Morphological Variation, Niche Divergence, and Phylogeography of Lizards of the *Liolaemus lineomaculatus* Section (Liolaemini) from Southern Patagonia

Author(s): M. Florencia Breitman, Marcelo F. Bonino, Jack W. Sites, Jr., Luciano J. Avila, and Mariana Morando

Source: Herpetological Monographs, 29(1):65-88.
Published By: The Herpetologists' League
DOI: http://dx.doi.org/10.1655/HERPMONOGRAPHS-D-14-00003
URL: http://www.bioone.org/doi/full/10.1655/HERPMONOGRAPHS-D-14-00003

Herpetological Monographs, 29, 2015, 65–88

DOI: 10.1655/HERPMONOGRAPHS-D-14-00003

MORPHOLOGICAL VARIATION, NICHE DIVERGENCE, AND PHYLOGEOGRAPHY OF LIZARDS OF THE *LIOLAEMUS LINEOMACULATUS* SECTION (LIOLAEMINI) FROM SOUTHERN PATAGONIA

M. Florencia Breitman[1,4,5], Marcelo F. Bonino[2], Jack W. Sites, Jr.[3], Luciano J. Avila[1], and Mariana Morando[1]

[1] *CENPAT–Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto Patagónico para el Estudio de Ecosistemas Continentales, Boulevard Almirante Brown 2915, U9120ACD, Puerto Madryn, Chubut, Argentina*

[2] *INIBIOMA (CONICET-UNCOMA), Laboratorio de Fotobiología, Centro Regional Universitario Bariloche, Quintral 1250, 8400, Bariloche, Río Negro, Argentina*

[3] *Brigham Young University, Life Sciences Building, Biology Department, Provo, UT 84602, USA*

Abstract: Patagonia is a biodiverse area of high conservation priority, and *Liolaemus* lizards comprise a large component of the endemic fauna. Recent molecular and morphological studies have revealed cryptic species in several *Liolaemus* groups, including the *Liolaemus lineomaculatus* section (22 species recognized to date), which is endemic to Patagonia. Despite being a conspicuous component of this area, little is known about the morphological, ecological, and genetic variation of lizards of the *L. lineomaculatus* section; moreover species limits and past demographic scenarios are still uncertain for many of these species. In this paper, we characterize the morphological, ecological (niche envelope), and genetic variability of the four southernmost species of the *L. kingii* group (*L. lineomaculatus* section). Our main goal is to clarify species boundaries (using integrative taxonomy) as well as to infer evolutionary and demographic histories. For this paper we used a total of 241 specimens, 195 of which were used for morphological analyses (10 morphometric, 10 meristic, and 7 qualitative characters) and 226 were sequenced for cytochrome *b*. We summarized ecological variation by using environmental data from 62 localities of occurrence in a geospatial modeling analysis to estimate current and past species niche envelopes and to test for niche similarity. We identified genetic lineages and evaluated differentiation among them at molecular, morphological, and niche envelope levels. Overall, we found support for the specific status of *L. baguali*, *L. escarchadosi*, and *L. sarmientoi* based on differentiation along each of these three levels. *Liolaemus tari* is also differentiated from the other species, even though we could not evaluate its niche envelope due to small sample size. We also show the first evidence of possible hybridization among some of these species and recognize a new candidate species.

Key words: Argentina; Biogeography; Evolution; Integrative taxonomy; *Liolaemus kingii* group

Liolaemus is an ecologically diverse and species-rich genus of lizards (Lobo et al. 2010; Breitman et al. 2011) distributed from Peru to Tierra del Fuego in temperate South America (8°S–54°S; Bottari 1975; Laurent 1990) and ranging in altitude from sea level to at least 5176 m (Aparicio and Ocampo 2010). The genus includes 260+ described species (Abdala and Quinteros 2014) and has been considered an interesting genus for studies of conservation, ecology, physiology, and phylogeography (Vega et al. 2000; Corbalán et al. 2011; Breitman et al. 2013; Kacoliris et al. 2013). The extensive morphological variation in *Liolaemus* has led to many taxonomic rearrangements since the genus was originally described by Wiegmann (1834; see Lobo et al. 2010 for a general overview); also, several molecular and morphological phylogenies (none including all species of the genus) have been recently proposed (Schulte et al. 2000; Lobo et al. 2010; Pyron et al. 2013; Olave et al. 2014).

The *Liolaemus lineomaculatus* section (including 22 species; some of them isolated, some sympatric, and some with partially overlapping distributions) is endemic to Patagonia and represents one of the most conspicuous groups of Patagonian vertebrates (Breitman et al. 2013; Abdala et al. 2014). Recent molecular phylogenies and a morphological revision are now available for the section (Breitman et al. 2011, 2013); both studies identify three main species groups: *L. lineomaculatus*, *L. magellanicus*, and *L. kingii*. Among those groups, a phylogeographic study of the *L. lineomaculatus* group has identified several candidate species (Breitman et al. 2012). A checklist with distributional data is also available for all lizard species from the southernmost province of Argentina (Breitman et al. 2014). Although these contributions represent significant advances, there is still a lack of information regarding species boundaries, past evolutionary scenarios, and demographic dynamics for most species of Patagonian lizards (Corbalán et al. 2011).

Patagonia is one of the last remote wild places on Earth. Because of its natural beauty, peculiar flora and fauna, and complex geological history, this region has attracted the attention of many naturalists (e.g., Darwin 1859; Moreno 1876; Ameghino 1906). Further, Patagonia has been assessed as a region with high endemism and high conservation priority (Corbalán et al. 2011). Desertification, caused mainly by overgrazing livestock and "mega-projects" (oil and mineral extraction and hydroelectric projects), is now considered a significant threat to Patagonian biodiversity (Paruelo and Aguiar 2003; Paruelo et al. 2006). Rigorous evaluations of biodiversity will provide the basis to develop realistic management and conservation projects that can minimize human impact. Evaluation of biodiversity to be used in conservation plans should be based on our best available evidence regarding taxonomy, species boundaries, distributions, and ecology (Mace 2004; Dayrat 2005; Schlick-Steiner et al. 2010).

Recently, the combination of the general lineage concept (de Queiroz 2005) with an "integrative taxonomy" (Dayrat 2005) methodological framework has provided a rigorous structure to evaluate species boundaries (Aguilar et al. 2013; Carstens et al. 2013; Miralles and Vences 2013). The strength of this approach is due to the delineation of species boundaries based on multiple and complementary lines of

[4] Present Address: University of Brasília, Department of Ecology, 70910-900 Brasília, DF, Brazil

[5] Correspondence: e-mail, florbreitman@gmail.com, breitman@unb.br

evidence including phylogeography, population genetics, comparative morphology, ecology, development, and behavior (Dayrat 2005; Aguilar et al. 2013; Carstens et al. 2013; Miralles and Vences 2013). The combination of at least two of these data types leads to lower error rates when assigning individuals to taxa (Schlick-Steiner et al. 2010). For example, a recent review of species delimitation in arthropods showed that when using only one line of evidence, failure rates for species delimitation ranged between 0.08 and 0.6, including 0.23 for morphology, 0.33 for mitochondrial DNA, 0.28 for nuclear DNA, and 0.6 for ecology, among others (Schlick-Steiner et al. 2010). Also, the same study showed that using at least three methodological approaches could reduce the mean failure rates significantly to an average error rate of 0.027 (Schlick-Steiner et al. 2010). The use of such a multidisciplinary framework has the final goal of providing good alpha-taxonomic documentation of diversity to critically underpin conservation programs addressing the biodiversity crisis (Schlick-Steiner et al. 2010; Arribas et al. 2012).

Several research projects focused on Patagonian taxa documented areas with high specific and genetic diversity from which some general biogeographic patterns are emerging (see review by Sérsic et al. 2011). However, only one study has explicitly assessed species limits in Patagonian lizards using an integrative taxonomic approach (Medina et al. 2013). Medina et al. (2013) included morphological and molecular data to corroborate and refine the species boundaries of lizards of the *Liolaemus kriegi* group of northwestern Patagonia.

Specifically for lizards of southern Patagonia included in the *Liolaemus kingii* group, phylogenetic relationships have been inferred using several markers and based on concatenation and species tree methods (Schulte and Moreno-Roark 2010; Breitman et al. 2011; Pyron et al. 2013; Olave et al. 2014). These studies have greatly advanced our understanding of the systematics of the group, but phylogenetic relationships are not completely resolved among the southernmost species of this group. Also, information regarding species' ecological and genetic variation remains limited; indeed, species boundaries are still uncertain for many individuals not collected from type localities (Cei 1986; Scolaro and Cei 1987, 1997; Breitman et al. 2013). Moreover, hypotheses about evolutionary scenarios and past demographic histories (from the field of phylogeography) are very limited and not strongly supported. Current knowledge about morphological variation in these species is mainly restricted to individuals collected from type localities, and a few studies show differentiation among species in color patterns, morphometric and meristic characters (Scolaro and Cei 1997; Breitman et al. 2013). These studies also showed high between-individual variation in morphological attributes within species and encouraged further study based on better geographic sampling (Breitman et al. 2013). For example, few studies include specimens collected throughout complete species ranges (which are often unknown), precluding accurate estimation of morphological, ecological, and genetic variability within and among widely distributed species.

In this paper we extend the earlier morphological study of Breitman et al. (2013) to a broader integrative phylogeographic study of the southernmost species of the *Liolaemus kingii* group. Specifically, we characterize ecological, genetic, and morphological variation in the southernmost species of the *L. kingii* group (*L. baguali*, *L. escarchadosi*, *L. sarmientoi*, and *L. tari*). Our goal here is not to solve phylogenetic relationships among the southernmost species of the *L. kingii* group (previously attempted using several nuclear genes); rather we present the first integrative evidence of species boundaries and hypothesize evolutionary and demographic histories of the recovered lineages.

Materials and Methods

Sampling Design

We collected a total of 241 specimens of the *Liolaemus kingii* group (Breitman et al. 2013) belonging to the *L. lineomaculatus* section (Schulte et al. 2000) from 43 localities (catalogued in the LJAMM-CNP herpetological collection of Centro Nacional Patagónico in Argentina). We also included 14 historical records of the focal species in this study from the literature (Bonino 2013) and we confirmed species identity after examining museum collections. We collected individuals across the distributional area of *L. baguali*, *L. tari*, *L. escarchadosi*, and *L. sarmientoi* (Fig. 1) in Santa Cruz Province south of the Central Plateau (south of ~48.5°S). We used several species as outgroups (Appendix I; Breitman et al. 2011) and sequenced a total of 226 individuals for this study. We used 195 individuals (153 adults and 42 juveniles, 100 females and 95 males; Appendix I) for morphological analyses and 62 locality records to perform niche envelope analyses. About 25% of the individuals used in the present study were employed in previous molecular and morphological studies (Breitman et al. 2011, 2013).

Genetic Data and Analyses

We extracted genomic DNA from liver using the Qiagen® DNeasy® 96 Tissue Kit, following the manufacturer's protocol. We amplified the 745-bp mitochondrial cytochrome *b* fragment (Kocher et al. 1989) for 226 individuals following the polymerase chain reaction and sequencing protocols of Morando et al. (2003). We edited sequences using SEQUENCHER v4.8 (TMGene Codes Corporation Inc. 2007) and translated sequences to amino acids to confirm the open reading frame. We performed alignments with MAFFT (Katoh et al. 2002) and deposited new sequences in GenBank (Accession numbers KP175352–KP175571, Appendix I). We used some previously published sequences mainly for outgroups (Appendix I).

We screened the 36 nuclear genes described in the Appendix S1 of Breitman et al. (2012), but did not find any genetic variation among our focal species; thus we used only the mitochondrial cytochrome *b* fragment. Despite limitations, we are confident in the value of this marker as a first-pass indicator of female population structure and identification of candidate species (Davis and Nixon 1992; Morando et al. 2003; Breitman et al. 2012; Aguilar et al. 2013). For our study, we are using gene trees and not species trees (Davis and Nixon 1992); in order to compensate the lack of species tree we included other lines of independent evidence (ecology and morphology).

We identified genetic lineages following Breitman et al. (2012) by (1) constructing a cytochrome *b* guide tree (see next paragraph) that includes all the ingroup haplotypes and outgroups; (2) identifying main clades/lineages (monophyletic

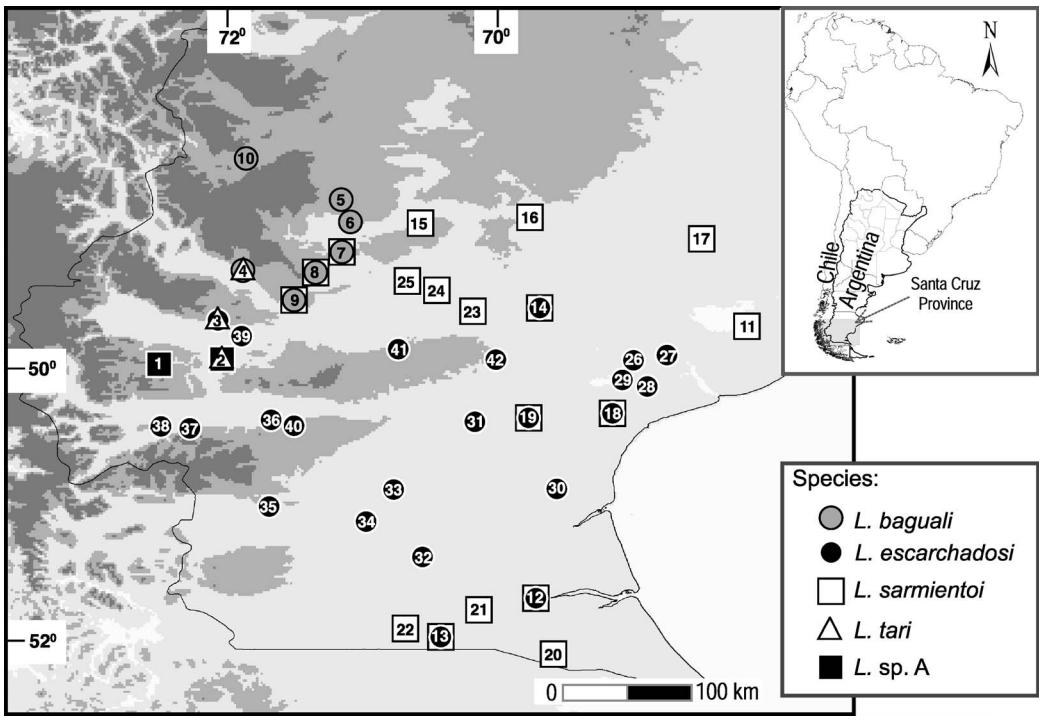

FIG. 1.—Map of southern Santa Cruz province showing the distribution of the focal species and the sampled localities. Areas above 500 and 1000 m are shaded in gray tones.

groups with approximately 3% genetic distances among them or more; see discussion in Breitman et al. 2011, 2012); (3) naming clades/lineages that include individuals collected from type localities as the nominal species; and (4) naming clades/lineages that include individuals collected in areas other than type localities (or samples with no clear taxonomic identity) as candidate species. Based on the recovered genetic lineages, we identified five groups corresponding to the four focal species plus a candidate species. In all cases we used *Liolaemus kingii* and one individual of each of the other focal/candidate species (collected from their type localities) as outgroups.

To generate the cytochrome *b* guide tree we used all the ingroup haplotypes (Supplementary Data I) and outgroups (Appendix 1); we selected the best-fit evolutionary model (TrN + I + G) using the corrected Akaike information criterion in JModelTest v0.1.1 (Posada 2008) and used it in the Bayesian analyses. We performed Bayesian analyses in MrBAYES v3.1.2 (Ronquist and Huelsenbeck 2003) using four heated Markov chains (default heating values) sampled at intervals of 1000 generations and run for 50 million generations. We used the equilibrium samples (after 25% burn-in) to generate a 50% majority-rule consensus tree and considered posterior probabilities > 0.95 as strong nodal support (Huelsenbeck and Ronquist 2001). We conducted maximum likelihood (ML) analyses in RAxML v7.0.4 (Stamatakis 2006), using a GTRGAMMA model of nucleotide substitution, based on 1000 rapid bootstrap analyses for the best ML tree; we inferred strong nodal support when bootstrap values > 70 (Hillis and Bull 1993; see their caveats). We ensured that mixing (traces with similar likelihoods) and convergence (effective sample sizes > 150) were reached before default program burn-in values, using the program TRACER v1.5.0 (Rambaut and Drummond 2009). For each main lineage, in order to have a more accurate estimation of branch lengths and relationships among individuals, we generated cytochrome *b* gene trees using Bayesian inference and ML analyses as previously described (using the following best-fit evolutionary models: for *Liolaemus baguali*, TIM2 + I; for *L. escarchadosi*, *L. sarmientoi*, and *L. tari*, TrN + I; and for *L.* sp. A, TPM2uf + I) and by rooting with a reduced set of outgroups (*L. kingii* and one individual of each one of the other lineages). We estimated pairwise cytochrome *b* genetic distances between species using Arlequin v3.11 (Excoffier et al. 2005).

For each lineage, we used a statistical parsimony algorithm to construct networks (Templeton et al. 1992) using TCS v1.21 (Clement et al. 2000) with the default connection significance (95%). For each lineage, we calculated standard molecular diversity indices (number of haplotypes, number of segregating sites, average number of differences between two random sequences, and haplotype and nucleotide diversity) using DnaSP v5.0 (Librado and Rozas 2009). Tajima's D and Fu's F_s (Tajima 1989; Fu 1997) are classical neutrality tests used to evaluate population demographic history, and both assume that populations have been in mutation-drift and migration-drift balance for a long period of time (Nei and Kumar 2000); when this is not the case due to sudden expansion, these indices usually have negative values. We also used the *R2* test because it is considered a sensitive indicator for detecting demographic growth using small sample sizes (Ramos-Onsins and Rozas 2002). We calculated Tajima's D, Fu's F_s, and *R2*, and we estimated the significance of these values using 5000 samples simulated under a coalescent algorithm in DnaSP.

Morphological Data and Analyses

From ethanol-preserved specimens, we evaluated variation for 10 morphometric (measured only in adults) and

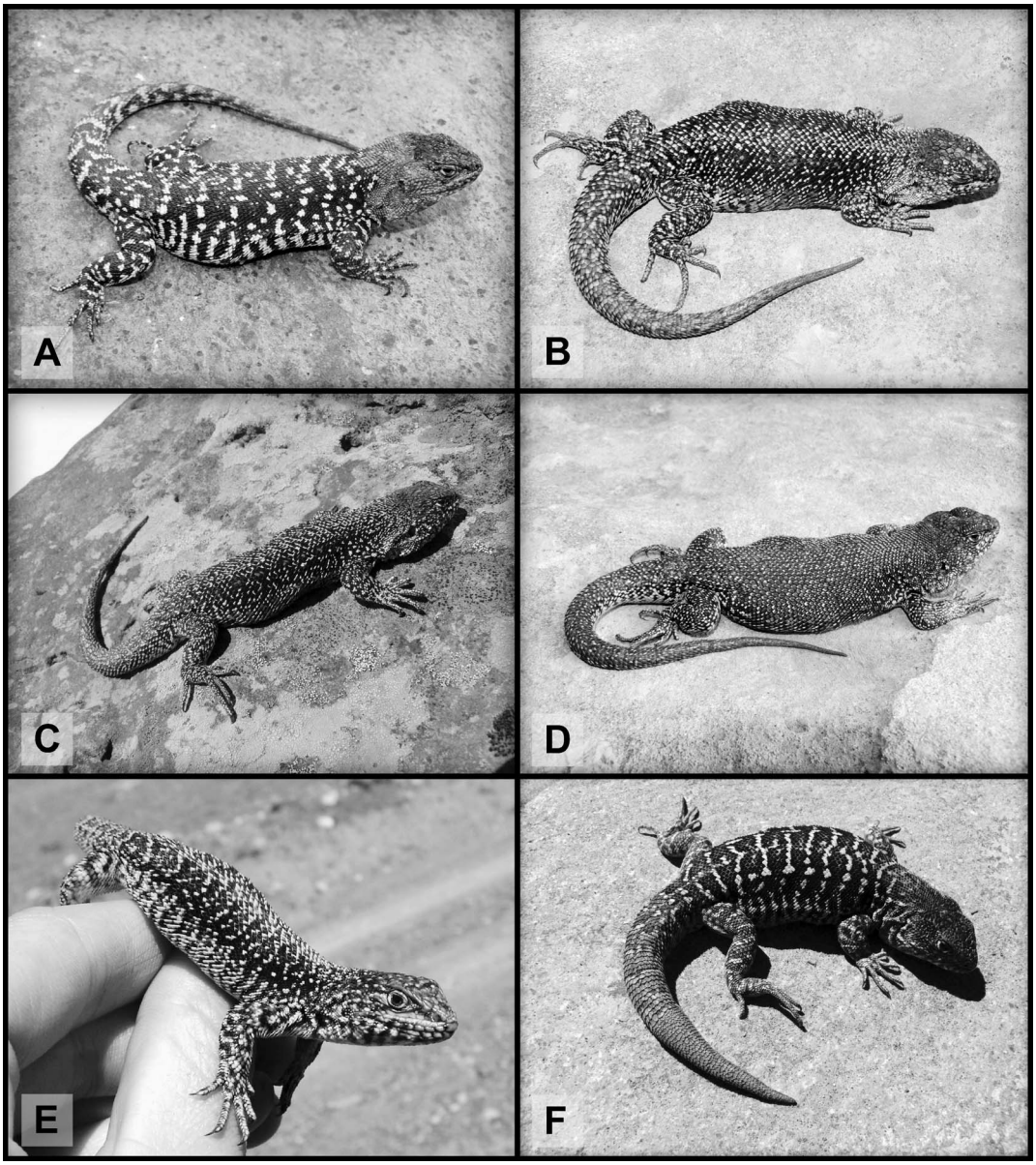

FIG. 2.—Photographs of adult males, collected in type localities, of the species used in this study: (A) *Liolaemus baguali* (SVL = 81 mm, LJAMM 9394; from Department of Lago Argentino, National Road 40, 27.3 km N of Tres Lagos, Sierras del Bagual), (B) *L. escarchadosi* (SVL = 80 mm, LJAMM 9335; from Department of Lago Argentino, Provincial Road 65, 43.5 km W of Provincial Road 17, 1 km S of Cerro Mank Aike), (C) *L. sarmientoi* (SVL = 84 mm, LJAMM 7205; from Department of Guer Aike, Laguna Azul, Reserva Geologica Provincial Laguna Azul, close to Estancia Monte Aymond), (D) *L. tari* (SVL = 101 mm, LJAMM 9400; Department of Lago Argentino, Punta del Lago plateau, toward Meseta Campo las Piedras, 7 km N of Estancia Punta del Lago), (E) *L.* sp. A (SVL = 70 mm, LJAMM 11548; from Department of Lago Argentino, National Road 40, 6 km S of La Leona hotel), and (F) *L. kingii* (SVL = 86 mm, LJAMM 7460; Department of Deseado, 5.5 km N of Puerto Deseado). Photos by C. H.F. Perez, N. Frutos, and L.J. Avila.

10 meristic (scale count) characters, as well as seven qualitative characters (only in adults) representing patterns of body coloration (e.g., Scolaro and Cei 1987, 1997; Abdala 2007; Escudero et al. 2012; Breitman et al. 2013). We took measurements to the nearest 0.1 mm using a Schwyz® electronic digital caliper and made scale counts using a stereoscopic microscope. Scale terminology, measurements, and chromatic states follow Smith (1946), Escudero et al. (2012), and Breitman et al. (2013). With few exceptions we made the measurements and scale counts on the right side of each specimen. We determined sex by the thickness of the base of the tail and the presence of precloacal pores (only in males); we identified adults by size and color patterns (Cei 1986; Breitman et al. 2013). Photographs of adult male individuals of the focal species are presented in Fig. 2.

We used the following morphometric characters: (1) snout–vent length (SVL; measured from the tip of the snout to the posterior margin of the precloacal scales); (2) fore–hind limb distance (taken from the armpit of the front leg to the anterior insertion of the hind limb); (3) foot length (measured ventrally along the fourth toe, from the base of the heel to the base of the claw); (4) radius-ulna length (measured from the elbow to the wrist); (5) hand length (the ventral length of the third finger from the base of the wrist to the base of the claw); (6) head width (distance between corners of the mouth); (7) head length (distance from the anterior edge of the auricular opening to the center of the

rostral scale); (8) rostral–nasal distance (distance between rostral and nasal scales); (9) rostral height (the longest vertical measure of the rostral scale); and (10) auditory meatus height (the longest vertical diameter of the auditory meatus). We used the following meristic characters: (1) scales around interparietal (number of scales in contact with the interparietal); (2) lorilabial scales (number of scales above the supralabial scales); (3) supralabial scales (number of scales of the right side of the upper edge of the mouth excluding the rostral scale); (4) infralabial scales (number of scales of the right side of the lower edge of the mouth, excluding the mental scale until the corner of the mouth; (5) midbody scales (number of scales around the body at the trunk); (6) dorsal scales (number of scales from the first nuchal scale to the line of scales between the preaxial margin of the hind limbs); (7) ventral scales (number of ventral scales from the mental scale—but excluding it—to the precloacal scales or the end of the cloacae); (8) Lamellae III finger (number of infradigital lamellae of the third finger); (9) Lamellae IV toe (number of infradigital lamellae of the fourth toe); and (10) precloacal pores (number of precloacal pores, at the lower edge of the cloacae).

We used the following qualitative characters: (1) dorsal stripe pattern (referring to the shape and size of white or yellow dorsal bands, perpendicular to the body axis) resolved in four categories: (1a) complete or slightly broken bands, (1b) dotted bands, (1c) irregular bands, (1d) indistinct or almost indistinct bands (these variables were illustrated and described as 0–20, 40, 60, and 80–100, respectively, in Scolaro and Cei 1987); (2) vertebral line (measure as the presence or absence of a single brownish-yellow dorsal line going from the nuchal region until the hind limbs; illustrated and described in Scolaro and Cei 1997); (3) dorsolateral lines (referring to the presence or absence of brownish-yellow dorsolateral lines going among the insertion of fore and hind limbs; illustrated and described in Scolaro and Cei 1997); (4) ventral variegation (measured as the presence or absence of any degree of ventral variegation—black and white spots); (5) ventral melanism (ventrally, from the mental scale to the cloacal region) and discriminated in five categories (illustrated and described in Escudero et al. 2012): (m0) no ventral melanism, (m1) melanism only in the gular zone, (m2) melanism only in the abdominal zone, (m3) melanism present in the ventral zone except in cloacal region and limbs, (m4) melanism present in the ventral zone except the limbs, (m5) melanism present in all the ventral zone; (6) presence/absence of red/orange scales in any part of the body (from digital photographs of specimens taken at the time of capture); and (7) head differentiation (measured as presence or absence of a different head coloration relative to body).

We performed morphological analyses separately using the meristic, morphometric, and qualitative data sets; we evaluated variation at intraspecific (between sex) and interspecific levels (among species/candidate species). We summarized morphological variation within species and between sexes using standard statistics (mean, range, and standard deviation) and transformed values of qualitative variables to percentages to reflect their prevalence between sexes and among species before being compared. We evaluated assumptions of variance homoscedasticity and normality for each morphometric and meristic variable using Levene and Shapiro–Wilks tests, respectively (Montgomery 1991); we evaluated the assumption of equality of the regression slopes using *F*-test in PAST v2.08 (Hammer et al. 2001).

We evaluated sexual dimorphism within species with either Student's *t* or Kruskal–Wallis tests (nonparametric tests) for the meristic data set and using analyses of covariance (ANCOVA) with SVL as a covariate for the morphometric data set. We used parametric or nonparametric multivariate analyses of variance (MANOVA, or NPMANOVA when assumptions were not met) to test for generalized differences between sexes within each species/candidate species in meristic and morphometric data sets (assumptions of MANOVA were only corroborated for the meristic data set of *Liolaemus sarmientoi*, confounding effect was not a problem because the design was almost balanced: 26 females and 25 males). Because we found sexual dimorphism in most of the species (see results), we separated data sets by sex (Vukov et al. 2006; Breitman et al. 2013; Medina et al. 2013) and analyzed them independently in the interspecific tests.

We evaluated interspecific differences in the meristic data set by performing one-way analysis of variance (ANOVA) using the Di Rienzo, Guzmán and Casanoves test (Di Rienzo et al. 2002); we checked assumptions of equal variance and normality as previously described (Montgomery 1991) and we used Kruskal–Wallis tests (Kruskal and Wallis 1952) with comparisons when assumptions were not met. For the morphometric data, we evaluated interspecific differences by using ANCOVA with SVL as the covariate (to control for the effect of size); we did not include *Liolaemus tari* and *L.* sp. A in this analysis due to their small sample sizes. We used ANOVA or Kruskal–Wallis tests to evaluate differences in SVL among species. We performed all the analyses in INFOSTAT® 2011 (Di Rienzo et al. 2011) and PAST v2.08.

Niche Envelope Data and Analyses

We used ecological niche modeling (ENM) analyses to characterize the bioclimatic niche envelope of each species, to test for niche divergence among species under current conditions, and to infer past distributions during the Last Glacial Maximum (LGM ~0.021 million years ago [mya]). The ENM approach is a widely used technique based on the association between large-scale climate data and known occurrence of the species under study; the test can identify the environmental variables (niche envelope) associated with population's persistence and thus hypothesized potential distributions (Aragón et al. 2010). Niche envelopes are not heritable traits and thus not valid for species delimitation, but divergence tests of niche models are used to visualize and quantify some components of ecological divergence between species (Wiens and Graham 2005; Arribas et al. 2012). Predictions made using ENM can be used as proxies for estimates of past or future putative habitable areas, under the assumption that past, current, and future ecological preferences are similar (Kozak et al. 2008; Colwell and Rangel 2009; Sánchez-Fernández et al. 2011). We did not interpret the areas predicted by ENM as the entirety of a species' distribution, because they might be overpredicted and also other factors (such us geographic barriers, historical factors, biotic interaction) can prevent occupancy of the

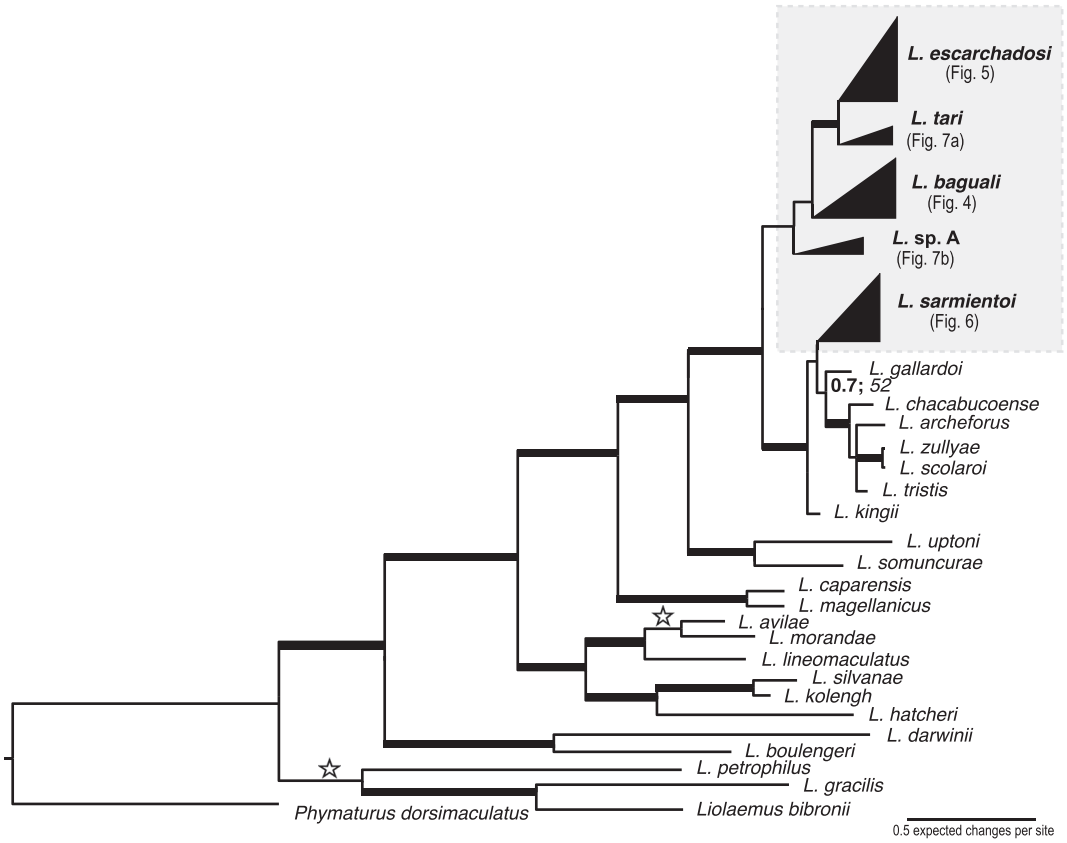

FIG. 3.—Cytochrome *b* gene tree showing phylogenetic relationships among the focal species (shown as collapsed branches in the gray inset) within the *Liolaemus lineomaculatus* section and other species of *Liolaemus* (relationships within species' individuals are presented in Figs. 4–7). *Phymaturus dorsimaculatus* was used as outgroup. Dark branches represent significant Bayesian and maximum likelihood (ML) support values; open stars denote nodes statistically supported only by ML. Support values higher than 0.7 posterior probability/50% bootstrap are shown.

complete putative ecological range (Jiménez-Valverde et al. 2008; Kozak et al. 2008; Aragón et al. 2010).

We could not conduct ENM analyses for *Liolaemus tari* and *L.* sp. A due to their small sample sizes (two and three localities, respectively), but we used 25 localities for *L. escarchadosi*, 19 localities for *L. sarmientoi*, and 18 localities for *L. baguali* (Appendix I). We modeled current and past distributions using MaxEnt v3.3.3e (Phillips et al. 2006), based on 19 bioclimatic variables available at the global meteorological database Worldclim. We used all variables (recognizing the overfitting risk) because when working with more than one species potential errors are constant (Munguía et al. 2008) and MaxEnt results are generally more stable using a greater number of variables (Elith and Leathwick 2009). We obtained present bioclimatic variables at a resolution of 1 km × 1 km and paleoclimatic data (LGM; ~0.021 mya) at a resolution of 2.5 arc-min; we resampled at 1-km × 1-km cells. MaxEnt has the ability to perform well when using a small number of presence data (Elith et al. 2006; Hernandez et al. 2006; Phillips et al. 2006). We ran MaxEnt using default parameters: a maximum of 500 iterations, convergence threshold of 0.00001, prevalence of 0.5, and a maximum of 10,000 randomly generated localities; we specified a cumulative output format to provide values between 0 and 100. Grid cells that predict the best conditions for the species according to the model have a cumulative value of 100, while accumulated values close to 0 correspond to predictions of inadequate conditions. We reclassified the projections obtained with MaxEnt to convert the continuous output into a map of presence–absence preventing the omission of known localities on output maps.

To evaluate the performance of each model, we used the area under the curve (AUC) of the receiver operating characteristics curve. The AUC reflects the proportion of correctly and incorrectly classified predictions across a range of probability thresholds (Pearce and Ferrier 2000); its value is interpreted as the probability that a random positive (presence) or negative (absence) point is correctly classified by the model (Phillips et al. 2006). Thus, AUC values are positively related to the predictive ability of the model (Manel et al. 2001). AUC values range between 0 and 1, with 1 indicating a perfect model; we accepted a model as correct when AUC > 0.75 (Elith 2002; Elith et al. 2006).

To test for niche divergence among species we calculated Schoener's *D* metric statistic (Schoener 1968) using ENMTools (Warren et al. 2010); this statistic measures the niche similarity between pairs of entities. Complementarily, we calculated the niche identity test using ENMTools; this test calculates habitat suitability scores (generated by ENMs) from two species and shows whether their ecological differences are significant. By comparing the overlap between ENMs generated from the actual data for each species (*D* statistic) to the null distribution obtained using the identity test, the program statistically evaluates whether ENMs produced by two populations or species are different. If Schoener's *D* statistic is smaller than the distribution generated by pseudoreplicates in the identity test, then the

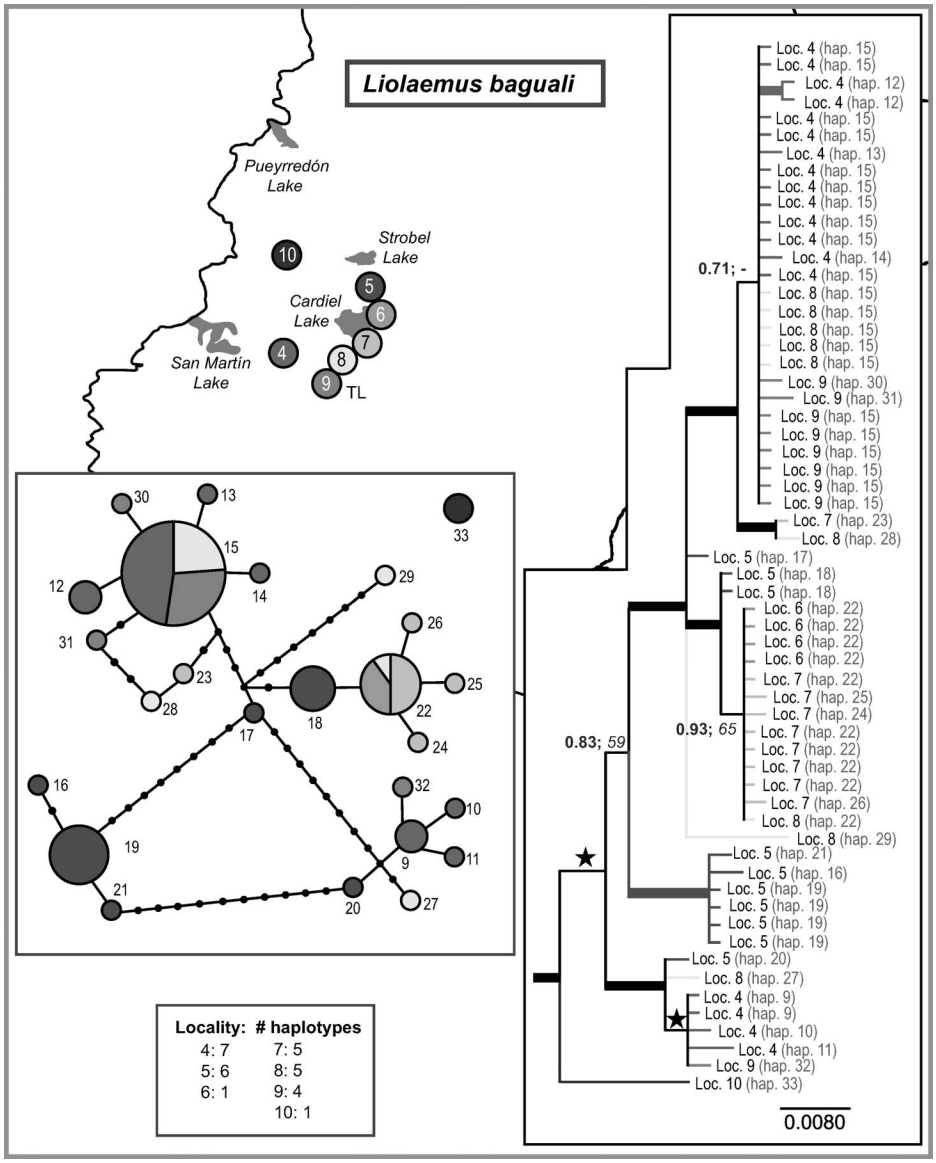

FIG. 4.—Distribution map (including locality numbers), cytochrome *b* gene tree, and network for *Liolaemus baguali* (color coded by locality; TL indicates type locality). Haplotypes and localities are shown on the tree; dark branches represent significant Bayesian and maximum likelihood support values, black stars denotes nodes statistically supported only by Bayesian analyses, and values higher than 0.7 posterior probability/50% bootstrap are shown. Haplotype numbers are shown in the network. Note that singleton Haplotype 33 is recovered at the base of the tree; this is the only sample collected in Locality 10.

hypothesis of niche identity must be rejected, meaning that both species occupy different ecological niches. We obtained confidence intervals (at $\alpha = 0.05$) from a null distribution generated using INFOSTAT® 2011 (Di Rienzo et al. 2011) and we compared the Schoener's *D* statistic of each pair of species with the lower limit of the confidence interval.

RESULTS

Genetic Analyses

Our complete cytochrome *b* matrix included a total of 106 different haplotypes ($n = 226$; length 745 bp; 116 informative sites; Supplementary Data I). The two phylogenetic analyses (Bayesian Inference and ML) inferred the

TABLE 1.—Pairwise genetic distances (expressed in %) among species/lineages. Values on the diagonal represent intragroup distances, values below the diagonal denotes corrected intergroup distances (intergroup distance − intragroup distance), and values above the diagonal represent uncorrected intergroup distances. Distances > 3% are shown in boldface.

	L. baguali	*L. escarchadosi*	*L. sarmientoi*	*L. tari*	*L.* sp. A
L. baguali	1.14	**4.51**	**5.97**	**4.17**	**4.68**
L. escarchadosi	**3.68**	0.54	**5.49**	2.63	**4.87**
L. sarmientoi	**4.91**	**4.73**	0.99	**6.11**	**6.28**
L. tari	**3.51**	2.27	**5.53**	0.18	**5.23**
L. sp. A	**3.95**	**4.45**	**5.63**	**4.99**	0.31

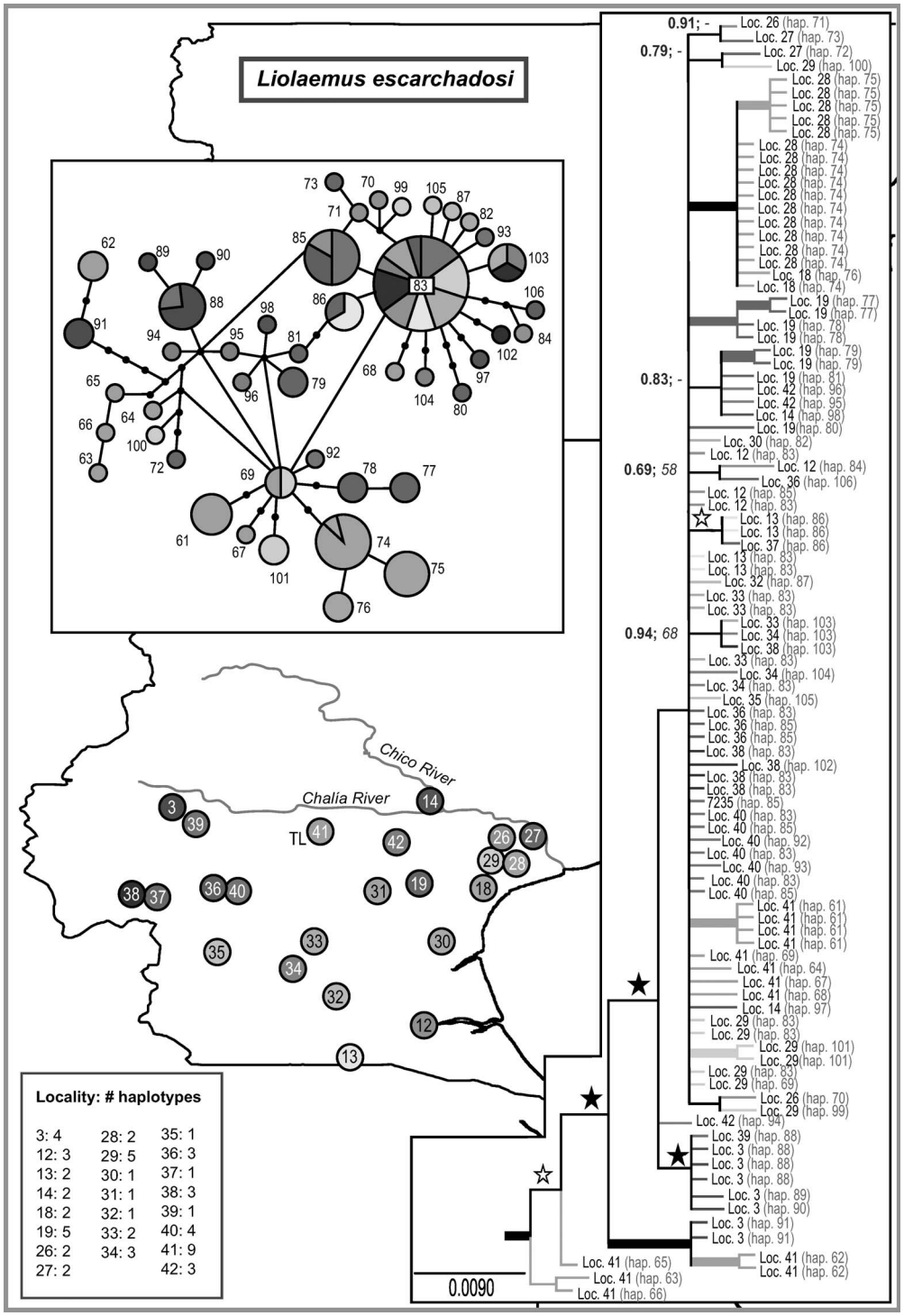

FIG. 5.—Distribution map (including locality numbers), cytochrome *b* gene tree, and network for *Liolaemus escarchadosi* (color coded by locality; TL indicates type locality). Haplotypes and localities are shown on the tree; dark branches represent significant Bayesian and maximum likelihood (ML) support values, black stars denotes nodes statistically supported only by Bayesian analysis, and open stars represent nodes statistically supported only by ML. Support values higher than 0.7 posterior probability/50% bootstrap are shown. Haplotype numbers are shown in the network.

same main clades and relationships within each lineage; topologies were concordant without any well-supported conflicts (collapsed tree in Fig. 3; complete gene tree in Supplementary Data II). We recovered five haploclades that correspond to four described species plus a novel lineage that we refer to as *Liolaemus* sp. A (Fig. 3). We present the network and phylogenetic results for each of these lineages in different figures (*L. baguali*, Fig. 4; *L. escarchadosi*, Fig. 5; *L. sarmientoi*, Fig. 6; *L. tari*, Fig. 7A; *L.* sp. A, Fig. 7B). Most of the pairwise genetic distances within each species were small (0.18–1.14%; Table 1). Uncorrected genetic distances among species were higher than 3% (4.17–6.28%), except for *L. escarchadosi* vs. *L. tari* (2.63%). We found high haplotype diversity and intermediate to low

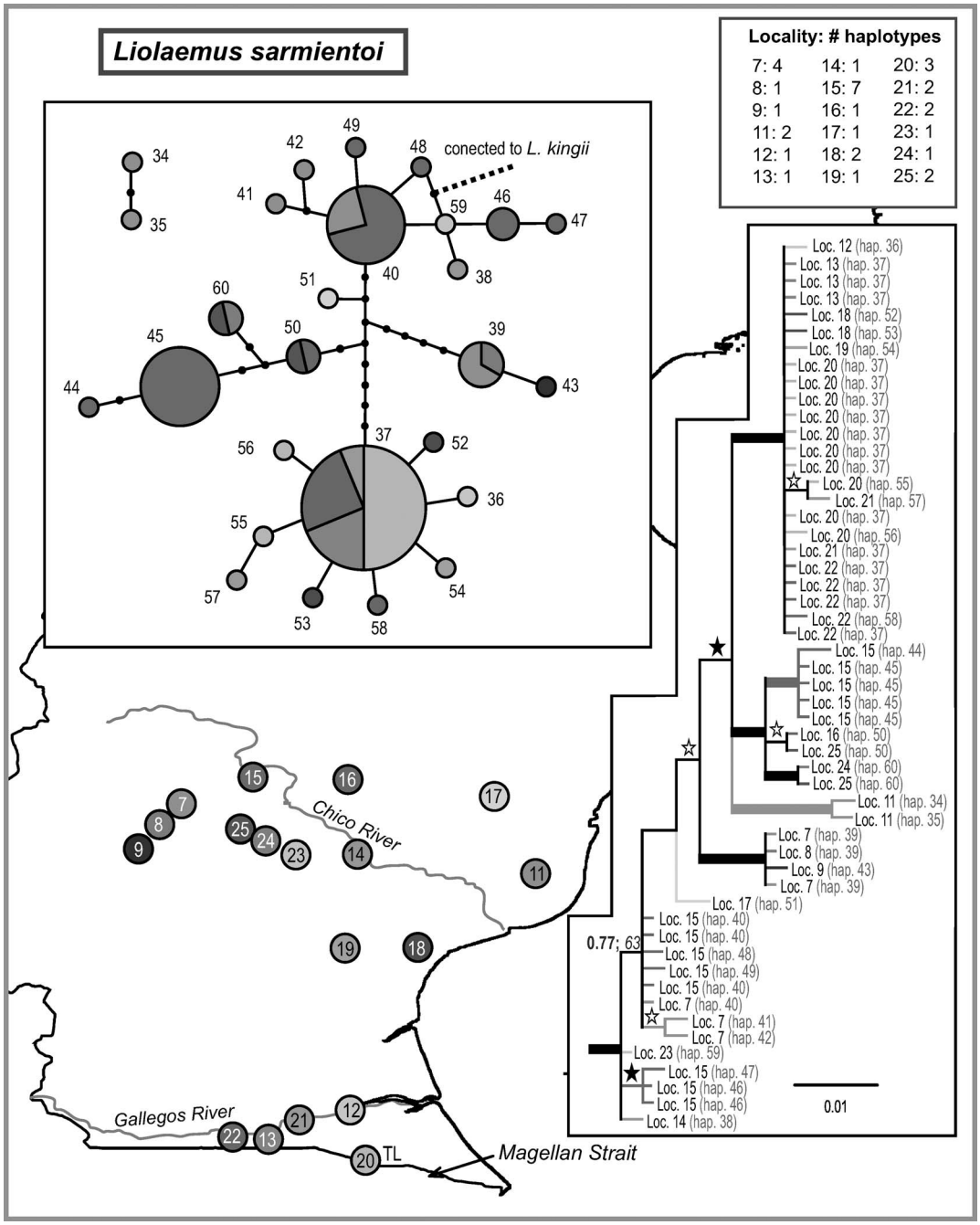

FIG. 6.—Distribution map (including locality numbers), cytochrome *b* gene tree and network for *Liolaemus sarmientoi* (color coded by locality; TL indicates type locality). Haplotypes and localities are shown on the tree; dark branches represent significant Bayesian and maximum likelihood (ML) support values, black stars denotes nodes statistically supported only by Bayesian analysis, and open stars represent nodes statistically supported only by ML. Support values higher than 0.7 posterior probability/50% bootstrap are shown. Haplotype numbers are shown in the network. Note that Haplotypes 34 and 35 were recovered forming an independent network, and both were collected at Locality 11.

nucleotide diversity in all species (Table 2). *Liolaemus tari*, *L.* sp. A, and *L. escarchadosi* had the lowest values of nucleotide diversity; *L. escarchadosi* had the highest value of haplotype diversity (Table 2). Neutrality tests were statistically significant only for *L. escarchadosi* ($Dt = -2.04$, $P = 0.0016$; $R2 = 0.0319$, $P = 0.0004$; *Fu's* $F_s = -40.78$, $P < 0.0001$). Network analyses recovered six different networks and one singleton (Figs. 4–7). In general, the number of networks was concordant with the number of clades in the gene trees with two exceptions. In the first case, *L. sarmientoi* samples were inferred in two networks; most of the samples were linked into one network except for two samples from Locality 11 that were linked separately (Fig. 6). In the second case, sample 11582 (belonging to *L. baguali*, Locality 10, Fig. 4) was a singleton not linked to the main network.

Network and gene tree results from *Liolaemus baguali* indicate that this species is genetically structured into four haploclades corresponding to clades inferred in the gene tree (Fig. 4). We inferred haplotypes from individuals of *L. baguali* in a structured tree, including well-supported clades and several singletons (Fig. 4). The northernmost localities

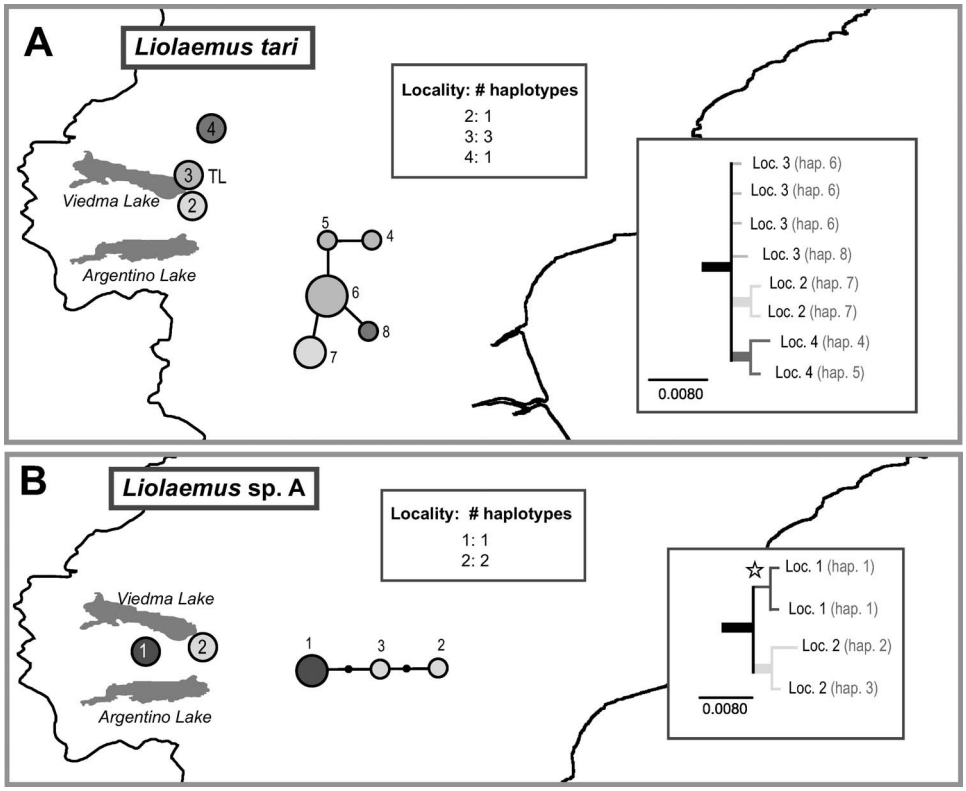

FIG. 7.—Distribution maps (including locality numbers), cytochrome *b* gene trees, and networks color coded by locality for: (A) *Liolaemus tari* (TL indicates type locality) and (B) *L.* sp. A. In both A and B, haplotypes and localities are shown on the tree; dark branches represent significant Bayesian and maximum likelihood (ML) support values, and open star denotes nodes statistically supported only by ML. Support values higher than 0.7 posterior probability/50% bootstrap are shown. Haplotype numbers are shown in the network.

(Localities 10 and 5) included well-differentiated haplotypes. The only individual (11582) collected in Locality 10 (the most geographically distant to the other localities) was a singleton inferred at the base of the tree (sister to all other haploclades) and outside of the network. Individuals collected in Locality 5 were genetically heterogeneous and were inferred in multiple well-supported haploclades throughout the tree (Fig. 4).

Individuals of *Liolaemus escarchadosi* are geographically and genetically structured (although less structured than *L. baguali*); they show signals of demographic expansion, marked in the southern part of its distribution (star-like connections in the network; Fig. 5). Individuals collected from the type locality (Locality 41) were genetically well-differentiated from each other and from the rest of the individuals; we inferred some individuals (9334, 9344, 9343) at deep nodes within the tree and in other haploclades within the larger polytomy. Similar deep structure was inferred for individuals from Localities 29 and 19 in well-supported clades (Fig. 5).

We inferred individuals of *Liolaemus sarmientoi* in a structured network with some star-like connections in the southern part of the distribution (Fig. 6). We inferred samples from *L. sarmientoi* in a structured tree (Fig. 6) with at least three well-supported clades. The individuals collected in Locality 11 (northeast of the Chico River) had very distinct haplotypes included in a different network but nested within the tree. All the individuals collected south of the Gallegos River (and a few individuals from Localities 18 and 19) formed a very well-differentiated haploclade deeply nested in the tree. We also inferred individuals from the westernmost localities (Localities 7–9) as a distinct haploclade. Individuals from Localities 14 and 15, and a few from Locality 7, formed two paraphyletic groups at the base of the tree (Fig. 6).

The clade including *Liolaemus tari* individuals was well supported (Fig. 7A) and haplotypes showed few differences among them. We inferred four individuals collected in the southwestern part of Santa Cruz (Localities 1 and 2) in

TABLE 2.—Values of standard molecular diversity indices.

	Sample size	Number of segregating sites	Number of haplotypes	Haplotype diversity	Nucleotide diversity ± SD	Average number of differences between two random sequences
L. baguali	62	56	25	0.86	0.01137 ± 0.00107	8.47
L. escarchadosi	101	56	46	0.94	0.00537 ± 0.0004	4.00
L. sarmientoi	53	43	27	0.90	0.00985 ± 0.00067	7.34
L. tari	8	4	5	0.86	0.00182 ± 0.00043	1.36
L. sp. A	4	4	3	0.83	0.00313 ± 0.00098	2.33

a strongly differentiated clade, here provisionally labeled as *Liolaemus* sp. A (Fig. 7B).

Morphological Analyses

We summarized and evaluated differentiation of morphometric and meristic variables within and among the following focal taxa (sample sizes in parentheses): *Liolaemus baguali* (morphometric: 16 females, 16 males; meristic: 24 females, 20 males), *L. escarchadosi* (morphometric: 20 females, 32 males; meristic: 46 females, 42 males), *L. sarmientoi* (morphometric: 21 females, 19 males; meristic: 26 females, 25 males), *L. tari* (morphometric: 3 females, 3 males; meristic: 4 females, 4 males), and *L.* sp. A (morphometric and meristic: 4 males). Due to their small sample size, we interpreted morphological results for *L. tari* and *L.* sp. A as hypotheses that need further testing. We summarized means (or ratios), standard deviations, and ranges of meristic and morphometric variables by sex for each species, as well as tests of intraspecific sexual dimorphism (Appendices II and III; Supplementary Data III). We summarized values of qualitative variables in Supplementary Data IV.

We observed sexual dimorphism among qualitative variables for ventral variegation, melanism, and dorsal stripe patterns in *Liolaemus tari*; melanism was sexually dimorphic in *L. escarchadosi* and *L. sarmientoi* (Supplementary Data IV). Qualitative characters showed high within-species variation and no clear differences among species; the exception to this was that the dorsal stripe pattern of *L. baguali* was the only more or less exclusive and homogeneous character within species (Supplementary Data IV). Almost all individuals of both sexes of *L. baguali* showed complete or slightly broken dorsal bands (Supplementary Data IV), while there was no typical pattern for the other species recovered from the qualitative variables analyzed in our study.

For the meristic data set, univariate sexual dimorphism was absent in *Liolaemus tari* and *L. baguali* but it was present in some variables of *L. escarchadosi* (supralabial scales, infralabial scales, dorsal scales, and lamelae IV toe) and *L. sarmientoi* (midbody scales and dorsal scales; Appendix II). We did not observe multivariate sexual dimorphism in *L. baguali* ($P = 0.3486$) but found that it was present in *L. escarchadosi* ($P = 0.0023$) and *L. sarmientoi* ($P = 0.0331$).

We found interspecific meristic differences among males and females of all species pairs, except for female *L. sarmientoi* vs. *L. escarchadosi*, male *L. tari* vs. *L. baguali* and *L. sarmientoi*, and male *L.* sp. A vs. *L. escarchadosi* (Appendix IV). We did not find any evidence for significant female differences among species in terms of scales around interparietal, lorilabial scales, supralabial scales, and infralabial scales. Overall, however, we found a higher number of interspecific differences among females relative to males.

For the morphometric data set, we detected univariate sexual dimorphism in several variables for all species (Appendix III). *Liolaemus escarchadosi* and *L. sarmientoi* showed sexual dimorphism at most variables (except for rostral–nasal distance, rostral height, and auditory meatus height). In contrast, *L. tari* and *L. baguali* were sexually dimorphic at only three variables: fore-hind limb distance, distance, head width, and head length. We only observed sexual dimorphism for SVL in *L. sarmientoi.* We observed multivariate sexual dimorphism in *L. baguali* ($P = 0.0181$), *L. escarchadosi* ($P = 0.0012$), and *L. sarmientoi* ($P < 0.0001$).

We tested for interspecific morphometric differences between *Liolaemus sarmientoi*, *L. baguali*, and *L. escarchadosi* using ANCOVA analyses (*L. tari* and *L.* sp. A were not tested due to small sample sizes). Assumptions of homoscedasticity were not confirmed for the head length variable in females or the foot length, head width, and rostral–nasal distance variables in males. We therefore do not present results or draw conclusions for tests whose assumptions were not met. Valid tests revealed significant differences in both sexes among the three species and are summarized in Appendix V. We did not find differences among species for rostral–nasal distance or rostral height in females, or among fore limb–hind limb distances, radius-ulna lengths, or rostral heights in males. We found significant differences in SVL only when comparing male *L. baguali* with *L. escarchadosi* and *L.* sp. A (Kruskal–Wallis $P = 0.0035$).

For *Liolaemus* sp. A, we were only able to evaluate morphological differences in males. We found that males of this candidate species showed significant differences in morphometric, squamation, and body patterns when compared with males of the other focal species (Appendix IV; Supplementary Data IV).

We identified nine individuals from four localities that were molecularly assigned (as described in the Materials and Methods section) to one of the four described species, but that had dorsal coloration morphotypes that were more similar to other sympatric species sampled from the same localities. One individual (9422, Haplotype 22, Locality 7; Figs. 1 and 4) in the *Liolaemus baguali* gene tree had a dorsal morphotype that was more similar to *L. sarmientoi* than to *L. baguali* (Fig. 2). Likewise, within the *L. escarchadosi* gene tree, we inferred three individuals (9401–9403, Locality 3, Figs. 1 and 5) having two haplotypes (88, 89) whose dorsal morphotypes were more similar to *L. tari* than to *L. escarchadosi* (Fig. 2). We inferred four individuals (9397, 9418, 9427, 9430; Haplotypes 39, 42, 43; Localities 7, 9; Figs. 1 and 6) in the *L. sarmientoi* gene tree that had morphotypes more similar to *L. baguali* than to *L. sarmientoi* (Fig. 2). And one individual (9349, Haplotype 8, Locality 4; Figs. 1 and 7) in the *L. tari* gene tree had a dorsal morphotype more similar to *L. baguali* than to *L. tari* (Fig. 2). In order to evaluate whether there were morphological signals of hybridization in these nine specimens, we compared their meristic and morphometric variables (only considering variables that showed significant interspecific differences in our other analyses) against the mean values of both sympatric species. We found that scale counts and measures were mostly similar to the species that the individuals were molecularly assigned to. However, four specimens had values of some variables that were more similar to the mean value of the species with which they shared the same dorsal morphotype: (1) Individual 9402 (ventral scales = 97), (2) Individual 9430 (auditory meatus height/SVL = 0.04027), (3) Individual 9418 (midbody scales = 77), and (4) Individual 9422 (head length/SVL = 0.20365).

Also in four localities (12–14 and 18; Figs. 1, 5, and 6) where *Liolaemus sarmientoi* and *L. escarchadosi* coexist, individuals were genetically assigned to one or the other of these species but, due to the interspecific morphological

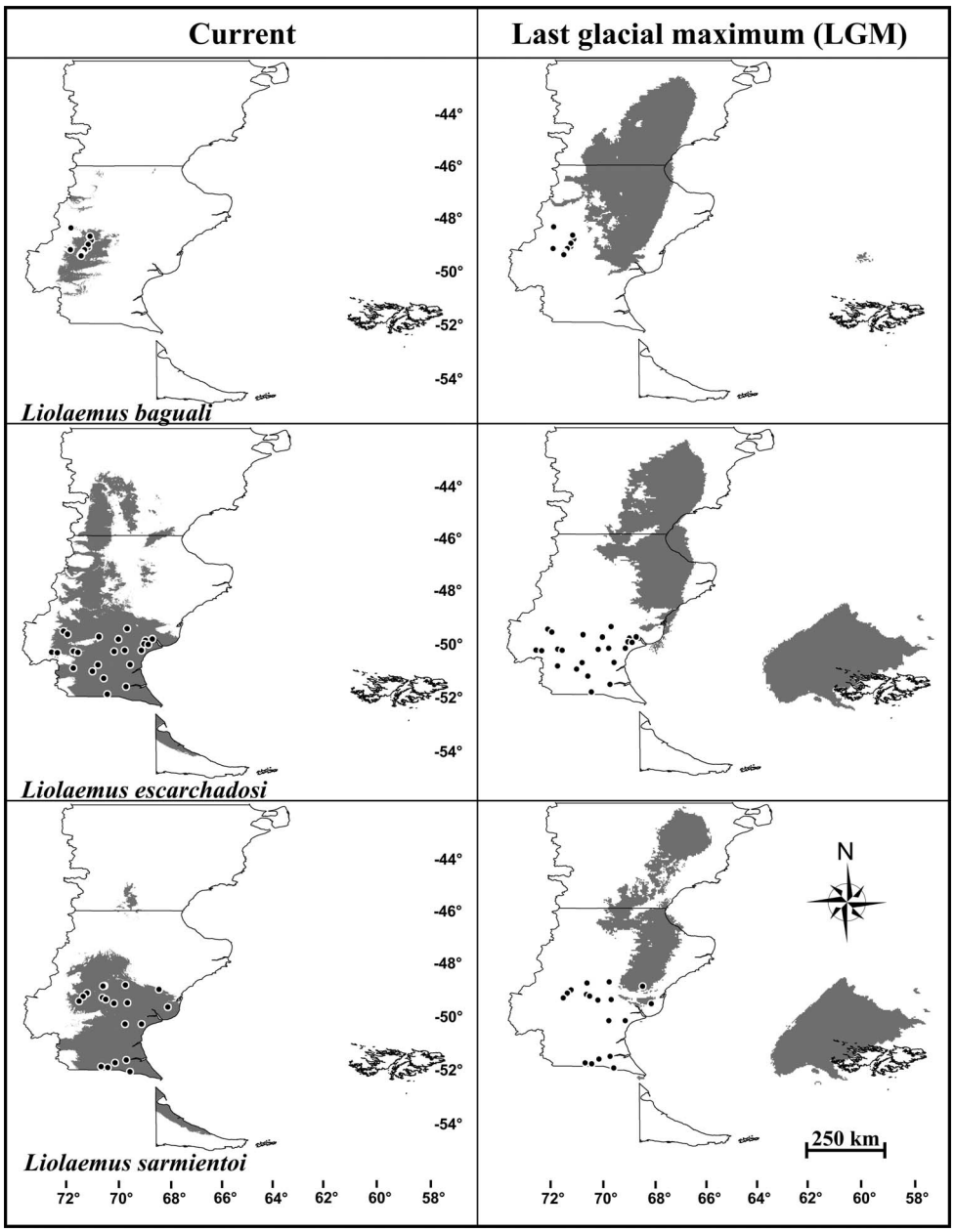

FIG. 8.—Current and paleoclimatic (Last Glacial Maximum) distributions projected for (A, B) *Liolaemus baguali*, (C, D) *L. escarchadosi*, and (E, F) *L. sarmientoi*; suitable environmental conditions are identified by gray shading, and sampled localities are shown in black.

similarity and high intraspecific morphological variability of these taxa, we were not able to make morphological assignments of these individuals.

Niche Models Analyses

We constructed ecological niche models for *Liolaemus baguali*, *L. escarchadosi*, and *L. sarmientoi* and used them to predict these species' current and LGM distributions. The AUC scores for these species models were > 0.95 (*L. baguali* AUC = 0.99, *L. escarchadosi* AUC = 0.96, *L. sarmientoi* AUC = 0.98), indicating that the models had high predictive ability. Current potential distributions for these three species mostly matched their known distributions, except the *L. escarchadosi* model that overpredicted this species range relative to its known range (Fig. 8). The projected palaeoclimatic distributions for these three species during the LGM showed the same general pattern of a northeastern Patagonian distribution (Fig. 8), but with extensive suitable habitat for *L. escarchadosi* and *L. sarmientoi* predicted over the continental shelf. Range shifts from LGM to the present indicated by these models were modest and WSW for *L. baguali*, more extensive and SSW for *L. sarmientoi*, and more displaced to the SW for *L. escarchadosi* (Fig. 8).

The degree of niche overlap between the three species pairs estimated by Schoener's *D* statistic (*Liolaemus baguali* vs. *L. escarchadosi* = 0.28, *L. baguali* vs. *L. sarmientoi* = 0.23, *L. escarchadosi* vs. *L. sarmientoi* = 0.65) was lower when compared with the lower bound calculated for the null model (*L. baguali* vs. *L. escarchadosi* = 0.67, *L. baguali* vs. *L. sarmientoi* = 0.69, *L. escarchadosi* vs. *L. sarmientoi* = 0.69; Supplementary Data V). Our results (Supplementary

Data V) indicate that all pairwise combinations of the species *L. baguali*, *L. escarchadosi*, and *L. sarmientoi* are more ecologically differentiated in climatic envelopes than expected by chance.

DISCUSSION

The goal of our paper was to characterize the morphological, ecological, and genetic variability of the four southernmost species of the *Liolaemus kingii* group (*L. baguali*, *L. escarchadosi*, *L. sarmientoi*, and *L. tari*); we used this information to estimate species boundaries and to hypothesize past evolutionary and demographic scenarios in these lizards. We used a multispecies data set including 241 lizards and 62 georeferenced localities; we found evidence supporting five lineages corresponding to four described species plus a candidate species. We present niche envelopes and the variation in morphological and genetic characters, and we compared results among lineages to offer preliminary hypotheses of species limits using an integrative taxonomic framework. A similar integrative approach was recently used to determine species limits in the *L. alticolor* group from Peru (Aguilar et al. 2013). We also present the first evidence of possible hybridization among species of the *L. kingii* group. Our results are relevant for the implementation of biodiversity conservation programs in Patagonia.

Species Boundaries

Based on cytochrome *b* data, five lineages were identified: *Liolaemus baguali*, *L. escarchadosi*, *L. sarmientoi*, *L. tari*, and *L.* sp. A. For *Liolaemus* an ~3% uncorrected genetic pairwise distance between clades represents a valid threshold for identifying putative species (Breitman et al. 2012). Specifically, for the *L. lineomaculatus* section this threshold was refined and estimated to be ~2.23% (Breitman 2013). These values represent average genetic differentiation among sister species of *Liolaemus* described based on morphology (Breitman 2013). Genetic distances among all species pairs included in this work were higher that 3% except between *L. escarchadosi* and *L. tari* (2.63%).

Overall, we found evidence from the three independent data sets (molecular, meristic, and morphometric, as well as divergence in niche envelopes) to support the specific status of *Liolaemus baguali*, *L. escarchadosi*, and *L. sarmientoi*. Small sample sizes did not allow us to perform statistically robust morphological or niche envelope analyses to compare *L.* sp. A and *L. tari* with the other species, but their genetic divergence, restricted distributions, and distinct in-life coloration (*L. tari* is characterized by a homogeneous brownish-gray coloration and *L.* sp. A has a pattern of small light dots scattered across the body with or without transverse bands, whereas the other species either have transverse bands, e.g., *L. baguali*, or a vertebral stripe, M.F. Breitman, personal observation; Fig. 2) indicate that both represent independent evolving lineages. We propose *L.* sp. A as a candidate species and we recommend that future taxonomic work, based on larger sample sizes and including comparisons with all described species of the *L. kingii* group, should be carried out before attempting a formal description of this lineage.

Intra- and Interspecific Ecological and Morphological Characterization

Ecological niche models provide evidence of niche divergence among all pairwise combinations of *Liolaemus baguali*, *L. escarchadosi*, and *L. tari*, which is consistent with their geographic distributions. However, the distributions of these species show some overlap (Fig. 1); *L. baguali* is found on the Asador Plateau and adjacent areas (western distribution), in some localities it is sympatric with *L. sarmientoi* and in others with *L. tari* (Fig. 1). *Liolaemus sarmientoi* has a more southern distribution; it is distributed peripherally to *L. escarchadosi* with some sympatric localities in the north, east, and south. Thus, in several localities, most of the studied species are sympatric with other species. The only two species that seem to be completely allopatric to each other are *L. baguali* and *L. escarchadosi*.

The distributions of the focal species do appear to be linked to different bioclimatic variables, as follows: (1) mean temperature of wettest quarter (29.2%), mean temperature of coldest quarter (18.7%), mean diurnal range (14.4%), and isothermality (14.3%) for *Liolaemus baguali*; (2) maximum temperature of the warmest month (50.7%), precipitation of the wettest quarter (16.2%), and mean of diurnal range (11.3%) for *L. escarchadosi*; and (3) maximum temperature of warmest month (34.6%), precipitation seasonality (12.9%), and mean temperature of coldest quarter (10.4%) for *L. sarmientoi.* Morphological characters also showed differences among species: (1) sexual dimorphism was detected in the meristic and morphometric data sets for *L. escarchadosi* and *L. sarmientoi*, but (2) it was only present in the morphometric data set for *L. baguali*, and (3) *L. sarmientoi* was the only species that showed sexual dimorphism in SVL (males bigger than females). We found significant differences in the regression slopes of head shape and body size in *L. sarmientoi* and *L. escarchadosi*; these results reflect the intersexual allometry existing in the species and highlight the different selection pressures that are acting in each sex (previously discussed in Breitman et al. 2013).

The species that showed the highest number of differences in the meristic data set (Appendix IV) was *Liolaemus baguali* relative to *L. escarchadosi* (males and females; midbody scales, dorsal scales, ventral scales, Lamellae III finger, Lamellae IV toe), but we also observed several differences when comparing females of *L. escarchadosi* with *L. sarmientoi* (infralabial scales, midbody scales, dorsal scales, ventral scales, Lamellae IV toe). We did not find meristic differences between females of *L. baguali* and *L. sarmientoi*. The morphometric data set showed several differences among species in both sexes, with *L. baguali* and *L. sarmientoi* showing the greatest number of differences among all species pairs (Appendix V).

The fact that we did not observe qualitative morphological differentiation among the focal species (Supplementary Data IV) reflects the challenge that researchers confront, not only in the field, but also if using only one class of data in species delimitation. For some species of the *Liolaemus kingii* group, field identification is difficult, especially in areas where two or more species are sympatric. Our findings of at least nine individuals (representing all the species) with one species' morphotype but with a mitochondrial haplotype of

its geographically adjacent species (from peripheral localities and with mostly terminal positions in the networks) suggest that interspecific hybridization might be affecting these populations; this has been observed in other closely related *Liolaemus* species (e.g., Olave et al. 2011). Out of those nine individuals, we found four that appear to show morphological evidence of hybridization; these individuals had some scale counts and/or measures similar to one sympatric species and others similar to the other sympatric species (for example, individual 9402 was genetically assigned to *L. escarchadosi* and had *L. tari*'s morphotype, but its midbody scale count was similar to *L. escarchadosi* while its ventral scale count was similar to *L. tari*; see results for the other individuals). In addition, sympatric individuals of *L. sarmientoi* and *L. escarchadosi* were genetically assigned to one or the other species but due to the similarity among those species and also the extensive morphological variation within each species, absolute morphological assignment of all individuals was not possible (Fig. 2). These species were formally described based on differences in color patterns (mainly background coloration, vertebral lines, and extent of melanism) and morphometric and meristic characters (Scolaro and Cei 1997). Although further morphological analyses (Breitman et al. 2013; this study) revealed differentiation among these species, they also showed that there is great variation in color patterns among individuals collected over their distributional range and even among individuals collected exclusively from type localities (Breitman et al. 2013). Thus, future studies including sympatric individuals of the focal species are needed in order to fully evaluate the correct taxonomic identification of those sympatric individuals. The inclusion of nuclear markers, behavioral characteristics, and quantification of color patterns of live organisms will improve species assignments (McKay et al. 2014), and will allow testing the extent of hybridization and/or mitochondrial introgression, and whether these processes contribute to the high level of phenotypic polymorphism found in these areas.

Comments on the Evolutionary History of *Liolaemus escarchadosi* and *L. sarmientoi*

Phylogenetic relationships among our focal species have been previously studied using several markers and based on concatenation and species tree methods (Schulte and Moreno-Roark 2010; Breitman et al. 2011; Pyron et al. 2013; Olave et al. 2014). These papers advanced our knowledge of the *Liolaemus lineomaculatus* section, but they did not completely resolve phylogenetic relationships among the focal species. These studies coupled with our results (see also Breitman et al. 2013) allow us to consider some aspects related to alternative phylogenetic placement of *L. escarchadosi* and *L. sarmientoi*. The proposals we develop in this section should be considered as a first round of hypotheses for which follow-up studies are needed.

Comparing previously published results for some species of the *Liolaemus lineomaculatus* section (Schulte and Moreno-Roark 2010; Bonino 2013; Breitman et al. 2013; Pyron et al. 2013; Olave et al. 2014; Bonino et al. 2015) with our results for *L. sarmientoi* and *L. escarchadosi* is particularly enlightening. There are five lines of evidence that support the hypothesis that these are sister species, but there is evidence for past or recent mitochondrial introgression/hybridization between *L. sarmientoi* and *L. kingii* (another species of the *L. lineomaculatus* section mainly distributed in Santa Cruz; Figs. 2, 3, and 9). First, phylogenetic relationships among these species differ depending on the data set and reconstruction method: analyses of mitochondrial markers showed that *L. sarmientoi* is closely related to *L. kingii* (inferred with strong support, Fig. 3; Schulte and Moreno-Roark 2010; Breitman et al. 2011; Pyron et al. 2013), but species tree methods (based on 11 nuclear and 2 mitochondrial genes) recovered *L. sarmientoi* and *L. escarchadosi* (together with the species *L. tari*) in a clade (although with no statistical support, 0.55 Pp; Olave et al. 2014); whereas *L. kingii* was recovered nested within other species of the *L. kingii* clade. Incongruence between different markers and phylogenetic reconstruction methods might reflect underlying evolutionary processes (Knowles and Kubatko 2010), including hybridization and incomplete lineage sorting (Funk and Omland 2003). Second, although the distributional areas of *L. sarmientoi*, *L. escarchadosi*, and *L. kingii* (Breitman et al. 2013) are different, they are sympatric at their distributions' borders (Fig. 9). *Liolaemus kingii* has the northernmost distribution, *L. escarchadosi* the southernmost, and *L. sarmientoi* is peripherally distributed to *L. escarchadosi*, but all three of these have contact areas (Fig. 9). Contact areas offer opportunities for past (indicated by paleoclimatic models) or present introgression and/or hybridization (especially in closely related or recently diverged species, where reproductive isolation might not yet be complete; Arnold 1997; Mallet 2007). Third, animals collected only from the species' type localities (Breitman et al. 2013) showed larger differences (meristic characters) between *L. sarmientoi* and *L. kingii* (males: lorilabial scales, midbody scales, dorsal scales, and ventral scales; females: dorsal and ventral scales) than those between *L. sarmientoi* and *L. escarchadosi* (males: supralabial scales; females: ventral scales). Fourth, current niche models predict highly overlapping (although statistically different) bioclimatically suitable areas for *L. sarmientoi* and *L. escarchadosi* (this study), whereas the current niche model for *L. kingii* (Fig. 10; Bonino 2013; Bonino et al. 2015) predicts a northern area that slightly overlaps with the predicted ranges of *L. sarmientoi* and *L. escarchadosi*. Fifth, paleoclimatic models predict extensive overlap among the three species in northeastern Santa Cruz and southeastern Chubut provinces (Figs. 8 and 10; Supplementary Data VI).

We predict that the use of a broader genetic data set analyzed in a species tree framework will increase the support for a sister group relationship between *Liolaemus sarmientoi* and *L. escarchadosi* and will provide further resolution of relationships within the *L. lineomaculatus* section. However, two other scenarios should be considered: (1) a hybrid origin for *L. sarmientoi* with *L. kingii* and *L. escarchadosi* as parental species and (2) *L. kingii* being the sister lineage of *L. sarmientoi*. In the second case, niche preferences and morphological similarities between *L. sarmientoi* and *L. escarchadosi* are not the result of common ancestry. The amplification of nuclear genes or SNPs in combination with available nuclear and mitochondrial genes (Breitman et al. 2011; Pyron et al. 2013; Olave et al. 2014) and novel methods (McTavish and Hillis 2014) should

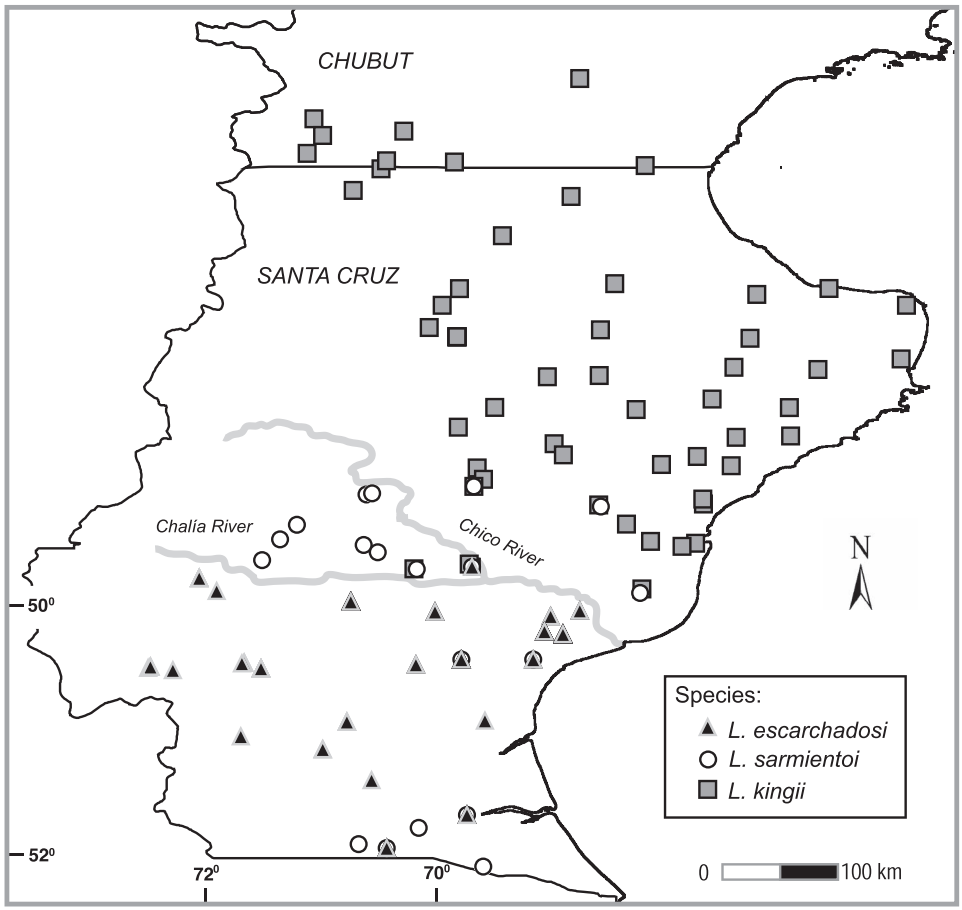

FIG. 9.—Geographic distribution of *Liolaemus escarchadosi*, *L. sarmientoi*, and *L. kingii*.

provide sufficient resolution to test the hybridization/introgression hypotheses postulated above. We think that it is important to emphasize these issues because they show evidence of a complex evolutionary history, which needs to be taken into consideration when areas of conservation priorities are delineated.

Pleistocene Cycles and Past Demographic Patterns

Current knowledge of Patagonian glacial cycles (from the late Miocene to the present) provides a solid theoretical framework to study the interactions between environmental changes and phylogeographic divergence/speciation from this region of South America (Rabassa et al. 2011). Glacial cycles identified in the Pleistocene include the Great Patagonian Glaciation (~1.68–1 mya), the Coldest Pleistocene Glaciation (0.7 mya), the Last Patagonian Glaciation (~0.180–0.140 mya), and the LGM (~0.025–0.016 mya; Rabassa et al. 2011). These glacial cycles were accompanied by changes in sea level, river volumes, and extent and duration of permafrost (or tundra) in Patagonia (Trombotto 2008; Rabassa et al. 2011). Phylogeographic studies of Patagonian taxa and the interplay that glacial cycles have had

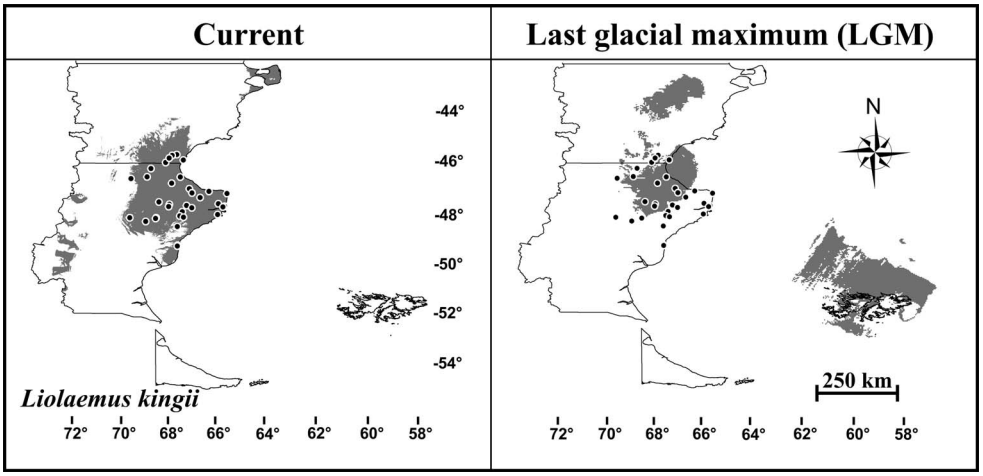

FIG. 10.—Current and paleoclimatic (Last Glacial Maximum) distributions projected for *Liolaemus kingii*.

in shaping their distributions and genetic patterns have increased in the last decade; most of them have shown that while some of the species appear to have moved north or east, others survived in situ in multiple refugia (Sérsic et al. 2011; Breitman et al. 2012; Premoli et al. 2012; Sede et al. 2012; Cosacov et al. 2013). Fixed (or almost fixed) differences in characters among populations or lineages might be considered evidence of lack of (or very low) gene flow (Davis and Nixon 1992), but in order to understand the evolutionary process underlying these patterns, integration of information regarding geological history and/or codistributed species is needed.

Liolaemus sp. A is restricted to the plateau located between the Viedma and Argentino lakes (Fig. 7a), an area also inhabited by another candidate species of the *L. lineomaculatus* group (Lineage 4; Breitman et al. 2012). The plateau was previously hypothesized as a refugium for different plant species; our results provide evidence for a refugium for two species of lizards from two different clades of the *L. lineomaculatus* section (Sérsic et al. 2011; Breitman et al. 2012). About 20% of this area is included in a national park (Parque Nacional Los Glaciares, information available at http://www.losglaciares.com) and is strictly protected, but we recommend protecting a much larger area because the region likely represents a refugium for multiple taxonomic groups besides lizards (Sérsic et al. 2011; Premoli et al. 2012; Sede et al. 2012; Cosacov et al. 2013).

Liolaemus escarchadosi is only found south of the Chico and Chalía rivers (Fig. 5), despite niche models predicting a wider distribution mainly in the northwestern territory of Santa Cruz (Fig. 8). This indicates that the Chico and Chalía rivers could represent geographic barriers for the distribution of this species, a result also hypothesized for plants (phylogenetic break 8 in Sérsic et al. 2011) and other lizard species (Breitman et al. 2012). In the southern part of the distribution (Fig. 5), we detected a signal of population expansion, indicating colonization toward an area that was hypothesized to be glaciated during the LGM (~0.017 mya; Rabassa et al. 2011).

Liolaemus baguali is distributed in the area between Pueyrredón, Cardiel, and San Martin lakes (Figs. 1 and 4) and overlaps in its distribution with *L. hatcheri*, another species from the *L. lineomaculatus* section (Breitman et al. 2012). This area (including the Asador Plateau) has also been hypothesized to be a refugium for plants and lizards (Sérsic et al. 2011; Breitman et al. 2012). Although populations of *L. hatcheri* are apparently much older (~10 mya; Breitman et al. 2012) than those of *L. baguali* (last common ancestor ~1.25 mya, diversification time ~0.5 mya; Breitman 2013), this later date is concordant with haploclade divergence within *L. hatcheri* (~1.30 mya). This concordance suggests that the period between the Great Patagonian Glaciation and the Coldest Pleistocene Glaciation was favorable for the colonization of new areas, and that *L. baguali* could have arrived at its actual southernmost distribution during those times. However, past climate conditions did not allow diversification of *L. baguali* (~0.5 mya) until after the Coldest Pleistocene Glaciations ended. Further, the paleodistribution model predicted a northwesternmost distribution with no presence in its current area, which is congruent with a later arrival of this species to this plateau. Further evidence for this source area from which colonization could have originated is suggested by one very distinct haplotype that has the northernmost distribution and an ancestral position in the gene tree, but additional sampling is needed in order to consider this a general pattern.

Liolaemus sarmientoi has a geographically fragmented distribution (Fig. 6) that is strongly structured genetically and has a terminal southern haploclade (in the area between Gallegos River and the Strait of Magellan; Fig. 6). This haploclade is separated from the other individuals by several mutational steps and has signals of a demographic expansion (Fig. 6), indicating recent colonization into this previously glaciated area (that might have been also recently colonized by *L. escarchadosi*). Finally, two haplotypes are recovered forming a different network that is confined to the easternmost part of its distribution (Fig. 6; Locality 11); future investigations should focus on understanding the role of the Chico River in shaping this pattern.

Our ecological niche models for *Liolaemus baguali*, *L. escarchadosi*, and *L. sarmientoi* predicted similar distributions during the LGM, indicating that these species may have been codistributed in a northern area relative to their current distributions (Fig. 8). However, only *L. sarmientoi* showed a statistically significant demographic expansion signal. For all three species, our models imply a great shift in their distributions (past vs. current), and for *L. escarchadosi* a small overlapping area among past and present predictions was observed (Fig. 8). In a recent study of *Liolaemus* lizards, the interplay between diffusion rate (geographical/range expansion) and demographic change (population growth; Camargo et al. 2013) showed that demographic increase can take place with or without geographical expansion or vice versa. These authors also showed that the diffusion rate for their focal species (*L. darwinii*) varied between a maximum of ~17 m/yr during the beginning of geographical expansion to a minimum of > 1 m/yr during demographic growth without range expansion. Those estimations are also supported by empirical ecological studies and fossil records (Albino 2005; Frutos and Belver 2007; see Camargo et al. 2013 and references therein). As a rough approximation, we calculated population movements as the distance from the center of a species' distribution between the ENM estimations for the LGM to the current distribution divided by 21,000 yr, obtaining the following dispersal estimates: *L. baguali* = ~18 m/yr, *L. escarchadosi* = ~21 m/yr, and *L. sarmientoi* = ~20 m/yr. Our estimated values are similar to those calculated for *L. darwinii* (Camargo et al. 2013); based on these we hypothesize scenarios of demographic movement that in one case (*L. sarmientoi*) was accompanied by demographic expansion and in two cases (*L. escarchadosi* and *L. baguali*) were not. Future work incorporating nuclear markers and integrating Bayesian relaxed models of spatial diffusion (Camargo et al. 2013) with those implemented in our paper, will provide a framework for testing the interplay between range expansion and population growth in these southern species.

Conclusion

The use of a multidisciplinary approach has the final goal of providing good alpha-taxonomic documentation of diversity to critically underpin conservation programs in response to the "biodiversity crisis," and in Patagonia this knowledge is urgently needed. Our contribution advances the morphological, genetic, and ecological knowledge of the four southernmost distributed species of lizards of the

Liolaemus kingii group. We provide support for species status of *L. baguali*, *L. escarchadosi*, *L. sarmientoi*, and *L. tari*, and we also recognize a new candidate species in southwestern Santa Cruz. The study of historical patterns gives an estimate of the adaptive capacity of a species relative to conservation of its environmental niche and where the areas of possible range shifts are located (Pauls et al. 2013). In this paper, we present past demographic and evolutionary hypotheses for these southern Patagonian lizards, which will be important for obtaining reliable estimations on how species might shift ranges according to future climate changes (Dépraz et al. 2008; Sinervo et al. 2010). Finally, the first evidence of possible hybridization among some of these species is discussed. We strongly encourage integration of our results into conservation plans for southern Patagonia and also encourage the use of an integrative framework for evaluating biodiversity in poorly known areas with high conservation priority.

Acknowledgments.—We thank R. Nieto and R. Neyro Martinez for help with laboratory work. We thank the people that helped with field collecting: N. Feltrin (in memoriam), M. Kozykariski, M. Nicola, C.H.F. Pérez, and C. Zanotti. We also thank J. Bagley, C. Aguilar Puntriano, A. Villalba Almendra, J. Nori, C. Medina, I. Minol, L. Welton, and several anonymous reviewers for useful comments and discussion. We thank the fauna authorities of Santa Cruz Province for collection permits. Financial support was provided by the following grants: PICT 2006-506 ANPCYT-FONCYT (LJA), ANPCYT FONCYT 33789 (MM), doctoral and postdoctoral fellowships (first and second authors) from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; first author). The NSF-PIRE award (OISE 0530267) supported collaborative research on Patagonian biodiversity to the following institutions (listed alphabetically): Brigham Young University, Centro Nacional Patagónico (Argentina), Dalhousie University, Instituto Botánico Darwinion (Argentina), Universidad Austral de Chile, Universidad de Concepción (Chile), Universidad Nacional del Comahue (Argentina), Universidad Nacional de Córdoba (Argentina), and the University of Nebraska; also the National Science Foundation Emerging Frontiers award (EF-1241885) supported collaborative research on lizard biodiversity and climate-driven extinctions to the following institutions (listed alphabetically): Brigham Young University, Ohio University, University of California Santa Cruz, and Villanova University.

Resumen: Patagonia es un área con gran biodiversidad y con prioridad alta de conservación, donde uno de los componentes mayoritarios de los vertebrados terrestres endémicos son las lagartijas del género *Liolaemus*. Recientemente, trabajos moleculares y morfológicos identificaron especies crípticas en diferentes grupos de especies de *Liolaemus*, incluyendo a la sección *Liolaemus lineomaculatus* (compuesta por 22 especies) que es endémica de Patagonia. A pesar de ser un componente conspicuo de Patagonia, se conoce muy poco acerca de la variación morfológica, ecológica y genética de las lagartijas de la sección *L. lineomaculatus*; también los límites de especies y los escenarios demográficos pasados de muchas de estas especies se encuentran poco estudiados. En este trabajo caracterizamos la variación morfológica, genética y ecológica de las cuatros especies mas australes del grupo *L. kingii* (sección *L. lineomaculatus*); nuestro objetivo principal es hipotetizar límites de especies (usando la taxonomía integrativa), la historia evolutiva y los escenarios demográficos pasados de estas especies. Para este trabajo usamos un total de 241 especímenes, 195 para análisis morfológicos (incluyendo 10 caracteres morfométricos, 10 merísticos y 7 cualitativos) y para 226 secuenciamos el gen citocromo *b*. Estudiamos la variación ecológica utilizando datos ambientales de 62 localidades de ocurrencia. Mediante un análisis de modelado geoespacial predecimos los nichos ecológicos presentes y pretéritos, y evaluamos la similitud de los mismos. En este trabajo identificamos linajes genéticos y evaluamos su grado de diferenciación a nivel molecular, morfológico y de nicho climático. Nuestros resultados muestran diferenciación en los tres niveles avalando las hipótesis de especies para *L. baguali*, *L. escarchadosi* y *L. sarmientoi*. A pesar de no haber podido evaluar el nicho climático de *L. tari*, encontramos evidencia de diferenciación morfológica y genética avalando también el nivel de especie de este linaje. Finalmente mostramos evidencia de posible hibridización entre algunas de estas especies, y reconocemos una nueva especie candidata.

Supplementary Data

Supplementary data associated with this article can be found online at http://dx.doi.org/10/1655/HERPMONOGRAPHS-D-14-00003.S1.

Literature Cited

Abdala, C.S. 2007. Phylogeny of the *boulengeri* group (Iguania: Liolaemidae, *Liolaemus*) based on morphological and molecular characters. Zootaxa 1538:1–84.

Abdala, C.S., and A.S. Quinteros. 2014. Los últimos 30 años de estudios de la familia de lagartijas más diversa de Argentina. Actualización taxonómica y sistemática de Liolaemidae. Cuadernos de Herpetolgía 28:55–82.

Abdala, C.S., D.E. Procopio, O.A. Stellatelli, A. Travaini, A. Rodríguez, and M.R. Ruiz Monachesi. 2014. New Patagonian species of *Liolaemus* (Iguania: Liolaemidae) and novelty in the lepidosis of the southernmost lizard of the world: *Liolaemus magellanicus*. Zootaxa 3866:526–542.

Aguilar, C., P.L. Wood, Jr., J.C. Cusi, A. Guzmán, F. Huari, M. Lundberg, E. Mortensen, C. Ramírez, D. Robles, J. Suárez, A. Ticona, V.J. Vargas, P.J. Venegas, and J.W. Sites, Jr. 2013. Integrative taxonomy and preliminary assessment of species limits in the *Liolaemus walkeri* complex (Squamata, Liolaemidae) with descriptions of three new species from Peru. ZooKeys 364:47–91.

Albino, A.M. 2005. A Late Quaternary lizard assemblage from the southern Pampean region of Argentina. Journal of Vertebrate Paleontology 25:185–191.

Ameghino, F. 1906. Les formations sédimentaires du Crétacé Superieur et du Tertiaire de Patagonia, avee an paralèlle entre leurs faunes mammalogiques et celles de I' ancient continent. Anales del Museo Nacional de Buenos Aires 15:1–568.

Aparicio, J., and M. Ocampo. 2010. Novedad zoogeográfica. *Liolaemus* grupo *montanus* Etheridge, 1995 (Iguania - Liolaemidae). Cuadernos de Herpetología 24:133–135.

Aragón, P., A. Baselga, and J.M. Lobo. 2010. Global estimation of invasion risk zones for the western corn rootworm *Diabrotica virgifera virgifera*: Integrating distribution models and physiological thresholds to assess climatic favorability. Journal of Applied Ecology 47:1026–1035.

Arnold, M.L. 1997. Natural Hybridization and Evolution. Oxford University Press, UK.

Arribas, P., C. Andújar, D. Sánchez-Fernández, P. Abellán, and A. Millán. 2012. Integrative taxonomy and conservation of cryptic beetles in the Mediterranean region (Hydrophilidae). Zoologica Scripta 42:182–200.

Bonino, M.F. 2013. Patrones y Procesos Fisioecológicos en Liolaemini (Squamata: Iguania) de la Patagonia. Ph.D. dissertation, National University of Cordoba, Argentina.

Bonino, M.F., D.L. Moreno Azócar, J.A. Schulte, II, and F.B. Cruz. 2015. Climate change and lizards: Changing species' geographic ranges in Patagonia. Regional Environmental Change 15:1121–1132.

Bottari, C.J. 1975. Sobre la presencia de *Liolaemus magellanicus* en Tierra del Fuego, Argentina (Reptilia, Iguanidae). Physis 34:211–213.

Breitman, M.F. 2013. Filogenia, Filogeografía y Patrones de Diversificación en la Sección *Liolaemus lineomaculatus* (Iguania: Squamata: Liolaemini) de América del Sur Austral (Patagonia). Ph.D. dissertation, National University of Cordoba, Argentina.

Breitman, M.F., L.J. Avila, J.W. Sites, Jr., and M. Morando. 2011. Lizards from the end of the world: Phylogenetic relationships of the *Liolaemus lineomaculatus* section (Squamata: Iguania: Liolaemini). Molecular Phylogenetics and Evolution 59:364–376.

Breitman, M.F., L.J. Avila, J.W. Sites, Jr., and M. Morando. 2012. How lizards survived blizzards: Phylogeography of the *Liolaemus lineomaculatus* group (Liolaemidae) reveals multiple breaks and refugia in southern Patagonia, and their concordance with other co-distributed taxa. Molecular Ecology 25:6068–6085.

Breitman, M.F., M. Morando, and L.J. Avila. 2013. Past and present taxonomy of the *Liolaemus lineomaculatus* section (Liolaemidae): Is the morphological arrangement hypothesis valid? Zoological Journal of the Linnean Society 168:612–668.

Breitman, M.F., I. Minoli, L.J. Avila, C.D. Medina, J.W. Sites, Jr., and M. Morando. 2014. Lagartijas de la provincia de Santa Cruz (Argentina): Distribución geográfica, diversidad genética y estado de conservación. Cuadernos de Herpetología 28:83–110.

Camargo, A., F.P. Werneck, M. Morando, J.W. Sites, Jr., and L.J. Avila. 2013. Quaternary range and demographic expansion of *Liolaemus darwinii* (Squamata: Liolaemidae) in the Monte Desert of Central Argentina using Bayesian phylogeography and ecological niche modelling. Molecular Ecology 15:4038–4054.

Carstens, B.C., T.A. Pelletier, N.M. Reid, and J.D. Satler. 2013. How to fail at species delimitation. Molecular Ecology 22:4369–4383.

Cei, J.M. 1986. Reptiles del centro, centro-oeste y sur de Argentina. Herpetofauna de las zonas áridas y semiáridas. Museo Regionale de Scienze Naturali 4:1–527.

Clement, M., D. Posada, and K.A. Crandall. 2000. TCS: A computer program to estimate gene genealogies. Molecular Ecology 9:1657–1659.

Colwell, R.K., and T.F. Rangel. 2009. Hutchinson's duality: The once and future niche. Proceedings of the National Academy of Sciences of the United States of America 106:19651–19658.

Corbalán, V., M.F. Tognelli, J.A. Scolaro, and S.A. Roig-Juñet. 2011. Lizards as conservation targets in Argentinean Patagonia. Journal for Nature Conservation 19: 60–67.

Cosacov, A., L.A. Johnson, V. Paiaro, A.A. Cocucci, F.E. Córdoba, and A.N. Sérsic. 2013. Precipitation rather than temperature influenced the phylogeography of the endemic shrub *Anarthrophyllum desideratum* in the Patagonian steppe. Journal of Biogeography 40:168–182.

Darwin, C. 1859. On the Origin of the Species by Means of Natural Selection: Or, The Preservation of Favored Races in the Struggle for Life. Murray, UK.

Davis, J.I., and K.C. Nixon. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. Systematic Biology 41: 421–435.

Dayrat, B. 2005. Towards integrative taxonomy. Biological Journal of the Linnean Society 85:407–415.

Dépraz, A., M. Cordellier, J. Hausser, and M. Pfenninger. 2008. Postglacial recolonization at a snail's pace (*Trochulus villosus*): Confronting competing refugia hypotheses using model selection. Molecular Ecology 17:2449–2462.

De Queiroz, K. 2005. A unified concept of species and its consequences for the future of taxonomy. Proceedings of the California Academy of Sciences 56:196–215.

Di Rienzo, J.A., A.W. Guzmán, and F. Casanoves. 2002. A multiple comparisons method based on the distribution of the root node distance of a binary tree. Journal of Agricultural, Biological, and Environmental Statistics 7:1–14.

Di Rienzo, J.A., F. Casanoves, M.G. Balzarini, L. González, M. Tablada, and C.W. Robledo. 2011. InfoStat. Available at http://www.infostat.com.ar. Archived by WebCite at http://www.webcitation.org/6NpTLyea1 on 26 August 2011.

Elith, J. 2002. Predicting the Distribution of Plants. Ph.D. dissertation, University of Melbourne, Australia.

Elith, J., and J.R. Leathwick. 2009. The contribution of species distribution modelling to conservation prioritization. Pp.70–93 in Spatial Conservation Prioritization: Quantitative Methods and Computational Tools (A. Moilanen, K.A. Wilson, and H.P. Possingham, ed.). Oxford University Press, UK.

Elith, J., C.H. Graham, R.P. Anderson, M. Dudík, S. Ferrier, A. Guisan, R.J. Hijmans, F. Huettmann, J.R. Leathwick, A. Lehmann, J. Li, L.G. Lohmann, B.A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J. McC. Overton, A.T. Peterson, S.J. Phillips, K. Richardson, R. Scachetti-Pereira, R.E. Schapire, J. Soberón, S. Williams, M.S. Wisz, and N.E. Zimmermann. 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29:129–151.

Escudero, P.C., I. Minoli, N. Frutos, L.J. Avila, and M. Morando. 2012. Estudio comparativo del melanismo en lagartijas del grupo *Liolaemus fitzingerii* (Liolaemini: *Liolaemus*). Cuadernos de Herpetología 26:79–89.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

Frutos, N., and L.C. Belver. 2007. Dominio vital de *Liolaemus koslowskyi* Etheridge, 1993 (Iguania: Liolaemini) en el noroeste de la provincia de La Rioja, Argentina. Cuadernos de Herpetología 21:83–92.

Fu, Y-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

Funk, D.J., and K.E. Omland. 2003. Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology Evolution and Systematics 34:397–423.

Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4:1–9.

Hernandez, P.A., C.H. Graham, L.L. Master, and D.L. Albert. 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. Ecography 29:773–785.

Hillis, D.M., and J.J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42:182–192.

Huelsenbeck, J.P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Jiménez-Valverde, A., J.M. Lobo, and J. Hortal. 2008. Not as good as they seem: The importance of concepts in species distribution modelling. Diversity and Distributions 14: 885–890.

Kacoliris, F.P., I. Berkunsky, and M.A. Velasco. 2013. Sex and size affect annual survival in threatened sand lizard. Herpetological Journal 23:59–62.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30:3059–3066.

Knowles, L.L., and L. Kubatko. 2010. Estimating Species Trees: Practical and Theoretical Aspects. Wiley-Blackwell, USA.

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Eduards, S. Pääbo, F.X. Villablanca, and A.C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proceedings of the National Academy of Science of the United States of America 86:6196–6200.

Kozak, K.H., C.H. Graham, and J.J. Wiens. 2008. Integrating GIS-based environmental data into evolutionary biology. Trends in Ecology and Evolution 23:141–148.

Kruskal, W.H., and W.A. Wallis. 1952. Use of ranks on one-criterion variance analysis. Journal of the American Statistical Association 47:583–621.

Laurent, R.F. 1990. Una especie apartada del género *Liolaemus* Wiegmann (Iguanidae, Lacertilia). Physis 39:79–84.

Librado, P., and J. Rozas. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Lobo, F.J., R.E. Espinoza, and S. Quinteros. 2010. A critical review and systematic discussion of recent classification proposals for liolaemid lizards. Zootaxa 2549:1–30.

Mace, G. 2004. The role of taxonomy in species conservation. Philosophical Transactions of the Royal Society of London B 359:711–719.

Mallet, J. 2007. Hybrid speciation. Nature 446:279–283.

Manel, S., H.C. Williams, and S. Ormerod. 2001. Evaluating presence–absence models in ecology: The need to account for prevalence. Journal of Applied Ecology 38:921–931.

McKay, B.D., H.L. Mays, Jr., C. Yao, D. Wan, H. Higuchi, and I. Nishiumi. 2014. Incorporating color into integrative biology: Analysis of the Varied Tit (*Sittiparus varius*) complex in East Asia. Systematic Biology 63:505–517.

McTavish, E.J., and D.M. Hillis. 2014. A genomic approach for distinguishing between recent and ancient admixture as applied to cattle. Journal of Heredity 105:445–456.

Medina, C.D., L.J. Avila, and M. Morando. 2013. Hacia una taxonomía integral: Poniendo a prueba especies candidatas relacionadas a *Liolaemus buergeri* Werner 1907 (Iguania: Liolaemini) mediante análisis morfológicos. Cuadernos de Herpetología 27:27–34.

Miralles, A., and M. Vences. 2013. New metrics for comparison of taxonomies reveal striking discrepancies among species delimitation methods in *Madascincus* lizards. PLoS ONE 8(7):e68242. DOI: 10.1371/journal.pone.0068242.

Montgomery, D.C. 1991. Diseño y Análisis de Experimentos. Grupo Iberoamérica, Mexico.

Morando, M., L.J. Avila, and J.W. Sites, Jr.. 2003. Sampling strategies for delimiting species: Genes, individuals, and populations in the *Liolaemus elongatus-kriegi* complex (Squamata: Liolaemidae) in Andean-Patagonian South America. Systematic Biology 52:159–185.

Moreno, F.P. 1876. Viaje a la Patagonia Austral. La Nación, Argentina.

Munguía, M., A.T. Peterson, and V. Sánchez-Cordero. 2008. Dispersal limitation and geographical distributions of mammal species. Journal of Biogeography 35:1879–1887.

Nei, M., and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, USA.

Olave, M., L.E. Martinez, L.J. Avila, J.W. Sites, Jr., and M. Morando. 2011. Evidence of hybridization in the Argentinean lizards *Liolaemus gracilis* and *Liolaemus bibronii* (Iguania: Liolaemini): An integrative approach based on genes and morphology. Molecular Phylogenetics and Evolution 61:381–391.

Olave, M., L.J. Avila, J.W. Sites, Jr., and M. Morando. 2014. Multilocus phylogeny of the widely distributed South American lizard clade *Eulaemus* (Liolaemini, *Liolaemus*). Zoologica Scripta 43:323–337.

Paruelo, J.M., and M.R. Aguiar. 2003. El impacto humano sobre los ecosistemas: El caso de la desertificación en Patagonia. Ciencia Hoy 13:48–59.

Paruelo, J.M., R.A. Golluscio, E.G. Jobbagy, M. Canevari, and M.R. Aguiar. 2006. Situación ambiental en la estepa Patagónica. Pp. 302–330, in La Situación Ambiental Argentina 2005, (A. Brown, U. Martinez Ortiz, M. Acerbi, and J. Corchera, ed.). Fundación Vida Silvestre, Argentina.

Pauls, S.U., C. Nowak, M. Bálint, and M. Pfenninger. 2013. The impact of global climate change on genetic diversity within populations and species. Molecular Ecology 22:925–946.

Pearce, J., and S. Ferrier. 2000. Evaluating the predictive performance of habitat models developed using logistic regression. Ecological Modeling 133:225–245.

Phillips, S.J., R.P. Anderson, and R.E. Schapire. 2006. Maximum entropy modelling of species geographic distributions. Ecological Modelling 190:231–259.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25:1253–1256.

Premoli, A.C., P. Mathiasen, M.C. Acosta, and V.A. Ramos. 2012. Phylogeographically concordant chloroplast DNA divergence in sympatric *Nothofagus s.s.* How deep can it be? New Phytologist 193:261–275.

Pyron, R.A., F.T. Burbrink, and J.J. Wiens. 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evolutionary Biology 2013:13–93. DOI: http://dx.doi.org/10.1186/1471-2148-13-93.

Rabassa, J., A. Coronato, and O. Martínez. 2011. Late Cenozoic glaciations in Patagonia and Tierra del Fuego: An updated review. Biological Journal of the Linnean Society 103:316–335.

Rambaut, A., and A.J. Drummond. 2009. Tracer v1.5. Available at http://beast.bio.ed.ac.uk/Tracer. Archived by WebCite® at http://www.webcitation.org/6cQ2zRt4o on 02 May 2015.

Ramos-Onsins, S.E., and J. Rozas. 2002. Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19:2092–2100.

Ronquist, F., and J.P. Huelsenbeck. 2003. MrBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 9:1572–1574.

Sánchez-Fernández, D., J.M. Lobo, P. Abellán, and A. Millán. 2011. Environmental niche divergence between genetically distant lineages of an endangered water beetle. Biological Journal of the Linnean Society 103:891–903.

Schlick-Steiner, B.C., F.M. Steiner, B. Seifert, C. Stauffer, E. Christian, and R.H. Crozier. 2010. Integrative taxonomy: A multisource approach to exploring biodiversity. Annual Reviews of Entomology 55:421–438.

Schoener, T.W. 1968. *Anolis* lizards of Bimini: Resource partitioning in a complex fauna. Ecology 49: 704–726.

Schulte, J.A., and F. Moreno-Roark. 2010. Live birth among iguanian lizards predates Pliocene–Pleistocene glaciations. Biology Letters 6:216–218.

Schulte, J.A., J.R. Macey, R.E. Espinoza, and A. Larson. 2000. Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: Multiple origins of viviparous reproduction and evidence for recurring Andean vicariances and dispersal. Biological Journal of the Linnean Society 69:75–102.

Scolaro, J.A., and J.M. Cei. 1987. A multivariate analysis of morphometric and exosomatic characters of iguanid lizards of the Patagonian *Liolaemus kingi* complex. Journal of Herpetology 21:343–348.

Scolaro, J.A., and J.M. Cei. 1997. Systematic status and relationships of *Liolaemus* species of the *archeforus* and *kingii* groups: A morphological and taxonumerical approach (Reptilia: Tropiduridae). Bollettino del Museo Regionale di Scienze Naturale 15:369–406.

Sede, M.S., M.V. Nicola, R. Pozner, and L.A. Johnson. 2012. Phylogeography and paleodistribution modeling in the Patagonian steppe: The case of *Mulinum spinosum* (Apiacea). Journal of Biogeography 39:1041–1057.

Sérsic, A.N., A. Cosacov, A.A. Cocucci, L.A. Johnson, R. Pozner, L.J. Avila, J.W. Sites, Jr., and M. Morando. 2011. Emerging phylogeographic patterns in plants and terrestrial vertebrates from Patagonia. Biological Journal of the Linnean Society 103:475–494.

Sinervo, B., F. Mendez-De la Cruz, D.B. Miles, B. Heulin, E. Bastiaans, M. Villagran-Santa Cruz, R. Lara-Resendiz, N. Martínez-Méndez, M.L. Calderon-Espinosa, R. Nelsi Mesa-Lázaro, H. Gadsden, L.J. Avila, M. Morando, I.J. De la Riva, P. Victoriano Sepulveda, C.F. Duarte Rocha, N. Ibargüengoytía, C. Aguilar Puntriano, M. Massot, V. Lepetz, T.A. Oksanen, D. Chapple, A. Bauer, W. Branch, J. Clobert, and J.W. Sites, Jr.. 2010. Erosion of lizard diversity by climate change and altered thermal niches. Science 328:894–899.

Smith, H.M. 1946. Handbook of Lizards. Comstock Publishing, USA.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.

Tajima, F. 1989. Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Templeton, A.R., K.A. Crandall, and C.F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633.

Trombotto, D. 2008. Geocryology of southern South America., 225–268, in The Late Cenozoic of Patagonia and Tierra del Fuego, (J. Rabassa, ed.). Elsevier, UK.

Vega, L.E., P.J. Bellagamba, and L.A. Fitzgerald. 2000. Long-term effects of anthropogenic habitat disturbance on a lizard assemblage inhabiting coastal dunes in Argentina. Canadian Journal of Zoology 78:1653–1660.

Vukov, T.D., G. Džukić, S. Lelo, L.J. Borkin, S.N. Litvinchuk, and L. Kalezić. 2006. Morphometrics of the yellow-bellied toad (*Bombina variegata*) in the Central Balkans: Implications for taxonomy and Zoogeography. Zoological Studies 45:213–222.

Warren, D.L., R.E. Glor, and M. Turelli. 2010. ENMTools: A toolbox for comparative studies of environmental niche models. Ecography 33:607–611.

Wiegmann, A.F.A. 1834. Beiträge zur Zoologie, gesammelt auf einer Reise um die Erde. Siebente Abhandlung. Amphibien. Nova Acta Physico-Medica, Academiae Caesare Leopoldino-Carolinae 17: Pp. 183–268, Plates XIII–XXII. [In German and Latin.]

Wiens, J.J., and C.H. Graham. 2005. Niche conservatism: Integrating evolution, ecology, and conservation biology. Annual Reviews of Ecology Evolution and Systematics 36:519–539.

WorldClim - Global Climate Data. Free climate data for ecological modeling and GIS. Available at http://www.worldclim.org/download. Archived by WebCite® at http://www.webcitation.org/6cQ3JVsVc on 20 October 2015.

Published on 4 December 2015

Appendix I

Specimens and localities of species presence used for this study. All individuals are deposited in the Centro Nacional Patagónico collection (LJAMM-CNP). We present locality numbers, latitude and longitude in parenthesis, GenBank accession numbers (in brackets) only for individuals used in molecular analyses (numbers starting with KP are novel for this study, numbers starting with JF were taken from previous studies), and sex (in parentheses) for individuals used in morphological analyses (M = male; F = female).

Liolaemus baguali: ARGENTINA: SANTA CRUZ: Locality 4, Department of Lago Argentino; 4 km N of Estancia Altavista going in the road of estancia San Adolfo, San Adolfo plateau, 8.6 km N of Provincial Road 31; 597–900 meters above sea level (masl; 49.17547°S, 71.87289°W; the datum for all the geographic coordinates = WGS84): 7261 [KP175356], 7262 [KP175357] (F), 7318 [KP175358] (M),7319 [KP175359] (M), 7320 [KP175360] (M), 7321 [KP175361] (F), 7322 [KP175362] (F), 7323 [KP175363] (F), 9347 [KP175364] (M), 9348 [KP175365] (M), 9350 [KP175366] (F), 9351 [KP175368] (F), 9352 [KP175369] (F), 9353 [KP175370], 9354 [KP175371], 9355 [KP175372], 9356 [KP175373], 9357 [KP175374]; Locality 5, Department of Río Chico; Lago Strobel plateau, toward the Strobel Lake, 31 km NW of National Road 40, 29 km NW of Provincial Road 29, 22 km NW of Estancia Las Coloradas; 849 masl (48.68556°S, 71.15017°W): 11564 [KP175375] (M), 11565 [KP175376] (M), 11566 [KP175377] (F), 11567 [KP175378], 11568 [KP175379] (M), 11569 [KP175380] (M), 11570 (F), 11571 [KP175381] (F), 11572 [KP175382] (F), 11573 [KP175383] (F), 11578 [KP175384] (F); Locality 6, Department of Río Chico; Cerro El Puntudo, entrance of Estancia Las Tunas, near the N coast of Cardiel Lake, 14 km W of National Road 40; 460 masl (48.82658°S, 71.09083°W): 7345 [KP175385] (F), 7346 [KP175386] (M), 7347 [KP175387], 7348 [KP175388]; Locality **7**, Department of Río Chico; National Road 40, 72.8 km N of Provincial Road 31 and National Road 288; 525 masl (49.10400°S, 71.19850°W): 9422 [KP175389] (M), 9423 [KP175390] (M), 9424 [KP175391] (M), 9426 [KP175392] (F), 9429 [KP175393] (F), 9433 [KP175394] (F), 9434 [KP175395], 9436 [KP175396] (M), 9437 [KP175397]; Locality 8, Department of Lago Argentino; National Road 40, rocky patch at 3 km of Estancia La Lucia, 54.8 km N of Tres Lagos, Cerro Cordon; 650–700 masl (49.23042°S, 71.34203°W): 7266 [KP175398], 7267 [KP175399] (F), 7268 [KP175400], 7270 [KP175401] (F), 7271 [KP175402], 7272 [KP175403],

7274 [KP175404] (M), 7275 [KP175405] (F), 7339 [KP175406] (M); Locality 9, Department of Lago Argentino; National Road 40, 27.3 km N of Tres Lagos, Sierras del Bagual; 475 masl (49.41025°S, 71.49953°W): 7229 [KP175407], 7230 [KP175408], 7231 [KP175409] (F), 7232 [KP175410] (F), 7233 [KP175411] (F), 7258 [KP175412] (M), 9394 [JF272766] (M), 9395 [KP175413] (M), 9396 [KP175414] (F); Locality 10, Department of Río Chico; Provincial Road 81, 57.1 km SW of National Road 40, toward Estancia Entre Rios; 709 masl (48.36017°S, 71.85264°W): 11582 [KP175367] (M). Other localities from bibliographic references (listed in Bonino 2013): (48.67792°S, 71.13256°W), (48.99°S, 71.2°W), (49.18°S, 71.34°W), (49.18494°S, 71.34578°W), (49.187139°S, 71.8755°W), (49.19°S, 71.34°W), (49.32°S, 71.45°W), (49.3333°S, 71.45°W), (49.37°S, 71.48°W), (49.386889°S; 71.498778°W), (49.42°S, 71.47°W).

Liolaemus escarchadosi: ARGENTINA: SANTA CRUZ: Locality 3, Department of Lago Argentino; Punta del Lago plateau, toward Meseta Campo las Piedras, 7 km N of Estancia Punta del Lago; 868 masl (49.56972°S, 72.04775°W): 9317 [KP175474] (F), 9401 [KP175475] (M), 9402 [KP175476] (M), 9403 [KP175477] (M), 9404 [KP175478] (F), 9409 [KP175479], 9410 [KP175480]; Locality 12, Department of Guer Aike; Provincial Road 5, 10 km W of Guer Aike, in rocky patches S of road; 66 masl (51.62617°S, 69.72164°W) 11484 [KP175446] (M), 11485 [KP175447] (M), 11486 [KP175448] (M), 11488 [KP175449]; Locality 13, Department of Guer Aike; rocky patch at the entrance of Campo Experimental INTA Potrok Aike, 8.4 km S of Provincial Road 52, margin E of Arroyo del Roble; 135 masl (51.91072°S, 70.41944°W): 11475 [KP175450] (M), 11477 [KP175451], 11478 [KP175452], 11479 [KP175453]; Locality 14, Department of Corpen Aike; Provincial Road 27, 103.1 km SE of Gobernador Gregores, close to National Road 288; 121 masl (49.47164°S, 69.67733°W): 7160 [KP175504] (F), 7162 [KP175505]; Locality 18, Department of Corpen Aike; National Road 3, 35.8 km S of entrance to Puerto Santa Cruz, 1 km N of Provincial Road 9; 357 masl (50.27572°S, 69.14756°W): 11465 [KP175435] (F), 11468 [KP175436] (M); Locality 19, Department of Corpen Aike; Provincial Road 9, 45.1 km W of National Road 3, after entrance of Estancia Santa Lucia; 372 masl (50.27661°S, 69.76817°W): 7189 [KP175437] (M), 7190 [KP175438] (M), 7192 [KP175439] (M), 7193 [KP175440], 7194 [KP175441], 7195 [KP175441], 7202 [KP175443] (M), 7203 [KP175444] (F); Locality 26, Department of Corpen Aike; National Road 288, 62.3 km E of Provincial Road 27, 14 km W of Comandante Luis Piedra Buena; 108 masl (49.90047°S, 68.99294°W): 7185 [KP175416] (F), 7186 [KP175417]; Locality 27, Department of Corpen Aike; National Road 3, 102 km SW of San Julian, 18 km NE of Comandante Luis Piedrabuena; 111 masl (49.84936°S, 68.73969°W): 11463 [KP175418] (M), 11464 [KP175419] (F); Locality 28, Department of Corpen Aike; National Road 288, 1 km E of National Road 3, 24 km W of Puerto Santa Cruz; 126 masl (50.05428°S, 68.88589°W): 7163 [KP175420] (M), 7164 [KP175421] (F), 7165 [KP175422] (M), 7166 (F), 7167 [KP175423] (F), 7168 [KP175424], 7169[KP175425] (F), 7170[KP175426] (M), 7171 [KP175427] (F), 7172 [KP175428] (F), 7173 [KP175429] (F), 7174 [KP175430] (M), 7175 [KP175431] (F), 7176 [KP175432] (F), 7187 [KP175433] (M), 7188 [KP175434] (F); Locality 29, Department of Corpen Aike; Provincial Road 17, 9.1 km SW of National Road 288, 11.1 km SW of Comandante Luis Piedrabuena; 54 masl (50.03100°S, 69.05036°W): 9307 [KP175506] (M), 9308 [KP175507] (M), 9309 [KP175508] (M), 9310 [KP175509] (M), 9311 [KP175510] (M), 9312 [KP175511] (F), 9313 [KP175512] (F), 9314 [KP175415] (F); Locality 30, Department of Guer Aike; Provincial Road 7, 3.5 km W of National Road 3, toward Estancia Domi Aike; 257 masl (50.80383°S, 69.56344°W): 11470 [KP175445] (M); Locality 31, Department of Corpen Aike; 2.1 km S of Provincial Road 9, toward Estancia Las Lagunas; 375 masl (50.31658°S, 70.16250°W): 9326 (F), 9327 (F), 9328 (F); Locality 32, Department of Guer Aike; 14.6 km W of Provincial Road 5, after entrance to Estancia San Jose, toward Estancia Corpie Aike; 199 masl (51.32122°S, 70.55033°W): 11494 [KP175454] (F); Locality 33, Department of Guer Aike; Provincial Road 2, 23.7 km N of Provincial Road 5, 25.7 km N of Esperanza; 314 masl (50.81603°S, 70.76186°W): 11489 [KP175455] (M), 11490 [KP175456] (F), 11491 [KP175457] (F), 11492 [KP175458] (F); Locality 34, Department of Guer Aike; Provincial Road 7, 64.8 km E of Tapi Aike, 14.9 km W of La Esperanza, at the south margin of Coyle River; 210 masl (51.05264°S, 70.97333°W): 7217 [KP175459] (M), 7218 [KP175460] (M), 7219 [KP175461] (F); Locality 35, Department of Guer Aike; National Road 40, 30.3 km N of Provincial Road 7 and Tapi Aike, close to Estancia El Manantial; 334 masl (50.93989°S, 71.68433°W): 7226 [KP175462]; Locality 36, Department of Lago Argentino; Provincial Road 9, 2 km E of National Road 40; 234 masl (50.30567°S, 71.65678°W): 7241 [KP175463] (F), 7242 [KP175464] (F), 7244 [KP175465], 11498 [KP175466]; Locality 37, Department of Lago Argentino; Provincial Road 15, 1 km S of El Calafate, Estancia Huyliche; 345 masl (50.36603°S, 72.27681°W): 11499 [KP175467] (F); Locality 38, Department of Lago Argentino; Provincial Road 11, 13 km W of El Calafate; 207 masl (50.34639°S, 72.48953°W): 11541 [KP175468] (F), 11543 [KP175469] (M), 11544 [KP175470] (F), 11545 [KP175471] (F), 11546 [KP175472] (F); Locality 39, Department of Lago Argentino; Provincial Road 23, 2.5 km W of National Road 40, toward El Chalten; 310 masl (49.68192°S, 71.89489°W): 7257 [KP175473] (F); Locality 40, Department of Lago Argentino; National Road 40, entrance to Laguna de los Escarchados, 29.4 km SE of Provincial Road 11 toward El Calafate; 819 masl (50.35158°S, 71.50989°W): 7234 (M), 7235 [KP175481] (M), 7236 [KP175482] (M), 7237 [KP175483] (M), 7238 [KP175484] (F), 7239 [KP175485] (F), 7240 [KP175486], 7245 [KP175487] (M), 7246 [KP175488] (F); Locality 41, Department of Lago Argentino; Provincial Road 65, 43.5 km W of Provincial Road 17, 1 km S of Cerro Mank Aike; 807 masl (49.77133°S, 70.72997°W): 9286 [KP175489] (F), 9319 [KP175490], 9334 [KP175491], 9335 [KP175492] (M), 9336 [KP175493] (M), 9337 (M), 9338 [KP175494] (M), 9339 (M), 9340 [JF272772], 9341 [KP175495] (F), 9342 [KP175496] (F), 9343 [KP175497] (F), 9344 [KP175498] (M), 9345 [KP175499] (M), 9346 [KP175500] (F); Locality 42, Department of Corpen Aike; Provincial Road 17, 96.5 km W of National Road 288, close to Estancia Cañadon Grande; 489 masl (49.85872°S, 69.99897°W): 9329 (M), 9330 [KP175501] (M), 9331 [KP175502] (F), 9332 [KP175503] (F). Other localities from bibliographic references (listed in Bonino 2013): (50.33983°S, 72.46919°W), (50.30658°S, 71.67452°W).

Liolaemus sarmientoi: ARGENTINA: SANTA CRUZ: Locality 7, Department of Río Chico; National Road 40, 72.8 km N of Provincial Road 31 and National Road 288; 525 masl (49.10400°S, 71.19850°W): 9418 [KP175521] (M), 9425 [KP175522] (F), 9427 [KP175513] (F), 9430 [KP175523] (F), 9432 [KP175524] (M); Locality 8, Department of Lago Argentino; National Road 40, rocky patch at 3 km of Estancia La Lucia, 54.8 km N of Tres Lagos, Cerro Cordon; 650–700 masl (49.23042°S, 71.34203°W): 7338[KP175525] (F); Locality 9, Department of Lago Argentino; National Road 40, 27.3 km N of Tres Lagos, Sierras del Bagual; 475 masl (49.41025°S, 71.49953°W): 9397 [KP175526] (F); Locality 11, Department of Corpen Aike; National Road 3, 49.4 km S of San Julian, close to the entrance of Estancia La Silvita; 161 masl (49.63214°S, 68.15253°W): 11448 [KP175514] (F), 11450 [KP175515] (M); Locality 12, Department of Guer Aike; Provincial Road 5, 10 km W of Guer Aike, in rocky patches S of road; 66 masl (51.62617°S, 69.72164°W): 11487 [KP175516] (F); Locality 13, Department of Guer Aike; rocky patch at the entrance of Campo Experimental INTA Potrok Aike, 8.4 km S of Provincial Road 52, margin E of Arroyo del Roble; 135 masl (51.91072°S, 70.41944°W): 11473 [KP175517] (M), 11474 [KP175518] (F), 11476 [KP175519]; Locality 14, Department of Corpen Aike; Provincial Road 27, 103.1 km SE of Gobernador Gregores, close to National Road 288; 121 masl (49.47164°S, 69.67733°W): 7161 [KP175520] (F); Locality 15, Department of Río Chico; Provincial Road 29, 35.5 km SW of Gobernador Gregores; 459 masl (48.84406°S, 70.59489°W): 9280 [KP175527], 9281 [KP175528], 11621 [KP175529] (M), 11622 [KP175530] (M), 11623 [KP175531] (M), 11624 [KP175532] (M), 11625 [KP175533] (M), 11626 [KP175534] (M), 11627 [KP175535] (F), 11628 [KP175536] (F), 11629 [KP175537] (F), 11630 [KP175538] (F), 11631 [KP175539], 11674 (F); Locality 16, Department of Río Chico; Provincial Road 12, 5 km N of Provincial Road 25, 40 km E of Gobernador Gregores; 331 masl (48.79814°S, 69.75300°W): 7143 [KP175540] (F); Locality 17, Department of Magallanes; intersection between Provincial Roads 25 and 77, close to Estancia Cerro Perdido; 241 masl (48.96869°S, 68.491167°W): 7121 [KP175541] (F); Locality 18, Department of Corpen Aike; National Road 3, 35.8 km S of entrance to Puerto Santa Cruz, 1 km N of Provincial Road 9; 357 masl (50.27572°S, 69.147556°W): 11466 [KP175542] (F), 11467 [KP175543] (M); Locality 19, Department of Corpen Aike; Provincial Road 9, 45.1 km W of National Road 3, after entrance of Estancia Santa Lucia; 372 masl (50.27661°S, 69.768167°W): 7196 [KP175544]; Locality 20, Department of Guer Aike; Laguna Azul, Reserva Geologica Provincial Laguna Azul, close to Estancia Monte Aymond; 146 masl (52.07472°S, 69.58128°W): 7197 (M), 7198 (M), 7199 (F), 7200 (F), 7201 [KP175545], 7204 [KP175546] (M), 7205 [KP175547] (M), 7206 [JF272782] (M), 7207 [KP175548] (F), 7208 [KP175549] (F), 7211 [KP175550] (M), 7212 [KP175551] (M), 7213 [KP175552], 7214 [KP175553]; Locality 21, Department of Guer Aike; National Road 40, 44.6 km SW of National Road 3, Estancia Las Buitreras; 62 masl (51.73603°S, 70.14117°W): 7215 [KP175554] (M), 7216 [KP175555] (M); Locality 22, Department of Guer Aike; National Road 40, 4.6 km of Estacion Gobernador Moyano; 160 masl (51.87658°S, 70.66375°W): 11480 [KP175556] (M), 11481 [KP175557] (M), 11482 [KP175558] (F), 11483 [KP175559] (F), 11533 [KP175560] (F); Locality 23, Department of Corpen Aike; Provincial Road 73, 89.7 km SE of National Road 40, 1 km from ex Hotel La Horqueta, 1 km NW of National

Road 291; 143 masl (49.49214°S, 70.18008°W): 9305 [KP175562] (M); Locality 24, Department of Corpen Aike; Provincial Road 73, 59.5 km SE of National Road 40; 315 masl (49.34217°S, 70.49442°W): 9301 [KP175561] (F); Locality 25, Department of Lago Argentino; Provincial Road 73, 48.5 km SE of National Road 40, toward ex Hotel La Horqueta; 318 masl (49.27981°S, 70.61567°W): 9285 (F), 9299 [KP175563] (M), 9300 [KP175564] (M). Other localities from bibliographic references (listed in Bonino 2013): (48.83172°S, 70.54263°W).

Liolaemus tari: ARGENTINA: SANTA CRUZ: Locality 2, Department of Lago Argentino; National Road 40, 6 km S of La Leona hotel, near the ex Estacion Astronomica Austral, 68.9 km N of Provincial Road 8; 249 masl (49.84778°S, 72.04083°W): 7251 [KP175570] (M), 7253 [KP175571] (M); Locality 3, Department of Lago Argentino; Punta del Lago plateau, toward Meseta Campo las Piedras, 7 km N of Estancia Punta del Lago; 868 masl (49.56972°S, 72.04775°W): 9400 [KP175566] (M), 9405 [KP175567] (F), 9406 [KP175568] (F), 9407 [JF272793] (F), 9408 [KP175569] (M); Locality 4, Department of Lago Argentino; 4 km N Estancia Altavista toward estancia San Adolfo, San Adolfo plateau, 8.6 km N of Provincial Road 31; 597–900 masl (49.17547°S, 71.87289°W): 9349 [KP175565] (F).

Liolaemus sp. A: ARGENTINA: SANTA CRUZ: Locality 1, Department of Lago Argentino; Provincial Road 69, 40.8 km W of National Road 40, 10–12 km W of Estancia La Herradura; 609 masl (49.89014°S, 72.50461°W): 11547 [KP175354] (M), 11548 [KP175355] (M); Locality 2, Department of Lago Argentino; National Road 40, 6 km S of La Leona hotel, near ex Estacion Astronomica Austral, 68.9 km N of Provincial Road 8; 249 masl (49.84778°S, 72.04083°W): 7250 [KP175352] (M), 7252 [KP175353] (M).

Outgroups (specific locality data are listed in Breitman et al. 2011): *Liolaemus archeforus* (46.96438°S, 71.10755°W): 9240 [JF272765]. *Liolaemus avilae* (47.09138°S, 71.02025°W): 9277 [JF272788]. *Liolaemus bibronii* (47.85033°S, 66.62216°W): 9897 [JF272767]. *Liolaemus boulengeri* (42.79 661°S, 70.95838°W): 3610 [JF272768]. *Liolaemus caparensis* (49.56972°S, 72.04775°W): 9388 [JF272789]. *Liolaemus chacabucoense* (47.19705°S, 71.58 583°W): 13049 [JF272769]. *Liolaemus darwinii* (40.34883°S, 65.04983°W): 10391 [JF272771]. *Liolaemus gallardoi* (47.99372°S, 71.68041°W): 9446 [JF272773]. *Liolaemus gracilis* (37.07494°S, 67.78544°W): 10517 [JF272774]. *Liolaemus hatcheri* (47.99372°S, 71.68041°W): 9491 [JF272775]. *Liolaemus kingii* (–47.71497°S, 65.83919°W): 9776 [JF272776]. *Liolaemus kolengh* (47.02105°S, 71.80883°W): 9300 [JF272777]. *Liolaemus lineomaculatus* (47.71 697°S, 65.84108°W): 7470 [JF272778]. *Liolaemus magellanicus* (52.35258°S, 68.38808°W): 6722 [JF272779]. *Liolaemus morandae* (45.62872°S, 67.68 433°W): 9678 [JF272787]. *Liolaemus petrophilus* (41.08775°S, 67.89072°W): 11121 [JF272780]. *Liolaemus scolaroi* (46.81286°S, 71.97822°W): 13033 [JF272783]. *Liolaemus silvanae* (46.96438°S, 71.10755°W): 9221 [JF272784]. *Liolaemus somuncurae* (41.39466°S, 66.95925°W): 6914 [JF272785]. *Liolaemus tristis* (46.98261°S, 69.79991°W): 9618 [JF272794]. *Liolaemus uptoni* (42.39180°S, 68.93331°W): 8426 [JF272795]. *Liolaemus zullyae* (46.84627°S, 71.87125°W): 7391 [JF272797]. *Phymaturus dorsimaculatus* (37.82055°S, 71.0866°W): 983 [JF272781].

Appendix II

Values of meristic variables in each species discriminated by sex. Mean ± SD (minimum–maximum) and *P* values of Student's *t* or Kruskal–Wallis tests are shown. For *Liolaemus* sp. A sexual dimorphism was not evaluated due to lack of females (—). Significant values are shown in boldface.

	Females	Males	All	*P*
L. escarchadosi				*P*
Scales around interparietal	6.54 ± 0.69 (5–8)	6.52 ± 0.77 (5–8)	6.53 ± 0.73 (5–8)	0.98
Lorilabial scales	4.96 ± 0.84 (4–8)	4.93 ± 0.78 (4–6)	4.94 ± 0.81 (4–8)	0.98
Supralabial scales	8.63 ± 0.8 (7–10)	8.19 ± 0.74 (6–9)	8.42 ± 0.8 (6–10)	**0.01**
Infralabial scales	5.3 ± 0.59 (4–7)	5.55 ± 0.55 (5–7)	5.42 ± 0.58 (4–7)	**0.04**
Midbody scales	64.52 ± 4.73 (56–81)	63.21 ± 5.28 (55–80)	63.9 ± 5.02 (55–81)	0.10
Dorsal scales	56.2 ± 3.53 (47–63)	54.14 ± 2.83 (50–60)	55.22 ± 3.36 (47–63)	**0.00**
Ventral scales	95.2 ± 6.3 (82–107)	92.5 ± 7.07 (80–112)	93.9 ± 6.78 (80–112)	0.05
Lamellae III finger	15.8 ± 1.05 (14–18)	15.52 ± 0.92 (13–17)	15.67 ± 0.99 (13–18)	0.10
Lamellae IV toe	20.15 ± 1.43 (17–24)	20.86 ± 1.39 (17–24)	20.49 ± 1.45 (17–24)	**0.02**
Precloacal pores	—	7.14 ± 1.09 (5–10)	—	—
L. tari				
Scales around interparietal	6.25 ± 0.5 (6–7)	7.75 ± 1.26 (6–9)	7 ± 1.2 (6–9)	0.06
Lorilabial scales	5.25 ± 0.96 (4–6)	5 ± 0 (5–5)	5.13 ± 0.64 (4–6)	0.42
Supralabial scales	8.25 ± 0.5 (8–9)	8 ± 1.63 (6–10)	8.13 ± 1.13 (6–10)	0.77
Infralabial scales	6.25 ± 0.5 (6–7)	5.5 ± 0.58 (5–6)	5.88 ± 0.64 (5–7)	0.28
Midbody scales	69.75 ± 5.56 (66–78)	69.25 ± 3.3 (65–73)	69.5 ± 4.24 (65–78)	0.88
Dorsal scales	59.75 ± 4.57 (54–65)	55.75 ± 4.19 (50–60)	57.75 ± 4.59 (50–65)	0.24
Ventral scales	104.3 ± 7.2 (97–113)	100.3 ± 5 (93–104)	102.3 ± 6.2 (93–113)	0.39
Lamellae III finger	15 ± 1.83 (13–17)	16.75 ± 0.96 (16–18)	15.88 ± 1.64 (13–18)	0.14
Lamellae IV toe	19.5 ± 2.08 (17–22)	21.5 ± 2.08 (19–24)	20.5 ± 2.2 (17–24)	0.22
Precloacal pores	—	7.25 ± 0.96 (6–8)	—	—
L. baguali				
Scales around interparietal	6.63 ± 0.77 (5–8)	6.4 ± 0.94 (5–9)	6.52 ± 0.85 (5–9)	0.23
Lorilabial scales	4.75 ± 0.74 (3–6)	5.55 ± 1.32 (4–8)	5.11 ± 1.1 (3–8)	0.05
Supralabial scales	8.42 ± 0.97 (7–11)	8.85 ± 0.88 (7–10)	8.61 ± 0.95 (7–11)	0.13
Infralabial scales	5.63 ± 0.88 (4–8)	5.65 ± 0.75 (4–7)	5.64 ± 0.81 (4–8)	0.83
Midbody scales	74.13 ± 4.52 (66–85)	73.6 ± 5.31 (63–85)	73.89 ± 4.84 (63–85)	0.72
Dorsal scales	61.83 ± 4.79 (51–69)	60.05 ± 4.56 (53–68)	61.05 ± 4.72 (51–69)	0.22
Ventral scales	100.3 ± 7.6 (85–112)	101 ± 6.1 (92–112)	100.8 ± 6.9 (85–112)	0.60
Lamellae III finger	17.21 ± 1.61 (14–20)	17.9 ± 1.83 (14–21)	17.52 ± 1.73 (14–21)	0.10
Lamellae IV toe	22.73 ± 1.35 (21–25)	22.7 ± 1.81 (19–25)	22.71 ± 1.57 (19–25)	0.88
Precloacal pores	—	7.67 ± 1.19 (6–10)	—	—
L. sarmientoi				
Scales around interparietal	6.81 ± 0.9 (5–9)	6.88 ± 1.13 (6–10)	6.84 ± 1.01 (5–10)	0.84
Lorilabial scales	5.08 ± 0.89 (4–7)	5 ± 0.91 (4–7)	5.04 ± 0.89 (4–7)	0.74
Supralabial scales	8.58 ± 0.7 (8–10)	8.6 ± 0.91 (7–10)	8.59 ± 0.8 (7–10)	0.77
Infralabial scales	6.31 ± 0.88 (5–8)	5.92 ± 0.76 (5–7)	6.12 ± 0.84 (5–8)	0.13
Midbody scales	70.65 ± 7.12 (60–86)	66.76 ± 5.83 (58–80)	68.75 ± 6.75 (58–86)	**0.03**
Dorsal scales	61.62 ± 5.97 (52–73)	57.76 ± 3.69 (52–65)	59.73 ± 5.31 (52–73)	**0.02**
Ventral scales	99 ± 7.61 (85–111)	95.6 ± 8.35 (78–109)	97.3 ± 8.1 (78–111)	0.12
Lamellae III finger	16.85 ± 1.49 (13–19)	17 ± 1.55 (14–20)	16.92 ± 1.51 (13–20)	0.71
Lamellae IV toe	22.38 ± 1.81 (18–26)	21.4 ± 1.91 (19–26)	21.9 ± 1.91 (18–26)	0.06
Precloacal pores	—	6.92 ± 1.47 (4–9)	—	—
L. sp. *A*				
Scales around interparietal	—	6.25 ± 1.26 (5–8)	6.25 ± 1.26 (5–8)	—
Lorilabial scales	—	5 ± 0.82 (4–6)	5 ± 0.82 (4–6)	—
Supralabial scales	—	8.5 ± 0.58 (8–9)	8.5 ± 0.58 (8–9)	—
Infralabial scales	—	5.75 ± 0.96 (5–7)	5.75 ± 0.96 (5–7)	—
Midbody scales	—	68 ± 4.24 (64–73)	68 ± 4.24 (64–73)	—
Dorsal scales	—	53 ± 2.16 (50–55)	53 ± 2.16 (50–55)	—
Ventral scales	—	97 ± 5.23 (93–104)	97 ± 5.23 (93–104)	—
Lamellae III finger	—	17 ± 1.41 (16–19)	17 ± 1.41 (16–19)	—
Lamellae IV toe	—	21.25 ± 1.26 (20–23)	21.25 ± 1.26 (20–23)	—
Precloacal pores	—	7.75 ± 1.89 (5–9)	7.75 ± 1.89 (5–9)	—

APPENDIX III

Values of morphometric ratios and snout–vent length (SVL) variables in each species discriminated by sex. Mean ± SD (minimum–maximum) and *P* values for analysis of covariance (for ratios) and analysis of covariance (only for SVL) are shown. For *Liolaemus* sp. A sexual dimorphism was not evaluated due to lack of females (—). Significant values are shown in boldface.

	Females	Males	All	*P*
L. escarchadosi	(n = 20)	(n = 32)		
SVL	74.7 ± 5.63 (64–88)	75.75 ± 6.51 (64–90)	75.35 ± 6.15 (64–90)	0.554
Distance fore–hind limbs/SVL	0.54 ± 0.07 (0.39–0.62)	0.5 ± 0.05 (0.38–0.61)	0.52 ± 0.06 (0.38–0.62)	**<0.001**
Foot length/SVL	0.25 ± 0.01 (0.23–0.29)	0.28 ± 0.02 (0.25–0.33)	0.27 ± 0.02 (0.23–0.33)	**<0.001**
Radius-ulna length/SVL	0.12 ± 0.01 (0.1–0.14)	0.13 ± 0.01 (0.11–0.16)	0.12 ± 0.01 (0.1–0.16)	**0.038**
Hand length/SVL	0.15 ± 0.01 (0.13–0.16)	0.16 ± 0.01 (0.13–0.19)	0.15 ± 0.01 (0.13–0.19)	**0.014**
Head width/SVL	0.18 ± 0.01 (0.16–0.22)	0.2 ± 0.02 (0.16–0.27)	0.19 ± 0.02 (0.16–0.27)	**<0.001**
Head length/SVL	0.23 ± 0.02 (0.2–0.28)	0.24 ± 0.02 (0.19–0.3)	0.24 ± 0.02 (0.19–0.3)	**<0.001**
Rostral–nasal distance/SVL	0.04 ± 0.005 (0.03–0.05)	0.04 ± 0.01 (0.03–0.05)	0.04 ± 0.01 (0.03–0.05)	0.057
Rostral height/SVL	0.02 ± 0.002 (0.01–0.02)	0.02 ± 0.003 (0.01–0.02)	0.02 ± 0.003 (0.01–0.02)	0.240
Auditory meatus height/SVL	0.04 ± 0.01 (0.03–0.05)	0.04 ± 0.01 (0.04–0.06)	0.04 ± 0.01 (0.03–0.06)	**0.001**
L. tari	(n = 3)	(n = 3)		
SVL	84.67 ± 5.51 (79–90)	83.67 ± 15.04 (74–101)	84.17 ± 10.15 (74–101)	0.919
Distance fore–hind limbs/SVL	0.63 ± 0.05 (0.59–0.68)	0.53 ± 0.11 (0.45–0.66)	0.58 ± 0.09 (0.45–0.68)	**0.018**
Foot length/SVL	0.3 ± 0.02 (0.28–0.31)	0.3 ± 0.03 (0.28–0.33)	0.3 ± 0.02 (0.28–0.33)	0.700
Radius-ulna length/SVL	0.14 ± 0.01 (0.13–0.15)	0.14 ± 0.02 (0.12–0.16)	0.14 ± 0.02 (0.12–0.16)	0.540
Hand length/SVL	0.16 ± 0.01 (0.15–0.17)	0.17 ± 0.02 (0.15–0.19)	0.16 ± 0.01 (0.15–0.19)	0.480
Head width/SVL	0.2 ± 0.02 (0.18–0.22)	0.24 ± 0.06 (0.2–0.31)	0.22 ± 0.04 (0.18–0.31)	**0.002**
Head length/SVL	0.26 ± 0.01 (0.25–0.27)	0.28 ± 0.04 (0.25–0.32)	0.27 ± 0.03 (0.25–0.32)	**0.023**
Rostral–nasal distance/SVL	0.04 ± 0.003 (0.04–0.04)	0.05 ± 0.01 (0.04–0.06)	0.04 ± 0.01 (0.04–0.06)	0.056
Rostral height/SVL	0.02 ± 0.001 (0.02–0.02)	0.02 ± 0.004 (0.02–0.02)	0.02 ± 0.002 (0.02–0.02)	0.340
Auditory meatus height/SVL	0.04 ± 0.003 (0.04–0.05)	0.04 ± 0.01 (0.04–0.05)	0.04 ± 0.005 (0.04–0.05)	0.650
L. baguali	(n = 16)	(n = 16)		
SVL	78.81 ± 8.98 (69–101)	84.25 ± 8.66 (69–100)	81.53 ± 9.11 (69–101)	0.091
Distance fore–hind limbs/SVL	0.58 ± 0.08 (0.46–0.79)	0.57 ± 0.09 (0.4–0.73)	0.57 ± 0.09 (0.4–0.79)	**<0.001**
Foot length/SVL	0.28 ± 0.02 (0.25–0.32)	0.3 ± 0.03 (0.24–0.33)	0.29 ± 0.02 (0.24–0.33)	0.064
Radius-ulna length/SVL	0.13 ± 0.02 (0.1–0.17)	0.14 ± 0.02 (0.12–0.17)	0.13 ± 0.02 (0.1–0.17)	0.490
Hand length/SVL	0.16 ± 0.02 (0.14–0.2)	0.18 ± 0.02 (0.14–0.21)	0.17 ± 0.02 (0.14–0.21)	0.196
Head width/SVL	0.2 ± 0.02 (0.17–0.24)	0.22 ± 0.03 (0.18–0.29)	0.21 ± 0.03 (0.17–0.29)	**0.017**
Head length/SVL	0.24 ± 0.02 (0.2–0.28)	0.26 ± 0.03 (0.23–0.31)	0.25 ± 0.03 (0.2–0.31)	**0.004**
Rostral–nasal distance/SVL	0.04 ± 0.004 (0.03–0.04)	0.04 ± 0.01 (0.02–0.05)	0.04 ± 0.01 (0.02–0.05)	0.502
Rostral height/SVL	0.02 ± 0.002 (0.01–0.02)	0.02 ± 0.002 (0.01–0.02)	0.02 ± 0.002 (0.01–0.02)	0.234
Auditory meatus height/SVL	0.04 ± 0.01 (0.03–0.06)	0.05 ± 0.01 (0.03–0.06)	0.05 ± 0.01 (0.03–0.06)	0.560
L. sarmientoi	(n = 21)	(n = 19)		
SVL	75.62 ± 5.82 (67–87)	80.11 ± 4.7 (69–90)	77.75 ± 5.72 (67–90)	**0.011**
Distance fore–hind limbs/SVL	0.58 ± 0.06 (0.45–0.67)	0.55 ± 0.05 (0.46–0.66)	0.56 ± 0.05 (0.45–0.67)	**<0.001**
Foot length/SVL	0.25 ± 0.01 (0.23–0.28)	0.28 ± 0.02 (0.26–0.31)	0.27 ± 0.02 (0.23–0.31)	**<0.001**
Radius-ulna length/SVL	0.11 ± 0.01 (0.09–0.15)	0.13 ± 0.01 (0.11–0.15)	0.12 ± 0.01 (0.09–0.15)	**0.025**
Hand length/SVL	0.14 ± 0.01 (0.12–0.17)	0.16 ± 0.01 (0.15–0.18)	0.15 ± 0.01 (0.12–0.18)	**<0.001**
Head width/SVL	0.18 ± 0.01 (0.16–0.21)	0.21 ± 0.02 (0.18–0.25)	0.19 ± 0.02 (0.16–0.25)	**<0.001**
Head length/SVL	0.21 ± 0.01 (0.18–0.25)	0.24 ± 0.02 (0.22–0.27)	0.22 ± 0.02 (0.18–0.27)	**<0.001**
Rostral–nasal distance/SVL	0.03 ± 0.004 (0.03–0.04)	0.04 ± 0.004 (0.03–0.05)	0.04 ± 0.004 (0.03–0.05)	0.098
Rostral height/SVL	0.02 ± 0.003 (0.01–0.02)	0.02 ± 0.003 (0.01–0.02)	0.02 ± 0.003 (0.01–0.02)	0.681
Auditory meatus height/SVL	0.05 ± 0.01 (0.03–0.06)	0.05 ± 0.005 (0.04–0.06)	0.05 ± 0.01 (0.03–0.06)	0.287
L. sp. A	(n = 0)	(n = 4)		
SVL	—	73.75 ± 5.68 (68–80)	73.75 ± 5.68 (68–80)	—
Distance fore–hind limbs/SVL	—	0.48 ± 0.03 (0.44–0.5)	0.48 ± 0.03 (0.44–0.5)	—
Foot length/SVL	—	0.3 ± 0.01 (0.29–0.3)	0.3 ± 0.01 (0.29–0.3)	—
Radiusulna length/SVL	—	0.12 ± 0.01 (0.11–0.14)	0.12 ± 0.01 (0.11–0.14)	—
Hand length/SVL	—	0.17 ± 0.02 (0.15–0.19)	0.17 ± 0.02 (0.15–0.19)	—
Head width/SVL	—	0.19 ± 0.02 (0.17–0.21)	0.19 ± 0.02 (0.17–0.21)	—
Head length/SVL	—	0.25 ± 0.02 (0.23–0.27)	0.25 ± 0.02 (0.23–0.27)	—
Rostral–nasal distance/SVL	—	0.04 ± 0.01 (0.04–0.05)	0.04 ± 0.01 (0.04–0.05)	—
Rostral height/SVL	—	0.02 ± 0.003 (0.01–0.02)	0.02 ± 0.003 (0.01–0.02)	—
Auditory meatus height/SVL	—	0.04 ± 0.0003 (0.04–0.04)	0.04 ± 0.0003 (0.04–0.04)	—

Appendix IV

Results for interspecific statistical tests of meristic variables discriminated by sex (females and males are shown above and below the diagonal, respectively). *P* values are reported when significant (from nonparametric Kruskal–Wallis tests), otherwise nonsignificant (NS) is reported. *Liolaemus* sp. A was only compared with males of other species (no females). Significant values are in boldface.

	L. baguali	*L. escarchadosi*	*L. sarmientoi*	*L. tari*
Infralabial scales				
L. baguali	—	NS	NS	NS
L. escarchadosi	NS	—	**<0.0001**	**<0.0001**
L. sarmientoi	NS	NS	—	NS
L. tari	NS	NS	NS	—
L. sp. A	NS	NS	NS	NS
Midbody scales				
L. baguali	—	**<0.0001**	NS	NS
L. escarchadosi	**<0.0001**	—	**<0.0001**	NS
L. sarmientoi	**<0.0001**	NS	—	NS
L. tari	NS	**<0.0001**	NS	—
L. sp. A	NS	NS	NS	NS
Dorsal scales				
L. baguali	—	**<0.0001**	NS	NS
L. escarchadosi	**<0.0001**	—	**<0.0001**	NS
L. sarmientoi	NS	**<0.0001**	—	NS
L. tari	NS	NS	NS	—
L. sp. A	**<0.0001**	NS	**<0.0001**	NS
Ventral scales				
L. baguali	—	**0.0101**	NS	NS
L. escarchadosi	**0.0002**	—	**0.0101**	**0.0101**
L. sarmientoi	**0.0002**	NS	—	NS
L. tari	NS	**0.0002**	NS	—
L. sp. A	NS	NS	NS	NS
Lamellae III finger				
L. baguali	—	**0.0004**	NS	**0.0004**
L. escarchadosi	**<0.0001**	—	NS	NS
L. sarmientoi	NS	NS	—	NS
L. tari	NS	NS	NS	—
L. sp. A	NS	NS	NS	
Lamellae IV toe				
L. baguali	—	**<0.0001**	NS	**<0.0001**
L. escarchadosi	**0.0125**	—	**<0.0001**	NS
L. sarmientoi	NS	NS	—	**<0.0001**
L. tari	NS	NS	NS	—
L. sp. A	NS	NS	NS	NS

Appendix V

Results for interspecific statistical tests of morphometric variables discriminated by sex (females and males shown above and below the diagonal, respectively). P values are reported when significant (from ANCOVA tests), otherwise NS (non significant) or ANM (assumptions not met) are reported.

	L. baguali	L. escarchadosi	L. sarmientoi
Distance fore–hind limbs			
L. baguali	—	NS	**0.03249**
L. escarchadosi	NS	—	**0.03249**
L. sarmientoi	NS	NS	—
Foot length			
L. baguali	—	**<0.0001**	**<0.0001**
L. escarchadosi	ANM	—	NS
L. sarmientoi	ANM	ANM	—
Radius-ulna length			
L. baguali	—	NS	**0.02886**
L. escarchadosi	NS	—	**0.02886**
L. sarmientoi	NS	NS	—
Hand length			
L. baguali	**—**	**<0.0001**	**<0.0001**
L. escarchadosi	**0.0008**	**—**	**<0.0001**
L. sarmientoi	**0.0008**	NS	—
Head width			
L. baguali	—	**0.0006**	**0.0006**
L. escarchadosi	ANM	—	NS
L. sarmientoi	ANM	ANM	—
Head length			
L. baguali	—	ANM	ANM
L. escarchadosi	NS	—	ANM
L. sarmientoi	**<0.0001**	**<0.0001**	—
Auditory meatus height			
L. baguali	—	NS	**0.000358**
L. escarchadosi	NS	—	**0.000358**
L. sarmientoi	**0.000291**	**0.000291**	—