Zoologica Scripta

Multilocus phylogeny of the widely distributed South American lizard clade *Eulaemus* (Liolaemini, *Liolaemus*)

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Submitted: 6 January 2014
Accepted: 25 February 2014
doi:10.1111/zsc.12053

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

The lizard genus *Liolaemus* and different clades within it have been the focus of several recent phylogenetic studies mainly based on morphology and mtDNA. Although there is general consensus for recognizing two clades (subgenera) within the genus, [*Liolaemus* (*sensu stricto*) and *Eulaemus*], phylogenetic relationships within each subgenus remain difficult to elucidate, given incomplete taxonomic sampling and large discordance between published studies. Here, new phylogenetic relationships for the *Eulaemus* subgenus are proposed based on the largest molecular data set ever used for this clade, which includes 188 individuals and 14 loci representing different parts of the genome (mtDNA, anonymous nuclear loci and nuclear protein-coding loci). This data set was analysed using two species tree approaches (*BEAST and MDC). Levels of discordance among methods were found, and with previously published studies, but results are robust enough to propose new phylogenetic hypotheses for the *Eulaemus* clade. Specifically well-resolved and well-supported novel hypotheses are provided within the *lineomaculatus* section, and we formally recognize the *zullyae* clade, the *sarmientoi* clade and the *hatcheri* group. We also resolve species relationships within the *montanus* section, and particularly within the *melanops* series. We found discordance between mitochondrial and nuclear trees and discussed alternative hypotheses for the *lineomaculatus* and *montanus* sections, as well as the challenge in resolving phylogenetic relationships for large clades in general.

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Introduction

In general, South America constitutes a biologically poorly known region (Beheregaray 2008), and in particular, this is the case of the lizard genus *Liolaemus*, which is a widely distributed group endemic to most of the temperate part of the continent. Its geographical distribution is associated with a wide range of climatic regimes: from the extremely arid Atacama desert (southern Peru) to temperate *Nothofagus* rainforests (Donoso-Barros 1966; Cei 1986; Etheridge & de Queiroz 1988; Etheridge 1995; Lobo 2001). *Liolaemus* includes approximately 239 described species (Breitman *et al.* 2013), but in 2003, one molecular study (Morando *et al.* 2003) suggested that the number of *Liolaemus* species could be as high as 320 (the number recognized at that time was 174) if scientists continued incorporating new approaches and continued to explore poorly known regions. During the last 10 years, approximately 65 new species have been described, and 79 have been proposed as candidate species in various phylogeographical papers (Morando *et al.* 2003, 2004, 2007; Breitman *et al.* 2012; Avila *et al.* 2004; Olave *et al.* 2012; Medina *et al.* 2013), which deserve further comprehensive studies.

Liolaemus and some clades within it have been the focus of several phylogenetic studies mainly based on morphology and mtDNA (e.g. Schulte *et al.* 2000; Avila *et al.* 2004; Espinoza *et al.* 2004; Morando 2004; Cruz *et al.* 2005;

Lobo 2005; Abdala 2007), but also based on few nuclear markers and limited taxon sampling (e.g. Morando *et al.* 2004; Avila *et al.* 2006; Breitman *et al.* 2011; Fontanella *et al.* 2012). Recently, Camargo *et al.* (2012) published a multilocus phylogeny of a clade within *Liolaemus* (*darwinii* group) based on 20 markers (18 nuclear) for 16 species, representing what at that time was the most densely sampled phylogenetic study, in terms of taxa and markers, for a clade within this genus. Although there is general consensus for recognizing two clades (subgenera) within the genus [*Liolaemus* (*sensu stricto*) and *Eulaemus*], phylogenetic relationships within each subgenus remain difficult to elucidate, due largely to incomplete taxon sampling and in the case of molecular based studies, few markers, which most probably are the cause of discordance between published studies. In particular, the *Eulaemus* subgenus is composed of 144 species (those described through January 2013) widely distributed in Argentina, and a more limited distribution in Chile (Schulte *et al.* 2000). One particular group (*wiegmannii* group) is also distributed along the coasts of Brazil and Uruguay (Etheridge 2000), and another (*montanus* group) extends north through Bolivia and much of Perú (Quinteros & Abdala 2011). While multiple studies consistently recover two large clades within this subgenus [the *lineomaculatus* section (Schulte *et al.* 2000; 21 species) and *montanus* section (Schulte *et al.* 2000; 123 species); e.g. Schulte *et al.* 2000; Morando *et al.* 2004; Avila *et al.* 2006; Abdala 2007; Fontanella *et al.* 2012; Pyron *et al.* 2013], relationships between several groups/complexes/clades included within the *montanus* section are not resolved (in this manuscript, we maintained all group names in their original taxonomic context defined in previous publications).

One recent study (Olave *et al.* 2012) focused on clarifying relationships between the main clades of *Eulaemus*. This study used explicit model-based approaches to compare the probability of observed gene tree discordance [deep coalescent events; Maddison (1997)] given different topologies previously proposed for this subgenus by Schulte *et al.* (2000), Avila *et al.* (2006), Abdala (2007), Fontanella *et al.* (2012), as well as two models describing the simultaneous divergence of clades. Olave *et al.* (2012) showed that the best-supported hypothesis to describe *Eulaemus* evolution includes two events of rapid simultaneous radiation of lineages, but this study did not focus explicitly on taxonomic issues in this clade. These hypotheses of rapid radiations explain why earlier studies were largely discordant: rapid radiations present very challenging scenarios to phylogenetic inference, and well-supported but strongly conflicting topologies may be recovered due to different data sources (molecular markers or morphological characters), methods of inference (i.e. Maximum Parsimony, Maximum Likelihood, Bayesian approaches) and/or different combinations of species sampled (Whitfield & Lockhart 2007).

Until recently, most molecular studies in *Eulaemus* were based on either a single locus (mtDNA) or the concatenation of multiple loci (e.g. Schulte *et al.* 2000; Morando *et al.* 2004, 2008; Cruz *et al.* 2005; Avila *et al.* 2006; Fontanella *et al.* 2012; Pyron *et al.* 2013). By concatenating gene sequences, only a single tree is estimated, when in reality phylogenies are comprised of multiple gene trees (Maddison 1997). Methods have now been developed to estimate species trees by accommodating the stochastic segregation of multiple independent loci (Knowles 2009; Knowles & Kubatko 2010). This allows the capture of relevant information, and consequently, species tree approaches are more accurate under challenging scenarios than traditional concatenated matrix analyses (Leaché & Rannala 2011).

In this study, we present a new hypothesis of phylogenetic relationships for the subgenus *Eulaemus* based on 188 individuals and 14 loci representing different parts of the genome [mtDNA, anonymous nuclear loci (ANL) and nuclear protein-coding loci (NPCL)], using two species tree approaches: *BEAST (Drummond & Rambaut 2007) and MDC (Than & Nakhleh 2009). Because we accepted the best-supported hypothesis for relationships between clades within the *montanus* section is a rapid radiation of these crown groups, here we focus on resolving relationships within some of these clades. In particular, we have fully sampled the *lineomaculatus* section (21 species) and the *melanops* series (25 species), and we present new evidence for the monophyly of each of these groups, as well as new hypotheses of relationships within each. We also have representatives of the *anomalus* (three of seven species), *wiegmannii* (seven of 12 species), *darwinii* (seven of 20 species) and *montanus* groups (seven of 59 species); this includes 70 of the 144 described species of the subgenus (as well as several candidate species), which to date represents the largest number of taxa and molecular markers used to estimate phylogenetic relationships within this subgenus.

Materials and methods

Field sampling

We included a total of 188 terminals of the subgenera *Eulaemus* and *Liolaemus*, sampled mostly from Argentina, with a small number from Chile and Brazil (Fig. 1). Specimens were collected by hand or noose, sacrificed by a pericardiac injection of sodium tiopenthal Abbot®/Pentovet®, dissected slightly to extract a sample of liver/muscle for molecular study, fixed in 10–20% formalin and later transferred to 70% ethanol. Tissues were stored in a freezer

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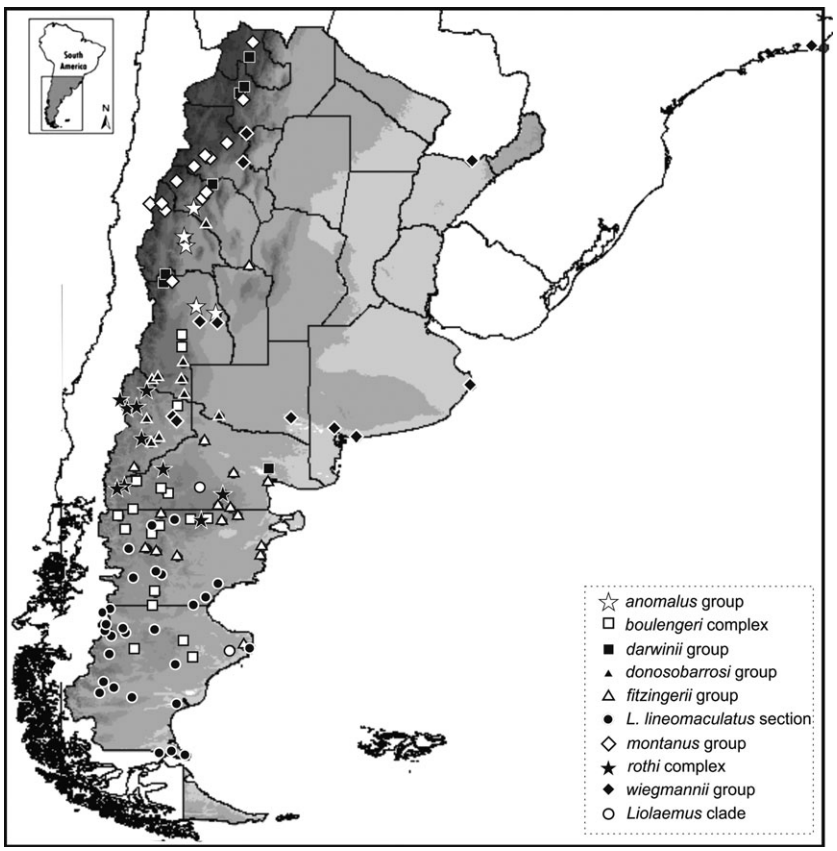

Fig. 1 Distribution map of samples. Each clade is represented by a particular symbol, as follows: anomalus group (white stars), boulengeri complex (white squares), darwinii group (black squares), donosobarrosi group (black triangles), fitizingeri group (white triangles), lineomaculatus section (black circle), montanus group (white romboids), rothi complex (black stars), wiegmannii group (black romboids) and outgroups of *Liolaemus* subgenus (white circles).

with 96% ethanol. Voucher specimens are deposited in the herpetological collections LJAMM-CNP of the Centro Nacional Patagónico, Puerto Madryn, Argentina (CENPAT–CONICET, http://www.cenpat.edu.ar/nuevo/colecciones03.html), and the Bean Life Science Museum, Brigham Young University (BYU; http://mlbean.byu.edu/Research Collections/Collections/ReptilesandAmphibians.aspx) (Table S1, available online).

Laboratory procedures

We sequenced two mitochondrial genes, four anonymous nuclear loci (ANL) and eight nuclear protein-coding loci (NPCL), a total of 14 genes. Sequences are deposited in GenBank (Table S2 available online, Accession Nos. KF966660-KF969205). Genomic DNA was extracted using the Qiagen®DNeasy® 96 Tissue Kit (Qiagen, Valencia, CA, USA) following the protocol provided by the manufacturer. For PCR and sequencing protocols, we followed Morando *et al.* (2003, 2004) for the mitochondrial genes [cyt-b (~800 bp) and 12S (~818 bp)], Camargo *et al.* (2012) for the four anonymous nuclear loci [ANL: A1D (~776 bp), A12D (~802 bp), A4B (~495 bp) and A9C (~758 bp)] and eight nuclear protein-coding loci (NPCL): EXPH5 [~901 bp], KIF24 [~535 bp], MXRA5 [~848 bp], (Portik *et al.* 2012), DNAH3 [~535 bp], PRLR [~501 bp], PNN [~902 bp], SNCAIP [~467 bp] (Townsend *et al.* 2008), CMOS [~530 bp] (Wiens *et al.* 1999). About 97% of sequences are original for this manuscript, and the remaining 3% of sequences were taken from Breitman *et al.* (2011) for the *L. lineomaculatus* section.

We amplified all nuclear genes using the touchdown PCR 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 mL total reaction volume). All sequences were edited using the program SEQUENCHER v4.8. (™Gene Codes Corporation Inc. 2007) and aligned with MAFFT (Katoh *et al.* 2002) based on 100 tree rebuilding iterations and a maxirate of 100. Protein-coding genes were translated to amino acids to check for codon errors, while alignments of ANL and the 12S regions were checked by eye and manually adjusted if necessary to maximize blocks of sequence identity. Missing data in all cases were coded as '?' (15.19% of the total data set was missing). For each gene, we selected the best-fitting model of evolution using JMODELTEST v0.1.1 (Posada 2008) using the Akaike criterion information (corrected)

(AICc). Because of the high mutation rate and saturation detected in cyt-b, we only used the 1st and 2nd codon positions when we used the full matrix. In all nuclear genes, recombination was tested using RDP: Recombination Detection Program v3.44 (Martin & Rybicki 2000; Heath *et al.* 2006).

Phylogenetic analyses

Study design. We used two species from the *Liolaemus* (*sensu stricto*) subgenus as outgroups – *L. petrophilus* and *L. bibronii* – and estimated species trees using two different approaches. We used the 'Minimizing Deep Coalescence' (MDC) dynamic programming (DP) algorithm implemented in PHYLONET package (Than & Nakhleh 2009), and *BEAST 1.6.2 (Drummond & Rambaut 2007). The MDC approach takes gene trees as input, which we obtained using MRBAYES v3.2 (Ronquist & Huelsenbeck 2003), and estimates the species trees by minimizing the number of deep coalescence of gene trees within the estimated species tree. In contrast, *BEAST is a Bayesian approach which uses DNA sequences to first estimate individual gene trees and then seeks the most likely species trees. We did not always get convergence with *BEAST using our full matrix (188 terminals, 14 loci), most probably because of large incongruence among gene trees coupled with a very large data matrix (i.e. too many parameters to be estimated by *BEAST; see section 3.4). Therefore, we only present results of full matrix of 188 taxa and 14 loci using the MDC approach. Note that here we accept the relationships between main groups of *Eulaemus* subgenus as proposed in Fig. 2, which includes two events of simultaneous radiation of lineages (i.e. hard polytomies).

In this article, we developed a computationally feasible subsampling approach (summarized in Table 1), in which we conducted separate analyses for the following partitions: 1-*L. lineomaculatus* section, 2-*L. montanus* section (composed of *wiegmannii*, *montanus*, *anomalus*, *darwinii* groups and two representatives of *L. melanops* series) and 3-*melanops* series (composed of *boulengeri* and *rothi* complexes, *fitzingerii* and *donosobarrosi* groups and one representative of *L. wiegmannii*). With the inclusion of two species of the *melanops* series for the *L. montanus* section analyses, we have included all recognized descendent lineages; thus, the impact on species tree estimation due to matrix reduction is minimized. We then ran *BEAST and MDC analyses for each of these three partitions. For species tree inferences using *BEAST, we ran 500×10^6 generations of MCMC and sampled at intervals of 50 000 generations (burnin 10%). Convergence was diagnosed by observation of ESS values >200 for each parameter estimated. We also ran separate analyses of mitochondrial loci using BEAST v1.6.2 and estimated species trees based only on nuclear loci for each partition using *BEAST. In both BEAST and *BEAST analyses, all priors were set as default values. Details of individuals used in phylogenetic analyses are summarized in Table S1 (available online).

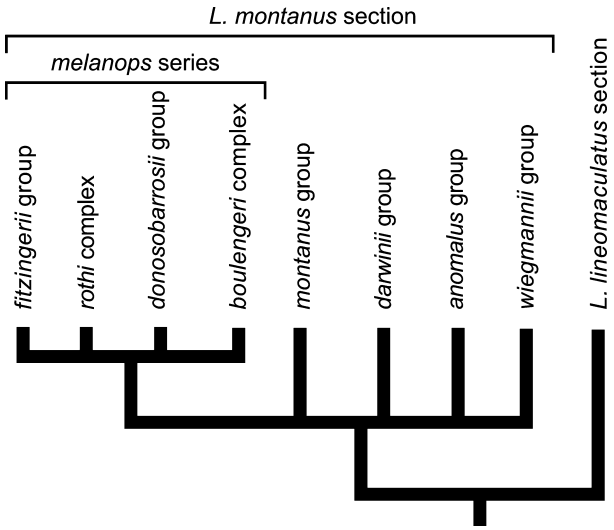

Fig. 2 Phylogenetic tree for the subgenus *Eulaemus*. This hypothesis was proposed by Olave *et al.* (2012) using explicit model-based approaches.

Table 1 Study design. We implemented the study design summarized here. Following the phylogenetic hypothesis illustrated in Fig. 2 (proposed by Olave *et al.* 2012), we split the full matrix (*Eulaemus* subgenus) into three different parts, including the *lineomaculatus* section, the *montanus* section (composed of *wiegmannii*, *montanus*, *anomalus*, *darwinii* groups and *melanops* series), and the *melanops* series (composed of *boulengeri* and *rothi* complexes, and *fitzingerii* and *donosobarrosi* groups). We implemented *BEAST for these three partitions and MDC approach for the full matrix (*Eulaemus* subgenus)

Matrix	Dimensions	Analyses performed
Eulaemus subgenus	$N = 188$; 14 loci	MDC
lineomaculatus section	$N = 52$; 13 loci	*BEAST
montanus section	$N = 61$; 14 loci	*BEAST
melanops series	$N = 82$; 14 loci	*BEAST

Gene trees. We conducted Bayesian analysis with four independent runs and two chains per run, for 10×10^6 generations of MCMC, and sampled at intervals of 1000 generations, and for each alignment, we used a burnin of the first 25% of the generations. These gene trees were used as the input files to perform MDC analyses.

We also concatenated both mitochondrial loci (cyt-b and 12S) and ran a Bayesian analysis in BEAST v1.6.2 using the

188 taxa. We ran 100×10^6 generations of MCMC and sampled at intervals of 10 000 generations with a burnin of 10%.

To quantify gene trees discordance, we calculated the distances between gene trees using the Penny and Hendy (1985) and Kuhner and Felsenstein (1994) methods implemented in the dist.topo function of the 'ape' library of the R package. The first method estimates strict topological distances, and the second one also includes branch lengths to estimate distances between trees. We also used the Phylonet package to calculate the number of deep coalescence events observed between each gene tree and the species tree estimated from the full matrix of 14 loci and 188 taxa (Table S1 available online; section 2.2.3). For both distance methods to evaluate gene tree discordance, as well as deep coalescence events counted between gene trees and the species tree, values equal to zero mean perfect congruence between trees, and higher values indicate incongruence.

Eulaemus *phylogeny*. Although we accept phylogenetic relationships of the subgenus *Eulaemus* as proposed in Fig. 2, we estimated a phylogeny based on MDC approach using a dynamic programming (DP) algorithm implemented in PhyloNet package (Than & Nakhleh 2009) based on the full matrix (Table 2; Table S1 available online).

Liolaemus lineomaculatus *section phylogeny*. We performed a *BEAST analysis using 13 genes (we excluded the nuclear gene MXRA5 because of the extensive missing data for this group) and 52 individuals (8,715 bp). We also recovered a species tree estimated using only the 11 nuclear loci and 82 taxa. We included candidate species that were previously included in Breitman *et al.* (2011) and used the same terminal label for each of these but added a 'B' (in reference to Breitman *et al.* 2011, for example *L.* sp. B4). We included two individuals per species in most cases.

Liolaemus montanus *section phylogeny*. We included representatives of the *anomalus*, *darwinii*, *wiegmannii* and *montanus* groups as focal taxa, and two individuals of *L. rothi* and *L.* sp. 4, representing the *melanops* series. We performed a *BEAST analysis using 14 genes and 57 individuals (9,436 bp). We divided the *L. wiegmannii* complex as *L. wiegmannii* (*sensu stricto*) and *L. wiegmannii* '1 to 3', following Avila *et al.* (2009), and we added a new candidate species, *L. wiegmannii* 4, to represent the *L. wiegmannii* complex. We also included *L. multimaculatus* and *L. multimaculatus* 1 as well as four other candidate species and used two to three individuals per species in most cases.

Liolaemus melanops *series phylogeny*. We included the *rothi* and *boulengeri* complexes and the *donosobarrosi* and

Table 2 Summary of matrices used following study design of Table 1. For the full matrix of the subgenus *Eulaemus*, the number of segregating sites (s.s.) is given; models of evolution were selected by AIC

			Mitochondrial loci		ANL				NPCL							
Matrix	Total length	*n*	cytb	12s	A1D	A4B	A9C	A12D	CMOS	DNAH3	EXPH5	KIF24	MXRA5	PNN	PRLR	SNCAIP
Eulaemus subgenus	8808 bp	188	528 bp	810 bp	749 bp	759 bp	411 bp	411 bp	481 bp	639 bp	811 bp	470 bp	827 bp	887 bp	431 bp	417 bp
			s. s. 285	s. s. 302	s. s. 202	s. s. 117	s. s. 97	s. s. 205	s. s. 68	s. s. 64	s. s. 165	s. s. 126	s. s. 110	s. s. 109	s. s. 100	s. s. 65
			GTR+I+G	SYM+G	HKY+G	K80 + G	K80+G	HKY+G	HKY+I+G	HKY+I+G	HKY+G	K80+G	HKY+G	HKY+I+G	JC	JC+G
L. lineomaculatus section	8715 bp	52	791 bp	870 bp	761 bp	515 bp	725 bp	673 bp	481 bp	740 bp	817 bp	491 bp	-	904 bp	453 bp	494 bp
			GTR+I+G	GTR+I+G	HKY+G	HKY+I	HKY+I	HKY+G	HKY+I	HKY+I	HKY+G	HKY+G		HKY+I	HKY+G	HKY+I
L. montanus section	9436 bp	57	710 bp	806 bp	768 bp	422 bp	757 bp	743 bp	522 bp	696 bp	836 bp	497 bp	847 bp	900 bp	468 bp	463 bp
			HKY+I+G	GTR+G	HKY+G	HKY+I	HKY+G	GTR+G	HKY+I	HKY+I	HKY+G	HKY+I+G	HKY+G	HKY+I+G	HKY+I	HKY+G
melanops series	9260 bp	82	805 bp	805 bp	770 bp	417 bp	742 bp	636 bp	505 bp	641 bp	850 bp	496 bp	835 bp	893 bp	443 bp	421 bp
			GTR+G	HKY+I+G	HKY+G	HKY+G	K80+I+G	GTR+G	HKY+I	GTR+I	HKY+G	HKY+I+G	HKY+I	HKY+I	HKY+G	K80+G

fitzingerii groups as focal taxa in this part of the study, and three individuals representing *L. wiegmannii* as outgroups. Here, we used a matrix of 82 taxa and 14 loci (9260 bp). We also recovered a species tree estimated using only the 12 nuclear loci and 82 taxa. We ran 100×10^6 generations of MCMC and sampled at intervals of 5000 generations (burnin 10%). As above, we included two to three individuals per species in most cases.

Results and discussion

Details of the data matrices and molecular evolution models used in this study are shown in Table 2. The MDC phylogenetic analysis of the full matrix (188 taxa and 14 loci) is shown in split Figs 3 (*L. lineomaculatus* section) and 4 (*L. montanus* section). *BEAST results per each matrix are shown in Figs 5 (*L. lineomaculatus* section), 6 (*L. montanus* section) and 7 (*melanops* series). Mitochondrial gene tree for full matrix is shown in Fig. S1 in supplementary material available online. Finally, species trees inferred from the nuclear loci alone are presented in Figs. S2 (*L. lineomaculatus* section), S3 (*L. montanus* section) and S4 (*melanops* series) in the online supplementary material. Deep coalescence calculation between each gene tree and species tree is presented in Table 3. Distances between gene trees are presented in Table S3 in supplementary information available online.

The L. lineomaculatus *section*

This is the sourthernmost distributed group of *Liolaemus*, reaching Tierra del Fuego and encompassing all of

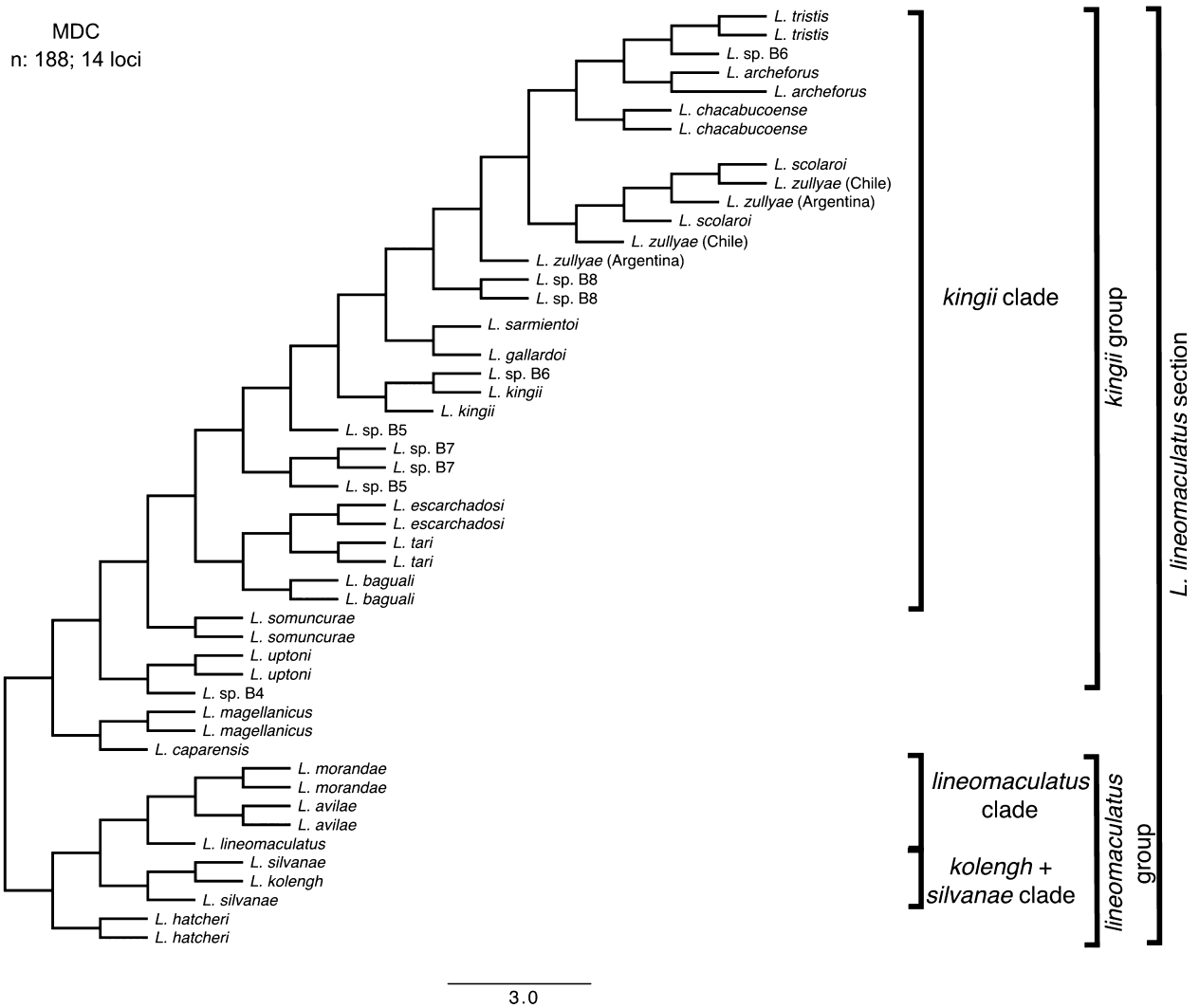

Fig. 3 *Eulaemus* phylogeny (*Liolaemus lineomaculatus* section). Species tree for the *Eulaemus* subgenus using MDC approach and full matrix of 14 loci and 188 taxa. The full species tree has been split into two figures (3 and 4); this is the *L. lineomaculatus* section.

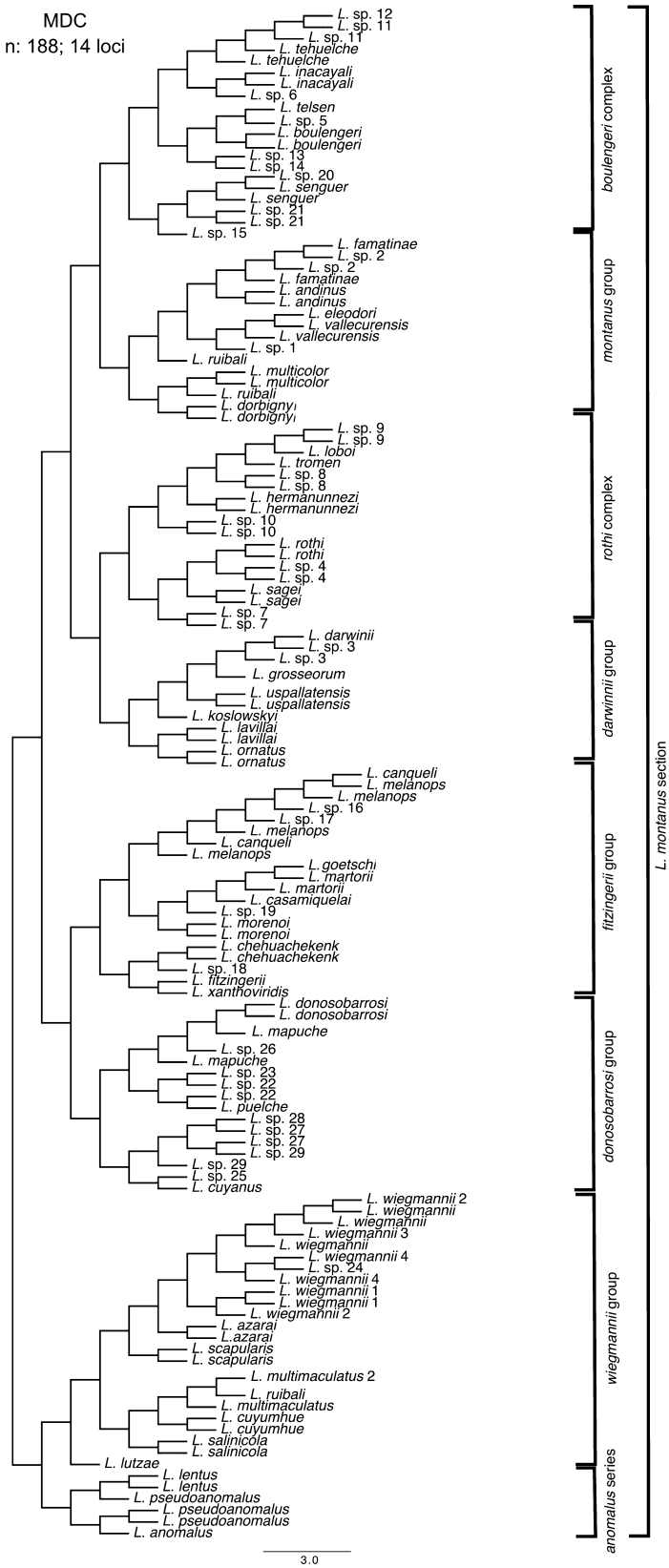

Fig. 4 *Eulaemus* phylogeny (*Liolaemus montanus* section). Species tree for the *Eulaemus* subgenus using MDC approach and full matrix of 14 loci and 188 taxa. The full species tree it is been split into two figures (3 and 4); this is the *L. motanus* section.

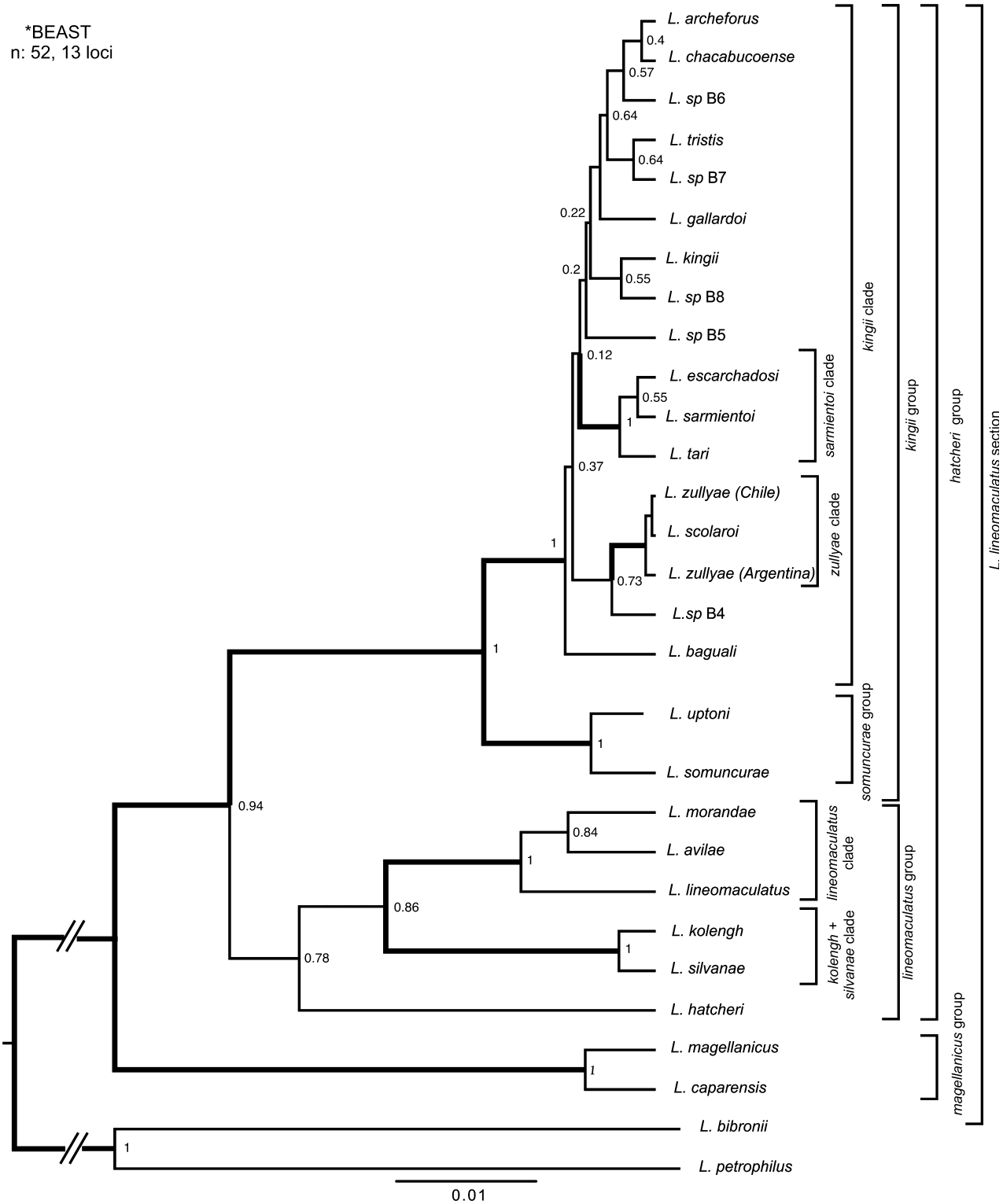

Fig. 5 *Liolaemus lineomaculatus* section phylogeny. Species trees estimated for the *L. lineomaculatus* section using *BEAST approach; bold branches represent nodal support >0.90.

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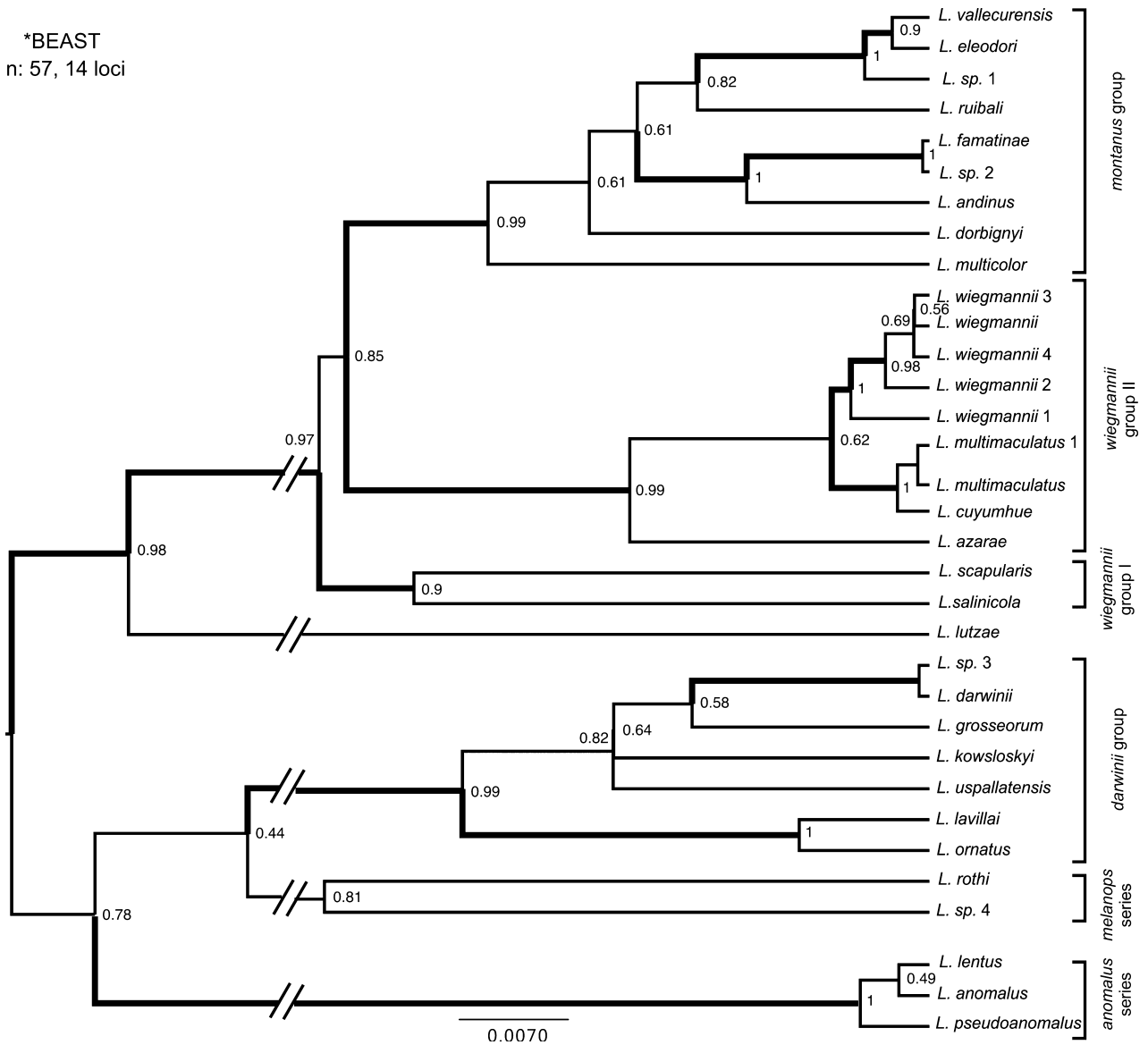

Fig. 6 *Liolaemus montanus* section phylogeny. Species trees estimated for the *L. montanus* section using *BEAST approach; bold branches represent nodal support >0.90.

Argentinian Patagonia (Fig. 1). Species from the *L. lineomaculatus* section are distributed across extremely heterogeneous landscapes with annual temperatures ranging from −20 °C to more than 40 °C, and during its evolution, this clade experienced a complex geological history including the uplift of the Andes, volcanism, marine introgressions and multiple cycles of glacial advance and retreat (Breitman *et al.* 2011). Our *BEAST species tree is shown in Fig. 5. We followed group names of Breitman *et al.* (2011, 2012, 2013) for clades with a PP >0.90. We used the same terminal label for each of these but added a 'B' (in reference to Breitman *et al.* 2011: *L.* sp. B4). We also named all strongly supported clades, including the: *zullyae* clade (PP = 0.97), *sarmientoi* clade (PP = 1) and the *hatcheri* group (PP = 0.94).

We recovered the three main clades previously recognized on both morphological and molecular evidence (Breitman *et al.* 2011, 2013) – the *kingii* (Cei 1986), *lineomaculatus* (Etheridge 1995) and *magellanicus* (Breitman *et al.* 2011) groups – although the *lineomaculatus* group had low statistical support [PP = 0.78].

The Breitman *et al.* (2011) phylogenetic study of this section was based on seven nuclear and two mitochondrial loci for one individual per species and using two different

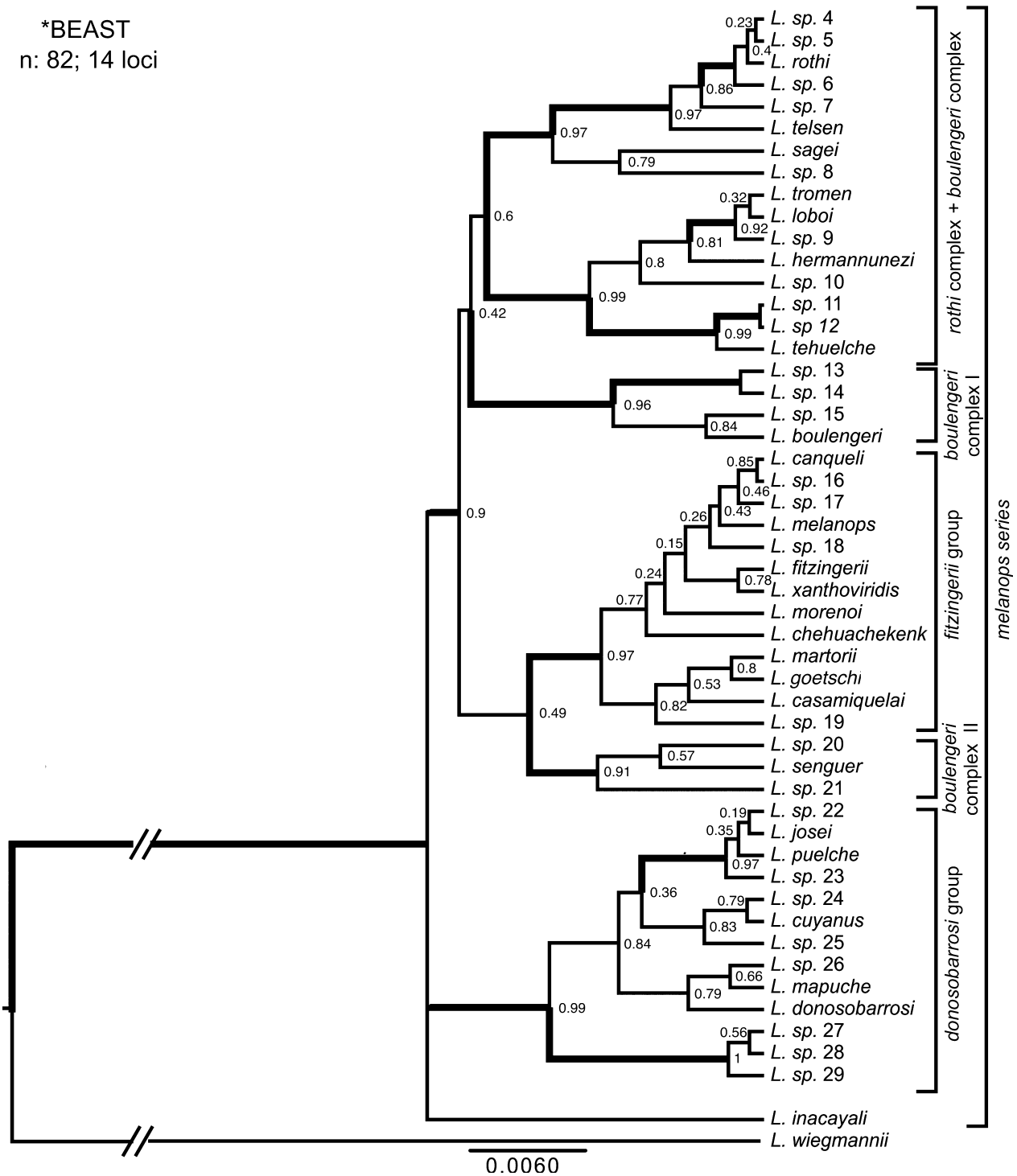

Fig. 7 *Melanops* series phylogeny. Species trees estimated for the *melanops* series using *BEAST approach. Bold branches represent nodal support >0.90.

approaches: the traditional concatenated analyses and a species tree approach (BEST). They recovered well-supported clades with the first approach, but many weakly supported nodes in their species tree analysis. Following Brito and Edwards (2009), Breitman *et al.* (2011) suggested that adding more individuals, loci or base pairs (Knowles 2009)

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Table 3 Number of deep coalescents observed between each gene tree and the species tree estimated using MDC approach and full matrix of 188 taxa and 14 loci

Locus	Deep coalescences
12S	123
CMOS	122
DNAH3	242
EXPH5	197
KIF24	243
A12D	340
A1D	228
A4B	242
A9C	139
MXRA5	125
PNN	117
PRLR	164
SNCAIP	122
cyt-b	201

could improve support values in a species tree analysis (see also Camargo *et al.* 2012). Here, we incorporated almost twice the number of individuals, and more nuclear loci, and we recovered a species tree topologically concordant with Breitman *et al.* (2011) but with higher support values for many nodes. Particularly, our *BEAST analysis (Fig. 5, compared with the Breitman *et al.* BEST tree, their Fig. 3A) resolved relationships between the three main clades *magellanicus*, *lineomaculatus* and *kingii* groups, and we also recovered the well-supported *sarmientoi* clade within the *kingii* clade.

However, the MDC analysis (Fig. 3) recovered the *magellanicus* group as sister clade of the *kingii* group, as well as *sarmientoi* clade as paraphyletic. Also, *L.* sp. B4 was recovered as sister taxon of *L. uptoni*, while *BEAST placed it within the *kingii* group. This last discrepancy was found by Breitman *et al.* (2011) between the concatenated and BEST analyses, and those authors hypothesized that hybridization and asymmetrical mtDNA introgression is a likely explanation for this pattern. Our mitochondrial and nuclear trees also showed discordances (Figs. S1 and S2 available online, respectively): the mitochondrial gene tree is concordant in this case with the MDC analysis (Fig. 3), while the nuclear species tree is concordant with *BEAST result using all loci (Fig. 5). Mitochondrial paraphyly could indicate present or past hybridization of species (Funk & Omland 2003), and this could explain different topologies recovered by different methods. This process has been suggested (Morando *et al.* 2004, 2007) and documented (Olave *et al.* 2011) in other clades of *Liolaemus* (*L. gracilis* and *L. bibronii*) and deserves further study for this particular case.

The L. montanus *section*

We performed both MDC and *BEAST analysis including 57 individuals representing the *anomalus*, *darwinii*, *montanus* and *wiegmannii* groups as focal groups, and two representatives of the *melanops* series as outgroups. *BEAST results recovered most of the main groups as well-supported clades (Fig. 6), including the *anomalus* series [PP = 1], the *darwinii* group [PP = 0.99] and the *montanus* group [PP = 0.99]. The exception is the *wiegmannii* group, which is recovered as paraphyletic, and with *L. lutzae* from Brazil as sister of the (*wiegmannii* group I + (*wiegmannii* group II + *montanus* group)) clade. We recovered two clades, *wiegmannii* groups I and II, with *wiegmannii* group II as the sister clade to the *montanus* group with moderate support (PP = 0.85), but we recovered the *wiegmannii* group as monophyletic in the MDC analysis (Fig. 4). Some studies based on morphology (Etheridge 1995, 2000; Abdala 2007), mtDNA (Schulte *et al.* 2000; Avila *et al.* 2009), morphology + mtDNA (Espinoza *et al.* 2004) and mtDNA + nDNA (Fontanella *et al.* 2012; Pyron *et al.* 2013) also recovered the *wiegmannii* group as monophyletic. Only one study based on behavioural data recovered the *wiegmannii* group as paraphyletic (Halloy *et al.* 1998). Given our MDC result and almost all previously published studies, we hypothesize that the *wiegmannii* group is monophyletic. Most probably the lack of complete taxon sampling, the high level of incongruence among gene trees (Table 3 and Table S3 available online), as well as the number of terminals in our matrix ($N = 57$), may have prevented *BEAST from fully resolving all relationships between the *wiegmannii* and *montanus* groups. The *wiegmannii* group includes 12 described species, all strictly arenicolous and no phylogenetic study has been published based on a complete taxon sampling of this group; its distribution covers a wide geographical area in Argentina, from northern Patagonia through northern Argentina (Fig. 1) and extending through coastal Uruguay and Brazil as far north as Rio de Janeiro. This group deserves detailed study.

Both *BEAST and MDC analyses recovered the *montanus* group as monophyletic (PP = 0.99) in agreement with previous analyses (Schulte *et al.* 2000; Espinoza *et al.* 2004; Avila *et al.* 2006; Abdala 2007; Fontanella *et al.* 2012; Pyron *et al.* 2013). This group includes 59 described species distributed from central Argentina and eastern Chile north through Bolivia and much of Perú, following the Andes (Quinteros & Abdala 2011). This group includes species inhabiting the highest elevations known for the genus, reaching 5176 m of altitude (Aparicio & Ocampo 2010). Similarly, as with the *wiegmannii* group, no phylogenetic study has been published that includes more than a few of the species recognized in the *montanus* group. We also have a limited sample of the suspected high species diversity for

this group (seven named taxa and two candidate species), so future studies will need to sharply increase the number of taxa, populations and loci to rigorously evaluate the evolutionary history of this group.

The *anomalus* series includes seven recognized species [three species were described by Abdala and Juarez Heredia (2013)], for which the basic biology is generally unknown due to the clade's restricted distribution, apparently very low population densities and extreme cryptic coloration (they are hard to find in the field). They usually live in saline environments (salt pans) at high elevations characterized by low vegetation cover, and the group is distributed from north-western to central-eastern Argentina (Abdala 2007). We recovered the *anomalus* clade in both analyses with strong support, in agreement with Abdala (2007) and Abdala and Juarez Heredia (2013). Here, *L. lentus* and *L. anomalus* are recovered as sister species, and *L. pseudoanomalus* as sister to these two species in the *BEAST analysis. However, these relationships are not well supported (PP = 0.49). MDC result showed *L. pseudoanomalus* as paraphyletic, as is also observed in the mitochondrial gene tree (Fig. S1 available online). Although the mitochondrial tree recovered *L. anomalus* as sister taxon of *L. lentus*, the nuclear species tree (Fig. S3 available online) did not resolve their relationships.

The *darwinii* group is distributed across the arid lands of the Monte Desert region of central and north-western Argentina (Fig. 1) and here was also recovered as monophyletic in both analyses (Fig. 6; PP = 0.99), in agreement with Abdala (2007), Avila *et al.* (2006) and Fontanella *et al.* (2012). Because a recent phylogenetic study of this group included 16 of 20 recognized species and 20 loci (Camargo *et al.* 2012), here we only included six representative species and recovered two clades: (*L. lavillai* + *L. ornatus*) and (*L.* sp. 3 + *L. darwinii*) (Fig. 6; PP = 1 and PP = 0.99 respectively), which is concordant with Camargo *et al.* (2012) results (the *ornatus* clade), but we could not resolve relationships between the other two species we sampled from this group.

We did not find support for most relationships between the main clades included in this partition, and the *BEAST well-supported (PP = 0.98) (*wiegmannii* + *montanus*) clade is discordant with the MDC tree. Although we recovered *L. rothi* + *L.* sp. 4 (representing the *melanops* series) as sister clade of the *darwinii* group and this clade as sister of the *anomalus* group, these relationships are not well supported (PP = 0.4 and PP = 0.78, respectively). Under a scenario of rapid radiation of lineages (Fig. 2), this is an expected pattern, given that relationships cannot be resolved due to high incongruence among gene trees.

The melanops *series*

Although the *melanops* series was not recovered as monophyletic by our MDC analysis (Fig. 4), this is an expected pattern under a hard polytomy scenario (Fig. 2), but each main clade (*boulengeri* complex, *donosobarrosi* group, *fitzingeri* group and *rothi* complex) was recovered as monophyletic in this analysis.

In particular, we recovered very low resolution in nuclear species tree (Fig. S4 available online). Our *BEAST analysis of the complete data set does resolve some within-group relationships (Fig. 7; higher PP in some nodes), but our *BEAST mitochondrial plus nuclear analyses recovered only limited resolution of among-group relationships within this *melanops* series. Removal of the mtDNA sequences reduced resolution considerably (Fig. S4 available online; nuclear gene tree). Both *BEAST and MDC analyses recovered the *donosobarrosi* and *fitzingerii* groups as monophyletic (Fig. 7; PP = 0.99).

The *donosobarrosi* group includes five described species distributed in north-western Argentina, while the *fitzingerii* group includes nine described species distributed from northern Monte ecotonal areas of the Somuncurá Plateau south across typical Patagonian Steppe through Santa Cruz Province (Fig. 1; Avila *et al.* 2006). Previous studies recovered the *donosobarrosi* and *fitzingerii* groups as monophyletic (Avila *et al.* 2006; Fontanella *et al.* 2012; Pyron *et al.* 2013), but Abdala (2007) recovered the *donosobarrosi* group as paraphyletic.

The *boulengeri* complex was previously recognized as a single species distributed across a linear distance of ~1200 km from north-western to south-eastern Patagonia. This region represents an ecologically and topographically complex landscape, and as originally described, the species *L. boulengeri* was characterized by extensive variation in morphology and coloration (Cei 1986). However, further studies based on better geographical sampling and new data sets resolved five species within this complex. In this study, as in similar studies of other clades (e.g. Avila *et al.* 2006; Abdala 2007), we identified several candidate species that need further research (sp. 13–15; Fig. 7). Similar to the *boulengeri* complex, *L. rothi* was recognized as a single species with extensive morphological variation, and later studies (Cei 1986) resulted in recognition of five species as part of the *rothi* complex. The *boulengeri* and *rothi* complexes are parapatrically distributed, with the *boulengeri* complex reaching a north-western distribution and the *rothi* complex expanding to south-eastern Patagonia (Fig. 1).

Although the *rothi* and *boulengeri* complexes were recovered as monophyletic in MDC analysis (Fig. 4), *BEAST recovered both as paraphyletic (Fig. 7), with *L. telsen* and *L. tehuelche* (considered as members of *boulengeri* complex) deeply nested within the *rothi* complex with strong support. Earlier studies by Abdala (2007) based on morphology, and Avila *et al.* (2006) with a subsample of molecular markers

(mtDNA) and taxa, reported extensive paraphyly for both complexes.

We also detected extensive paraphyly in the nuclear gene trees (Fig. S4 available online). Paraphyly could result from incomplete lineage sorting or hybridization, or a combination of these (Funk & Omland 2003). As noted above, hybridization was recently reported for other *Liolaemus* species (Olave *et al.* 2011), and unpublished data for the *boulengeri* and *rothi* complexes also suggest hybridization. This could explain why *BEAST cannot recover the monophyly of these two complexes even with complete taxon sampling and a larger number of independent loci. However, detailed integrative studies will be necessary to test these and other hypotheses. Alternatively, we may have used too large of a matrix (=82 taxa) which, when associated with high gene tree incongruence (Table 3 and Table S3 available online), is problematic for this type of analysis (Leaché & Rannala 2011). We detected two levels of discordance in this part of the study: one among main clades and another within clades (see polytomies in Fig. 2). This condition presents a very challenging scenario for reconstruction of relationships within each main clade; thus, further analyses are warranted.

The challenging Eulaemus *phylogeny and species tree methods*

Here, we propose new phylogenetic hypotheses for the subgenus *Eulaemus*, using recently developed species tree analyses (*BEAST and MDC), which take into account the stochastic segregation of independent loci. Although we have used the largest molecular data set currently available (14 loci and 188 taxa), we still found some limitations in resolving relationships between and within the main clades (Figs 3–5). Adding more data (taxa and loci) probably would help to resolve relationships within the main clades. However, adding more data also increases the number of parameters to be estimated and would also likely increase discordance among gene trees due to incomplete lineage sorting (ILS). Phylogenetic estimation of species trees therefore becomes more challenging (Than & Nakhleh 2010).

The level of ILS depends on both time since divergence (τ) and population size (N_e), where smaller τ and higher N_e increase ILS (Knowles *et al.* 2007; Yang & Rannala 2010; Leaché & Rannala 2011). Thus, the rapid radiation hypothesis that was proposed for this group (Fig. 2) constitutes a very challenging scenario, given internodes of $\tau \approx 0$. Also, recent divergence of species within main clades provides higher expected patterns of ILS. This is quantitatively reflected in high number of deep coalescence events detected between gene trees and species trees (Table 3) and distances between gene trees (Tables S3 available online). Thus, to reconstruct phylogenetic relationships for the *Eulaemus* clade, methods need to deal with gene tree discordance at two levels: ancestral discordance among main clades given the rapid radiation of lineages and within main clades given the recent divergence of species.

Under this challenging scenario, phylogenetic inferences are more likely to fail in recovering the real relationships, but species tree analyses perform better than traditional concatenated matrices (Leaché & Rannala 2011), because they can accommodate ILS in their models (Knowles 2009; Knowles & Kubatko 2010). Given these issues, we strongly encourage researchers to use species tree methods for future phylogenetic studies of this genus.

Acknowledgements

We thank F. Breitman, M. Kozykariski, C. Medina, N. Feltrin, C.H.F. Perez, N. Frutos, M. Nicola, R. Martinez, C. Zanotti, S. Reese and K. Temus for assistance in field collections. We thank A. Camargo for help in the laboratory and data analyses. We also thank other members of the Grupo de Herpetología Patagónica for assistance in animal curation procedures. We thank L. Vega and F. Kacoliris for *L. multimaculatus* tissue samples and Miguel Trefaut Rodriguez for *L. lutzae* tissue samples. Financial support was provided by Grants PICT 2006-506 ANPCYT-FONCYT (LJA), ANPCYT-FONCYT 33789 (MM), and a doctoral fellowship (MO) from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Kennedy Center for International Studies, the Department of Biology and the M.L. Bean Life Science Museum of BYU, and NSF-PIRE award (OISE 0530267) for support of collaborative research on Patagonian Biodiversity granted to the following institutions (listed alphabetically): Brigham Young University, Centro Nacional Patagónico (AR), Dalhousie University, Instituto Botánico Darwinion (AR), Universidad Austral de Chile, Universidad de Concepción, Universidad Nacional del Comahue, Universidad Nacional de Córdoba, and University of Nebraska. We thank Dr. Keith Crandall for continuing support. We thank the fauna authorities from Buenos Aires, Chubut, Santa Cruz, Neuquén, Catamarca, Córdoba, Corrientes, Jujuy, La Pampa, La Rioja, Salta, San Juan, San Luis, Tucumán, Mendoza and Río Negro provinces for collection permits.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Mitochondrial tree of *Eulaemus*. The mitochondrial tree was estimated using Bayesian inference: bold branches represent nodal support >0.90.

Fig. S2. *Liolaemus lineomaculatus* section phylogeny based on nuclear loci. Species trees estimated for the *L. lineomaculatus* section using *BEAST approach; bold branches represent nodal support >0.90.

Fig. S3. *Liolaemus montanus* section phylogeny based on nuclear loci. Species trees estimated for the *L. motanus* section using *BEAST approach; bold branches represent nodal support >0.90.

Fig. S4. *Melanops* series phylogeny based on nuclear loci. Species trees estimated for the *melanops* series using *BEAST approach; bold branches represent nodal support >0.90.

Table S1. List of individuals employed.

Table S2. Genbank accession numbers.

Table S3. Distances calculated between gene trees, using Penny and Hendy (1985; white cells) and Kuhner and Felsenstein (1994; grey cells) methods implemented in dist.topo function of 'ape' library of R package.