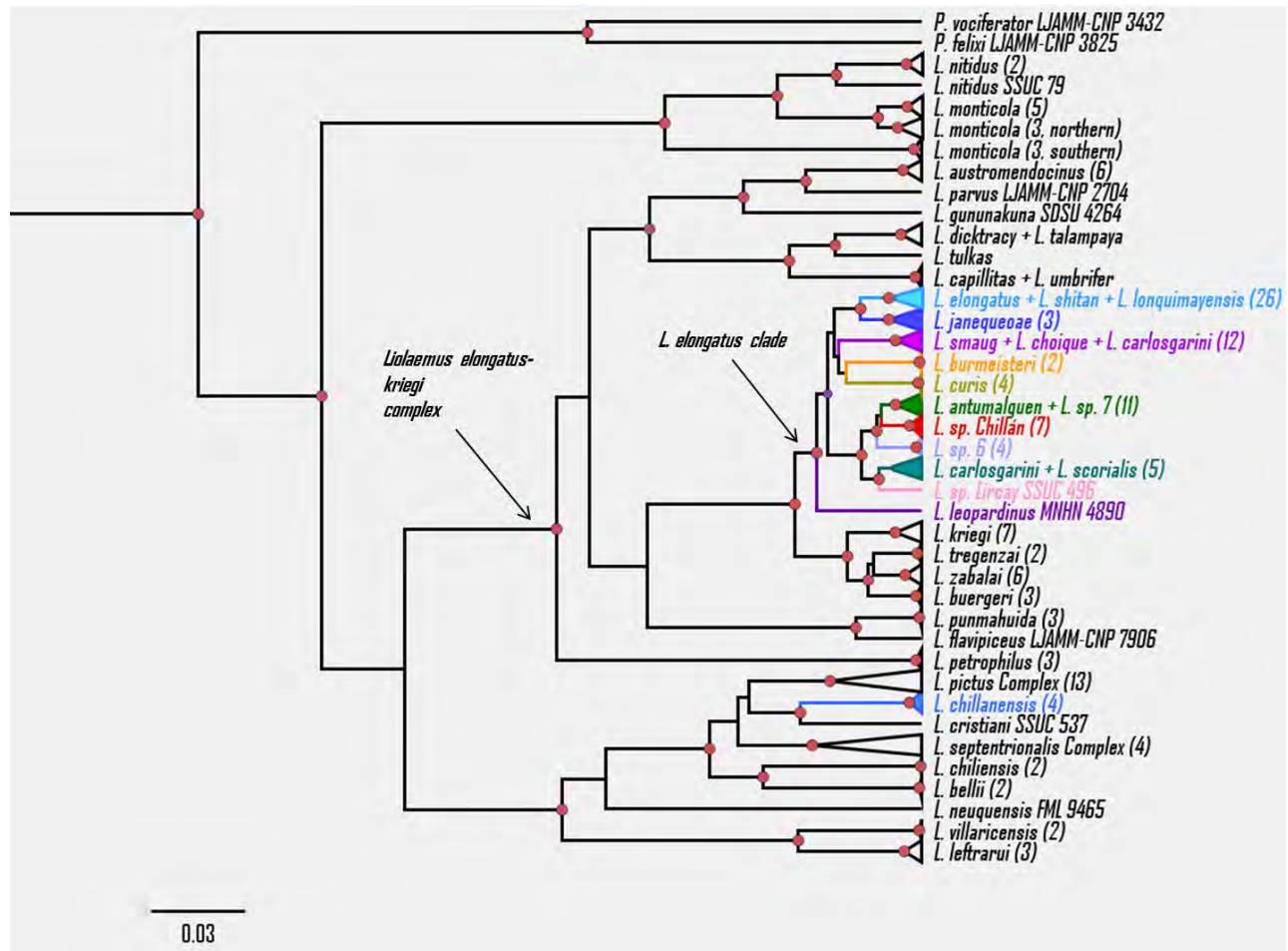
Fig. 5. Maximum Clade Credibility ultrametric tree (MCC) with species delimitation found through the Generalized Mixed Yule Coalescent model (GMYC). Lineages of the *L. elongatus* clade and *L. chillanensis* are in color. Red circle on the node denotes posterior probabilities > 0.95. Scale shows the number of changes per site. Numbers between parentheses indicate the number of sequences of the collapsed nodes.

Table 2. Eigenvalues, the percentage of the total variance and the cumulative percentage of variance for PC1-3, and the correlation of each variable (residuals of a regression with the SVL)

	PCA		
Eigenvalues	PC1	PC2	PC3
Eigenvalue per Component	2.41	1.25	0.94
% of var.	40.29	20.86	15.69
Cum. % of var.	40.29	61.16	76.86
Eigenvectors	PC1	PC2	PC3
Head Length (HL)	0.885	-0.220	-0.081
Head Height (HH)	0.753	0.209	0.373
Head Width (HW)	0.790	-0.133	0.153
Axilla-Groin Distance (AGD)	-0.579	0.373	0.393
Foot Length (FL)	0.225	0.559	-0.750
Arm Length (AL)	0.277	0.831	0.237
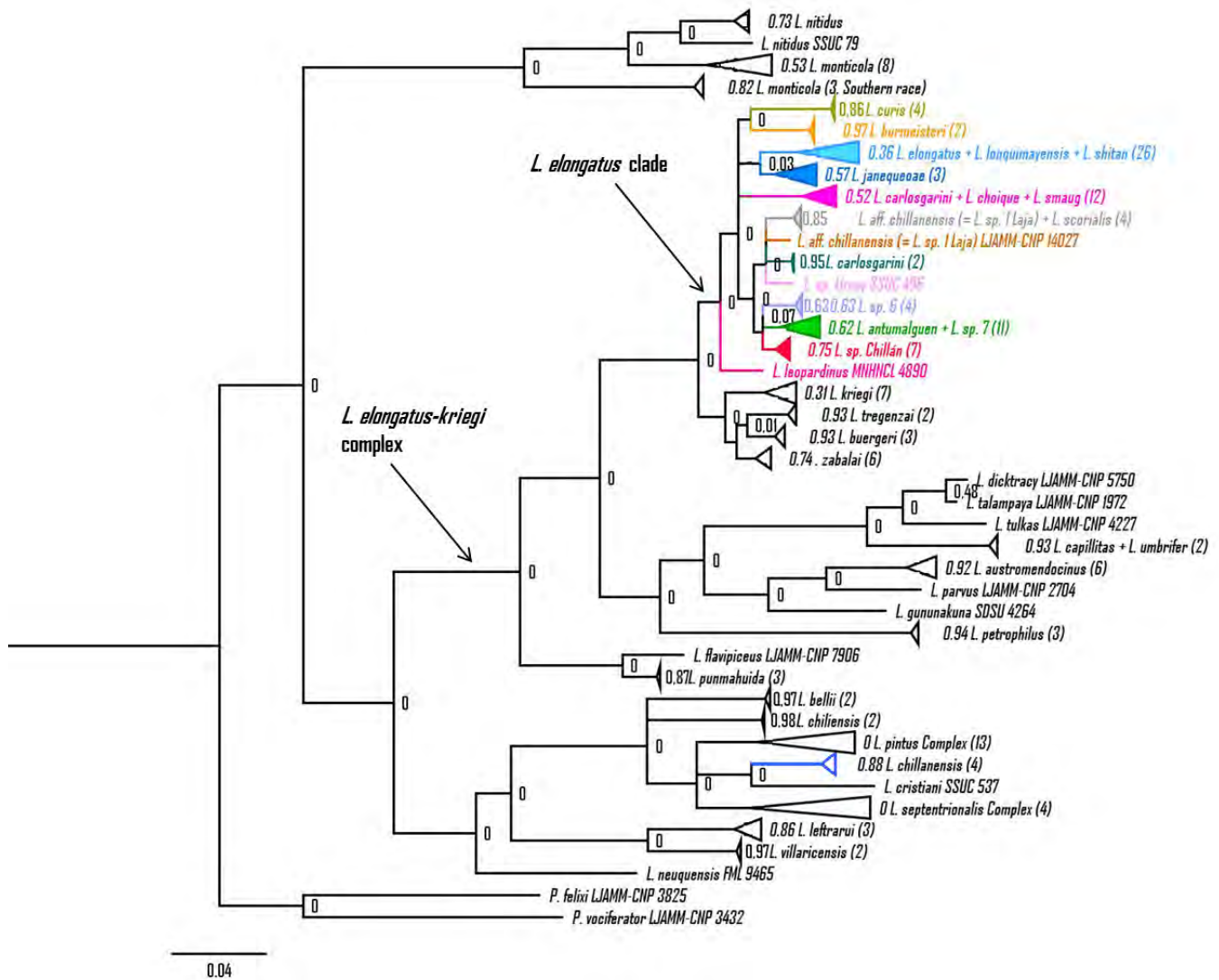
Fig. 6. Bayesian Poisson Tree Processes (bPTP) with highest posterior probability supported delimitation. Zero in the deep nodes denotes that no samples through MCMC recover all sequences as a single species. Values in the collapsed nodes denote the posterior probability to support each species. Scale shows the number of changes per site. Numbers between parentheses indicate the number of sequences of the collapsed nodes.

Table 3. Results of the Kruskal-Wallis test (*H*) with degrees of freedom (*d.f.*) among species of the *L. elongatus* clade and *L. chillanensis* and the *post hoc* comparison (Dunn's test). Only significant results are provided

	Kruskal-Wallis test			Dunn's test
	H	*P*	*d.f.*	
Midbody scales	62.15	< 0.01	8	*L.* sp. Chillán > *L. antumalguen*, *L. curis*, *L.* sp. 6; *L. janequeoae* > *L. antumalguen*, *L. curis*, *L.* sp. 6; *L. carlosgarini* > *L. antumalguen*, *L. curis*, *L.* sp. 6
Dorsal scales	58.46	< 0.01	8	*L. chillanensis* > *L. curis*, *L. elongatus*, *L.* sp. 6; *L. janequeoae* > *L. curis*, *L. elongatus*, *L.* sp. 6; *L. scorialis* > *L. curis*, *L.* sp. 6
Ventral scales	56.51	< 0.01	8	*L.* sp. Chillán > *L.* sp. 6; *L. chillanensis* > *L.* sp. 6; *L. elongatus* > *L.* sp. 6; *L. scorialis* > *L.* sp. 6; *L. janequeoae* > *L. antumalguen*, *L. curis*, *L.* sp. 6

related in our phylogeny) in the number of midbody scales, but does not differ from *L. carlosgarini* or *L. scorialis*.

Based in this, we proposed that *Liolaemus* sp. Chillán need to be recognized as full species because the *Cyt-b* samples of it form a monophyletic lineage; *L.* sp. Chillán also can be statistically differentiated and shows no overlap in morphological space with regards to its most closely related taxa (*L. antumalguen* and *L.* sp. 6).

Liolaemus antonietae sp. n.
(Figs. 2, 8)
urn:lsid:zoobank.org:pub:A4F4AEED-1AB0-48E7-88DD-C2711FBACED2

2009 *Liolaemus monticola chillanensis*, Torres-Pérez, Méndez, Benavides, Moreno, Lamborot, Palma & Ortiz. Biol. J. Linn. Soc., 96: 635.
2015 *Liolaemus chillanensis*, Escobar-Huerta, Santibáñez & Ortiz. Gayana, 79: 95.
2016 *Liolaemus* sp. Chillán Troncoso-Palacios, Díaz, Puas, Riveros-Riffo & Elorza. Zookeys, 632:127
2017 *Liolaemus chillanensis* Medina, Avila, Sites & Morando. J. Zool. Syst. Evol. Res., 55: 238.

Material examined: Holotype: SSUC Re 697, male (Fig. 8A-B). Termas de Chillán, Biobío Region, Chile (36°54'S,71°24'W, 1766 m). Collected by J. Troncoso-Palacios, H. Díaz and F.A. Urra. January 9, 2014.

Paratypes: SSUC Re 695-96, 698-99 (Fig. 8C-G), male, female, male, female, respectively. Same data as the holotype. SSUC Re 700, male. Laguna del Huemul, Shangrila, Biobío Region, Chile (36°52'S, 71°28'W, 1955 m). Collected by F.A. Urra. December 2014.

Specimens used in morphological and color variation: MZUC 38086 and 38090, males. Termas de Chillán.

Specimens used only in color variation: MZUC 28251, 28254 and 28569, three males; and MZUC 28249 one female. Termas de Chillán.

Diagnosis: Here we provide a diagnosis in regards of all species in the *L. elongatus* clade plus *Liolaemus chillanensis* - previously confused with *L. antonietae* - and *L. monticola* - which has a similar color pattern. *Liolaemus antonietae* is a medium size *Liolaemus* (max. SVL = 77.6 mm), with many midbody scales (86-98; 91.5 ± 3.8), lateral dark
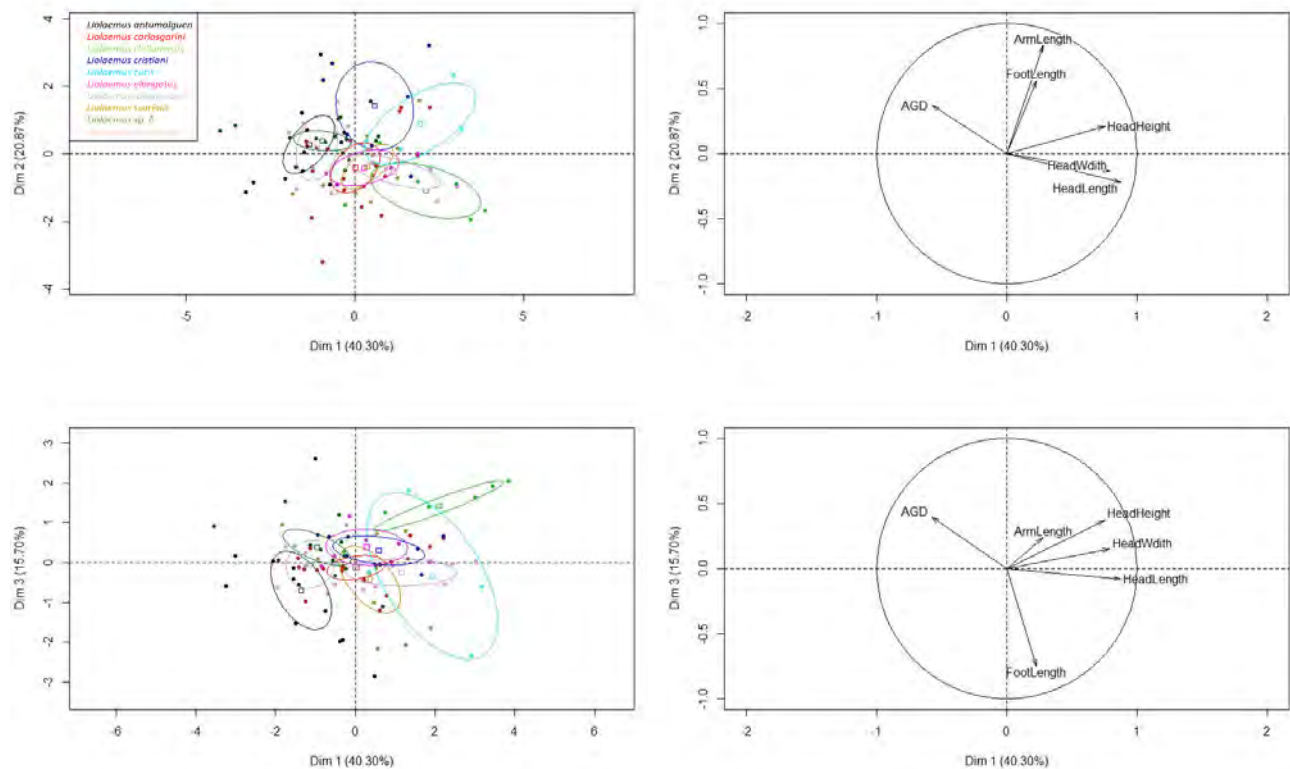
Fig. 7. Principal Component Analysis (PCA) plots. On the left panels, the individuals are colored according to their species as shown on the legend on the top left corner. Ellipses represent the 95% confidence interval around the centroid for each species. On each axis, the PC is labelled according to its number and the percentage of the total variance that PC explains. On the right panels are the variables graphs, which illustrate the contribution of each variable to the construction of the axes.

band, absence of dorsal melanism or black ventral color. The males have precloacal pores and it is the only member of the *L. elongatus* clade that can exhibit arboreal behavior.

Liolaemus antonietae differs from *L. chillanensis* because this species has olive-brown or bluish-brown dorsal color, olive or bluish ventral color and lacks precloacal pores, whereas *L. antonietae* never has olive or bluish coloration and the males have precloacal pores. *Liolaemus antonietae* resembles *L. monticola* in that both species have a brown dorsal color and dark lateral band. However, phylogenetic evidence shows that *L. monticola* does not belong in the *L. elongatus-kriegi* complex (Fig. 4). Moreover, *L. monticola* is smaller (max. SVL = 65.6 mm) and has fewer midbody scales (54-66) than *L. antonietae* (max. SVL = 77.6 mm; midbody scales = 86-98).

Liolaemus antonietae is closely related to *L. antumalguen*, but is smaller (max. SVL = 77.6 mm vs. max. SVL = 107.8 mm) and has more midbody scales (86-98 vs. 72-82, Table 3). Moreover, *L. antumalguen* has black ventral coloration, and several specimens have black transversal dorsal spots or a very melanic dorsal coloration, whereas *L. antonietae* has whitish or gray ventral coloration and totally lacks black transversal dorsal spots.

Liolaemus antonietae has more midbody scales (86-98 vs 76-90), and is larger (max. SVL = 77.6 mm vs max. SVL = 69.9 mm) than *L. scorialis*. Dorsal coloration in *L. scorialis* is noticeably darker than in *L. antonietae*, with a marked dark occipital band, which is absent in *L. antonietae*.

Liolaemus antonietae differs from *L. carlosgarini* because this latter species features a yellowish brown dorsal coloration and a conspicuous dark occipital band, traits absent in *L. antonietae*. All males of *L. antonietae* have precloacal pores, whereas only 50% of *L. carlosgarini* males have precloacal pores.

Liolaemus curis has a yellowish dorsal coloration accompanied by black transversal dorsal spots or an overall melanic dorsal coloration, and less midbody scales (68-76) than *L. antonietae* (Table 3).

Liolaemus antonietae is smaller (max. SVL = 77.6 mm vs. max. SVL = 90.7 mm) than *L. choique*, and although we have no data of *L. choique* for statistical analysis, the midbody scale count ranges show almost no overlap (86-98 vs. 74-88, Table 1). Also, *L. choique* has yellowish or black dorsal coloration, whereas *L. antonietae* has light brown dorsal coloration.

Liolaemus antonietae differs from *L. elongatus*

because this last features a marked occipital and lateral bands or dorsal melanism, whereas only some specimens of *L. antonietae* have an inconspicuous occipital band and no specimen has dorsal melanism.

The dorsal pattern of *Liolaemus antonietae* resembles the pattern of *L. smaug*. However, males of *L. antonietae* have light brown dorsum, whereas males of *L. smaug* have a bright golden yellow dorsal color. Females and males of *L. antonietae* have white dots on the dorsum, a trait only found on males of *L. smaug*.

Liolaemus antonietae differs from *L. janequeoae*, because the latter species does not feature any dorsal pattern apart of few black or white dots and is smaller than *L. antonietae* (max. SVL 66.9 vs 77.6 mm).

Liolaemus antonietae differs from *L. leopardinus*, because it lacks the leopard-like dorsal spots on dorsum and tail present in *L. leopardinus.*

Liolaemus antonietae is smaller (max. SVL = 77.6 mm) than *L. crandalli* (max. SVL = 93.4 mm), but both exhibit a similar color pattern. However, *L. crandalli* has blackish dorsal head coloration, which is light brown in *L. antonietae*. According to the phylogenetic analysis of Avila et al. (2015) this species is the sister taxon of *L. smaug* + *L. choique*, whereas in our phylogeny *L. antonietae* is not closely related to *L. smaug* or *L. choique*.

Although we have no scale count data of *L. burmeisteri* to perform a statistical analysis, the midbody (70-81) and ventral scale count ranges (99-110) show no overlap with *L. antonietae* (86-98, 118-131, respectively). Additionally, almost all specimens of *L. burmeisteri* lack a dorsal pattern, whereas *L. antonietae* always features a dorsal pattern (vertebral line, paravertebral dark spots and white dots).

Description of the holotype: Male. SVL = 61.2 mm. Horizontal diameter of the eye: 2.88 mm. Subocular length: 3.8 mm. Length of the fourth supralabial: 2.5 mm. Head length (from the anterior border of the auditory meatus to the tip of the snout): 14.3 mm. Head height (at the level of ear openings): 6.1 mm. Head width (distance between the two ear openings): 11.8 mm. Neck width: 11.7 mm. Interorbital distance: 6.3 mm. Ear-eye distance: 4.8 mm. Internasal scales distance: 1.8 mm. Ear width: 1.4 mm. Ear height: 1.8 mm. Axilla-groin distance: 24.4 mm. Body width: 17.2 mm. Forelimb length: 23.2 mm. Hindlimb length: 38.6 mm. Tail length (not autotomized): 99.2 mm, with relation tail length/SVL = 1.6. Rostral scale,

Fig. 8. *Liolaemus antonietae* sp. nov., all from type locality. (A-B) Holotype, male (SSUC Re 697), (C-E) Paratypes males (SSUC Re 698 and 695) and (F-G) Paratype female (SSUC Re 699).

wider (2.8 mm) than high (1.1 mm).

Two postrostrals. Four internasals. Interparietal is pentagonal, with a small central spot marking the position of the parietal eye. The interparietal is smaller than the parietals, and is surrounded by six scales. Nine scales between the interparietal and rostral. Seventeen scales between the occiput and the rostral (Hellmich index; Lobo, 2005). Orbital semicircles are complete and formed by thirteen scales on both sides. Five supraoculars on the right side and four on the left. Six superciliary scales. Frontal area divided into five scales, from back to front: 2, 1 and 2. Preocular separated from the lorilabials by one loreal scale. Two scales between nasal and canthal. Nasal in contact with the rostral, surrounded by six scales (excluding the rostral). One row of lorilabials between the supralabials and the subocular. Six supralabials, the fourth is curved upward without contacting the subocular. Five infralabials scales. Pentagonal mental scale, in contact with four scales. Five pairs of post-mental shields, the second is separated by two scales. Temporal slightly keeled, subimbricated. Nine temporal scales between the level of superciliary scales and the rictal level. Four scales on the anterior edge of the ear, slightly projected, which do not cover the auditory meatus. Differentiated auricular scale, which is narrow and elongated. Forty-six gular scales between the auditory meatuses. The lateral neck fold is "Y" shaped. There is a ventrolateral fold running from the axilla to the groin. Midbody scales: 90. Dorsal scales are lanceolated, subimbricated, keeled (without mucrons) and with interstitial granules. Dorsal smaller than the ventrals. Dorsal scales: 74. Ventral scales are rounded, smooth, imbricated and without interstitial granules. Ventral scales: 118. Four precloacal pores. Supra-femoral scales are lanceolated, imbricated and smooth or slightly keeled. Infra-femoral scales are lanceolated or rhomboidal, smooth and imbricated. Supra-antebrachials scales are lanceolated, imbricated and keeled. Infra-antebrachials are rounded or rhomboidal, imbricated and smooth. Dorsal scales of the first third of the tail are rhomboidal, imbricated, keeled and mucronated. Ventral scales the first third of the tail are lanceolated, smooth and imbricated. Lamellae of the fingers: I: 10, II: 14, III: 20, IV: 23 and V: 12. Lamellae of the toes: I: 10, II: 18, III: 22, VI: 29 and V: 19.

Color of the holotype in life: The head has a light brown dorsal color, with few dispersed dark brown spots. The head has a similar shade as the dorsum. The subocular is whitish with a vertical black line at the posterior end. The cheeks are whitish. The temporal zone is light brown with a dark brown stripe on the middle. The dorsum is light brown. There is a fragmented vertebral line. There are 12 series of black spots on the paravertebral fields, running from the occiput to the base of the tail. The flanks are light brown with a black lateral band that goes from the upper portion of the shoulder to the groin. There are several white dots dispersed on this black lateral band. There are white dots dispersed between the black lateral band and the paravertebral fields. The area between the belly and the dark band on the flank is yellowish with dispersed white dots. The limbs are brown, with dispersed black dots on the fore limbs and dispersed black and whitish dots on the hind limbs. The tail has a light brown dorsal coloration, with dark rings. Ventrally, the throat, the belly, the limbs and the tail are light grey, with some whitish scales dispersed. Inconspicuous yellowish color on the cloaca and thighs. Precloacal pores are orange.

Variation: In six males (including holotype): SVL: 61.2-77.6 mm. Axilla-groin distance: 24.4-29.3 mm. Head length: 14.3-18.8 mm. Head width: 11.7-15.3 mm. Head height: 6.1-9.6 mm. Leg length: 38.6-45.7 mm. Arm length: 23.2-26.3 mm. Foot length: 18.9-22.5 mm. Tail length: 99.2-120.0 mm (two specimens, autotomized in the others). Tail length/SVL = 1.6. In two females: SVL: 56.6-70.5 mm. Axilla-groin distance: 24.9-31.8 mm. Head length: 13.4-15.4 mm. Head width: 10.5-12.2 mm. Head height: 6.3-7.3 mm. Leg length: 36.9-38.7 mm. Arm length: 22.0-24.7 mm. Foot length: 18.8-20.2 mm. Tail length: 99.0-110.0 mm (no autotomized), with relation tail length/SVL = 1.6-1.8.

The scalation variation in *Liolaemus antonietae* is as follows. Midbody scales: 86-98 (91.5 ± 3.8). Dorsal scales: 71-78 (74.4 ± 2.3). Ventral scales 118-131 (123.6 ± 5.4). Fourth finger lamellae: 21-24 (23.0 ± 1.1). Fourth toe lamellae: 28-30 (28.8 ± 0.8). Supralabial scales: 6-7 (6.2 ± 0.4), the fourth curved upward. Infralabial scales: 4-5 (4.7 ± 0.5). Interparietal scale pentagonal or hexagonal, bordered by 5-8 scales (5.8 ± 1.2). Nasal and rostral always in contact. Precloacal pores in males: 3-5. Precloacal pores are absent in females.

Males and females have a similar color patterns to the holotype, with small variations. Some specimens have no distinguishable vertebral line, while others have an inconspicuous occipital

band. Some specimens have diffuse dark rings on the dorsal surface of the tail. In specimens with regenerated tail, there is a vertebral line on the regenerated zone. Some males have no yellowish coloration on the cloaca and thighs and have whitish color on the ventral surface of the throat, belly, limbs and the tail, with inconspicuous gray spots dispersed. Females have marked dark spots on the throat and belly and the white dorsal dots are less abundant than males, even totally absent in one female.

Distribution and natural history: Known from two localities in the Biobío Region, Chile: Termas de Chillán (type locality, 36°54'S, 71°24'W, 1,766 m, Fig. 9) and Laguna del Huemul, Shangrila (36°52'S, 71°28'W, 1,955 m), 8 km NW from the type locality. Unlike other species of the *L. elongatus* clade, which are mainly saxicolous (Morando et al. 2003; Avila et al. 2015), *L. antonietae* is mostly arboreal, which was seen basking on fallen trunks and on trees up approximately 4 m high. Few specimens were observed basking on rocks. Furthermore, we observed a very peculiar gregarious behavior, in which 18 specimens were observed coming out of the surrounding vegetation and entering to the same hollow of a stand tree (*Nothofagus* sp.) at sunset, in a time frame no longer than an hour. The vegetation in the type locality is dominated by *Chusquea* sp. and *Nothofagus* sp. It is an abundant lizard. At the date of capture (January), one female had three embryos and the other had several small oocytes. No remains were found in the stomach, but remnants of insects and plants along with parasitic nematodes were observed inside the intestine. In both localities, *L. antonietae* was found in syntopy with snake *Tachymenis chilensis* and lizards *L. chillanensis*, *L. septentrionalis* and *L. tenuis*, being recently recorded also *Phymaturus vociferator* (Urra et al. 2017).

Etymology: This species is named after Antonieta Labra Lillo, a prominent Chilean herpetologist who has made significant contributions to the eco-physiology and behavior of lizards, especially to the study of the influence of chemical signals on their behavior. She has also edited the book "Herpetología de Chile", which had a great impact on the Chilean herpetology. We propose the common name "Antonieta's lizard" in English and "Lagarto de Antonieta" in Spanish. JTP gratefully thanks AL for years of teaching and collaboration.

DISCUSSION

We describe a new species of *Liolaemus* belonging to the *L. elongatus* group, *Liolaemus antonietae*, which was previously identified as *L. chillanensis* by Torres-Pérez et al. (2009) and followed by Escobar-Huerta et al. (2015b). The species was probably misidentified because the definition of *L. chillanensis* itself was ambiguous. The description was written in German (Müller and Hellmich 1932), while most researchers who worked on this species have been Spanish speakers. It is also difficult to obtain this publication (to our knowledge there is no official digital copy available). Moreover, the holotype of *L. chillanensis* is lost (Franzen and Glaw 2007). Here we provide several color pictures of *L. chillanensis* in life, showing the variation in color, and highlight the features that diagnose it, based on the distinctive characteristics that Müller and Hellmich (1932) describe for the holotype. Additionally, we add a *Cyt-b* sequence from *L. chillanensis* to GenBank and we note that the sequences from GenBank currently assigned to *L. monticola* ssp. (AY850623, AY850624, AY730670, AY529904, AY850625, AY529903) are indeed assignable to *L. chillanensis.*

The identity of several specimens assigned to *L. chillanensis* has been confusing. Avila et al. (2010 2012) used a *Cyt-b* sequence of "*L. chillanensis*" (voucher LJAMM-CNP 3434) without locality data, but according to Medina et al. (2017), this specimen was collected in Laguna del Maule, Maule Region, Chile, and is assignable to *L. carlosgarini*. Recently, Troncoso-Palacios et al. (2015) made a mistake regarding this, because they point out that the "*L. chillanensis*" *Cyt-b* sequence used by Avila et al. (2010 2012) was generated by Torres-Pérez et al. (2009), which is incorrect because both works used different vouchers. Additionally, Avila et al. (2015) used a *Cyt-b* sequence of *L.* aff. *chillanensis* (vouchers LJAMM-CNP 14027 and 14029) from 14.2 km W Refugio del Laja and assigned it to the *L. elongatus-kriegi* complex, a result contrary to that obtained by us for *L. chillanensis.* One species in the *L. elongatus-kriegi* complex, *L. scorialis*, was described recently from Laja by Troncoso-Palacios et al. (2015) and it is possible that this publication was not included as background source of data in Avila et al. (2015). In fact, both sequences of *L.* aff. *chillanensis* were placed in the same node as *L. scorialis* in our BI analysis (Fig. 4) and bPTP analysis delimited *L. scorialis* and one sequence
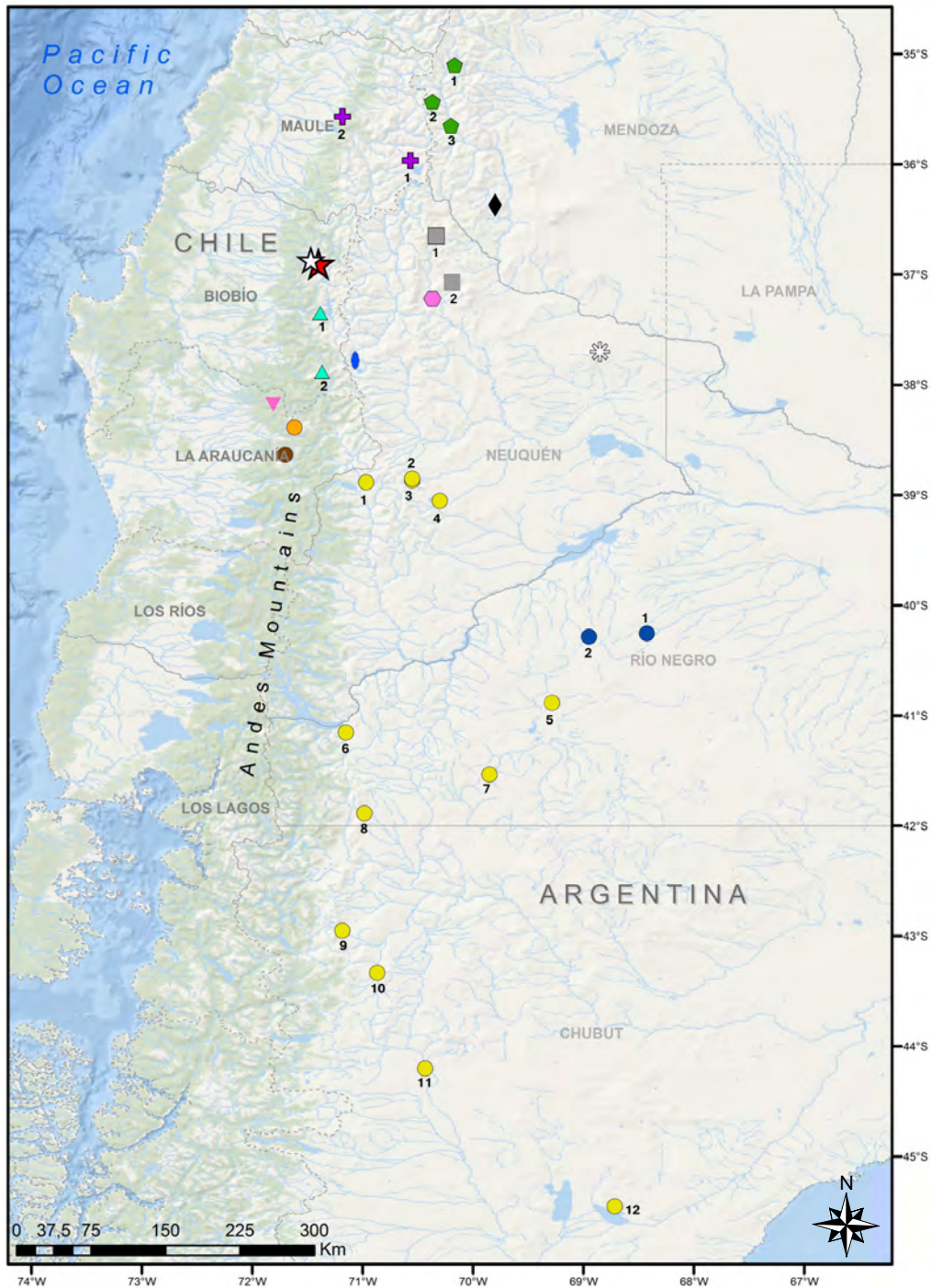
Fig. 9. Distributional map for *Liolaemus antonietae* sp. nov. along with geographically proximate species of the *L. elongatus* clade. Stars: *Liolaemus antonietae* sp. nov (red = Termas de Chillán, type locality; white = Shangrila). Green pentagon: *L. smaug* (1 = near Las Leñas, 2 = between Las Loicas and Peteroa Volcano, 3 = near Las Loicas). Lilac cross: *L. carlosgarini* (1 = Maule Lagoon, 2 = Lircay). Black diamond: *L. choique* (Paso el Choique). Gray square: *L. antumalguen* (1= Domuyo volcano, 2= Tromen Volcano). Pink haxagon: *L. burmeisteri* (Caepe Malal). White asterisk: *L. crandalli* (Auca Mahuida Volcano). Green triangle: *L. scorialis* (1 = Laja Lagoon, 2 = La Mula Lagoon). Orange circle: *L. elongatus* (formerly *L. lonquimayensis*, Lonquimay Volcano). Brown circle: *L. elongatus* used for morphology and DNA analyses (Llaima volcano). Yellow circles: *L. elongatus* used for DNA analyses (1 = Pampa de Lonco Luan, 2 = Primeros Pinos, 3 = Portal La Atravesada, 4 = Laguna Blanca, 5 = near Ingeniero Jacobacci, 6 = San Carlos de Bariloche, 7 = Ojo de Agua, 8 = El Maiten, 9 = Esquel, 10 = Tecka, 11 = Gobernador Costa and 12 = Los Manantiales). Blue circles: *L. shitan* (1 = Estancia Piedras Blancas, type locality, 2 = near San Antonio del Cuy). Pink inverted triangle: *L. janequeoae* (Tolhuaca). Blue ellipse: *L. sp. 6 (Copahue Volcano).

of *L.* aff. *chillanensis* as the same species (Fig. 6). This is congruent with Medina et al. (2017), since they list LJAMM-CNP 14027 and 14029 as *L.* sp. 1 and include "12 taxa… one being described (*L.* sp. 1, Esquerré, personal communication)" in their *L. elongatus* clade analysis (p. 239). In fact, Troncoso-Palacios, Díaz, Esquerré and Urra, described *L. scorialis* (Troncoso-Palacios et al. 2015) from the Laja surroundings.

Medina et al. (2017) found low *Cyt-b* distance between the taxon that they refer as "*L. chillanensis*" (here described as *L. antonietae*) and the lineage composed of *L. antumalguen* + *L.* sp. 7, but stated that both lineages were recover as full species in the species delimitation analysis. Moreover, Medina et al. (2017) shows in the "Supporting information Table 2" that *L. antumalguen* + *L.* sp. 7 do not share *Cyt-b* haplotypes with "*L. chillanensis*". Congruent with this, our species delimitation analyses (GMYC and bPTP) found *L. antonietae* (referred as *L. chillanensis* in Medina et al. 2017) as a lineage at the species level, monophyletic in regards to the most closely related lineage (*L. antumalguen* + *L.* sp. 7). Additionally, the PCA shows that *L. antonietae* and *L. antumalguen* do not share the same morphological space; some meristic variables show statistical differentiation and striking differences in coloration were found. All these support the recognition of this taxon as an independently evolving lineage but the low *Cyt-b* distance suggests that *L. antonietae* is a young species.

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Appendix 1. Specimens examined. Museum codes are as follow: MNHNCL (Museo Nacional de Historia Natural, Chile), MRC (Museo Regional de Concepción), MZUC (Museo de Zoología of Universidad de Concepción) and SSUC (Colección de Flora y Fauna Patricio Sánchez Reyes, Pontificia Universidad Católica de Chile). (download)

Appendix 2. Specimens used for phylogenetic analysis. (download)