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Genetic divergence of Chilean long-tailed snake (*Philodryas chamissonis*) across latitudes: conservation threats for different lineages

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ABSTRACT

Aim The Chilean long-tailed snake (*Philodryas chamissonis*) has a wide distribution across different latitudes and ecosystems in Chile ranging from the south of the Atacama Desert (26° S) to the extremely humid Valdivian temperate rain forest (40° S). Throughout this vast distribution, which is isolated by the Andes mountain range on the east and the Pacific Ocean on the west, there are biogeographical boundaries and large geographical barriers that must have played an important role in the distribution of genetic diversity within this species. This study aimed at elucidating the evolutionary history of *P. chamissonis* in Chile by analysis of mitochondrial DNA sequences.

Location Chile (29°41′–38°23′ S).

Methods We extracted DNA from 66 tissue samples collected across different latitudes and amplified and sequenced the mitochondrial DNA control region and the NADH dehydrogenase subunit 4 gene for phylogenetic and population analysis.

Results Four distinct haplogroups were identified for *P. chamissonis*. These are highly consistent with a latitudinal geographic pattern, different ecosystems and the increase in topography towards central Chile. Three of the four haplogroups are concentrated in central Chile (33° S latitude) where the highest herpetofaunal diversity of the country is found. The Maipo River acts as historical geographical barrier for the species influenced by Pleistocene glaciation cycles, leading to a marked phylogeographical boundary. A strong population structure was found for the species ($\Phi_{st} = 0.78$, $P < 0.0001$), with a high haplotype diversity ($h = 0.97 \pm 0.01$) and nucleotide diversity ($\pi = 0.0151 \pm 0.0077$).

Main conclusions At least three evolutionarily significant units (ESUs) were designated for the species, and these should be taken into account for conservation plans. Three of the four haplogroups found within *P. chamissonis* are already threatened because their distribution along central Chile overlaps with most of the largest cities in the country.

Keywords

Conservation, evolutionarily significant unit (ESU), geographical barrier, *Philodryas chamissonis*, phylogeography, snake.

INTRODUCTION

The fauna of Chile has been isolated for millions of years by the Andes Mountains in the east, the Pacific Ocean in the west, the Atacama Desert in the north and the Drake Passage in the south (Donoso-Barros, 1966). Because of this, Chile has a small diversity of reptiles compared with other countries with similar climates (Greene & Jaksic, 1992), but high species endemism in multiple groups (Smith-Ramírez, 2004). Although a large variety of habitats in Chile are sufficiently rich to harbour a

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great number of snakes, only seven species of snakes are present in the country, among them six terrestrial species (Vidal *et al.*, 2008) and a marine elapid species that lives in the Pacific Ocean around Easter Island. The rest of the species belongs to the Dipsadidae, subfamily Xenodontinae (Zaher *et al.*, 2009). Between two and six xenodontine species currently allocated in the genera *Philodryas* (Wagler, 1830) and *Tachymenis* (Wiegmann, 1836) have been recognized in Chile by different authors (Gigoux, 1940; Donoso-Barros, 1966; Donoso Barros, 1970; Thomas, 1976; Veloso & Navarro, 1988; Nuñez & Jaksic, 1992; Vidal *et al.*, 2008).

The genus *Philodryas* is endemic to South America and is distributed from 8° N to 42° S in the complete width of the South American continent (Thomas, 1976) with a total of 15–22 species (Zaher *et al.*, 2008). The most widely distributed and best known species of terrestrial snake in Chile is the long-tailed snake, *Philodryas chamissonis* (Wiegmann, 1835). Its distribution extends from the city of Copiapo (26° S) south to Valdivia (40° S) (Donoso-Barros, 1966) and from sea level to 2300 m altitude (Mella, 2005). Except for an isolated and questionable record by Thomas (1976) for Uspallata, Mendoza Province, all records of *P. chamissonis* have been on Chilean territory, thus rendering this species endemic. The Chilean long-tailed snake can be identified by its characteristic colour pattern, having a central brown dorsal stripe limited by two white stripes that run down the length of the snake (Donoso-Barros, 1962). This species is also usually recognized by its medium size of up to approximately 220 cm in total length (Greene & Jaksic, 1992), and more specifically by the length of its tail, which is one-third of its body length (Mella, 2005). Cytologically, *P. chamissonis* has a $2n = 36$ karyotype with 16 macrochromosomes and 20 microchromosomes, which is relatively common in most 'colubrid' snake species (Moreno *et al.*, 1987).

Because of its extensive distribution, *P. chamissonis* inhabits a very diverse set of habitats from the southern parts of the driest desert of the world to the very cold and humid Valdivian temperate rain forest (Donoso-Barros, 1966). Consequently, *P. chamissonis* is found across most of the six areas of endemism that have been reported for the Chilean herpetofauna and across one biogeographical break around 30° S (Vidal *et al.*, 2009). Moreover, its distribution encompasses ten ecological regions in three major ecological zones, i.e. Desert, Mediterranean and Oceanic (Veloso & Navarro, 1988). As *P. chamissonis* has such an extensive latitudinal distribution, environmental gradients might generate clinal genetic variation; however, geographical barriers would be expected to generate abrupt genetic divergences (Avise, 2000). The heterogeneity of the habitats found in Chile, with distinct latitudinal and altitudinal ecological and climatic gradients, entails that this snake is adapted to a variety of different habitats.

Given the narrow Chilean continental shelf with an average width of 180 km and its extension across different latitudes, there are many large rivers that cross the complete width of the country (e.g., Aconcagua River, Bio-Bio River, Maipo River). Historically, the water volume of most of the central and southern Chilean rivers was influenced by Pleistocene climatic changes. Throughout the last glacial maximum (LGM, 23,000–17,000 years ago), an ice sheet covered southern Chile from 56° to 35° S in the Andes Mountains and 42° S at the coastal lowlands (Clapperton, 1993; McCulloch *et al.*, 2000). As the rivers mostly originate at 3000 m a.s.l. and depend on deglaciation, water volumes were historically significantly larger than today, forming a potential barrier to species dispersal (Lamborot & Eaton, 1997). Quaternary glaciation periods led to the reduction in many species distributions to lower latitudes followed by post-glacial expansion leaving a signature of reduced genetic diversity (Hewitt, 2000; Provan & Bennett, 2008). Thus, colder temperatures during this time period in the southern area of the country could have had a deep impact on populations of ecothermic species such as *P. chamissonis*. Furthermore, Chile is characterized by its extreme topographic relief which comprises not only the Andes in the east but also a large coastal mountain range extending from the north to Chiloe. The topography also changes latitudinally, increasing towards north and central Chile compared with the southern regions of the country. As *P. chamissonis* only reaches up to 2000 m a.s.l. under current climates (Veloso & Navarro, 1988), a large mountain range could limit the exchange of individuals between populations.

Here, we document the phylogeographical pattern of *P. chamissonis* across its distribution based on sequence analyses of mitochondrial control region (CR) and NADH dehydrogenase subunit 4 gene (ND4). In our study, we focused on four main questions: Is there concordance between the geographical distribution of *P. chamissonis* lineages and the different Chilean ecoregions? What was the effect of Pleistocene glaciations on *P. chamissonis* populations? Is there an effect of topography and hydromorphology on *P. chamissonis* populations? Is there concordance between the known herpetofaunal biogeographical breaks and the distribution of *P. chamissonis* lineages?

METHODS

Sample collection

A total of 66 samples were collected over 3 years (2006–2009) from carcasses found on highways and rural roads, or in the form of blood or tail-tip samples from live individuals obtained during fieldwork. Sampling covered most of the known distribution of the species (29°41′–38°23′ S latitude; Table 1; Fig. 1). All samples were preserved in absolute ethanol. All relevant data for each sample were recorded, such as geographic location, morphological characteristics, sex and date on which the sample was collected. A blood sample from *Philodryas trilineat*a (Burmeister, 1861) was obtained from Buin Zoo in Chile for use as an outgroup.

PCR and sequencing

DNA was extracted using the DNeasy blood and tissue kit (Qiagen Inc., Valencia, CA, USA), and mitochondrial DNA

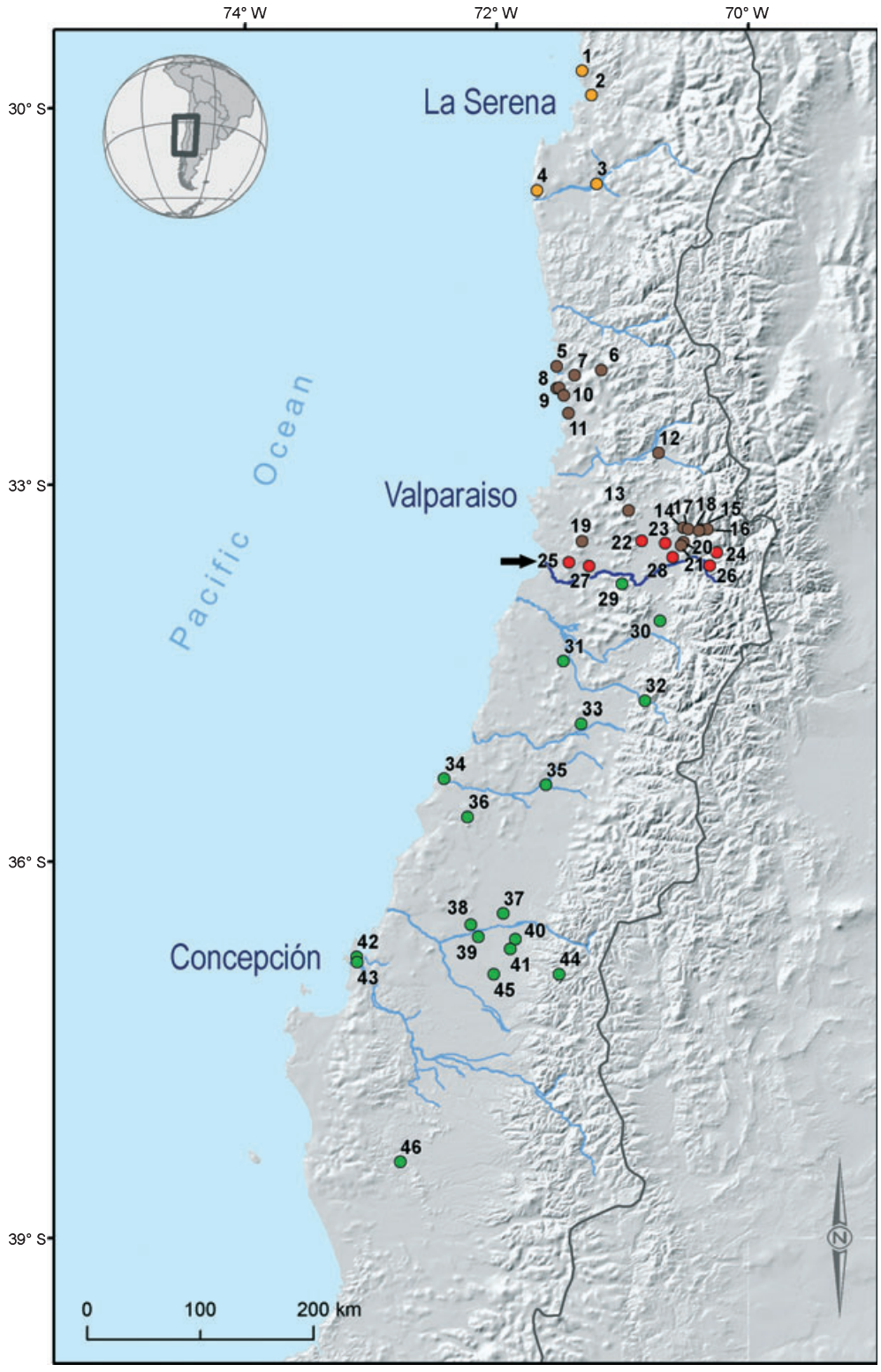

Figure 1 Map of Chile with the distribution of the haplotypes found (haplogroups represented by different colours), and the main cities are indicated (La Serena, Valparaiso and Concepción) and the arrow indicates the Maipo River.

control region (CR) sequences of 751 bp were amplified from all 66 individuals by polymerase chain reaction (PCR), using the primers L16577 (Burbrink *et al.*, 2000) and H690 (Kumazawa *et al.*, 1996). NADH dehydrogenase subunit 4 (ND4) sequences of 567 bp were amplified and sequenced for 17 individuals using the primers DW1641 and DW1642 (Janzen *et al.*, 2002). PCR amplification for both mtDNA regions comprised an initial denaturing step at 95 °C for 10′, followed by a touchdown

Table 1 All localities grouped by phylogenetic analysis according to the geographical location, followed by site number (no) (Fig. 1), geographical location, sample size, control region (CR) and ND4 haplotypes.

Site no.	Locality name	Latitude	Longitude	Sample size	CR haplotype	GenBank accession No.	ND4 haplotype	GenBank accession No.
1	Caleta Arrayán	29°41′ S	71°19′W	1	PCH01	HM639914		
2	La Serena	29°53′ S	71°14′W	1	PCH02	HM639915	NPC01	HM639951
3	Ovalle	30°36′ S	71°12′W	1	PCH03	HM639916	NPC02	HM639952
4	Fray Jorge	30°39′ S	71°40′W	1	PCH04	HM639917		
5	Palo Colorado	32°03′ S	71°31′W	2	PCH05	HM639918		
6	Tilama	32°05′ S	71°10′W	1	PCH06	HM639919		
7	Gualguali	32°07′ S	71°22′W	1	PCH06	HM639919		
8	Puquen	32°13′ S	71°31′W	1	PCH07	HM639920		
9	Los Molles	32°13′ S	71°30′W	1	PCH08	HM639921		
10	La Ballena	32°17′ S	71°27′W	1	PCH09	HM639922		
11	Caleta La Ligua	32°25′ S	71°25′W	1	PCH10	HM639923	NPC03	HM639953
12	San Felipe	32°44′ S	70°42′W	1	PCH09	HM639922		
13	Chicauma	33°12′ S	70°57′W	1	PCH05	HM639918	NPC03	HM639953
14	La Dehesa	33°20′ S	70°30′W	1	PCH11	HM639924		
15	Villa Paulina	33°21′ S	70°22′W	1	PCH12	HM639925		
16	Farellones	33°21′ S	70°19′W	3	PCH12	HM639925	NPC04	HM639954
17	Arrayan	33°21′ S	70°28′W	1	PCH12	HM639925	NPC04	HM639954
18	La Hermita	33°22′ S	70°23′W	2	PCH12, 13	HM639925–HM639926		
19	Cuesta Ibacache	33°27′ S	71°19′W	1	PCH06	HM639919		
20	Mahuida	33°27′ S	70°30′W	1	PCH11	HM639924		
21	Peñalolén	33°29′ S	70°31′W	1	PCH14	HM639927		
22	Pudahuel	33°26′ S	70°50′W	1	PCH15	HM639928	NPC05	HM639955
23	Parque O'Higgins	33°27′ S	70°39′W	1	PCH16	HM639929	NPC06	HM639956
24	Los Maitenes	33°32′ S	70°15′W	1	PCH17	HM639930	NPC07	HM639957
25	Cuncumén	33°37′ S	71°25′W	1	PCH15	HM639928	NPC05	HM639955
26	Camino Lagunillas	33°38′ S	70°18′W	1	PCH18	HM639931		
27	Laguna Esmeralda	33°38′ S	71°15′W	3	PCH15, 16, 19	HM639928-29, HM639932	NPC06	HM639956
28	Puente Alto	33°38′ S	70°36′W	1	PCH16	HM639929		
29	NW Laguna Aculeo	33°47′ S	71°00′W	1	PCH20	HM639933		
30	Rancagua	34°05′ S	70°42′W	1	PCH21	HM639934		
31	Las Cabras	34°24′ S	71°28′W	1	PCH22	HM639935	NPC08	HM639958
32	Rio Tinguiririca	34°43′ S	70°49′W	1	PCH23	HM639936		
33	Curicó	34°54′ S	71°19′W	2	PCH24, 25	HM639937, HM639938		
34	Constitución	35°20′ S	72°25′W	1	PCH26	HM639939		
35	Talca	35°23′ S	71°36′W	2	PCH27, 28	HM639940, HM639941		
36	Comuna Empedrado	35°38′ S	72°13′W	1	PCH29	HM639942		
37	San Carlos	36°24′ S	71°56′W	1	PCH30	HM639943		
38	San Nicolás	36°30′ S	72°12′W	1	PCH31	HM639944		
39	Chillán	36°36′ S	72°04′W	11	PCH30–35	HM639943–HM639948	NPC09 to 11	HM639959-HM639961
40	Coihueco	36°37′ S	71°50′W	3	PCH35	HM639948		
41	Pinto	36°41′ S	71°53′W	1	PCH32	HM639945	NPC10	HM639960
42	Portezuelo	36°45′ S	73°06′W	1	PCH36	HM639949		
43	Tanilvoro	36°48′ S	73°06′W	1	PCH30	HM639943		
44	Las Trancas	36°53′ S	71°30′W	1	PCH30	HM639943		
45	El Carmen	36°53′ S	72°01′W	1	PCH37	HM639950		
46	Galvarino	38°23′ S	72°45′W	1	PCH36	HM639949		

sequence at 95 °C for 15″, 60–50 °C for 30″, 72 °C for 45″, with two cycles at each annealing temperature, and 35 amplification cycles at 95 °C for 15″, 50 °C for 30″, 72 °C for 45″, followed by a final extension step of 30′ at 72 °C. PCRs were performed in a total volume of 35 μl containing 2 μl of DNA, 1× reaction buffer, 1.4 mM of $MgCl_2$, 200 μM of each dNTP, 0.4 μM of each primer and 0.8 units of Taq DNA polymerase Platinum (Invitrogen®, Sao Paulo, SP, Brazil).

PCR products were visualized by ethidium bromide in a 0.8% agarose gel and were purified and sequenced by

Macrogen, Korea. The sequences of all *P. chamissonis* mtDNA haplotypes were deposited in GenBank (CR: accession numbers HM639914–HM639950; ND4: accession numbers HM639951–HM639961) along with those for *P. trilineata* (CR: HM639962; ND4: HM639963).

Population analysis

Forward and reverse sequences were aligned with Proseq v. 2.91 (Filatov, 2002). Polymorphic sites and haplotypes were identified by eye using CLUSTAL X v.1.81 (Thompson *et al.*, 1997). Genetic diversity was evaluated by calculating nucleotidic diversity (π), haplotype diversity (h) and the number of substitutions (D_A) using the program ARLEQUIN v.3.11 (Excoffier *et al.*, 2005). Genetic differences between populations and groups were examined by analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) using Arlequin. To detect signatures of population expansions, we calculated Tajima's D neutrality statistic (Tajima, 1989) and Fu's Fs value (Fu, 1997) for each phylogenetic clade. Mismatch distributions were calculated using Arlequin with 1000 bootstrap replicates. Correlations between geographical and genetic distance of 26 groups were assessed using the Mantel test (Mantel, 1967) implemented with Arlequin.

Phylogenetic analysis

To study relationships between different CR haplotypes, we used the median joining network (MJN) created by NETWORK v.4 (Bandelt *et al.*, 1999). *Philodryas trilineata* was chosen as an outgroup for CR and ND4 phylogenetic reconstruction. The appropriate model of substitution was selected using Akaike information criterion (AIC) in the program MODELTEST version 3.06 (Posada & Crandall, 1998) implemented by PAUP v4.10 (Swofford, 2000). The model selected for CR was TVM+I+G, while the model selected for ND4 was TrN+I. Bayesian analysis (BA) was performed using MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001) using the general type of the best fit model parameters defined for the data set. Four independent Markov chains, each starting with random trees for each of four simultaneous chains, were run for five million generations. To create a consensus tree with the highest levels of phylogenetic structure, we used FIGTREE v.1.2.2 (Rambaut, 2009). Divergence between clades was calculated using MEGA v.2.1 (Kumar *et al.*, 2004).

RESULTS

Phylogenetic and phylogeographical analysis

A total of 751 bp of CR revealed 37 haplotypes in the 66 individual snakes, defined by 44 polymorphic sites including 7 indels, 28 transitions and 12 transversions. Median joining network (MJN) revealed four distinctive clusters corresponding to different geographic locations: (i) North (29°41′–30°39′ S); (ii) Central-North (32°04′–33°29′ S); (iii) Central (33°26′–33°38′ S); (iv) South (33°47′–38°23′ S). No haplotypes were shared among the highly divergent groups. The South group was separated from the Central group by seven mutational steps, while the largest separation was between the Central group and the Central-North group with nine mutational steps. Moreover, the North group was separated from the Central-North group by eight mutational steps (Fig. 2).

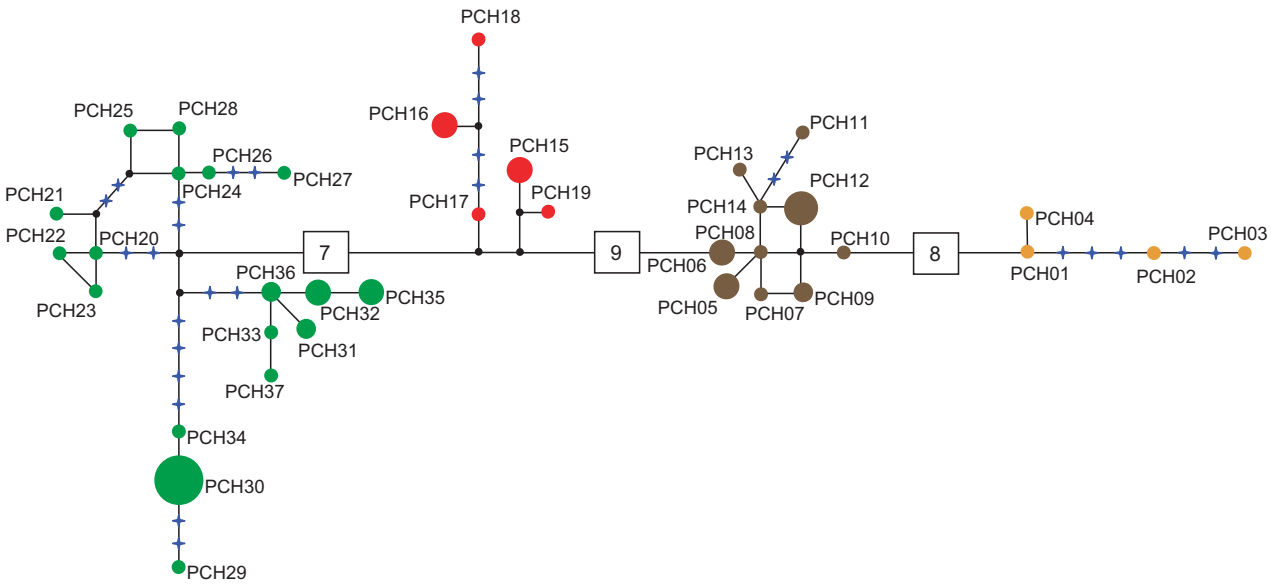

Figure 2 Median joining network (MJN) of *Philodryas chamissonis* for control region sequences. South clade is represented by green colour, Central in red, Central-North in brown and North in orange. Two to four mutation positions are marked with blue crosses. More than four mutation sites are indicated with a square with the corresponding number of mutated positions.

BA phylogenetic trees for CR encompass four major distinctive haplogroups; however, the Central-North haplotypes form a basal polytomy (Fig. 3). Four subclades are described along the South subclade and are highly consistent with the latitudinal geographic distribution of *P. chamissonis*. Higher divergence values were found between the South and North clades (1.9%), and between the South and Central-North clades (1.9%) followed by both the Central and North clades and the Central and Central-North clades (1.6%; Table 2). Finally, the least divergent clades are North and Central-North and the Central and South clades, both with 1.4% divergence. These divergence values are inferior compared with the *P. chamissonis* and *P. trilineata* divergence value of 9.8%. Moreover, the divergence values within each of these four clades were between 0.3% and 0.6%. A similar phylogenetic tree topology was obtained in analyses of 567 bp of a more conserved mtDNA gene, ND4, in which 11 haplotypes among 17 snakes were defined by 20 polymorphic sites (Fig. 4). ND4 phylogenetic reconstruction yielded the same four haplogroups as the analyses of CR.

High haplotype diversity ($h = 0.97 \pm 0.01$) and nucleotide diversity ($\pi = 0.0151 \pm 0.0077$) were found for the species. Haplotypic diversity for each of the four haplogroups was high ranging between 0.83 and 1; while nucleotide diversity ranged between 0.0030 and 0.0077 (Table 3). Using AMOVA, a high population structure was found considering 26 populations and four groups ($\Phi_{st} = 0.78, P < 0.0001$) as seen with MJN. Pairwise F_{st} among the four groups was high, varying between 0.59, $P < 0.0001$ (Central and South) and 0.80, $P < 0.0001$ (Central-North and Central). A pattern of isolation by distance (IBD) was found including all locations ($R^2 = 0.23, P < 0.0001$). However, higher correlation was found within the South group ($R^2 = 0.42, P < 0.013$) possibly indicating the reduced presence of a barrier within groups compared with across locations. The remaining groups did not show a significant correlation.

No evidence of recent population expansion was found for *P. chamissonis* using Tajima's *D* neutrality statistic ($D = 0.81$, $P = 0.82$); however, a significant Fu value ($Fs = -9.57$; $P = 0.02$) was observed. When the samples were separated into four groups, no significant Tajima values were observed (Table 3). However, the Fu value was significant for both Central-North and South clades showing a negative value which can indicate recent population expansion. Mismatch distribution was multimodal; however, three of the four separate groups were unimodal without the presence of a signature of recent expansion for the North, Central-North and South groups. The Central group was the only one that had a multimodal distribution.

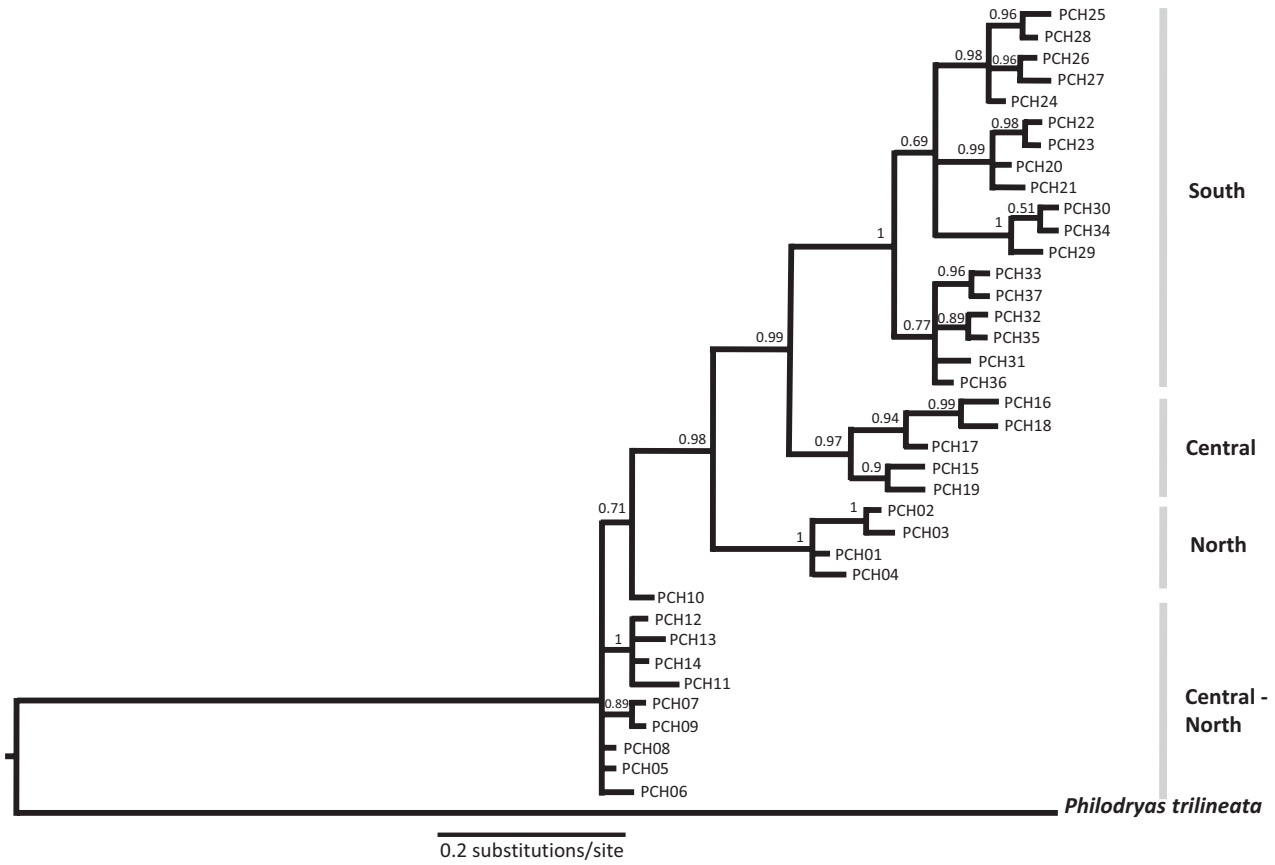

Figure 3 Bayesian phylogenetic reconstruction from the mtDNA control region for *Philodryas chamissonis*. Posterior support value is shown for nodes.

Table 2 Divergence between *Philodryas chamissonis* clades, within clades and outgroup *P. trilineata* obtained from CR markers (lower diagonal) and ND4 marker (upper diagonal).

	P. chamissonis					
	North	Central-North	Central	South	*P. trilineata*	Within Group
P. chamissonis						
North	–	0.010	0.019	0.016	0.098	0.002
Central-North	0.014	–	0.015	0.013	0.099	0.004
Central	0.016	0.016	–	0.011	0.113	0.005
South	0.019	0.019	0.014	–	0.108	0.005
P. trilineata	0.096	0.094	0.096	0.101	–	X
Within Group	0.003	0.003	0.005	0.006	X	–

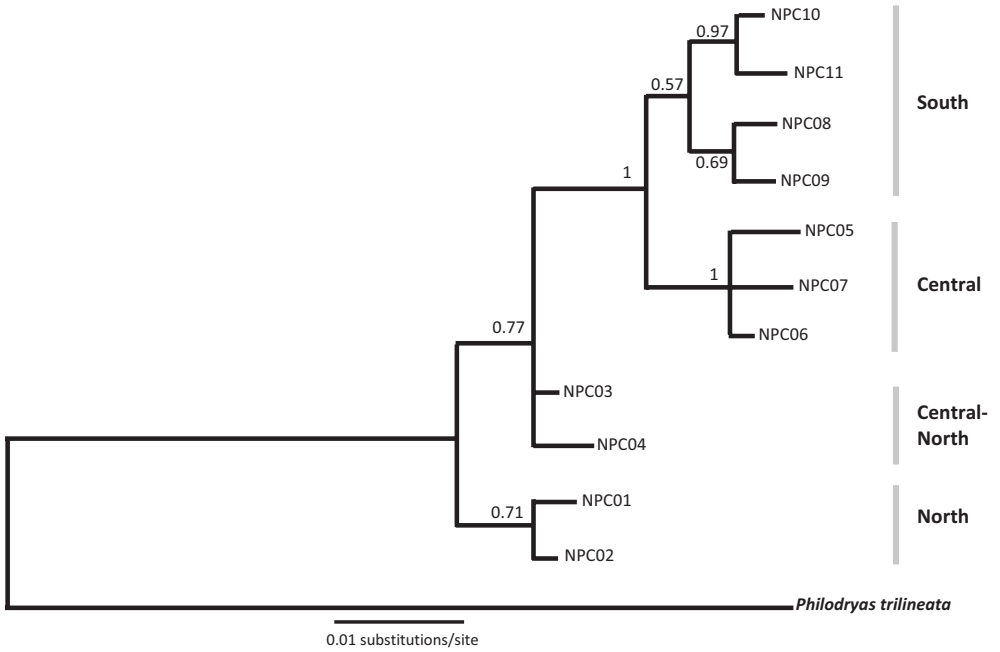

Figure 4 Bayesian phylogenetic reconstruction from the mtDNA ND4 for *Philodryas chamissonis*. Posterior support value is shown for nodes.

Table 3 Defined groups followed by sample size (*N*), number of haplotypes (Hap), polymorphic sites (*S*), haplotype (*h*) and nucleotide (π) diversity, Tajimas's *D* and Fu's *F*s neutrality test, and Mismatch distribution demographic (SSD) and spatial (SSD*).

				Genetic Diversity				Mismatch distribution	
Clade	*N*	Hap	*S*	*h*	π	Tajima's *D*	Fu's *F*s	pSSD	pSSD*
North	4	4	7	1 ± 0.18	0.0051 ± 0.0038	1.09; 0.86	−0.82; 0.15	0.31	0.29
Central-North	21	10	8	0.89 ± 0.05	0.0030 ± 0.0019	−0.20; 0.47	−3.65; 0.03*	0.008	0.005
Central	9	5	8	0.83 ± 0.10	0.0047 ± 0.0030	1.60; 0.97	−0.53; 0.61	0.42	0.85
South	32	18	19	0.91 ± 0.04	0.0077 ± 0.0042	0.56; 0.75	−4.69; 0.04*	0.008	0.07
Total	66	37	45	0.97 ± 0.01	0.0151 ± 0.0077	0.81; 0.82	−9.57; 0.02*	0.30	0.05

DISCUSSION

Based on morphological data, Thomas (1976) describes the centre of origin for the genus *Philodryas* on the eastern slope of the central Andes. The author states that the members of the Tachymenoides complex (*P. chamissonis*, *P. tachymenoides* and *P. simonsi*) arrived on the west coast in southern Peru. During the Miocene, the Andes in this region had lower altitudes than

the present; therefore, since the Miocene the Andes uplift has been on the order of 2300–3500 m (Gregory-Wodzicki, 2000). Today, the lowest passes in this region rise up to 4600 m and act as a barrier for these species (Thomas, 1976). The formation of Atacama Desert was responsible for the isolation of *Philodryas* spp. on the south-western Andes from the other members of the genus spurring allopatric speciation of *P. chamissonis* (Thomas, 1976). The colonization of the species in the Chilean territory occurred from north to south as also suggested by our data. Four divergent groups (North, Central-North, Central and South) were supported by the MJN of the CR sequences for *P. chamissonis* along most of its geographical range (29°41′–38°23′ S). Moreover, the phylogenetic reconstruction (CR and ND4 gene; Figs 3 and 4) for *P. chamissonis* suggests a basal position of northern haplogroups related to southern haplogroups. These are consistent with the latitudinal geographical distribution of the haplotypes and the species evolutionary history.

The distribution of *P. chamissonis* encompasses most of the extreme and diverse ecosystems found in Chile, from the dry Atacama Desert to the Valdivian temperate rain forest. On ectothermal species, this large climatic range, in addition to the different ecosystems, can impose strong selective forces driving intraspecific divergence and contributing to restricted gene flow across different environments. Indeed, the different ecosystems across the distribution of *P. chamissonis* are highly consistent with the geographical distribution of haplogroups described in our study. The North and Central-North haplogroups belong to one large ecological region according to Di Castri (1968); this so-called Mediterranean region possesses a large variety of habitats that are mainly characterized by sclerophyllic bushy vegetation with dry xerophytic thorn shrubs (Di Castri, 1968; Torres-Pérez *et al.*, 2007). However, the North haplogroup (29°41′–30°39′ S latitude) is mostly characterized by a desert and semi-desert habitat (Villagrán & Hinojosa, 2005). The Central and South haplogroups belong to two ecological regions, the Mediterranean region and the Oceanic region that comprises the Valdivian temperate rain forest (Di Castri, 1968). Therefore, haplotypes from the South haplogroup represent the largest distribution of *P. chamissonis* and occupy the most diverse and heterogeneous habitat compared with more northern populations. Furthermore, with an annual mean of 13 °C the temperature in the southernmost area of the South haplogroup is very low for an ectothermal species. Although the latitudinal distribution across different ecosystems plays an important role in the divergence of *P. chamissonis*, boundaries between haplogroups were, in some cases, highly marked and limited by a few kilometres only, as discussed later.

North and Central-North phylogeographical boundaries (30° S latitude)

Along the distribution of *P. chamissonis*, a boundary between areas of herpetofaunal endemism around 30° S has been described (Vidal *et al.*, 2009). This biogeographical boundary is consistent with the divergence of the North and Central-North haplogroups of *P. chamissonis*. The North haplogroup (supported by MJN and BA) ranges between 29°41′ and 30°39′ S, while the Central-North clade ranges between 32°04′ and 33°29′ S. Moreover, the 30° S latitude is also a marked biogeographical limit for terrestrial vegetation characterized by a clear ecosystem change from hyperarid deserts and semi-deserts in the north to the Mediterranean region which ranges from 30° to 38° S latitude (Villagrán & Hinojosa, 2005). South of this biogeographical boundary, the southern cone of South America is marked by a climatic and vegetation contrast on both sides of the Andes, with a continuous strip of arid climate crossing the continent from NW towards SE, known as *Arid Diagonal of South America* (Villagrán & Hijojosa, 1997).

Central-North and Central phylogeographical boundaries (33° S latitude)

Along the central region of Chile, we can find at least three divergent haplogroups within the 33° S latitude. The high diversity of *P. chamissonis* lineages found in central Chile is congruent with the overall highest values of herpetofaunal richness and endemism from 31° to 44° S (Vidal *et al.*, 2009). The Central group haplotypes have a restricted geographical distribution compared with the other haplogroups. Nevertheless, the Central group had a large amount of genetic diversity and a multimodal mismatch distribution emphasizing the heterogeneity of this region. Similar results were described in a comparative phylogeography study of three Chilean lizards, revealing deep phylogeographical structure of *Liolaemus tenuis* and *L. lemniscatus* within the mesomorphic zone in central Chile relative to more southern parts of their ranges (Victoriano *et al.*, 2008). According to these authors, topography effects increase towards the north of the species' distribution where the altitude of the Andes increases. The phylogeographic boundary between Central-North and Central haplogroups of *P. chamissonis* could be influenced in a similar manner by the geological topology of the region. On the other hand, the Central haplogroup is limited in the south by the Maipo River as further discussed.

Central and South phylogeographical boundaries (33° S latitude)

The southern clade is a consistent haplogroup with a wide distribution from the southern area of the Maipo River (33°47′ S) to the southern limit of the species' distribution (38°23′ S), spanning a distance of 534 km. A variety of different habitats is found along this area, changing from a Mediterranean ecosystem in central Chile to the Valdivian temperate rain forest (Di Castri, 1968). The Maipo River is the main geographical barrier isolating the southern haplogroup from the more northern haplogroup. Although ice cover during the LGM (18,000–20,000 ya) reached up to 43° S in the coastal region and 35° S in the Andes (Clapperton, 1993;

McCulloch *et al.*, 2000), the influence of the ice sheets towards the northern habitats extended approximately to the latitude of Santiago (33° S). Consequently, the water volume originating from the Andes was significantly higher than today. During LGM, the glacier of Maipo Valley was particularly well developed, reaching the central Valley in the southern area of Santiago (Lamborot & Eaton, 1997). Thus, allopatric distribution seems to account for genetic divergence between geographically isolated haplogroups on both sides of the Maipo River, where no haplotype is shared to this date. Similar results of isolation were described for an endemic Chilean lizard, *Liolaemus monticola*, where the Maipo River acts as a barrier separating two chromosomal races (Lamborot & Eaton, 1997; Lamborot *et al.*, 2003), and phylogeographic analysis based on cytochrome b sequences revealed two reciprocally monophyletic clades north and south of the Maipo River (Torres-Pérez *et al.*, 2007). Although the Aconcagua River also represents a more recent barrier to gene flow for *L. monticola* leading to different clades (Torres-Pérez *et al.*, 2007), no genetic divergence in this area was observed for *P. chamissonis*. Rivers acting as geographical barriers for gene flow in reptile species have been described in other phylogeographical studies. For example, two clades of the American rat snake (*Pantherophis obsoletus* complex) are separated by the Mississippi River that also separates various populations of fish, amphibians and other reptiles in the USA (Burbrink *et al.*, 2000). Grazziotin *et al.* (2006) found a similar case in the South American pitviper *Bothrops jararaca* complex, but in this case the authors believed that it was very difficult to assess the historical importance of the Paranapanema River as a barrier for the species, even though the putative genetic barrier between both clusters corresponded to the present course of the River.

Although the lowlands of Chile and the majority of *P. chamissonis*' distribution were not covered by ice sheet during LGM, colder temperatures in the southern area could have had a deep impact on populations. Glaciations apparently influenced the southern distribution of the Chilean lizards *L. tenuis* and *L. pictus,* leading to a reduced genetic diversity because of founder effects (Victoriano *et al.*, 2008). In the case of *P. chamissonis*, the *Fs* value was significant for the southern haplogroup, suggesting an evidence of recent population expansion. In accordance with these data, populations on the northern distribution south of the Maipo River could have expanded as suitable habitat spread further south when temperatures increased during the Holocene. On the other hand, colder temperatures in the southern area of the country during the LGM were not sufficient to cause a reduction in genetic diversity in *P. chamissonis* towards the south, which can be also evidenced by the MJN which differs from a star-like topology found in cases of recent expansion, and a lack of signature observed by the mismatch distribution and the Tajima. At present, *P. chamissonis* is also distributed along low-temperature environments such as high altitudes of 2364 m a.s.l. where a few samples were collected, or high latitudes around 40° S on the south limit of its distribution (Donoso-Barros, 1966).

Conclusions and conservation implications

Our study is a significant contribution to the few phylogeographical studies from the southern hemisphere (Beheregaray, 2008) and the understanding of the biogeography of the region, as well as to the knowledge of *P. chamissonis*, which is classified as data deficient (IUCN, 2010). The four main haplogroups found for *P. chamissonis* along Chile are characterized by different ecosystems, a biogeographical limit (30° S) and the limit between two herpetofaunal areas of endemism, an increase in geological topography towards central Chile, and the Maipo River as a geographical barrier influenced by Pleistocene glaciation cycles. Consequently, based on the reciprocal monophyly concept (Moritz, 1994), at least three evolutionarily significant units (ESUs) can be defined and considered for management and conservation actions. However, further studies including biparentally inherited markers are necessary to better understand the four major evolutionary lineages and the contact zones, mainly if dispersal in *P. chamissonis* is sex biased.

Moreover, a greater amount of haplogroups found for *P. chamissonis* in central Chile (33° S) overlaps with most of the large cities of the country. Snakes are usually directly persecuted and killed by humans or indirectly by dog attacks or vehicle traffic. In our study, 68% of the samples collected were specimens found dead because of human activity. Many injured snakes are taken to rescue centres in different areas of the country. After recovery, these specimens are reintroduced into the wild, but mostly without taking into account their origin. Thus, we propose that the different ESUs should be taken into consideration when reintroducing individuals in their natural habitat to avoid the possibility of exogamic depression as observed in other snake species (e.g., Rawlings & Donnellan, 2003).

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