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Lizards from the end of the world: Phylogenetic relationships of the *Liolaemus lineomaculatus* section (Squamata: Iguania: Liolaemini)

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ABSTRACT

The *Liolaemus lineomaculatus* section is a geographically widely distributed group of lizards from the Patagonian region of southern South America, and includes 18 described species representing the most southerly distributed *Liolaemus* taxa (the genus includes 228 species and extends from Tierra del Fuego north to south-central Peru). Despite high species diversity, the phylogenetic relationships of this section are unknown. In the present work we sampled all described species in the *L. lineomaculatus* section as well as currently undescribed candidate species to reconstruct the first complete phylogenetic hypothesis for the clade. Our data set included four anonymous nuclear loci, three nuclear protein-coding loci, and two mitochondrial genes. We compared results obtained with three different phylogenetic methods for the concatenated data set (Maximum Parsimony, Maximum Likelihood and Bayesian Inference) with a coalescent-based species tree approach (BEST), and recovered congruent, strongly-supported topological arrangements across all methods. We identified four main clades within the *L. lineomaculatus* section: the *lineomaculatus*, *magellanicus*, *somuncurae*, and *kingii + archeforus* groups, for which we estimated divergence times. We discuss the taxonomic implications of these results and how the future integration of phylogeographic, niche modeling and morphological approaches will allow testing biogeographical hypotheses in this clade.

1. Introduction

Lizards have been used as model organisms for testing many ecological and evolutionary hypotheses at different levels (populations, communities) and at multiple spatial and temporal scales (reviewed in Camargo et al., 2010). In some regions, chiefly Australia, Europe, and North America, baseline taxonomic and phylogenetic knowledge is sufficient to support detailed hypothesis-driven studies that have provided important insights into general ecological and evolutionary processes (reviewed in Camargo et al., 2010). However, in other areas of the world, alpha diversity, basic taxonomic knowledge, and distributions of regional lizard faunas are insufficient to support more synthetic studies. Thus lizards provide an excellent example of "Linnean" and "Wallacean" shortfalls, which means respectively incomplete knowledge of species and their distributions (Lomolino, 2004). These can be rectified only by intensive and careful field work, followed by morphological, molecular, and ecological studies. In the Patagonian region of southern South America, the *Liolaemus lineomaculatus* section is characterized by these Linnean and Wallacean shortfalls.

The genus *Liolaemus* is one of the most ecologically diverse and species-rich genera of lizards on earth, with 228 recognized species (Lobo et al., 2010a; and the recently described *Liolaemus chacabucoense*, Núñez and Scolaro, 2009, *Liolaemus casamiquelai*, Avila et al., 2010a, *Liolaemus antumalguen*, Avila et al., 2010b, *Liolaemus cazianiae* and *Liolaemus halonastes*, Lobo et al., 2010b). Moreover, the true diversity of the genus may be as much as twice this number or more by some estimates (Morando et al., 2003). *Liolaemus* is distributed over a wide geographic area spanning a large range of latitudinal (14° ± 30′–52° ± 30′S), altitudinal (0–4,500 m) and climatic regimes, from the extremely arid Atacama Desert to temperate *Nothofagus* rainforest (Cei, 1986, 1993; Donoso-Barros, 1966; Etheridge and de Queiroz, 1988; Etheridge and Espinoza, 2000; Frost and Etheridge, 1989; Hellmich, 1951; Lobo, 2001). Two main groups were proposed by Laurent in 1983 within *Liolaemus*, based on a set of morphological characters (number of precloacal pores, tail length, and position of the nasal scales): *Liolaemus sensu stricto* (or the "Chileno group", mainly distributed in Chile) with 91 described species, and *Eulaemus* (or the "Argentino group" largely confined to Argentina) with 137 described species (Lobo et al., 2010a). Laurent's hypothesis has been supported by several recent molecular and morphological studies (Abdala, 2007; Cruz et al., 2005; Espinoza et al., 2004; Morando, 2004; Schulte et al., 2000).

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The *L. lineomaculatus* section includes 18 described species. Based on morphological characters, it is commonly divided in three groups: (1) the *archeforus* group (Cei, 1986) with eight described species: *Liolaemus archeforus*, *Liolaemus sarmientoi*, *Liolaemus gallardoi*, *Liolaemus zullyae*, *Liolaemus tari*, *Liolaemus scolaroi*, *Liolaemus escarchadosi* and *L. chacabucoense*; (2) the *kingii* group (Cei, 1986) with five described species: *Liolaemus kingii*, *Liolaemus sumuncurae*, *Liolaemus baguali*, *Liolaemus tristis* and *Liolaemus uptoni*; and (3) the *lineomaculatus* group (Etheridge, 1995) with four described species: *L. lineomaculatus*, *Liolaemus hatcheri*, *Liolaemus silvanae* and *Liolaemus kolengh*. One other species, *Liolaemus magellanicus*, is recognized as a member of the *L. lineomaculatus* section, but it is not clearly assigned to any of these groups.

Etheridge (1995) assigned species from the *L. lineomaculatus* section to the *Liolaemus sensu stricto* group on the basis of morphological evidence, but later several authors found evidence for placing the *L. lineomaculatus* section into the *Eulaemus* group. In the first quantitative phylogenetic analysis of the genus, Young Downey (1998) recovered four species of the *L. lineomaculatus* section (*L. lineomaculatus*, *L. kingii*, *L. archeforus* and *L. silvanae*) as forming the sister clade to the *montanus* section (both members of the *Eulaemus* group), based on allozyme data. Schulte et al. (2000) found similar results based on mitochondrial DNA (mtDNA) sequences of three species (*L. lineomaculatus*, *L. somuncurae* and *L. magellanicus*), and used the name "*L. lineomaculatus* section" to identify this clade. A similar clade (*L. kingii*, *L. magellanicus* and *L. lineomaculatus*), was also recovered by Morando (2004) based on mtDNA and nuclear DNA (nucDNA).

The *L. lineomaculatus* section is among the least studied of all *Liolaemus* groups, with a majority of species known only from type localities and described from limited material, with poor diagnoses and limited justification (see Lobo et al., 2010a, e.g.: Núñez and Scolaro, 2009; Pincheira-Donoso and Núñez, 2005; Pincheira-Donoso et al., 2008a,b). Further, the group has the most southerly distribution of the genus, ranging from northern Patagonia south to the tip of the continent, and across the Strait of Magellan to Tierra del Fuego (Abdala and Lobo, 2006). This region has been subjected to a complex geological history including the uplift of the Andes, volcanism, marine introgressions, and extreme climatic oscillations driven by cyclic glaciations–deglaciations (Rabassa et al., 2005; Rabassa, 2008). Furthermore, species from the *L. lineomaculatus* section are distributed across extremely heterogeneous landscapes (annual temperatures vary from −20 °C to more than 40 °C); thus, its phylogenetic/phylogeographic history has also likely been complex and interesting. A well-resolved and well-supported phylogenetic hypothesis for this group can contribute to studies of its evolutionary history and how this compares with the histories of other co-distributed taxa (Azpilicueta et al., 2009; Coronato et al., 1999; Cosacov et al., 2010; Lessa et al., 2010; Marchelli et al., 1998; Marchelli and Gallo, 2004, 2006; Markgraf, 1983; Markgraf et al., 1995; Morando et al., 2007; Muellner et al., 2005; Villagran, 1991). Further, ecological, physiological, and behavioral studies, some focused on adaptations to cold climates for some *Eulaemus* species (Ibargüengoytía et al., 2002, 2010; Jacksic and Schwenk, 1983; Kozykariski et al., 2008; Medina and Ibargüengoytía, 2010; Pincheira-Donoso et al., 2008a, 2009a,b), can in the future be evaluated within a more inclusive evolutionary context for the *L. lineomaculatus* section.

No phylogenetic hypothesis exists for most species of the *L. lineomaculatus* section; thus, *our main objective is to provide a well-supported phylogeny for the entire clade. We use a multi-locus molecular data set, and compare the inferred topologies across multiple phylogenetic reconstruction methods*. Our sampling includes all 18 described species represented by specimens from their type localities in most cases, and eight distinct molecular lineages that may represent undescribed species ("candidate species"; Morando et al., 2003) included in the *L. lineomaculatus* section. We sequenced two mitochondrial gene regions, three nuclear protein-coding genes, and four anonymous nuclear loci for all named and candidate species. We then performed phylogenetic analyses based on different partitions of the concatenated sequences (all genes separately, all nuclear genes, all mitochondrial genes, and all genes combined), using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) methods. We also used a coalescent-based species tree approach (Liu and Pearl, 2007), because several studies have demonstrated that concatenation methods can recover inaccurate topologies under some conditions (incomplete lineage sorting, hybridization/introgression, gene duplication, horizontal gene transfer, and gene tree error estimation) (Degnan and Rosenberg, 2009; Heled and Drummon, 2009; Kubatko and Degnan, 2007; Liu and Pearl, 2007; Maddison, 1997; Pamilo and Nei, 1988). Coalescent-based species tree methods can accommodate gene tree heterogeneity caused by incomplete lineage sorting into species tree estimation (Degnan and Rosenberg, 2009; Edwards, 2009; Liu and Pearl, 2007; Rannala and Yang, 2003), and BEST is among the few methods that directly infer the evolutionary history of the species rather than gene trees (Liu et al., 2009). Few studies have used this combination of methods in lizards (Fujita et al., 2010; Leaché, 2009, 2010; Wiens et al., 2009).

2. Materials and methods

2.1. Taxon sampling

The *L. lineomaculatus* section includes 18 recognized species; 16 of the type localities are located in Argentina and two in Chile. Samples were collected from 15 of the Argentina type localities and one in Chile (*L. scolaroi*; Fig. 1). We could not collect samples from the type localities of *L. magellanicus* and *L. chacabucoense*, but we included samples collected 50 km and 200 km east of their type localities, respectively. In both cases, the morphological characters of our specimens matched those of the vouchers described from their respective type localities. We also included individuals from eight candidate species (they represent different lineages with more than three percent of molecular distance with other described species, and morphological differences) that are currently being studied by our research group. We also included a sample of *L. zullyae* from Chile, as it was found in sympatry with *L. scolaroi*, and although males from both species are considerably different, the uncorrected pairwise cyt-b distance between them is zero.

To test monophyly of the *L. lineomaculatus* section and its phylogenetic position within *Liolaemus*, we selected five other species of the genus as outgroups, including *Liolaemus boulengeri* and *Liolaemus darwinii* from the *montanus* section (member of *Eulaemus* clade), and *Liolaemus bibronii*, *Liolaemus gracilis* and *Liolaemus petrophilus* from *L. sensu stricto* clade. We rooted all trees using two species of *Phymaturus*, the sister genus to *Liolaemus* (Etheridge, 1995; Lobo et al., 2010a). This rooting scheme permitted us to test both monophyly of the *L. lineomaculatus* section and its affinity with either the *Eulaemus* clade or the *L. sensu stricto*. We used a total of 65 lizards from a wide geographic area (Fig. 1); details of the specimens and localities are summarized in Appendix A.

Two individuals collected in the same locality were chosen as representatives of each terminal taxon to check for mistakes. We constructed NJ trees on cyt-b sequences to confirm that in all cases individuals from the same locality were conspecific, and then we usually selected one specimen for further amplification of all markers. In a few cases some genes did not amplify for one individual, so we included the conspecific from the same locality to complete the data set, and in three cases where this second animal (and others from that locality) did not yield a PCR product, we used another

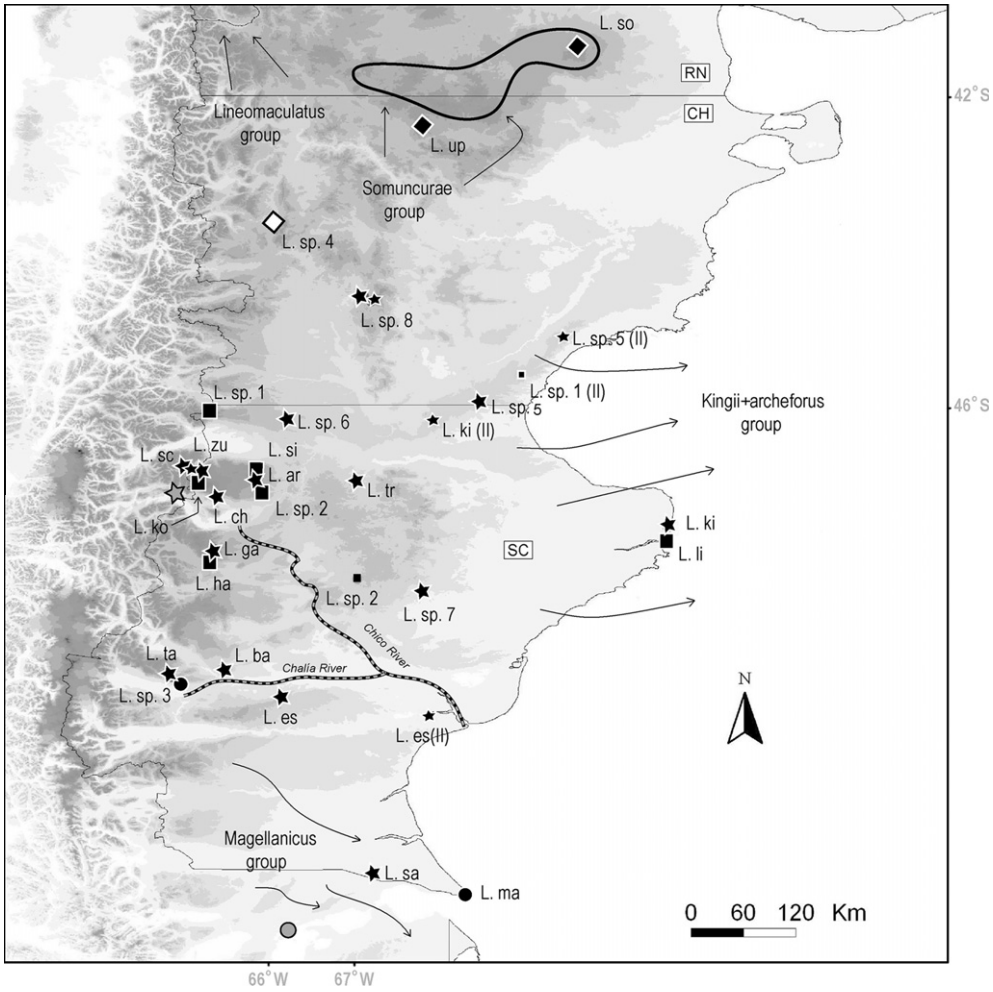

Fig. 1. Distribution map for species of the *Liolaemus lineomaculatus* section sampled for this study. Squares: *lineomaculatus* group (L. li: *L. lineomaculatus*, L. ha: *L. hatcheri*, L. si: *L. silvanae*, L. ko: *L. kolengh*, *L.* sp.1 and *L.* sp. 2); circles: *magellanicus* group (L. ma: *L. magellanicus*, *L.* sp. 3); black diamonds: *somuncurae* group (L. so: *L. somuncurae*, L. up: *L. uptoni*), and white diamond: *L.* sp. 4 (partial support for its inclusion on this group); stars: *kingii + archeforus* group (L. ki: *L. kingii*; L. sa: *L. sarmientoi*, L. es: *L. escarchadosi*, L. ba: *L. baguali*, L. ta: *L. tari*, L. ga: *L. gallardoi*, L. ch: *L. chacabucoense*, L. sc: *L. scolaroi*, L. zu: *L. zullyae*, L. ar: *L. archeforus*, L. tr: *L. tristis*, *L.* sp. 5, *L.* sp. 6, *L.* sp.7, *L.* sp. 8). Gray symbols indicate non-sampled type localities; larger size symbols identify sampled type localities, while small symbols and (II) show additional sampled localities. Chalía and Chico Rivers are indicated with gray and black lines. Arrows indicate the proposed refugia for the *lineomaculatus*, *magellanicus* and *kingii + archeforus* groups; in the *somuncurae* group arrows and the circled area indicate the Somuncurá Plateau, a possible refugium for this group. RN: Río Negro Province, CH: Chubut Province, SC: Santa Cruz Province.

individual from a nearby locality (and identified as a conspecific in the cyt-b NJ tree; see Appendices A and B for details on voucher specimens amplified for each species and gene).

2.2. Gene sampling

We collected new sequence data for seven nuclear genes (three protein-coding loci [NPCL], and four anonymous loci [ANL]) and two mitochondrial genes (cytochrome *b* and 12S). Our gene sampling was based on a screening of published sets of nuclear primers (Gamble et al., 2008; Kocher et al., 1989; Saint et al., 1998; Townsend et al., 2008; Wiens et al., 1999), and on a non-published set of ANL primers developed by A. Camargo (Personal Communication) for lizards of the *L. darwinii* complex (members of the *montanus* section of *Eulaemus*). We screened primers for 36 genes and selected the most informative for different hierarchical levels of divergence in the focal group of this study. These genes include the NPCL Cmos, ACM4tg, and PRLR (Gamble et al., 2008; Saint et al., 1998; Townsend et al., 2008); and the anonymous fragments LDA8F, LDA1D, LDA9C, LDA9E (Camargo, Personal Communication).

2.3. Molecular data

Genomic DNA was extracted using the Quiagen® DNeasy® 96 Tissue Kit for animal tissues following the protocol provided by the manufacturer. Protocols for PCR and sequencing procedures follow Morando et al. (2003, 2004) for 12S and cyt-b, Avila et al. (2004) for the NPCL, and the touchdown cycle described by Noonan and Yoder (2009), with standard reaction conditions (per sample: 2 μl dNTPs (1.25 mM), 2 μl 5× Taq buffer, 1 μl each primer (10 μM), 1 μl MgCl (25 mM), and 0.1 μl Taq DNA polymerase (5 U/μl; Promega Corp., Madison, WI); 14 μl total reaction volume) for the ANL genes. All sequences (ANL, NPCL and mitochondrial) were edited and aligned using the program Sequencher v4.8. (™Gene Codes Corporation Inc., 2007) and checked by eye to maximize blocks of sequence identity, except for the mitochondrial fragment 12S, for which we used Clustal X (Higgins and Sharp, 1988, 1989; Thompson et al., 1997) for alignment. We identified some indels in the following loci: LDA1D: three indels (three, four and five bp); LDA8F: six indels (three, four, five, eight, 13, and 51 bp); LDA9C: three indels (12, 14, and 16 bp); and LDA9E: three indels of one bp plus two larger indels (two and 80 bp). The 12S

fragment included only small indels; nine single and one two bp in length. We confirmed open reading frame in all protein-coding genes by translation into amino acids. Missing data in all cases were coded as "?", and sequences are deposited in GenBank (Accession Nos. JF272765–JF273049). For each gene we selected the best-fitting model using JModelTest v0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) using the corrected Akaike information criterion (Table 1). In all nuclear genes, recombination was tested and excluded using RDP: Recombination Detection Program v3.44 (Heath et al., 2006; Martin and Rybicki, 2000).

2.4. Gene partitions and data congruence

To accommodate the possibility of third-base saturation, we split the cyt-b data into two partitions; the (a) 1st + 2nd positions and (b) the 3rd position, and then used JModeltest v0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) to select the appropriate model of evolution for each partition. Similar models were selected for both partitions (TPM2uf + G and TIM2 + I + G for a and b, respectively), and we estimated Bayesian trees for both using the same search parameters as used for mtDNA analyses (below). Topologies were concordant, but with different levels of resolution, suggesting that the third base does not present a saturation problem. We therefore considered the addition of more parameters unnecessary and did not further partition the cyt-b data set. When deciding whether combining gene partitions prior to phylogenetic analyses is valid, it is important for investigators to use an objective test. Several studies have shown that the incongruence length difference test (ILD; also the partition homogeneity test in PAUP*) may be biased as a test of congruence between gene partitions (Barker and Lutzoni, 2002; Cunningham, 1997; Yoder et al., 2001). Therefore, we assessed levels of incongruence among gene partitions using a method similar to Westneat and Alfaro (2005). We calculated MP jackknife, ML bootstrap, and BI posterior probability trees for each gene partition, then compared congruence and incongruence of strongly supported clades across trees. Although some partitions showed only weak resolving power, there was no conflict among partitions; all well-supported clades were similar across all trees, so we combined all data partitions in subsequent analyses.

2.5. Phylogenetic analyses

Phylogenetic relationships were inferred from concatenated sequences using MP, ML, and BI methods. Parsimony analyses were conducted using TNT (Goloboff et al., 2003), based on a traditional search re-sampling the matrix with jackknife (36 removal probability) and with 1000 replicates for single-genes, and with 10,000 replicates for the concatenated matrixes. Likelihood analyses for individual loci were conducted using RAxML v7.0.4 (Stamatakis, 2006), based on 1000 rapid bootstrap analyses for the best ML tree. For concatenated analyses we used PAUP v4.0b4b (Swofford, 2001) to run 10,000 bootstrap pseudoreplicates (Felsenstein, 1985), with strong nodal support being inferred for bootstrap values $\geqslant 70$ (Hillis and Bull, 1993; with caveats).

Separate Bayesian analyses were conducted for each gene and for the partitioned concatenated matrix (using partitions previously identified for each gene) using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). Each analysis used four heated Markov chains (using default heating values) run for 5 million generations for individual genes, and 50 million generations for the partitioned concatenated analyses, with Markov chains sampled at intervals of 1000 generations. The equilibrium samples (after 25% of burn-in) were used to generate a 50% majority-rule consensus tree, and posterior probabilities (*Pp*) were considered significant when $\geqslant 0.95$ (Huelsenbeck and Ronquist, 2001).

To reconstruct a species tree incorporating the multispecies coalescent approach, we used the hierarchical Bayesian model implemented in BEST v2.2 (Edwards et al., 2007; Liu and Pearl, 2007; Liu et al., 2008). Two separate analyses were run for 70 million generations (sampling every 1000 generations). The gene mutation prior was set to 0.2 and 1.8 (Castillo-Ramírez et al., 2010). The prior distribution for the effective population size was modeled using an inverse gamma distribution over a broad range of Θ priors, with mean values of $\Theta = 0.015$, $\Theta = 0.0105$, $\Theta = 0.105$, and $\Theta = 0.6$ ($\beta = 0.03$, $\beta = 0.021$, $\beta = 0.21$, and $\beta = 0.12$, respectively, while holding α constant at $\alpha = 3$). We excluded the first 50% of trees as burn-in, even though likelihood values appeared to reach stationarity much earlier (~5%). Posterior probability values for species relationships were obtained by summarizing the post-burn-in posterior distribution of species trees with a 50% majority-rule consensus tree. As above, we considered clades with $Pp > 0.95$ to be strongly supported; however, we are aware that the relationship between *Pp* from BEST and the probability of a species tree clade being correctly reconstructed remains under-explored (Wiens et al., 2009).

To ensure that convergence was reached before default program burn-in values, we evaluated convergence of Bayesian MCMC phylogenetic analyses (MrBayes and BEST) by examining likelihood and parameter estimates over time in Tracer v1.5.0 (Rambaut

Table 1
Nucleotide substitution models selected (out of 88 candidate models) for all the genes/partitions with the corrected Akaike information criterion. Parsimony-informative-characters (P-I-C) and Parsimony-non-informative-characters (P-N-I-C) for ingroup species are shown, with outgroups used for the phylogenetic inference of each gene/partition.

Gene/partition	Length (bp)	Evolution model	Nst-rates	P-I-C	P-N-I-C	Nature	Outgroup
LDA8F	673	TPM1uf + G	6 – Gamma	26	31	Nuclear non-coding	*L. boulengeri*
LDA1D	749	HKY + G	2 – Gamma	16	22	Nuclear non-coding	*L. boulengeri*
LDA9C	706	TPM3uf + G	6 – Gamma	30	26	Nuclear non-coding	*L. petrophilus*
LDA9E	676	TPM2uf + G	6 – Gamma	27	14	Nuclear non-coding	*L. petrophilus*
ACM4tg	431	TIM2 + G	6 – Gamma	8	5	Nuclear	*Phymaturus*
Cmos	480	TPM2 + I	6 – Equal	8	2	Nuclear	*Phymaturus*
PRLR	465	HKY + I	2 – Equal	7	8	Nuclear	*Phymaturus*
Cyt-B	804	TrN + I + G	6 – Gamma	191	56	Mitochondrial	*Phymaturus*
1 + 2 Position	536	TPM2uf + G	6 – Gamma	32	15	Mitochondrial	*Phymaturus*
3 Position	268	TIM2 + I + G	6 – Gamma	159	41	Mitochondrial	*Phymaturus*
12S	881	TIM3 + I + G	6 – Gamma	100	32	Mitochondrial	*Phymaturus*
Mitochondrials	1685	GTR + I + G	6 – Gamma	291	88	Cyt-B + 12S	*Phymaturus*
Nuclear non-coding	2804	TPM3uf + G	6 – Gamma	99	93	LDA8F + LDA1D + LDA9C + LDA9E	*L. petrophilus*
Nuclear coding	1376	TrN + I + G	6 – Gamma	31	15	ACM4tg + Cmos + PRLR	*Phymaturus*
Nuclears	4180	TIM3 + I + G	6 – Gamma	130	108	Nuclear coding + non-coding	*Phymaturus*
All	5865	GTR + I + G	6 – Gamma	421	196	Nuclear + mitochondrial + coding	*Phymaturus*

and Drummond, 2009). All parameters had effective sample sizes (ESS) greater than 200, and most were greater than 300 upwards to over 15,000; thus, most runs had at least several hundred independent samples from the MCMC chains, a good indication that the analyses adequately sampled the posterior distributions.

2.6. Comparisons

To evaluate between-method differences in our results, we compared gene trees recovered by the three concatenation approaches (MP, BI, ML) and did the same for topologies for the mtDNA locus, combined nuclear loci, and the combined mtDNA plus nucDNA data sets. We compared topologies recovered from analyses based on the concatenated matrix for all genes with those recovered with BEST. Lastly, we discuss our results in the context of earlier non-phylogenetic morphological hypotheses and the limited molecular phylogenetic hypotheses available for the *L. lineomaculatus* section.

2.7. Divergence time analysis

We provide a "first pass" temporal calibration by estimating divergence times between the main clades of *L. lineomaculatus* section and between *Liolaemus* (*sensu stricto*) and *Eulaemus*. We performed a Likelihood ratio test (LRT) using JModeltest v0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) to test for deviation from a strict molecular clock, and then applied a "standard" 2% sequence divergence per million years with a standard deviation of 0.14 to our cyt-b matrix. Because the cyt-b data do not conform to the strict molecular clock (LRT = 1335.463051, $P < 0.01$), we used BEAST v1.6.1 with a relaxed uncorrelated lognormal clock model (Drummond and Rambaut, 2007). Two independent analyses were performed for 75 million generations and sampled every 1000 generations, with a GTR model of nucleotide substitution with gamma distributed rate variation among sites (determined from jModeltest, Posada, 2008), and assuming a Yule tree prior. The ESS for parameter estimates and convergence were checked using Tracer v1.5 (Raumbaut and Drummond, 2009). We excluded the first 10% of trees as burn-in and almost all parameters had ESS greater than 500 (except for "prior" that was 198.828). The value of dating the splits in main clades of this complex allows us to hypothesize possible scenarios under which lineages have diverged; however, we do recognize the limitations of our approach (Graur and Martin, 2004), and interpret our results cautiously (Hillis et al., 1996).

3. Results

3.1. Lineages recovered

We recovered phylogenetic hypotheses that were highly concordant across methods implemented in this study. In all single-gene and concatenated analyses, results obtained with different methods (MP, ML, BI) were topologically very similar, and none recovered any strongly supported conflicting nodes. In almost all cases MP topologies were less resolved than model-based methods (BI and ML), while the performance between the last two was similar.

Concatenation algorithms provide a generally well-resolved picture of higher-level relationships in the *L. lineomaculatus* section (Fig. 2). Four main clades are recovered in a pectinate topology with strong support in almost all cases (*Pp* = 1, MP jackknife and ML bootstrap >95%): (1) the *lineomaculatus* group, including four described species plus two candidate species, recovered as the sister clade of the rest of the species; (2) the *magellanicus* group (*L. magellanicus* + *L.* sp. 3); (3) the *somuncurae* group, defined here as (*L. somuncurae* + *L. uptoni*); and (4) the *kingii* + *archeforus* group, including all remaining species plus five candidate species. No support was found for the hypothesized (from morphological data) *kingii* and *archeforus* groups as two different clades (Fig. 2).

Based on combined nuclear and mitochondrial markers, we recovered the *lineomaculatus* group as sister clade of ((*magellanicus*) (*somuncurae* (*kingii* + *archeforus*)) (Fig. 2). The trees recovered with mtDNA vs. nuDNA were congruent (not shown) with three exceptions: (1) within the *lineomaculatus* group the mtDNA tree recovered a clade ((*L. lineomaculatus* + *L.* sp. 1) + *L.* sp. 2) which was contradicted in the nuDNA tree (the topology is the same as in Fig. 2b); (2) *L. sarmientoi*, in the mtDNA tree is recovered within the (*L. kingii* + *L.* sp. 7 + *L.* sp. 6) clade, while in the nuDNA tree it is recovered in the (*L. escarchadosi* + *L. tari*) clade; and (3) *L.* sp. 4, in the mtDNA tree the species is recovered within the *somuncurae* group, while in the nuDNA tree it is recovered in the *kingii* + *archeforus* clade.

3.2. Comparisons

At a more inclusive level, the *L. lineomaculatus* section was recovered with strong support for monophyly, and strongly supported as the sister clade to the *montanus* section, corroborating earlier hypotheses that they are part of the *Eulaemus* group.

Trees recovered with different priors using BEST were always congruent. The combined BEST analysis (allDNA_BEST) was in general concordant with the allDNA_con topology, but support values were considerably lower (Fig. 2). The only statistically supported incongruence between these analyses was the relationship of *L.* sp. 4 which was recovered as the sister group to the (*L. somuncurae* + *L. uptoni*) clade in all concatenated analyses, but was nested in the (*kingii* + *archeforus*) clade in the BEST analyses. We are not confident in resolving this relationship in favor of the coalescent vs. concatenated analyses, so we consider the phylogenetic position of this species in need of further study.

3.3. Divergence time estimation

The divergence time estimation between *Liolaemus* (*sensu stricto*) and *Eulaemus* was 18.50 million years ago (Mya) (95% HPD = 13.50 – 23.82) during the Early Miocene, right after the uplift of the southern Andes (~23 Mya; Ramos, 1989) started. Estimated divergence between the *lineomaculatus* and *montanus* sections puts this split in the Middle Miocene (~14.36 Mya; 95% HPD = 10.25 – 18.64), and the split between *lineomaculatus* group and the (*magellanicus* (*somuncurae* (*kingii* – *archeforus*))) clade at the Late Miocene (~8.46 Mya; 95% HPD = 6.26 – 10.84). Divergence between the *magellanicus* and (*somuncurae* (*kingii* + *archeforus*)) clades is estimated at Late Miocene/Early Pliocene (~5.87 Mya; 95% HPD = 4.26 – 7.62); while divergence between *somuncurae* and (*kingii* + *archeforus*) groups is estimated at the Early Pliocene (~4.25 Mya; 95% HPD = 3.17 – 5.48). Divergence times between taxa from the *kingii* + *archeforus* group are estimated for Late Pliocene and during the Pleistocene between 2.2 and 0.0199 Mya with a 95% HPD of (1.57 – 2.85) and (0.00 – 0.056), respectively.

4. Discussion

4.1. Relationships of the L. lineomaculatus section

Based on analyses of representatives from described and candidate species of the *L. lineomaculatus* section sequenced for nine loci, and tested with several other congeneric species representing a diversity of other clades of *Liolaemus*, we found strong support for

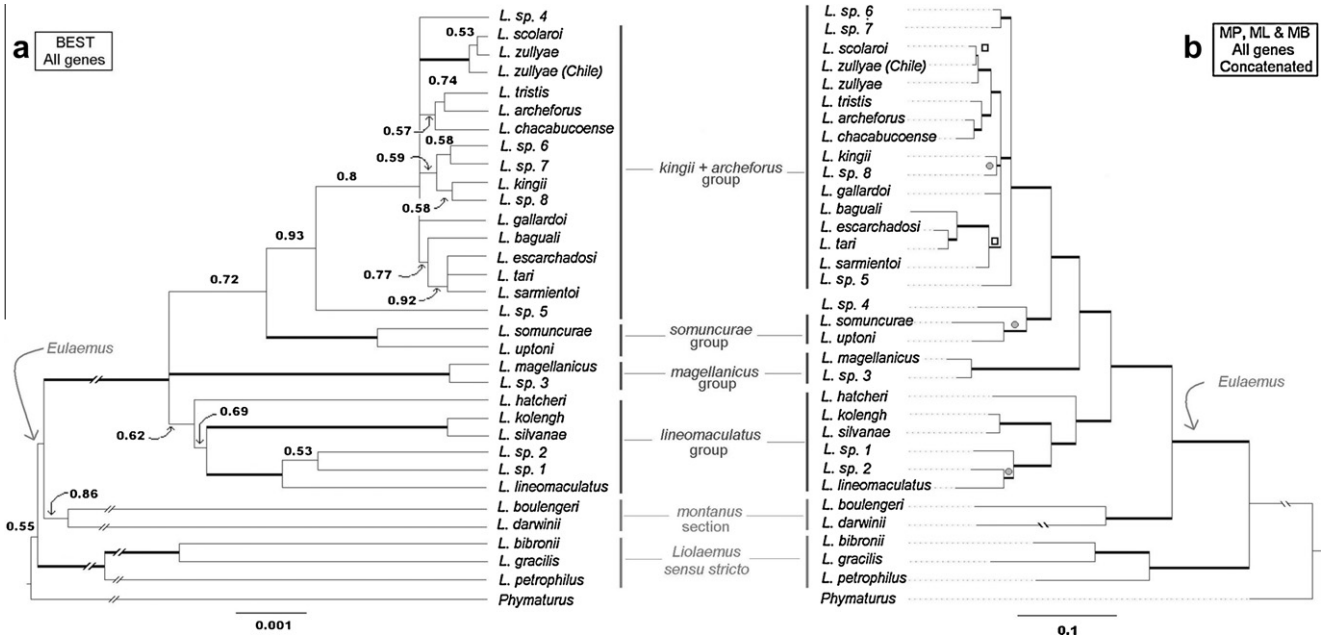

Fig. 2. On the left (a) consensus tree from BEST, all genes analyses. Posterior probability values higher than 0.5 are shown, bold branches show clades with *Pp* > 0.95. On the right (b) Bayesian tree, representing concatenated analyses and summarizing information from MP and ML methods. Nodes with high support from three methods (MP jackknife and ML bootstrap >0.70; *Pp* > 0.95) are identified by bold branches; open squares show nodes with weak MP support, and open circles nodes with weak MP and ML support.

monophyly of the group (Fig. 2). We recovered the *L. lineomaculatus* section as the sister clade to the *montanus* section, with an estimated Middle Miocene divergence, and included within the *Eulaemus* clade, a relationship congruent with previous molecular phylogenetic studies. For example, the mtDNA study of Schulte et al. (2000), based on 57 *Liolaemus* species including three representatives of the *L. lineomaculatus* section, also recovered the *L. lineomaculatus* section as part of the *Eulaemus* clade. Moreover, this same sister-group relationship between the *L. lineomaculatus* section and the *montanus* section has been recovered by many recent studies (Avila et al., 2004; Cruz et al., 2005; Espinoza et al., 2004; Morando, 2004; Morando et al., 2003; Schulte et al., 2004). Interestingly, the cold-adapted lizards of the *L. lineomaculatus* section are the southernmost-distributed of the diverse *Eulaemus* clade, which extends north to southern Peru and east to coastal habitats in Brazil. In this context, our study provides a critical first step towards a well-supported multi-locus phylogeny of the more inclusive clade *Eulaemus*, which will provide opportunities to test several thermal-adaptation hypotheses in the future.

Historically, the *L. lineomaculatus* section has been in taxonomic flux. Laurent (1985, and see also Laurent, 1995) proposed the subgenus *Rhytidodeira* Girard (1858; including *L. archeforus*, *L. gallardoi*, *L. kingii*, *L. sarmientoi*, and *L. somuncurae*), and designated *Proctotretus kingii* Bell as the type species of the group despite the fact that Donoso-Barros (1970) had previously designated *L. bibronii* as the type species for *Rhytidodeira*. This taxonomic disagreement led Pincheira-Donoso and Núñez (2005) to consider the use of *Rhytidodeira* inappropriate and to propose a new subgeneric name: *Donosolaemus*, for the same species group (excluding *L. bibronii*). Pincheira-Donoso and Núñez (2005; p. 32) listed six characters to justify recognition of this new subgenus, three of which are based on the absence of characters that are widespread in other *Liolaemus* groups (see Cei, 1993; Etheridge, 1995; Etheridge and Espinoza, 2000), and three others that are present in different *Liolaemus* groups (see Etheridge, 1995; Laurent, 1983). However, we agree with Lobo et al. (2010a) that this argument is invalid and we discourage the use of this name. Schulte et al. (2000) used the name "*L. lineomaculatus* section," and Espinoza et al. (2004) referred to the group as the "*lineomaculatus* clade." Since there is no clear justification for its status as a separate subgenus (Lobo et al., 2010a), we follow Schulte's nomenclature and call this clade the *L. lineomaculatus* section.

As noted above, very few studies have included even a modest number of species from this section; thus, it is not surprising that relationships within the *L. lineomaculatus* section are poorly known. The first molecular study was based on allozymes and included four species of this section (Young Downey, 1998), and the first DNA-sequence study of Schulte et al. (2000) included only three species. Espinoza et al. (2004) published an ecological study on the origins of herbivory, based on a *Liolaemus* phylogeny generated from a combination of morphological and molecular characters (some characters taken from the literature and new data presented by those authors). Espinoza et al. (2004) included 12 species of the *L. lineomaculatus* section (*L. archeforus*, *L. zullyae*, *L. gallardoi*, *L. sarmientoi*, *L. baguali*, *L. tari*, *L. escarchadosi*, *L. kingii*, *L. magellanicus*, *L. lineomaculatus*, *L. silvanae*, and *L. hatcheri*), which were recovered as the sister clade to the *L. montanus* section. Although there are no support values associated with their tree, the recovered relationships are congruent with those recovered in this work, with the exception of the position of *L. kingii*; Espinoza et al. (2004) recovered this species outside the clade comprising the rest of the (*kingii* + *archeforus*) clade, while we found strong support for its position nested within this clade. We could not find locality data for the *L. kingii* samples used by Espinoza et al. (2004), and given the wide distribution of this taxon and the fact that it is likely a species complex (Breitman et al., unpublished data), it is possible that their sample represents a different species.

We found support with BEST and concatenated analyses for recognition of four major groups within the *L. lineomaculatus* section: (1) the *lineomaculatus* group, including *L. lineomaculatus*, *L. hatcheri*, *L. silvanae* and *L. kolengh*, plus candidate species *L.* sp.

1 and *L.* sp. 2 (with a Late Miocene divergence from the rest of the section); (2) the *magellanicus* group including *L. magellanicus* and *L.* sp. 3 (Late Miocene/Early Pliocene divergence from the (*somuncurae*, *kingii* + *archeforus*) clade); (3) the *somuncurae* group including *L. somuncurae* and *L. uptoni* (Early Pliocene divergence from *kingii* + *archeforus* group); and (4) the *kingii* + *archeforus* group including *L. baguali*, *L. escarchadosi*, *L. tari*, *L. sarmientoi*, *L. scolaroi*, *L. zullyae*, *L. tristis*, *L. archeforus*, *L. chacabucoense*, *L. kingii*, *L. gallardoi*, plus four candidate species (*L.* sp. 5, *L.* sp. 6, *L.* sp. 7 and *L.* sp. 8; divergence times between these species are Late Pliocene and during Pleistocene). Relationships between the *lineomaculatus*, *magellanicus*, and *somuncurae* + (*kingii* + *archeforus*) groups are not resolved by BEST analyses, but with all concatenated analyses we recover a pectinated topology with strong support for the following structure: (*lineomaculatus* group (*magellanicus* group (*somuncurae* group (*kingi* + *archeforus* group)))) (Fig. 2b).

4.2. Discordances between BEST and concatenated trees

The BEST species tree recovers a deep trichotomy between the *lineomaculatus*, *magellanicus* and (*somuncurae* + *kingii* + *archeforus*) clades, which might reflect geographic fragmentation in which three or more lineages differentiated more or less simultaneously from a common ancestor. If this was the most plausible hypothesis, then we should have recovered the same history with the concatenation analyses, but they all recover strongly supported and well-resolved topologies. Moreover, separate mtDNA and nuDNA concatenated analyses recover this same topology; thus, we suggest that our dataset is insufficient for BEST to resolve this trichotomy. Absence of resolution by BEST might be due to an insufficient number of individuals, loci, alleles, base pairs (Brito and Edwards, 2009), and/or locus quality (Knowles, 2009). These limitations may be further compounded by unknown demographic issues including past and/or present gene flow, ancestral population sizes, and branch lengths between nodes (time between speciation events) (Carling and Brumfield, 2007; Castillo-Ramírez et al., 2010; Eckert and Carstens, 2008; Maddison and Knowles, 2006; Camargo et al., 2011).

Liolaemus sp. 4 was recovered as the sister group to the (*L. somuncurae* + *L. uptoni*) clade in all concatenated analyses, but was nested in the (*kingii* + *archeforus*) clade in the BEST analyses. This incongruence could be an example of fully resolved branching order due to the mtDNA locus over-riding the nuclear signal in the all genes concatenated analyses (because mitochondrial genes should sort to monophyly four times faster than any single nuclear locus, Ballard and Whitlock, 2004). With concatenated nuclear genes only, we recovered with strong support the same results as with all genes with BEST (*L.* sp. 4 nested into the *kingii* + *archeforus* clade), while with mitochondrial genes we recovered this species nested into the *somuncurae* group. This observation is consistent with a past hybridization hypothesis with asymmetrical mtDNA gene flow from one of these clades into another (common in animals; Funk and Omland, 2003), but further study is needed to rule out other alternatives, and establish directionality of introgression if this hypothesis is supported. The lower level of resolution in the shallower parts of the phylogenetic trees, particularly with BEST approach, could also simply reflect a lack of information from more recent divergence events without sufficient time for differentiation. Avila et al. (2006) suggested that for the *fitzingerii* clade (included in *Eulaemus*), which has a partially overlapping distribution with the *kingii* + *archeforus* group, glacial advances most probably pushed populations to the east (when the sea level was lower), fostering fragmentation and recent divergence events. A similar history could have affected lineages from this group, with "incipient" species dispersing back to the west during interglacials, and possibly contacting and hybridizing with other "incipient" species.

4.3. The morphological hypothesis

There are three frequently referenced morphological groups within the *L. lineomaculatus* section, including the *lineomaculatus*, *kingii* and *archeforus* groups (Cei, 1986; Etheridge, 1995); all are recognized on the basis of meristic characters (scale counts) and disjunct geographic distributions. Our study recovered the *lineomaculatus* group, but we do not have support for the "traditional" *kingii* and *archeforus* groups recognized in these earlier studies. Moreover, our evidence places the species distributed on and adjacent to the Somuncurá Plateau into a different lineage (*somuncurae* group) external to the clade comprising the rest of the species of the *kingii* + *archeforus* group. The incorrect assumption of the monophyly of a group will mislead other researchers (behaviorists, ecologists, etc.), but so far only one ecological study has been based on most of the species from the *L. lineomaculatus* section (Espinoza et al., 2004), and it was not based on the "traditional" morphological hypothesis. On the basis of support for the clades recovered in this study, from multiple unlinked gene regions and across different methods and optimality criteria, we suggest that researchers interested in comparative ecological/evolutionary studies in *Eulaemus* adopt the topology presented here as the best-supported working hypothesis.

4.4. Post-hoc biogeographical hypotheses

Although we only used individuals collected from (or near) type localities, our findings indicate that some distributional remarks and post-hoc biogeographical hypotheses are warranted. The *lineomaculatus* group is widely distributed over a large area in Santa Cruz and Chubut Provinces, ranging from the coast west to the Andes and north to central Neuquén Province (Cei, 1986; Christie, 2002; IIbargüengoytía et al., 2001; Williams, 1997; Fig. 1). The *lineomaculatus* group was recovered as the sister taxon to a clade comprising the rest of the section, while the next clade (*magellanicus* group) is restricted to the southernmost area of Santa Cruz Province (south of the Rio Chico) and in Tierra del Fuego (Fig. 1). The *somuncurae* group is restricted to the northernmost part of the distribution of this section (mainly on the Somuncurá Plateau; Cei, 1986), while the sister clade to the *somuncurae* group (the *archeforus* + *kingii* group) is distributed in southern Chubut and Santa Cruz Provinces. The concatenated analyses also provide strong support for one clade (*L. scolaroi*, *L. zullyae*, *L. tristis*, *L. archeforus* and *L. chacabucoense*) confined to a relatively small area between the Buenos Aires–General Carrera and Cochrane–Posada–Pueyrredón Lakes of northwestern Santa Cruz Province (around 46°S). A second clade (*L. tari*, *L. escarchadosi* and *L. baguali*) inhabits plateaus of the upper Santa Cruz River basin, in southwestern Santa Cruz Province (around 50°S; Fig. 1).

Our phylogenetic hypotheses, taken in combination with these distributional patterns and previous hypotheses of ancestral refugia for other Patagonian taxa (see below), allow us to hypothesize that during glacial advances, ancestral populations of the four main clades (*lineomaculatus*, *magellanicus*, *somuncurae* and *kingii* + *archeforus* groups) likely persisted in the north (Somuncurá Plateau) (42°S), south (55°S), east (46–50°S) and west (40°S) of their current distributions. Specifically, we suggest that the *lineomaculatus* clade (*L.* sp. 1, see above) persisted in southwestern Neuquén Province (around 40°S, arrows in Fig. 1), a refugial region proposed for other taxa (Azpilicueta et al., 2009; Cosacov et al., 2010; Marchelli et al., 1998; Marchelli and Gallo, 2004, 2006; Morando et al., 2007; Muellner et al., 2005; Villagran, 1991). For the *magellanicus* clade, we hypothesize a refugium south of the Chalía and Chico Rivers and/or in the southeastern Tierra del Fuego (55°S, arrows in Fig. 1). This is also coincident with a recent study that

identified a phylogeographic break in populations of the plant *Calceolaria polyrhiza* at the Chico River, and the authors hypothesized the presence of a refugium south of this River (Cosacov et al., 2010). Other authors have hypothesized a refugium in southeastern Tierra del Fuego (55°S) (Coronato et al., 1999; Markgraf, 1983; Markgraf et al., 1995), or a break in populations of rodents at the Strait of Magellan (Lessa et al., 2010). Given these findings, the hypotheses of such a refugium for the *magellanicus* group could be tested by sampling these lizards from Tierra del Fuego.

We suggest that ancestral lineages of the *kingii* + *archeforus* clade could have persisted in refugial areas now under sea level along the eastern margin of Patagonia (arrows in Fig. 1). These refugia have been suggested previously for others organisms (Avila et al., 2006; Cosacov et al., 2010; Huck et al., 2009; Mraz et al., 2007; Pinceel et al., 2005; Ronikier et al., 2008), as reduced ocean volumes during glacial advances (Hulton et al., 2002) shifted the Atlantic Patagonian coastline four degrees to the east (Auer, 1956). This new land may have offered suitable habitats to escape permafrost conditions during glacial times in what is now southern Patagonia (Jakob et al., 2009), especially for species that are today restricted to low-elevation areas on the eastern margin of Patagonia. One paleo-modeling study of niche in *L. petrophilus* identified a Last Glacial Maximum (LGM) refugium on the shallow continental shelf E of Patagonia at latitude 41–43°S (Fontanella et al., 2011). Lastly, for the *somuncurae* clade we hypothesize a refugium on the Somuncurá Plateau (42°S), based on a phylogeographic analysis that found evidence for a North-to-South colonization pattern (Breitman, unpublished data).

Our divergence estimates suggest that speciation events within the *kingii* + *archeforus* clade occurred during the Late Pliocene and during Pleistocene, perhaps during the glacial advances of the Great Patagonian Glaciation (GPG; 1.2–1.0 Mya; Rabassa, 2008). During this largest Patagonian glaciation, ice tongues extended to the Atlantic Ocean in the continental area south of the Gallegos River (southern Santa Cruz Province), for the first time in the Cenozoic (Clapperton, 1993; Rabassa et al., 2000). This event would have had a massive impact on the abundance and distribution of lizards and other terrestrial taxa. However, the divergence events that gave origin to the *lineomaculatus*, *magellanicus*, *somuncurae* and *kingii* + *archeforus* clades, were deeper in the past (Miocene), and we cannot yet compare our divergence estimation times with other studies (see above) because most of these have been focused on Quaternary events and very few have presented molecular-divergence estimates (e.g. Cosacov et al., 2010).

5. Future directions

The chronology of southern South American glaciations is one of the best known in the world, due in part to precision dating (40Ar/39Ar) of volcanic rocks associated with glacial deposits (Rabassa et al., 2005). This record shows that some glacial events were synchronized with those in the Northern Hemisphere (Heusser and Heusser, 2006), while others were not (Schaefer et al., 2006). The earliest glaciations occurred in the late Miocene/early Pliocene (~7.0–5.0 Mya), followed by at least eight glaciations from Middle- to Late-Pliocene, and then 14–16 further glaciations after the GPG (Rabassa et al., 2005). In the context of this complex but fascinating geological history, lizards of the *L. lineomaculatus* section can provide one model system for testing a number of evolutionary hypotheses. For example, what might have been the locations of glacial refugia for these lizards, and did the multiple post-glacial re-colonizations occur from one or several refugia via one or multiple dispersal events? How well do lizard phylogeographic histories match those of co-distributed populations of small mammals, flowering plants, or other taxa currently under study? Has this clade experienced different speciation rates relative to other co-distributed clades, and how do patterns of species diversification relate to their current distributions and ecology? Molecular, morphological and ecological/geographical data are being used to conduct integrative phylogeographic analyses to delimit species boundaries within this clade (in addition to alpha taxonomic studies and new species descriptions), and to reconstruct a temporal sequence of demographic histories. We present here the first step for this work by delimiting well-supported clades within the *L. lineomaculatus* section and suggesting some possible biogeographic scenarios for future hypothesis testing.

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Appendix A. Species and individuals used in this study with information on author and year of description. Sampling localities with geographic coordinates are provided; all samples were collected in Argentina except for *L. scolaroi and L. zullyae* (both collected in Chile).

Species	Descriptor (year)	LJAMM – CNP	Locality	South	West
L. archeforus	Donoso-Barros and Cei (1971)	9238, 9240, 9242	Santa Cruz. Dto. Lago Buenos Aires. Puesto Lebrun (ahora Puesto Viejo) 27.3 km W casco Estancia La Vizcaina. Meseta del Lago Buenos Aires	−46.96438	−71.10755
L. baguali	Cei and Scolaro (1983)	9394, 9395	Santa Cruz. Dto. Lago Buenos Aires. Sierra del Bagual, camino 1 km E de Ruta Nacional 40	−49.41025	−71.49952
L. chacabucoense	Núñez and Scolaro (2009)	13,049, 13,048, 13,050	Santa Cruz. Dto. Lago Buenos Aires. Ruta Provincial 41, 35.3 km E Paso Roballos, cerca Rio Correntoso	−47.19705	−71.58583
L. escarchadosi	Scolaro and Cei, 1997	7163	Santa Cruz. Dto. Corpen Aike. Ruta Nacional 288, 1 km E empalme Ruta Nacional 3, 24 km W Puerto Santa Cruz	−50.05427	−68.88588
		9340	Santa Cruz. Dto. Lago Argentino. Ruta Provincial 65, 43.5 km W empalme Ruta Provincial 17, 1 km S Cerro Mank Aike	−49.77133	−70.72997
L. gallardoi	Cei and Scolaro (1982)	9446, 9454	Santa Cruz. Dto. Rio Chico. Estancia Cerro Beltza, 12 km N Ruta Provincial 37	−47.99372	−71.68041
L. hatcheri	Stejneger (1909)	9485, 9489, 9491	Santa Cruz. Dto. Rio Chico. Estancia Cerro Beltza, 12 km N Ruta Provincial 37	−47.99372	−71.68041
L. kingii	Bell (1843)	9776	Santa Cruz. Dto. Deseado. 5.5 km N Puerto Deseado	−47.71497	−65.83919
		10,157	Santa Cruz. Dto. Deseado. Ruta Provincial 16, 42.1 km N Las Heras, 2 km W Estancia Sarai	−46.20961	−68.78733
L. kolengh	Abdala and Lobo (2006)	7300	Santa Cruz. Dto. Lago Argentino. Camino a Los Antiguos, 15.6 a 21 km N paso Roballos	−47.02105	−71.80883
L. lineomaculatus	Boulenger (1885)	7470, 7471	Santa Cruz. Dto. Deseado. 5.5 km N Puerto Deseado por camino costero	−47.71697	−65.84108
L. magellanicus	Hombron and Jacquinot (1847)	6722	Santa Cruz. Dto. Guer Aike. Reserva Provincial Cabo Vírgenes, 3 km S Faro.	−52.35258	−68.38808
L. sarmientoi	Donoso-Barros (1973)	7204, 7206	Santa Cruz. Dto. Guer Aike. Laguna Azul, Reserva Geológica Provincial Laguna Azul, cerca de estancia Monte Aymond	−52.07472	−69.58127
L. scolaroi	Pincheira-Donoso and Núñez (2005)	13,033, 13,034	XI Region de Aysen, Chile Chico. Camino a Reserva Jeinimeni, 49 km SW empalme camino Los Antiguos – Chile Chico, 4 km NE entrada a Reserva Jeinimeni, 1 km NE Rio Jeinimeni	−46.81286	−71.97822
L. silvanae	Donoso-Barros and Cei (1971)	9221	Santa Cruz. Dto. Lago Argentino. Puesto Lebrun (ahora Puesto Viejo) 27.3 km W casco Estancia La Vizcaina. Meseta del Lago Buenos Aires	−46.96438	−71.10755
L. somuncurae	Cei and Scolaro (1981)	6911, 6914	Río Negro. Dto. 9 de Julio. 65.6 km destacamento policial El Rincon, cerca de cerro Corona, entre cerro Corona Grande y cerro Corona Chico	−41.39466	−66.95925
L. tari	Scolaro and Cei (1997)	9407	Santa Cruz. Dto. Lago Argentino. Meseta basáltica Punta del Lago, camino a Meseta Campo las Piedras, 7 km N Estancia Punta del Lago	−49.56972	−72.04775
L. tristis	Scolaro and Cei (1997)	9618, 9619	Santa Cruz. Dto. Lago Buenos Aires. Ruta Provincial 39, 7.5 km N Estancia La Maria, 16 km S Arroyo Piramides	−46.98261	−69.79991
L. uptoni	Scolaro and Cei (2006)	8426	Chubut. Dto. Gastre. Ruta provincial 4, 58, 3 km W Gan Gan	−42.39180	−68.93331
L. zullyae (LT)	Cei and Scolaro (1996)	7391	Santa Cruz. Dto. Lago Buenos Aires. Camino paso Roballos – Los Antiguos, 49.1 km N puente metálico sobre el Río Ghio	−46.84627	−71.87125
		8894	Chubut. Dto. Senguer. Ruta Nacional 40, 26 km N Alto Río Senguer	−44.80608	−70.70691
L. zullyae (Chile)		13,039, 13,040	XI Region de Aysen, Chile Chico. Camino a Reserva Jeinimeni, 17 km NE entrada a Reserva Jeinimeni	−46.77986	−71.80261

Appendix A (*continued*)

Species	Descriptor (year)	LJAMM – CNP	Locality	South	West
L. sp. 1		9258	Santa Cruz. Dto. Lago Buenos Aires. Laguna de los Gendarmes, Ruta Provincial 45, camino a El Portezuelo, 87.6 km NW Perito Moreno	−46.09952	−71.68269
		9678	Chubut. Dto. Escalante. Ruta Provincial 37, 2.5 km W empalme Ruta Nacional 3	−45.62872	−67.68433
L. sp. 2		9275, 9277	Santa Cruz. Dto. Lago Buenos Aires. Meseta Lago Buenos Aires, 18.7 SW Puesto Lebrun	−47.09138	−71.02025
		9542	Santa Cruz. Dto. Lago Buenos Aires. Camino vecinal a Estancia La Morocha, 5.1 km NW ex Hotel Dos Manantiales, NW Ruta Provincial 12	−48.25236	−69.78072
L. sp. 3		9388	Santa Cruz. Dto. Lago Argentino. Meseta basáltica Punta del Lago, camino a Meseta Campo las Piedras, 7 km N Estancia Punta del Lago	−49.56972	−72.04775
L. sp. 4		9183	Chubut. Dto. Languineo. Ruta Nacional 40, 16.1 km S Tecka	−43.62991	−70.84088
L. sp. 5		9202	Chubut. Dto. Escalante. Ruta Nacional 3, 70.2 km SW Garayalde	−43.62991	−70.84088
		9205	Chubut. Dto. Escalante. Estación Holdich (abandonada)	−45.96663	−68.19958
L. sp. 6		13,053, 13,055	Santa Cruz. Dto. Lago Buenos Aires. Ruta Nacional 40, 39.7 km N empalme Ruta Provincial 43, Cordon El Pluma	−46.18225	−70.66791
L. sp. 7		9814, 9815, 9999	Santa Cruz. Dto. Magallanes. Ruta Provincial 77, 77.7 km NW empalme Ruta Provincial 25, 2 km N Estancia Vega Grande	−48.40952	−68.93452
L. sp. 8		8898	Chubut. Dto. Paso de Indios. Ruta Provincial 23, 65.1 km E empalme Ruta Provincial 20, camino a Estancia Los Flamencos	−44.59741	−6969058
		9190	Chubut. Dto. Paso de Indios. Ruta Provincial 23, 77.6 km E empalme Ruta Provincial 20, 1 km SE Estancia Los Flamencos	−44.66616	−69.6062
L. boulengeri		10,177, 10,178	Santa Cruz. Dto. Deseado. Ruta Provincial 16, 42.1 km N Las Heras, 2 km W Estancia Sarai	−46.20961	−68.78733
		3610	Santa Cruz. Dto. Cushamen. Ruta Provincial 12 y embarcadero La Cancha	−42.79661	−70.95838
L. darwinii		10,392, 10,391	Río Negro. Dto. San Antonio. Gran Bajo del Gualicho. 42, 4 km NW San Antonio Oeste, por Ruta Provincial 2	−40.34883	−65.04983
L. bibronii		9896	Santa Cruz. Dto. Deseado. 5.5 km N Puerto Deseado	−47.71497	−65.83919
		9897, 9898	Santa Cruz. Dto. Deseado. Ruta Provincial 47, 55.4 km SW Tellier, 3 km S puente sobre Rio Deseado, en empalme Ruta Provincial 89	−47.85033	−66.62216
L. gracilis		10,517	La Pampa. Dto. Puelén. Ruta Provincial 16, 23, 6 km W empalme Ruta Nacional 151	−37.07494	−67.78544
L. petrophilus		11,121	Rio Negro. Dto. 9 de Julio. Ruta Provincial 8, 34, 8 km S Los Menucos (camino a Prahuaniyeu)	−41.08775	−67.89072
P. dorsimaculatus	983, 982	Neuquén. Dto. Ñorquín. Copahue		−37.82055	−71.0866
P. patagonicus	3205	Chubut. Dto. Gaiman. Ruta Nacional 25, 40 km WSW Dolavon		−43.45438	−66.12119

Appendix B. Voucher individuals from LJAMM – CNP collection used for sequencing each gene. Missing data is shown with boldface questions marks.

Species	12S	LDA8F	ACM4tg	Cmos	Cyt-b	LDA1D	LDA9C	LDA9E	PRLR
L. archeforus	9240	9240	9240	9240	9240	9238	9240	9242	9240
L. baguali	9395	9395	9395	9395	9394	9395	9395	9395	9395
L. chacabucoense	13,049	13,049	13,048	13,050	13,049	13,049	13,049	13,049	13,049
L. escarchadosi	9340	9340	9340	9340	9340	9340	9340	7163	9340
L. gallardoi	9446	9446	9446	9454	9446	9446	9446	9454	9446
L. hatcheri	9491	9491	9491	9489	9491	9491	9491	9485	9491
L. kingii	9776	9776	9776	9776	9776	9776	9776	10,157	9776
L. kolengh	7300	7300	7300	7300	7300	7300	7300	7300	7300
L. lineomaculatus	7470	7471	7470	7470	7470	7470	7470	7470	7470
L. magellanicus	6722	6722	6722	6722	6722	6722	6722	6722	6722
L. sarmientoi	7206	7206	7204	7206	7206	7206	7204	7206	7206
L. scolaroi	13,034	13,034	13,034	13,034	13,033	13,033	13,033	13,034	13,033
L. silvanae	9221	9221	9221	9221	9221	9221	9221	9221	9221
L. somuncurae	6914	6914	6914	6914	6914	6914	6911	6911	6914
L. tari	9407	9407	9407	9407	9407	9407	9407	9407	9407
L. tristis	9618	9618	9618	9619	9618	9618	9618	9618	9618
L. uptoni	8426	8426	8426	8426	8426	8426	8426	8426	8426
L. zullyae	7391	7391	8894	7391	7391	7391	7391	7391	7391
L. zullyae (Chile)	13,039	13,039	13,039	13,039	13,039	13,040	13,039	13,039	13,039
L. sp. 1	9678	9678	9678	9678	9678	9678	9678	9258	9678
L. sp. 2	9277	9277	9277	9277	9277	9277	9275	9542	9277
L. sp. 3	9388	9388	9388	9388	9388	9388	9388	9388	9388
L. sp. 4	9183	9183	9183	9183	9183	9183	9183	9183	9183
L. sp. 5	9202	9202	9202	9205	9202	9202	9202	9202	9202
L. sp. 6	13,053	13,053	13,053	13,055	13,053	13,053	13,053	13,053	13,053
L. sp. 7	9814	9814	9814	9815	9814	9999	9814	9814	9814
L. sp. 8	9190	9190	9190	8898	9190	9190	9190	9190	9190
L. darwinii	10,392	10,391	10,391	10,392	10,391	10,391	10,392	10,391	10,391
L. boulengeri	10,177	10,178	10,177	10,177	3610	10,177	10,177	10,177	3610
L. bibronii	9897	**????**	9896	9896	9897	**????**	9896	9898	9896
L. gracilis	10,517	**????**	10,517	10,517	10,517	**????**	**????**	10,517	10,517
L. petrophilus	11,121	**????**	11,121	11,121	11,121	**????**	11,121	11,121	**????**
P. patagonicus (3205) and *P. dorsimaculatus* (982/3).	983	**????**	982	3205	983	**????**	**????**	**????**	983

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