

Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata: Tropicuridae) from the central-southern Andean range

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Abstract

In this study, we used a recently developed supertrees method to test for shared phylogeographical signal in partially overlapping geographical ranges of lizards of the genus *Liolaemus* from the Andean Range in south-central Chile. We reconstruct mtDNA gene trees for three partially codistributed species (*Liolaemus tenuis*, *L. lemniscatus* and *L. pictus*), and our sampling effort is sufficient to allow statistical tests of shared signal between the combinations *L. tenuis*–*L. pictus*, and *L. tenuis*–*L. lemniscatus*. For both combinations, standardized maximum agreement subtrees scores showed statistically significant signal for shared pattern in regions of overlap, as evaluated by randomization tests ($P < 0.001$ and < 0.05 , respectively). The matrix representation with parsimony tree obtained from the combination of the three different gene trees revealed concordant phylogeographical associations of all species, and was consistent with the geographical association of intraspecific haploclades with three Chilean bioclimatic zones. A multidimensional scaling analysis of several climate variables showed highly significant differences among these zones, which further suggests that they may have contributed to similar patterns of intraspecific divergence across all three species. In the mesomorphic zone in Central Chile, the species *L. tenuis* and *L. lemniscatus* may have codiverged in response to shared orogenic vicariant events, which likely predominated over climatic events associated with cycles of glacial advance and retreat. In the hygromorphic zone in southern Chile, however, glacial cycles likely predominated in structuring the phylogeographical histories of *L. tenuis* and *L. pictus*, although important ecological differences between these two caution against broad generalizations at this point.

Keywords: Andes, Chile, comparative phylogeography, *Liolaemus*, lizard, maximum agreement subtrees, mtDNA

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Introduction

Comparative phylogeography is becoming an increasingly important subdiscipline of biogeography focused on reconstruction of the evolutionary history of ecological associations and communities on a regional scale (Bermingham &

Moritz 1998; Arbogast & Kenagy 2001). The methods of comparative phylogeography typically do this in two steps; the first is to reconstruct the evolutionary histories of two or more codistributed species within a region of interest, and then determine whether some parts of these shared histories reflect common responses to the same historical events. While the first step of this general approach (phylogenetic inference) is relatively well-developed, the actual comparative component of evaluating evolutionary histories of multiple species for signal of a shared response to the same historical events (orogenies, marine

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transgressions, glacial cycles, volcanism, etc.) has until recently been largely descriptive and qualitative. This is beginning to change as new methodologies permit tests of *a priori* hypotheses in regions where sufficient background information permits the formulation of explicit alternatives that can be distinguished with statistical rigor (Carstens *et al.* 2005). Continued refinement of methods will be extremely useful for exploring different models of speciation and other historical processes (Carstens & Richards 2007), and also for identifying areas of endemism, high species richness, and geographical foci of evolutionary processes for conservation planning (Moritz 2002; Rissler *et al.* 2006).

Ideal study systems include taxa that share broadly overlapping distributions and a common history in the same region for a sufficient time to accumulate signal tracking the history (Zink 2002). In these cases, congruent patterns of evolutionary divergence will be evident in the phylogenies of the codistributed species if the shared signal has not been erased by more recent, species-specific idiosyncratic events. The strongest evidence for shared signal requires divergence in both spatial and temporal contexts (Donoghue & Moore 2003).

One challenge to obtaining estimates of spatial divergence has been the requirement that taxa be codistributed, but recent methods accommodate statistical tests of shared phylogeographical signal between taxa having only partially overlapping geographical distributions using a supertree approach (Lapointe & Rissler 2005; see Bininda-Emonds 2004 for a general review of supertrees). Here, we apply this approach (LR05 hereafter) to three species of *Liolaemus* from the complex landscapes comprising the Pacific flank of the Andean Range in south-central Chile. This requires that we: (i) obtain well-resolved and strongly supported hypotheses of relationships within each of the three species of *Liolaemus* based on reasonable sampling throughout their ranges; and (ii) use these genealogies to implement the LR05 approach to see if any combinations of species show significant spatial codivergence. Even with a finding of spatial codivergence, we recognize that we cannot discriminate among all possible alternative explanations for a shared pattern. Rather, we consider this a 'hypothesis-generating' study in the context described by Avila *et al.* (2006); in poorly known taxa and/or regions for which background data are insufficient to generate plausible alternative *a priori* hypotheses, 'first pass' studies are needed to generate one or more plausible hypotheses. These may then be followed by studies that require collection of multiple classes of data [i.e. nuclear genes (Carsten & Knowles 2007), bioclimatic or palaeo-ecological variables (Hugall *et al.* 2002; Carstens & Richards 2007), etc.], and then specifying and testing a number of alternative historical hypotheses that approximate biological reality (but not so many that spurious findings become likely; Johnson & Omland 2004).

The study region and target species

The western slope of the central Chilean Andes exhibits some historical and physical features suitable for these kinds of studies because the region is characterized by extreme topographic relief, high species endemism in multiple groups (Smith-Ramirez 2004), and it has experienced a complex palaeoclimatic and geomorphological history. Some examples are the tectonic uplift of the Andes over the last 23 million years, beginning during the Miocene and continuing to the present (Ramos 1989), and periodic global cooling during the Pleistocene (1.8 million years ago to 14 000 years ago) which produced multiple shifts in climate, landscape, and sea level. At maximum advance during this last glacial period, an ice sheet covered an extensive region of southern Chile and would have excluded much of the terrestrial biota from a large part of the southern Andes (Fig. 1). Several species of squamate reptiles now occur in this area, and many of these have almost certainly experienced the same palaeoclimatic and geomorphological histories.

The lizard genus *Liolaemus* Wiegmann 1834, is represented in Chile by 76 recognized species, four of which are polytypic and give a total of 85 taxa (Pincheira-Donoso & Núñez 2005). The heterogeneity of the Chilean territory, with pronounced latitudinal and altitudinal climatic and ecological gradients, implies that widely distributed species of *Liolaemus* are adapted to very different environmental regimes. The high species richness, high endemism, and the frequently observed extensive intraspecific morphological variation in widely distributed species collectively suggests that this genus has been a dynamic group in an evolutionary sense – it has likely speciated extensively in response to a combination of recent and ancient tectonic and climatic changes in this part of South America. Several species of *Liolaemus* are abundant and have sympatric and sometimes syntopic distributions (Donoso-Barros 1966; Núñez & Jaksic 1992), and Chilean species represent several distinct clades of the genus (Schulte *et al.* 2000). Furthermore, many species differ in key features of their life histories (habitat preference, parity mode, etc.), so closely related and distantly related species, with similar and different ecologies, often occur in sympatry. This collection of attributes makes the group well-suited for comparative phylogeographical studies.

Here, we chose three focal species for comparative phylogeographical study because of their accessibility in the field, well-defined geographical distributions, and a high level of geographical codistribution between different combinations of species. The species include: *Liolaemus tenuis* (Dumeril & Bibron 1837), *Liolaemus pictus* (Dumeril & Bibron (1837), and *Liolaemus lemniscatus* (Gravenhorst 1838). These species are phylogenetically members of the same clade (i.e. the subgenus *Liolaemus*), but *L. lemniscatus* and *L. tenuis* are much more closely related to each other than either is to *L. pictus* (Schulte *et al.* 2000). Of these, the arboreal

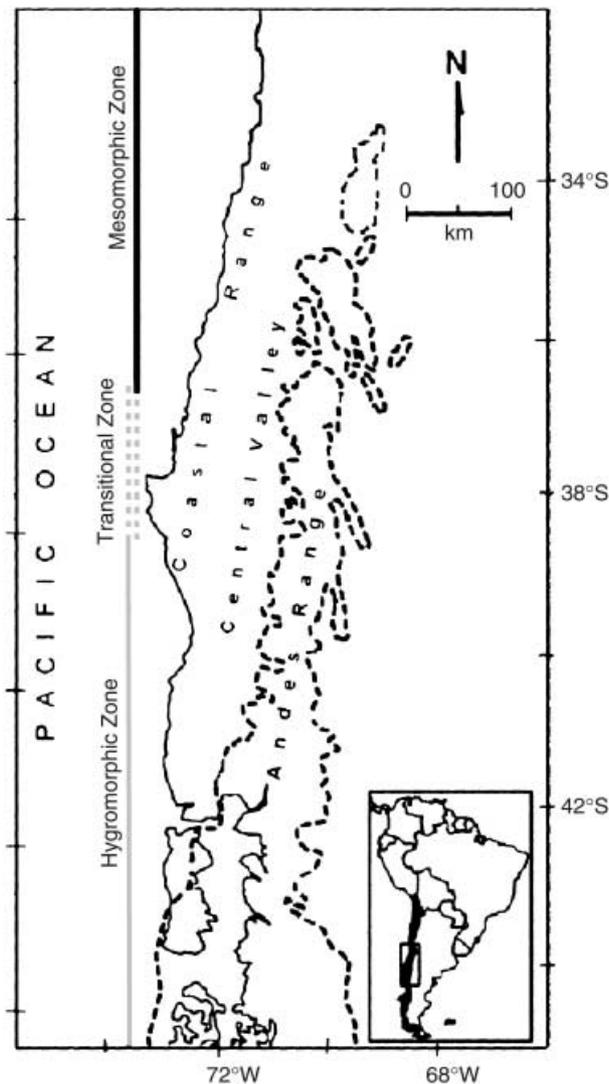


Fig. 1 Distribution of the ice shield during the LGM (dashed line) and the approximate latitudinal extent of the three Chilean biogeographical zones (mesomorph, transitional, and hygromorphic) considered in this study.

L. tenuis has the largest distribution, ranging from Coquimbo (29°58'S, 71°21'W) south to Los Lagos (39°51'S, 72°50'W; Fig. 2a), and altitudinally extending from sea level to 1800 m (Donoso-Barros 1966; Vidal *et al.* 2004, 2005a); this distribution covers bioclimatic and ecological conditions ranging from Mediterranean to sub-Andean (Di Castri 1968), and includes areas that were glaciated during the last glacial maximum (LGM; Lowell *et al.* 1995). Its distribution is associated with grassland and shrub habitats in the mesomorph zone (Donoso-Barros 1966), and the species is oviparous (Ceï *et al.* 2003).

The more terrestrial *L. lemniscatus* (also oviparous) is associated with warmer and drier environments than *L. tenuis* (Lambrot & Ortiz 1990; Pincheira-Donoso & Nuñez 2005),

and ranges from La Serena (30°S) south to the Biobío River (38°S; Fig. 2c), and altitudinally from 250 to 1400 m. Finally, *L. pictus* ranges from the Biobío region (~37°S) south to Chiloé Island (43°S; Fig. 2b), and ecologically from ~200–1700 m. It is an arboreal lizard found in the *Nothofagus* forest from the transitional to hygromorphic zones in Chile, and the conifer woodlands of southern Neuquén and northern Río Negro provinces in Argentina (Martins *et al.* 2004). This species is viviparous (Donoso-Barros 1966; Ceï *et al.* 2003; Vidal *et al.* 2005b), and likely most tolerant of the three species to cold and humidity. This species is the most likely candidate to persist during the LGM fragmentation (Vidal *et al.* 2005b).

These species overlap latitudinally from the Biobío region (~37°S) south to Llanquihue (~42°S), and two (*L. tenuis* and *L. lemniscatus*) overlap from Coquimbo (~30°S) to the Biobío River (Fig. 2). In combination, this represents in a shared latitudinal distribution of over 1000 km.

Materials and methods

Taxon sampling

Lizards were collected in the field by hand or noose, euthanized, and preserved as museum vouchers in the Monte L. Bean Life Science Museum at Brigham Young University (BYU), and in the Museo de Zoología, Universidad de Concepción (MZUC). We collected a total of 278 lizards from 61 localities (Fig. 3a) in the following species proportions: 144 individuals of *Liolaemus tenuis* (from 41 localities), 82 *Liolaemus pictus* (15 localities), and 52 *Liolaemus lemniscatus* from 14 sites (Table 1; Fig. 3a). Three species (*Liolaemus chiliensis*, *Liolaemus cyanogaster* and *Liolaemus monticola*) were chosen as outgroup taxa on the basis of relationships reported by Schulte *et al.* (2000). Table 1 summarizes the number of individuals collected per locality and other distributional information for each species considered in this study.

Laboratory protocols

Total genomic DNA was extracted from muscle tissue preserved in 96% ethanol, following the protocol developed by Fetzner (1999), and using the QIAGEN DNeasy tissue kit according to the standard protocol. Three microlitres of extraction products were separated by electrophoresis on a 1% agarose gel to assess DNA quality, and samples were diluted if necessary before polymerase chain reaction (PCR) amplification. Two mitochondrial gene regions were amplified via PCR in a 'cocktail' containing 2.0 µL of template DNA (approximate concentration estimated on a 2% agarose gel), 8 µL of dNTPs (1.25 mM), 4 µL of 10× *Taq* buffer, 4 µL of each primer (10 µM), 4 µL of MgCl (25 mM), 24 µL of distilled water and 0.25 µL of *Taq* DNA polymerase (5 U/µ) from Promega Corp. The gene regions used include

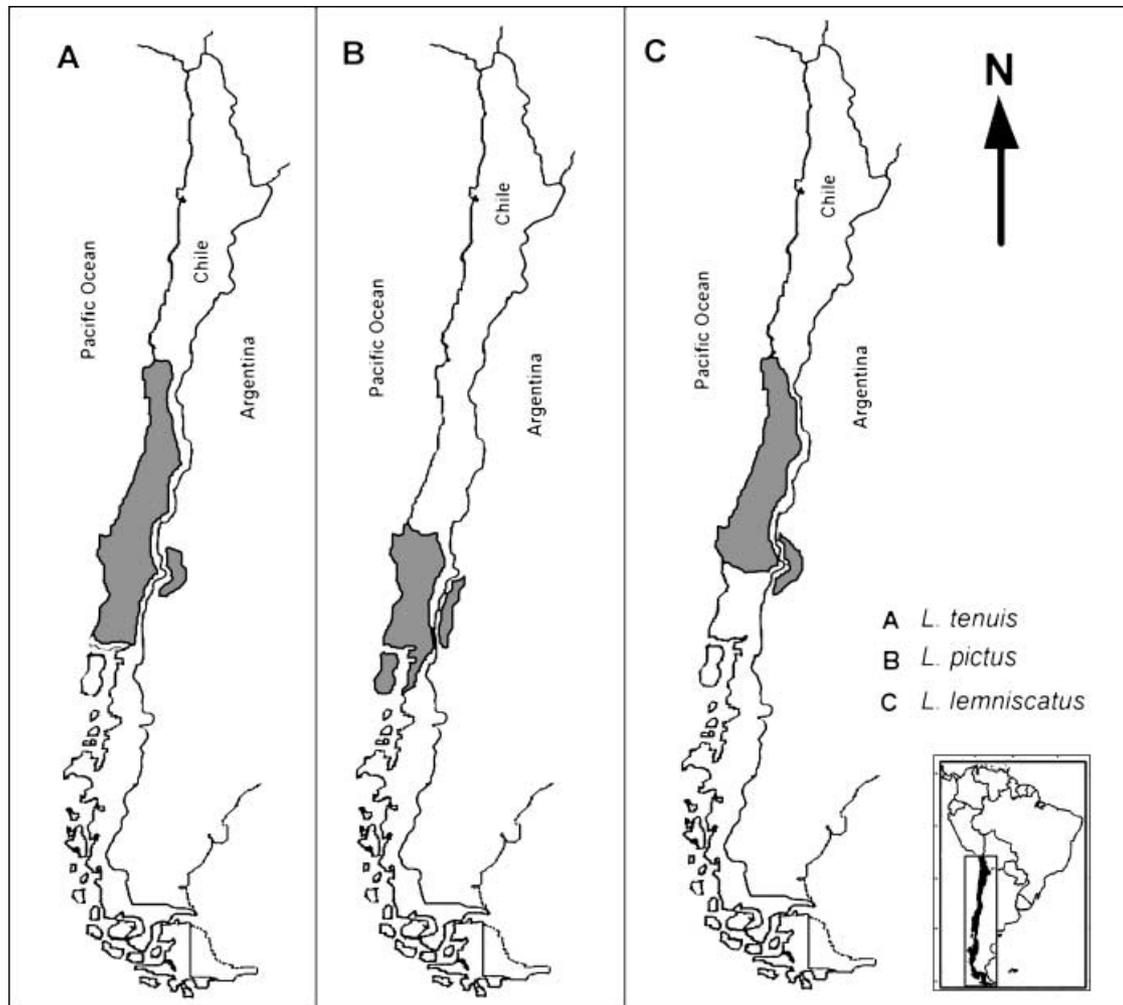


Fig. 2 Geographical distribution of the three species of *Liolaemus* analysed in this study.

(i) a fragment of ~760 bp of the cytochrome *b* (*cyt b*) gene, and (ii) a fragment of ~740 bp of the 12S gene. For *cyt b*, we used light strand primers GluDGL (5'-TGACTTGAARAA CCAAYCGTTG-3'; Palumbi 1996), F1 (5'-TGAGGACARATAT CHTTYTGRGG-3'), and heavy strand *cyt b* 3 (5'-GGCAA ATAGGAARTATCATTG-3'; Palumbi 1996). For the 12S gene, we used the 12e (5'-GTRCGCTTACCWTGTTACG ACT-3') and tPhe (5'-AAAGCACRGCCTGAAGATGC-3') primers of Wiens *et al.* (1999).

Double-stranded PCR products were checked by electrophoresis on 2% agarose gels, purified using a GeneClean III kit (BIO 101, Inc.), and directly sequenced bidirectionally using the PerkinElmer ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction (PE Applied Biosystems). Excess Dye Terminator was removed with CentriSep spin columns (Princeton Separations Inc.), and sequences then fractionated by polyacrylamide gel electrophoresis on a ABI PRISM 377 automated DNA sequencer (PE Applied Biosystems) at the DNA Sequencing Center at Brigham Young University.

Sequence alignments

Sequences were edited and aligned with SEQUENCHER 4.2 (Gene Codes Corp. Inc. 1995), and the protein coding region *cyt b* was translated into amino acids for confirmation of alignment. Divergence was lower for the 12S fragment and the number of indels was small, typically single base differences, except for one 6-bp indel in *L. pictus*. The alignment of this region was performed with CLUSTAL_X (Thompson *et al.* 1997) using default settings for gap and mismatch penalties, with subsequent manual adjustments that maintain base-pair identity in conserved nucleotide blocks. Missing data were coded as '?'. Sequences were deposited in GenBank under accession numbers EU649101–EU649113. Additional sequences for 12S of *L. tenuis* (code AY662055) and *cyt b* and 12S of *L. pictus* (codes AY367791 and AY367820, respectively) from Alumine Province of Neuquen, Argentina, were obtained from GenBank. Recent studies on Argentinian groups of

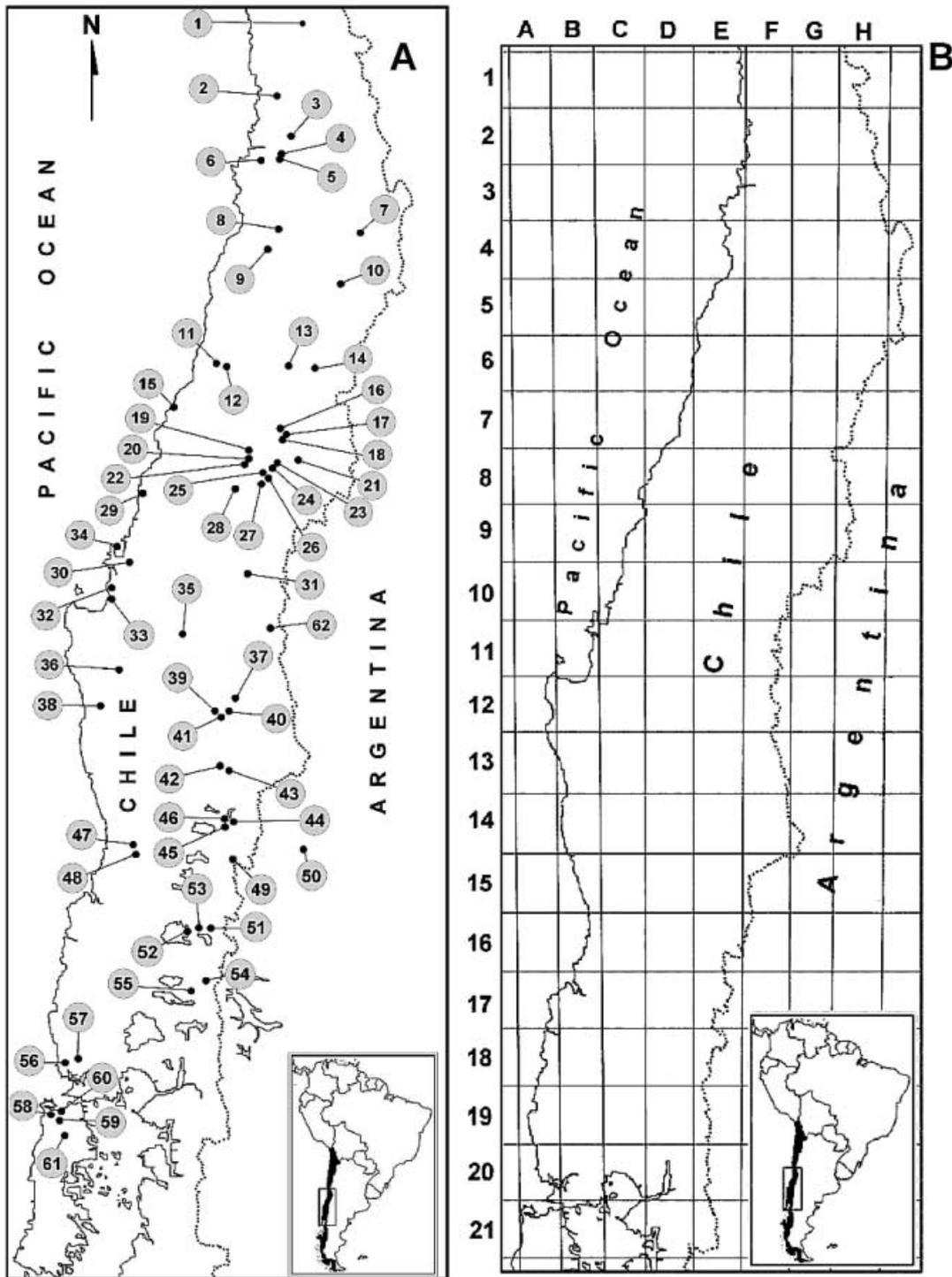


Fig. 3 (a) Geographical distribution of the samples included in this study; black dots identify localities, and locality numbers correspond to those summarized in Table 1. (b) Grid for areas used in the supertrees analysis; each square is a half degree on a side, and these are identified by letter × number codes (i.e. G1, F2) which are summarized in Table 1.

Liolaemus (Morando *et al.* 2003, 2004, 2007; Avila *et al.* 2004, 2006) show that mtDNA *cyt b* and 12S gene regions were both informative at intraspecific and interspecific levels.

Gene tree phylogenies for individual species

Phylogenetic analyses followed in part our earlier studies of *Liolaemus* (Morando *et al.* 2003, 2004; Avila *et al.* 2006);

Table 1 List of localities, sample sizes, and grid codes for the samples used in the supertrees analysis; site numbers are plotted in Fig. 3a, and area codes in Fig. 3b

Species	Site no.	Locality name	Geographical coordinates	Sample size	Area code	
<i>Liolaemus tenuis</i>	1	Salamanca	31°46'S, 70°57'W	3	G1	
	2	La Ligua	32°28'S, 71°30'W	4	F2	
	4	Cementerio Olmue	32°59'S, 71°11'W	7	F3	
	5	Olmue	33°00'S, 71°12'W	1	F3	
	6	Quilpue	33°02'S, 71°25'W	5	E3	
	7	Cajon del Maipo	33°39'S, 70°4'W	1	H4	
	8	Melipilla	33°42'S, 71°13'W	7	F5	
	10	Coya	34°12'S, 70°32'W	4	G5	
	12	Hualañe	34°58'S, 71°49'W	5	E7	
	13	Romeral	34°58'S, 71°7'W	4	F7	
	14	Los Queñes	34°59'S, 70°49'W	8	G7	
	15	Constitucion	35°19'S, 72°25'W	2	D8	
	17	Vilches	35°35'S, 71°5'W	4	F8	
	18	Alto Vilches	35°35'S, 71°25'W	1	F8	
	19	Yerbas Buenas	35°45'S, 71°34'W	3	E9	
	20	Linares	35°50'S, 71°34'W	4	E9	
	22	SurOeste Linares	35°51'S, 71°35'W	4	E9	
	24	Embalse Ancoa	35°54'S, 71°17'W	1	F9	
	26	Longavi	35°56'S, 71°23'W	1	E9	
	28	Norte de Parral	35°58'S, 71°28'W	1	E9	
	29	Cobquecura	36°08'S, 72°46'W	6	C9	
	30	Nonguen	36°47'S, 72°55'W	4	C11	
	31	Las Trancas	36°53'S, 71°34'W	5	E11	
	33	Lota	37°05'S, 73°10'W	1	B11	
	34	Isla Quiriquina	37°20'S, 72°30'W	8	B10	
	35	Los Angeles	37°23'S, 72°1'W	3	E12	
	37	Pemehue	38°03'S, 71°43'W	4	E13	
	38	Lanahue	38°07'S, 73°16'W	4	B13	
	39	Victoria	38°10'S, 71°57'W	6	E13	
	40	Malleco	38°10'S, 71°48'W	1	E13	
	41	Malleco, Las Mentas	38°12'S, 71°50'W	4	E13	
	42	Cherquenco	38°40'S, 71°53'W	5	E14	
	43	Volcan Llaima	38°41'S, 71°48'W	1	E14	
	44	Huerquehue	39°11'S, 71°45'W	1	E15	
	46	Ribera Lago Caburgua	39°10'S, 71°49'W	2	E16	
	47	La Capilla	39°25'S, 72°53'W	5	C16	
	48	Calquinco	39°28'S, 72°51'W	6	C16	
	49	Puesco	39°32'S, 71°43'W	1	E16	
	50	Alumine, Argentina	39°25'S, 70°53'W	1	ARG	
	51	Los Llollles	40°11'S, 72°0'W	1	E18	
	52	Ranco	40°13'S, 72°16'W	6	D18	
	<i>Liolaemus lemmiscatus</i>	3	Ocoa	32°49'S, 71°5'W	4	F5
		4	Cementerio Olmue	32°59'S, 71°11'W	1	F3
		9	Rinconada Maipú	33°52'S, 71°20'W	3	F5
		10	Coya	34°12'S, 70°32'W	2	G5
		11	Hualañe-Vichuquen	34°56'S, 71°55'W	4	E7
		14	Los Queñes	34°59'S, 70°49'W	10	G7
		16	Cementerio Vilches	35°33'S, 71°12'W	8	F8
		21	Camino a Rangue	35°50'S, 71°0'W	4	F5
		23	Robleria	35°51'S, 71°15'W	1	F9
		24	Embalse Ancoa	35°54'S, 71°17'W	1	F9
		25	Precordillera Linares	35°55'S, 71°22'W	3	F9
27		Longavi Sur	35°57'S, 71°15'W	1	E9	
32		Coronel	37°01'S, 73°8'W	5	B11	
33		Lota	37°05'S, 73°10'W	5	B11	

Table 1 Continued

Species	Site no.	Locality name	Geographical coordinates	Sample size	Area code
<i>Liolaemus pictus</i>					
	36	Nahuelbuta	37°47'S, 73°3'W	3	B13
	41	Malleco, Las Mentas	38°12'S, 71°50'W	10	E13
	43	Volcan Llaima	38°41'S, 71°48'W	11	E14
	45	Huerquehue Sur	39°12'S, 71°46'W	2	E16
	50	Alumine, Argentina	39°25'S, 70°53'W	1	ARG
	51	Los Llollles	40°11'S, 72°0'W	3	E18
	52	Ranco	40°13'S, 72°16'W	1	D18
	53	Maihue	40°12'S, 72°6'W	2	E18
	54	Puyehue	40°41'S, 72°0'W	4	E19
	55	Antillanca	40°46'S, 72°12'W	11	D19
	56	Sur Oeste Estaquillas	41°27'S, 73°44'W	9	A20
	57	Estaquillas	41°26'S, 73°41'W	3	A20
	58	Este de Ancud	41°55'S, 73°54'W	7	A21
	59	Sur de Ancud	41°55'S, 73°50'W	7	A21
	60	Ancud	41°56'S, 73°53'W	2	A21
	61	Belben	42°04'S, 73°45'W	6	A21
Outgroups:					
<i>Liolaemus cyanogaster</i>	29	Cobquecura	36°08'S, 72°46'W	2	C9
<i>Liolaemus chiliensis</i>	31	Las Trancas	36°53'S, 71°34'W	1	E11
<i>Liolaemus monticola</i>	62	Volcan Antuco	37°21'S, 71°6'W	1	F12

redundant haplotypes were identified using COLLAPSE (version 1.1; available at: http://biology.byu.edu/faculty/kac/crandall_lab/computer.html), and removed from their respective data matrices. Both single gene regions and the combined data set (~1500 bp for both gene regions) were used for Bayesian and maximum-likelihood (ML) phylogenetic analyses on the nonredundant sequence matrixes. Trees obtained from analyses of individual gene regions and combined data sets showed almost identical topologies, and none recovered strongly supported topological conflicts (Wiens 1998) within any species, so only results of the combined analyses are included here. The program MODELTEST (version 3.04; Posada & Crandall 1998) was used to select the best-fitting model of evolution (Akaike criterion; Posada & Buckley 2004), for each gene region within each species. Two models were selected for *cyt b*; for *L. tenuis*, the model was TrN + I + Γ , while for *L. lemniscatus* and *L. pictus* the model was K81uf + I + Γ . For 12S (run separately and in the combined data set), the general time reversible model with invariable and variable sites with a discrete gamma distribution (GTR + I + Γ ; Yang 1994) was selected as the best-fit model for all species. All ML analyses were based on heuristic searches with 10 random addition replicates, maxtrees = 100, and tree-bisection-reconnection (TBR) branch-swapping algorithm, as implemented in PAUP (Swofford 2002). ML bootstrap (MLB) proportions were obtained by performing five separate searches with 20 random addition replicates each, and then combining the total 100 pseudoreplicates to obtain one MLB value.

Bayesian analyses were performed using MRBAYES 2.0 (Huelsenbeck & Ronquist 2001), based on the same models used for the ML searches. For each species, we carried out runs for 5 million generations, with four incrementally heated chains, and then sampled at intervals of 1000 generations to include 5000 data points. Stationarity was estimated by plotting (with Microsoft Excel 2003) the log-likelihood scores against generation time, and assuming stationarity when the curves flattened out. This phase was reached between 30 000 and 40 000 generations, depending on the species. Then, we discarded the first 30–40 trees (burn-in), and the 50% majority-rule trees were obtained from the remaining 4970–4960 data points with the purpose of obtaining the posterior probability (PP) values. To avoid local entrapment on a sub-optimal peak in the tree space, we performed two independent analyses, and compared these for convergence to similar log-likelihood mean values (Huelsenbeck & Bollback 2001; Leaché & Reeder 2002). We also compared the posterior probabilities for individual clades obtained from the separate analyses for congruence to ensure convergence of the two analyses. All phylogenetic analyses were performed on an IBM Sp2 supercomputer in the BYU supercomputing facility.

Additionally, we estimated several genetic diversity indexes for each species of *Liolaemus* by using all the sequences, both for each area indicated in Fig. 3b, and for each main clade obtained from the phylogenetic analysis. We estimated the haplotype (= gene) diversity (H_d ; Nei 1987, p. 180) and nucleotide diversity (π , Nei 1987, p. 257) using DNASP 4.10 and MEGA 3.1, respectively.

Comparative phylogeography among species

We used the phylogeographical hypotheses (individual phylogenetic trees) of the three species derived from the combined data sets, as described above, to test for topological congruence between relevant combinations of codistributed species. The LR05 method first uses maximum agreement subtrees to represent overlapping geographical regions represented in individual gene trees, and this requires that original data (gene trees for each species) be recoded to define common regional units. We did this by subdividing the sampling area into a grid system delimited as 0.25 degree square latitude/longitude squares, and coding each square with letters (longitude arrangement) and numbers (latitude arrangement; Fig. 3b). The sampling area includes three zones described in general biogeographical classifications of the Chilean territory (Pisano 1954; Mann 1964; Fig. 1a), so we further subdivided the grid into three zones corresponding to the mesomorphic zone (c. 29°S to 36°S; rows 1–9 in Fig. 3b); transitional zone (c. 36°S to 38°S; rows 10–13 in Fig. 3b); and hygromorphic zone (c. 38°S to 40°S; rows 14–21 in Fig. 3b). The geographical origins of all unique haplotypes were used to define intraspecific area cladograms for each species, where different areas coded in Fig. 3b were represented by one unique haplotype.

In each gene tree, all strongly supported [PP > 0.95 (Leaché & Reeder 2002); MLB > 70 (Hillis & Bull 1993)] reciprocally monophyletic haploclades were pruned to a single representative sequence for each area, and then the individual haplotype names were replaced with their respective area names. The most basal haplotype in each clade was designated the semaphoront taxon. Using PAUP* (Swofford 2002), a maximum agreement subtree (MAST) was established for each pairwise combination of area trees; MASTs are appropriate to compare trees bearing different numbers of 'leaves' representing partially overlapping regions (grids in this study; see Lapointe & Rissler 2005). For each pairwise comparison (of individual gene trees), the size of each MAST was used as a congruence index (the larger the MAST, the more congruent the two area trees are). These values were normalized for each comparison by dividing the size of the respective MAST by the number of areas common to both trees, and then compared to the distribution frequency of the MAST scores of 1000 randomly generated trees (Markov model) with an equal number of shared areas as the original MAST. The concordance of area trees was tested using TREEMAP 1.0b (Page 1994) by treating co-occurring taxa as host–parasite associations. Congruence of area trees was inferred when the test was significant (i.e. larger than randomly generated MAST scores, 95%, for $P = 0.05$). In order to test whether coalescent events among codistributed taxa are correlated, times to coalescence (of codistributed taxa) for codivergent nodes (in units of genetic distance) were plotted

using TREEMAP 1.0 (Page 1994). Each pair of codiverging nodes were compared using Spearman's rho (two-tailed) test where N is the number of pairs of coalescing nodes ($\alpha = 0.05$).

Pairs of area trees that were statistically more concordant than expected by chance alone were combined using matrix representation with parsimony (MRP with triple fit; see Bininda-Emonds 2004 for other kinds of supertrees); MRP was chosen because it is not biased by the shape of the input trees (Wilkinson *et al.* 2005). The supertree summarizes geographical areas that correspond to clade breaks in multiple taxa with unique ecological and life-history characteristics, providing evidence for a shared history of vicariant isolation or selection. This analysis was implemented using RADCON version 1.1.6 (Thorley & Page 2000). Because influential physical factors may have played a role in generating such patterns, a second prediction made by the LR05 method is that, if multiple source trees are congruent (i.e. if they show signs of shared historical processes), then phylogeographical regions defined in a supertree will be statistically different with respect to climatic variables. In these tests, absence of congruence among source trees would lead to a supertree with no phylogeographical signal and in turn, with no relationship to climatic factors (Lapointe & Rissler 2005, p. 291).

To quantify climate variables for the geographical areas considered in the supertree, we used a multivariate analysis to summarize six variables recorded from 85 meteorological stations located throughout the study area (taken from Amigo & Ramírez 1998). In areas represented by more than one weather station, we used the most centrally located station in each geographical area represented in the supertree. The climatic variables are: T , annual mean temperature; M , mean daily maximum temperature of the coldest month; m , mean daily minimum temperature of the coldest month; I_c , index of continentality or thermal amplitude (calculated as the difference between the daily mean temperatures of the hottest and coldest months); P , mean annual precipitation; and T_p = above zero temperature index (the sum of all the monthly mean temperatures greater than 0°C, multiplied by 10).

A nonmetric multidimensional scaling (MDS) analysis, derived from Euclidean distance similarity matrices, was used to visualize dissimilarities between the same areas included in the supertree (based in the six climatic variables). All data were log-transformed and normalized before analysis. Each area was assigned to one of the three classical biogeographical zones for Chile, and this feature was considered an independent factor in the analysis. The results were further tested for significance using analysis of similarity (PRIMER version 6 ANOSIM; Clarke & Gorley 2005), which is analogous to a univariate ANOVA and reflects differences between treatment groups (the three Chilean biogeographical zones) in contrast to differences among

replicates within groups (the R statistic). Under the null hypothesis ('no difference between groups'), $R = 0$, and this was tested by a nonparametric permutation; there should be little or no effect on the average R value if the labels identifying which replicates belong to which samples are randomly rearranged (Clarke 1999). Finally, the PRIMER version 6 BIO-ENV routine linking multivariate patterns was used to identify 'influential parameters' on the arrangement obtained from MDS. This is a nonparametric correlation between two distance matrices. We obtained one matrix of distances between geographical areas in the supertree topology from the number of steps between areas in the nonrooted supertree, and log-transformed these numbers before the analysis. The second matrix was based on the same log-transformed and normalized climatic data used in the previous MDS. The BIO-ENV method assigns hierarchical correlation values both to each individual climatic variable and for combinations of them.

Results

General patterns of variation

We obtained 68 unique haplotypes (combined gene regions) for *Liolaemus tenuis*, and 59 and 37 nonredundant haplotypes for *Liolaemus pictus* and *Liolaemus lemniscatus*, respectively. The ratio of unique haplotypes relative to the total number of individuals sampled for each species was 0.46 for *L. tenuis*, 0.71 for *L. pictus*, and 0.71 for *L. lemniscatus*. Weighting these indicators by the total geographical area sampled (number of localities) gives a lower estimate of mtDNA haplotype variability in *L. tenuis* (an average of 1.7 haplotypes per locality), compared to *L. lemniscatus* (2.64), and *L. pictus* (3.93).

Both the haplotype diversity (H_d) and the nucleotide diversity (π) values for each main clade and for each species are shown in Table 2 (values for each individual area are summarized in Appendix). *Liolaemus tenuis* showed the lowest H_d value (0.928), while estimates were identical and higher for *L. lemniscatus* and *L. pictus*, respectively. Different relative values were obtained for π , which was highest in *L. tenuis* ($\pi = 0.074$) and lowest in *L. pictus*

($\pi = 0.051$). When calculated for the major clades in each species (Table 2), a pattern of reduced genetic diversity in southern clades was detected in all three species (clades B2 in *L. lemniscatus* and *L. pictus*, and B in *L. tenuis*). The highest levels of variation were detected in clades B1 in *L. lemniscatus* and *L. pictus*, and in clade A1 in *L. tenuis*. The lowest genetic diversity was obtained for the southern localities (Clade B) of *L. tenuis* ($H_d = 0.624$; $\pi = 0.002$).

Intraspecific phylogeographical structure

In all three species, the intraspecific consensus trees obtained from Bayesian analyses of individual gene regions and the combined data recovered nearly identical topologies, and these trees were also topologically virtually identical to those derived from the ML analyses. In cases of non-identical topologies, differences were due either to unresolved parts of some gene trees, or conflicting structure with weak nodal support (as estimated from PP and MLB values). We conclude that there is no evidence for significant conflict between gene regions or methods within any of the species (following Wiens 1998), and present here the ML topologies with both MLB and PP estimates of nodal support. In all three cases, alternative structure of outgroup rooting options always recovered monophyletic ingroup haplotypes with strong support (MLB = 100; PP = 1.0).

Figure 4 presents the consensus of two ML trees ($\ln L = -7299.4293$) for *L. tenuis*, which is also representative of the majority rule consensus of 9977 Bayesian trees (mean $\ln L = -6981$). Two large haploclades were recovered, the most strongly supported of which includes haplotypes from the mesomorphic and transitional zones (clade A; MLB = 79; PP = 0.99). The second less well-supported clade (B) includes haplotypes from the hygromorphic Zone (including one site in Argentina), some transitional zone haplotypes and two from the mesomorphic locality (E7 square). Within this arrangement, inclusion of the E7 area (clade B) was uniquely inconsistent with its geographical location, but placement of E7 in this clade receives only moderate support (MLB = 83, PP = 0.76). The subclade monophyletic for haplotypes from the hygromorphic and transitional Zones is strongly supported (MLB = 95; PP = 1.0).

Table 2 Haplotype (gene) diversity/nucleotide diversity (π ; \pm S.D.), for the main clades in the three species of *Liolaemus*; clades are identified in figures 4 to 6

Clade	<i>L. lemniscatus</i>	<i>L. tenuis</i>	<i>L. pictus</i>
A	0.900 \pm 0.161/0.022 \pm 0.003	0.981 \pm 0.005/0.060 \pm 0.005	0.953 \pm 0.031/0.019 \pm 0.003
A1		0.969 \pm 0.010/0.040 \pm 0.005	
A2		0.952 \pm 0.019/0.052 \pm 0.006	
B	0.988 \pm 0.008/0.058 \pm 0.005	0.624 \pm 0.072/0.002 \pm 0.001	0.985 \pm 0.006/0.029 \pm 0.004
B1	0.986 \pm 0.010/0.049 \pm 0.004		0.972 \pm 0.012/0.028 \pm 0.004
B2	0.778 \pm 0.091/0.002 \pm 0.001		0.952 \pm 0.096/0.008 \pm 0.002
Total sp	0.985 \pm 0.007/0.066 \pm 0.005	0.928 \pm 0.017/0.074 \pm 0.007	0.985 \pm 0.006/0.051 \pm 0.005

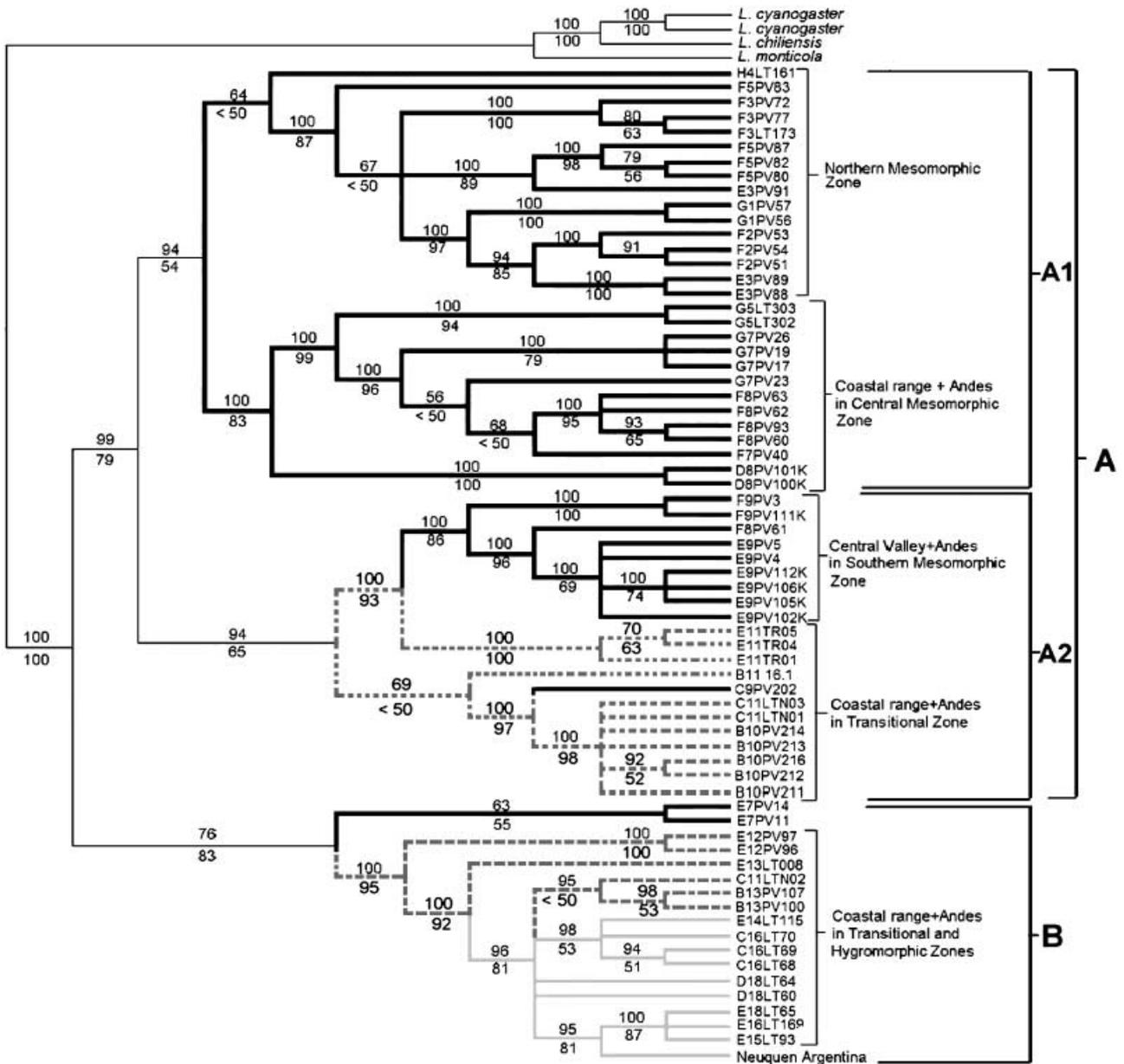


Fig. 4 Mitochondrial gene tree for *Liolaemus tenuis* based on ML ($\ln L = -7299.4293$) and Bayesian analyses, constructed from the *cyt b* and 12S regions. Numbers above and below branches represent the posterior probabilities and ML bootstrap values, respectively. Black, dashed, and light grey branches identify samples from the mesomorphic, transitional, and hygromorphic zones, respectively.

Within clade A two nested clades are recovered (A1 and A2 in Fig. 4) with strong PP but weak MLB support. Clade A1 includes exclusively haplotypes from the mesomorphic zone (MLB = 54, PP = 0.94), while clade A2 includes a mixture of haplotypes from the transitional zone (B10, B11, C11 and E11 areas), and haplotypes coming from the Central Valley + Andes in the southern mesomorphic zone (F8, F9 and E9 areas; MLB = 65, PP = 0.94). Within the transition zone, haplotypes from the Andean and Coastal ranges have not attained reciprocal monophyly. Within

the clade A1, we recovered subclades consisting of haplotypes exclusive to the northern mesomorphic zone (G1, F2, E3, F5 and H4 areas), and those exclusive to the Coastal and Andean ranges in the central mesomorphic zone (G5, G7, F7, F8 and D8). This last node was strongly supported (MLB = 83, PP = 1.0).

The gene tree for *L. lemniscatus* (Fig. 5) recovers two well-supported clades; the smaller of these (clade A) includes three haplotypes from the area F5 and the northernmost haplotype from area F3, localities from the transverse

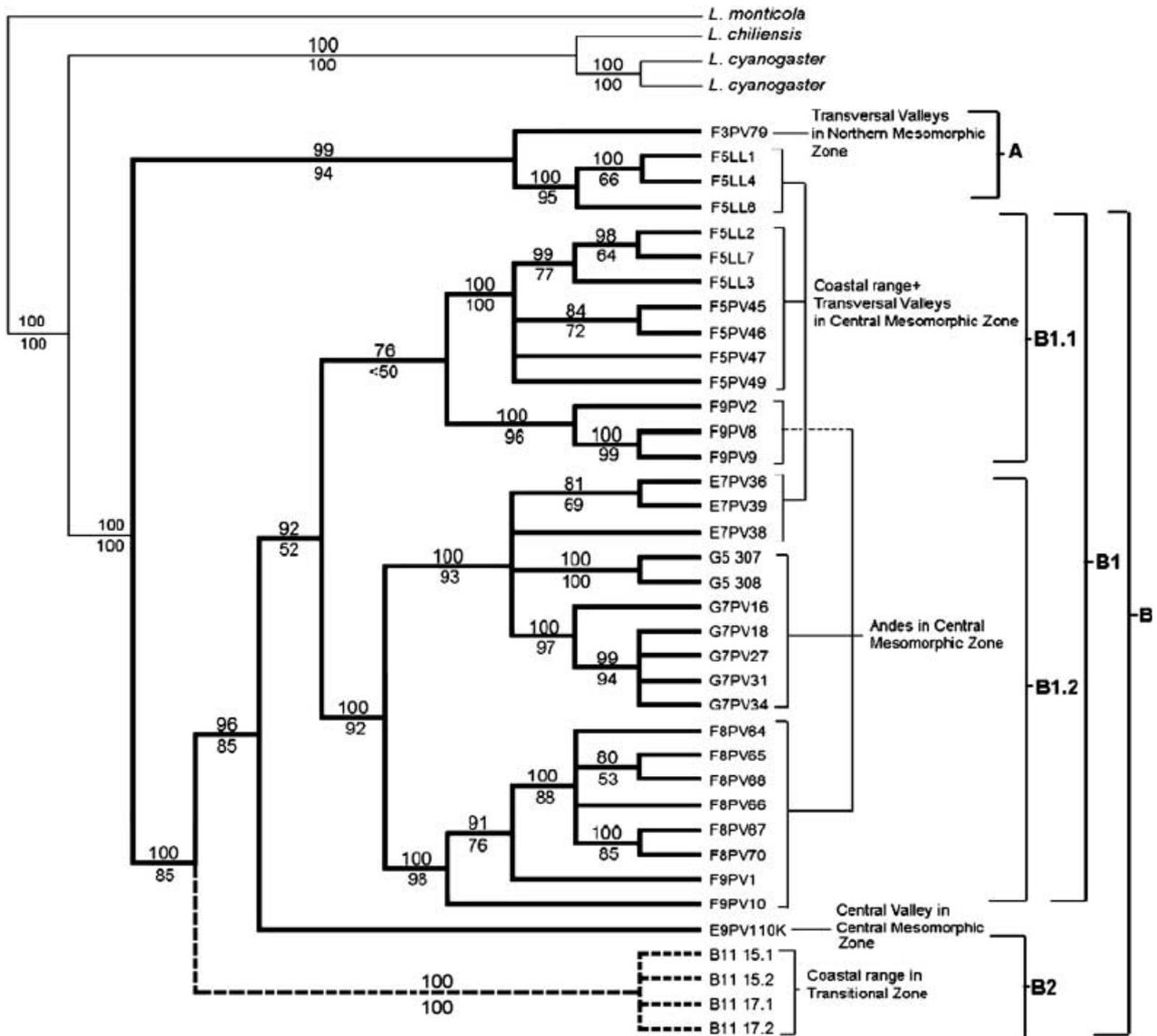


Fig. 5 Mitochondrial gene tree for *Liolaemus lemmiscatus* based on ML (lnL = -6195.8893) and Bayesian analysis; numbers above and below branches, and branch shading are as found in Fig. 4.

valleys of the northern mesomorphic zone (MLB = 94, PP = 0.99). The second clade (clade B; MLB = 85, PP = 1.0) resolves two well-supported subclades that are reciprocally monophyletic with respect to geography. Clade B2 (MLB = 100, PP = 1.0) includes only haplotypes from the Coastal Range in the transitional zone (area B11). Clade B1 is well supported (MLB = 85, PP = 0.96) and includes 29 unique haplotypes detected in this species, but none has sorted to monophyly by geographical locality. Subclade B1.1 (weakly supported) for example, includes haplotypes from the Coastal Range, transverse valleys, and the Andean Range in the central mesomorphic zone (areas F5 and F9). Similarly, subclade B1.2 (more strongly supported) is a mix of geographical areas: it includes haplotypes from the Coastal

Range, transverse valleys (both represented by area E7), and Andean Range (areas G5, G7, F8, and F9) of the central mesomorphic zone. Two well-supported haploclades within subclade B1.2 correspond to latitudinal regions previously recognized as distinct bioclimatic zones; one represents a northern subgroup restricted to an area between 34°S and 35°S latitude (E7, G5, G7), and the second includes haplotypes from areas F8 and F9, approximately between 35°S and 36°S latitude. A unique haplotype from the Central Valley in the central mesomorphic zone is the sister to clade B1.

All analyses for *L. pictus* recover two highly divergent haploclades (A and B; Fig. 6). Clade A includes haplotypes from the Coastal Range, the Andean Range in the transitional zone, and more northern Andean areas from the hygro-

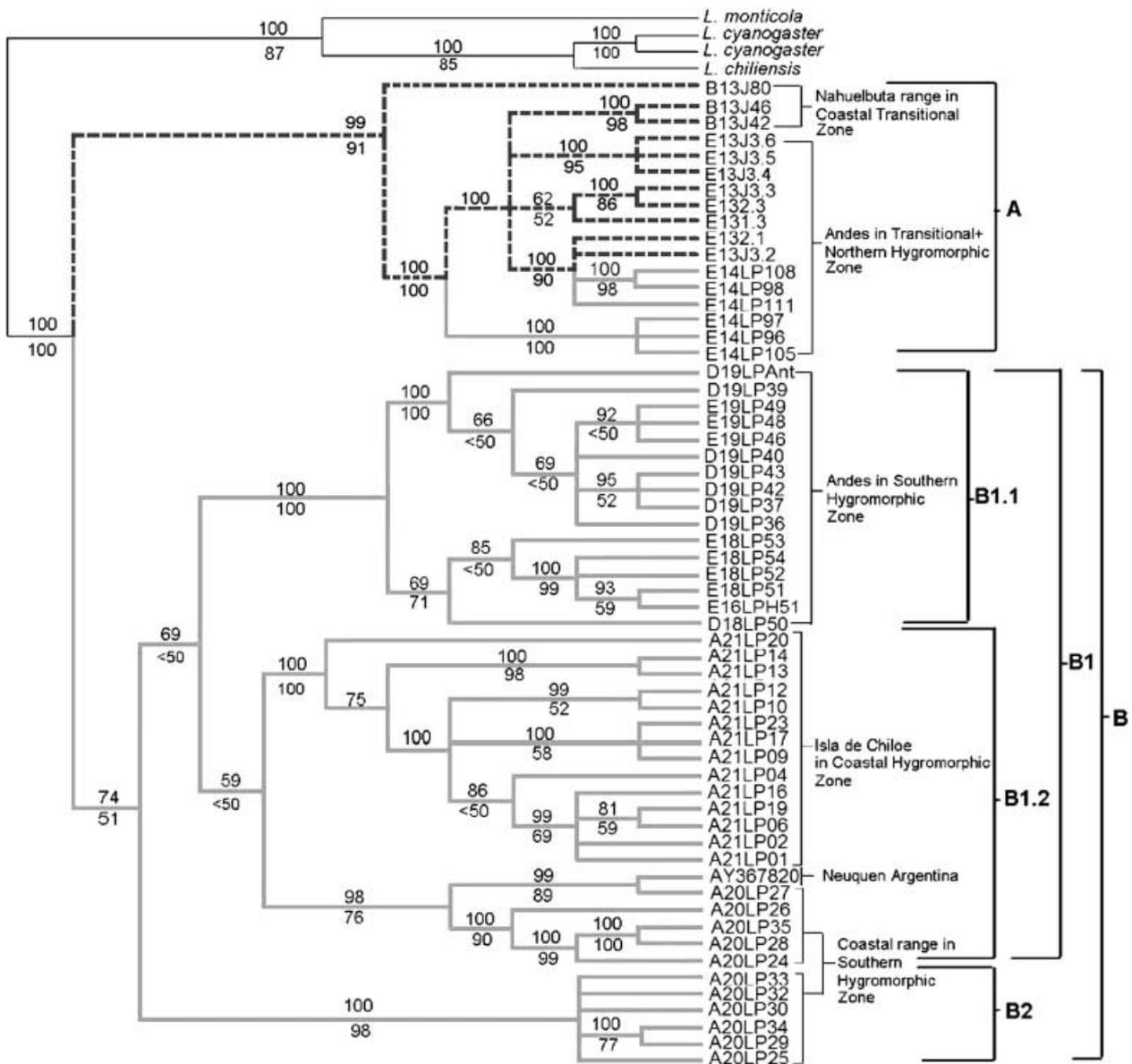


Fig. 6 Mitochondrial gene tree for *Liolaemus pictus* based in ML ($\ln L = -5816.3776$) and Bayesian analysis; numbers above and below branches, and branch shading are as found in Figs 4 and 5.

morphic zone, with strong support (MLB = 91, PP = 0.99). Clade B is comprised of haplotypes that are distributed exclusively in the hygromorphic zone, representing Andean and Coastal areas, albeit with weak support (MLB = 51, PP = 0.74). Within clade B, six haplotypes from the mainland coastal area near Chiloé Island (A20; clade B2), are strongly supported as distinct from all other sequences in clade B (MLB = 98, PP = 1.0). The monophyly of clade B1 receives almost no support (MLB < 50, PP = 0.69), but several of its subclades do. For example, clade B1.1 is comprised exclusively of Andean haplotypes from the southern hygromorphic zone (areas D18, D19, E16 and E18; Fig. 6;

MLB = 100, PP = 1.0), and another includes all A21 haplotypes from Chiloé Island in the Coastal hygromorphic zone. Clade B1.1 is weakly recovered as the sister clade to a haplotype including the area A20 sequences from the coastal mainland adjacent to Chiloé Island, and the haplotype from Neuquen, Argentina.

Comparative phylogeography

The size of the three area trees (areas coded in Fig. 3) was 30 in *L. tenuis*, 11 in *L. pictus* and 10 in *L. lemniscatus*, for a total of 34 areas represented overall. There were no areas

	<i>Liolaemus tenuis</i>	<i>Liolaemus pictus</i>	<i>Liolaemus lemniscatus</i>
<i>L. tenuis</i>	0.71 ($P < 0.001$)	0.60 ($P < 0.05$)
<i>L. pictus</i>	7
<i>L. lemniscatus</i>	10	0

Table 3 Normalized MAST values (probability values in parentheses) of the randomization analysis (above diagonal); numbers below the diagonal are the number of shared areas in the sampling design

shared by all three species trees, but *L. tenuis* shared 10 localities with *L. lemniscatus* and seven with *L. pictus*; *L. pictus* and *L. lemniscatus* did not co-occur at any of our sampling locations. Plots of times to coalescence in units of genetic distance for correlated codivergence events were not significant. Table 3 summarizes standardized MAST scores for the two species pairs sampled from regions of overlap. The mean value for the two standardized MAST scores is 0.66; the comparison between *L. tenuis* and *L. lemniscatus* shows the higher value (0.71), and is highly significant by the randomization test ($P < 0.001$). The *L. tenuis*–*L. pictus* standardized MAST value is 0.60, also significant ($P < 0.05$). The respective MAST for both comparisons are shown in Fig. 7(a,b). Both show a general trend of closely related haplotypes occupying proximal geographical areas. One exception is area D18 in the *L. tenuis*–*L. pictus* MAST, which is associated with the northern areas (B13 and E14). Because both comparisons gave significant P values, we used them to generate a supertree representing the 34 sampled geographical regions.

The MRP tree obtained from the combination of the three individual area trees is shown in Fig. 7c, and summarizes the concordant phylogeographical associations of the species among the 34 areas sampled. This MRP tree was considered in the interpretation of the climatic variables analysed for three Chilean bioclimatic zones (mesomorphic, transitional and hygromorphic) defined by Mann (1964) and Pisano (1954). The MRP tree obtained from the *Liolaemus* haplotypes shows a topology clearly consistent with these *a priori* defined bioclimatic zones. One haplotype occupies many areas of the mesomorphic zone (root 1). Specifically, those from the northern and central areas constitute a clade (D8 to H4), latitudinally ranging from approximately 32°S to 35°30'S. A second group includes areas from the southern mesomorphic zone (C9, E9, F9, E11) and some of those from the coastal transitional zone (B10, B11 and C11; root 2). The third group in the MRP tree includes all other areas from the transitional zone (Andean transitional zone, E12, B13 and E13), all areas from the hygromorphic zone, and the peripheral Argentinean site (root 3). The single geographical inconsistency detected is the association of the E7 area from mesomorphic zone within this third group, but in the topology of the MRP tree, this area is near the root of this group and close to the transitional areas in the second group.

The ANOSIM analysis of the climatic variables reveals highly significant differences among groups of geographical

areas from the three bioclimatic zones (global $R = 0.436$; $P < 0.001$). Both comparisons involving the mesomorphic zone were statistically different; the mesomorphic–hygromorphic comparison showed $R = 0.463$ ($P < 0.001$), and the mesomorphic–transitional comparison gave $R = 0.438$ ($P < 0.05$); there was no significant association for the hygromorphic–transitional comparison ($R = 0.295$; $P > 0.05$). These results suggest more pronounced climatic differences between the mesomorphic and transitional zones than between the hygromorphic and transitional zones. Figure 8 presents the spatial arrangement of the 32 supertree geographical areas in an MDS three-dimensional plot representing a multivariate summary of the climatic variables. The stress value for MDS was low (0.03), corresponding to a good fit for this kind of analysis (i.e. little distortion between the original data and the scaling axes). The clusters of points in the graph are generally consistent with the latitudinal position of the localities, and consequently with the Chilean bioclimatic zones. The transitional points (squares) are clustered between the mesomorphic and hygromorphic zones, and the similarity patterns for the climatic variables among areas correspond to the genetic structure of the species phylogeographies represented in the supertree. Spearman rank correlations derived from the BIO-ENV routine indicated that five of the six weather parameters essentially captured the full MDS profile, and showed relatively high correlation values between the supertree distance matrix and the climate matrix (global $R = 0.527$, $P < 0.001$; Table 4).

Discussion

General patterns of divergence

We detected patterns of evolutionary relationships for each *Liolaemus* species that are consistent with Chilean geography. Independently, for the three *Liolaemus* species analysed, the topologies reflect latitudinal gradients. The general pattern for *L. tenuis*, the most broadly distributed, is a higher genetic diversity, longer branch lengths and greater population structure in the mesomorphic zone, while the opposite is observed for the periglacial and glacial populations. *Liolaemus lemniscatus* is mainly distributed in the Mesomorphic zone and its level of divergence between haplotypes suggests a similar history to the northern codistributed area of *Liolaemus tenuis*. Clades of both species show a general

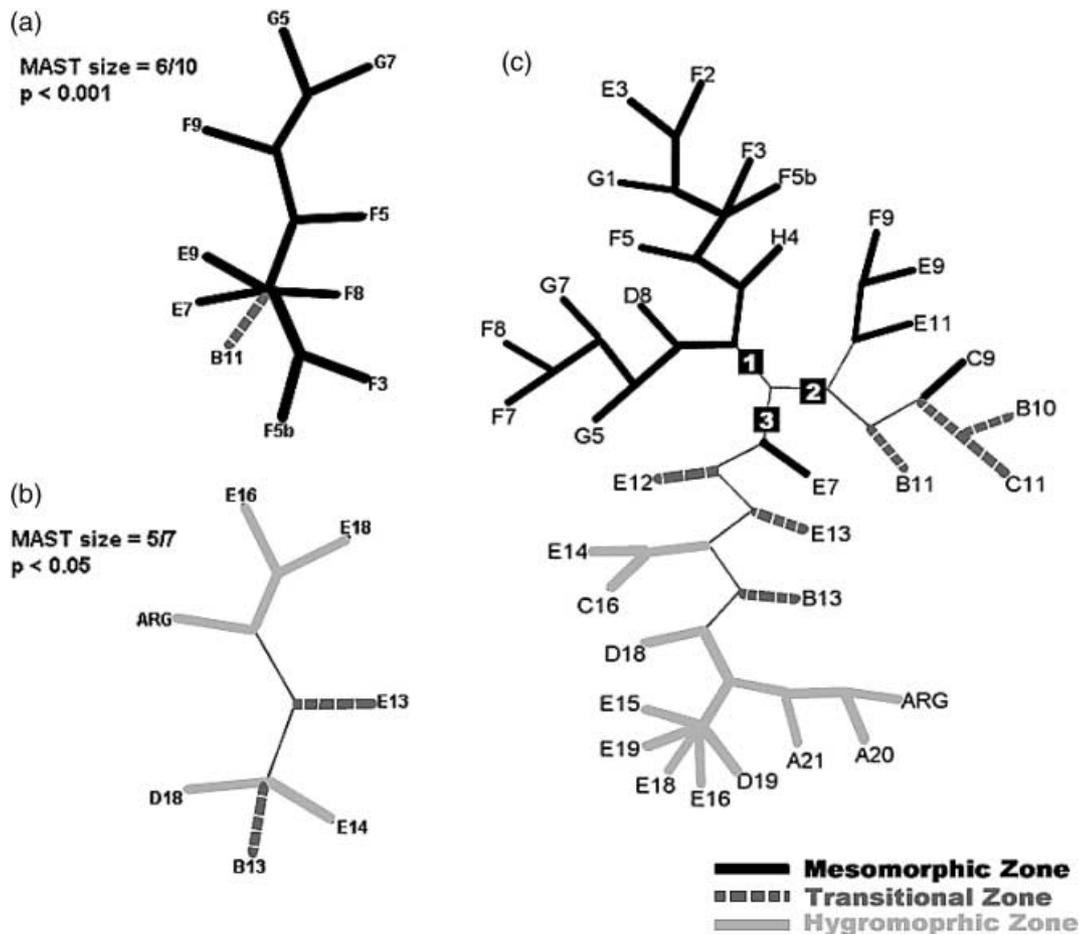


Fig. 7 MAST for pairwise combinations of area trees between *Liolaemus tenuis*–*Liolaemus lemniscatus* (a), and *L. tenuis*–*Liolaemus pictus* (b). C presents the MRP tree obtained from the combination of the three individual area trees; area codes are as described in Table 1. The main clades are labelled with numbers from 1 to 3, and branch shading is as found in Figs 4–6.

pattern of higher gene and/or nucleotide diversity in northern (A clades) vs. southern (B clades) localities (Table 2). Finally, *Liolaemus pictus*, the most southern species, is characterized by diversity and branch lengths that are curiously higher than the codistributed populations of *L. tenuis*. The southernmost populations of *L. pictus*, which are definitively distributed in glaciated areas, are characterized by branch lengths and genetic diversity indexes consistent with a reduced effect of glacial cycles on patterns of genetic variation. All this suggests a complex history that requires additional corroborative evidence for resolution, but broadly similar patterns — deep phylogeographical structure in northern populations and high genetic similarity on both sides of the Andes in southern populations — have been reported in two other terrestrial species; the conifer *Fitzroya cupressoides* (Allnut *et al.* 1999), and forest-dwelling rodents of the *Abrothrix olivaceous/xanthorinus* complex (Smith *et al.* 2001), and in the freshwater fish *Percichthys trucha* (Ruzzante *et al.* 2006).

Our gene tree for *L. tenuis* recovered a close relationship between the eastern Argentinean populations of and southern Chilean populations from the Continental Chiloé (clade B; Fig. 3), where it is probable that several low elevation gaps connected biota from both sides of the Andes at these latitudes. Similarly, within the fish *P. trucha*, deep mtDNA haplotype divergence between western vs. eastern drainages is evident in northern regions (38°), presumably because of a history dominated by vicariant events in this part of the range. Further south, *P. trucha* is characterized by the absence of deep divergence between eastern and western populations, and at approximately 40°S several western drainages include haplotypes that are more closely related to some eastern-drainage haplotypes than they are to those in other western populations. Ruzzante *et al.* (2006) interpreted this pattern as possibly due to historical gene flow followed by a recent cessation of former *trans*-Andean watershed connections in southern regions. In contrast, genetic patterns in northern populations of

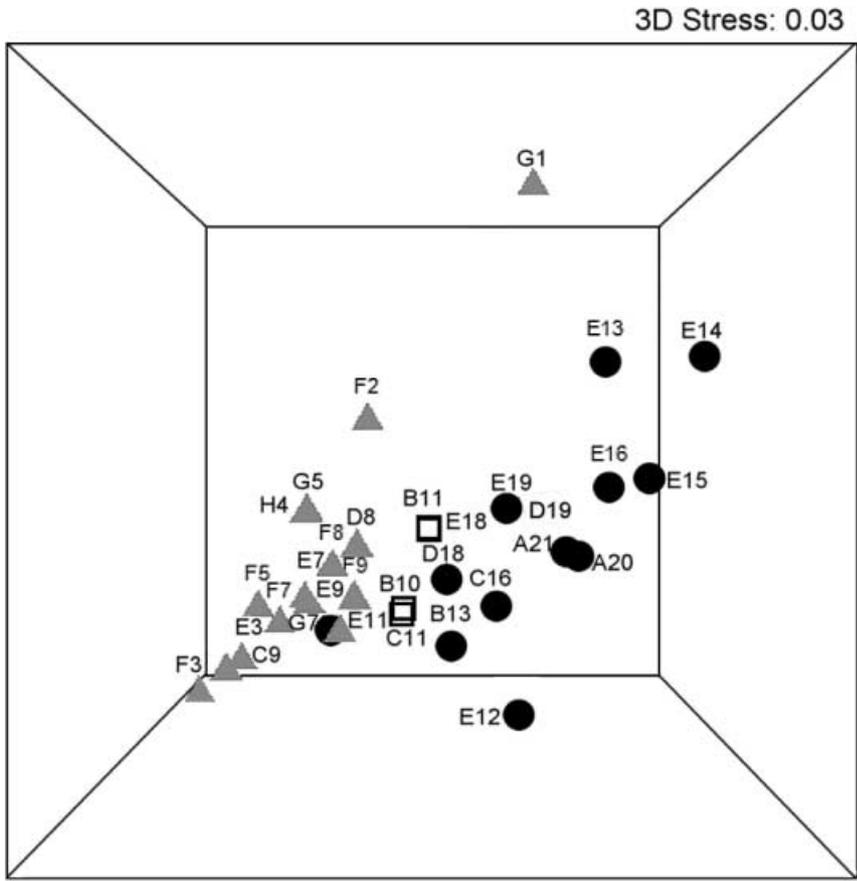


Fig. 8 Multidimensional scaling analysis summarizing six climate variables (see text for details) for the three Chilean biogeographical zones. Triangles, mesomorphic zone; squares, transitional zone; and circles, hygromorphic zone; area codes are as described in Table 1.

Table 4 Spearman rank correlations (PRIMER version 6 BIO-ENV) between areas supertree distances and weather variables based distances. Variables: (1) *T*, annual mean temperature; (2) *M*, mean daily maximum temperature of the coldest month; (3) *m*, mean daily minimum temperature of the coldest month; (4) *I_c*, index of continentality or thermal amplitude (calculated as the difference between the daily mean temperatures of the hottest and coldest months); (5) *P*, mean annual precipitation

Number of variables	Correlation	Selections
2	0.527	1,5
2	0.527	2,5
3	0.527	1,2,5
3	0.527	1,4,5
3	0.527	2,4,5
3	0.527	3,4,5
4	0.527	1,2,4,5
4	0.527	1,3,4,5
4	0.527	2,3,4,5
5	0.527	1,2,3,4,5

P. trucha suggest relatively stable habitats and limited dispersal (Ruzzante *et al.* 2006), and this pattern is broadly replicated in two of the three species of *Liolaemus* in this study.

Comparative phylogeography

Our results are consistent with the hypothesis that two paired combinations of three species of *Liolaemus* included in this study have responded in parallel to shared historical process sufficient to influence their phylogeographical structure. In other words, these species (*L. tenuis*, *L. lemniscatus*) have been codistributed for a sufficiently long time to possibly have evolved along similar demographic trajectories in response to the same historical events. Among other things, natural selection and/or vicariant events associated with recent cycles of glaciation (Heusser 1981; van Geel *et al.* 2000) and floral changes associated with shifting climates (Villagrán & Hinojosa 1997), may have driven parallel divergence in these lizards. The genetic and geographical structure of the populations described by the area supertree is consistent with this hypothesis.

In the northern range of the *Liolaemus* species studied here (mesomorphic zone), our results show significant phylogeographical congruence between *L. lemniscatus* and *L. tenuis*. Because this region should have been less directly impacted by late Pleistocene glaciation cycles (McCulloch *et al.* 2000), and because both of these species show deep phylogeographical structure in this region relative to more southern parts of their ranges, we suggest that older tectonic/

orogenic events, or perhaps very old climatic events (onset of Pleistocene changes) may have most strongly influenced the histories of each species. Specifically, the occurrence of a complex mix of localized transverse valleys bisected by high mountains, and the complete isolation of the Andean and Coastal ranges, coupled with less dramatic climatic oscillations resulting from cycles of glacial advance/retreat further south (Hollin & Schilling 1981), may have forged deep phylogeographical splits and allowed the persistence of old haplotypes at some localities. These northern populations segregate for both old and newer (derived) haplotypes (especially *L. tenuis*) relative to more southern populations.

The second significant comparison of this study includes more southern populations of *L. tenuis* and its overlap in part with *L. pictus*; heterospecific populations in this region may have shared a history of unstable and recurrent changes associated with repeated younger Pleistocene glaciations. Three or four glacial events are recorded from this region, all of which strongly influenced vegetation dynamics (e.g. Pastorino & Gallo 2002), and probably influenced microevolutionary processes in most of the associated biota. These glacial cycles operated for approximately 2 million years during the Pleistocene, from which the LGM began ~20 000–18 000 bp and finished 14 000–10 000 bp. During the LGM, the ice sheet was about 2000 km long and followed the distribution of the Andes range, from 34°S to 56°S (Fig. 1). At its maximum extent, the ice sheet extended from sea level at 43°S (Hollin & Schilling 1981), and to the north the ice sheet's influence extended to the approximate latitude of Santiago, where it descended to 500 m (Solbrig 1976). This climatic/geological scenario is consistent with the levels of divergence and genetic variation detected for one of the two species distributed in southern Chile. Specifically *L. tenuis*, the most widely distributed species sampled, showed lower values both for the haplotype and nucleotide diversity for the clade distributed from 38°S to the southern edge of its distribution (Clade B; Fig. 4). This clade was characterized by the shortest branch lengths of all terminals (tree not shown), and by a few and very similar haplotypes distributed across a broad area that was strongly influenced by the ice shield. We hypothesize that this signature reflects a relatively recent range expansion at these latitudes after the retreat of the ice shield.

All data suggest that the climate was colder and more humid than at present during glacial advances, and that this influence was strongest in the southern region and decreased in effect from south-to-north to the transitional zone (Villagrán 2001). The general model for changes in the biotic distributions during these glacial–postglacial cycles suggests that glacial effects led to local extinctions, fragmentation, isolation, and rearrangements in assemblages of species, forcing many taxa into refugia in the north, northwest, or in the east in the Patagonian Steppe

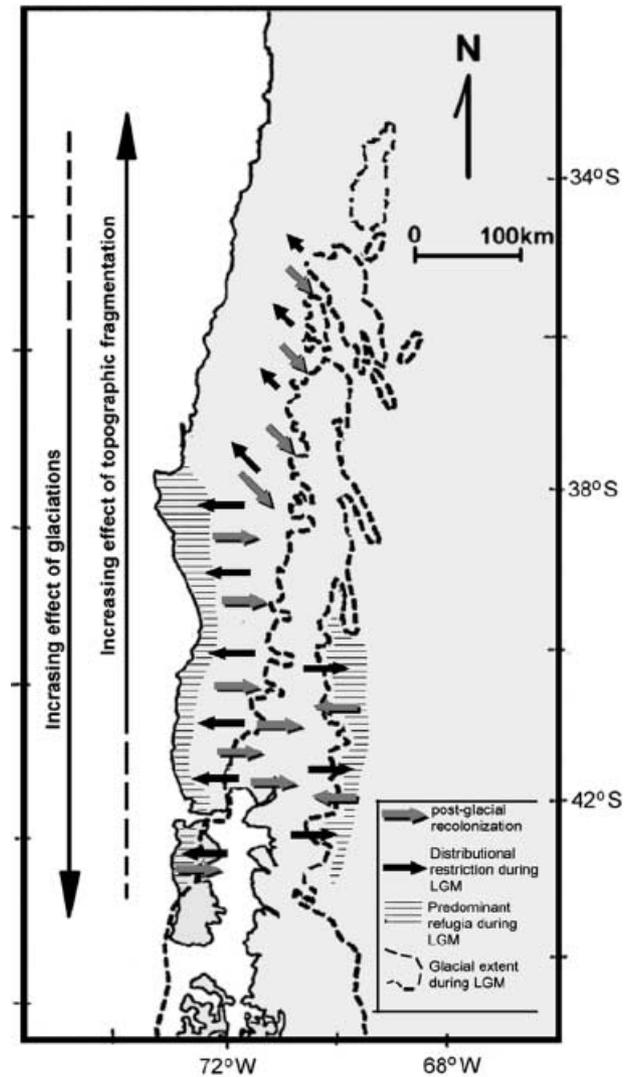


Fig. 9 Distribution of major refugial areas postulated for the LGM, and generalized dispersal routes postulated for the three species of *Liolaemus* considered in this study.

(Muellner *et al.* 2005). Recent evidence for low-elevation Pleistocene refugia was presented by Premoli *et al.* (2000, 2003), who suggested that a great part of the Chilean Patagonian biota was likely pushed into coastal areas between 38°S and 43°S (Fig. 9). As one example, the Andean *Nothofagus* forest was likely pushed north and from high to low altitudes with glacial advances, and it has only recently colonized the central valleys (Villagrán & Hinojosa 1997; Villagrán 2001).

The demonstration of spatially congruent evolutionary histories shared among two combinations of the *Liolaemus* species studied here implies a sufficiently long ecological association between these taxa (in parts of their ranges) for tectonic and/or climatic factors to generate congruent phylogeographical patterns. We are encouraged by these

results, because this study is a very broad assessment of patterns across a topographically and climatically extremely variable landscape, and it ignores the fact that, even for congeners, there are sufficient life-history differences among the *Liolaemus* species studied here to confound phylogeographical patterns at finer scales of resolution. For example, the effect of colder climatic conditions in southern Chile must be a significant factor in causing extinctions or density reductions in local populations of ectothermal terrestrial vertebrates in general. While the effects of glaciations in southern populations of *L. tenuis* and *L. pictus* should result in a shared phylogeographical pattern in this region, important differences between the two are almost certainly responsible for the more complex patterns in this part of the range. *Liolaemus tenuis* has a broader distribution than *L. pictus*, and the former generally occupies more open habitats and forest edges, while *L. pictus* is more strongly associated with forest habitat. This difference alone suggests that *L. tenuis* likely has higher thermal requirements, and it is also oviparous whereas *L. pictus* is viviparous, which would also favour *L. pictus* in cooler environments. Our study was not designed to distinguish among such variables to make inferences about ecological factors that might explain divergence, or absence thereof, within and between species in this group, and we mention these here simply to recognize the limits of this study.

The most powerful property of comparative phylogeography is the use of information from different codistributed clades — ideally with very different ecologies — presumed to share a history of similar climatic and geological processes; these represent independent replications in a natural experiment of evolution (Zink 2002; Zamudio & Savage 2003). Many studies have analysed the comparative phylogeography of codistributed taxa from independent lineages, with the aim of detecting common evolutionary processes, but until recently the most common approach consisted of qualitative and visual comparisons of individual gene trees (Avice 2000). More recently statistical methods have been used to quantitatively test alternative *a priori* hypotheses of congruence in phylogeographical patterns of sympatric taxa (e.g. Carstens *et al.* 2005), but these approaches are problematic in taxa with only partially overlapping distributions. A major advantage of the method used here (Lapointe & Rissler 2005) is that MRP trees can be compared in paired statistical tests appropriate to species sharing partially overlapping ranges.

This is the first comparative phylogeographical study in southern South America using statistical methods to test for the occurrence of areas with common evolutionary history, but much remains to be done in follow-up studies. For example, our analysis of climate variables suggests that the previously defined bioclimatic zones have some explanatory power for natural selection to drive population divergence, but more detailed niche modelling efforts will

need to be integrated with geographical range information for stronger inferences on the role of habitat differences in driving speciation (Graham *et al.* 2004). More intensive sampling will provide more details on potential areas of overlap which can be evaluated with additional new tests of shared history (Zhang *et al.* 2006), and these can be linked to expanded genetic studies with nuclear markers to test long-term demographic stability that might be expected in refugial areas (Hutchinson & Templeton 1999; Knowles & Richards 2005). Finally, tests for codivergence in space must be linked to tests for codivergence in time (Hickerson *et al.* 2006); such studies in this part of the world will provide a southern hemisphere perspective to the comparative phylogeographical studies of the effects of Quaternary glacial cycles in Europe and North America (Hewitt 2000; Moreno *et al.* 2001). The approach will also yield more general patterns with the inclusion of more taxa, and these studies are in progress.

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Appendix

Haplotype (gene) diversity/nucleotide diversity (π) (\pm SD), for each analysed area, and total values for the three species of *Liolaemus*. n/c, values not calculated

Area	<i>L. lemniscatus</i>	<i>L. tenuis</i>	<i>L. pictus</i>
1G		0.667 \pm 0.314/0.001 \pm 0.001	
2F		0.833 \pm 0.222/0.012 \pm 0.003	
3E		0.700 \pm 0.218/0.017 \pm 0.003	
3F	n/c	0.607 \pm 0.164/0.003 \pm 0.001	
4H		n/c	
5F	0.982 \pm 0.046/0.059 \pm 0.007	0.714 \pm 0.181/0.009 \pm 0.002	
5G	1.000 \pm 0.500/0.008 \pm 0.003	0.500 \pm 0.265/0.003 \pm 0.001	
7E	0.833 \pm 0.222/0.007 \pm 0.002	0.600 \pm 0.175/0.003 \pm 0.001	
7F		0.000 \pm 0.000/0.000 \pm 0.000	
7G	0.867 \pm 0.071/0.005 \pm 0.001	0.750 \pm 0.139/0.002 \pm 0.001	
8D		1.000 \pm 0.500/0.005 \pm 0.003	
8F	0.929 \pm 0.084/0.004 \pm 0.001	1.000 \pm 0.126/0.036 \pm 0.005	
9C		0.000 \pm 0.000/0.000 \pm 0.000	
9E	n/c	0.864 \pm 0.072/0.004 \pm 0.001	
9F	1.000 \pm 0.126/0.058 \pm 0.007	1.000 \pm 0.500/0.008 \pm 0.003	
10B		0.893 \pm 0.086/0.012 \pm 0.003	
11B	0.778 \pm 0.091/0.002 \pm 0.001		
11C		0.833 \pm 0.222/0.084 \pm 0.009	
11E		0.700 \pm 0.218/0.002 \pm 0.001	
12E		0.667 \pm 0.314/0.001 \pm 0.001	
13B		0.500 \pm 0.265/0.001 \pm 0.001	1.000 \pm 0.272/0.046 \pm 0.006
13E		0.343 \pm 0.128/0.001 \pm 0.000	0.956 \pm 0.059/0.010 \pm 0.002
14E		0.533 \pm 0.172/0.001 \pm 0.001	0.800 \pm 0.114/0.013 \pm 0.003
15E		n/c	
16C		0.564 \pm 0.134/0.002 \pm 0.001	
16E		0.667 \pm 0.314/0.002 \pm 0.001	1.000 \pm 0.500/0.014 \pm 0.004
18D		0.600 \pm 0.215/0.001 \pm 0.001	n/c
18E		n/c	0.900 \pm 0.161/0.006 \pm 0.002
19D			0.818 \pm 0.119/0.002 \pm 0.001
19E			0.833 \pm 0.222/0.003 \pm 0.001
20A			0.985 \pm 0.040/0.028 \pm 0.004
21A			0.909 \pm 0.047/0.006 \pm 0.001
Total sp	0.985 \pm 0.007/0.066 \pm 0.005	0.928 \pm 0.017/0.074 \pm 0.007	0.985 \pm 0.006/0.0512 \pm 0.005