

Genetic diversity and insular colonization of *Liolaemus pictus* (Squamata, Liolaeminae) in north-western Patagonia

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Abstract The Chilotan Archipelago and surrounding areas of north-western Patagonia (41°–43°S, 72°–74°W) offer a unique opportunity to study the interplay between the recent genetic and paleoenvironmental evolution on temperate rainforest environments. Previous studies in this region have postulated that land biota persisted west of the Patagonian ice sheet, in ice-free low-elevation regions of the mainland, and the north-western portion of Isla Grande de Chiloé during Quaternary ice ages. In this study, we analysed the phylogeographical structure (Cytochrome b) of the iguanid lizard *Liolaemus pictus* to estimate their genetic structure in response to glacial–interglacial cycles and colonization routes. We found that populations from the mainland and Isla Grande de Chiloé do not share haplotypes and, thus, are divergent haplogroups. This divergence might reflect an ancient isolation, much older than the last glaciation. Moreover, the existence of four divergent haplogroups among *L. pictus* populations in the mainland suggests the persistence of multiple isolated populations during the last glaciation. Our results also indicate that the colonization of small islands occurred from several source sites, located both in the mainland and in Isla Grande de Chiloé, after the Last Glacial Maximum.

Key words: archipelago, Chile, Cytochrome b, glacial cycle, lizard, phylogeography.

INTRODUCTION

Archipelagean biota offer useful models for examining evolutionary hypotheses, including those pertaining to repeated cycles of colonization and extinction, adaptation, and drift-induced population differentiation (Juste *et al.* 2001; Hille *et al.* 2003). Depending on their geological/environmental history, different processes can be considered as the main drivers that underlie the observed evolutionary patterns. Tectonic and glacioeustatic variations have altered the surface areas and degrees of insularity in many archipelagos (Cook *et al.* 2001; Hille *et al.* 2003; Parmakelis *et al.* 2003), whereas in other cases, direct glacial influence has imposed additional variation (Cook *et al.* 2001; Fleming & Cook 2002; Harlin-Cognato *et al.* 2006).

The geographical distribution of genetic diversity among species in high and middle latitude regions has often been associated with Late Pleistocene glacial cycles (Hewitt 1996, 1999), which involve the following processes: (i) fragmentation of a widespread ancestral species into refugia during periods of increased glacial cover, (ii) subsequent genetic divergence

among small isolated populations, followed by (iii) range expansion during glacial shrinkage. Typically, the last (beginning approx. 125 000 years ago) or second to last (approx. 250 000 years ago) of such cycles are invoked (Klicka & Zink 1997). In several cases, the role of glacial cycles has been demonstrated to promote or maintain divergence (Hewitt 1996, 1999; Schmitt & Seitz 2001; Janzen *et al.* 2002; Liebers & Helbig 2002).

Physical setting and paleoenvironmental history of north-western Patagonia

The Chilotan Archipelago includes Isla Grande de Chiloé (IGC) and a series of small islands located on the Seno Reloncaví, Golfo de Ancud and Golfo de Corcovado eastern interior seas, as well as along the western margin of the SE Pacific Ocean (Fig. 1). The Andes mountain range in continental Chiloé marks the eastern limit of the interior seas, with deep glacial valleys and fjords often mantled by glacial and volcanic deposits. The Andes establishes an effective climatic and biological boundary, harbouring glaciers on the highest summits, some of which have been active

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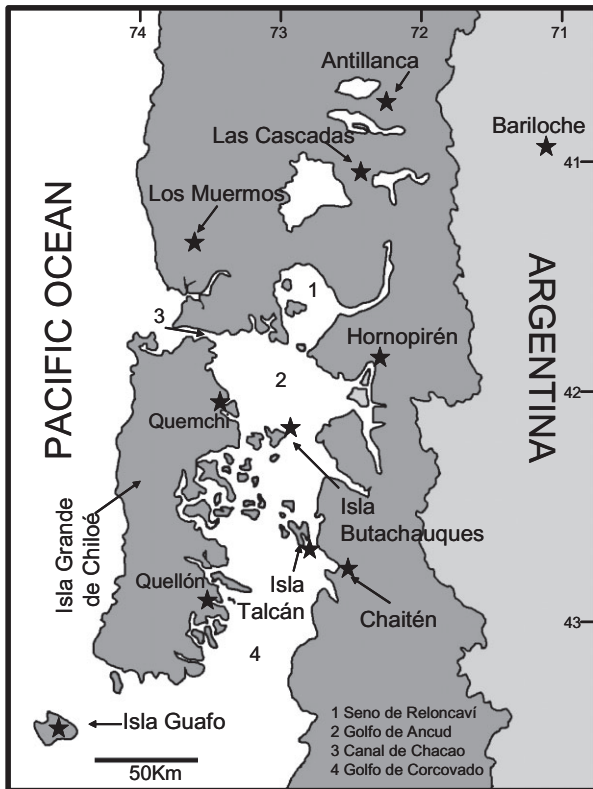


Fig. 1. The geographical distribution of *Liolaemus pictus* included in this study within the archipelago area. Sequences from Bariloche locality representing the subspecies *Liolaemus pictus argentinus* were obtained from GenBank. *Liolaemus pictus septentrionalis* subspecies was not incorporated in the map for being geographically distant from the study area (35°36'S, 71°03'W).

volcanoes during the Quaternary Period. The north-eastern tip of IGC is separated from the mainland by the Canal de Chacao, a 25-km-long seaway oriented along an east-to-west axis, having relatively shallow depths (≤ 80 m) and narrow width (approx. 2.5 km).

North-western Patagonia has been affected by repeated glaciations during Plio-Pleistocene times, which have shaped its present physical and biological configuration (Villagrán *et al.* 1986; Denton *et al.* 1999; Moreno & León 2003). During the Last Glacial Maximum (LGM) (approx. 26 000–17 500 cal yr BP (calendar years before present)), piedmont glacier lobes covered the western Andean slopes, the interior seas, vast areas of the intermediate depression, as well as the southern and eastern portions of IGC (Mercer 1976; Heusser & Flint 1977). Extreme glacial conditions terminated with a warm pulse at approx. 17 500 cal yr BP that led to a rapid glacier withdrawal from the lowlands (Denton *et al.* 1999), reaching temperatures similar to the present within approx. 2000 years (Heusser *et al.* 1999; Moreno *et al.* 1999;

Lamy *et al.* 2007). During the culmination of the LGM, global sea surface level (SSL) was about 120 m below the modern coastline, establishing a land bridge between IGC and the mainland along the exposed continental shelf (Heusser 1982; Villagrán *et al.* 1986; Formas 1989; Moreno *et al.* 1994; Formas & Brieva 2000). As Patagonian glaciers withdrew into the Andes, a series of small islands located east of IGC were either uncovered or formed sometime between approx. 17 500 and 14 700 cal yr BP, judging from minimum radiocarbon dates for ice-free conditions in continental Chiloé (Heusser 1990; Denton *et al.* 1999). This deglacial trend was contemporaneous with a sustained SSL rise that persisted until approx. 7000 cal yr BP, when modern levels were achieved at a global scale. This deglacial SSL rise featured distinct accelerations in response to the collapse of land-based ice sheet during the so-called meltwater pulses 1a and 1b, dated at approx. 14 600 and 11 500 cal yr BP, respectively. About 2/3 of the total SSL recovery during the last deglaciation was completed by the end of these events (Fairbanks 1989; Lambeck & Chappell 2001; Siddall *et al.* 2003). Assuming limited isostatic rebound and coastal erosion, the modern insularity of the Chilotan archipelago may have been achieved by approx. 7000 cal yr BP. Additionally, biological interchange through land bridges was possible: (i) between IGC and the mainland, between approx. 26 000 and ≥ 7000 cal yr BP; and (ii) between IGC, the mainland and the archipelago, between $\leq 17 500$ and ≥ 7000 cal yr BP, depending on the bathymetry of the interior sea. More recent dispersal events could have occurred via rafting to/from the archipelago. Trans-Andean biological connections between 41°–43°S could have occurred at times of limited ice cover during the warmest intervals of interglacials (Moreno *et al.* 1994; Moreno 2000; Stern 2004; Whitlock *et al.* 2007).

Liolaemus lizards are broadly represented in South America (Donoso-Barros 1966; Frost & Etheridge 1989) with more than 160 extant species found mainly in Argentina and Chile (Etheridge & Espinoza 2000; Schulte *et al.* 2000). Most Chilean species of the genus *Liolaemus* have been defined on the basis of morphological criteria, with very few studies that document their genetic variation (Vásquez 2001; Torres-Pérez *et al.* 2003; Vidal *et al.* 2004; Victoriano *et al.* 2008). The heterogeneity of the Chilean territory, with pronounced climatic and ecological gradients along latitudinal and altitudinal transects, implies that widely distributed species of *Liolaemus* face different environmental conditions (Victoriano *et al.* 2008; Vidal 2008). This genus has been considered a dynamic group, in an evolutionary sense, considering high species richness and endemism, along with the extensive intraspecific morphological variation observed in widely distributed species (Victoriano *et al.* 2008;

Table 1. Site location and number of *Liolaemus pictus* specimens sequenced in this study and diversity parameters estimated from mtDNA *Cyt b* sequences

Places	Subspecies	Locality	<i>n</i>	K	H	S	Π	D	Fu
Mainland	<i>L. p. pictus</i>	Antillanca	25	12	0.843	14	2.3	-1.28	-5.40*
		Las Cascadas	5	5	1.000	28	11.6	-1.02	-0.15
		Los Muermos	12	11	0.985	36	13.2	0.50	-2.05
		Hornopirén	18	4	0.575	3	0.7	-0.72	-1.09
		Chaitén	7	4	0.714	4	1.1	-1.42	-1.22
Isla Grande de Chiloé	<i>L. p. chilensis</i>	Bariloche	2	-	-	-	-	-	-
		Northern area: Quemchi	31	10	0.871	14	3.7	-1.23	-3.45*
Small islands	<i>L. p. codoceae</i>	Southern area: Quellón	20	12	0.911	22	4.1	-1.29	-3.55*
		Isla Butachauques	14	6	0.813	23	6.0	-0.73	2.09
		Isla Guafo	5	2	0.400	5	2.0	-1.12	2.64
Total	<i>L. p. talcanensis</i>	Isla Talcán	17	5	0.757	35	13.4	1.06	9.04*
			154	67	0.975	98	16.6	-0.34	-18.42*

* $P < 0.05$. Number of individuals (*n*); number of haplotypes (K), haplotypic diversity (H), number of polymorphic sites (S), mean number of pairwise sequences differences (Π), Tajima test (D), Fu's F_s test (Fu). -, not applicable.

Vidal *et al.* 2009). *Liolaemus pictus* is one of the southernmost distributed lizard species in Chile (37°–43°S), including mainland and island populations. Six subspecies of *L. pictus* have been described on the basis of morphological traits and geographical distribution (Donoso-Barros 1966, 1970; Urbina & Zúñiga 1977; Pincheira & Núñez 2005), four of which are restricted to the Chilotan archipelago (41°–43°S, Fig. 1, Table 1). It is thus expected that the genetic relationship between populations located in the archipelago and the mainland are relevant for explaining their morphological differences.

Ice-free, lowland regions west of the Andean glacial lobes have been proposed as potential areas where terrestrial biota could have lingered during Quaternary ice ages. These include the north-western third of IGC, the lowlands adjacent to the Coastal Range in the mainland, and the intervening sector of exposed continental shelf, which might have served as a corridor between the former areas. If this was indeed the case, we predict that a unique gene pool could have persisted in these 'western refugia' during ice ages and that genetic diversity spilled into areas formerly covered by glaciers. Modern phylogeography should reflect this process, exhibiting a relative homogeneity in genetic structure and a gradient of genetic diversity from refugia to more recently deglaciated areas. Here, we present a phylogeographical study using mtDNA data from mainland and island populations of *L. pictus* to assess the possible role of paleoenvironmental changes associated with Quaternary glaciations (the last 2 million years) on the geographical and genetic structuring of this group. We specifically aimed to test the hypothesis that populations from the mainland and IGC were confined to putative glacial refugia during the LGM. Moreover, an analysis of the possible

colonization routes into the archipelago was also included.

METHODS

Sampling and laboratory protocols

Tissue samples of 154 lizard tails were obtained from 11 mainland and insular localities (Fig. 1; Table 1). Total genomic DNA was isolated from samples using a standard phenol chloroform extraction protocol. The partial Cytochrome b (*Cyt b*, 535 bp) gene was amplified and sequenced with two primers designed from tRNA-Glu and tRNA-Thr sequences of the iguanid lizards *Iguana iguana* (AN: NC002793) and *Sceloporus occidentalis* (AN: NC005960) (LioGluFor: AAACCACCGTTGTTATTCAACTA and LioThrRev: CCATCTTTGGCTTACAAGACCAGAG). DNA sample dilutions were performed (100 ng μL^{-1}) for polymerase chain reaction (PCR) amplification. The *Cyt b* gene region was amplified via PCR in 25 μL of reaction volume containing 0.3 μL Taq (Invitrogen), 2.5 μL of PCR Buffer 10 \times , 1.6 μL MgCl_2 50 mmol L^{-1} , 2 μL dNTPs 100 mmol L^{-1} , 0.5 μL of each primer (10 pmol), and 1 μL of template DNA. The thermal cycling amplification conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of strand denaturation at 94°C for 30 s, annealing at 53.3°C for 1 min and 30 s, primer extension at 72°C for 1.5 min, and a final 10-min extension at 72°C. Size of PCR products was checked by comparing with a 100 bp DNA ladder (Invitrogen) in 2% agarose gel. Amplified DNA was purified with the QIAquick PCR Kit according to the supplier's protocol (Qiagen, USA). Finally, all samples were sequenced in the forward and reverse direction with an automated DNA-sequencer. Sequence alignments were accomplished using Proseq (Filatov 2002), and checked by eye. For phylogenetic reconstruction, partial sequences of *Liolaemus pictus argentinus* (accession number (AN): AY367791

and AY173795) from Bariloche and out-groups (*Liolaemus tenuis* (AN AY851742), *Liolaemus chillanensis* (AN AY850622) and *Liolaemus villarricensis* (AN AY850629)) were obtained from GenBank. Additionally, *Liolaemus pictus septentrionalis* subspecies ($n = 4$) from Altos de Vilches (35°36'S, 71°03'W) was incorporated as an out-group.

Phylogeography of the mainland, Isla Grande de Chiloé and small islands

For this analysis, we focused on the phylogeographical pattern on the mainland and sites on IGC, including the small islands that were formed after the LGM (Butachauques and Talcán islands), in order to reveal recent colonization events from older processes. We computed a median-joining network, using the Network 4.5.0.0 software (Bandelt *et al.* 1999) to decipher the relationships and geographical partitioning among haplotypes. To assess mitochondrial genetic diversity within *L. pictus*, we calculated the number of haplotypes (K), number of polymorphic sites (S), nucleotide diversity (H) and number of pairwise differences (II) using the DNAsp software (Rozas *et al.* 2003). Analyses of molecular variance (AMOVA, Excoffier *et al.* 1992) were performed using the SAMOVA software (Dupanloup *et al.* 2002) to study the proportion of total genetic variation attributable to different hierarchical levels, based on the geographical distribution of haplotypes. Several groupings of populations were tested to maximize the among-group component of molecular variance, that is, to determine the maximum degree of phylogeographical structure present in the data (Liebers & Helbig 2002). The genetic structure defined by SAMOVA and their significance level were evaluated by 1000 permutations of populations among groups (Dupanloup *et al.* 2002).

We carried out mismatch analysis (with pairwise distances) as a final assessment of population demographic histories. To see whether the populations have undergone a sudden population growth, we compared the mismatch distribution of pairwise differences among haplotypes with expectations of a sudden-expansion model (Rogers 1995). The goodness of fit between the observed and expected mismatch distribution was tested by a parametric bootstrap approach that uses the sum of squared deviations between observed and expected mismatch distribution as a test statistic, as implemented in Arlequin 2.0 (Schneider *et al.* 2000). Additionally, we tested whether the pattern of observed polymorphism within populations was consistent with a neutral equilibrium Wright–Fisher model using Fu's F_s (Fu 1997). This statistic takes on a negative value with an excess of rare haplotypes. Such a finding may occur under scenarios of background selection, selective sweeps or population expansions. However, F_s has proved to be the most sensitive statistic with respect to demographic expansion (Fu 1997; Ramos-Onsins & Rozas 2002). Tajima's D statistic was calculated to distinguish between a DNA sequence evolving neutrally *versus* one evolving under a non-random process such as selection, demographic expansion–contraction or introgression (Tajima 1989). Fu's F_s and Tajima's test were computed using the program DNAsp (Rozas *et al.* 2003). The time of divergence (t) between the two lineages was calculated according to theory as $\text{Pi inter}/2u$, where Pi inter is the mean

number of haplotype differences between the groups and u is the number of substitutions per sequence per million years. We used the two substitution rates proposed by Torres-Pérez *et al.* (2007) and Morando *et al.* (2004), 1.4 and 2.5% Ma⁻¹, to estimate the divergence times in the *L. pictus* complex.

Maximum parsimony (MP) and maximum likelihood (ML) criteria were implemented, using PAUP* 4.0b1 (Swofford 2002), to determine the phylogenetic position of haplotypes on the recently colonized island. For MP analysis, all characters were equally weighted, and a search was performed with 1000 replicates and maxtrees set to 5000, followed by tree-bisection reconnection branch swapping. For ML, the selected TIM + I + G model of evolution was estimated using the ModelTest software version 3.7 (Posada & Crandall 1998), and based on the Akaike Information Criterion under a heuristic search with 10 replicates and tree-bisection reconnection branch swapping. Support for the resulting clades was estimated with nonparametric bootstrapping (Felsenstein 1985), using 1000 pseudoreplicates. Bootstrap values >90% are considered strong support for a clade (Hillis & Bull 1993). We used partial sequences of *L. tenuis*, *L. chillanensis* and *L. villarricensis* as out-groups, and included *L. p. septentrionalis* (from Alto de Vilches) and *L. p. argentinus* (from Bariloche) within the in-group.

RESULTS

We obtained 535-bp-long *Cyt b* sequences of 154 *L. pictus* specimens collected from 11 localities (Table 1). Five main haplogroups within *L. pictus* were assorted by median-joining networks (Fig. 2). The first haplogroup (HG-1) included individuals from the mainland localities called Antillanca and Las Cascadas. A second haplogroup (HG-2) included all specimens from Hornopirén, Chaitén and Bariloche, and one haplotype from Los Muermos. A third haplogroup (HG-3) included IGC (both the northern and southern populations) and Isla Guafo. The latter haplogroup did not share haplotypes with the mainland. The fourth haplogroup (HG-4) represented haplotypes from Los Muermos and the last haplogroup (HG-5) included sequences from Las Cascadas and Los Muermos. When the small islands were considered, haplotypes from Isla Talcán were assigned to HG-1 and HG-2, corresponding to the Antillanca-Las Cascadas and Hornopirén–Chaitén clades, respectively. Most of the samples collected from Isla Butachauques were within HG-5, while one haplotype was assorted to HG-3 (IGC).

The number of haplotypes per locality ranged between four (Hornopirén and Chaitén) and 19 (IGC). Eighty-nine nucleotide sites (18.3%) were found to be polymorphic. The mean number of pairwise sequence differences ranged from 0.7 to 13.2 (Table 1). Mismatch distribution showed a unimodal wave in Chiloé–Guafo Island and unimodal with low peaks in 21 and 25 pairwise differences in Antillanca-Las Cascadas localities as predicted for a population

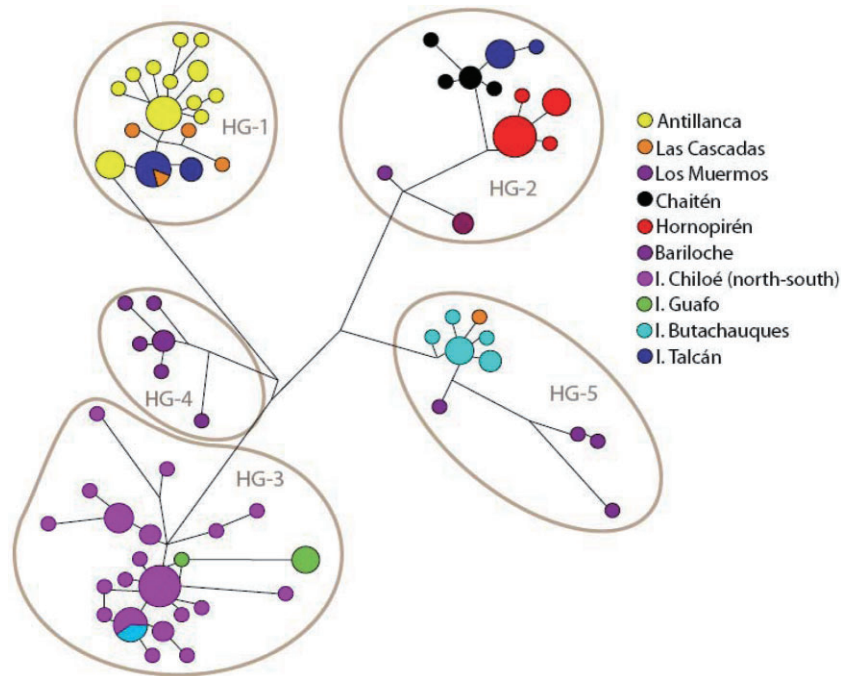


Fig. 2. Median-joining networks of mitochondrial Cytochrome b (*Cyt b*) haplotypes of *Liolaemus pictus* populations. Circle sizes are proportional to frequency. The smallest circle corresponds to one individual. North and South samples of Isla Grande de Chiloé were similarly coloured because their haplotypes are closely related.

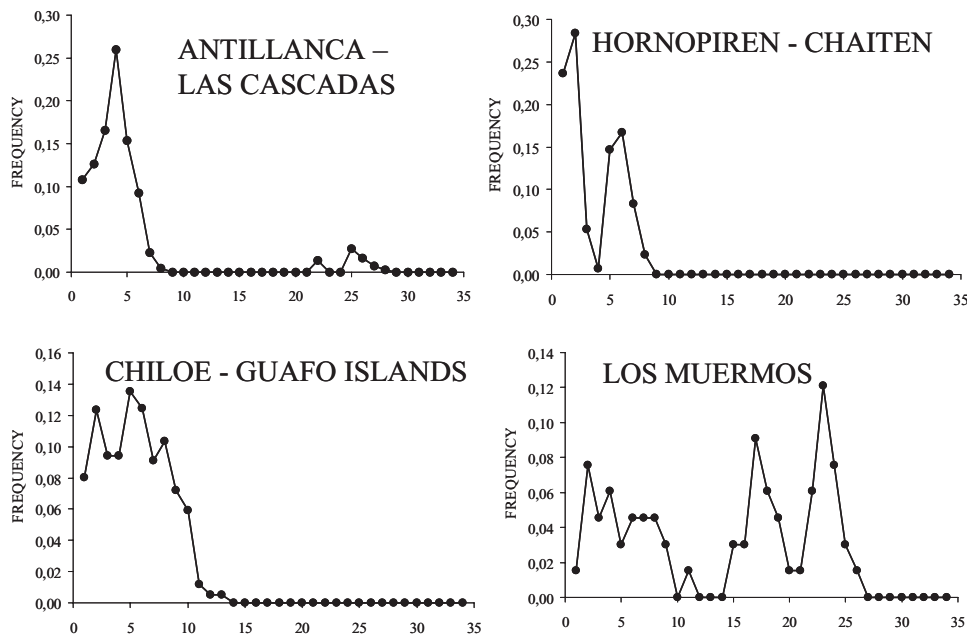


Fig. 3. Mismatch distribution of pairwise differences between sequences of *Liolaemus pictus* from different groups (obtained by analysis of molecular variance).

that has undergone demographic expansion, but Hornopirén-Chaitén locality was bimodal wave, and the wave to Los Muermos was multimodal suggesting an absence of demographic change in different times

(Fig. 3) (Rogers & Harpending 1992). Additionally, Fu's F_s supported the demographic expansion for Antillanca, and IGC (north and south) localities rejecting stasis at the 0.01 significance level (Table 1).

Table 2. Molecular variance analysis for seven *Liolaemus pictus* populations in six different models or groups

Model	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	% variance	
							Among intergroup	Within locality
2	Antillanca Las Cascadas I. Chiloé N I. Chiloé S I. Guafo Los Muermos	Hornopirén Chaitén					38.33	16.59
3	Antillanca Las Cascadas	Hornopirén Chaitén	I. Chiloé N I. Chiloé S Los Muermos				57.80	18.17
4	Antillanca Las Cascadas	Hornopirén Chaitén	I. Chiloé N I. Chiloé S I. Guafo	Los Muermos			70.47	19.00
5	Antillanca Las Cascadas	Hornopirén Chaitén	I. Chiloé N I. Chiloé S	I. Guafo	Los Muermos		72.66	19.51
6	Antillanca Las Cascadas	I. Chiloé N I. Chiloé S	Hornopirén	Chaitén	I. Guafo	Los Muermos	71.90	19.81

Population on the Chiloé Island was divided into North (N) and South (S) populations. All models showed high significance, $P < 0.001$.

The F_s values were mostly negative as well, but for Isla Guafo, Isla Butachauques and Isla Talcán, these values were positive. Tajima's test showed negative and non-significant ($P < 0.05$) values for all localities, except Los Muermos and Isla Talcán (Table 1). A nested analysis of variance was maximized with a model of five groups applied to the *L. pictus* localities, which accounted for 73% of the molecular variance among groups and 19.5% within localities (Table 2). Using a mutation rate of 2.5% for saurian *Cyt b* (Crochet *et al.* 2004) and 1.4% for ectothermic vertebrates (Avice *et al.* 1998), we estimated a divergence time of approx. 860 000 cal yr BP and 1 500 000 cal yr BP, respectively, between populations from IGC and the adjacent mainland.

The MP analyses recovered 541 equally parsimonious trees (Length = 361, Consistency Index = 0.66, Retention Index = 0.86), and a strict consensus tree was generated (Fig. 4). The ML analyses recovered one tree ($\ln L = -1759.61617$) and, because MP and ML analyses produced very similar results, a single tree is presented herein (Fig. 4). A *L. pictus* haplotype tree, rooted with sequences from *L. tenuis*, *L. villarricensis* and *L. chillanensis*, revealed two main clades supported by a 100% bootstrap as follows: a clade (belonging to the *L. p. septentrionalis* subspecies) with individuals of the northernmost distributional range of the species, and another clade with the in-group (from localities around and within the Chilotan archipelago). In this last group, five clades were strongly supported by bootstrap values >90%. A first clade (HG-1) included haplotypes from Antillanca and Las Cascadas, both

described as *Liolaemus pictus pictus* (Donoso-Barros 1966), and from Isla Talcán previously assigned to *Liolaemus pictus talcanensis* by Urbina and Zúñiga (1977) and Pincheira and Núñez (2005). A second clade (HG-2) included mainland haplotypes attributed to *L. p. pictus* (Hornopirén, Chaitén and one haplotype from Los Muermos), haplotypes categorized as *L. p. argentinus* (Bariloche), as well as haplotypes from Isla Talcán (*L. p. talcanensis*). A third clade (HG-3) included all localities from IGC assigned to *Liolaemus pictus chiloensis*, two haplotypes from Isla Guafo, and one haplotype from Isla Butachauques corresponding to *Liolaemus pictus codoceae*. A fourth clade (HG-4) only included haplotypes from Los Muermos (assigned to *L. p. pictus*). Finally, a fifth clade (HG-5) included haplotypes from Los Muermos, one from Las Cascadas (*L. p. pictus*), and others from Isla Butachauques (*L. p. codoceae*) (Fig. 4).

DISCUSSION

Contrary to our expectations of a simple predictable geographical pattern of genetic diversity, the mtDNA data suggest a complex phylogeographical history of *L. pictus*. Our results indicate that populations from IGC and the adjacent mainland exhibit high levels of genetic divergence (Fig. 2) that could predate several glaciations during the Late Pleistocene. This is supported by the multimodal curve progression of mismatch distribution in Antillanca-Las Cascadas, Hornopirén-Chaitén and Los Muermos localities and

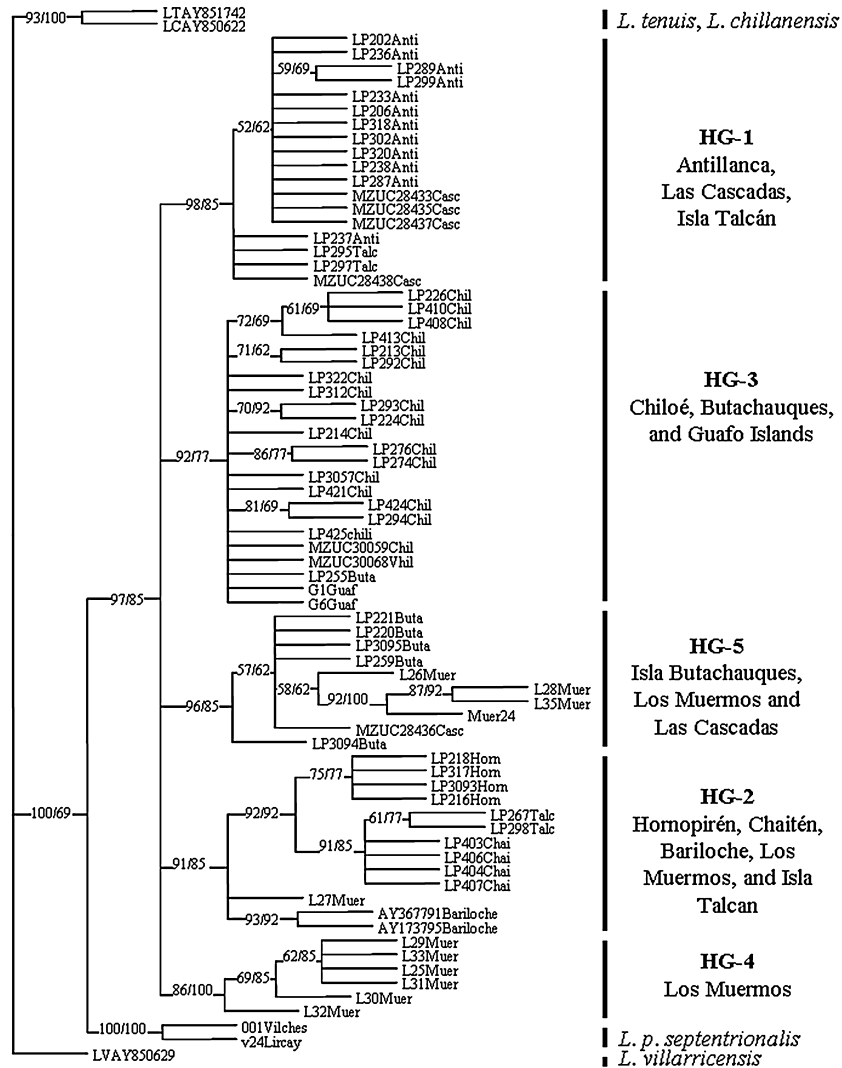


Fig. 4. Single maximum likelihood (ML) tree ($\ln L = -1759.61617$) coincident with maximum parsimony (MP) tree for non-redundant Cytochrome b haplotypes of the *Liolaemus pictus* populations. Numbers in the branches represent bootstrap for MP and ML.

negative value of Fu's F_s at some localities, although values for Guafo, Butachauques and Talcán islands were positive.

We calculated a divergence time of approx. 860 000 cal yr BP between populations from IGC and the adjacent mainland, based on a mutation rate of 2.5% for saurian *Cyt b* (Crochet *et al.* 2004). Regardless of the chosen mutation rate (range 0.5 to 2.5%; Avise *et al.* 1998; Schulte *et al.* 2000; Crochet *et al.* 2004), our results strongly suggest that an ancient lineage split migrated into IGC during the waxing and waning of early Pleistocene glaciations. These results challenge our expectation of genetic connectivity between the putative refugia from IGC and the mainland along the exposed continental shelf during sea level minima, associated with the most recent Quater-

nary glaciations. Our results indicate that *L. pictus* populations that currently occupy the Andean foothills originated from geographically differentiated gene pools, thus suggesting that postglacial colonization occurred from multiple sources and not from a single 'western refuge'. We propose that the highly divergent and geographically contiguous lineages from Antillanca-Las Cascadas and Hornopirén-Chaitén-Bariloche must have remained in separate ice-free regions during glaciations. Our data suggest at least three Late Pleistocene sources: (i) north-western IGC; (ii) a northern source located in ice-free sectors of the Longitudinal Valley adjacent to Antillanca; and (iii) a source located in ice-free regions east of the Andes. Among such, we propose north-western IGC as a true refuge, suggesting that populations of *L. pictus* could

have remained isolated over repeated glacial and interglacial phases. In contrast, colonization from the northern and eastern sources discussed above could represent shifts in the geographical range driven by Pleistocene glaciations. However, such interpretation must be carefully considered and alternative explanations can be proposed. In fact, the patterns observed in *L. pictus* could be due to multiple genetic and demographic processes including reduced dispersal and small effective population sizes which are well known to create breaks in phylogeographical signal without a corresponding break in geography (Irwin 2002). Moreover, the use of a single maternally inherited molecule to describe population histories has led to some uncertainty over whether the mitochondrial gene trees obtained in hundreds of phylogeographical studies are representative of the histories of the relevant species or populations (see Edwards & Bensch 2009).

The divergence time among these lineages clearly predates Late Pleistocene glaciations, which can be said to be analogous to the populations located in IGC and the adjacent mainland. The fact that this divergence was maintained throughout the Pleistocene suggests the following: (i) cycles of population contraction occurred repeatedly in separate mainland subregions during glaciations, and (ii) population expansion and reshuffling of ancestral geographical ranges during interglacials were insufficient to cause a significant blurring of this differentiation. Physical, climatic or biological barriers to postglacial expansion might have contributed to this highly structured phylogeographical pattern. Moreover, the high levels of divergence among haplotypes in several populations (e.g. Los Muermos) suggest the possibility that their origin might predate the Middle Pleistocene (Klicka & Zink 1997; Cook *et al.* 2001). This is consistent with the findings of long branch lengths in phylogenetic trees and high genetic diversity indexes in the southernmost populations of *L. pictus* located at approx. 40°S (Victoriano *et al.* 2008). For Victoriano *et al.* (2008) results are interpreted as indicators of a reduced effect of glacial cycles on patterns of genetic variation, and they proposed that extensive areas in the Coastal Range acted as refugia for *L. pictus* during the Pleistocene.

Our results from Isla Talcán, Isla Butachauques and Isla Guafo suggest that these islands were colonized from mainland sources, as well as from IGC. Data from Isla Talcán suggest intermingling of two highly divergent and geographically contiguous 'mainland lineages', namely, the Antillanca-Las Cascadas in the North and the Hornopirén-Chaitén in the South. Although these lineages do not share haplotypes, both are present in the island. However, only one haplotype is currently found on the adjacent mainland, namely, Hornopirén-Chaitén. In contrast, the Antillanca-Las

Cascadas haplotypes are absent along an approx. 200-km stretch in the Andean region, between Las Cascadas and Chaitén. One explanation for the presence of both lineages in the island might be based on two successive colonization phases, associated with shifts in their distributional range. First, we postulate an initial colonization phase of Isla Talcán by populations belonging to the HG-1 group that inhabited the Chaitén region to the east, followed by a relatively recent northward contraction in its geographical distribution. A secondary phase features range expansions into the same mainland sector by populations belonging to HG-2 and the subsequent colonization of Isla Talcán. We speculate that the source of those immigrants might be populations originating from glacial populations located on the eastern flank of the Andes (*L. p. argentinus*). The geographical and temporal discontinuity in these phases might result from climatic/biogeographical shifts, as well as volcanic disturbance on the land biota during the Holocene. Supporting evidence comes from paleoclimate studies which document a southward shift in the distribution of thermophilous Valdivian trees (e.g. *Eucryphia cordifolia*) during a multi-millennial warm-dry interval, prominent ice recession to the Andean headwaters, lake-level lowering, and intense fire activity driven by warmer-than-present air temperatures and weakening of the westerly winds in the early Holocene (Whitlock *et al.* 2007; Abarzúa & Moreno 2008; Moreno *et al.* 2010). This regional-scale phenomenon was followed by a change in opposite direction over the last approx. 7800 years, which led to an expansion of hygrophilous, cold-resistant North Patagonian trees in north-western Patagonia (Moreno 2004). We speculate that the geographical distribution of *L. pictus* populations, belonging to HG-1, responded in a similar manner throughout the Holocene. Second, intensive disturbance by fire and volcanic activity on the Andean foothills (Moreno 2000; Naranjo & Stern 2004; Stern 2004; Villarosa *et al.* 2006) at the beginning of the Holocene, coupled with profound landscape and vegetation changes (Moreno 2004; Abarzúa & Moreno 2008), might have temporarily decimated lizard populations in the Chaitén area. This may have allowed replacement of HG-1 by HG-2 clades. Isla Talcán could have escaped the disturbance effects thanks to its isolation from the mainland, along with its larger distance to the active volcanic centres.

Specimens collected in Isla Butachauques are genetically related to those inhabiting the adjacent northern areas of the mainland, that is, Los Muermos and Las Cascadas, and on a lesser extent to those in the IGC. These results suggest a simple colonization process from the neighbouring land areas of this island exposed during deglaciation. In the same manner, *L. pictus* from Isla Guafo are closely related to specimens in IGC.

Implications for paleogeography and biogeography

A puzzling paradox emerges when considering the calculated antiquity of the splitting of *Liolaemus* populations from the mainland and IGC, as well as the biological exchanges that occurred during times of lowered SSL during Quaternary glaciations along the exposed continental shelf (Moreno *et al.* 1994). If the genetically based estimated time for such disjunction is correct, then *Liolaemus* lizards were unable to utilize the land bridge over an interval that included at least the eight most recent glacial cycles. Likely explanations for this apparent contradiction involve the following scenarios: (A) the current shallow configuration of the continental shelf is a very recent phenomenon and glacioeustatic variations associated with glacial–interglacial cycles over the last 860 000–1 500 000 years were of insufficient magnitude to expose land bridges between the mainland and IGC; (B) intense meltwater production, along with extensive glacial extents during the most recent glaciations, established fluvio-glacial barriers for the dispersal of small land vertebrates along the exposed continental shelf between the mainland and IGC. Because explanation A is incompatible with the colonization of IGC by *Liolaemus* lizards via a land bridge, we advocate two alternative scenarios: (1) *L. pictus* individuals from the mainland reached IGC as waif dispersals, approx. 860 000–1 500 000 years ago, or (2) lizards were able to colonize IGC during an interval in which Patagonian glaciers reached a limited extent and SSL lowering did not expose vast areas to allow the formation of land bridges between the mainland and IGC, except a narrow fringe within the area of the Canal Chacao. Support for scenario 2 comes from deep-sea oxygen isotope stratigraphies, which show the predominance of glacial cycles occurring within 41 000-year periodicities, followed by the establishment of dominant approx. 100 000-year cycles over an interval of 650 000–950 000 years ago (Raymo & Nisancioglu 2003). Because of their much shorter duration, glaciations dominated by 41 000-year periodicities must have led to a much smaller Patagonian ice sheet and attenuated SSL depressions when compared with the most recent ice cycles, which occurred within 100 000-year periodicities. This theoretical condition could have facilitated the migration of *Liolaemus* lizards into IGC through a land bridge connected to the mainland, under limited SSL depression and in the absence of major meltwater streams originating from the north-western sector of the Patagonian ice sheet, which could have impeded their transit.

We have no explanation as to why *Liolaemus* lizards were able to reach Isla Guafo, Isla Butachauques and Isla Talcán as waif dispersals following the LGM, but were unable to get to IGC through the same mecha-

nism over a much larger period (approx. 860 000 years ago) and considering a much shorter distance. Future studies on other land vertebrates having different dispersal capabilities over marine or fluvial barriers might provide insights into this problem. Taxonomic implications of these results are discussed in Appendix S1.

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SUPPORTING INFORMATION

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

Appendix S1. Taxonomic implications of the results.