



## Evidence of body size and shape stasis driven by selection in Patagonian lizards of the *Phymaturus patagonicus* clade (Squamata: Liolaemini)



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### ABSTRACT

During the speciation process sibling lineages accumulate differences in time (e.g. genetic, morphological, and/or ecological). Phenotypic traits such as size or shape, however, could experience rapid changes or show stasis depending on their role in survival and reproduction. The clade *Phymaturus patagonicus* includes 26 species characterized by a conservative morphology, and all inhabit rock crevice microhabitats in arid environments. In this study we quantify levels of morphological divergence (size and shape) among the multiple species relative to interspecific molecular divergence, and show that most species have not diverged significantly in size and/or shape to permit unambiguous species diagnosis with morphological data alone. The influence of stabilizing selection for an adaptive optimum in body size and head shape was detected for 13 of the 16 variables analyzed in an Ornstein-Uhlenbeck model. The strict dependence of these species to rock-crevice microenvironments likely explains the observed morphological stasis across the many species of the *Phymaturus patagonicus* group.

### 1. Introduction

The speciation process leads to divergence of lineages in time and the generation of new independent entities known as species. Over time sister lineages will diverge at the genetic and morphological levels, and in ecological niche space, among other factors (de Queiroz, 2007). However, these differences may accumulate at different rates, and depending on the specific environment, the new species will diverge in some of these attributes while retaining similarities in others. For example, recent studies of birds (Campagna et al., 2012) and fishes (Elmer et al., 2010) revealed rapid morphological divergence relative to molecular divergence between closely-related species. In another case study of sunflowers, speciation was driven along ecological axes, and differences were detected only at specific genes relevant to environmental differences, against a background of little or no divergence in neutral genes and floral morphology (Andrew and Rieseberg, 2013).

Non-coding regions of DNA are expected to evolve at “neutral” rates in the absence of selection, and should accumulate differences in a time-dependent manner (molecular clocks, Zuckerkandl and Pauling, 1965). In contrast, phenotypic traits could experience either rapid change or extreme stasis over time, depending on the ecological context

of the speciation process (Erwin, 2007; Smith et al., 2011). In particular, body size is a fundamental character in the animal biology that may covary with ecological, physiological, or other life history traits (fecundity, age at maturity, metabolic efficiency, and thermoregulation; Sookias et al., 2012). Thus, body size has complex interactions with fitness (LaBarbera, 1989). For example, a larger size could increase success in obtaining food, defending a home range, and reproduction success (Hone and Benton, 2005), against trade-offs with cuckoldry or predation. On the other hand, shape, another phenotypic attribute that is less often studied than size, seems to be important in different life history traits, including sexual reproduction (e.g. Brandt et al., 2016; Kaliontzopoulou et al., 2010; Vidal et al., 2005, 2006), social interactions (Kaliontzopoulou et al., 2012), and/or feeding (Florio et al., 2012).

The South American lizard genus *Phymaturus* is an interesting system to study body size and shape evolution. These lizards are characterized morphologically by having a flattened head and body, lateral nuchal skin folds, and tail with regular whorls of spines scales (Etheridge, 1995). Across the whole genus ( $n \approx 48$  species; Scolari et al., 2016), the majority of the species are not diagnosed by clear morphological differences, but largely by differences in color pattern

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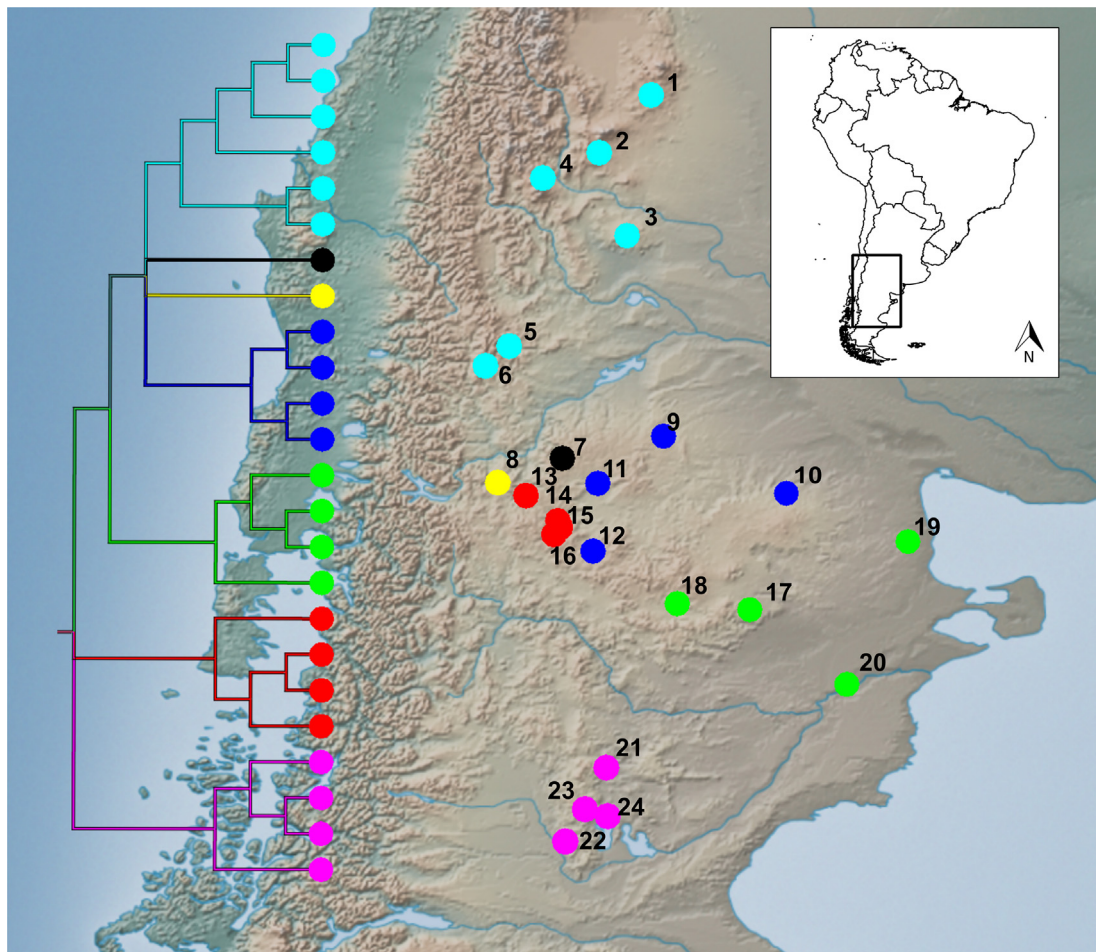
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**Fig. 1.** Species tree inferred with BEAST using combined mitochondrial and nuclear DNA sequences (modified from Morando et al., 2013). References: *payuniinae* group (cyan): *P. nevadoi* (1), *P. payuniinae* (2), *P. sitesi* (3), *P. delheyi* (4), *P. zapalensis* (5), *P. rahuensis* (6); *P. cacivioi* (black, 7); *P. tenebrosus* (yellow, 8); *somuncurensis* group (blue): *P. ceii* (9), *P. somuncurensis* (10), *P. sinervoii* (11), *P. etheridgei* (12); *spurcus* group (red): *P. manuelae* (13), *P. spurcus* (14), *P. spectabilis* (15), *P. excelsus* (16); *calcogaster* group (green): *P. calcogaster* (17), *P. camilae* (18), *P. yachanana* (19), *P. patagonicus* (20); *indistinctus* group (magenta): *P. felixi* (21), *P. indistinctus* (22), *P. videlai* (23), *P. castillensis* (24). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and/or size (e.g. Lobo and Quinteros, 2005a); and body shape has been studied in only a few species (González Marín et al., 2016a, 2016b).

The *Phymaturus patagonicus* clade includes 26 species (Fig. 1), all with restricted geographic distributions along Patagonian Steppe environments of southern South America from 35°S in southern Mendoza, to 45°S in southern Chubut province (Ceï 1986, 1993; Morando et al., 2013). All species are viviparous (Schulte et al., 2000; Ceï et al., 2003), primarily herbivorous (Espinoza et al., 2004) and strictly saxicolous, inhabiting mainly volcanic plateaus and peaks (Ceï, 1986). These lizards take advantage of rock crevices to hide from predators (Pietrek et al., 2009), and for thermoregulation (Ibargüengoytía et al., 2008; Vidal et al., 2010). The strict dependency on these microenvironments makes *Phymaturus* a good candidate clade in which to study the evolution of body size and shape, since both traits are likely to be constrained in order to allow these lizards to take advantage of the rock fissures. That is, if the morphology of these lizards favors the use of cracks for the survival, then natural selection could “drive” the evolution of morphology to an adaptive optimum, and with it a decrease in interspecific variation of these traits (i.e. stabilizing selection, Olave et al., 2017); this is our working hypothesis. Thus, absence of pronounced interspecific variation in these traits is expected among these closely-related species (Lobo and Quinteros, 2005a).

In contrast, levels of genetic divergence among species of this clade are likely to be less constrained (at least for neutral markers). At the molecular level, it is expected that given enough time and relative to

population size, at some point diverging lineages (species) will reach reciprocal monophyly in absence of interspecific gene flow (Degnan and Rosenberg, 2009; Rosenberg, 2013). Further, *Phymaturus* species are characterized by long generation times compared to other co-distributed lizards (e.g. the sister genus *Liolaemus*, geckos of the genus *Homonota*), since they do not reach sexual maturity until ~7–8 years (Piantoni et al., 2006). Thus, longer generation times can “stretch” incomplete lineage sorting (ILS; Degnan and Rosenberg 2009; Rosenberg, 2013) over longer absolute times. *Phymaturus* is also characterized by small populations with restricted distributions (Abdala et al., 2012; Minoli et al., 2015), which would accelerate “allele sorting” to reciprocal monophyly. This attribute of *Phymaturus* may “cancel out” the allele-sorting effects of longer generation times. Further, post-divergence hybridization and gene flow may also delay “allele sorting” (Mallet, 2005, 2007), and this phenomenon has been reported for several species groups in *Liolaemus* (e.g. Olave et al., 2017; Camargo et al., 2012).

Interspecific hybridization has been suggested as a possibility for some species of *Phymaturus patagonicus* clade inhabiting northwestern Patagonia (Lobo et al., 2012b; Morando et al., 2013). In this study we analyzed interspecific variation in body size and head shape within this clade to compare levels of genetic divergence with respect to morphological divergence, and evaluate the role of natural selection in the evolution of body size and head shape. We show that even when there are clear genetic differences among all species, changes in body size and

head shape are extremely limited. We detect strong selection acting to maintain the body size and shape at an evolutionary optimum, and we discuss the role of these phenotypic traits in *Phymaturus* survival in relation to the conservative life history.

## 2. Material and methods

### 2.1. Field work and material examined

We examined 808 specimens representing 24 of the 26 described species of the *Phymaturus patagonicus* clade; we did not include the recently described species *P. curivilcun* (Scolaro et al., 2016) and *P. desuetus* (Scolaro and Tapari, 2009) because there are no specimens available. We used specimens deposited in the LJAMM-CNP herpetological collection of the Instituto Patagónico para el Estudio de los Ecosistemas Continentales, Consejo Nacional de Investigaciones Científicas y Técnicas (IPEEC-CONICET), Puerto Madryn, Argentina. All specimens were collected by hand, euthanized by a pericardiac injection of sodium tiopentotal Pentovet®, fixed in 10–20% formalin and stored in 70% ethanol. We also examined the type material deposited in several herpetological collections (Appendix A).

Within the *P. patagonicus* clade, we recognized the five taxonomic groups proposed by Morando et al. (2013): *calcogaster*, *indistinctus*, *payunia*, *somuncurensis* and *spurcus* groups. Because there is no clear support for the placement of either *P. tenebrosus* or *P. cavivioi* within any of these groups (*P. sp.* 18 and *P. sp.* 19, in Morando et al., 2013), we included *P. tenebrosus* in the *spurcus* group and *P. cavivioi* in the *somuncurensis* group, based on their geographic proximity to these respective species' groups.

### 2.2. Molecular data

Genomic DNA was extracted using the Qiagen® DNeasy® Tissue Kit following the protocol provided by the manufacturer. The mitochondrial *cyt-b* gene was amplified by PCR following the protocols of Morando et al. (2003, 2004) for mitochondrial genes (*cyt-b* [~800 bp]). All sequences were edited and aligned using the program Sequencher v4.8. (™Gene Codes Corporation Inc. 2007). In addition, we used a total of 11 nuclear loci extracted from GenBank used in the phylogenetic study by Morando et al. (2013), detailed in Table 1 (Appendix B). Nuclear genes were phased with the DnaSp program, thus doubling the amount of data in the matrix and avoiding ambiguities in heterozygous individuals.

### 2.3. Morphometric data

We took linear measurements with a digital caliper (to the nearest 0.1 mm) as follows: snout-vent length (SVL; from tip of snout to vent), axilla-groin distance (AGD; distance from axilla to groin from posterior

edge of forelimb insertion to anterior edge of hindlimb insertion), head length (HL; distance between anterior edge of auditory meatus and snout tip), head width (HW; taken from the temporal regions), head height (HH; from highest parietal point to the throat), foot length (FL; from tip of toe of the 4th toe to heel), tibia length (TL; greatest length of tibia, from knee to heel), arm length (AL; from tip of toe of the 3rd toe to elbow), rostral-pineal length (RPL, distance between rostral and pineal scale), nostril-eye length (NEL, shortest distance from nostril to the eye), and nasal width (DEN, distance between nostrils).

We used a Principal Component Analysis (PCA) to present a visualization of differences and similarities between species of each group to identify which variables contribute most to the morphometric variation (Claude, 2008; Abdi et al., 2013; Minoli et al., 2014). We also implemented a multivariate analysis of variance (MANOVA) and Hotelling contrasts to test for significant differences between species. We performed statistical analyses using R 3.1.3 (R Development Core Team, 2014), FactoMiner 1.21 package (Lê et al., 2008; Husson et al., 2013) for PCA, and INFOSTAT (Di Rienzo et al., 2016) for MANOVA tests. We also used the morphometric data in a joint Bayesian inference of species boundaries with the molecular data in the program iBPP (Solís-Lemus et al., 2015).

### 2.4. Geometric data

We used a geometric morphometric approach to capture variation in form and to provide a solid statistical framework for studying the general properties of body shape (Bookstein, 1991; Adams et al., 2004; Kaliontzopoulou, 2011). We used a digital camera (Lumix Fz 60, resolution 16 MP 24×) to capture head shape (dorsal view), and employed the program Make-Fan6 (Sheets, 2005) to produce equidistant ratios that established the bases for drawing the contours. We digitized eight landmarks (L) and 13 semi-landmarks (SL) on the dorsal right side of the head with the tpsDig2 version 2.16 software (Rohlf, 2010a), and tpsUtil version 1.52 (Rohlf, 2012). We positioned landmarks on the following sites: snout (front end of the rostrum), posterior margin of the right nostril, front edge of the right eye (first supraocular scale), posterior edge of the right eye (last supraocular scale), median superciliary scale (up to the midline between the first and last supraocular scale), median circumorbital scale (up to midline between the first and last supraocular scale), pineal scale, and the last scale of the neck fold (up to the pineal scale) (Appendix C). We eliminated the effects of rotation, translation and scaling (Rohlf and Slice, 1990) with Generalized Procrustes Analysis using tpsRelw version 1.49 software (Rohlf, 2010b) for semi-landmarks, and MorphoJ version 2.0 (Klingenberg, 2011) for landmarks. For these analyses, we established *a priori* groups corresponding to recognized species within each group (Morando et al., 2013). We analyzed shape variation implementing a canonical variate analysis (CVA).

Lastly, data from the geometric analyses were used in a joint

**Table 1**

Summary of the sequences used in this study. Marker: gene used. Length: length of sequence in base pairs, n: number of specimen used, Author: data source, GB: GenBank accession numbers.

Marker	Length (bp)	n	Author	GB
Cytb	742	170	Morando et al. (2013)	JX969009–JX969028/JX969029–JX969059
cmos	523	23	Morando et al. (2013)	JX969517–JX969533/JX969534–JX969563
NT3	541	22	Morando et al. (2013)	JX969595–JX969614/JX969564–JX969594
PRLR	533	22	Morando et al. (2013)	JX969497–JX969516/JX969466–JX969496
PNN	1004	21	Morando et al. (2013)	JX969420–JX969436/JX969437–JX969465
Phy38	735	20	Morando et al. (2013)	JX969123–JX969142/JX969143–JX969170
Phy41	576	21	Morando et al. (2013)	JX969315–JX969334/JX969335–JX969364
Phy60	917	20	Morando et al. (2013)	JX969171–JX969185/JX969186–JX969215
Phy64	631	17	Morando et al. (2013)	JX969111–JX969122/JX969365–JX969389
Phy84	616	22	Morando et al. (2013)	JX969216–JX969235/JX969236–JX969266
Phy87	737	22	Morando et al. (2013)	JX969390–JX969419
Phy89	632	21	Morando et al. (2013)	JX969267–JX969285/JX969286–JX969314

Bayesian inference of species boundaries with the genetic data in the program iBPP (Solís-Lemus et al., 2015). For these analyses, we extracted values from PC1-5 scores from the geometric analyses (Huang and Knowles, 2016).

## 2.5. Evaluating the level of molecular and morphological divergence

We evaluated the level of genetic vs phenotypic divergence among *P. patagonicus* species using the program iBPP (Solís-Lemus et al., 2015). This program was originally developed to test species boundaries based on both genetic and morphological data in an integrative model-based Bayesian framework. Here, following Olave et al. (2017), we compared the level of genetic and morphological divergence observed among lineages. We performed two combined analyses: 1 – molecular data with morphometric data, and 2 – molecular data with geometric data; we then analyzed each data set independently: 3 – molecular data, 4 – morphometric data, and 5 – geometric data. We also subdivided the morphological dataset for males and females and ran independent analyses for both sexes.

This program uses the reversible-jump Markov Chain Monte Carlo algorithm (rjMCMC) that calculates the posterior probabilities of the existence of each node in a given phylogeny as a guide tree. The program sequentially collapses internal nodes, and calculates the posterior probability of each  $\tau$  equal to or different from 0. The models explored are labeled with 0 and 1 at each node, specifying collapsed or split nodes, respectively; our approach specifies alternative models ranging from 111 (four species) to 000 (single species). We included a prior distribution of  $\theta$  and  $\tau = G(2, 2000)$ , which leads to a mean = 0.001, and left default values of  $\sigma^2$  and  $\kappa = 0$ , thus the priors are non-informative and the program estimates them. We used the species tree proposed by Morando et al. (2013; their Fig. 6b) as guide tree. For the unresolved cases (e.g. the *P. payunia* group), we used the Bayesian hypothesis (Fig. 5 in Morando et al., 2013), which is congruent with other published phylogenetic trees (Lobo et al., 2012a, 2018). The modified species tree based on mitochondrial and nuclear DNA sequences from Morando et al. (2013) was represented by GenGIS software (version 2.4.0) and is depicted in Fig. 1.

We ran MCMC analyses for > 100,000 generations, sampled every 10,000 steps (10% burnin), and performed independent analyses for each of the five groups within the *P. patagonicus* clade. *Phymaturus tenebrosus* and *P. cavioi* were not included in this analysis because there is no statistical support for their inclusion in any of the groups (Morando et al., 2013).

We interpreted values with posterior probability > 0.95 as strong support in favor of a speciation event (Leaché and Fujita, 2010). To generate PC plots with mitochondrial data we used the RGL package in R to visualize differences and similarities among species.

## 2.6. Estimating morphological evolution

We used the species tree proposed by Morando et al. (2013) to study the evolution of the morphological variables (morphometric and geometric), and to evaluate the fit of different models of morphological evolution using the GEIGER package in R (Harmon et al., 2008). We tested a total of five models: Brownian motion (BM), Ornstein-Uhlenbeck (OU), Early-burst (EB), trend and kappa. These models include both neutral evolution and selection, and we used the corrected Akaike Criterion (AICc) to select the best-fit model.

## 3. Results

### 3.1. Interspecific molecular divergence within the *P. patagonicus* clade

The combined analyzes of molecular + morphometric data with iBPP estimated PP > 0.95 for each node in the five groups, except *P. spurcus* and *P. spectabilis* (red terminals in Fig. 1), where PP = 0.93

(Fig. 2D). Topologies for the molecular + geometric data inferred PP > 0.95 for all nodes (Fig. 2A–E), and results of the molecular-only analyses remain unchanged for all groups (PP > 0.95 for each node; Fig. 2A–E).

The principal component analyses performed with the cyt-b gene does not show overlap between species for any of the five groups (Fig. 3A–E). Interestingly, some individuals of *P. castillensis* (Fig. 3B) and *P. spectabilis* (Fig. 3E) were inferred as highly different. The inter-specific comparisons show that *P. rahuensis* and *P. sitesi* are closely related to *P. nevadoi* and *P. payunia*, but without overlapping (Fig. 3C).

### 3.2. Body size and head shape differences among the *Phymaturus patagonicus* species

We present the results of iBPP analyses using the full morphological dataset (Fig. 2) and for females (Fig. S1) and males (Fig. S2) separately (see Supplementary Material). Given that all results are highly consistent, here we describe only the results of the full dataset (Fig. 2). The results of iBPP based on morphometric data inferred nodes with a wide range of values (Fig. 2A–E), all of them with lower support in comparison to the results from the molecular dataset. Lower values were inferred for the *spurcus* group (PP = 0.03–0.12; Fig. 2D), while the *payunia* group is resolved with higher values (PP = 0.89–1.0; Fig. 2A). Our results are variable for the rest of the groups. The *calcogaster* group is characterized by intermediate values (PP = 0.45–0.60; Fig. 2B), with *P. patagonicus* inferred with the highest values (PP = 1; Fig. 2B). Within the *somuncurensis* group values ranged from a low of PP = 0.19 for *P. ceii* / *P. somuncurensis* (Fig. 2C), to PP = 1 for the rest of the group (Fig. 2C). Similarly, values within the *indistinctus* group ranged from (PP = 0.47; Fig. 2E) for *P. videali* / *P. indistinctus* (PP = 0.47) to (PP = 1) for the rest of group.

Pairwise geometric divergence values were low for most groups (PP = 0.00–0.70; Fig. 2A–E; within which the *indistinctus* group had the lowest value), followed by *spurcus* group (PP = 0.00–0.02; within which the *P. spurcus* / *P. spectabilis* comparison was lowest), and, the *calcogaster* group (PP = 0.00–0.03; Fig. 2B; within which the *P. calcogaster* / *P. camilae* was lowest). The highest values were estimated for the *somuncurensis* group (PP = 0.49–0.94; Fig. 2C; with *P. ceii* / *P. somuncurensis* showing the lowest divergence), followed by the *payunia* group (PP = 0.10–0.70; Fig. 2A; with *P. nevadoi* / *P. payunia* showing the lower value).

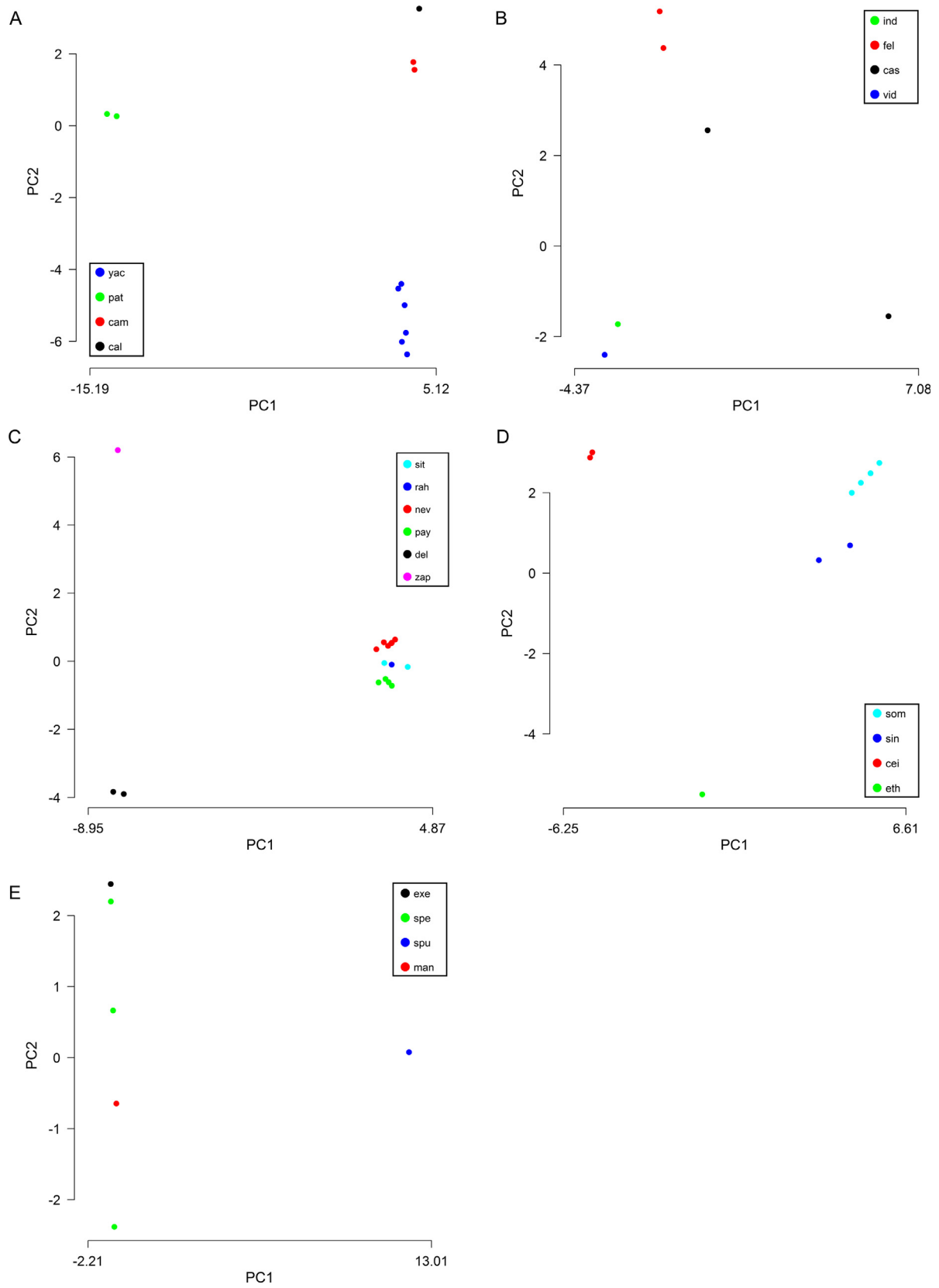
The MANOVA analyses identified significant differences for all combinations of species pairs ( $p < 0.0001$ ), but Hotteling contrasts showed overlap between species in three groups: (i) *P. spectabilis* × *P. excelsus* × *P. spurcus* and *P. tenebrosus* × *P. manulae*, (ii) *P. sitesi* × *P. delheyi* × *P. nevadoi* × *P. payunia*, and (iii) *P. somuncurensis* × *P. etheridgei* (Table 2). These three groups also overlapped in PCA confidence ellipses; overlap is almost 100% between *P. sitesi* with *P. zapalensis* and *P. payunia* (Fig. 4C), *P. etheridgei* and *P. ceii* with *P. somuncurensis* (Fig. 4D), and *P. spectabilis* with *P. excelsus* and *P. spurcus* (Fig. 4E). The only non-overlapping pair of groups are *P. calcogaster* (Fig. 4A) and *P. indistinctus* (Fig. 4B). The PCA summarizing all continuous variables indicated that most of the overall variation could be explained by the first three components (Table 3).

Finally, some species within groups differed significantly in head shape (Mahalanobis distance,  $p < 0.0001$ , Table 4). The CVA scatter plot resolved species as isolated groups (Fig. 5A, D and E); overlap was limited but not significant between *P. indistinctus* and *P. videlai* (Fig. 5B), and *P. nevadoi* and *P. payunia* (Fig. 5C). In Fig. 5, the wire-frame graph shows the shape variation along each axis; values are highest in the temporal (Sl: 13–17) and occipital regions (Sl: 18–21).

### 3.3. Morphological evolution

Two models best explained morphological evolution in the *Phymaturus patagonicus* clade (Table 5): thirteen morphometric





**Fig. 3.** Principal component analysis (PCA) of mitochondrial and nuclear genes. References: *payunia* (A), *calcogaster* (B), *somuncurensis* (C), *spurus* (D) and *indistinctus* (E) groups.

**Table 2**

Summary statistics and Hotteling contrast results for species of the *Phymaturus patagonicus* clade: *calcogaster* (a), *indistinctus* (b), *payunia* (c), *somuncurensis* (d), and *spurcus* (e) groups. Means sharing the same letter are not significantly different ( $p > 0.05$ ).

Species	Variables											n	Class	
(a)														
	SVL	HL	HW	HH	DEN	NEL	RPL	AGD	AL	TL	FL			
<i>P. yachanana</i>	83.48	14.62	14.69	8.37	2.38	4.72	10.58	40.85	24.88	16.27	22.59	20	A	
<i>P. patagonicus</i>	87.83	15.12	15.62	9.4	2.36	4.61	10.88	45	27.11	16.85	24.36	37		B
<i>P. camilae</i>	88.57	15.24	15.57	8.94	2.53	5.06	10.97	43.24	25.69	16.37	23.46	41		C
<i>P. calcogaster</i>	87.91	14.96	15.52	8.98	2.5	4.69	10.69	43.67	25.83	16.48	23.26	50		D
(b)														
<i>P. videlai</i>	86.76	14.91	15.24	9.08	2.43	4.81	10.63	43.85	25.16	15.32	22.3	23	A	
<i>P. indistinctus</i>	83.68	14.57	15.16	9.03	2.49	4.78	10.56	40.47	25.49	16.08	23	27		B
<i>P. felixi</i>	81.21	14.09	14.48	8.44	2.36	4.39	10.08	40.5	23.39	14.75	20.58	38		C
<i>P. castillensis</i>	88.99	15.35	15.73	9.37	2.72	5.03	10.8	43.86	27.09	17.17	24.74	42		D
(c)														
<i>P. zapalensis</i>	85.11	14.29	14.53	8.87	2.27	4.08	10.33	42.58	24.47	16.09	22.75	31	A	
<i>P. rahuensis</i>	86.32	15.13	15.73	9.06	2.76	4.75	10.57	43.42	24.78	16.88	22.8	15		B
<i>P. payunia</i>	85.02	13.59	13.71	8.79	2.12	4.1	9.97	41.87	24.57	16.08	22.99	20		C
<i>P. delheyi</i>	87.87	14.06	14.11	8.94	2.06	4.34	10.49	43.81	26.52	16.82	24.65	18		D
<i>P. sitesi</i>	83.69	13.93	14.19	8.71	2.15	4.15	10.05	41.38	25.6	15.91	23.05	15		D
<i>P. nevadoi</i>	87.23	14.08	14.21	8.83	2.44	4.38	10.21	44.07	25.78	16.51	23.88	25		D
(d)														
<i>P. sinervo</i>	89.89	15.42	15.7	9.33	2.8	5.11	11.13	44.46	26.41	17.52	23.87	46	A	
<i>P. ceii</i>	87.41	14.91	14.98	8.85	2.6	4.69	10.78	42.96	26.15	16.54	23.28	42		B
<i>P. cacivio</i>	93.17	16.09	16.3	9.13	2.9	5.15	11.2	46.19	27	17.93	24.28	27		C
<i>P. somuncurensis</i>	86.23	14.95	15.29	8.92	2.6	4.71	10.65	42.56	25.36	16.4	22.67	31		D
<i>P. etheridgei</i>	86.03	15.05	14.97	9	2.62	4.8	10.75	42.33	25.26	16.1	23	45		D
(e)														
<i>P. manuelae</i>	92.32	15.72	16.26	9.53	2.63	4.67	11.12	47.21	27.28	17.36	24.45	9	A	B
<i>P. spurcus</i>	87.95	15.2	15.29	8.84	2.56	4.78	10.69	44.08	25.87	16.49	23.6	32	A	C
<i>P. tenebrosus</i>	89.83	15.57	16.07	9.34	2.81	4.95	11.11	44.97	26.27	17.01	24.26	29		B
<i>P. spectabilis</i>	87.63	15.06	15	8.76	2.71	4.82	10.73	44.15	25.25	16.21	23.01	55		C
<i>P. excelsus</i>	86.23	15.14	14.82	8.63	2.57	4.71	10.52	42.54	25.43	16.23	23.51	36		C

variables fit an Ornstein-Uhlenbeck model (OU), and the other three variables (DEN, RPL and PC3) fit a Brownian model (BM).

## 4. Discussion

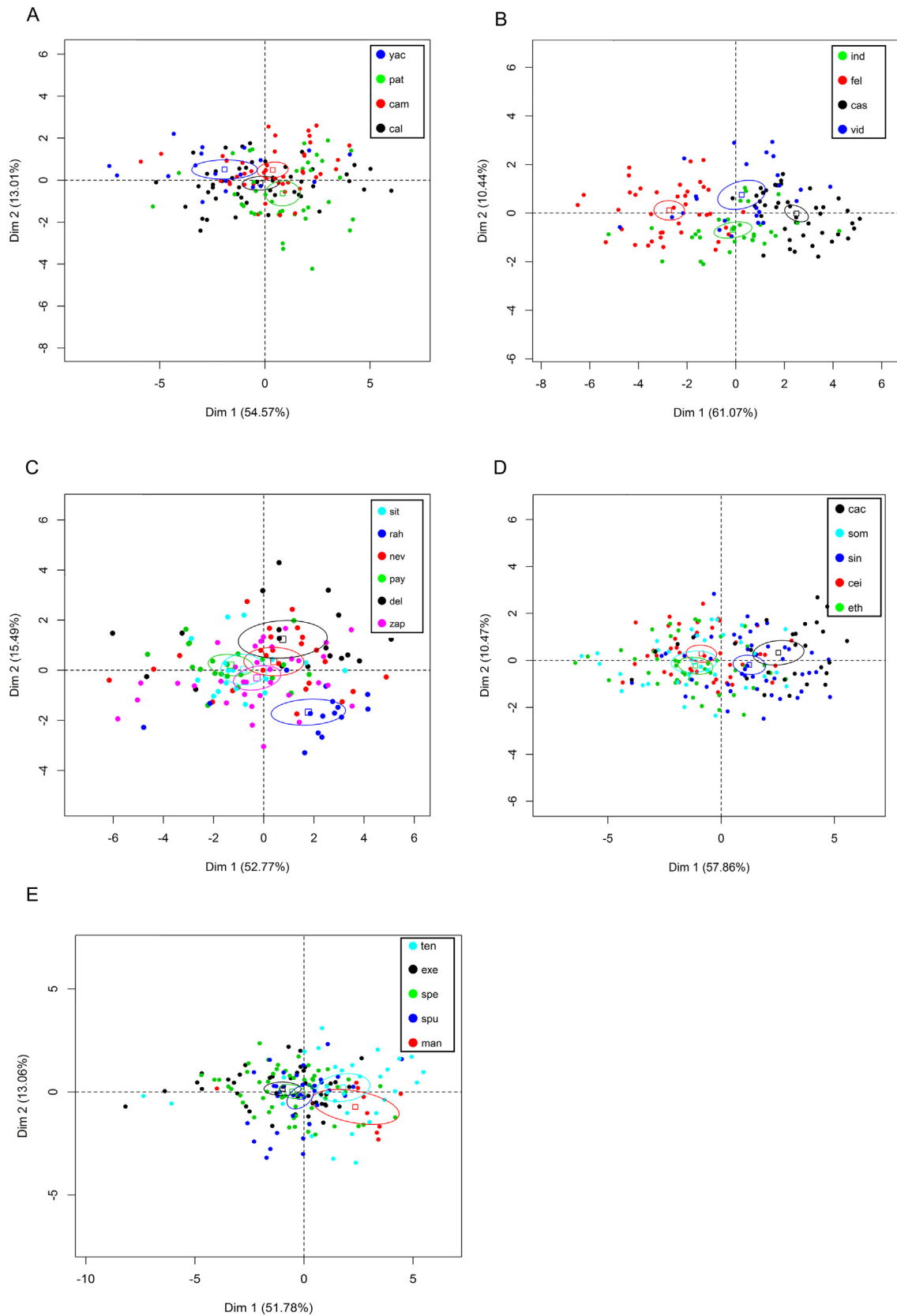
### 4.1. Lack of size and shape differences among *Phymaturus* species: the role of natural selection

Diversification studies have been carried out at different levels (e.g. genetic vs morphological, or ecological vs genetic), showing as a result differences in diversification rates, which are not always correlated (Adams et al., 2009). The genus *Phymaturus* is a good model within which to quantify interspecific genetic and morphological divergence; all are characterized by a conserved and apparently specialized morphology for using rock cracks as refuge (Pietrek et al., 2009). Here we show within-clade differences in molecular vs morphological divergence; the former show conventional “clock-like” divergence, while the morphological characters (size and shape) are highly conserved. Analyses based on molecular (Figs. 2 and 3) and combined molecular + morphological data (Fig. 2) show clear differences among species within each of the groups; probably in the combined analyses the results could be largely due to the genetic data. This is expected when enough time has passed since the divergence of species, and the pattern of incomplete lineage sorting has been (at least partially) overwritten (Maddison, 1997; de Queiroz, 2007).

In contrast with genetic data, interspecific differences in body size and head shape were lower within the *P. patagonicus* clade, for both metric (Fig. 4; Table 3) and geometric data (Fig. 5; Table 4). Tests for body size differences were significant (Table 2) but with extensive overlap in three of the five groups (Table 2C-D; Fig. 4C-D), with differences in the *calcogaster* and *indistinctus* groups (Table 2A, B; Fig. 4A, B). Furthermore, the iBPP result based on the morphological data does not reflect differences between these two groups (Fig. 2B and E), or

almost any of the others (Fig. 2C and D), except the *payunia* group (Fig. 2A). Analyses based on geometric data did not find differences for any of these species (Fig. 2A and D). Morphological differences may reflect life history traits (e.g. habitat use, competition or sexual selection), thus the evolution of morphology is likely driven by more than one factor. For *Phymaturus* species, body size and head shape have been used to delimit species (Avila et al., 2014; González Marín et al. 2016a), for which they proved to be good estimators, but little is known about how these phenotypic traits change through time. Our results reveal the lack of interspecific differences in body size and head shape among most of the species of the *Phymaturus patagonicus* group.

Absence of morphological divergence for the relatively old *P. patagonicus* group (25 mya, Schulte, 2013) seems unlikely to be random. Our results (Table 5) revealed that patterns of body size and head shape evolution in the *P. patagonicus* clade were best explained by Ornstein-Uhlenbeck models (OU) in most cases. The Brownian Motion (BM) model (simple diffusion) includes random fluctuations through time (Felsenstein, 1988; Harmon et al., 2010), and it only fits best two traits (DEN, RPL), as well as the third principle component (PC3). In contrast, the OU model is the best fit for 13 of the 16 morphological variables included here; consistent with a history of stabilizing selection driving the population to an adaptive optimum (Butler and King, 2004; Harmon et al., 2010). Similar tests of morphological evolution have been carried out in *Liolaemus*, and 50% of the analyzed variables (most associated with locomotor performance) are explained by the OU model (Tulli et al., 2016), suggesting stabilizing selection. A recent study of the *Liolaemus rothi* complex focused on nine continuous variables, and showed a “trend” model as the best fit, suggesting a linear rate of selection (increment and decrement) for most of them (Olave et al., 2017). Reaney et al. (2018) also found that the OU model fits better the evolution of SVL in *Phymaturus*. The authors found that the mode of lineage diversification in *Phymaturus* seems to follow a non-adaptive radiation, and attributed this to the clade's conservative life history,



**Fig. 4.** Principal component analysis (PCA) of continuous variables. Confidence 95% ellipses were considered around the species individuals. References: *payunia* (A), *calcogaster* (B), *somuncurensis* (C), *spurcus* (D) and *indistinctus* (E) groups.



**Table 3**  
Principal Component Analysis (PCA) results based on continuous variables. Numbers in bold are variables that contribute most to the axis.

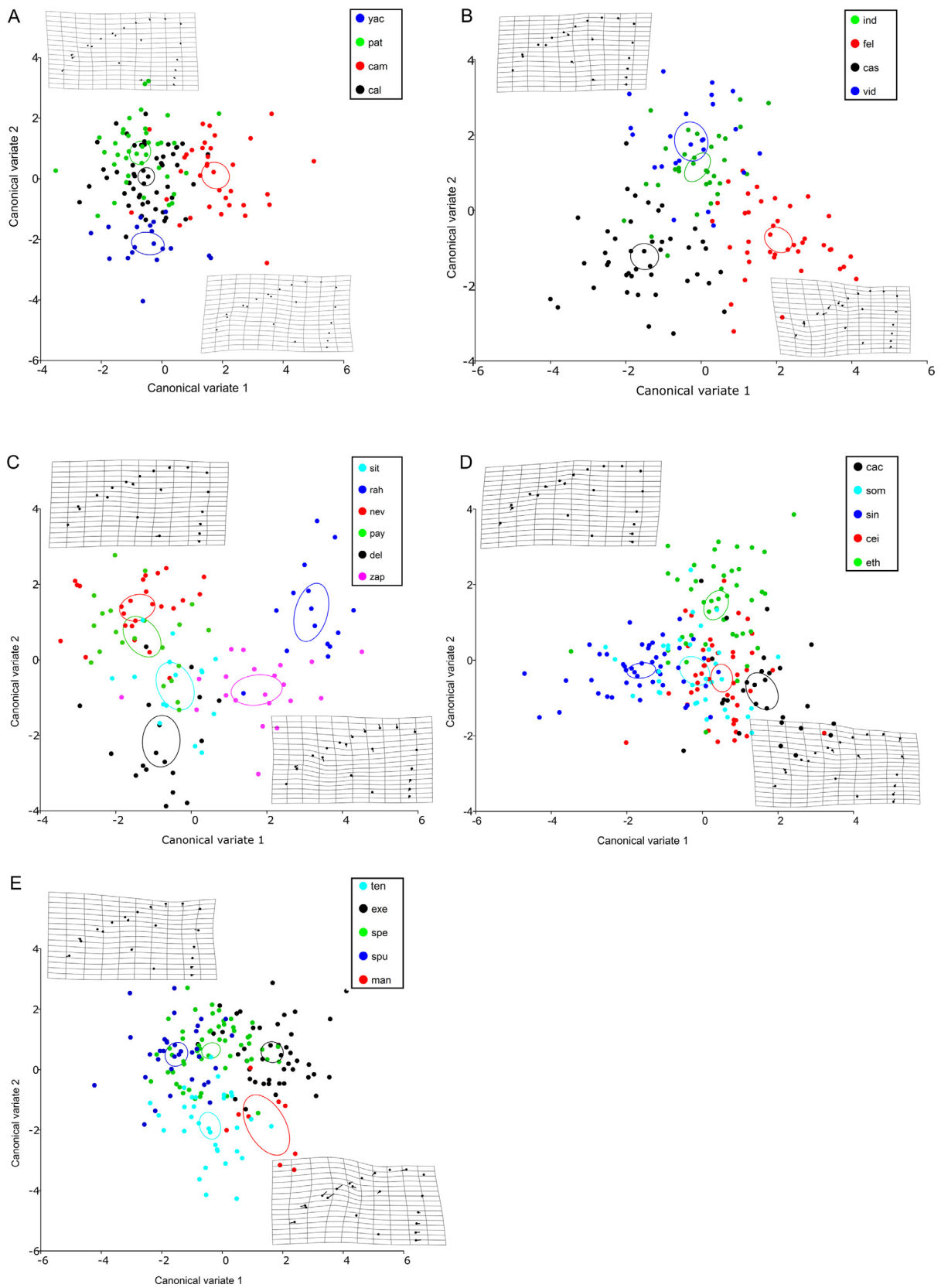
	Eigenvalues			Eigenvectors										
	Eigenvalue per component	% of Variance	Cumulative % of variance	SVL	HL	HW	HH	DEN	NEL	RPL	AGD	AL	TL	FL
<i>calcogaster</i> group														
PC1	6.00	54.57	54.57	9.01	<b>13.56</b>	<b>13.15</b>	12.04	3.39	3.26	10.49	3.34	10.44	11.39	9.93
PC2	1.43	13.01	67.57	12.25	0.42	0.09	0.02	18.49	<b>22.61</b>	3.74	<b>35.01</b>	5.44	1.88	0.04
PC3	1.14	10.36	77.93	18.58	0.08	1.04	3.31	14.05	17.81	0.01	20.81	10.15	0.92	13.26
<i>indistinctus</i> group														
PC1	6.72	61.07	61.07	9.76	<b>12.63</b>	<b>10.74</b>	10.52	4.28	9.06	10.72	3.55	10.38	9.03	9.33
PC2	1.15	10.44	71.51	<b>22.81</b>	0.02	1.23	0.92	0.07	0.58	0.47	<b>61.51</b>	1.89	4.63	5.87
PC3	0.77	6.99	78.49	0.46	0.68	3.18	2.38	87.89	0.10	4.72	0.14	0.05	0.20	0.19
<i>payunia</i> group														
PC1	5.80	52.77	52.77	9.39	<b>13.72</b>	<b>11.27</b>	9.79	4.73	7.43	13.12	4.11	7.27	12.26	6.92
PC2	1.70	15.49	68.25	11.36	3.26	6.47	2.61	<b>20.62</b>	<b>8.23</b>	0.35	15.38	<b>16.21</b>	0.03	15.47
PC3	1.02	9.24	77.50	14.54	0.19	0.01	4.65	6.60	1.53	0.34	43.41	7.93	6.23	14.56
<i>somuncurensis</i> group														
PC1	6.37	57.86	57.86	9.96	<b>12.53</b>	<b>11.95</b>	7.98	6.95	7.87	9.48	5.43	8.89	11.04	7.90
PC2	1.15	10.47	68.34	<b>24.41</b>	0.76	0.79	11.11	3.30	9.70	4.51	<b>41.74</b>	3.33	0.21	0.15
PC3	0.98	8.93	77.27	2.91	0.14	0.65	0.01	2.90	10.50	8.93	11.45	27.13	0.43	34.95
<i>spurcus</i> group														
PC1	5.70	51.78	51.78	10.50	<b>14.07</b>	<b>13.31</b>	13.03	3.83	4.61	13.03	5.93	8.26	10.69	2.74
PC2	1.44	13.06	64.84	16.17	0.25	0.47	1.16	<b>25.66</b>	24.00	0.00	<b>26.91</b>	2.69	0.52	2.16
PC3	1.02	9.32	74.16	2.72	0.08	0.07	0.28	8.91	13.90	0.60	4.65	2.87	5.13	60.80

especially the viviparous reproduction. This mode of reproduction is characteristic of other cold-climate lizards (Guillette, 1993), and might act as barriers to their radiation across warmer climates, where oviparous species predominate, including several viviparous groups (*Lio-laemus*) widely distributed throughout different Patagonia environments that, unlike *Phymaturus*, occur in a great diversity of different environments. In cases of relatively deeply divergent groups, such as the *P. patagonicus* clade, this is expected if a correlated suite of morphological characters key for survival is maintained by stabilizing selection.

Previous ecomorphological studies show that, in addition to morphology, some species of *Phymaturus* show aspects of their life histories that are also conserved (Abdala et al., 2014; Tulli et al., 2011, 2016), including reproduction (viviparous, Schulte et al., 2000; Cei et al., 2003), niche (Debandi et al., 2012), diet (Espinoza et al., 2004), thermal biology (Cruz et al., 2009), and habitat use (Tulli et al., 2011, 2016). Specifically, flattened bodies typically characterize saxicolous lizards (e.g. *Tropidurus semitaeniatus*, Pelegrin et al., 2017; *Sauromalus obesus*, Stebbins, 1985; *Platysaurus intermedius*, Howard and Hailey,

**Table 4**  
Canonical variate analysis performed between species of each groups. Results are reported as Mahalanobis distances after 10,000 permutation runs. All distances were significant (p < 0.0001).

<i>calcogaster</i> group					
	<i>P. calcogaster</i>	<i>P. camilae</i>	<i>P. patagonicus</i>		
<i>P. camilae</i>	25,338	0			
<i>P. patagonicus</i>	21,159	27,323	0		
<i>P. yachanana</i>	27,829	32,178	30,238		
<i>indistinctus</i> group					
	<i>P. castillensis</i>	<i>P. felixi</i>	<i>P. indistinctus</i>		
<i>P. felixi</i>	35,799	0			
<i>P. indistinctus</i>	31,688	32,976	0		
<i>P. videlai</i>	35,592	37,275	29,848		
<i>payunia</i> group					
	<i>P. delheyi</i>	<i>P. nevadoi</i>	<i>P. payunia</i>	<i>P. sitesi</i>	<i>P. rahuensis</i>
<i>P. nevadoi</i>	3845	0			
<i>P. payunia</i>	38,596	31,063	0		
<i>P. sitesi</i>	31,116	34,256	30,218	0	
<i>P. rahuensis</i>	53,064	49,504	50,588	46,225	0
<i>P. zapalensis</i>	37,903	42,221	4,005	34,126	36,353
<i>somuncurensis</i> group					
	<i>P. cacivioi</i>	<i>P. ceii</i>	<i>P. etheridgei</i>	<i>P. sinervoi</i>	
<i>P. ceii</i>	23,835	0			
<i>P. etheridgei</i>	27,569	22,593	0		
<i>P. sinervoi</i>	3316	25,356	26,632	0	
<i>P. somuncurensis</i>	27,666	21,097	23,697	23,487	
<i>spurcus</i> group					
	<i>P. excelstus</i>	<i>P. manuelae</i>	<i>P. spectabilis</i>	<i>P. spurcus</i>	
<i>P. manuelae</i>	36,349	0			
<i>P. spectabilis</i>	22,898	39,291	0		
<i>P. spurcus</i>	32,412	43,882	18,844	0	
<i>P. tenebrosus</i>	31,935	36,029	26,647	29,111	



**Fig. 5.** Canonical variate analysis with wireframe graph of geometric morphometric variables. Confidence 95% ellipses were considered around the species individuals. References: *payuniae* (A), *calcogaster* (B), *somuncurensis* (C), *spurcus* (D) and *indistinctus* (E) groups.

**Table 5**  
AICc morphological evolution results. Selected models are shown with an asterisk for each variable.

Variables	Models				
	BM	OU	EB	Trend	Kappa
SVL	139.35	131.22*	137.45	135.05	137.29
HL	126.78	126.16*	129.58	127.18	128.40
HW	130.50	128.91*	133.29	130.44	132.30
HH	131.21	125.81*	134.00	130.29	134.00
DEN	118.24*	119.25	121.03	118.96	121.03
NEL	125.59	125.41*	128.38	126.13	127.22
RPL	127.66*	127.76	130.46	128.30	129.06
AGD	156.89	132.41*	142.64	139.23	141.95
AL	138.42	132.05*	141.22	137.52	141.16
TL	136.01	130.60*	138.81	135.25	138.58
FL	135.84	130.25*	138.63	135.16	138.63
PC1	453.49	452.23*	456.28	454.62	456.26
PC2	596.64	596.23*	599.44	597.46	597.49
PC3	597.98*	597.98	600.77	598.89	600.40
PC4	553.42	549.00*	556.22	553.40	555.59
PC5	545.76	543.21*	548.56	546.15	548.52

1999), which are hypothesized to facilitate the use of rock crevices as refuges from predators (Pietrek et al., 2009), as well as for thermoregulation (Ibargüengoytía et al., 2008; Vidal et al., 2010; Eloi and Leite-Filho, 2013). In terms of thermoregulation, since species of the *P. patagonicus* clade live in a climate characterized by dry cold, with winter temperatures up to  $-22.8^{\circ}\text{C}$  (Conti, 1998), then the thermal properties of the rocks may help increase their body temperatures (Krenz, 2008; Vidal et al., 2010). Further, a refuge in the cracks can serve as a defense mechanism, because these saxicolous lizards are characterized by short limbs, and seem to be more specialized for climbing than running (Tulli et al., 2011, 2016), so that their escape from predators could be slow. This particular environment (e.g. ecological niche conservatism) can act as mechanism (stabilizing selection) for morphological stasis.

Thus, the strict dependency of *Phymaturus* to volcanic plateaus could be driving the evolution of size and head shape to an adaptive optimum (Table 5), and possibly constrain interspecific variation among closely related species (Figs. 4 and 5). Likewise, the minimum phenotypic divergence between sister lineages (low PP values; Fig. 2) in morphometric and geometric data, reinforces the idea of conserved morphological evolution.

#### 4.2. Taxonomic comments on species of the *Phymaturus patagonicus* clade

The number of described *Phymaturus* species has tripled in the last 10 years (Abdala and Quinteros, 2014), most of which were diagnosed with limited morphological differences. This has generated much discussion about the validity of some species (Lobo and Quinteros, 2005b; Lobo et al., 2010, 2012b; Avila et al., 2014). Here, we applied a coalescent-based species delimitation method, and show strong molecular differences among all described species of the *P. patagonicus* group. This

## Appendix A

List of specimens studied, sampled locations and geographic coordinates. The acronyms correspond to the following herpetological collections: LJAMM-CNP (Centro Nacional Patagónico, Puerto Madryn, Argentina), UNCo (Universidad Nacional de Comahue, San Carlos de Bariloche, Río Negro), DC-JMC (José Miguel Cei, Universidad Nacional de San Luis, San Luis, Argentina), JAS-DC (José Alejandro Scolaro, Centro Nacional Patagónico, Puerto Madryn, Argentina), MCN (Museo de Ciencias Naturales, Universidad Nacional de Salta, Argentina), MLP.S/R (Museo de La Plata, Universidad Nacional de La Plata, La Plata, Argentina), FML (Fundación Miguel Lillo, Tucumán).

*Phymaturus cavivioi* (24): ARGENTINA: RÍO NEGRO: Pilcaniyeu Department: Provincial Road 67, 19.7 km NE Cañadon Chileno ( $-40.795$ ;  $-69.881$ ): LJAMM-CNP 16148–16156, 16158–16160. El Cuy Department: Provincial Road 67, 20 km NE Mencúé ( $-40.325$ ;  $-69.439$ ): LJAMM-CNP 16162–16168. Provincial Road 67, 19.2 Km NE Mencúé: ( $-40.325$ ;  $-69.438$ ): LJAMM-CNP 5541. Provincial Road 67. 20 Km S Mencúé ( $-40.568$ ;  $-69.750$ ): LJAMM-CNP 5547–49. Provincial Road 67. 37.9 km SWE junction Provincial Roads 6 and 74 ( $-40.316$ ;  $-69.368$ ): LJAMM-CNP 6996–7003.

includes the *P. spurcus* group, characterized by a history of uncertainty about species boundaries (Lobo et al., 2012a,b; Avila et al., 2014; Corbalán et al., 2016). Earlier studies have hypothesized interspecific hybridization (based on molecular data; Morando et al., 2013), or morphological polymorphism within a single species, based on color pattern (Avila et al., 2014) and geographically proximal distributions (Lobo and Quinteros, 2005b). More recently, Corbalán et al. (2016) considered *P. excelsus* and *P. spectabilis* as synonyms of *P. spurcus* based on a single locus (COI). Similarly, Becker et al. (2018) based on three mtDNA (COI, ND1, ND2) and eight transfer RNAs proposed that these three species are conspecific. The limitation of these two studies is that they are almost exclusively based on mtDNA. Here, we provide molecular support for divergence for the species *P. spectabilis*, *P. spurcus* and *P. excelsus* (PP = 0.93–95; Fig. 2) analyzing multiple loci simultaneously, that provides much greater power to resolve boundaries between species than those using a single locus (Fujisawa et al., 2016). Nonetheless, it is necessary to analyze these three species at a population level with genomic data (e.g. SNPs, Camargo and Sites 2013) to fully understand their evolutionary history and species limits, which most probably include multiple events of hybridization.

We suggest that the lack of correlation between species' molecular diversification and morphological changes shown here (Fig. 2), highlights the limitations of focusing on morphometric data (linear and geometric) to delimit species in some groups. This is especially important within clades for which there is evidence that natural selection may have played a strong role in the evolution of body size and shape. We advocate an integrative approach (Dayrat, 2005; Padial and de la Riva, 2007; Padial et al., 2010) as the best strategy for species delimitation studies.

## 5. Conclusion

We found evidence of natural selection favoring the evolution of body size and head shape to an adaptive optimum in species of the *Phymaturus patagonicus* clade. This interpretation explains the observed lack of interspecific differences for these traits, even when most species can be delimited/diagnosed by clear genetic differences. Our findings provide clues to understand how natural selection operates during the speciation processes, and how different interspecific attributes within a clade could vary at different rates.

## Acknowledgments

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*Phymaturus calcogaster* (46): ARGENTINA: CHUBUT: Telsen Department: Road between Cona Niyeu and Telsen, 40.4 km Provincial Road 8 (–41.800; –65.150): LJAMM-CNP 13235–13237. Provincial Road 8, Quele Cura-Sierra Colorada (–42.227; –66.362): LJAMM-CNP 15384–15389. Provincial Road 8, Quele Cura-Sierra Colorada (–42.227; –66.361): LJAMM-CNP 6550–6553. Road to Laguna de las Vacas, 16.2 km Provincial Road 4 (–42.517; –67.359): LJAMM-CNP 6855–6857, 8125–8144. Laguna de las Vacas (–42.479; –67.365): MLP 5130–5135, 5352–5354. Laguna de las Vacas, SE of lagoon (–42.499; –67.351): UNSa 4295–4298, 4301–4304. Quele Cura (–42.183; –66.366): JAS 1095–1097, 1154–1156.

*Phymaturus camilae* (48): ARGENTINA: CHUBUT: Telsen Department: Provincial Road 4, 18 km E Provincial Road 11 (–42.517; –67.966): LJAMM-CNP 6101–6102. Provincial Road 67, 17.7 km N Gan Gan (–42.405; –68.257): LJAMM-CNP 3408. Provincial Road 67, 31.4 km N Gan Gan (–42.388; –68.159): LJAMM-CNP 3446, 3450. Provincial Road 4, 2 Km E Gan Gan (–42.529; –68.018): LJAMM-CNP 5592. Provincial Road 67, 20.7 km N Gan Gan (–42.400; –68.166): LJAMM-CNP 6492. E Gan Gan (–42.518; –68.270): LJAMM-CNP 6746–6748. Provincial Road 67, 17.6 km N Gan Gan (–42.424; –68.285): LJAMM-CNP 6771–6778. Provincial Road 67, 16 km N Gan Gan (–42.424; –68.286): LJAMM-CNP 6901. Provincial Road 67, 19.5 km N Gan Gan (–42.420; –68.239): LJAMM-CNP 7482–7485. Provincial Road 67, 20 km Gan Gan (–42.420; –68.238): LJAMM-CNP 7608–7614. Provincial Road 67, 22.6 km S Gan Gan (–42.696; –68.232): LJAMM-CNP 8186–8188. Gastre Department: Cerro Navidad, 3 km S Provincial Road 4, 40 km W Gan Gan: LJAMM-CNP 6106. Santana Sacanana (–42.465; –68.726): JAS 1957–1962, 2076–2080, 2101, 2118, 2125.

*Phymaturus castillensis* (43): ARGENTINA: CHUBUT: Sarmiento Department: Sierra del Castillo, 5 km W Provincial Road 24 (–45.139; –69.173): LJAMM-CNP 15505–15524, 15612. La Juanita, Provincial Road 24–58 km N of Sarmiento (–45.141; –69.175): MLP 1219, 1234, 5441–5444. La Juanita, Provincial Road 24 a 58 km N de Sarmiento (–45.141; –69.175): UNSa 3960–3969, 3975–3978. NW lagoon Colhue Huapi: UNSa 869-1, 869-2.

*Phymaturus ceii* (46): ARGENTINA: RÍO NEGRO: 25 de Mayo Department: Provincial Road 6, 8.4 km S Colan Conue: LJAMM-CNP 6804–6807. Provincial Road 8, 17 km S San Antonio del Cuy (–40.287; –68.459): LJAMM-CNP 1626–1630. Provincial Road 8. 17 km S San Antonio del Cuy (–40.283; –68.450): UNSa 898, 910–915. El Cuy Department: Provincial Road 6, 45.4 km SW Provincial Road 8 (–40.293; –68.924): LJAMM-CNP 6803. Provincial Road 6, 72.79 km SW El Cuy (–39.712; –67.080): LJAMM-CNP 15236. Provincial Road 74, 5 km N Provincial Road 67 (–40.284; –68.975): LJAMM-CNP 15828–15836. Provincial Road 6, 45.4 km SW Provincial Road 8 (–40.293; –68.924): LJAMM-CNP 6820–6822, 6845–6846. Chasicó, S El Cuy (–40.383; –69.009): MLP 5289–5293, 5917–5918. Provincial Road 6. (–40.346; –68.980): UNSa 3913, 3916, 3920, 3939–3942.

*Phymaturus delheyi* (19): ARGENTINA: NEUQUÉN: Pehuenches Department: Northern Tromen Volcano massif, Butacó Creek, on Provincial Road 37 (–36.983; –69.983): MLP 2609–2611. Provincial Road 37, stream Butacó (–36.988; –70.000): LJAMM-CNP 5220, 7655–7659, 8516, 15936–15944.

*Phymaturus etheridgei* (13): ARGENTINA: RÍO NEGRO: 25 de Mayo Department: Provincial Road 76, 57 km S Ingeniero Jacobacci (–41.755; –69.361): LJAMM-CNP 3590–3592, 3638–3645, 3681, 3688–3689, 3707, 5897, 6548–6549. Provincial Road 76, 43 km N Moligüe (–41.579; –69.392): FML 8435, 23495–23500. Provincial Road 76, between Ing. Jacobacci and Moligüe (–41.579; –69.392): JAS 1907–1916. Provincial Road 76, 37 km S National Road 23 (–41.599; –69.3784): MLP 5930, 5932. 43 km N Moligüe (–41.583; –69.370): UNSa 3109–3112. Provincial Road 76, entre Ing. Jacobacci y Moligüe (–41.580; –69.390): UNSa 4305–4308.

*Phymaturus excelsus* (48): ARGENTINA: RÍO NEGRO: Ñorquinco Department: National Road 6, 1.5 km N Ojo de Agua: FML 9320, 21454, 2059–2062. Ojo de agua: JAS 1127–1135, 1851, 1855, 1861–1862, 1869, 1882, 2068–2071, 2105–2107, 2122–2123. Provincial Road 6, 1 km NW Ojo de Agua: (–41.542; –69.859): LJAMM-CNP 3534, 3622–3626, UNSa 1385–1388, 1582–1588.

*Phymaturus felixi* (45): ARGENTINA: CHUBUT: Paso de Indios Department: JAS 1924, 1931–1938. Provincial Road 24, 110 km S Paso de Indios (–44.517; –69.190): LJAMM-CNP 3717, 3823–3837, 3882–3883. Provincial Road 24, 3 km N Mallin Angosto (–44.529; –69.181): LJAMM-CNP 9172–9173, 9187–9188. Provincial Road 24, Paso de Indios (–44.352; –69.408): MLP 5915. Provincial Road 24, 108 km S Paso de Indios: UNSa 1279–1282. Provincial Road 24, 84.5 km S Paso de Indios (–44.452; –69.297): UNSa 3980–3985, 3988–3991.

*Phymaturus indistinctus* (32): ARGENTINA: CHUBUT: Sarmiento Department: Sierras San Bernardo, Cañadon de las Pulgas: DC-JMC 329–331. National Road 26, 3.5 km W Puerta del Diablo (–45.462; –69.714): LJAMM-CNP 15538–15555. Provincial Road 20, Pampa Lehman (–45.409; –69.868): LJAMM-CNP 8198. Provincial Road 20, 4 km N Provincial Road 22 (–45.431; –69.840): UNSa 2238, 2272, 2651. Provincial Road 20, 19 km W Los Manatiales (–45.450; –69.700): UNSa 685,687, 3943–3947, 3954.

*Phymaturus manuelae* (9): ARGENTINA: RÍO NEGRO: Pilcaniyeu Department: National Road 23, 4.8 km SE Comallo (–41.044; –70.216): LJAMM-CNP 5447–5448, 5589. National Road 23, 26 km W Comallo (–41.022; –70.544): MLP 5370–5371. Between Pilcaniyeu and Las Bayas, Pilcaniyeu (–41.203; –70.690): UNSa 3929–3933.

*Phymaturus nevadoi* (25): ARGENTINA: MENDOZA: Malargüe Department: Provincial Road 180, 22.9 km N junction Provincial Road 186, 3.1 km S La Ventana post (–35.929; –68.616): LJAMM-CNP 4431–4433. Provincial Road 186, 25.5 km NE Mina Ethel, 4 km S Marfil Post (–35.928; –68.616): LJAMM-CNP 7933–7938, LJAMM-CNP 16418–16424. Provincial Road 186 (–35.929; –68.540): UNSa 3652–3659.

*Phymaturus patagonicus* (32): ARGENTINA: CHUBUT: Gaiman Department: National Road 25, 40 km SW Dolavon (–43.454; –66.121): LJAMM-CNP 3205–3210, 15151–15167. Hills next to the road to Boca Toma, 16.2 km S junction Ruta Nacional 25, road gate to 28 de Julio (–43.450; –65.917): LJAMM-CNP 14219. 30 km W Dolavon: DC-JMC 332–35, 842. 40 km W Dolavon: JAS 1124–1126, 1160–1164, MLP 777, 778.

*Phymaturus payuniaie* (24): ARGENTINA: MENDOZA: Malargüe Department: unnamed road, 2.8 km E junction Provincial Road 183, 13.8 km E road to El Clavado Post, Payún Plateau (–36.665; –69.281): LJAMM-CNP 4436–4438. Provincial Road 183, piedmont of Volcán Payún Liso (–36.488; –69.371): LJAMM-CNP 7975–7977. 20 km S Payún Liso, 60 km SE Mantecilla (–36.313; –69.901):

DC-JMC 807, 808. Piedmont of Payún Liso (–36.313; –69.901): DC-JMC 99. Provincial Road 183, 16 km S Volcan Payun (–36.672; –69.270): UNSa 3648–3651, 3665, 3669–3679.

*Phymaturus rahuensis* (14): ARGENTINA: NEUQUÉN: Catán Lil Department: of Provincial Road 46, 25 km E Rahue, 6 km junction Provincial Road 24, Bajada de Rahue, La Jardinera stream (–39.388; –70.732): LJAMM-CNP 16117–16129. Rocky cliffs on of Provincial Road 46, 25 km E Rahue, 6 km junction Provincial Road 24, Bajada de Rahue, La Jardinera stream (–39.388; –70.732): LJAMM-CNP 5378–5379.

*Phymaturus sinervoii* (49) ARGENTINA: RÍO NEGRO: 25 de Mayo Department: Provincial Road 6, 61 km N Ing. Jacobacci (–41.036; –70.408): JAS 1818–1825, 1840, 1844, 1888, 1891, 1897–1898, 1901–1906. Provincial Road 6, 65 km N Ing. Jacobacci (–40.890; –69.298): LJAMM-CNP 15238, 15855–15869. Provincial Road 6, 64 Km NE Ing. Jacobacci (–40.890; –69.299): LJAMM-CNP 6542–6547. Provincial Road 6, 61 km N Ing. Jacobacci (–41.036; –70.408): MLP 5660–5664, 5920–5922, 5929.

*Phymaturus sitesi* (20): ARGENTINA: NEUQUÉN: Pehuenches Department: Auca Mahuida mountain near Cerro de las Antenas, Auca Mahuida Natural Protected Area (–37.717; –68.917), MLP.S 2605–2608, LJAMM-CNP 10464–10468. Riscos Altos, 38.6 km S junction Provincial Road 6, Auca Mahuida Natural Protected Area (–37.702; –68.931): LJAMM-CNP 12297–12298. Park Ranger Post, 38.6 km S junction Provincial Road 6, Auca Mahuida Natural Protected Area Neuquén (–37.702; –68.858): LJAMM-CNP 12157, 12189, 12190, 12214.

Road SW Park Ranger Post, Auca Mahuida Natural Protected Area (–37.711; –68.886): LJAMM-CNP 13402. Añelo Department: Auca Mahuida Natural Protected Area, Communication Station Site, South Slope, Auca Mahuida Natural Protected Area (–37.776; –68.893): LJAMM-CNP 12311–12312.

*Phymaturus somuncurensis* (37): ARGENTINA: RÍO NEGRO: 9 de Julio Department: 65.6 km police station El Rincon, near Corona Hill, between Corona Grande Hill and Corona Chico Hill (–41.394; –66.959): LJAMM-CNP 6022, 6826–6832. N laguna Paraguay, 937 m de Provincial Road 60 (–41.361; –66.940): LJAMM-CNP 15806–15021. Meseta de Somuncurá (–41.199; –66.893): LJAMM-CNP 4453–4456. Valcheta Department: Meseta del Somuncurá: MLP 1646–1651. Laguna Raimundo (–40.950; –66.370): DC-JMC 337–8, 832-3.

*Phymaturus spectabilis* (62): ARGENTINA: RÍO NEGRO: Ñorquinco Department: Provincial Road 6, 17.4 km NE Ojos de Agua, 28 km SW Ingeniero Jacobacci, (–41.448; –69.776): LJAMM-CNP 3600–3605, 3617–3621, 3634–3636. Ing. Jacobacci, paraje Yuquiche: JAS 1033–1038, 1041–1042, 1048–1063, 1091, 1252–1256, 1265–1266, 1556–1558. Provincial Road 6 (–41.427; –69.751): MLP 5343–5346, 5880–5883. 25 de Mayo Department: 28 km S Ing. Jacobacci (–41.601; –69.517): UNSa 1203-07, 1212.

*Phymaturus spurcus* (30): ARGENTINA: RÍO NEGRO: 25 de Mayo Department: National Road 23, hill near Estancia Huanuluan, 25.1 km W Ing. Jacobacci (–41.365; –69.808): LJAMM-CNP 3630. National Road 23, 25 km W Ing. Jacobacci, Estancia Huanuluan (–41.365; –69.811): LJAMM-CNP 15870–15871. 1.5 km N ojo de agua: FML 21452-53, 21494. National Road 23, Estancia Huanuluan (–41.360; –69.810): UNSa 1238–1240, 1845–1848, 1590, 1596. Estancia Huanuluan (–40.500; –68.216): JAS-DC 822–831, 1257–1263, 1809, 1857, 1858.

*Phymaturus tenebrosus* (33): ARGENTINA: RÍO NEGRO: Pilcaniyeu Department: National Road 40, Cerro Alto: LJAMM-CNP 8681, FML 10060–10062, 22073–22074, JAS 811, 824, 832–835, 1103, 1247, 1250. National Road 40, 20 km S Cerro Alto (–40.877; –70.574): LJAMM-CNP 15837–15847. National Road 40, 20 km S Cerro Alto (–40.901; –70.611): UNSa 1263–1265, 1270–1273, 3934.

*Phymaturus videlai* (24) ARGENTINA: CHUBUT: Sarmiento Department: Buen Pasto JAS 1146, 1149–1150. Provincial Road 23, 3 km E Buen Pasto (–45.068; –69.423): LJAMM-CNP 15558–15566, 15572–15578. Buen Pasto, 85 km NW Sarmiento (–45.069; –69.423): MLP 5438–5439. Buen Pasto, 85 km NW Sarmiento (-45.070; -69.424): UNSa 4203–4204, 4207.

*Phymaturus yachanana* (16): ARGENTINA: RÍO NEGRO: San Antonio Department: Rocky cliffs of Sierra Grande mountain, behind the Sierra Grande town (–41.616; –65.383): LJAMM 8203–8204. Valcheta Department: Establecimiento La Polvareda, Sierra Grande (–41.558; –66.499): LJAMM-CNP 3233–3238, 3259–3263. Estancia Cecchi, 24.8 km W Arroyo Los Berros (–41.470; –66.322): LJAMM – CNP 3317–19.

*Phymaturus zapalensis* (32): ARGENTINA: NEUQUÉN: Catán Lil Department: Provincial Road 46, 9.5 km SW gate Laguna Blanca National Park (–39.135; –70.429): LJAMM-CNP 8067–8074. Laguna Teru, near Zapala (–38.855; –70.483): DC – JMC 8. Catán Lil (–38.932; –69.962): PH – Co 42, 44–46. Catán Lil (–38.961; –69.977): PH – Co 37, 39. Catán Lil (–38.956; –69.959): PH – Co 60–66. Provincial Road 46, 9.5 km S Laguna Blanca (–39.134; –70.430): UNSa 3845–3853, 3867.

**Appendix B**

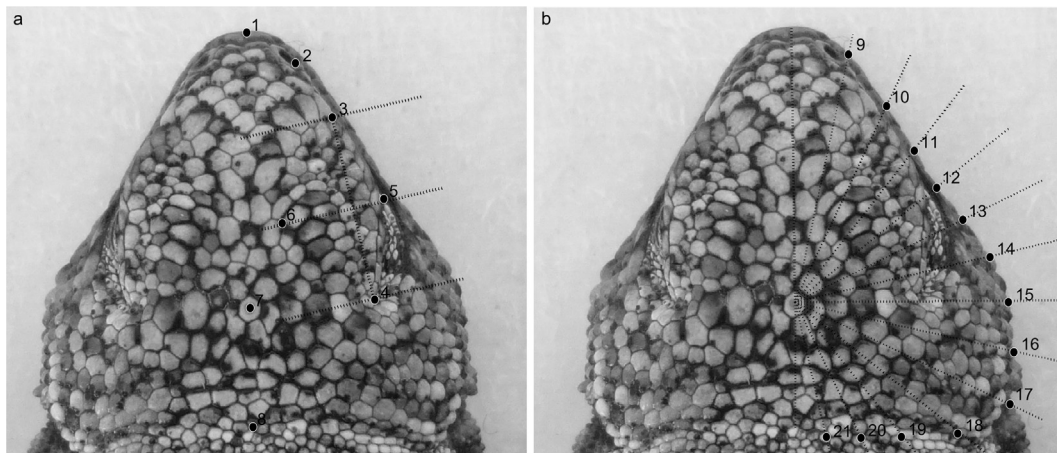
List of gene-sequenced specimens, the LJAMM-CNP column specifies the voucher number and is completed with an asterisk for the sequence of that individual and the geographical coordinates of the sampled locations. The voucher numbers in bold are specimens with newly generated Cyt-b sequences.

Species	LJAMM-CNP	Cyt- b	cmos	NT3	PRLR	PNN	Phy38	Phy41	Phy60	Phy64	Phy84	Phy87	Phy89	Locality
<i>P. castillensis</i>	<b>15503, 15506, 15612</b>	*	–	–	–	–	–	–	–	–	–	–	–	–45.138; –69.173
<i>P. calcogaster</i>	6855, 6857, 8125–8132	*	–	–	–	–	–	–	–	–	–	–	–	–41.500; –66.650
<i>P. camilae</i>	<b>6856</b> 3407 3408	* * *	* – *	* – *	* – *	* – *	* – *	* – *	* – *	* – *	* – *	* – *	* – *	–42.500; –67.359 –42.405; –68.257 –42.405; –68.257
	6771–6778, 6901 7482–7485	* *	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	–42.424; –68.285 –42.420; –68.239
<i>P. ceii</i>	1584 1916, 2727, 2728, 6851, 6852	* * *	* – –	* – –	* – –	* – –	* – –	* – –	* – –	* – –	* – –	* – –	* – –	–40.286; –68.459 –40.286; –68.458
<i>P. delheyi</i>	5221 7654–7659	* *	* –	* –	* –	* –	* –	* –	* –	* –	* –	* –	* –	–36.988; –70.000 –36.989; –69.999
<i>P. etheridgei</i>	3589–3592, 3681, 3688, 3689, 6548, 6549	* * *	– – –	– – –	– – –	– – –	– – –	– – –	– – –	– – –	– – –	– – –	– – –	–41.755; –69.361
	5897	*	* –	* –	* –	* –	* –	* –	* –	* –	* –	* –	* –	–41.752; –69.359
<i>P. excelsus</i>	2136–2137, 2266, 2355, 2356, 2652–2654 2265	* * * *	– – – *	– – – *	– – – *	– – – *	– – – *	– – – *	– – – *	– – – *	– – – *	– – – *	– – – *	–41.542; –69.859 –41.542; –69.859

<i>P. felixi</i>	3717	*	*	*	*	*	*	*	*	*	*	*	-44.517; -69.190	
	3823, 3824,	*	-	-	-	-	-	-	-	-	-	-	-44.517; -69.190	
	3826–3837, 3883	*	*	*	*	*	*	*	*	*	*	*	-44.517; -69.190	
<i>P. indistinctus</i>	3825	*	*	*	*	*	*	*	*	*	*	*	-44.517; -69.190	
	2124	*	*	*	*	*	*	*	*	*	*	*	-45.462; -69.714	
	2393, <b>15538</b> , <b>15549</b>	*	-	-	-	-	-	-	-	-	-	-	-45.462; -69.714	
<i>P. manuelae</i>	5448	*	*	*	*	*	-	-	-	*	*	*	-41.044; -70.216	
	5589	*	-	-	-	-	-	-	-	-	-	-	-41.044; -70.216	
<i>P. nevadoi</i>	4431	*	*	*	*	*	*	*	*	*	*	*	-35.929; -68.616	
	4432, 4433	*	-	-	-	-	-	-	-	-	-	-	-68.616; -35.929	
	7933–7938	*	-	-	-	-	-	-	-	-	-	-	-68.616; -35.928	
<i>P. patagonicus</i>	3205	*	*	*	*	*	*	*	*	*	*	*	-43.454; -66.121	
	3206–3209, <b>15151</b> , <b>15152</b> , <b>15157</b>	*	-	-	-	-	-	-	-	-	-	-	-43.454; -66.121	
	<i>P. payunia</i>	4436	*	-	-	-	-	-	-	-	-	-	-	-36.665; -69.281
4437		*	*	*	*	*	*	*	*	*	*	*	-36.665; -69.281	
4438, 7976, 7977		*	-	-	-	-	-	-	-	-	-	-	-36.488; -69.371	
7975		*	*	*	*	*	*	*	*	*	*	*	-36.488; -69.371	
<i>P. rahuensis</i>	5378, <b>16117</b> , <b>16121</b> , <b>16128</b>	*	-	-	-	-	-	-	-	-	-	-	-39.388; -70.733	
	5379	*	*	*	*	*	*	*	*	*	*	*	-39.388; -70.733	
<i>P. sinevoi</i>	6542,	*	-	-	-	-	-	-	-	-	-	-	-40.890; -69.299	
	6544–6546, <b>15855</b> , <b>15859</b> , <b>15865</b>	*	*	*	*	*	*	*	*	*	*	*	-40.890; -69.299	
	6543	*	*	*	*	*	*	*	*	*	*	*	-37.723; -68.888	
<i>P. sitesi</i>	10367	*	*	*	*	*	*	*	*	*	*	*	-37.723; -68.888	
	10368,	*	-	-	-	-	-	-	-	-	-	-	-37.723; -68.888	
	10465–10468, 10554–10556, 10561	*	*	*	*	*	*	*	*	*	*	*	-41.199; -66.893	
	4453	*	*	*	*	*	*	*	*	*	*	*	-41.199; -66.893	
<i>P. somuncur-ensis</i>	4454–4456, <b>10958</b> , <b>15806</b> , <b>15811</b> , <b>15820</b>	*	-	-	-	-	-	-	-	-	-	-	-41.199; -66.893	
	<i>P. spectabilis</i>	3600	*	*	*	*	*	-	*	-	*	*	*	-41.448; -69.776
3601–3605, 3617–3621		*	-	-	-	-	-	-	-	-	-	-	-41.448; -69.776	
3586		*	*	*	*	*	*	*	-	*	*	*	-41.365; -69.808	
<i>P. spurcus</i>	3627, 3629, 3630	*	-	-	-	-	-	-	-	-	-	-	-41.365; -69.808	
	<i>P. videlai</i>	9084–9086, <b>15558</b> , <b>15566</b> , <b>15571</b> , <b>15572</b> , <b>15578</b>	*	-	-	-	-	-	-	-	-	-	-	-45.069; -69.460
<i>P. yachanana</i>		3233, 3235–3238	*	-	-	-	-	-	-	-	-	-	-	-41.558; -66.499
		3234	*	*	*	*	*	*	*	*	*	*	*	-41.558; -66.499
	8203–8205	*	-	-	-	-	-	-	-	-	-	-	-41.470; -66.322	
	<b>14366–14368</b>	*	-	-	-	-	-	-	-	-	-	-	-41.612; -65.393	
<i>P. zapalensis</i>	8067	*	*	*	*	*	*	*	*	*	*	-	-39.135; -70.429	
	8068–8074	*	-	-	-	-	-	-	-	-	-	-	-39.135; -70.429	

Appendix C

Dorsal view of the head with landmarks (L) and 13 semi-landmarks (Sl). References: (a) 1- snout, 2- posterior margin of the nostril, 3- first supraocular scale, 4- last supraocular scale, 5- median superciliary scale, 6- median circumorbital scale, 7- pineal scale, 8- last scale of the neck fold, (b) 9–21 (semi-landmarks).



## Appendix D. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ympev.2018.08.019>.

## References

- Abdala, C.S., Acosta, J.L., Acosta, J.C., Álvarez, B.B., Arias, F., Avila, L.J., Blanco, M.G., Bonino, M., Boretto, J.M., Brancatelli, G., Breitman, M.F., Cabrera, M.R., Cairo, S., Corbalán, V., Hernando, A., Ibarguengoytia, N.R., Kacolicis, F., Laspiur, A., Montero, R., Morando, M., Pelegrin, N., Fulvio Pérez, C.H., Quinteros, A.S., Semhan, R.V., Tedesco, M.E., Vega, L., Zalba, S.M., 2012. Categorización del estado de conservación de las lagartijas y anfisbenas de la República Argentina. *Cuadernos de Herpetología* 26 (1), 215–248.
- Abdala, C.S., Quinteros, A.S., 2014. Los últimos 30 años de estudios de la familia de lagartijas más diversa de Argentina. Actualización taxonómica y sistemática de Liolaemidae. *Cuadernos de Herpetología* 28 (2), 55–82.
- Abdala, V., Tulli, M.J., Russell, A.P., Powell, G.L., Cruz, F.B., 2014. Anatomy of the crus and pes of neotropical iguanian lizards in relation to habitat use and digitally based grasping capabilities. *Anatomical Rec.* 297 (3), 397–409.
- Abdi, H., Williams, L.J., Valentin, D., 2013. Multiple factor analysis, principal component analysis for multitable and multiblock data sets. *Wiley Interdiscip. Rev. Comput. Stat.* 5 (2), 149–179.
- Adams, D.C., Rohlf, F.J., Slice, D.E., 2004. Geometric morphometrics, ten years of progress following the revolution. *Italian J. Zool.* 71 (1), 5–16.
- Adams, D.C., Berns, C.M., Kozak, K.H., Wiens, J.J., 2009. Are rates of species diversification correlated with rates of morphological evolution? *Proc. Roy. Soc. B, Biol. Sci.* 276, 2729–2738.
- Andrew, R.L., Rieseberg, L.H., 2013. Divergence is focused on few genomic regions early in speciation, incipient speciation of sunflower ecotypes. *Evolution* 67 (9), 2468–2482.
- Avila, L.J., Perez, C.H.F., Minoli, I., Morando, M., 2014. A new lizard of the *Phymaturus* genus (Squamata: Liolaemidae) from Sierra Grande, northeastern Patagonia, Argentina. *Zootaxa* 3793, 99–118.
- Bookstein, F.L., 1991. *Morphometric Tools for Landmark Data, Geometry and Biology*. Cambridge University Press, New York.
- Becker, L.A., Boretto, J.M., Cabezas-Cartes, F., Márquez, S., Kubisch, E., Sclolaro, J., Sinervo, B., Ibarguengoytia, N.R., 2018. An integrative approach to elucidate the taxonomic status of five species of *Phymaturus* Gravenhorst, 1837 (Squamata: Liolaemidae) from northwestern Patagonia, Argentina. *Zool. J. Linn. Soc.* 1–15.
- Brandt, R., Barros, F.C., Noronha, C., Tulli, M.J., Kohlsdorf, T., 2016. Sexual differences in locomotor performance in *Tropidurus catalanensis* lizards (Squamata: Tropiduridae) body shape, size and limb musculature explain variation between males and females. *Biol. J. Linn. Soc.* 118, 598–609.
- Butler, M.A., King, A.A., 2004. Phylogenetic comparative analysis, a modeling approach for adaptive evolution. *Am. Nat.* 164 (6), 683–695.
- Camargo, A., Avila, L.J., Morando, M., Sites Jr, J.W., 2012. Accuracy and precision in species tree estimation: an empirical evaluation of performance in lizards of the *Liolaemus darwini* group (Squamata: Liolaemidae) under varying sub-sampling designs. *Syst. Biol.* 61, 272–288.
- Campagna, L., St. Clair, J.J., Loughheed, S.C., Woods, R.W., Imberti, S., Tubaro, P.L., 2012. Divergence between passerine populations from the Malvinas-Falkland Islands and their continental counterparts, a comparative phylogeographical study. *Biol. J. Linn. Soc.* 106 (4), 865–879.
- Camargo, A., Sites, J.W., 2013. Species delimitation: a decade after the renaissance. *En: The species problem: ongoing issues*. InTech Open Access Publisher.
- Cei, J.M., 1986. *Reptiles del centro, centro-oeste y sur de la Argentina. Herpetofauna de las zonas áridas y semiáridas*. Monograph IV. Museo Regionale di Scienze Naturali Torino, pp. 1–527.
- Cei, J.M., 1993. *Reptiles del Noroeste, Nordeste y Este de la Argentina. Herpetofauna de las Selvas subtropicales, Puna y Pampas*. Monograph XIV. Museo Regionale di Scienze Naturali Torino, pp. 1–949.
- Cei, J.M., Videla, F., Vicente, L., 2003. From oviparity to viviparity: A preliminary note on the morphometric differentiation between oviparous and viviparous species assigned to the genus *Liolaemus* (Reptilia, Squamata, Liolaemidae). *J. Zool. Syst. Evol. Res.* 41, 152–156.
- Claude, J., 2008. *Morphometrics with R*. Springer, New York.
- Conti, H.A., 1998. Característica climática de la Patagonia. In: Correa, M.N. (Ed.), *Flora Patagónica*. Colección científica del INTA, Buenos Aires, Argentina, pp. 31–47.
- Cruz, F.B., Belver, L., Acosta, J.C., Villavicencio, H.J., Blanco, G., Cánovas, M.G., 2009. Thermal biology of *Phymaturus* lizards: evolutionary constraints or lack of environmental variation? *Zoology* 112 (6), 425–432.
- Corbalán, V., Debandi, G., Sclolaro, J.A., Ojeda, A., 2016. DNA Barcoding of *Phymaturus* lizards reveals conflicts in species delimitation within the patagonicus clade. *J. Herpetol.* 50 (4), 654–666.
- Dayrat, B., 2005. Towards integrative taxonomy. *Biol. J. Linn. Soc.* 85 (3), 407–415.
- Debandi, G., Corbalán, V., Sclolaro, J.A., Roig-Juñent, S.A., 2012. Predicting the environmental niche of the genus *Phymaturus*: Are *palluma* and *patagonicus* groups ecologically differentiated? *Austral. Ecol.* 37 (3), 392–400.
- Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends Ecol. Evol.* 24 (6), 332–340.
- de Queiroz, K., 2007. Species concepts and species delimitation. *Syst. Biol.* 56 (6), 879–886.
- Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2016. *Grupo InfoStat*. Universidad Nacional de Córdoba, Argentina, FCA.
- Etheridge, R., 1995. Redescription of *Ctenoblepharys adspersa* Tschudi, 1845, and the taxonomy of Liolaeminae (Reptilia, Squamata, Tropiduridae). *American Museum Novitates*, pp.3142.
- Elmer, K.R., Kusche, H., Lehtonen, T.K., Meyer, A., 2010. Local variation and parallel evolution: morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. *Philos. Trans. Royal Soc. London B, Biol. Sci.* 365 (1547), 1763–1782.
- Eloi, F.J., Leite-Filho, E., 2013. Competing for a place in the sun, a short study with *Tropidurus hispidus* and *Tropidurus semitaeniatus* (Squamata: Tropiduridae). *Revista Nordestina de Biologia* 21, 59–69.
- Erwin, D.H., 2007. Disparity: morphological pattern and developmental context. *Palaeontology* 50 (1), 57–73.
- Espinosa, R.E., Wiens, J.J., Tracy, C.R., 2004. Recurrent evolution of herbivory in small, cold-climate lizards: breaking the ecophysiological rules of reptilian herbivory. *PNAS* 101 (48), 16819–16824.
- Felsenstein, J., 1988. Phylogenies and quantitative characters. *Annu. Rev. Ecol. Syst.* 19, 445–471.
- Florio, A.M., Ingram, C.M., Rakotondravony, H.A., Louis, E.E., Raxworthy, C.J., 2012. Detecting cryptic speciation in the widespread and morphologically conservative carpet chameleon (*Furcifer lateralis*) of Madagascar. *J. Evol. Biol.* 25 (7), 1399–1414.
- Fujisawa, T., Aswad, A., Barraclough, T.G., 2016. A rapid and scalable method for multilocus species delimitation using Bayesian model comparison and rooted triplets. *Syst. Biol.* 65 (5), 759–771.
- Guillette, L.J., 1993. The evolution of viviparity in lizards. *Bioscience* 43 (11), 742–751.
- González Marín, A., Pérez, C.H.F., Minoli, I., Morando, M., Avila, L.J., 2016a. A new lizard species of the *Phymaturus patagonicus* group (Squamata: Liolaemini) from northern Patagonia, Neuquén, Argentina. *Zootaxa* 4121 (4), 412–430.
- González Marín, A., Morando, M., Avila, L.J., 2016b. Morfología lineal y geométrica en un grupo de lagartijas patagónicas del género *Phymaturus* (Squamata, Liolaemini). *Revista Mexicana de Biodiversidad* 87 (2), 399–408.
- Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E., Challenger, W., 2008. GEIGER,

- investigating evolutionary radiations. *Bioinformatics* 24, 129–131.
- Harmon, L.J., Losos, J.B., Jonathan Davies, T., Gillespie, R.G., Gittleman, J.L., Bryan Jennings, W., Purvis, A., Ricklefs, R.E., Schluter, D., Schulte II, J.A., Seehausen, O., Sidlauskas, B.L., Torres-Carvajal, O., Weir, J.T., Mooers, A., 2010. Early bursts of body size and shape evolution are rare in comparative data. *Evolution* 64 (8), 2385–2396.
- Hone, D.W.E., Benton, M.J., 2005. The evolution of large size, how does Cope's rule work? *Trends Ecol. Evol.* 20, 4–6.
- Howard, K.E., Hailey, A., 1999. Microhabitat separation among diurnal saxicolous lizards in Zimbabwe. *J. Trop. Ecol.* 15 (3), 367–378.
- Huang, J.P., Knowles, L.L., 2016. The species versus subspecies conundrum, quantitative delimitation from integrating multiple data types within a single Bayesian approach in Hercules beetles. *Syst. Biol.* 65 (4), 685–699.
- Husson, F., Josse, J., Lê, J., Mazet, J., 2013. *FactoMineR*, Multivariate exploratory data analysis and data mining with R. R package version 1.21.
- Ibargüengoytia, N.R., Acosta, J.C., Boretto, J.M., Villavicencio, H.J., Marinero, J.A., Krenz, J.D., 2008. Field thermal biology in *Phymaturus* lizards, comparisons from the Andes to the Patagonian steppe in Argentina. *J. Arid Environ.* 72 (9), 1620–1630.
- Kalioztopoulou, A., Carretero, M.A., Llorente, G.A., 2010. Intraspecific ecomorphological variation, linear and geometric morphometrics reveal habitat-related patterns within *Podarcis bocagei* wall lizards. *J. Evol. Biol.* 23 (6), 1234–1244.
- Kalioztopoulou, A., 2011. Geometric morphometrics in herpetology, modern tools for enhancing the study of morphological variation. *Basic Appl. Herpetol.* 25, 5–32.
- Kalioztopoulou, A., Adams, D.C., Van der Meijden, A., Perera, A., Carretero, M.A., 2012. Relationships between head morphology, bite performance and ecology in two species of *Podarcis* wall lizards. *Evol. Ecol.* 26 (4), 825–845.
- Klingenberg, C.P., 2011. MorphoJ, an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* 11, 353–357.
- Krenz, J.D., 2008. Field thermal biology in *Phymaturus* lizards, comparisons from the Andes to the Patagonian steppe in Argentina. *J. Arid Environ.* 72 (9), 1620–1630.
- LaBarbera, M., 1989. Analyzing body size as a factor in ecology and evolution. *Annu. Rev. Ecol. Syst.* 20, 97–117.
- Lê, S., Josse, J., Husson, F., 2008. *FactoMineR*, An R package for multivariate analysis. *J. Stat. Softw.* 25 (1), 1–18.
- Leaché, A.D., Fujita, M.K., 2010. Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). In: *Proceedings of the Royal Society of London B, Biological Sciences*, 277(1697), 3071–3077.
- Lobo, F., Quinteros, A.S., 2005a. A morphology-based phylogeny of *Phymaturus* (Iguania, Liolaemidae) with the description of four new species from Argentina. *Papéis Avulsos de Zoologia (São Paulo)* 45 (13), 143–177.
- Lobo, F., Quinteros, A.S., 2005b. Taxonomic studies of the genus *Phymaturus* (Iguania, Liolaemidae), Redescription of *Phymaturus patagonicus* Koslowsky 1898, and revalidation and redescription of *Phymaturus spurcus* Barbour 1921. *J. Herpetol.* 39 (4), 533–540.
- Lobo, F., Abdala, C.S., Valdecantos, S., 2010. Taxonomic studies of the genus *Phymaturus* (Iguania, Liolaemidae), description of four new species. *South Am. J. Herpetol.* 5 (2), 102–126.
- Lobo, F., Cruz, F., Abdala, C.S., 2012a. Multiple lines of evidence show that *Phymaturus agilis* Scolari, Ibargüengoytia and Pincheira-Donoso, 2008 is a junior synonym of *Phymaturus spectabilis* Lobo and Quinteros, 2005. *Cuadernos de Herpetología* 26 (1), 21–27.
- Lobo, F., Abdala, C.S., Valdecantos, S., 2012b. Morphological diversity and phylogenetic relationships within a South-American clade of iguanian lizards (Liolaemidae, *Phymaturus*). *Zootaxa* 3315, 1–41.
- Lobo, F., Barrasso, D.A., Paz, M., Basso, N.G., 2018. Phylogenetic relationships within a patagonian clade of reptiles (Liolaemidae: Phymaturus) based on DNA sequences and morphology. *J. Zool. Syst. Evol. Res.* 1–21.
- Maddison, W.P., 1997. Gene trees in species trees. *Syst. Biol.* 46 (3), 523–536.
- Mallet, J., 2005. Hybridization as an invasion of the genome. *Trends Ecol. Evol.* 20 (5), 229–237.
- Mallet, J., 2007. Hybrid speciation. *Nature* 446 (7133), 279–283.
- Minoli, I., Morando, M., Avila, L.J., 2014. Integrative taxonomy in the *Liolaemus fitzingerii* complex (Squamata, Liolaemini) based on morphological analyses and niche modeling. *Zootaxa* 3856 (4), 501–528.
- Minoli, I., Morando, M., Avila, L.J., 2015. Reptiles of Chubut province, Argentina: richness, diversity, conservation status and geographic distribution maps. *ZooKeys* 498, 103–126.
- Morando, M., Avila, L.J., Sites, J.W., 2003. Sampling strategies for delimiting species, Genes, individuals, and populations in the *Liolaemus elongatus-kriegi* complex (Squamata: Liolaemidae) in Andean-Patagonian South America. *Syst. Biol.* 52, 159–185.
- Morando, M., 2004. Sistemática y filogenia de grupos de especies de los géneros *Phymaturus* y *Liolaemus* (Squamata: Tropiduridae: Liolaeminae) del oeste y sur de Argentina. Universidad Nacional de Tucumán, Argentina, Tesis de Doctorado.
- Morando, M., Avila, L.J., Perez, C.H.F., Hawkins, M.A., Sites, J.W., 2013. A molecular phylogeny of the lizard genus *Phymaturus* (Squamata: Liolaemini): implications for species diversity and historical biogeography of southern South America. *Mol. Phylogenet. Evol.* 66 (3), 694–714.
- Olave, M., Avila, L.J., Sites, J.W., Morando, M., 2017. Hidden diversity within the lizard genus *Liolaemus*, genetic vs morphological divergence in the *L. rothi* complex (Squamata: Liolaeminae). *Mol. Phylogenet. Evol.* 107, 56–63.
- Padial, J.M., De La Riva, I., 2007. Integrative taxonomists should use and produce DNA barcodes. *Zootaxa* 1586 (1), 67–68.
- Padial, J.M., Miralles, A., De la Riva, I., Vences, M., 2010. The integrative future of taxonomy. *Front. Zool.* 7, 16.
- Pelegri, N., Mesquita, D.O., Albinati, P., Caldas, F.L.S., de Queiroga, Barbosa, Cavalcanti, L., Costa, T.B., Falco, D.A., Galdino, J.Y.A., Tucker, D.B., Garda, A.A., 2017. Extreme specialization to rocky habitats in *Tropidurus* lizards from Brazil, Trade-offs between a fitted ecomorph and autoecology in a harsh environment. *Austral. Ecol.* 42 (6), 677–689.
- Piantoni, C., Ibargüengoytia, N.R., Cussac, V.E., 2006. Age and growth of the Patagonian lizard *Phymaturus patagonicus*. *Amphibia-Reptilia* 27 (3), 385–392.
- Pietrek, A.G., Walker, R.S., Novaro, A.J., 2009. Susceptibility of lizards to predation under two levels of vegetative cover. *J. Arid Environ.* 73 (4), 574–577.
- Reaney, A.M., Saldarriaga-Córdoba, M., Pincheira-Donoso, D., 2018. Macroevolutionary diversification with limited niche disparity in a species-rich lineage of cold-climate lizards. *BMC Evol. Biol.* 18 (1), 16.
- R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: <http://www.R-project.org/>.
- Rohlf, F.J., Slice, D.E., 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool.* 39, 40–59.
- Rohlf, F.J., 2010a. tpsDig version 2.16. Department of Ecology and Evolution, State University of New York at Stony Brook.
- Rohlf, F.J., 2010b. Relative warps version 1.49. Department of Ecology and Evolution, State University of New York at Stony Brook.
- Rohlf, F.J., 2012. tpsUtility program version 1.52. Department of Ecology and Evolution, State University of New York at Stony Brook.
- Rosenberg, N.A., 2013. Discordance of species trees with their most likely gene trees, a unifying principle. *Mol. Biol. Evol.* 30 (12), 2709–2713.
- Schulte, J.A., Macey, J.R., Espinoza, R.E., Larson, A., 2000. Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. *Biol. J. Linn. Soc.* 69, 75–102.
- Schulte, J.A., 2013. Undersampling taxa will underestimate molecular divergence dates: an example from the South American lizard clade Liolaemini. *Int. J. Evolut. Biol.* 2013, 1–12.
- Scolaro, J.A., Tapari, F.O., 2009. Una nueva especie del género *Phymaturus* del 'grupo *patagonicus*' en los afloramientos rocosos del sudoeste de la provincia de Río Negro, Patagonia Argentina (Reptilia: Iguania: Liolaemidae). *Naturalia Patagónica* 5 (1), 80–93.
- Scolaro, J.A., Corbalán, V., Tappari, O.F., Obregón Streitenberger, L., 2016. Lizards at the end of the world: a new melanic species of *Phymaturus* of the *patagonicus* clade from rocky outcrops in the northwestern steppe of Chubut province, Patagonia Argentina (Reptilia: Iguania: Liolaemidae). *Boletín del Museo Nacional de Historia Natural* 65, 137–152.
- Sheets, H.D., 2005. MakeFan, a tool for drawing alignment “fans” at equal angular spacing.
- Smith, K.L., Harmon, L.J., Shoo, L.P., Melville, J., 2011. Evidence of constrained phenotypic evolution in a cryptic species complex of agamid lizards. *Evolution* 65 (4), 976–992.
- Solis-Lemus, C., Knowles, L.L., Ané, C., 2015. Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution* 69, 492–507.
- Sookias, R.B., Butler, R.J., Benson, R.B.J., 2012. Rise of dinosaurs reveals major body-size transitions are driven by passive processes of trait evolution. *Proc. Royal Soc. London B* 279, 2180–2187.
- Stebbins, R.C., 1985. *Western Reptiles and Amphibians*. Houghton Mifflin Company, New York, pp. 336.
- Tulli, M.J., Abdala, V., Cruz, F.B., 2011. Relationships among morphology, clinging performance and habitat use in Liolaemini lizards. *J. Evol. Biol.* 24 (4), 843–855.
- Tulli, M.J., Cruz, F.B., Kohlsdorf, T., Abdala, V., 2016. When a general morphology allows many habitat uses. *Integrative Zool.* 11 (6), 483–499.
- Vidal, M.A., Ortiz, J.C., Ramírez, C.C., Lambort, M., 2005. Intraspecific variation in morphology and sexual dimorphism in *Liolaemus tenuis* (Tropiduridae). *Amphibia-Reptilia* 26, 343–351.
- Vidal, M.A., Veloso, A., Méndez, M.A., 2006. Insular morphological divergence in the lizard *Liolaemus pictus* (Liolaemidae). *Amphibia-Reptilia* 27, 103–111.
- Vidal, M.A., Habit, E., Victoriano, P., González-Gajardo, A., Ortiz, J.C., 2010. Thermoregulation and activity pattern of the high-mountain lizard *Phymaturus paluma* (Tropiduridae) in Chile. *Zoologia* 27 (1), 13–18.
- Zuckerandl, E., Pauling, L., 1965. Evolutionary divergence and convergence in proteins. *Evol. Genes Proteins* 97, 97–166.