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Integrative taxonomy in the *Liolaemus fitzingerii* complex (Squamata: Liolaemini) based on morphological analyses and niche modeling

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Abstract

It has long been considered sufficient a single method or only a descriptive diagnosis to propose a new species. Recently, many works have proposed new theoretical paradigms to consider multiple sources of evidence to support the hypothesis of new taxa within an integrative approach. Despite this, many new described species continue to be merely descriptive and without any reproducible statistical analysis to support these descriptions. We tested whether five species described as members of a species complex of the lizard genus *Liolaemus* from Patagonia, can be differentiated based on classical morphometric analyses and ecological niche modeling. Individuals were sampled from their type localities. Our results showed that the univariate tests and Principal Component Analyses (PCA) were more accurate to differentiate species compared to the Linear Discriminant Analyses (LDA). However, there were almost no morphometric differences between two of the analyzed species. Major differences were found in bioclimatic variables of four of the species through Maxent ENMs and PCA using the original worldclim variables. Our results partially support the hypothesis that species can be differentiated by classical morphometric analyses, and found a strong support for the hypothesis that these taxa can be differentiated through their bioclimatic niches. These two approaches based on repeatable statistical basis, can supplement qualitative descriptive diagnoses of new species of the genus *Liolaemus*.

Key words: ecological niche modeling, integrative taxonomy, lizards, morphology, operational criteria, species' limits

Introduction

One of the major challenges systematists and taxonomists face when describing new taxa is to clearly advocate a species concept and implement associated delimitation methods, which implies a strong theoretical background as well as a variety of analytical methods. Several contributions have been written concerning species definition (e.g., de Queiroz 2005, 2007; Camargo & Sites 2013) and de Queiroz (1998) listed numerous species concepts; currently most evolutionary biologists agree that species are separately evolving lineages of populations or metapopulations (de Queiroz 2007; Padial *et al.* 2010). An important aspect to consider is the operational criteria used to delimit species (Sites & Marshall 2004), which is one of the main focus of discussion among systematists, because scientists give priority to different operational criteria depending on their working systems (de Queiroz 2007; Yeates *et al.* 2011). The species concept and operational criteria used for delimiting species (whether it is explicit or not), have a major impact on systematic and taxonomic arrangements (Sites & Crandall 1997), and also have a great impact on conservation and management strategies, especially for groups with a large number of species (Camargo *et al.* 2010).

Integrative taxonomy (Dayrat 2005) is currently the working paradigm that provides the best theoretical basis for hypothesizing new species, implementing more than one line of evidence. This framework is described as the science that is intended to delineate the units of the diversity of life from multiple and complementary perspectives, such as phylogeography, comparative morphology, population genetics, ecology, development, behavior, etc. (Dayrat 2005). The main theoretical concept is to employ more than one line of evidence to hypothesize new taxa (e.g., Schlick-Steiner *et al.* 2010) and three alternative protocols have been proposed: integration by accumulation, by congruence and by consensus, and each of them has advantages and disadvantages to delimit species (Padial *et*

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al. 2010). The integration by accumulation is based on the assumption that divergences in any of the organism' attributes that constitute taxonomic characters, can provide evidence for the existence of a new species; and defends the view that the only way for true integration is to allow any source of evidence (even a single one) to support species discovery. The congruence approach is defined on the basis that differences in two or more taxonomic characters is adequate to validate a new species. The integration by accumulation approach may over-estimate the number of species by identifying distinct species where there may be only intraspecific character variation. On the contrary, integration by congruence is a highly stringent approach that might under-estimate the number of species by being unable to detect cryptic or young species. The consensus protocol for integrative taxonomy is a general working protocol that combines advantages of cumulative and congruence approaches (Padial *et al.* 2010). As a result of the difficulties in delineating closely related species, the integrative taxonomy framework by consensus is considered the most suitable and adequate approach to analyzed cryptic species and species complexes (Padial & De la Riva 2009; Padial *et al.* 2010).

Using multiple methodological approaches is considered the more robust operational criteria to hypothesize new species, and is much more robust than implementing a single method. Few works have combined molecular, morphological and ecological methods to hypothesized new species (e.g., Sanders *et al.* 2006; Leaché *et al.* 2009; Blankers *et al.* 2012, Ahmadzadeh *et al.* 2013), whereas others have proposed new species based on two approaches (e.g., Wiens & Penkrot 2002; Malhotra & Thorpe 2004; Rivera *et al.* 2011; Florio *et al.* 2012). Nonetheless, the great majority of published works have proposed new species using a single approach (e.g., Kaliontzopoulou *et al.* 2005; Passos *et al.* 2009) and also, in some contributions there is only one type of data used (e.g., morphological) but without any hypothesis tested with statistical analyses (Avila 2003; Abdala & Lobo 2006; Scolaro & Tapari 2009), which may lead to incorrect results that, constantly generates systematic-taxonomic rearrangements and discussions (e.g., *Liolaemus* genus, Lobo *et al.* 2010). These permanent systematic changes and new proposals are very common in *Liolaemus*, a highly diverse genus for which many synonymizations and redescriptions have been published (Etheridge 1998; Quinteros & Lobo 2009; Nori *et al.* 2010).

Liolaemus is a lineage that underwent a major speciation process, which is reflected in the constant rearrangements of systematic proposals and also by a constant description of new species. This South American genus of lizards with more than 230 described species (Breitman *et al.* 2011a; Abdala *et al.* 2012a), includes cryptic species (Lobo & Espinoza 2004; Pincheira-Donoso *et al.* 2007a), species complexes (Morando *et al.* 2003; Avila *et al.* 2006) and, in some cases, species with extensive geographic ranges that are accompanied by phenotypic and clinal variations (Pincheira-Donoso *et al.* 2007b, 2008; Escudero *et al.* 2012). The majority of these taxa were described based only on descriptive morphology without statistical analyses and most of the disputes on the validity of some species may be due to the lack of agreement on which lines of evidence are required to consider a lineage as a new species (de Queiroz 2007).

This issue is particularly relevant for the *Liolaemus fitzingerii* group, which includes taxa diagnosed and described based only on descriptive morphology, without statistical analyses. The *fitzingerii* group (*sensu* Avila *et al.* 2006; partially equivalent to '*fitzingerii* clade' Abdala 2007) is distributed from northern Neuquén and Río Negro provinces to southern Santa Cruz province (Escudero *et al.* 2012) and comprises two species complexes: *fitzingerii* and *melanops* (*sensu* Avila *et al.* 2006, 2010; Escudero *et al.* 2012). To test if classical morphometric analyses and ecological niche modeling can differentiate closely related *Liolaemus* species, we considered the five species currently included within the *fitzingerii* complex: *Liolaemus fitzingerii*, *L. xanthoviridis* (Cei & Scolaro 1980), *L. chehuachekenk* (Avila *et al.* 2008) and the recently described *L. camarones* and *L. shehuen* (Abdala *et al.* 2012b). Several controversial taxonomic arrangements have been proposed for this species complex since the 1970's (Donoso-Barros & Cei 1971; Cei 1973; Cei & Scolaro 1977; Scolaro & Cei 1977) to the present (Abdala *et al.* 2012a; 2012b). The main problem related to species limits and diversity in the *L. fitzingerii* complex, may be linked to the proliferation of species concepts and operational criteria throughout these last three decades. Furthermore, some papers did not include a species concept or an operationally criterion and taxonomic changes were made without any analyses, solely based on taxonomic authority (e.g., Cei & Scolaro 1983). Most probably, this has led the same authors to several subsequent papers with continuous changes on the taxonomic identity of this species complex (see Cei & Scolaro 1983; Scolaro *et al.* 1985).

A great step forward in *Liolaemus* alpha taxonomy, would be to estimate (or re-evaluate) species boundaries based on the integrative taxonomy framework including more than one approach with reproducible statistical analyses to propose and diagnose new taxa. As we discussed above, if the species can be differentiated by an

integrative taxonomy approach, the currently diagnosed *Liolaemus* species should present clear differences with more than one methodological approach. The objective of this work is to review and assess the accuracy of the most common morphological and niche modeling analyses as additional approaches coupled to traditional species diagnosis (see Aguilar *et al.* 2013) to detect differences between closely related species, using the *Liolaemus fitzingerii* species complex as an example to answer the following questions:

1. Can current described species be distinguished from each other based on commonly used morphological traits?

2. Which morphological and ecological traits contribute the most to differentiate the diagnosed taxa?

3. Can we detect diagnostic traits for each taxa that are useful for species delimitation?

In this work, we performed an extensive review and assessment of morphological and ecological variation across this species complex and we implemented standardized methods for data acquisition and treatment. Based on these results, we propose to adopt the integrative taxonomy approach to review *Liolaemus* species complexes and describe new taxa.

Material and methods

Field work, examined material and species concept. Several surveys were carried out from January 2000 to January 2013 during spring-summer seasons along the complete geographic distribution of the *Liolaemus fitzingerii* species complex, which spans over the Chubut and Santa Cruz provinces in Patagonia, Argentina (Escudero *et al.* 2012). Specimens were collected by hand after visual spotting. Latitude, longitude and elevation were determined by a Garmin GPS 12™ Global Position Device. After capture, lizards were euthanized by a pericardiac injection of sodium thiopenthotal Pentovet®, fixed in 10–20 % formalin and later transferred to 70 % ethanol (Simmons 2002). Samples are deposited in the herpetological collections of Monte L. Bean Life Science Museum-Brigham Young University (BYU; Provo, USA), Museo de La Plata (MLP; La Plata, Argentina), Fundación Miguel Lillo (FML; Tucumán, Argentina) and Centro Nacional Patagónico (LJAMM-CNP; Puerto Madryn, Argentina). We included a total of 223 specimens from 53 localities (Fig. 1, Appendix) from the five type localities and surrounding areas. In this study, we followed the General Lineage Species Concept according to de Queiroz (1998) and the integrative taxonomy framework by consensus (Padial *et al.* 2010). We considered as recognition criteria to distinguish a putative taxon from the others the presence of one or more exclusive differences in each implemented method.

Morphological analyses. We used a total of 82 adult males and 65 adult females from 27 localities (Appendix) and for most cases we included at least ten individuals of each sex from each species (Fig. 1). To select morphological variables, we searched for literature focused on species descriptions of the *Liolaemus fitzingerii* complex (Cei & Scolaro 1980; Avila *et al.* 2006, 2008, 2010; Abdala 2007; 2012a; 2012b), and we included a total of 11 continuous and 9 discrete characters from adult fixed specimens. Scale terminology and measurements follow Smith (1946). All bilateral characters were measured on the right side of each specimen, and when this was not possible (e.g., lack of a member) they were taken on the left side. Scale counts were performed using a stereoscopic microscope Stemi DV4 Zeiss® and continuous biometric variables were recorded using an electronic Schwyz® caliper to the nearest 0.01 mm, and included: SVL, snout vent length (measured from the anterior tip of the rostral scale to vent); AGD, axillae groin distance (measured from the posterior edge of the forelimb insertion to the anterior edge of the hindlimb insertion); HL, head length (measured from the anterior edge of the auditory meatus to the anterior tip of the rostral scale); HW, head width (measured between both edges of the two auditory meatus); HD, head depth (measured from the parietal surface to the throat, considered at the anterior border of both auditory meatus); DBN, distance between nostrils (measured between the inner edges of both nostrils); NED, nostril eye distance (measured from the most anterior superciliares and preocular scales to the anterior tip of the rostral scale); RPD, rostral-parietal distance (measured from the posterior tip of interparietal scale to the junction with both parietal scales); FLL, fore limb length (measured from the elbow to the most distal lamellae of the third toe); TL, tibial length (measured from the knee to heel); HLL, hind limb length (measured from the heel to the most distal lamellae of the fourth toe). The meristic variables registered were: LLS, number of lorilabial scales; SLS, number of supralabial scales; ILS, number of infralabial scales; SCM, number of scales in contact with mental scale; L4T, number of lamellae of the fourth toe; SAMB, number of scales around midbody; DS, number of dorsal scales; VS, number of ventral scales; PCP, number of cloacal pores.

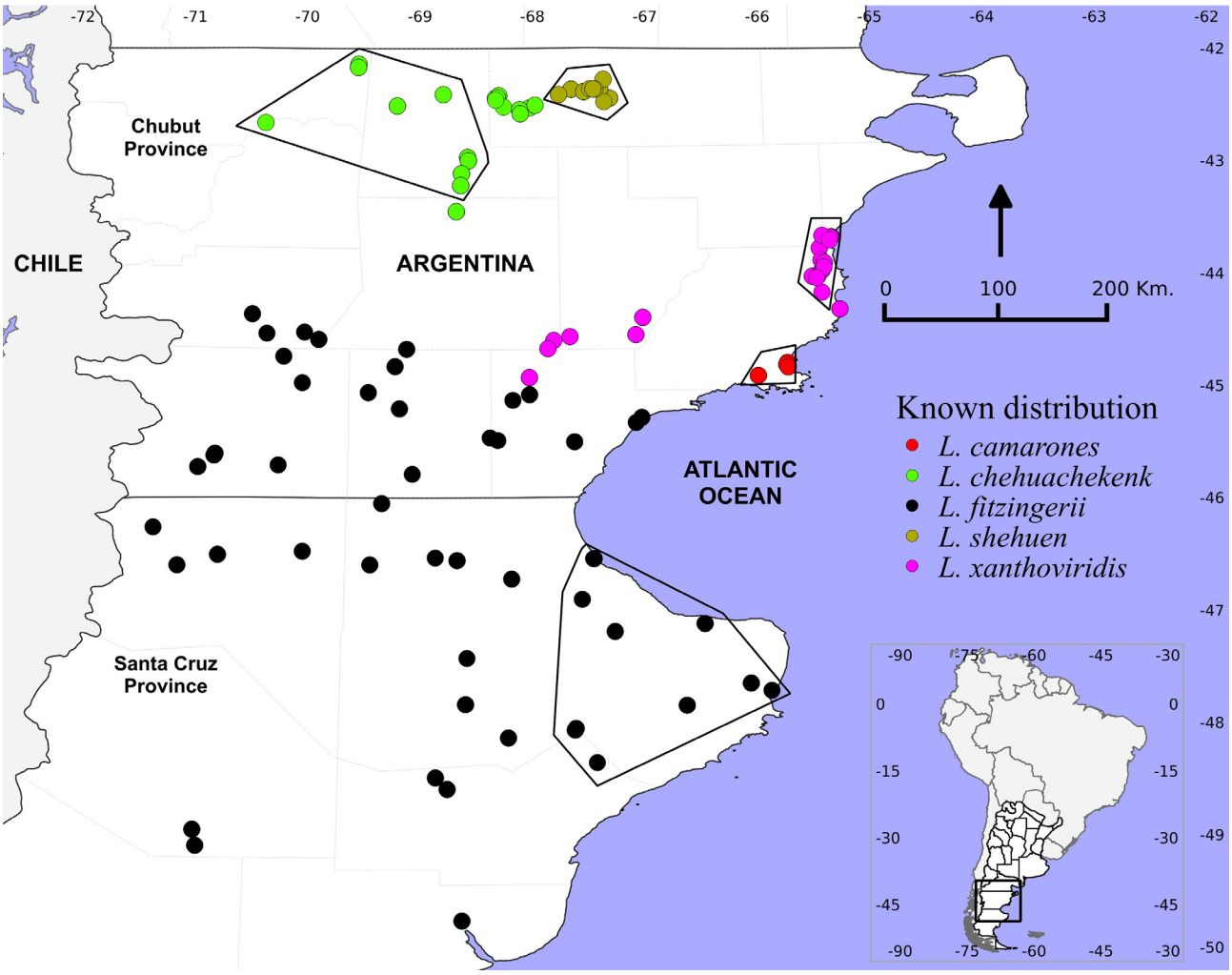

FIGURE 1. Known geographic distribution for the studied species and sampled locations selected (inside polygons) from type localities and surroundings for each species.

Statistical analyses. We tested for morphological differences among the five species. Male and female specimens were treated separately in all analyses to avoid potential bias caused by sexual dimorphism (Verrastro 2004; Laspiur & Acosta 2007). We calculated descriptive statistics from each one of the morphological characters most commonly used and evaluated the accuracy of statistical analyses frequently used to evaluate differences between species. As exploratory analyses, we performed Principal Components Analysis (PCA) including both continuous and meristic characters and Lineal Discriminant Analysis (LDA). To decide how many PCA axes we considered in analysis, we used "The Scree Test", "Proportion of Variance Accounted For" criteria and components that had a minimum of 10% of the variation (O'Rourke & Hatcher 2013). We considered for these PCA results as a correlated variable to the PCs, all those which present a $P < 0.05$. The LDA test assumes that the variables are independent, with homogeneity of covariance matrices and normally distributed. These assumptions were tested with Spearman Correlations, Barlett and Multivariate and Univariate Normality tests. Considering these assumptions, all morphological continuous variables were tested: raw data, log-transformed and each variable divided by SVL. Once assumptions were met, we carried out LDA on the continuous variables standardized by SVL. We excluded from all analysis SCM from males because all individuals present 4 scales, and PPC from the female's data set because we did not record any.

Further on, we used all the other variables to perform univariate analyses. When Marginality Principles (Claude 2008) were validated, we used Analysis of the Covariance (ANCOVA) on the continuous variables with SVL as covariate to adjust all size-correlated characters, to test for significant differences among species. When the variable was not influenced by SVL, we performed an Analysis of Variance (ANOVA). When parametric P values were significant ($P \leq 0.05$), multiple post hoc comparisons were performed using Tukey's honestly significant

difference (HSD) test for unequal sample size (Miller & Haden 2006; Yandell 1997). Homoscedasticity and normality assumptions were checked with Levenne (Zar 2010) and Shapiro-Wilks tests (Claude 2008). When these assumptions were not met, we performed a nonparametric Kruskal-Wallis test on the meristic variables with multiple post hoc comparisons (Conover 1999). All statistical analyses were performed in R 2.15.2, we used the FactoMiner 1.18 package (Lê *et al.* 2008; Husson *et al.* 2013) for PCA analyses and the MASS package (Venables & Ripley 2002) for LDA.

Environmental niche models (ENMs). We analyzed ecological differences between species using ENMs and data from samples collected at type localities and surrounding areas. We revised a total of 191 geographical records from 44 localities (Appendix). Several previous works analyzed the real potential of environmental niche modeling using low numbers of species records and remarked its importance for limited species distributions (Anderson *et al.* 2002; Pearson *et al.* 2007). Models for each species were created using a total of 44 locality records: *L. chehuachekenk* (n = 9), *L. fitzingerii* (n = 11), *L. shehuen* (n = 10), *L. xanthoviridis* (n = 14). To lessen the possibility of inflating validation statistics by including localities that are not spatially independent (Hampe 2004; Luoto *et al.* 2005) or autocorrelated localities, we conservatively removed from the training data set all localities situated within 5 km of the test locality for each jackknife model. Based on the substantial local variation in topography and climatic conditions that exists in the studied area (as shown in our environmental layers), we considered localities separated by at least 5 km to exhibit sufficient potential variation as to be considered spatially independent. We removed duplicated coordinates records per species and excluded *L. camarones* because the number of localities (n = 2) was not enough to perform a robust analysis, thus for this analysis, we included four of the five species of this complex (Fig. 1).

To model and compare each taxa, we used Maxent 3.3.3k (Phillips & Dudík 2008) and presence-only data to model species distributions. Like other niche-based models constructed from presence-only data, the predicted distribution describes suitability in ecological (environmental and climatological) space, which is then projected onto geographic space revealing a prediction of the geographic distribution of the taxon of interest (Phillips *et al.* 2006). For our analyses, we used 19 environmental variables for current conditions (1950–2000) and an altitude variable all of ~ 30 arc-second resolution from the studied area (WorldClim—Global Climate Data; http://www.worldclim.org/tiles.php?Zone=43). The included bioclimatic and topographic variables were: ALT = Altitude, BIO1 = Annual Mean Temperature, BIO2 = Mean Diurnal Range (Mean of monthly (maximum temp–minimum temp), BIO3 = Isothermality (BIO2/BIO7)*(100), BIO4 = Temperature Seasonality (standard deviation*100), BIO5 = Max Temperature of Warmest Month, BIO6 = Minimum Temperature of Coldest Month, BIO7 = Temperature Annual Range (P5–P6), BIO8 = Mean Temperature of Wettest Quarter, BIO9 = Mean Temperature of Driest Quarter, BIO10 = Mean Temperature of Warmest Quarter, BIO11 = Mean Temperature of Coldest Quarter, BIO12 = Annual Precipitation, BIO13 = Precipitation of Wettest Month, BIO14 = Precipitation of Driest Month, BIO15 = Precipitation of Seasonality (Coefficient of Variation), BIO16 = Precipitation of Wettest Quarter, BIO17 = Precipitation of Driest Quarter, BIO18 = Precipitation of Warmest Quarter, BIO19 = Precipitation of Coldest Quarter. These variables data were imported into DIVA GIS and we extracted values by points per species localities. In order to decide the variables model assessment (considering the small numbers of localities used for these species), an initial Maxent Jackknife test (Pearson *et al.* 2007) was performed for all the variables and checked which ones contributed most. Also, for pairs of variables that were highly correlated, we chose the variable considered biologically more meaningful (related to the studied organism) in addition to a correlation criterion (Rissler & Apodaca 2007; Debandi *et al.* 2012). Correlation matrices were then generated for all 19 variables per species record within each of two general climatic categories: temperature (BIO1–11) and precipitation (BIO12–19). Altitude was considered for all models. Pearson Correlation Coefficient was used with a ≥ 0.75 threshold to identify and remove highly correlated variables (Rissler *et al.* 2006; Debandi *et al.* 2012; Kershaw *et al.* 2013). Considering the entire 43 tile, fourteen variables were chosen and used in Maxent models including ALT, BIO12, BIO13, BIO15, BIO16, BIO17, BIO18, BIO19, BIO2, BIO3, BIO4, BIO6, BIO8, BIO9. Finally each climate layer was entered into Maxent as ASCII raster grid. For each species, Maxent was run considering the following features: Linear features, response curve, pictures predictions and a Jackknife test for variable importance. We assigned 20 % of the presence points to test the model and 80 % of the data to train the model and set "Apply threshold rule to equal training sensitivity & specificity". To evaluate model performance for each species, we used AUC values (Area Under the Receiver Operating Characteristics Curve) with the summarize results of the 5-fold cross-validation (e.g., Yu *et al.* 2013; Lyu & Sun 2014). The AUC values range from 0.5 for

models with no predictive power to 1.0 for models with perfect predictive power (Swets 1988). We considered AUC values greater than 0.9 denote ‘‘very good’’ predictive power, values between 0.8 and 0.9 denote ‘‘good’’ predictive power and values between 0.7 and 0.8 indicate ‘‘useful’’ predictive power (Swets 1988). Although AUC has known limitations as a measure of model performance (Franklin 2009), it still is the most used analysis. Other selected algorithms were: Replicates 5, Crossvalidate, while the remaining options were left on default values. Bil and Logistic were chosen as file-format output. All .bil output files were transformed to raster format with DIVA-GIS 7.5 (Hijmans *et al.* 2001) and maps were done considering the original output of legend´s interval range and colours from Maxent with Quantum GIS 1.8 (Quantum GIS Geographic Information System 2013). Suitability values range from 0 (unsuitable habitat) to 1.0 (highly suitable habitat), with 0.5 representing habitat suitability at typical presence locations.

Additionally, to examine the overall levels of divergence in the ecological niche, we conducted principal component analysis (PCA) for each species with the BIOCLIM and altitude values extracted for each climate layer. We considered for PCA results as a correlated variable, all those which presented a $P \leq 0.00001$ and a correlation higher than 60 %. To determine whether separation in the ecological niche was statistically significant we used multivariate analysis of variance (MANOVA) with PCA axis scores as dependent variables and species as the fixed factors (Rissler & Apodaca 2007; Rivera *et al.* 2011). Additionally, we performed a MANOVA on PCA axis scores, with pairwise comparisons by sub-setting the four species to analyze overall differences among them. All statistical analyses were performed with R 3.0.2 (R Core Team 2014).

Results

Morphological analyses

We detected greater differences between species in the analyses of continuous variables than of discrete ones. Comparisons among females showed more differences than between males, with univariate or multivariate analyses.

Descriptive analyses. Interspecific comparisons with basic descriptive statistics for both sexes showed that continuous and meristic variables overlap (mean, standard deviation and rank, Tables 1–2).

Multivariate analyses. For the principal component analysis (PCA) for both sexes, we retained the first three components. These three components for males explained 63.64 % of the morphological variation; while for females they explained 72.40 % of the variation (Table 3). In both sexes, the continuous variables presented high loadings and correlation values for the first component, whereas discrete variables presented high correlations for the second and third components (Table 3). The graphical representation of male individuals' space in PC1–PC2 and PC1–PC3 with 95 % confidence ellipse around barycenter (the mean vector of each category, see Abdi *et al.* 2013) of the species (Fig. 2, left panel), presented a clear overlap between *Liolaemus camarones* and *L. shehuen* with the other three species. In the variables' space (Fig. 2, right panel), all the continuous variables were highly correlated in PC1 except for DBN, HW and NED, in PC2 highly correlated were VS, SAMB, LLS, and in PC3 the highly correlated were DS and PCP. For females, the PC1–PC2 graph (Fig. 3) showed clear overlap and proximity of barycenters between all species, with the exception of *L. xanthoviridis*; while the PC1–PC3 graph presented a clear non overlapping ellipses and barycenters for *L. chehuachekenk* and *L. xanthoviridis*, but showed an overlap between *L. fitzingerii*, *L. camarones* and *L. shehuen*. The variable’s space (Fig. 3, right panel) showed that PC1 had a high correlation between all continuous variables except for NED, and in PC2 all meristic variables were highly represented; and in PC3 SAMB, ILS, DS and L4T were well represented.

The prior probabilities of the linear discriminant analysis (LDA) between males (N = 82) were: *Liolaemus camarones* (0.15854), *L. chehuachekenk* (0.23171), *L. fitzingerii* (0.15854), *L. shehuen* (0.28049), *L. xanthoviridis* (0.17073). The 84.21 % of the variation is explained by the first two axes of the discriminant analysis. The 95 % confidence ellipses showed a clear overlap between *L. camarones* and *L. fitzingerii* (Fig. 4.1). *Liolaemus chehuachekenk* had the higher error rate in specimen classification (11 of 19 individuals, error = 57.89 %), followed by *L. camarones* (4 of 13 individuals, error = 30.77 %), *L. fitzingerii* (3 of 13 individuals, error = 23.08 %), *L. xanthoviridis* (3 of 14 individuals, error = 21.43 %) and *L. shehuen* (3 of 23 individuals, error = 13.04 %). Males presented a total of 24 misclassified cases (29.27 %) from 82 specimens analyzed (Table 4).

TABLE 1. Descriptive summary of continuous variables for the *Liolaemus fitzingerii* species complex. Mean, standard deviation, minimum and maximum values are presented in mm. References: SVL: snout vent length; AGD: axillae groin distance; HL: head length; HW: head wide; HD: head deep; DBN: distance between nostrils; NED: nostril eye distance; RPD: rostral – parietal distance; FLL: fore limb length; TL: tibia length; HLL: hind limb length; N: total of specimens.

	Liolaemus camarones		*Liolaemus chehuachekenk*		*Liolaemus fitzingerii*		*Liolaemus shehuen*		*Liolaemus xanthoviridis*	
SEX	males	females	males	females	males	females	males	females	males	females
SVL	94.22±7.34 (82.22–103.80)	85.22±17.04 (52.02–107.36)	94.97±9.14 (71.20–103.62)	91.75±7.59 (77.44–101.28)	87.37±15.53 (59.10–106.65)	82.82±18.15 (47.12–102.14)	85.39±5.7 (78.31–99.84)	78.63±8.74 (64.16–90.91)	81.24±8.25 (66.50–94.32)	66.28±11.95 (44.11–81.49)
AGD	42.05±4.27 (35.48–48.92)	40.53±8.91 (22.62–52.29)	43.33±5.21 (31.96–50.70)	42.78±4.81 (31.73–50.19)	39.35±8.08 (26.28–51.16)	38.56±10 (19.05–49.82)	38±3.5 (32.30–43.27)	36.43±5.01 (29.55–44.88)	36.82±4.67 (29.06–45.38)	30.44±6.62 (20.21–40.96)
HL	18.85±1.43 (16.28–20.69)	16.13±2.80 (11.15–20.39)	19.47±1.89 (14.35–22.19)	17.88±1.21 (15.63–19.58)	17.27±2.66 (12.48–20.57)	15.35±2.51 (10.51–18.93)	17.18±1.08 (15.90–19.59)	15.41±1.52 (13.47–17.87)	16.98±1.56 (14.18–19.28)	13.40±1.70 (9.70–15.77)
HW	17.03±1.77 (13.92–19.09)	14.15±2.76 (9.41–18.04)	15.33±2.55 (11.32–19.07)	12.91±1.31 (10.26–15.64)	14.72±2.73 (10.04–18.70)	13.27±2.41 (8.49–16.06)	14.74±1.17 (13.10–17.42)	13.53±2.04 (11.44–16.56)	12.36±1.35 (10.07–14.57)	10.46±1.63 (7.48–12.64)
HD	12.99±1.41 (10.20–15.05)	10.85±2.30 (7.26–14.07)	12.23±1.5 (8.37–13.93)	10.43±0.95 (8.30–11.67)	11.38±2.23 (7.69–14.61)	9.99±1.85 (6.34–12.56)	10.81±0.84 (9.69–12.36)	9.69±1.48 (8.27–12.33)	10.69±1.37 (8.68–12.57)	8.12±1.55 (5.03–10.65)
DBN	3.60±0.34 (2.99–4.07)	3.23±0.47 (2.53–3.98)	3.39±0.52 (2.06–4)	2.98±0.40 (2.10–3.64)	3.35±0.5 (2.39–3.96)	3.05±0.47 (2.13–3.66)	3.23±0.32 (2.57–3.88)	3.09±0.29 (2.76–3.55)	2.98±0.28 (2.55–3.33)	2.60±0.40 (1.85–3.15)
NED	4.38±0.22 (3.95–4.64)	3.99±0.51 (3.12–4.85)	4.59±0.4 (3.49–5.32)	4.46±0.37 (3.80–4.95)	4.11±0.46 (3.13–4.69)	3.72±0.50 (2.87–4.47)	3.94±0.27 (3.45–4.66)	3.64±0.34 (3.14–4)	4.39±0.33 (3.95–4.89)	3.55±0.57 (2.39–4.45)
RPD	15.28±1.15 (13.31–17.22)	13.33±1.99 (9.45–16.41)	14.98±1.29 (11.66–16.61)	13.84±1.01 (11.84–15.12)	14.18±2.13 (10.42–16.74)	12.95±2.11 (8.97–15.98)	13.25±0.65 (12.27–14.77)	12.50±1.11 (11.16–14.12)	13.22±1.04 (11.19–14.63)	10.98±1.50 (8.12–12.83)
FLL	24.66±1.38 (22.26–26.53)	21.60±3.02 (14.91–25.55)	24.66±2.17 (18.91–27.20)	22.17±1.49 (18.80–24.37)	22.99±3.82 (15.73–26.81)	21.13±4.22 (11.52–25.81)	22.89±2.26 (14.19–25.81)	21.23±1.44 (18.76–24)	21.69±1.7 (18.96–23.95)	17.81±2.73 (11.79–20.47)
TL	19.10±1.14 (17.01–20.65)	16.10±2.46 (11.05–19.46)	18.84±1.78 (13.60–21.32)	17±1.38 (13.55–19.19)	17.31±2.73 (12.09–20.13)	15.69±2.88 (9.49–18.70)	17.23±1.02 (15.93–19.39)	15.29±1.10 (13.55–17.47)	16.53±2.06 (12.87–19.31)	13.27±2.24 (8.89–15.79)
HLL	24.55±1 (22.19–25.72)	21.44±2.61 (15.93–25.14)	24.72±1.89 (19.55–27.28)	22.55±1.35 (19.67–24.04)	23.64±3.08 (17.15–26.41)	21.54±3.17 (14.17–24.49)	23.38±1.24 (21.58–26.26)	20.72±1.46 (18.35–23.81)	21.88±1.68 (18.89–24.07)	18.22±2.48 (12.89–21.01)
N	13	10	19	15	13	12	23	10	14	18

TABLE 2. Descriptive summary of discrete variables for the *Liolaemus fitzingerii* species complex. Mean, standard deviation, minimum and maximum values are presented. References: LLS: lorilabial scales; SLS: supralabial scales; ILS: infralabial scales; SCM: scales in contact with mental scale; L4T: lamellae of the fourth toe; SAMB: scales around midbody; DS: dorsal scales; VS: ventral scales; PCP: cloacal pores; N: total of specimens.

	Liolaemus camarones		*Liolaemus chehuachekenk*		*Liolaemus fitzingerii*		*Liolaemus shehuen*		*Liolaemus xanthoviridis*	
SEX	males	females	males	females	males	females	males	females	males	females
LLS	10.31±0.48 (10–11)	9.60±0.84 (8–11)	10.26±1.45 (8–13)	10.33±0.98 (9–12)	9.69±1.18 (7–11)	10.17±0.94 (9–12)	9.57±1.78 (7–12)	10.80±1.03 (9–12)	9.64±0.93 (8–11)	9.39±1.04 (8–11)
SLS	9±1 (8–11)	8±0.47 (7–9)	8.58±1.17 (6–10)	8.87±0.74 (8–11)	8.46±0.88 (7–10)	8.50±0.67 (8–10)	8.39±1.03 (7–10)	8.60±0.84 (7–10)	8.21±0.58 (7–9)	8.06±0.80 (7–9)
ILS	6.92±0.64 (6–8)	6.50±0.71 (6–8)	6.32±0.95 (5–9)	6.40±0.51 (6–7)	7.08±0.64 (6–8)	7.08±0.51 (6–8)	6.65±0.65 (5–8)	6.90±0.99 (5–8)	6.36±0.5 (6–7)	6.61±0.61 (6–8)
SCM	4±0 (4–4)	4±0 (4–4)	4±0 (4–4)	4.20±0.56 (4–6)	4±0 (4–4)	4.17±0.58 (4–6)	4±0 (4–4)	4.40±0.84 (4–6)	4±0 (4–4)	4.22±0.65 (4–6)
L4T	26.69±1.75 (24–30)	26.30±1.34 (24–29)	28.11±1.94 (25–32)	27.73±1.94 (24–31)	27.38±1.26 (25–29)	26.92±1.56 (24–29)	29±1.6 (26–32)	28.40±1.26 (26–30)	27.86±2.03 (25–31)	26.94±1.95 (24–32)
SAMB	73.23±3.63 (68–81)	72.90±2.81 (69–77)	71.16±3.61 (63–80)	71.13±3.96 (65–79)	72.85±3.29 (68–77)	72.42±3.18 (68–78)	69.52±3.27 (64–78)	73.20±4.16 (69–79)	69.29±2.64 (63–73)	69.44±3.84 (61–76)
DS	78.46±3.31 (73–83)	80.60±2.41 (78–84)	77.63±3.99 (72–86)	81.60±6.23 (72–96)	76.92±4.42 (69–85)	78±2.95 (74–82)	75.57±4 (69–83)	77.30±3.80 (72–85)	80.14±5.02 (72–88)	81.17±3.33 (73–85)
VS	115.31±7.18 (104–125)	119.80±4.08 (116–127)	114.37±6.45 (101–125)	120.87±5.41 (113–132)	117.69±4.57 (110–126)	119.58±6.73 (110–131)	117.30±4.6 (110–130)	122.30±6.65 (113–133)	119.50±5.59 (110–130)	120.50±3.87 (114–127)
PCP	8.85±0.9 (7–10)	0±0 (0–0)	8.58±1.26 (5–11)	0±0 (0–0)	8.69±0.75 (8–10)	0±0 (0–0)	8.35±1.03 (7–11)	0±0 (0–0)	8.50±0.94 (7–10)	0±0 (0–0)
N	13	10	19	15	13	12	23	10	14	18

TABLE 3. Results of Principal Component Analysis performed with all morphometric variables. References: Eig. Comp.: Eigenvalues per component; % Var.: Percentage of Variance; Cum. % Var.: Cumulative Percentage of Variance; SVL, snout vent length; AGD, axillae groin distance; HL, head length; HW, head wide; HD, head deep; DBN, distance between nostrils; NED, nostril eye distance; RPD, rostral–parietal distance; FLL, fore limb length; TL, tibial length; HLL, hind limb length; LLS, lorilabial scales; SLS, supralabial scales; ILS, infralabial scales; SCM, scales in contact with mental scale; L4T, lamellae of the fourth toe; SAMB, scales around midbody; DS, dorsal scales; VS, ventral scales; PCP, cloacal pores.

Males					
PC1		PC2		PC3	
Eig. Comp.	9.03	Eig. Comp.	1.60	Eig. Comp.	1.46
% Var.	47.55	% Var.	8.43	% Var.	7.66
Cum. % Var.	47.55	Cum. % Var.	55.98	Cum. % Var.	63.64
Correlations		Correlations		Correlations	
SVL	0.98043 ($P < 0.00001$)	VS	0.70830 ($P < 0.00001$)	DS	0.68509 ($P < 0.00001$)
RPD	0.96046 ($P < 0.00001$)	SAMB	0.55444 ($P < 0.00001$)	L4T	0.49986 ($P < 0.00001$)
HL	0.95393 ($P < 0.00001$)	ILS	0.38544 ($P = 0.00035$)	LLS	0.48751 ($P < 0.00001$)
TL	0.95312 ($P < 0.00001$)	DS	0.29312 ($P = 0.00753$)	SLS	0.34228 ($P = 0.00165$)
HD	0.94839 ($P < 0.00001$)	DBN	0.25476 ($P = 0.02090$)	VS	0.32558 ($P = 0.00284$)
AGD	0.89093 ($P < 0.00001$)	PCP	0.23350 ($P = 0.03475$)	PCP	-0.49714 ($P < 0.00001$)
HLL	0.88236 ($P < 0.00001$)	NED	-0.28274 ($P = 0.01006$)		
HW	0.86914 ($P < 0.00001$)	LLS	-0.52724 ($P < 0.00001$)		
FLL	0.86024 ($P < 0.00001$)				
DBN	0.81419 ($P < 0.00001$)				
NED	0.69047 ($P < 0.00001$)				
SAMB	0.24568 ($P = 0.02610$)				
ILS	0.21754 ($P = 0.04961$)				
L4T	-0.22894 ($P = 0.03856$)				
Females					
PC1		PC2		PC3	
Eig. Comp.	10.10	Eig. Comp.	2.07	Eig. Comp.	1.58
% Var.	53.17	% Var.	10.89	% Var.	8.34
Cum. % Var.	53.17	Cum. % Var.	64.06	Cum. % Var.	72.40
Correlations		Correlations		Correlations	
RPD	0.98365 ($P < 0.00001$)	L4T	0.59323 ($P < 0.00001$)	DS	0.44009 ($P = 0.00024$)
SVL	0.98114 ($P < 0.00001$)	VS	0.58998 ($P < 0.00001$)	L4T	0.43991 ($P = 0.00025$)
TL	0.96497 ($P < 0.00001$)	SAMB	0.53194 ($P = 0.00001$)	NED	0.38899 ($P = 0.00136$)
AGD	0.95943 ($P < 0.00001$)	LLS	0.50617 ($P < 0.00002$)	SCM	0.26415 ($P = 0.03348$)
HD	0.95794 ($P < 0.00001$)	ILS	0.48997 ($P < 0.00003$)	HW	-0.29218 ($P = 0.01819$)
HLL	0.95514 ($P < 0.00001$)	SLS	0.45275 ($P < 0.00015$)	DBN	-0.32833 ($P = 0.00758$)
HL	0.95462 ($P < 0.00001$)	DS	0.39710 ($P < 0.00106$)	SAMB	-0.53266 ($P < 0.00001$)
FLL	0.95110 ($P < 0.00001$)	SCM	0.35530 ($P < 0.00368$)	ILS	-0.63554 ($P < 0.00001$)
HW	0.91666 ($P < 0.00001$)				

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TABLE 3. (continued)

Correlations	Correlations	Correlations
DBN	0.83695 ($P < 0.00001$)	
NED	0.77277 ($P < 0.00001$)	
LLS	0.38211 ($P = 0.00168$)	
SAMB	0.37717 ($P = 0.00195$)	
SLS	0.37521 ($P = 0.00207$)	
VS	0.29014 ($P = 0.01905$)	

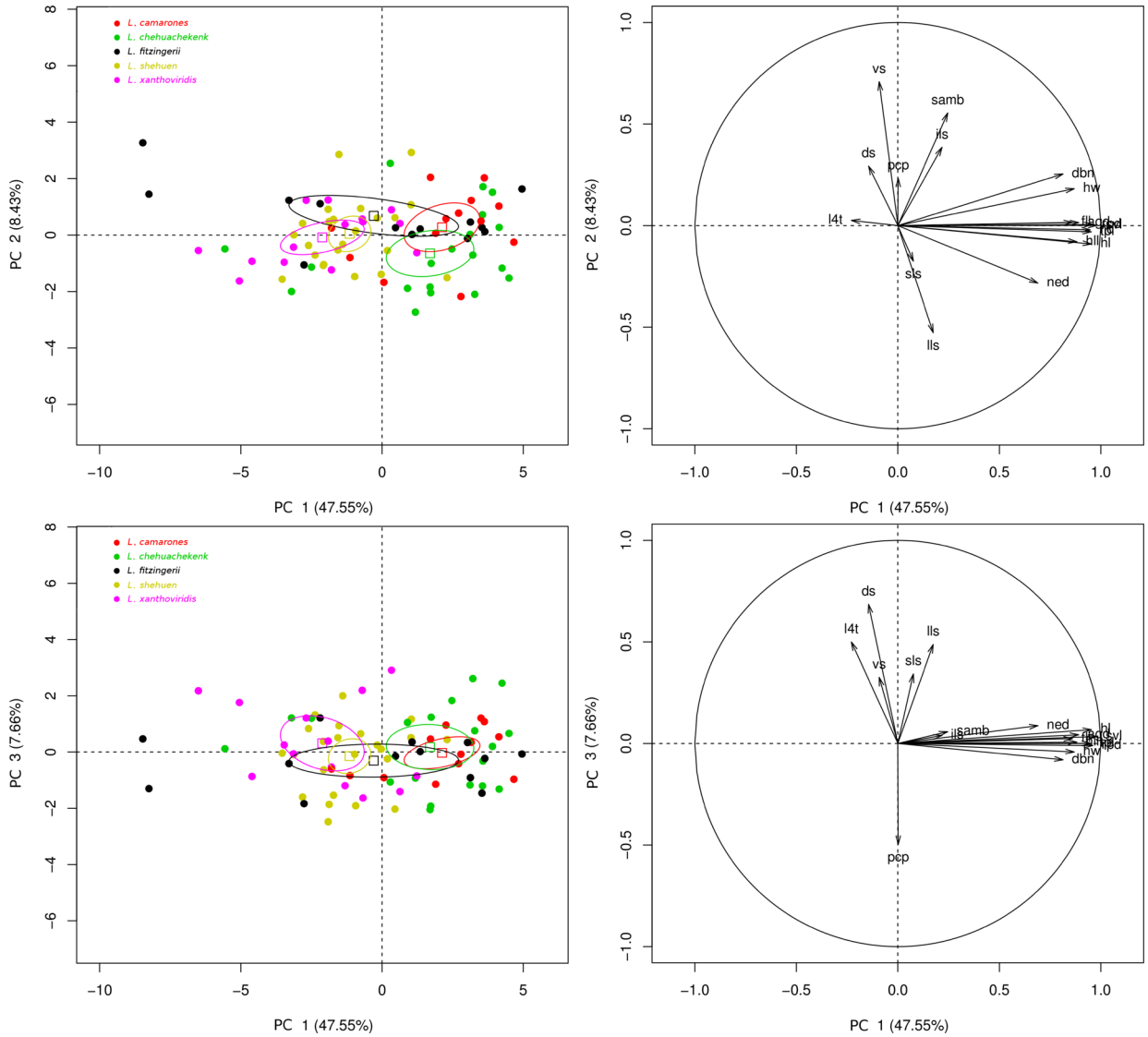

FIGURE 2. Principal Component Analysis of males including all morphological variables. Three first components and ellipses of 95 % confidence around the species barycenter are shown.

The prior probabilities of the linear discriminant analysis for females (N = 65) were: *Liolaemus camarones* (0.15385), *L. chehuachekenk* (0.23077), *L. fitzingerii* (0.18462), *L. shehuen* (0.15385), *L. xanthoviridis* (0.27692). The 79.38 % of the variation is explained by the first two axes of the discriminant analysis. The 95 % confidence ellipses showed a clear overlap between *L. camarones*, *L. fitzingerii* and *L. xanthoviridis* (Fig. 4.2). *Liolaemus camarones* had the higher classification error rate (4 of 10 individuals, error = 40 %), followed by *L. fitzingerii* (4 of 12 individuals, error = 33.33 %), *L. chehuachekenk* (3 of 15 individuals, error = 20 %), *L. xanthoviridis* (3 of 18

individuals, error = 16.67 %) and *L. shehuen* (1 of 10 individuals, error = 10 %). Females presented a total of 15 (23.08 %) misclassified individuals from the 65 analyzed (Table 4).

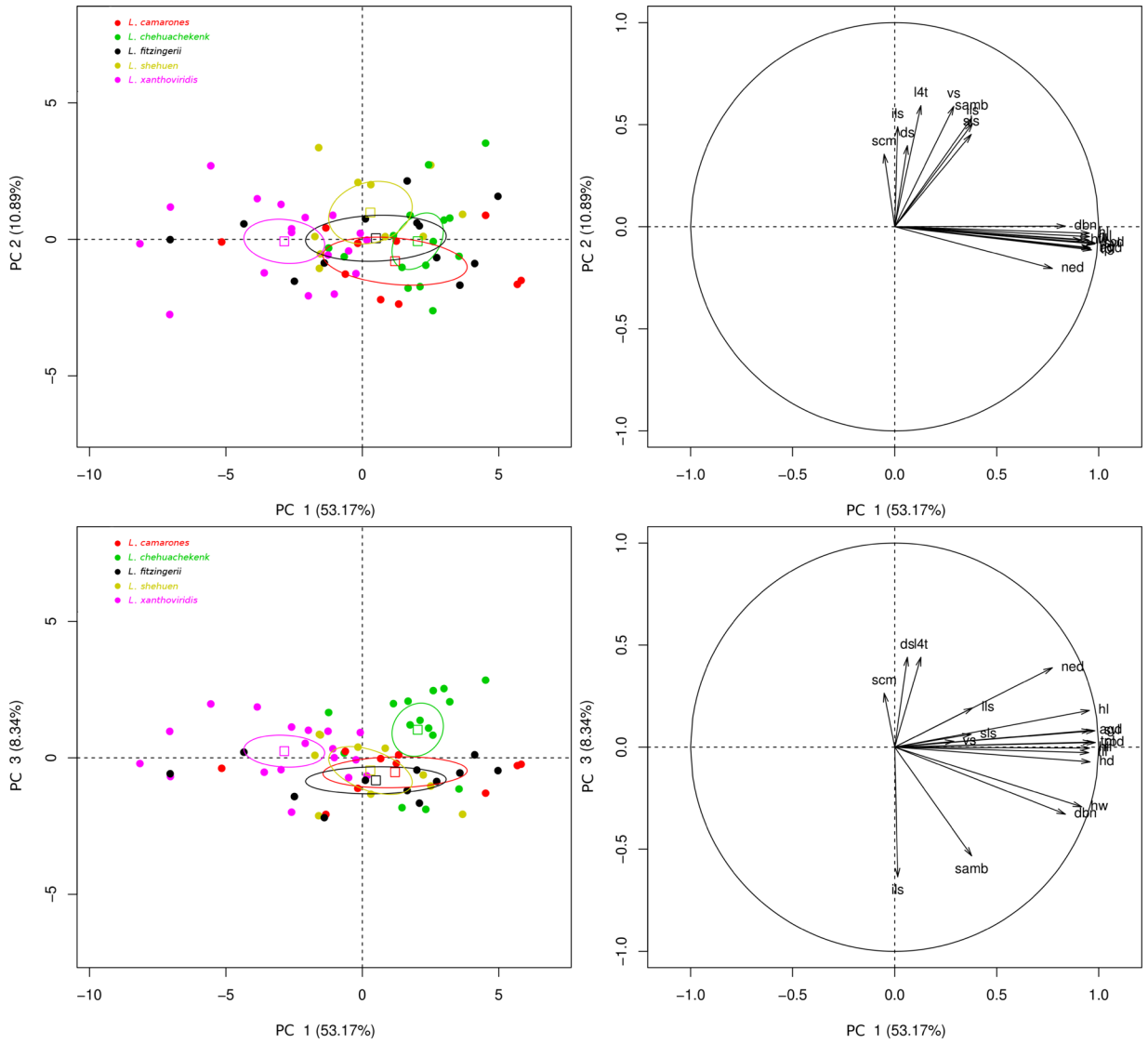

FIGURE 3. Principal Component Analysis of females including all morphological variables. Three first components and ellipses of 95 % confidence around the species barycenter are shown.

Univariate analyses. We analyzed nineteen morphological variables for males, and thirteen showed significant differences (9 continuous, 4 discrete, N = 82); for females, eleven (7 continuous, 4 discrete, N = 65) out of eighteen variables showed differences. Variables that differed between males of the five species were significant for the overall ANOVAs for SVL ($F_{4,77}$ = 6.363; P = 0.00018), HL ($F_{4,77}$ = 7.243; P = 0.00005), HW ($F_{4,77}$ = 9.885; P < 0.00000), DBN ($F_{4,77}$ = 4.435; P = 0.00280), NED ($F_{4,77}$ = 11.071; P < 0.00000); whereas overall ANCOVA was significant for HD ($F_{5,76}$ = 5.8705; P = 0.00036), and RPD ($F_{5,76}$ = 9.1433; P < 0.00000). The outcome of Kruskal Wallis tests showed significant differences for FLL ($H_{(4, n=82)}$ = 21.2636, P = 0.00028), HLL ($H_{(4, n=82)}$ = 22.9823, P = 0.00013), ILS ($H_{(4, n=82)}$ = 15.2861, P = 0.00414), L4T ($H_{(4, n=82)}$ = 14.0891, P = 0.00702), SAMB ($H_{(4, n=82)}$ = 15.6178, P = 0.00358), and DS ($H_{(4, n=82)}$ = 9.8432, P = 0.04315). The variables that showed differences among females were significant for the overall ANOVA for SVL ($F_{4,60}$ = 8.6332; P = 0.00001), HW ($F_{4,60}$ = 7.6244; P = 0.00005), HD ($F_{4,60}$ = 6.3487; P = 0.00025), DBN ($F_{4,60}$ = 4.8941; P = 0.00176). The overall of Kruskal Wallis tests showed significant differences for HL ($H_{(4, n=65)}$ = 29.9506, P = 0.00001), NED ($H_{(4, n=65)}$ = 22.9193, P = 0.00013), FLL ($H_{(4, n=65)}$ = 24.9967, P = 0.00005), LLS ($H_{(4, n=65)}$ = 13.1887, P = 0.01039), SLS ($H_{(4, n=65)}$ = 12.2417, P = 0.01564), L4T ($H_{(4, n=65)}$ = 10.2872, P = 0.03586), DS ($H_{(4, n=65)}$ = 10.7674, P = 0.02931). Variables with

significant difference ($P \leq 0.05$) in the post hoc comparisons are summarized in a crosstab for both sexes (Tables 5–6).

TABLE 4. Individual classification from LDA analysis with continuous variables adjusted by SVL. References: A: *Liolaemus camarones*, B: *L. chehuachekenk*, C: *L. fitzingerii*, D: *L. shehuen*, E: *L. xanthoviridis*.

Males								
Species	A	B	C	D	E	True n	Misclassified specimens	Error %
A	9	0	2	2	0	13	4	30.77
B	0	8	1	6	4	19	11	57.9
C	2	0	10	1	0	13	3	23.08
D	2	1	0	20	0	23	3	13.04
E	0	2	1	0	11	14	3	21.43
Females								
Species	A	B	C	D	E	True n	Misclassified specimens	Error %
A	6	0	1	0	3	10	4	40
B	0	12	0	3	0	15	3	20
C	3	0	8	0	1	12	4	33.33
D	0	0	1	9	0	10	1	10
E	0	0	3	0	15	18	3	16.67

TABLE 5. Results of multiple univariate post hoc comparisons among males from the *Liolaemus fitzingerii* species complex. Only significant differences ($P \leq 0.05$) between variables are shown. References: SVL: snout vent length, HL: head length, HW: head wide, HD: head deep, DBN: distance between nostrils, NED: nostril eye distance, RPD: rostral—parietal distance, FLL: fore limb length, TL: tibial length, HLL: hind limb length, ILS: infralabial scales, L4T: lamellae of the fourth toe, SAMB: scales around midbody, DS: dorsal scales;*: ANCOVA test, $^{\&}$: ANOVA test, $^{+}$: Kruskal-Wallis test.

Species	*L. camarones* (n = 13)	*L. chehuachekenk* (n = 19)	*L. fitzingerii* (n = 13)	*L. shehuen* (n = 23)	*L. xanthoviridis* (n = 14)
L. camarones	-	HD*, ILS^{+}, L4T^{+}	HW$^{\&}$, HD*, RPD*	HW$^{\&}$, HD*, NED$^{\&}$, RPD*, FLL^{+}, HLL^{+}, L4T^{+}, SAMB^{+}, DS^{+}	SVL$^{\&}$, HL$^{\&}$, HW$^{\&}$, HD*,DBN$^{\&}$, RPD*, FLL^{+}, HLL^{+}, ILS^{+}, SAMB^{+}
L. chehuachekenk	HD*, ILS^{+}, L4T^{+}	-	HL$^{\&}$, HD*, NED$^{\&}$, RPD*, ILS^{+}	SVL$^{\&}$, HL$^{\&}$, HD*, NED$^{\&}$, RPD*, FLL^{+}, HLL^{+}	SVL$^{\&}$, HL$^{\&}$, HW$^{\&}$, HD*,DBN$^{\&}$, RPD*, FLL^{+}, HLL^{+}
L. fitzingerii	HW$^{\&}$, HD*, RPD*	HL$^{\&}$, HD*, NED$^{\&}$, RPD*, ILS^{+}	-	RPD*, L4T^{+}, SAMB^{+}	HW$^{\&}$, HD*, RPD*, FLL^{+}, HLL^{+}, ILS^{+}, SAMB^{+}
L. shehuen	HW$^{\&}$, HD*, NED$^{\&}$, RPD*, FLL^{+}, HLL^{+}, L4T^{+}, SAMB^{+}, DS^{+}	SVL$^{\&}$, HL$^{\&}$, HD*, NED$^{\&}$, RPD*, FLL^{+}, HLL^{+}	RPD*, L4T^{+}, SAMB^{+}	-	HW$^{\&}$, NED$^{\&}$, HLL^{+}, HLL^{+}, DS^{+}
L. xanthoviridis	SVL$^{\&}$, HL$^{\&}$, HW$^{\&}$, HD*,DBN$^{\&}$, RPD*, FLL^{+}, HLL^{+}, ILS^{+}, SAMB^{+}	SVL$^{\&}$, HL$^{\&}$, HW$^{\&}$, HD*,DBN$^{\&}$, RPD*, FLL^{+}, HLL^{+}	HW$^{\&}$, HD*, RPD*, FLL^{+}, HLL^{+}, ILS^{+}, SAMB^{+}	HW$^{\&}$, NED$^{\&}$, HLL^{+}, HLL^{+}, DS^{+}	-

TABLE 6. Results of multiple univariate post hoc comparisons among females from the *Liolaemus fitzingerii* species complex. Only significant differences ($P \leq 0.05$) between variables are shown. References: SVL: snout vent length, HL: head length, HW: head wide, HD: head deep, DBN: distance between nostrils, NED: nostril eye distance, FLL: fore limb length, LLS: lorilabial scales, SLS: supralabial scales, L4T: lamellae of the fourth toe, DS: dorsal scales;[*]: ANCOVA test, [&]: ANOVA test, [+]: Kruskal-Wallis test.

Species	*L. camarones* (n = 10)	*L. chehuachekenk* (n = 15)	*L. fitzingerii* (n = 12)	*L. shehuen* (n = 10)	*L. xanthoviridis* (n = 18)
L. camarones	-	HL^{+}, NED^{+}, SLS^{+}, L4T^{+}		NED^{+}, LLS^{+}, SLS^{+}, L4T^{+}, DS^{+}	SVL$^{\&}$, HL^{+}, HW$^{\&}$, HD$^{\&}$, DBN$^{\&}$, NED^{+}, FLL^{+}
L. chehuachekenk	HL^{+}, NED^{+}, SLS^{+}, L4T^{+}	-	HL^{+}, NED^{+}, DS^{+}	HL^{+}, NED^{+}, DS^{+}	SVL$^{\&}$, HL^{+}, HW$^{\&}$, HD$^{\&}$, NED^{+}, FLL^{+}, LLS^{+}, SLS^{+}
L. fitzingerii		HL^{+}, NED^{+}, DS^{+}	-	L4T^{+}	SVL$^{\&}$, HL^{+}, HW$^{\&}$, HD$^{\&}$, FLL $^{+}$, DS^{+}
L. shehuen	NED^{+}, LLS^{+}, SLS^{+}, L4T^{+}, DS^{+}	HL^{+}, NED^{+}, DS^{+}	L4T^{+}	-	HL^{+}, HW$^{\&}$, DBN$^{\&}$, FLL^{+}, LLS^{+}, L4T^{+}, DS^{+}
L. xanthoviridis	SVL$^{\&}$, HL^{+}, HW$^{\&}$, HD$^{\&}$, DBN$^{\&}$, NED^{+}, FLL^{+}	SVL$^{\&}$, HL^{+}, HW$^{\&}$, HD$^{\&}$, NED^{+}, FLL^{+}, LLS^{+}, SLS^{+}	SVL$^{\&}$, H^{+}L, HW$^{\&}$, HD$^{\&}$, FLL^{+}, DS^{+}	HL^{+}, HW$^{\&}$, DBN$^{\&}$, FLL^{+}, LLS^{+}, L4T^{+}, DS^{+}	--

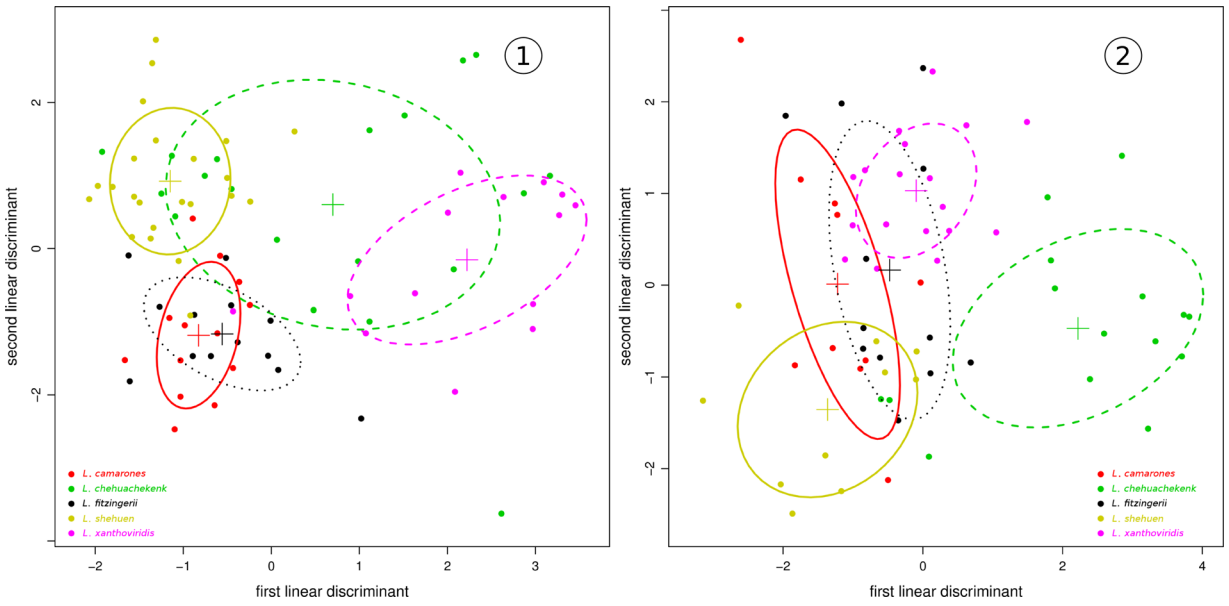

FIGURE 4. Lineal Discriminant Analysis with all continuous variables standardized by SVL. Two first discriminant axis and ellipses of 95 % confidence are shown. References: Males 1), Females 2).

Environmental niche models (ENM)

Maxent. We present maps of probabilistic habitat suitability for each species, determined by their respective Maxent point-wise mean model (Fig. 6). After the variable selection process, the variables that the four species had in common were BIO3 and ALT. Relative contributions of the environmental variables averages over replicates runs to the Maxent model per species are shown in Fig. 5. The variable ALT contributed 31.7 % to the *Liolaemus fitzingerii* model, 12.4 % to *L. xanthoviridis*, 8.2 % to *L. shehuen*, and 1.9 % to *L. chehuachekenk*. The variable BIO3 although considered in the four models, had little contribution to them. The environmental variables used for all species' models that had variable levels of contribution were BIO16 in *L. chehuachekenk* (10.8 %) and *L.*

shehuen (0 %), BIO4 in *L. chehuachekenk* (30 %) and *L. shehuen* (6.6 %), ALT in *L. fitzingerii* (31.7 %), *L. xanthoviridis* (12.4 %), *L. chehuachekenk* (1.9 %) and *L. shehuen* (8.2 %).

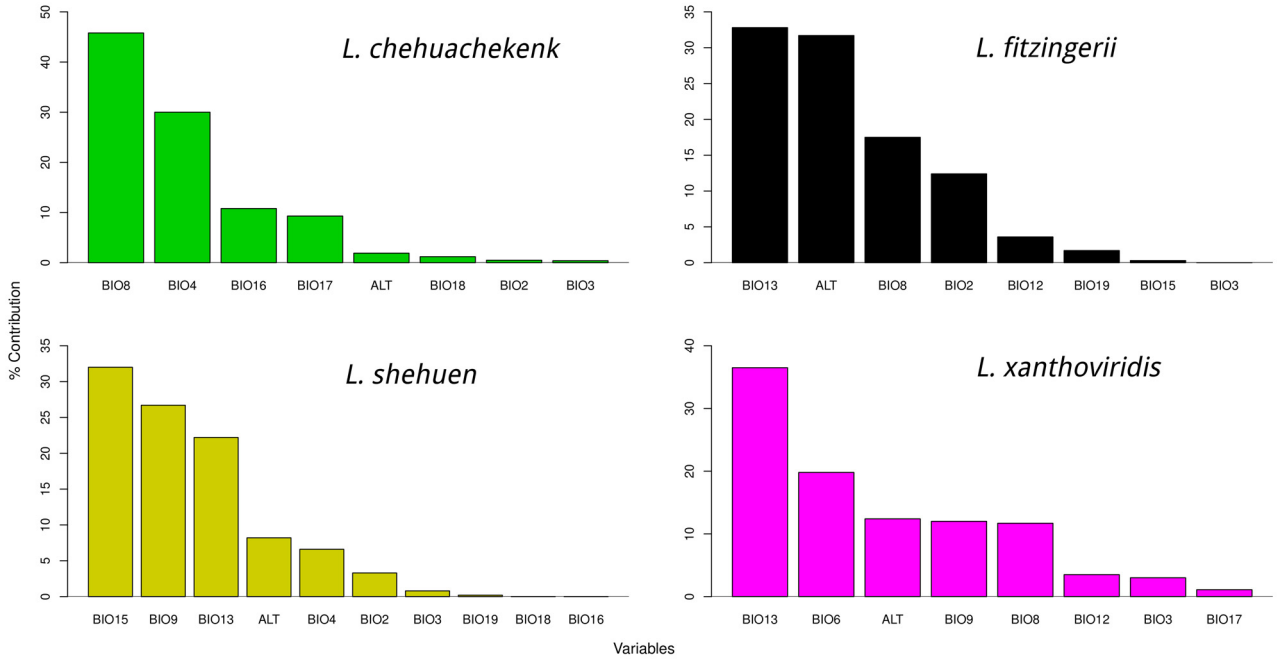

FIGURE 5. Relative contributions of the environmental variables to the Maxent models. Values shown are averages over replicate runs.

The average test AUC on training data for *Liolaemus chehuachekenk* for the replicate runs was 0.973 (± 0.024 SD). The mean response and the standard deviation of how each environmental variable affects the Maxent prediction for this species, showed that BIO2, BIO3, BIO4 and ALT had the highest variation (results nor shown); meanwhile the highest relative contributions to the model from averages over replicate runs were from BIO8, BIO4, BIO16 and BIO17 (Fig. 5). The results of the Jackknife tests on variable importance showed that the environmental variable with highest gain when used in isolation was BIO18, which consequently appeared to have the most useful information by itself (graphics not shown). The environmental variable that decreases the gain the most when omitted was BIO8, which therefore appeared to have higher proportion of information not present in the other variables. The average test AUC on training data for the replicate runs in *L. fitzingerii* was 0.982 (± 0.008 SD), and the mean response and the standard deviation on how each environmental variable affects the Maxent prediction showed that BIO8 and BIO2 had the highest variation. The highest values of relative contributions to the model from averages over replicate runs were from BIO13, ALT, BIO8 and BIO2 (Fig. 5). The Jackknife test output of variable importance showed BIO13 as the environmental variable with highest gain when used in isolation, thus it appeared to have the most useful information by itself. The environmental variable ALT is the variable that decreases the gain the most when omitted, thus ALT had the most information that was not present in other variables. The average test AUC on training data for the replicate runs of *L. shehuen* was 0.988 (± 0.002 SD) and the mean response and the standard deviation on how each environmental variable affects the Maxent prediction, showed that BIO3 had the highest variation values. The highest relative contributions to the model from averages over replicate runs were BIO15, BIO9 and BIO13 (Fig. 5). The Jackknife test of variable importance showed that BIO13 was the environmental variable with highest gain when used in isolation, thus it appeared to have the most useful information by itself. The environmental variables that decrease the gain the most when omitted were BIO9, BIO15 and ALT; hence those variables appeared to have information that was not present in the other variables. The average test AUC on training data for the replicate runs in *L. xanthoviridis* was 0.997 (± 0.001 SD). The mean response and the standard deviation of how each environmental variable affects the Maxent prediction showed that ALT had the highest variation. The highest relative contributions to the model from averages over replicate runs were from BIO13, BIO6, ALT, BIO9 and BIO8 (Fig. 5). The results of the Jackknife test of variable importance indicated that the environmental variable with highest gain when used in isolation was

BIO13, which consequently appeared to have the most useful information by itself. The environmental variables that decreased the gain the most when omitted were BIO6, BIO9 and BIO8, thus those appeared to have information that was not present on the other variables.

Principal components analysis. The principal component analysis including species with all environmental variables, revealed that the first three components explained 88.67% (PC1 = 45.03 %, PC2 = 27.32 %, PC3 = 16.32 %) of the variation. The individuals' space showed that PC1 contrasts *Liolaemus chehuachekenk* and *L. shehuen* with *L. fitzingerii* and *L. xanthoviridis*, and that PC2 contrasts *L. chehuachekenk* and *L. fitzingerii* with *L. shehuen* and *L. xanthoviridis* (Fig. 7, left panel). We graphically present the differences between species' localities with 95 % confidence ellipses around barycenter of each species, which showed a clear separation between them (Fig. 7, left panel). The first principal component depicted a strong positive correlation with BIO6, BIO11, BIO14, BIO18 and BIO17, and negative correlations with BIO3, BIO4, BIO7, BIO2 and ALT. Positive correlations for PC2 correlations are more strongly represented by BIO10, BIO8, BIO1, BIO5 and negative correlations for BIO15, BIO12, BIO13, BIO16, BIO19, while for PC3 was strongly correlated with BIO15, BIO18 and BIO17 (Table 8).

In the variables' space, PC1 contrasts mostly precipitation variables (BIO18, BIO14, BIO6 and BIO17) with temperature and altitude variables (ALT, BIO3 and BIO9) with an negative correlation. The variables' interactions for PC2 contrasts temperature that were negatively correlated with precipitation (BIO10 vs. BIO15; BIO5 with BIO12, BIO13, BIO16 and BIO19). The PC3 showed a negative correlation between BIO5 vs. BIO14, BIO17 and BIO18. The barycenter values of the environmental variables per species are shown in Table 7.

TABLE 7. PCA barycenter values from environmental variables per species. References: ALT: Altitude, BIO1: Annual Mean Temperature, BIO2: Mean Diurnal Range (Mean of monthly (max temp–min temp), BIO3: Isothermality (P2/P7)*(100), BIO4: Temperature Seasonality (standard deviation*100), BIO5: Max Temperature of Warmest Month, BIO6: Min Temperature of Coldest Month, BIO7: temperature Annual Range (P5–P6), BIO8: Mean Temperature of Wettest Quarter, BIO9: Mean Temperature of Driest Quarter, BIO10: Mean Temperature of Warmest Quarter, BIO11: Mean Temperature of Coldest Quarter, BIO12: Annual Precipitation, BIO13: Precipitation of Wettest Month, BIO14: Precipitation of Driest Month, BIO15: Precipitation of Seasonality (Coefficient of Variation), BIO16: Precipitation of Wettest Quarter, BIO17: Precipitation of Driest Quarter, BIO18: Precipitation of Warmest Quarter, BIO19: Precipitation of Coldest Quarter, A: *Liolaemus camarones*, B: *L. chehuachekenk*, C: *L. fitzingerii*, D: *L. shehuen*, E: *L. xanthoviridis*. Units: Altitude: meters, Temperature: Celsius degrees, Precipitation: mm.

Variable	B	C	D	E
ALT	799.89	100.27	812.60	188.15
BIO1	9.50	10.81	10.16	12.38
BIO2	13.40	10.16	13.81	11.74
BIO3	49.12	46.73	48.87	47.43
BIO4	543.23	463.50	563.31	503.74
BIO5	24.72	22.72	25.64	25.48
BIO6	-2.57	0.98	-2.61	0.72
BIO7	27.29	21.74	28.25	24.76
BIO8	3.37	5.57	5.89	7.02
BIO9	15.19	12.51	15.97	9.65
BIO10	16.19	16.28	17.06	18.43
BIO11	2.82	5.05	3.24	6.12
BIO12	192.44	213.27	176.80	182.77
BIO13	29.00	27.47	19.70	24.23
BIO14	7.78	11.87	10.10	9.92
BIO15	42.56	29.32	21.16	31.83
BIO16	78.33	76.47	54.00	63.69
BIO17	26.33	39.00	34.70	32.08
BIO18	30.11	43.07	38.90	39.23
BIO19	67.44	66.33	46.30	52.15

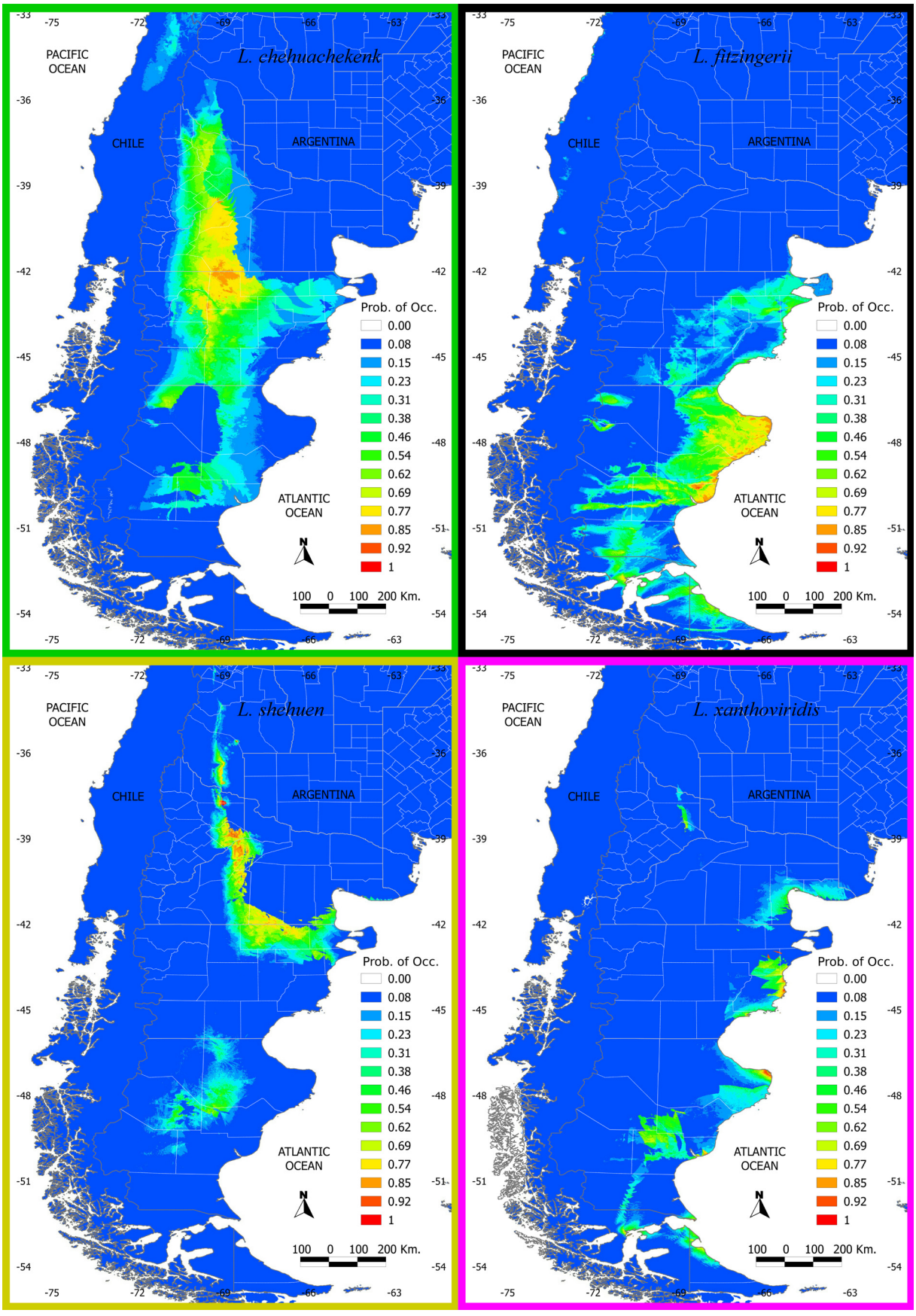

FIGURE 6. Probabilistic maps of habitat suitability for each species, determined by maximum entropy modelling.

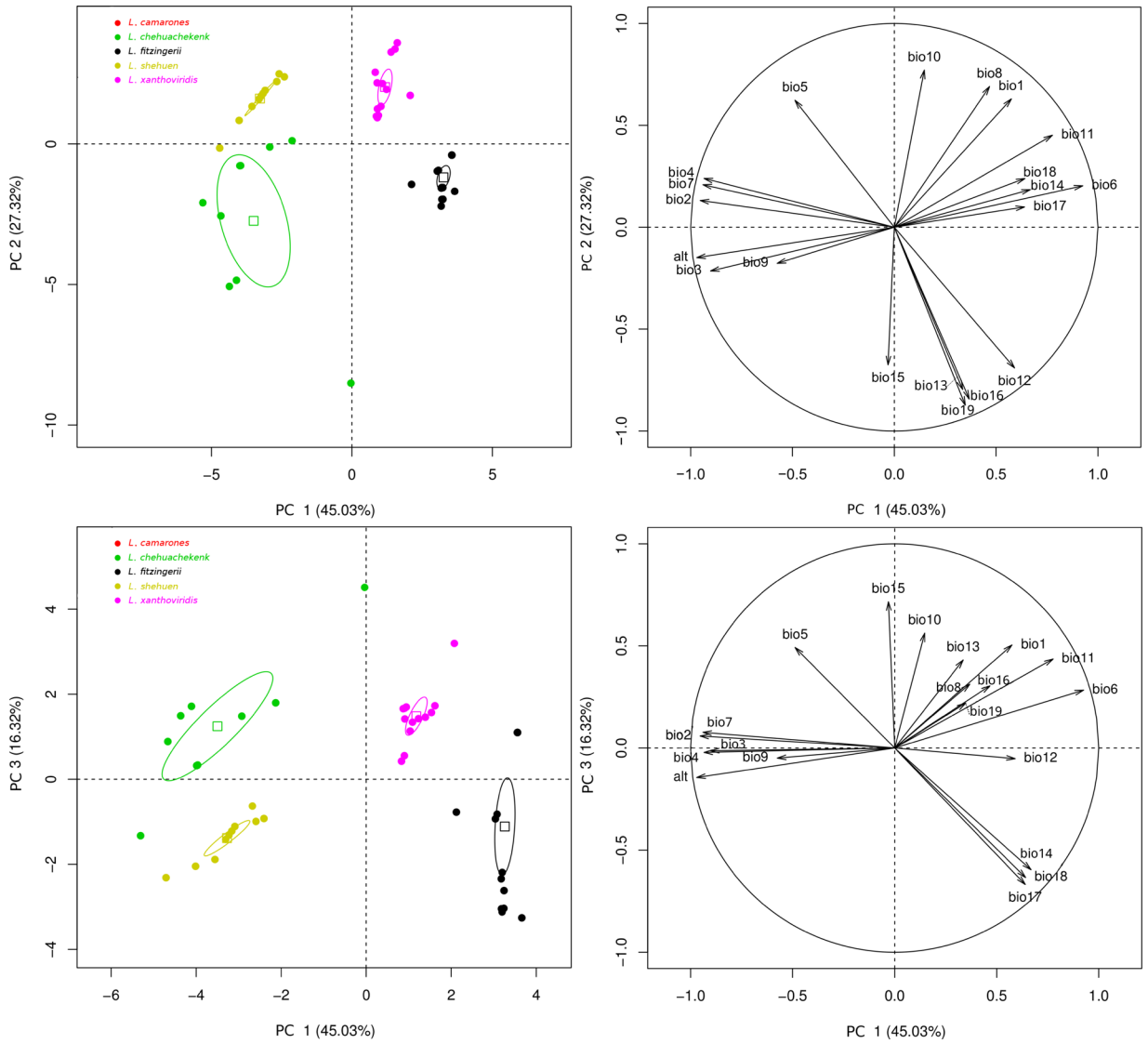

FIGURE 7. Principal Component Analysis of species localities with 19 Bioclim and altitude variables. Three first components are shown for the localities's and variables's spaces. Ellipses of 95 % confidence were plotted around the species's localities.

TABLE 8. Results of the correlations between the PCs and the variables obtained from the PCA performed with the values of BIOCLIM and altitude.

PC1			PC2			PC3		
Variable	correlation	*P* value	Variable	correlation	*P* value	Variable	correlation	*P* value
BIO6	92.55%	≤ 0.00001	BIO10	77.12%	≤ 0.00001	BIO15	71.64%	≤ 0.00001
BIO11	77.65%	≤ 0.00001	BIO8	69.06%	≤ 0.00001	BIO18	-63.46%	≤ 0.00001
BIO14	66.80%	≤ 0.00001	BIO1	63.00%	≤ 0.00001	BIO17	-66.85%	≤ 0.00001
BIO18	64.16%	≤ 0.00001	BIO5	62.33%	≤ 0.00001			
BIO17	63.97%	≤ 0.00001	BIO15	-67.53%	≤ 0.00001			
BIO3	-90.25%	≤ 0.00001	BIO12	-69.10%	≤ 0.00001			
BIO4	-93.44%	≤ 0.00001	BIO13	-79.65%	≤ 0.00001			
BIO7	-93.97%	≤ 0.00001	BIO16	-84.29%	≤ 0.00001			
BIO2	-95.34%	≤ 0.00001	BIO19	-87.38%	≤ 0.00001			
ALT	-97.01%	≤ 0.00001						

Manova. The overall MANOVA analysis using PC scores and species as categorical variables showed differences between species in the bioclimatic and topographic variables (Wilks´ $\lambda = 0.00752$, $F_{3, 43} = 71.695$, $P \leq 0.00001$). We detect statistically significant separation between species when the PC axis considered species as factor (PC1 axis: $F_{3, 43} = 199.19$, $P < 0.00001$; PC2 axis: $F_{3, 43} = 27.043$, $P < 0.00001$; PC3 axis: $F_{3, 43} = 15.683$, $P < 0.00001$). Furthermore, pairwise comparisons by subsetting species showed differences between all the pairs for the four species (*L. chehuachekenk* vs. *L. fitzingerii*: Wilks´ $\lambda = 0.04135$, $F_{1, 22} = 154.19$, $P < 0.00001$); *L. chehuachekenk* vs. *L. shehuen*: Wilks´ $\lambda = 0.10447$, $F_{1, 17} = 42.859$, $P < 0.00001$); *L. chehuachekenk* vs. *L. xanthoviridis*: Wilks´ $\lambda = 0.05556$, $F_{1, 20} = 102$, $P < 0.00001$); *L. fitzingerii* vs. *L. shehuen*: Wilks´ $\lambda = 0.00380$, $F_{1, 23} = 1832.4$, $P < 0.00001$); *L. fitzingerii* vs. *L. xanthoviridis*: Wilks´ $\lambda = 0.04301$, $F1_{1, 26} = 177.99$, $P < 0.00001$); *L. shehuen* vs. *L. xanthoviridis*: Wilks´ $\lambda = 0.01212$, $F_{1, 21} = 516.03$, $P < 0.00001$).

Discussion

The discovery of cryptic species considering multiple approaches is decisive for correct classification and biodiversity conservation (Beheregaray & Caccone 2007; Schlick-Steiner *et al.* 2010). This is the first detailed integrative analysis that combines extensive statistical analyses based on external morphology and environmental niche models (ENMs) between all species of the *Liolaemus fitzingerii* complex. While morphometric analyses were able to detect some differences between the five taxa, the ENMs showed clear differences between the four taxa that were possible to compare. The continuous and lepidosis characters presented some differences between the species; the morphological tests had dissimilar performance in detecting them and were non-conclusive in terms of supporting the diagnosis for some of the species. The ENMs allowed a clear spatial differentiation between four of the species' potential distributions and also their probable suitable habitats as the set of individual environmental variables important for the presence of each species were different. Precipitation of Wettest Quarter (BIO16), Temperature Seasonality (BIO4) and Altitude were the variables in common for the analyzed taxa, that contributed most to the models of the four species. In addition to this, an important variable was Mean Temperature of Wettest Quarter (BIO8) which had an important contribution for three of the four taxa. The Precipitation of Wettest Month (BIO13) was the variable with the highest gain when used in isolation for three species models. Only the *L. chehuachekenk* model presented Precipitation of Warmest Quarter (BIO18) as the variable with the highest gain when used in isolation.

Morphological analyses. In the *Liolaemus* taxonomic literature, it is usual to find new species descriptions (Ocampo *et al.* 2012) with descriptive values (often in tables) as the only evidence to support the new species hypothesis. Our results for this type of basic statistical approach, showed that they do not contribute to detect clear differences between species of this complex. Many continuous and discrete variables had mean, SD, minimum and maximum values that clearly overlap, not showing differences among taxa, a pattern that is commonly found in other *Liolaemus* complexes (Abdala 2005; Scolaro & Cei 2006; Nori *et al.* 2010). Also it has been found that these continuous morphological variables have large latitudinal variation (Cruz *et al.* 2005; Pincheira-Donoso *et al.* 2007b, 2008), and present high phenotypic plasticity influenced by the environment (Cruz *et al.* 2005; Naya & Bozinovic 2006; Canale & Henry 2010). Thus, we consider that using these parameters as the only operational criteria to delimit species without other kind of statistical, repeatable and objective analyses, coupled with no other evidence rather than subjective and qualitative observed differences (e.g., coloration patterns), is useless to propose a robust hypothesis and diagnosis of a new *Liolaemus* species.

Previous works that have analyzed morphological variables using principal component analysis for delimiting *Liolaemus'* species are scarce (Breitman *et al.* 2011b; Aguilar *et al.* 2013). The principal component analysis is used to find in a multivariate context, a set of standardized orthogonal linear combinations that together explain the variation in the original variables (Crawley 2007). This analysis explains differences between individuals, but not between levels of a factor and these variables must be strongly correlated (Luo *et al.* 1999; Harlow 2005). The results of PCA for males and females in the space of individuals, showed three partially overlapping groups considering as a reference the 95% ellipses of confidence (Figs. 2–3, left panels). These results do not present conclusive evidence to differentiate the five species in the individual's space. In congruence with this, similar results were published for *Liolaemus* (Breitman *et al.* 2011a; Aguilar *et al.* 2013) and other taxa (see Barata *et al.* 2012; Ahmadzadeh *et al.* 2013; Camp *et al.* 2013). On the contrary, PCA is a great tool for understanding what

variables are the most that contribute to the morphological variation (Claude 2008), as well as the interactions between variables (Abdi *et al.* 2013). Continuous variables explained most of the morphological variation in PC1 for both sexes (DBN, HW, NED), while the variation in PC2 and PC3 was mainly explained by the lepidosis variables (SAMB, L4T, VS; Table 3, Fig. 2–3). According to Iezzoni & Pritts (1991), the PCA could be used to assess which variables explain most variability between individuals and use them to make other post hoc comparative analyses; while Berner (2011), considers that PCA-based approaches are inappropriate for size correction and should be abandoned in favor of methods using univariate general linear models, with an adequate independent body size metric as covariate.

Taxonomic studies focusing on closely related species and new species descriptions frequently include discriminant analysis (e.g., Scolaro *et al.* 1985; Passos *et al.* 2009; Medina *et al.* 2013), and levels of misclassification are variable depending on the study group (for *Liolaemus e*xamples, see Breitman *et al.* 2013). Linear Discriminant Analysis is often used to emphasize differences between groups with the weights given by the prior, which may differ from their prevalence in the dataset (Venables & Ripley 2002; McLachlan 2004) and find linear combinations of variables that describe intergroup differences (Claude 2008). The LDA for continuous variables standardized by SVL, were not robust enough to detect the five taxa *a priori* included in this group. The misclassification rate was high and showed a clear graphic overlap between some species (*L. camarones* and *L. fitzingerii* both sexes, Fig. 4, Table 4). Also, LDAs showed the lowest classification error rate for both sexes of *L. xanthoviridis*, but were not effective to classify and assign specimens of *L. chehuachekenk* and *L. shehuen*, especially between males (Fig. 4, Table 4). On the contrary, these last two species compared with univariate tests showed major differences in continuous variables. The species of the *L. fitzingerii* complex were moderately discriminated with LDAs analyses performed with standardized continuous variables, thus under this statistically context, this kind of characters are not completely useful for diagnosing cryptic or closely related species.

Several works have analyzed morphological differences between lizard populations using univariate analyses (Lamborot *et al.* 2003; Metzger & Herrel 2005; Pincheira-Donoso *et al.* 2007a; Pincheira-Donoso & Scolaro 2007), but only a few manuscripts used them for a new species description (Vega *et al.* 2008; Breitman *et al.* 2011a; 2011b). This kind of tests allow to analyze simple measurements individually, and they are very easily implemented with little knowledge (Claude 2008); they also allow to make adjustments to overcome biases from other variables, fulfill statistical assumptions (Harlow 2005), and show which variables are different among the species. The univariate results and comparisons among males, showed that most of the differences were mostly represented by continuous variables; and some species presented only few differences in head size (e.g., three variables for *Liolaemus fitzingerii* vs. *L. camarones*, Table 5). On the contrary, multiple univariate comparisons between females showed at least one significant difference for lepidosis variables in each comparison, except for *L. fitzingerii* vs. *L. camarones*, that had no differences (Table 6).

Based on morphological analyses implemented here, some species showed clearly different morphologies, while others were almost not possible to differentiate. 1—The species with more differences across comparisons were: *Liolaemus xanthoviridis* (PCA, LDA and univariate analyses with differences in 16 variables), *L. shehuen* (LDA and univariate analyses with differences in 14 variables) and *L. chehuachekenk* (PCA, LDA only for females and univariate analyses with differences in 14 variables). *Liolaemus xanthoviridis* showed for both sexes, the lowest LDA classification error rate and the univariate results showed that it has the largest number of variables with significant differences compared to the other species. 2—Species that although it was difficult, were detected as statistically different are: *L. fitzingerii* from *L. chehuachekenk* (PCA, LDA, and univariate analyses with differences in six variables) and *L. shehuen* (LDA and univariate analyses with differences in three variables). 3—*Liolaemus fitzingerii* and *L. camarones* may differ in color pattern and some descriptive measures, but almost none of the variables were statistically different. These two taxa showed an overlap in PCA and LDA for individuals of both sexes, coupled with univariate analyses that showed no significant differences between females, while males only differed in three head variables (HW, HD and RPD). Although coloration pattern could be a useful diagnostic character, if it is not analyzed through appropriate and reproducible analyses (e.g., Corso *et al.* 2012; Teasdale *et al.* 2013), subjective detected differences on coloration are not strong evidence to hypothesize new *Liolaemus* taxa, especially for species complexes (see Escudero *et al.* 2012). With all the classical morphological analyses implemented here, we detected more significant differences between females than among males, even though males are usually used to detect differences and describe new lizard taxa for this and other related *Liolaemus* complexes.

Although in recent years, numerous lizard papers have approached morphological studies based on multiple statistical analyses within the integrative taxonomy paradigm (e.g., Barata *et al.* 2012; Kaliontzopoulou *et al.* 2012; Vasconcelos *et al.* 2012; Ahmadzadeh *et al.* 2013), this approach has been scarcely implemented in *Liolaemus* lizards (Aguilar *et al.* 2013; Breitman *et al.* 2013). If we compare the performance of the three types of statistical morphological analyses we implemented for the *Liolaemus fitzingerii* group, our PCA results are in agreement with Claude (2008) and Harlow (2005) as they proved to be a robust analysis for highly correlated variables, especially when performed with associated *p*-values (Lê *et al.* 2008). Therefore, to identify the variables that best delimit *Liolaemus* species, we consider PCA a better and more adequate multivariate approach than LDA, to test differences between continuous variables. Moreover, in agreement with Berner (2011) we consider that using a prior exploration through a PCA followed by univariate analyses would be the most appropriate approach to find morphological differences between taxa. Most of the variability detected by our PCA analysis was explained by continuous variables, which were also the ones with more variation in the univariate analyses. Although it would be tempting to recommend their use, continuous variables should be used with caution, since previous works on lizards reported a strong association with different temperatures and latitudes (see Oufiero *et al.* 2011). On the contrary, if the goal of the study is to classify individuals of previously defined groups or *Liolaemus* species, we consider that this analysis is relatively difficult to use with the original variables, because its usage should be thorough and careful to meet all the required assumptions. Another constraint in this regard, is that the LDA analysis is to classify individuals (Claude 2008), rather than determine which variables differentiate the taxa. The LDA was used for comparing specimens that are geographically separated, diagnosed and designated *a priori* as different species, thus while the graphics may seem conclusive for separating taxa, the misclassification rates were high for some of them. Consequently, we consider that LDA should be used and interpreted with care and cannot be presented as the single morphological analysis to compare taxa and should not be use as the solely analysis to support a hypothesis of a new *Liolaemus* species.

Only a few works used univariate analyses of continuous variables (Pincheira-Donoso *et al.* 2007a; Pincheira-Donoso & Scolaro 2007; Vega *et al.* 2008), or combined continuous and discrete variables (Breitman *et al.* 2011a; 2011b), for the assessment of species boundaries and diagnosis of new *Liolaemus* species. There are also some morphological studies on lizards that applied statistical analyses to detected differences in closely related species, and in order to perform parametric tests (i.e., ANCOVAs, MANOVAs), have standardized of all variables by SVL (e.g., Kaliontzopoulou *et al.* 2005), but without specifying if this decision was validated on previous corroboration of bias produced by variable interactions. As a final consideration, we consider that diagnosis of new *Liolaemus* taxa increase their power when they incorporate this univariate analyses (e.g., Breitman *et al.* 2011a; 2011b), with prior models testing the influence or collinearity within morphometric variables, and in combination with other lines of evidence, they are very useful to support a new species hypothesis.

Environmental niche modeling analyses. The usage of ENMs for delimiting species in the *Liolaemus* taxonomic literature is scarce (Fontanella *et al.* 2012; Aguilar *et al.* 2013), although they have been used more in its sister genus *Phymaturus* (Debandi *et al.* 2012; Scolaro *et al.* 2013). The environmental niche models presented rely on the principle of maximum entropy (Phillips *et al.* 2006) to calculate the most likely distribution of the studied taxa based on presence records (Elith *et al.* 2011). Therefore modeled area of potential distribution of each species, represent a set of unique environmental and climatic conditions for their type localities and surroundings. The species of the *L. fitzingerii* complex for which it was possible to implement ENMs analyses (all except *L. camarones*), showed clear ecological differences between them. Although the sampling scheme we used for selecting localities for the ENM analyses could have biased the results, we consider that our decision was based on the difficulty of assigning certain individuals to a particular taxon, thus we feel confident on the inferences we can make based on these results. Although our work represents a great advance in the knowledge of the ENMs for this species complex, the complete picture is still limited, since more sampling is needed to analyze a potential spatial overlap of *L. camarones* with the other taxa.

Previous findings showed a remarkable evolutionary flexibility of thermal biology for *Liolaemus* genus (Espinoza *et al.* 2004). The Mean Temperature of Wettest Quarter was the most important contributor to three of the four species models. These species of lizards are excellent thermoregulators (Medina *et al.* 2012), so this bioclimatic variable might be an important factor in selecting microhabitat (Rodríguez-Serrano *et al.* 2009) and feeding habits (Espinoza *et al.* 2004). Recently, phylogeneticaly based analyses, suggested that modifications of thermal physiology and behavioral compensation of thermal ecology, including microhabitat selection is wide

spread in *Liolaemus* (even in sister species, see Rodríguez-Serrano *et al.* 2009). The Precipitation of Wettest Month is also a variable with high contribution for three of the four studied species. On the contrary, previous works did not find a phylogenetic signal for rainfall between closely related *Liolaemus* species (Medina *et al.* 2012). The precipitation influence on *Liolaemus* species have not been studied from an eco-physiological approach as has been done with the temperature, hence we consider necessary future works to evaluate how these type of variables could influence ecophysiological processes, activities or their microhabitat (e.g., foraging and oviposition).

Both the ENMs, and statistical analyses (PCA and MANOVA) performed on bioclimatic variables, showed that these four species have differences in environmental conditions characterizing their ecological niches. Many studies have used ecological niche models to assess differences between close taxa (Gvoždík *et al.* 2008; Crespi *et al.* 2010; Rivera *et al.* 2011; Scolaro *et al.* 2013). Moreover, a few studies on contact areas between species (Martínez-Freiría *et al.* 2008) and based on few localities (Pearson *et al.* 2007) were able to find differences in bioclimatic variables. Consequently, we consider that this kind of analyses could contribute to the effort of detecting differences between very closely related taxa within the integrative taxonomy paradigm.

Integrative taxonomy. In summary, the results of multivariate and univariate morphological analyses based on continuous and meristic variables for both sexes, showed moderate differences in four species (*Liolaemus chehuachekenk, L. fitzingerii, L. shehuen* and *L. xanthoviridis*) out of the five included in the *L. fitzingerii* complex. Additionally, the ENMs also differentiated these four species from each other. *Liolaemus camarones* is only known from its type locality, which precluded niche model analyses, and all the surrounding areas are considered as part of the distribution range of three of the other species of this complex; and morphological analyses implemented here did not detect statistically significant differences from the other four species of this complex. Thus, based on the integrative taxonomy approach, our combined morphological results and environmental niche models strongly support the species' status of four previously described taxa within the *L. fitzingerii* complex and no support was found for the hypothesis of *L. camarones* being a different species. This work has demonstrated the utility of repeatable and objective analyses within the integrative taxonomy paradigm for a species complex of the lizard genus *Liolaemus*, providing robustness to hypothesis testing and diagnoses.

The main challenge for implementation of multiple repeatable analyses to support the diagnosis of a new species most probable is the selection of variables, analyses and operational criteria. This decision could lead to different results, especially on taxa with closely related or cryptic species (see Bickford *et al.* 2007; Vasconcelos *et al.* 2012) with wide geographic distribution, or with the use of the term "morphospecies" (*sensu* Krell 2004). In consonance with such problems, several papers presented the current problems of describing new species as a stage of taxonomic crisis (Dayrat 2005; Agnarsson & Kuntner 2007; Wägele *et al.* 2011) and several methods' reviews, as well as new theoretical proposals have been postulated to deal with the challenge of delimitating species (Marshall & Sites Jr 2003; Padial *et al.* 2010). Morphological analyses performed with the authors' commitment to give appropriate treatment of variables and validation of statistical assumptions, undoubtedly contribute to test the validity of new taxa hypotheses and also to the repeatability that science advocates (see Kaiser *et al.* 2013). Furthermore, the ENMs methods are widely used to find bio ecological differences between taxa (e.g., Rivera *et al.* 2011; Debandi *et al.* 2012; Wooten & Gibbs 2012), and based on the results presented here we consider that if used as additional analyses, they may contribute to differentiate cryptic species. These complementary analyses associated to species descriptions, are needed to sustain robust new species hypotheses and taxonomic changes, since this basic information has major impact on biogeographic (Corbalán & Debandi 2008; Vera-Escalona *et al.* 2010) and conservation (Corbalán *et al.* 2011; Katzner *et al.* 2011a; 2011b) studies. Recent analyses that included museum-based collections data showed numerous cases of lizard population extinctions worldwide (Sinervo *et al.* 2010), which coupled with the taxonomic crisis (Agnarsson & Kuntner 2007; Wägele *et al.* 2011), enhance the value of the results of the integrative taxonomy approach presented here and will make a useful contribution to new described *Liolaemus* taxa in the future.

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APPENDIX. Specimens examined. Methods applied are given in bold capitals, country in plain capitals, provinces in italics capitals, departments in regular italics, species in bold italics and localities in plain text.

MORPHOLOGY: ***Liolaemus camarones*** (n = 23):—ARGENTINA: *CHUBUT*: *Florentino Ameghino*: Bahía Camarones, Playa Elola (44°50'19.0"S 65°43'23.0"W): LJAMM-CNP 2464, 2500, 2502-4. 1 Km S from entrance Playa Elola Road (44°48'56.3"S, 65°44'43.3"W): LJAMM-CNP 15128-30. 3 Km S from entrance Playa Elola Road (44°49'55.0"S, 65°44'12.8"W): LJAMM-CNP 15122-4, 15131-35. Provincial Route 1, 31 km S Camarones, 1 km E La Isabel Ranch entrance (44°55'55.1"S, 65°59'19.0"W): LJAMM-CNP 11736-39, 11744-46. **MORPHOLOGY & ENM**: ***Liolaemus chehuachekenk*** (n = 34):—ARGENTINA: *CHUBUT*: *Cushamen*: Provincial Route 13, 8 km N El Molle (42°09'02.7"S, 69°33'37.0"W): LJAMM-CNP 5926-32. *Gastre*: Provincial Route 58, 13.5 km N El Escorial (42°59'34.5"S, 68°35'35.6"W): LJAMM-CNP 12356, 12383-90, 12393, 12395-96, 12398-99, 12409-12, 12959. Provincial Route 4, 47.6 km W Gan Gan (42°25'58.6"S, 68°48'35.8"W): LJAMM-CNP 6742-43. Provincial Route 49, 30 km S Gastre (42°31'02.5"S, 69°12'8.5"W): LJAMM-CNP 5961-65. *Languiñeo*: Provincial Route 12, 3 km E Gualjaina river bridge (42°40'50.6"S, 70°22'20.1"W): LJAMM-CNP 8851. ***Liolaemus fitzingerii*** (n = 25):—ARGENTINA: *SANTA CRUZ: Deseado:* 1 Km W Tellier (47°39'12.5"S, 66°03'05.8"W): LJAMM-CNP 2918-20, 4891. Provincial Route 14, 3.9 km E junction Provincial Route 68, 2.4 km E El Polvorin Ranch (47°07'03.4"S, 66°28'46.9"W): LJAMM-CNP 9681-90, 9692. Provincial Route 47, 55.4 km SW Tellier, 3 km S over Deseado river bridge (47°51'01.2"S, 66°37'19.8"W): LJAMM-CNP 9828-31. National Route 3, junction with Deseado river (47°12'38.0"S, 67°16'47.6"W): LJAMM-CNP 2891-92, 4875-77. Provincial Route 43, 30 km S Pico Truncado (46°54'27.7"S, 67°33'21.3"W): LJAMM-CNP 4612. ***Liolaemus shehuen*** (n = 33):—ARGENTINA: *CHUBUT*: *Telsen*: Laguna de Vaca path, 3.5 km S junction Provincial Route 4 (42°27'52.6"S, 67°19'51.6"W): LJAMM-CNP 6943-48, 6950-52, 6961. Laguna de Vaca road, 2 km S junction Provincial Route 4 (42°23'20.2"S, 67°33'41.3"W): LJAMM-CNP 11023-37. Provincial Route 4, 65.5 Km W Telsen (42°22'03.8"S, 67°39'22.0"W): LJAMM-CNP 5520, 5521-25, 5665. Provincial Route 4, 80 km W Telsen (42°25'55.0"S, 67°46'4.0"W): LJAMM-CNP 6883. ***Liolaemus xanthoviridis*** (n = 32):—*ARGENTINA: CHUBUT: Florentino Ameghino*: Provincial Route 1, 1 Km S Dos Pozos (43°55'37.0"S, 65°24'10.0"W): LJAMM-CNP 2220, 2284-85, 2527-30. Provincial Route 1, 10 Km S Dos Pozos (43°59'53.0"S, 65°25'26.0"W): LJAMM-CNP 2427-28, 2505-08, 2658. Provincial Route 1, 2.5 Km N Dos Pozos (43°53'15.0"S, 65°26'51.0"W): LJAMM-CNP 2221-2222. Provincial Route 1, 12 Km S Dos Naciones ranch (43°47'53.5"S, 65°27'49.3"W): LJAMM-CNP 2418, 2689. Provincial Route 1, 18 km S Dos Pozos Postal Office (44°02'01.4"S, 65°28'43.5"W): LJAMM-CNP 14341-42. Provincial Route 32, 4 Km from junction Provincial Route 2 (44°02'01.0"S, 65°31'37.0"W): LJAMM-CNP 2204. *Rawson*: Isla Escondida Bay (43°41'55.0"S, 65°20'23.0"W): LJAMM-CNP 2201-03, 2487, 2551. Isla Escondida beach (43°41'04.4"S, 65°21'57.8"W): LJAMM-CNP 14350. Isla Escondida beach (43°41'04.4"S, 65°20'29.2"W): LJAMM-CNP 14351-55. **ENMs:** ***Liolaemus chehuachekenk*** (n = 19):—ARGENTINA: *CHUBUT*: *Cushamen*: Provincial Route 13, 8 km N El Molle (42°10'24.9"S, 69°33'51.3"W): BYU 48202-03, FML 15105-06, MLP.S 2535-36. *Gastre*: Provincial Route 50, 10 km N El Escorial (43°00'00.2"S, 68°34'14.1"W): LJAMM-CNP 5939, 5936-38. Provincial Route 58, 39.6 km NE junction Provincial Route 40 (43°07'05.0"S, 68°38'54.2"W): LJAMM-CNP 8832-34. Provincial Route 58, 23 km SW junction Provincial Route 59 (43°14'31.6"S, 68°38'20.0"W): LJAMM-CNP 8825-30. ***Liolaemus fitzingerii*** (n = 18):—*ARGENTINA: SANTA CRUZ: Deseado:* National Route 3, 10 Km S Caleta Olivia (46°33'43.8"S, 67°27'3.6"W): LJAMM-CNP 2895-97, 4879. National Route 3, 6 km N Tres Cerros (48°03'00.8"S, 67°37'38.3"W): LJAMM-CNP 4637-39. National Route 3, 7 Km N Tres Cerros (48°04'05.9"S, 67°37'50.7"W): LJAMM-CNP 2871-72. National Route 3, 6 km N Tres Cerros (48°04'03.2"S, 67°37'5.6"W): LJAMM-CNP 4675-78. National Route 3, 10 Km S Caleta Olivia (46°33'43.8"S, 67°27'3.6"W): BYU 47299, 47300. National Route 3 at Km 2107, ~7 Km N Tres Cerros (48°04'05.9"S, 67°37'50.7"W): BYU 47295-96. *Magallanes:* Provincial Route 47, 19.5 km S junction Provincial Route 87 (48°22'42.1"S, 67°25'18.8"W): LJAMM-CNP 9983. ***Liolaemus shehuen*** (n = 12):—ARGENTINA: *CHUBUT: Telsen:* Laguna de Vaca (42°29'45.1"S, 67°22'53.7"W): LJAMM-CNP 3225. Laguna Sepaucal, path from road to Colonia Sepaucal (42°17'45.6"S, 67°22'17.5"W): LJAMM-CNP 3223-24. Provincial Route 4, 41.6 Km W Telsen (42°22'6.9"S, 67°24'7.9"W): LJAMM-CNP 5596-99. Road, 45.2 Km W Telsen (42°22'01.7"S, 67°27'38.8"W): LJAMM-CNP 5466. Provincial Route 4, 53.5 km W Telsen, Mallin Grande Ranch (42°22'54.8"S, 67°28'42.0"W): LJAMM-CNP 6738-40. Provincial Route 4, 74 km E Gan Gan (42°22'48.2"S, 67°29'29.1"W): LJAMM-CNP 5465. ***Liolaemus xanthoviridis*** (n = 26):—*ARGENTINA: CHUBUT: Florentino Ameghino*: Provincial Route 1, 11 km S Dos Pozos, 2 km S La Perla Ranch y Punta Tombo entrance (43°57'57.7"S, 65°24'21.2"W): LJAMM-CNP 14475-84. Provincial Route 1, 2 km S juntion Provincial Route 32, Santa Magdalena ranch (44°03'03.5"S, 65°28'14.9"W): LJAMM-CNP 14497. 20 Km S Provincial Routes 32 y 1 junction (44°10'27.0"S, 65°25'22.0"W): MLP.S 2460. *Rawson*: Provincial Route 1, junction Bahia Isla Encondida road (43°40'09.4"S, 65°25'25.6"W): LJAMM-CNP 14486-89. Isla Escondida Bay (43°41'55.0"S, 65°21'4.5"W): LJAMM-CNP 2485, 2488. Isla Escondida Bay (43°42'29.5"S, 65°21'22.9"W): LJAMM-CNP 14485. Isla Escondida Bay (43°41'55.0"S, 65°21'4.5"W): BYU 48119; MLP.S 2461.