Phylogeography of a Patagonian lizard and frog: Congruent signature of southern glacial refuges

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Abstract Pleistocene glaciations produced significant increases in continental ice cover in polar and mid-latitude temperate areas, sea-level declines and shifts and reshuffling of biomes, all of which promote either isolation, coalescence or fragmentation in the distribution of land biota. If populations of several taxa have been co-distributed for a prolonged time, and if the periods between perturbation or vicariance processes have been more or less stable, it is expected that divergence patterns of closely related and ecologically similar species will be congruent because of their similar biological and demographic characteristics. Based on this premise, we analysed the phylogeographic structure (*cytochrome b*) of *Liolaemus pictus* and *Batrachyla leptopus*, two widely co-distributed lizard and frog species, respectively, in the Chiloé Archipelago of southern Chile, to decipher their genetic structure in response to a common climatic and environmental history. Haplotype network analysis and Bayesian inference suggest an evolutionary pattern of genetic diversity for the two species that is consistent with the Quaternary glacial history of southern Chile, and suggests a complex phylogeographic history in the *Liolaemus* and *Batrachyla* species. High-divergence levels among haplotypes in some island populations of the archipelago also suggest genetic connectivity between putative refuges from Chiloé Island and the mainland along the exposed continental shelf during sea level minima associated with the most recent Quaternary glaciations. Our results are consistent with our hypothesis that two species have responded to parallel historical events in which the historical process during the last glacial maximum (approximately 41°S) has been sufficient to influence their phylogeographic structure.

Key words: archipelago, *Batrachyla leptopus*, cytochrome b, *Liolaemus pictus*, phylogeography.

INTRODUCTION

Archipelagic 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 geologic/environmental history, different processes can be considered as the main promoters that underlie the observed evolutionary patterns (Hille *et al.* 2003; Parmakelis *et al.* 2003; Harlin-Cognato *et al.* 2006). Comparative phylogeography has recently emerged as a method for determining the role of historical events and demographic processes (Zink 1996; Taberlet *et al.* 1998) including island effects (Cook *et al.* 2001). Comparison of multiple-species phylogeographic patterns in a region allows inferences to be made on the differential effects of large-scale historical events (Bermingham & Moritz 1998; Arbogats & Kenagy 2001). Previous work has demonstrated that if the populations of these taxa have been co-distributed for a prolonged time and have been more or less stable in the periods between the processes of perturbation or barrier generation, it is

reasonable to expect congruence among their patterns of divergence (Lourie *et al.* 2005). This approximation allows elucidating relationships among lineages, detecting past demographic events and estimating demographic change parameters (Rosenberg & Nordborg 2002; Crespi *et al.* 2003). Several studies have shown congruent phylogeographic patterns in plants, invertebrates and vertebrates, and have led to the interpretation that Pre-Quaternary and Quaternary geological events have been important driving forces in the evolutionary history of Patagonian lineages (Núñez *et al.* 2011; Sérsic *et al.* 2011). According to Breitman *et al.* (2012) and Barber *et al.* (2012), some lineages have persisted in different refuges during glacial cycles in Patagonia, and some phylogeographic breaks and/or refuges are shared among plants, rodents and lizards. A similar result was found in the otter *Lontra provocax* in western Patagonia comparing two southern populations of this species (Vianna *et al.* 2011).

It is proposed that the lowlands may have functioned as potential refuges for terrestrial biota, which would have persisted during the Quaternary glaciations and served as natural corridors for them. Ice cover increased during glacial periods and may have suppressed the exchange of terrestrial biota through the Andes. However, there could have been trans-Andean connections between 41 and 43°S during the warm interglacial period

doi:10.1111/aec.12325

(Moreno *et al.* 1994). Glacial and vegetation changes during the Holocene epoch (the last approx. 11 700 years) are mainly associated with climate variability and secondarily with volcanic and paleofire disturbance throughout the region (Whitlock *et al.* 2007; Jara & Moreno 2014; Moreno *et al.* 2015). The area between 41 and 43°S is located in the southern portion of the Southern Volcanic Zone where Stern (2004) recognized 13 active volcanic centres. The catastrophic effect of volcanism on the biota is expected to be more pronounced in sectors adjacent to these Andean centres, so that the geographical distribution of genetic diversity among species, in regions associated with glacial cycles during the Late Pleistocene, is drastically changed (Hewitt 1996, 1999; Klicka & Zink 1997; Stern 2004). In several cases, the role of glacial cycles has been demonstrated to promote or maintain divergence (Hewitt 1996, 1999; Knowles 2001; Schmitt & Seitz 2001; Janzen *et al.* 2002; Liebers & Helbig 2002). Other cases of genetic divergence have shown that they predate Quaternary glaciations, that is, more than 2 million years ago (Crespi *et al.* 2003; Morando *et al.* 2004; Torres-Pérez *et al.* 2007).

The Chiloé Archipelago in northwest Patagonia includes Chiloé Island (the largest island in the archipelago) and a series of small islands in the interior sea located east of Chiloé Island (Fig. 1; Vidal *et al.* 2012a). The mainland sector east of the interior includes the Andes Cordillera, which maintains glaciers on the highest summits and contains several active volcanoes. This area of Patagonia was affected by repeated glaciations during the Plio-Pleistocene (Denton *et al.* 1999), which gave origin to the current biogeographical conformation. The Last Glacial Maximum (approx. 26 000 to 17 500 year BP) is the most recent of those intervals with thick and extensive glacial cover.

The heterogeneity of the Chilean territory, with pronounced climatic and ecological gradients along latitudinal and altitudinal transects, implies that widely distributed reptile and amphibian species face different environmental conditions (Victoriano *et al.* 2008; Vidal 2008). In northwest Patagonia, various species endemic to Chile are found in the Chiloé Archipelago (41–43°S); among them are some species of the genera *Liolaemus* and *Batrachyla*. *Liolaemus pictus* is one of the southernmost lizard species found in Chile (37–43°S), featuring mainland and island populations. Six subspecies of *L. pictus* have been described based on morphological traits and geographical distribution (Donoso-Barros 1966, 1970; Urbina & Zúñiga 1977; Pincheira & Núñez 2005), and four of these are restricted to the Chiloé Archipelago. A taxonomic study of these subspecies by Young (1998) did not define them clearly, and Lobo (2001) proposed raising the subspecies to full species
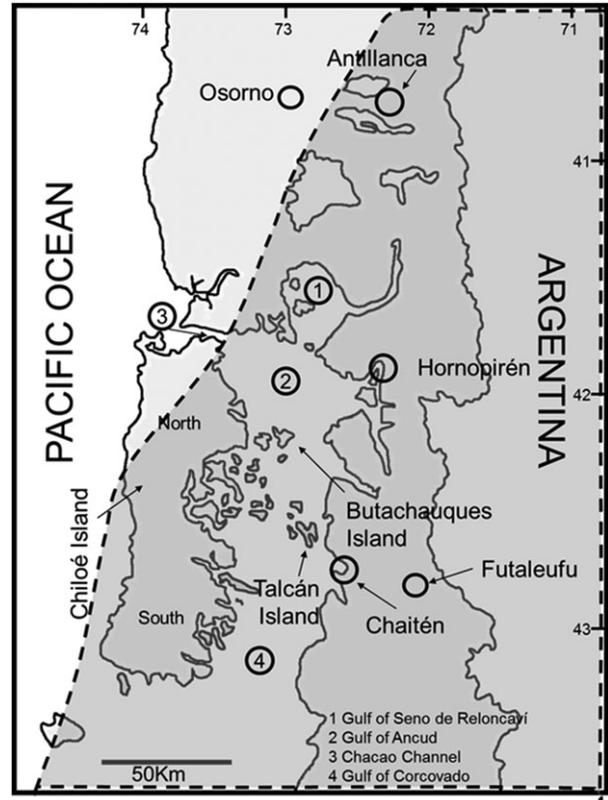

Fig. 1. Geographic distribution of *Liolaemus pictus* and *Batrachyla leptopus* included in this study within the archipelago. Shaded area represents the last glaciation limit. Open circles correspond to localities studied (Table 1).

status. Vidal *et al.* (2012a), however, suggested that disparities between mtDNA haplotypes and subspecific designations could partly be due to selection on morphology. A critical comparison of the two approaches is therefore required to determine whether distinct haplogroups denote separately evolving morphological lineages. The frog *Batrachyla leptopus* is widely distributed (37–45°S), including mainland and island populations (Capurro 1960; Global Amphibian Assessment 2003). Formas and Brieva (2000) suggested that the genetic distances between populations of *B. leptopus* in Chiloé Island and the mainland could be explained by the establishment of the Chacao Channel seaway, which may have acted as a recent barrier to gene flow; this also indicates less genetic variability in island populations. Relative sea level fluctuations associated with glacial-interglacial cycles promoted intermittent connections between the islands and the mainland at multi-millennial time scales. This situation may have allowed gene flow between these populations during low sea level intervals (Formas & Brieva 2000;

Vidal *et al.* 2009) although gene flow information has not been confirmed.

Here, we present a phylogeographic study using mtDNA data from mainland and island populations of *L. pictus* (lizard) and *B. leptopus* (frog) to assess the possible role of paleoenvironmental changes associated with Quaternary glaciations (approximately the last 2 million years) on the geographic and genetic structuring of these co-distributed groups. Our specific aim is to test the hypothesis that lizard and frog populations from the mainland and Chiloé Island were confined to putative glacial refuges during the Last Glacial Maximum. An analysis of the possible colonization routes into the archipelago is also discussed for both species.

METHODS

Study area, sample collection and DNA extraction

Lizards and frogs were collected in the field manually or with a noose, euthanized and preserved as museum voucher specimens in the Museum of Zoology of the *Universidad de Concepción* and Herpetological Collection of the Department of Cellular and Genetic Biology of the Universidad de Chile. The *Servicio Agrícola y Ganadero de Chile* (Chilean Agriculture and Livestock Service) permitted the capture of the animals; the specimens were euthanized to provide sufficient material for a comprehensive study of the species (geometric morphometrics, stable isotopes, digestive enzymes, diet and molecular analysis), considering the lack of information about these species. Many other specimens used in this study came from the Museum of Zoology of the Universidad de Concepción collection. Specimens totalled 181 from both species in seven

localities (Fig. 1): 127 *L. pictus* and 54 *B. leptopus* individuals. Table 1 summarizes the number of individuals collected per locality.

Total DNA was isolated from samples by modifying the salt extraction method described by Aljanabi and Martinez (1997). A fragment of the cytochrome b (Cyt b) gene for each lizard specimen 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: AAACCACCGTTGTTAT TCAACTA and LioThrRev: CCATCTTTGGCTTACAAG ACCAGAG) (Vidal *et al.* 2012b). Partial Cyt b of each frog was amplified and sequenced with two primers, (GAACTAA TGGCCCACACWWTACGNAA) and CytbAR-H (TAWA AGGGTCTTCTACTGGTTG) (Goebel *et al.* 1999). DNA sample dilutions were performed (100 ng mL^{-1}) for polymerase chain reaction (PCR) amplification. For lizards, the Cyt b gene region was amplified by PCR in 25 µL reaction volume consisting of 0.3 µL Taq (Invitrogen), 2.5 µL PCR Buffer 10×, 1.6 µL MgCl$_2$ 50 mM, 2 µL dNTPs 100 mM, 0.5 µL of each primer (10 pmol) and 1 µL template DNA. The thermal cycling amplification conditions were the following: initial denaturation at 94 °C for 5 min, 35 cycles of strand denaturation at 94 °C for 30 s, annealing at 53.3 °C for 1 min and 18 s, primer extension at 72 °C for 1 min and 30 s and a final 10 min extension at 72 °C. For the frogs, the Cyt b gene region was amplified by PCR in 15 µL of reaction volume consisting of 0.16 µL Taq (Invitrogen), 1.64 µL PCR Buffer 10×, 1.1 µL MgCl$_2$ 50 mM, 0.75 µL dNTPs 100 mM, 0.16 µL of each primer (10 pmol) and 3 µL template DNA. The thermal cycling amplification conditions were the following: initial denaturation at 94 °C for 1 min, 41 cycles of strand denaturation at 94 °C for 40 s, annealing at 44 °C for 1 min and 50 s, primer extension at 72 °C for 1 min and a final 7 min extension at 72 °C.

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

Location	Locality																
		Liolaemus pictus							*Batrachyla leptopus*								
		n	*K*	*H*	*π*	*S*	∏	D	Fu	*n*	*K*	*H*	*π*	*S*	∏	D	Fu
Mainland	Antillanca (Osorno)	25	13	0.843	0.0044	14	2.3	−1.28	−5.40*	9	5	0.889	0.0116	21	8.5	0.49	2.57
	Hornopirén	25	4	0.575	0.0033	3	0.7	−0.72	−1.09	4	2	0.667	0.0109	8	2.2	4.15	4.15
	Chaitén (Futaleufú)	8	4	0.714	0.0016	4	1.1	−1.42	−1.22	4	3	0.833	0.0055	6	3.0	−0.81	0.73
Chiloé Island	Northern area: Quemchi	18	9	0.871	0.0057	14	3.7	−1.23	−3.45*	7	3	0.544	0.0067	3	0.8	−1.35	−0.23
	Southern area: Quellón	20	10	0.911	0.0073	22	4.1	−1.29	−3.55*	19	9	0.731	0.0008	28	4.2	−1.89	−0.81
Small islands	Butachauques Island	14	12	0.813	0.0112	23	6.0	−0.73	2.09	4	2	0.500	0.0018	2	1.0	−0.71	1.09
	Talcán Island	17	5	0.757	0.0251	35	13.4	1.06	9.04*	7	6	0.952	0.014	20	9.0	0.60	−0.02
Total		127	57	0.966	0.0296	75	15.8	0.22	−6.05	54	25	0.872	0.0110	57	8.0	−1.27	−5.35

*P < 0.05. D, Tajima test; Fu, Fu test; *H*, haplotypic diversity; *K*, number of haplotypes; *n*, number of individuals; *S*, number of polymorphic sites; ∏, mean number of pairwise sequences differences; *π*, nucleotide diversity.

The size of PCR products in both cases was checked by comparing with a 100 bp DNA ladder (Invitrogen) in 2% agarose gels. Amplified DNA was purified with the QIA quick 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 edited with Proseq (Filatov 2002) and visually checked. We also verified the sequences with the DnaSP 3.53 (Rozas *et al.* 2003) program to translate them into amino acids.

Data analysis

We computed a median-joining network with the Network 4.6.0.0 software (Bandelt *et al.* 1999) to infer relationships and geographical partitioning among haplotypes. To assess mitochondrial genetic diversity in *L. pictus* and *B. leptopus*, we calculated the number of haplotypes (K), number of polymorphic sites (S), haplotype (H) and nucleotide (π) diversity and the number of pairwise differences (Π) with the DnaSP v5 software (Librado & Rozas 2009). We also tested the pattern of observed polymorphism within populations to determine whether it was consistent with a neutral equilibrium Wright–Fisher model using Fu's Fs (Fu 1997). This statistic has a negative value when there is an excess of rare haplotypes. Such a finding can occur under scenarios of background selection, selective sweeps or population expansions. Fs 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 Fs and Tajima's test were computed with the DnaSP v5 program (Librado & Rozas 2009).

The Bayesian approach was implemented to determine the phylogenetic relationships of gene trees. Non-redundant sequences of *L. pictus* ($n = 57$ sequences) and *B. leptopus* ($n = 50$ sequences) were used in this analysis. For phylogenetic reconstruction of *L. pictus*, two species were chosen as outgroup taxa, and their sequences were obtained from GenBank (*L. tenuis* Accession number (AN): AY851742, *L. chillanensis* AN: AY850622), while two specimens of *B. taeniata* were chosen as outgroup taxa for phylogenetic reconstruction of *B. leptopus*. The appropriate nucleotide substitution model (GTR + I + G) was estimated in both cases with the ModelTest 3.7 software (Posada & Crandall 1998) based on the Akaike Information Criterion. Phylogenetic trees were reconstructed with the Bayesian Markov Chain Monte Carlo approach implemented in the BEAST 1.6.2 program (Drummond & Rambaut 2007), which provides a flexible framework for hypothesis testing with time-structured molecular sequence data (Rambaut *et al.* 2009). We used the uncorrelated relaxed clock to run this analysis. The analysis was run for 50 million generations, and 1000 generations were retained, resulting in a file of 10 000 trees. The first generations (20%) were removed as burn-in. All trees below the observed stationary level were discarded. These trees

were summarized in a maximum *a posteriori* tree with TreeAnotator (part of the BEAST package) and visualized in FigTree v1.2.2.

RESULTS

We obtained 930 bp Cyt b sequences of 127 *L. pictus* specimens collected from seven localities (Table 1). Four main haplogroups within *L. pictus* were grouped by median-joining networks (Fig. 2). The first haplogroup (HG-1) included individuals from Antillanca and two haplotypes from Talcán Island (TI). A second haplogroup (HG-2) included all the specimens from Hornopirén and Chaitén and two haplotypes from TI. A third haplogroup (HG-3) included Chiloé Island (both northern and southern populations), one haplotype from Talcán Island and one from Butachauques Island (BI). The fourth haplogroup (HG-4) was composed of haplotypes from BI. It is important to note that haplotypes from Talcán Island were assigned to HG-1, HG-2 and HG-3, which corresponded to the Antillanca and Hornopirén–Chaitén clades and CI, respectively, while haplotypes
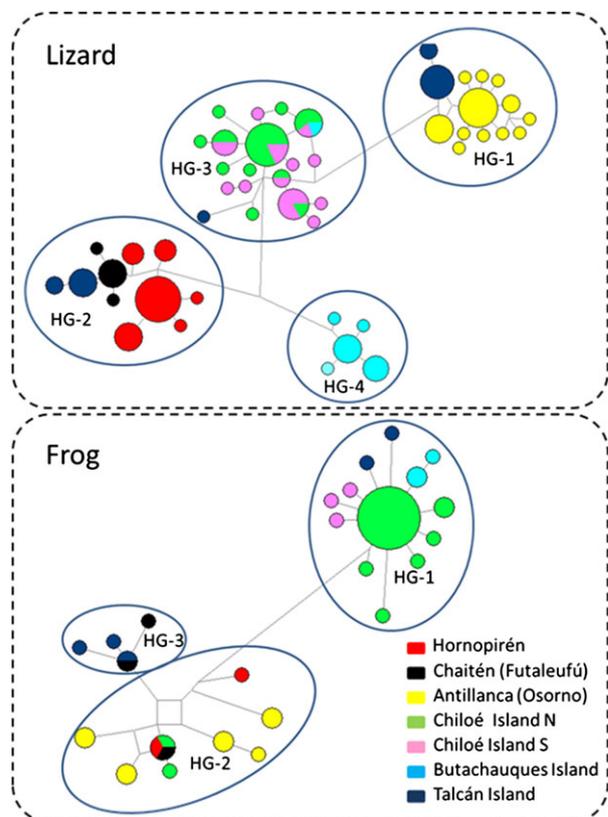

Fig. 2. Median-joining networks of mitochondrial cytochrome b (Cyt b) haplotypes of *Liolaemus pictus* and *Batrachyla leptopus* populations. Circle size in the network is proportional to haplotype sampling frequency.

from Butachauques Island were grouped in HG-3 and HG-4, respectively. The number of haplotypes per locality ranged between four (Hornopirén and Chaitén) and 12 (CI-South). A total of 75 nucleotide sites (8%) were found to be polymorphic. The mean number of pairwise sequence differences ranged from 0.7 to 13.4, which corresponded to nucleotide diversity ranging from 0.00075 to 0.0144 (Table 1). Fu's Fs also supported demographic expansion for the Antillanca and Chiloé Island (north and south) localities, thus rejecting stasis at the 0.01 significance level (Table 1). The Fs values were also mostly negative, but this value was positive for BI. Tajima's test showed negative non-significant ($P < 0.05$) values for all localities, with the exception of Talcán Island (Table 1). An *L. pictus* tree of 57 non-redundant haplotypes rooted with the sequences of *L. tenuis* and *L. chillanensis* revealed four principal clades, which were all supported by high posterior probabilities ($P = 0.99$). The first includes all the haplotypes of the northernmost mainland locality studied, Antillanca, and two haplotypes from Talcán Island (HG-1). The second clade gathers all the haplotypes from mainland Hornopirén and two other haplotypes from Talcán Island (HG-2). A third clade includes all the haplotypes from Chiloé Island (northern and southern populations), one haplotype from Talcán Island and two haplotypes from BI; this suggests that both islands were colonized from CI. Finally, a fourth clade includes only haplotypes from Butachauques Island (Fig. 3).

We obtained 731 bp Cyt b sequences of 54 *B. leptopus* specimens collected from seven localities (Table 1). Three main haplogroups in *B. leptopus* are demonstrated by median-joining networks (Fig. 2). The first haplo group (HG-1) includes individuals from the island localities CI-North and CI-South as well as several individuals from Talcán Island and BI. A second haplogroup (HG-2) consisted of all the specimens from Osorno and Hornopirén and some individuals from TI, whereas the last haplogroup (HG-3) involved some haplotypes from Talcán Island and sequences from Futaleufú, which is east of the archipelago and adjacent to the island localities.

The number of haplotypes per locality ranged from 2 (Hornopirén and BI) to 9 (CI-South). A total of 57 nucleotide sites (4.2%) were polymorphic, and the mean number of pairwise sequence differences ranged from 0.8 to 9.0 (Table 1). Fu's Fs also supported demographic expansion for CI-North and CI-South localities by rejecting stasis at the 0.01 significance level (Table 1). The Fs values were also mostly negative, but were positive for mainland localities. Tajima's test showed negative and non-significant ($P < 0.05$) values for Chaitén, Chiloé Island (north and south) and Butachauques Island (Table 1). Two main haplogroups in *B. leptopus* recovered by the Bayesian approach in Talcán Island are found on both the mainland and CI, indicating that this island was colonized from both sites at two different times. Butachauques Island is nested in the Chiloé Island haplogroup, which reinforces the idea that colonization came from Chiloé Island (Fig. 4).

DISCUSSION

Our results suggest that populations from Chiloé Island and the mainland exhibit high levels of genetic divergence in both species, based on the number of mutational steps in the haplotype networks and Bayesian inference. If our results based on mtDNA represent the evolutionary history of these species in an accurate manner, then they challenge the idea of genetic connectivity between the putative refuges in Chiloé Island and the mainland along the exposed continental shelf during sea level minima associated with the most recent Quaternary glaciations (Denton *et al.* 1999). In this context, the role of glacial cycles as promoters or inhibitors of divergence in many animal groups is the subject of debate (Hewitt 1996, 1999; Knowles 2001; Schmitt & Seitz 2001; Janzen *et al.* 2002; Liebers & Helbig 2002) because the last glacial maximum does not always explain the divergence (Crespi *et al.* 2003; Morando *et al.* 2004). On the other hand, post-glacial colonization reveals other mechanisms associated with the current distribution of species because the way in which previously glaciated areas are colonized can vary according to the intrinsic characteristics of species or topographic changes produced by the glacial event (Hewitt 1996). If divergence is associated with glacial periods in which populations were reduced to single or multiple allopatric refuges, this should be reflected in the regional structure of genetic variation (Premoli *et al.* 2002). In contrast, if divergence is associated with successive founder events during the colonization of previously glaciated areas, recently founded populations would have been derived from a source population and would show reduced levels of diversity (Hewitt 1999; Knowles 2001).

Our results indicate that the *L. pictus* and *B. leptopus* populations originated from geographically differentiated gene pools, thus suggesting that post-glacial colonization came from at least two sources and not from a single 'western refuge'. We propose that the highly divergent and separated lineages from Antillanca (Osorno) and Chaitén (Futaleufú) remained in separate ice-free regions during glaciations. These results are similar to those found by Vidal *et al.* (2012a,b) for *L. pictus*, given that the evolutionary history of the species is evidently the same. Although the results are similar, it is important to note that the sequences used in this study are longer (additional 395 bp), and fewer localities were studied, which should be consistent with the geographical distribution of *B. leptopus*. Our data suggest at least two sources: northwest Chiloé Island and a source probably
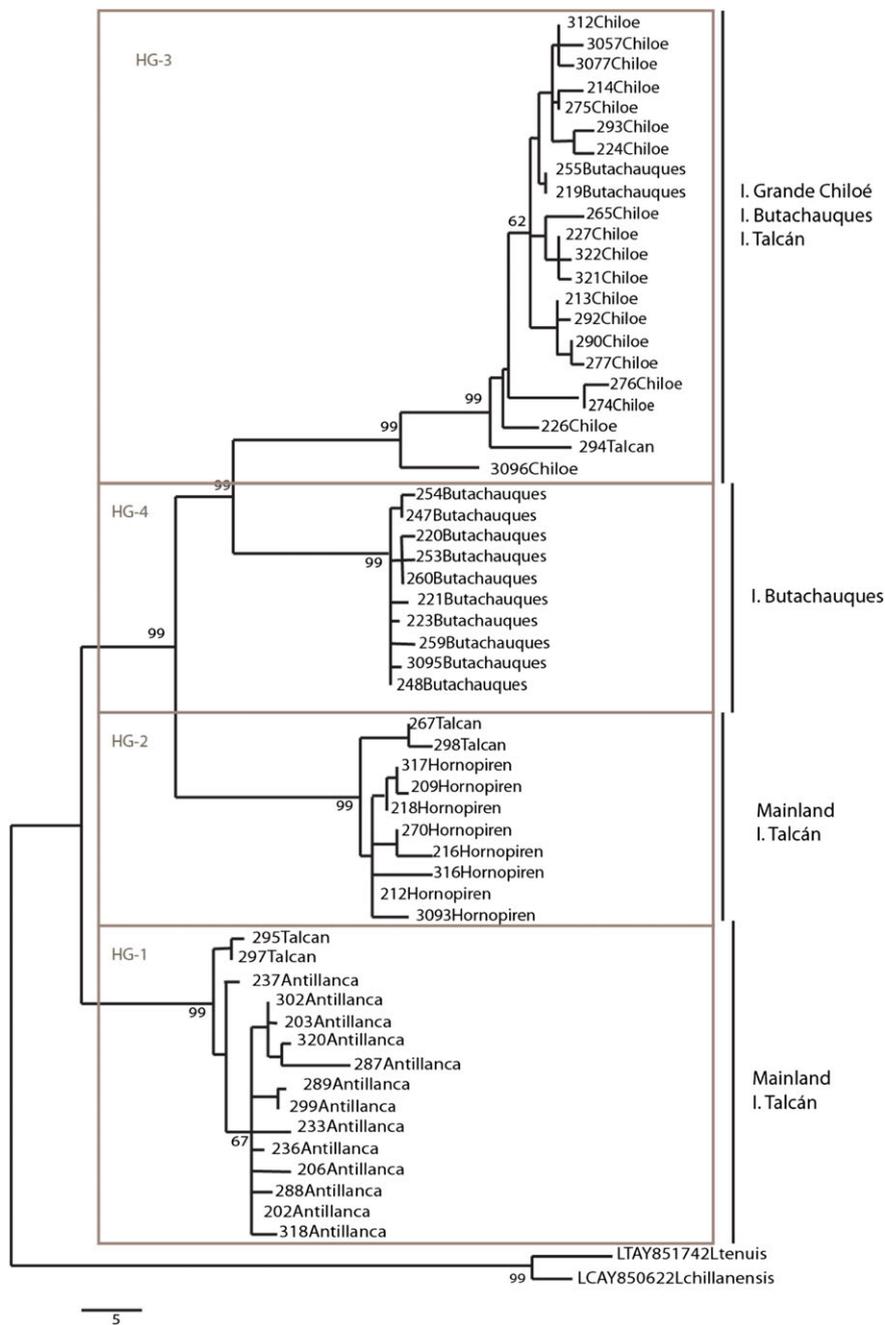
Fig. 3.   Bayesian inference for the *Liolaemus pictus* lizard obtained from the cytochrome b gene. Numbers on branches indicate support based on subsequent probabilities for Bayesian analyses.

located in sectors beyond the ice margin east of the Andes in Argentina (colonization from Chaitén (Futaleufú) to IT). Nevertheless, additional sampling is needed from eastern Andean sectors. As previously described for the lizard *L. pictus* (Vidal *et al.* 2012b), we propose northwest Chiloé Island as a true refuge, suggesting that populations of both species could have remained isolated over repeated glacial and interglacial phases. We calculated a divergence time of approx. 1.1 million year BP between

populations from Chiloé Island and the mainland based on a mutation rate of 0.8% for Cyt b in *Batrachyla* (Mueller, 2006). Our results suggest that an ancient lineage split migrated to Chiloé Island during the waxing and waning of mid-Pleistocene glaciations. Similar results were found in *L. pictus* by Vidal *et al.* (2012b); this suggests that the same historical event would have generated similar demographic effects causing similar evolutionary trajectories. Our results from Talcán Island and
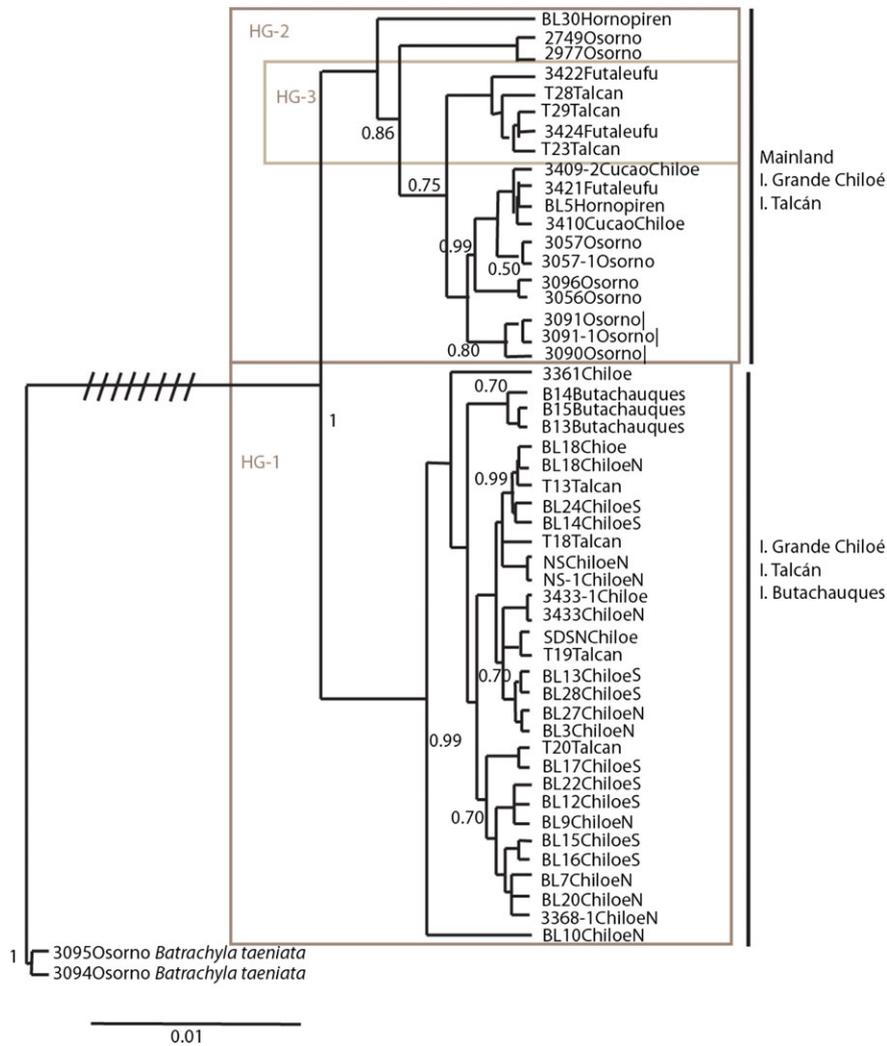
Fig. 4. Bayesian inference for the *Batrachyla leptopus* frog obtained from cytochrome b gene. Numbers on branches indicate support based on subsequent probabilities for Bayesian analyses.

Butachauques Island suggest that these islands were colonized from mainland sources as well as from CI. Data from Talcán Island suggest the intermingling of two highly divergent and geographically contiguous 'mainland lineages' for the lizard, that is, Antillanca in the north and Chaitén–Hornopirén in the south. For the frogs, highly divergent haplogroups were Chaitén in the south and Chiloé Island in the archipelago.

Although these lineages do not share haplotypes in *L. pictus*, both haplotypes are present on the island in both species. One explanation for the presence of both lineages on the island could be due to two successive colonization phases associated with shifts in their distribution range. Vidal *et al.* (2012a) suggested that the geographical and temporal discontinuity in *L. pictus* could have resulted from climatic/biogeographical shifts as well as volcanic disturbance of the land biota during the Holocene. There is supporting evidence

from paleoclimate studies that document a southward shift in the distribution of thermophilous Valdivian trees (e.g. *Eucryphia cordifolia*) and intense fire activity during a multi-millennial warm–dry interval, prominent ice recession to the Andean headwaters, lake level lowering and weakening of the westerly winds in the early Holocene (Moreno *et al.* 2010). Specimens collected on Butachauques Island are genetically related to those inhabiting the adjacent northern areas of the mainland in *L. pictus* (Vidal *et al.* 2012a) and to a lesser extent to those on Chiloé Island in *B. leptopus*. These results suggest a simple colonization process from the island's neighbouring land areas exposed before deglaciation.

The results of this study are consistent with our hypothesis that the *Liolaemus* and *Batrachyla* species have responded in similar ways to glacial and eustatic forcing over the last glacial cycles. The patterns observed in

L. pictus and *B. leptopus* 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 the phylogeographic signal without a corresponding break in geography (Irwin 2002), and previously suggested for *L. pictus* and *L. cyanogaster* in the same area (Vidal *et al.* 2012b). It is important to note that the objective of comparative phylogeography is to describe evolutionary divergence patterns in co-distributed populations of different taxa (Bermingham & Moritz 1998; Avise 2000); if the populations of these taxa have been co-distributed for a prolonged time and have been more or less stable in the periods between the processes of perturbation or barrier generation, it is reasonable to expect their divergence patterns to be congruent (Lourie *et al.* 2005). This is important when comparing co-distributed species that show similar reproductive and evolutionary rates and vagility (Vidal *et al.* 2012b); however, in this case, *Liolaemus* lizards and *Batrachyla* frogs differ in their biology, so that congruence in our results is highly relevant.

There has been much discussion on the use of mitochondrial DNA to describe the population history of species, because it would be unrepresentative of the true histories of natural populations (Edwards & Bensch 2009). We worked under the assumption that both species are defined and that gene trees are closely related to their evolutionary history. According to Vidal *et al.* (2012b), other species belonging to *Eupsophus* (Núñez *et al.* 2011), *Pristidactylus* and other species are co-distributed with *Liolaemus* and *Batrachyla* in the same archipelago; an examination of variation at a number of nuclear loci is required to determine if our results are an accurate representation of the evolutionary history of these species, so future studies of this co-distribution could help to understand the evolutionary processes on the community level and the mechanisms of differentiation in south-central Chile.

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