Biological Journal of the Linnean Society, 2018, **123**, 712–727. With 4 figures.

Phylogeography of the Chilean red cricket *Cratomelus armatus* (Orthoptera: Anostostomatidae) reveals high cryptic diversity in central Chile

FERMÍN M. ALFARO[1,2†], CARLOS P. MUÑOZ-RAMÍREZ[3,4†], ÁLVARO ZÚÑIGA-REINOSO[5], STEVEN A. TREWICK[6] and MARCO A. MÉNDEZ[5*]

[1]*Laboratorio de Entomología Ecológica, Departamento de Biología, Facultad de Ciencias, Universidad de La Serena, Casilla 554, La Serena, Chile*
[2]*Instituto de Investigación Multidisciplinar en Ciencia y Tecnología, Universidad de La Serena, La Serena, Chile*
[3]*Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Concepción, Chile*
[4]*Centro de Investigación en Biodiversidad y Ambientes Sustentables (CIBAS), Universidad Católica de la Santísima Concepción, Concepción, Chile*
[5]*Laboratorio de Genética y Evolución, Facultad de Ciencias, Departamento de Ciencias Ecológicas, Universidad de Chile, Santiago, Chile*
[6]*Ecology Group, Massey University, Private Bag 11-222, Palmerston North, New Zealand*

Received 28 November 2017; revised 2 February 2018; accepted for publication 2 February 2018

We analysed the phylogeographical history of the red cricket *Cratomelus armatus* (Orthoptera: Anostostomatidae) from central and southern Chile using 248 mitochondrial DNA *COI* sequences. Phylogenetic analyses revealed multiple lineages that were highly structured geographically. The two main lineages (north and south) were parapatric, with a contact zone at the latitude of Concepción (~36.6°S), and have an estimated divergence time of 2 Mya. Deep divergence and a species delineation analysis suggest that these lineages should be considered as different species. The north lineage exhibited four well-supported subclades whose divergence times occurred during the Largest Patagonian Glaciation between 0.84 and 1.1 Mya. Signals of demographic expansion in southern areas indicate a more recent history for the south lineage (southern Chile). A positive correlation between latitude and genetic distances between populations suggests postglacial colonization of southern areas. Bayesian estimations of population size over time placed a bottleneck at ~150 kya. Our results support a role for glaciations in shaping contrasting patterns of genetic diversification in *C. armatus*. More intensive past glaciations may have promoted diversification in central Chile, whereas subsequent glaciations, with stronger impacts in southern areas, could have constrained diversification in southern Chile. We discuss the taxonomic implications of our findings and hypothesize a contrasting role for glaciation on patterns of genetic diversification in central and southern Chile.

ADDITIONAL KEYWORDS: cryptic diversity – glaciations – high genetic diversity – mitochondrial DNA – multiple refugia.

INTRODUCTION

Pleistocene climatic oscillations had important consequences for species distributions, genetic diversity and the evolutionary history of taxa, particularly in temperate regions (Avise, 2000; Hewitt, 2000, 2004; Sérsic *et al.*, 2011). Phylogeographical research has revealed that isolation and postglacial expansion from refugia are among the most important processes explaining genetic patterns in the Northern Hemisphere (Avise, 2000; Hewitt, 2004). In contrast to the vast phylogeographical research conducted in temperate regions of North America and Europe, phylogeographical studies on taxa from temperate regions of South America have accumulated at a much lower pace (Beheregaray, 2008;

*Corresponding author. E-mail: mmendez@uchile.cl
†These authors contributed equally to this work.

Turchetto-Zolet *et al.*, 2013), limiting our understanding of the processes driving biodiversity patterns in this region. Predictably, Pleistocene glaciations and the Andean orogenesis have played important roles in shaping biodiversity in southern South America (Ruzzante *et al.*, 2006; Sérsic *et al.*, 2011). However, inferred evolutionary histories of taxa in this region appear more complex than previously assumed, with variation in phylogeographical patterns indicating mixed responses to environmental change (Sérsic *et al.*, 2011; Victoriano *et al.*, 2012).

A general pattern found in several phylogeographical studies conducted in central and southern Chile is a decrease of genetic diversity towards higher latitudes (e.g. Rodríguez-Serrano, Cancino & Palma, 2006; Himes, Gallardo & Kenagy, 2008; Victoriano *et al.*, 2008). This pattern has supported the idea of glacial refugia in northern areas with more stable climatic conditions that allowed the persistence of taxa during the Last Glacial Maximum (LGM) and recolonization and expansion into southern areas after glaciations (Palma *et al.*, 2005; Lessa, D'Elía & Pardiñas, 2010). Although this pattern has been observed in several taxa (see Sérsic *et al.*, 2011), some studies have found patterns that are in contrast to this general trend and seem to reveal a more complex scenario. For instance, studies analysing patterns of genetic diversity in fish (Unmack *et al.*, 2009), lizard (Vera-Escalona *et al.*, 2012) and freshwater crustacean (Xu *et al.*, 2009) species have shown weak or no relationship between genetic diversity and glacial impact. Indeed, some of these studies (Xu *et al.*, 2009) have also found high genetic diversity in areas that were putatively covered by the ice sheet, suggesting a more complex scenario, with potential cryptic refugia within the hypothesized boundaries of the ice sheet.

These contrasting patterns only emphasize that it is still too soon to make generalizations, and more research needs to be conducted to gain a better understanding of the phylogeographical complexity of the region. In addition, the strong taxonomic bias in phylogeographical studies conducted in the region, which have focused on terrestrial vertebrates and plants (Beheregaray, 2008; Sérsic *et al.*, 2011; Turchetto-Zolet *et al.*, 2013), has limited a more general understanding of the historical processes impacting diversity in southern Chile. In particular, the most diverse group of animals on Earth, the insects, has so far received little attention in temperate areas of South America (but see Zúñiga-Reinoso *et al.*, 2016).

In this study, we examined the phylogeography of a large cricket that is widely distributed in central and southern Chile, the Chilean red cricket or 'grillo rojo' (*Cratomelus armatus* Blanchard, 1851, Anostostomatidae) with the aim of investigating patterns of genetic diversity across extensive glaciated and unglaciated areas of southern South America for the first time in an insect species.

Cratomelus armatus is endemic to central and southern Chile (from 34 to 45°S), living in environments from the coast to the Andes Range (Elgueta, Cammouseight & Carbonnel, 1999; Alfaro *et al.*, 2015). This distribution encompasses the biogeographical subregions of Central Chile and Sub-Antartica and the biogeographical provinces of Santiago, Maule and the Valdivian Forest (Morrone, 2006). This wide distribution includes areas of southern Chile that were glaciated and not glaciated during the Pleistocene, making this species a suitable model to study the impact of glacial cycles. *Cratomelus armatus* are important in forest trophic webs as predators of a range of small invertebrates and as prey for small to large carnivores (Jaksić *et al.*, 1990; Jimenez *et al.*, 1990). The species is flightless and has been documented as highly territorial, with cannibalistic behaviour and fossorial habits (Angulo, 2001) that may suggest high site fidelity and perhaps constrained vagility. The extensive geographical distribution across various environmental conditions, some of which are considered old and climatically stable, and the potentially low to moderate dispersal capability predict high phylogeographical structure.

Here, we used mitochondrial DNA (hereafter mtDNA) to investigate the phylogeographical history of *C. armatus* and to analyse patterns of lineage diversification and genetic diversity across its entire geographical distribution. We test the predictions of high genetic diversity and structure in populations occupying areas that are considered to have been ecologically stable through the Pleistocene (northern and west-central; Fig. 1) and low genetic diversity in areas that were glaciated, as a consequence of postglacial colonization. As unglaciated areas are considered more stable, we also predict high demographic stability, and therefore genetic patterns consistent with constant population size over time. Glaciated areas (southern areas) are expected to show signals of population expansion and recent bottlenecks.

MATERIAL AND METHODS

Taxon sampling

We examined a total of 248 individuals of *C. armatus* from 46 localities in Chile (Table 1, Fig. 1). We also included in the phylogenetic analyses as outgroups five additional individuals of the congeneric species *Cratomelus integer*, which is parapatric with *C. armatus* to the north (Table 1, Fig. 1). The sampled area encompasses most of the known geographical

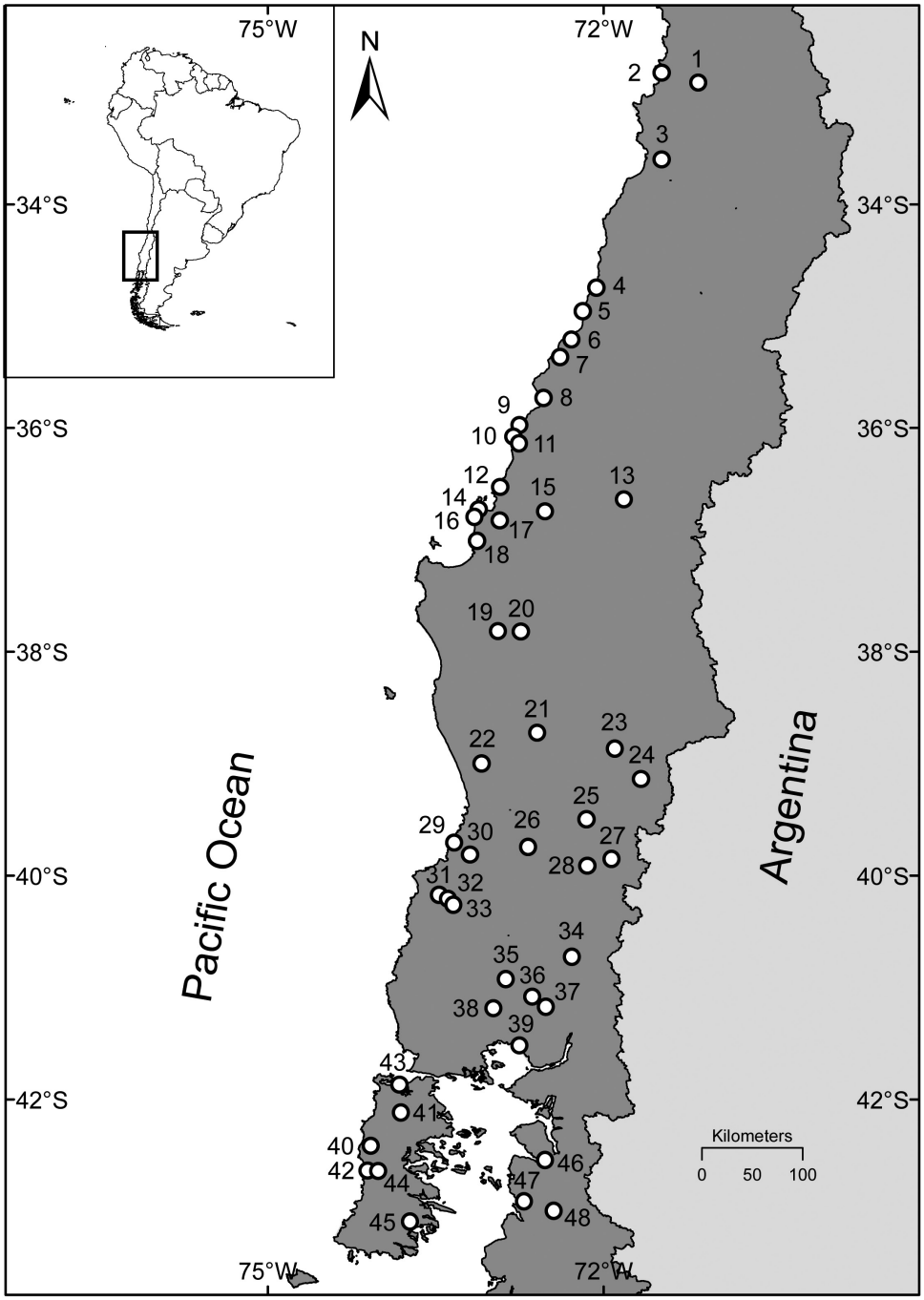

Figure 1. Sampling localities of *Cratomelus armatus* used in the study. The numbers represent the identification of localities (see Table 1).

distribution of the species, which extends over 1200 km, from the Cardenal Caro Province in the north (O'Higgins, 34°S) to the southern Palena Province (Los Lagos, 42°S; Fig. 1). For species identification, we followed Gorochov (1999). The collected individuals were fixed and stored in 99% ethanol and deposited in the repository of the Laboratorio de Genética y Evolución, Universidad de Chile, Chile (GEVOL), the collection of Laboratorio de Entomología Ecológica, Universidad de La Serena, Chile (LEULS) and the Phoenix group collection, Massey University, New Zealand.

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

DNA was extracted from muscle tissue using a modified salt extraction method (Jowett, 1986;

Table 1. Sampling locations and genetic diversity estimated for the populations of *Cratomelus armatus*

Site no.	Locality	Latitude	Longitude	*N*	π	*Hd*
Cratomelus integer						
1	Parque Nacional La Campana	–32.9167	–71.1500	1	–	–
2	Ritoque	–32.8254	–71.4790	1	–	–
3	Leyda	–33.6017	–71.4799	3	–	–
Cratomelus armatus						
4	Llico	–34.7463	–72.0650	6	0.01987	0.8
5	Iloca	–34.9576	–72.1844	4	0.00261	0.8333
6	Putú	–35.2116	–72.2856	7	0.0056	0.9524
7	Constitución	–35.3665	–72.3867	4	0.00436	1
8	Reserva Nacional Federico Albert	–35.7316	–72.5366	6	0.0027	0.8
9	Tregualemu	–35.9767	–72.7519	12	0.00147	0.7879
10	Buchupureo	–36.0795	–72.8038	3	0.01307	0.6667
11	Cobquecura	–36.1396	–72.7541	6	0.00044	0.3333
12	Pingueral	–36.5288	–72.9240	1	–	–
13	Coihueco	–36.6399	–71.8163	10	0.02891	0.9778
14	Talcahuano	–36.7304	–73.1145	5	0.03791	0.8
15	Coyanco	–36.7461	–72.5202	3	0.00959	1
16	Hualpén	–36.7965	–73.1541	3	0	0
17	Laguna Pineda	–36.8279	–72.9261	12	0.00705	0.9697
18	Coronel	–37.0097	–73.1310	2	0	0
19	Parque Nacional Nahuelbuta	–37.8164	–72.9429	10	0.0075	0.7111
20	Vegas Blancas	–37.8223	–72.7387	6	0.00166	0.8667
21	Cerro Ñielol	–38.7271	–72.5921	7	0.00952	0.9048
22	Teodoro Schmidt	–38.9995	–73.0908	5	0.00523	0.4
23	Vilcún	–38.8672	–71.8966	1	–	–
24	Huerquehue	–39.1386	–71.6664	1	–	–
25	Lican Ray	–39.4964	–72.1526	4	0.00588	0.5
26	Malihue (Fundo St. Olga)	–39.7470	–72.6735	5	0.00471	0.9
27	Neltume	–39.8511	–71.9256	10	0.00206	0.7778
28	Reserva Nacional Mocho Choshuenco	–39.9138	–72.1461	7	0.00735	0.9524
29	Parque Oncol	–39.7085	–73.3316	1	–	–
30	Valdivia	–39.8138	–73.1935	5	0.00549	0.9
31	La Unión	–40.1740	–73.4699	8	0.00616	0.9643
32	Parque Nacional Alerce Costero	–40.2119	–73.3905	6	0.0034	0.9333
33	San Juan de la Costa	–40.2616	–73.3394	7	0.00411	0.7143
34	Parque Nacional Puyehue	–40.7262	–72.2829	13	0.00446	0.8846
35	Puerto Octay	–40.9282	–72.8718	6	0.00331	0.7333
36	Cascadas (Cascadas bridge)	–41.0850	–72.6347	10	0.00726	0.8667
37	Volcán Osorno	–41.1746	–72.5152	5	0.00549	0.9
38	Punta Larga	–41.1874	–72.9826	4	0.00741	1
39	Parque Katalapi	–41.5200	–72.7506	3	0.00087	0.6667
40	Chiloe (near Parque Nacional Chiloé)	–42.4172	–74.0786	6	0.00401	0.8667
41	Chiloé (Puntra)	–42.1192	–73.8111	1	–	–
42	Chiloé (Cucao)	–42.6398	–74.1078	3	0.00436	0.6667
43	Chiloé (Ancud)	–41.8718	–73.8233	1	–	–
44	Chiloé (north point of Huillinco Lake)	–42.6432	–74.0115	9	0.00806	0.8889
45	Chiloé (Yaldad)	–43.0927	–73.7293	6	0.00784	0.8
46	Caleta Leptepú	–42.5445	–72.5219	4	0.00065	0.5
47	Chaitén	–42.9131	–72.7097	4	0.00261	0.5
48	El Amarillo	–42.9987	–72.4431	6	0.00131	0.6

Hd, haplotype diversity; *N*, sample size; π, nucleotide diversity.

Sunnucks & Hales, 1996). We amplified partial sequences of the mtDNA gene cytochrome oxidase I (*COI*) using primers C1-J-2183 (5′-CAACATTTATTTTGATTTTTTGG-3′) and TL2-N-3014 (5′-AATTCCGCACATTGCCTAATCATTA-3′) (Simon *et al.*, 1994). Previous studies with related crickets have shown that this marker provides good resolution for phylogeographical analysis (e.g. Trewick & Morgan-Richards, 2004; Pratt, Morgan-Richards & Trewick, 2008; Brettschneider *et al.*, 2009; Chappell, Trewick & Morgan-Richards, 2012). The reaction mixture included 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM each primer, 1 U Taq polymerase (Invitrogen, Carlsbad, CA, USA) and 50–100 ng total DNA. The thermal protocol for the PCR was 94 °C for 5 min, followed by 36 cycles of 94 °C for 45 s, 45–50 °C for 30 s and 72 °C for 60 s, with a final extension at 72 °C for 2 min. The PCR products were visualized in agarose gels and sequenced using the same primers. DNA sequences were edited and aligned in BIOEDIT v7.0.5.2 (Hall, 1999) and translated into amino acids in Mega 6 to check for codon stops and frame shifts that could indicate alignment errors and the potential presence of nuclear copies of the mitochondrial gene (Numts; Song, Moulton & Whiting, 2014). Levels of substitution saturation were analysed with Xia's test (Xia *et al.*, 2003) in DAMBE, version 5.1.5 (Xia & Xie, 2001). All sequences are available in GenBank (MG202165–MG202417).

Time-calibrated genealogy and species delimitation analysis

A dated genealogical reconstruction was estimated by Bayesian inference with the program BEAST version 2.4.4 (Bouckaert *et al.*, 2014). We included all the sequences of *C. armatus* and the sequences of the congeneric species *C. integer* as an outgroup (MG202413–MG202417). The HKY + G evolutionary model was selected as the best-fit model using the program jModelTest version 0.1.1 (Posada, 2008) using the Bayesian information criterion. The strict-clock model was selected as the molecular clock prior, using a substitution rate of 0.017 substitutions per site per million years, following recently published rates for insects (Papadopoulou, Anastasiou & Vogler, 2010) and orthopterans (Allegrucci, Trucci & Sbordoni, 2011; Kaya & Çiplak, 2016).

We conducted two independent analyses to check for consistency in the results. Each analysis was run for 50 million generations, sampling trees every 10 000 generations. The program Tracer v. 1.6 (Rambaut & Drummond, 2009) was used to visualize the traces of the Markov chain Monte Carlo runs and to check that the effective sample size of model parameters were > 200 (indication of convergence). Sampled trees were summarized using the maximum clade credibility criteria in TreeAnnotator v2.4.4 (distributed as part of BEAST), discarding the first 20% of the trees as burn-in. The summarized tree was visualized and edited for illustration purposes using Figtree v1.4.2 (Rambaut, 2008). Complementarily, to examine haplotype relationships and the frequency distribution across space we constructed a haplotype network using the median joining algorithm (Bandelt, Forster & Röhl, 1999) implemented in PopArt v1.7.1 (Leigh & Bryant, 2015).

Following the results from the phylogenetic analyses, we conducted a barcode gap analysis to look for evidence of species-level differences between major clades using the automatic procedure ABGD described by Puillandre *et al.* (2012). This analysis uses the barcode gap, which is the gap observed when divergence among individuals of the same species is smaller than divergence among individuals from different species, to automatically find groups that might correspond to different potential species. A range of prior intraspecific divergence values from 0.001 to 0.1 was assayed (in ten steps), applying a relative gap width (X) of 1.5 and using three options for genetic distance (JC69, K80 and simple distance). This analysis was performed through the Web server of ABGD (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html).

Analyses of genetic diversity and demography

To estimate genetic diversity across the distribution of *C. armatus*, we estimated several diversity statistics, namely haplotype diversity (*Hd*), nucleotide diversity (π) and genetic distance among geographically close populations for all sampling localities, using the program DnaSP v5.10.01 (Librado & Rozas, 2009). Given that phylogenetic analyses revealed multiple lineages, demographic analyses were conducted separately for each of the two main lineages that were supported as distinct by the ABGD analysis (see the results section below and Fig. 2). This approach was preferable to compare the north and south areas of the species distribution, impacted in a different manner by glaciations. Signals of demographic expansion and/or stability were evaluated in several ways. We first estimated Fu's *Fs* (Fu, 1997) and Tajima's *D* statistics, evaluating their significance using 1000 simulations in Arlequin v3.5.1.3 (Excoffier & Lischer, 2010). Significantly negative values for these statistics imply recent population expansion, whereas values not significantly different from zero are interpreted as evidence of stable population size. We used Bayesian skyline plot (BSP) analyses implemented in the program BEAST v1.8.0 (Drummond *et al.*, 2012) to investigate population changes over time. This coalescent approach uses genealogical information to estimate important population genetic

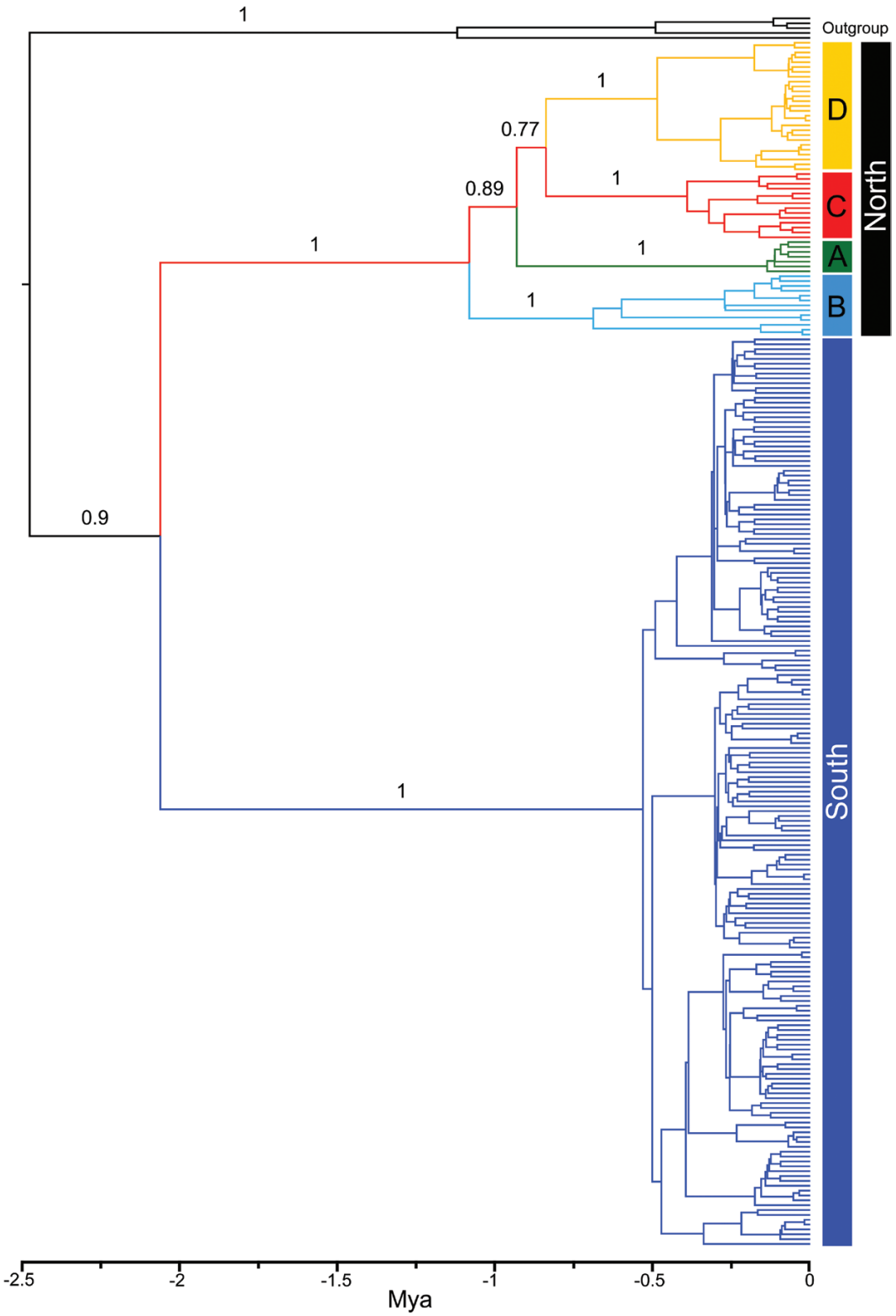

Figure 2. Bayesian phylogenetic tree for *Cratomelus armatus* based on mitochondrial DNA. Numbers on nodes are Bayesian posterior probabilities, and the colour of branches represents the different lineages (see Fig. 3).

parameters to reconstruct the effective population size (N_e) over time using a Bayesian statistical approach. Priors and settings were kept as in the dated genealogical analyses, but using the coalescent Bayesian skyline option as the tree prior. We ran 100 million iterations, sampling every 10 000 iterations to generate 100 000 parameter estimates for two independent runs. Likelihood values of demographic plots for each genetic group were visualized with the program Tracer v1.5.0 (Rambaut & Drummond, 2009).

Complementarily, a Mantel test (Mantel, 1967) was also performed to compare patterns of isolation by distance between the north and south lineages. Impacted areas that are not under equilibrium will show weak or no correlation between geographical distance and genetic structure, whereas areas that have been more stable will show a stronger correlation. The Mantel test was conducted in Alleles In Space (AIS; Miller, 2005).

Testing the impact of glaciations on genetic diversity and structure

We conducted various tests to assess the impact of glaciation on genetic diversity. We performed correlation tests between measures of genetic diversity and latitude. The general model suggests that the extent of glaciation increased with latitude, predicting a decrease in genetic diversity from north to south. We first looked for correlation between genetic diversity and latitude with all localities, as the distribution of populations strongly follows a latitudinal gradient. However, the presence of distinct lineages in our phylogenetic analyses suggested that these should be treated separately, so we then focused the analysis on populations of the southernmost clade (south of 37°S), which presented different degrees of exposure to glacial coverage.

Three measures of genetic diversity were also used to assess for geographical correlation, namely nucleotide diversity (π), haplotype diversity and the average number of pairwise differences between populations. Analysis using pairwise genetic distances and latitude used only pairwise distances of one decimal degree or less (~111 km) to represent local diversity. We used the midpoint between the compared localities to obtain a single latitude value for pairwise comparisons. Finally, to visualize the variation of genetic distances across the distribution of the species better, we performed a genetic landscape shape interpolation analysis with the program AIS (Miller, 2005).

RESULTS

We obtained a 765 bp alignment of mtDNA *COI* gene sequences comprising 167 variable sites and a total of 117 haplotypes from 248 individuals. The sequences presented low saturation as indicated by Xia's test [index of substitutional saturation (Iss) < critical Iss (Iss.c); $P < 0.0001$], which supported their suitability for phylogenetic inference. The nucleotide frequencies were 31.16% (A), 37.87% (T/U), 16.09% (C) and 14.88% (G); the overall transition/transversion ratio (R) was 3.69, a value that is typical of mtDNA data sets (nuclear data sets are usually below $R = 2$; Nei & Kumar, 2000). This transition/transversion ratio, along with the absence of internal codon stops and frame shifts in the sequence alignment, suggests the absence of nuclear copies of mtDNA in our data set.

Genealogical reconstruction and estimation of divergence times

The Bayesian topology supported the existence of two highly divergent lineages (north and south clades; Fig. 2), with an estimated divergence of 2.06 Mya [95% high posterior density (HPD): 1.54–2.5 Mya], that come into contact at the latitude of Concepción (~36.4°S). Indeed, two sampling sites, Coihueco (13) and Talcahuano (14) (see Table 1), harboured individuals of both lineages. The north lineage, which encompasses samples collected from Llico (34°S) to Coronel (37°S), was the most diverse, with four lineages showing high geographical structure (lineages A–D; Fig. 2). The geographical limits of these lineages broadly coincide with three major river systems, the Mataquito, Maule and Itata rivers. Although relationships between subclades A–D were not well resolved, geographical structure and high branch support for these clades support their recognition as differentiated groups. The divergence times for these lineages ranged from 1.08 to 0.49 Mya (see Table 2), with an estimated age for the most recent common ancestor (MRCA) of 1.08 Mya (95% HPD: 0.84–1.36 Mya). In contrast to the north lineage, the south lineage showed shallow phylogenetic relationships and little geographical structure, despite being

Table 2. Divergence time estimates with 95% HPD confidence intervals for clades based on mitochondrial DNA *COI* of *Cratomelus armatus*

Clade	MRCA (Mya)	95% HPD
Clade A	0.14	0.05–0.24
Clade B	0.39	0.25–0.54
Clade C	0.49	0.32–0.66
Clade D	0.69	0.46–0.91
Clades BC	0.84	0.61–1.04
Clade A(BC)	0.93	0.69–1.15
North (ABCD)	1.08	0.84–1.36
South	0.53	0.38–0.67
North–South	2.06	1.54–2.5

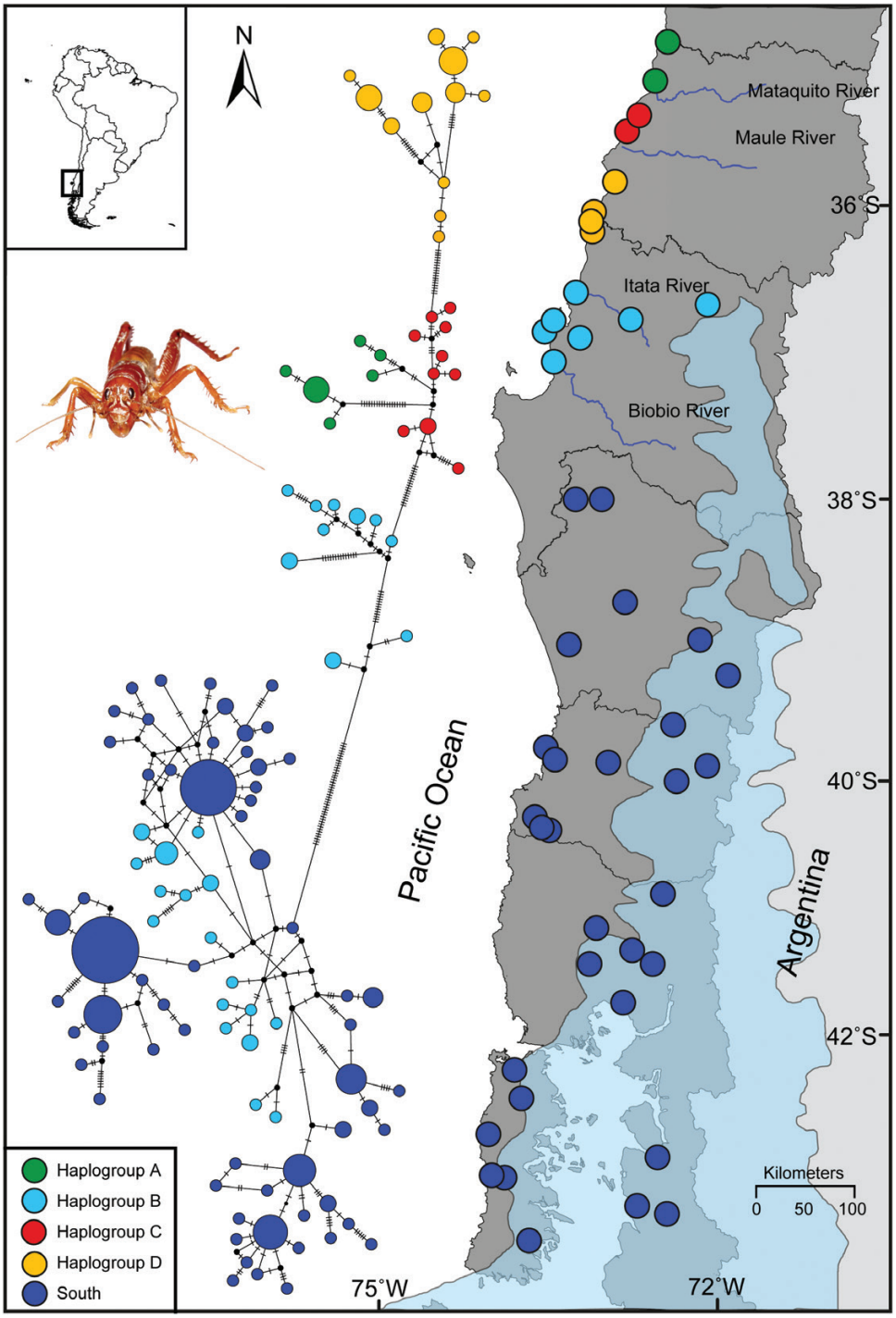

Figure 3. Median joining network of mitochondrial DNA of *Cratomelus armatus* from central and southern Chile, showing geographical distribution of haplogroups. The haplotypes are represented by circles whose sizes are proportional to their frequencies. Colours represent the different geographical groups where haplotypes are present. The light blue shape shows the extent of the ice cover during the Last Glacial Maximum (Clapperton, 1993).

distributed across a larger geographical area. The estimated age of the southern lineage was ~50% younger than the age of the northern lineage, with a time to the MRCA of 0.53 Mya (95% HPD: 0.38–0.67 Mya).

The haplotype network was consistent with the genealogical reconstruction and showed a complex structure with five main haplogroups separated by a large number of nucleotide substitutions (Fig. 3).

Cratomelus armatus with haplogroup B haplotypes came from localities north of the Biobio River, whereas those with group D haplotypes were associated with localities south of the Maule River (between the Maule and Itata rivers). Haplogroup C was associated with localities south of the Mataquito River, whereas haplogroup A was associated with localities north to the mouth of the Mataquito River. Individuals collected in localities south of the Biobio River (37–43°S) belonged to the south lineage. The four haplogroups north of the Biobio River were represented by haplotypes at similar frequencies that did not form star-like topologies. In contrast, the south lineage included a few haplotypes at high frequency, each with numerous rare derived haplotypes differing by one to a few nucleotide substitutions. This star-like topology is often associated with recent demographic expansions.

Genetic differentiation and species delimitation analysis

Genetic distances between the north and south clades and between subclades within the north clade were fairly high (Table 3), revealing high cryptic diversity within the nominal species *C. armatus*, especially in its northern range (from the Biobio River to the north). The average (uncorrected) number of pairwise differences between the north and the south lineages was 52.34 (p-distance = 0.068). The average number of pairwise differences between clades A, B, C and D (subclades within the north clade) ranged from 22.7 (p-distance = 0.03) to 31.5 (p-distance = 0.041), with the smallest difference being between clades A and C and the largest between B and D (Table 3).

The automatic barcode gap species delimitation analysis (ABGD) using all the available distance options (JC69, K80 and simple distance) produced consistent results (see Supporting Information, Fig. S1 for full results). We therefore refer only to the results obtained with the K80 evolutionary model. The results showed a multimodal pairwise genetic distance distribution with a clear, wide barcode gap located in the distance range 0.05–0.06 (Supporting Information, Fig. S1A). Furthermore, the method detected two stable candidate species regardless of the distance option chosen, with estimated prior maximum divergence of intraspecific diversity (P) as large as 6%. These putative species corresponded to the two main lineages found in the Bayesian genealogical analyses (north and south).

Table 3. Average number of pairwise differences (uncorrected) between haplogroups based on mitochondrial DNA *COI* of *Cratomelus armatus* (see Fig. 3)

Haplogroup	A	C	D	B	South
A	–	22.71	28.20	29.01	54.24
C	0.03	–	24.89	26.51	51.65
D	0.037	0.032	–	31.50	52.91
B	0.038	0.035	0.041	–	50.60
South	0.071	0.068	0.069	0.066	–

Below diagonal, genetic divergence between clades (p-distance).

Patterns of genetic diversity and demographic analyses

Considering all the sampling sites, both nucleotide diversity (π) and haplotype diversity (*Hd*) showed no correlation with latitude (Supporting Information, Fig. S2A, B), against expectations of a major glacial impact on genetic diversity towards high latitudes. Genetic diversity (nucleotide and haplotype diversity) was relatively similar across the distribution of *C. armatus*, except for three localities where nucleotide diversity was markedly high (i.e. outliers; Supporting Information, Fig. S3): Llico, Coihueco and Talcahuano. The high nucleotide diversity in these three localities can be explained by the presence of haplotypes from different lineages (see Fig. 3).

Regardless of the inclusion or exclusion of these location samples, correlation analyses showed no significant relationship between latitude and genetic diversity, either for π ($R = -0.311$, $P = 0.12$ including admixed localities, and $R = 0.2$, $P = 0.34$ excluding admixed localities) or for *Hd* ($R = -0.024$, $P = 0.91$ including admixed localities, and $R = -0.04$, $P = 0.85$ excluding admixed localities; see Supporting Information, Fig. S2). In contrast, genetic distance among geographically close populations did show a tendency to decrease with latitude, as can be seen in the landscape genetic analysis (Fig. 4). This pattern is explained, at least in part, by the richer lineage diversity and phylogeographical structure present in the north and central portions of the species distribution, clearly evident in the genealogy and network (Figs 2, 3).

Given that genealogy and species delineation analyses suggested two lineages of species status within the nominal *C. armatus* distribution, it makes sense to treat these lineages separately for further analyses. For the case of patterns of genetic diversity vs. latitude, only the south lineage was evaluated because it is distributed in an area where glaciations were more relevant during the most recent glacial periods. Neither nucleotide diversity ($R = -0.041$, $P = 0.87$) nor haplotype diversity ($R = -0.42$, $P = 0.073$) decreased with latitude for the south lineage (Supporting Information, Fig. S2), in contrast to our expectations. Genetic distance among populations, however, did show a significant north–south decrease ($R = -0.57$,

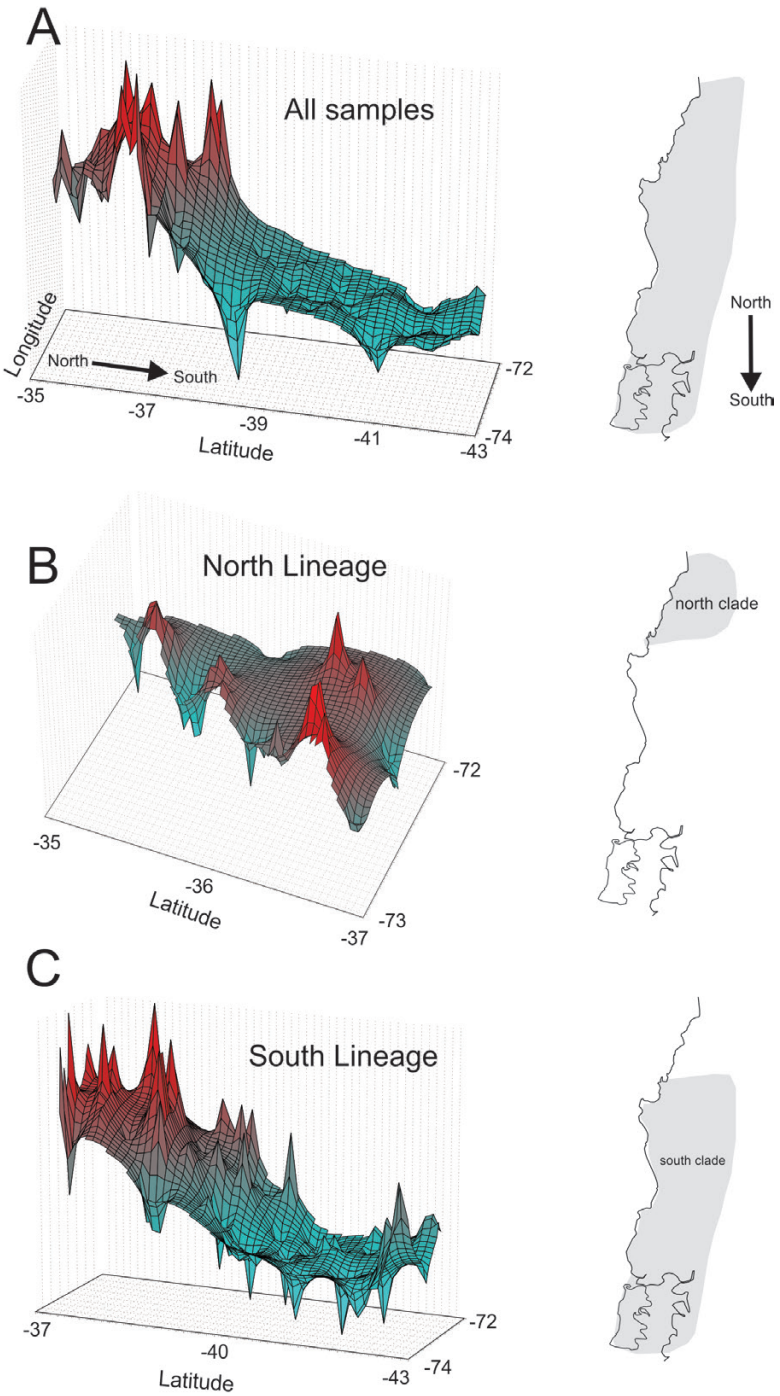

Figure 4. Genetic landscape shape interpolation analyses for the distribution of *Cratomelus armatus* based on mtDNA of all samples (A), north lineage samples (B) and south lineage samples (C). The *x*- and *y*-axes correspond to the coordinates of the geographical locations of the samples examined in this study.

$P = 0.000$), indicating a more homogeneous (less structured) distribution of genetic diversity in southern areas (Supporting Information, Fig. S4). The landscape genetic analysis confirmed this latitudinal pattern by showing deeper valleys towards higher latitudes in the interpopulation genetic diversity landscape (shown in Fig. 4C). Strikingly, this visualization also showed a moderate increase in among-population genetic distances in Chiloé, one of the southernmost areas included in this study.

Demographic analyses indicated different demographic histories for the north and south lineages. For the north lineage, both neutrality tests, *Fs* and Tajima's *D*, showed no significant departures from zero, indicating stable population size and long-term population history (Table 4). The Mantel test also showed a high and significant correlation between geographical and genetic distances ($R = 0.72$, $P < 0.001$) for the north lineage. In contrast, for the south lineage the *Fs* and Tajima's *D* tests showed negative and significant departures from zero, suggesting past bottlenecks and a more recent population history (Table 4). Consistently, the Mantel test for this group showed a weak (although significant) correlation between genetic distance and geographical distance ($R = 0.17$, $P < 0.001$).

The Bayesian skyline plot analyses showed consistent patterns with the above tests. For the north clade, the analysis inferred constant population size over time (Supporting Information, Fig. S5A), whereas for the south clade it inferred a demographic expansion over time (Supporting Information, Fig. S5B) that started ~100–150 kya and coincided with the OIS 6 glacial event, the most extensive in the past half million years (Mortyn *et al.*, 2003). Altogether, these results strongly suggest a more recent demographic history for the southern lineage, consistent with a scenario of lower stability in southern Chile.

DISCUSSION

Deep divergence supports cryptic species

The deep divergence between the north and south lineages and the ABGD analysis revealed that these lineages most probably represent two different species. In other regions harbouring a richer diversity of Anostostomatid crickets, such as South Africa and Australasia, the analysis of molecular data (*COI* gene) has proved useful to gain a better understanding of interspecific vs. intraspecific variation (e.g. Trewick & Morgan-Richards, 2004; Pratt *et al.*, 2008; Brettschneider *et al.*, 2009; Chappell *et al.*, 2012).

Table 4. Results of the neutrality test for the two principal lineages (north and south) of *Cratomelus armatus*

Lineage	*N*	Tajima's *D*	*P*-value	Fu's *Fs*	*P*-value
North	61	0.38	0.72	–3.4	0.18
South	187	–1.94	0.00*	–24.6	0.00*

N is the number of individuals.
*Statistically significant at the 0.05 level.

Allegrucci *et al.* (2013), analysing several weta species from New Zealand, showed that genetic distances within species ranged from 0.3 to 1.7%, whereas genetic distances between species ranged from 1.9 to 9.3%. Trewick (2008), who analysed genetic diversity within the *Sigaus* species complex, found genetic distances to range from 3 to 8.3% between major haplogroups, suggesting the need for further taxonomic work. Likewise, within the South African species *Libanasidus vittatus*, genetic divergence of 3.3% between clades was reported by Brettschneider *et al.* (2009), and a further examination of morphological data found several differences that suggested new species/lineages. Several studies have found that divergences in the range of 2–3% for insects are consistent with traditional taxonomic limits (Hajibabaei *et al.*, 2006; Lefébure *et al.*, 2006). Our results, which show between-clade genetic distances ranging from 3 to 6.9%, indicate a long history of isolation between major clades and suggest the possibility of a complex of cryptic species within what is currently known as *C. armatus*. Whether all these divergent lineages have evolved reproductive isolation, and therefore become different species, is a question that requires further investigation. Nevertheless, the deep divergence between samples from the north and the south of the distribution of *C. armatus* (a p-distance of 0.07) indicates a long history of isolation between these lineages and strongly suggests distinct species. These lineages are parapatric at the latitude of Concepcion, with some localities harbouring individuals from both lineages (Talcahuano and Coihueco). It is likely that the Biobio River (part of the largest river network in Chile and whose mouth discharges near Concepcion) had historically contributed to reduce gene flow between the north and south lineages. However, the fact that these lineages are in contact in some localities suggests that migration across the river is not fully interrupted, and therefore gene flow must be restricted by some other reproductive barriers apart from geographical ones (e.g. ecological, behavioural, genetics). It has been recorded that *C. armatus* displays strong territoriality, with aggressive behaviour that commonly results in cannibalism in controlled experiments (Angulo, 2001). This type of behaviour has been associated with geographical segregation and parapatric distributions in some taxa (e.g. Nevo, Naftali & Guttman, 1975; Arif, Adams & Wicknick, 2007; Jankowski, Robinson & Levey, 2010), but further studies are required to test whether this factor plays a role in maintaining the parapatry between the north and south lineages of *C. armatus*. Nevertheless, molecular evidence is strong in suggesting that these lineages should be considered two different species; therefore, we recommend detailed morphological descriptions to describe them formally.

The northern lineage, with its higher diversity of lineages, may well represent more cryptic species, because divergences between subclades are > 3%. However, detailed ecological and morphological studies, as well as additional molecular markers, would be necessary to test this hypothesis.

Regardless of our current ability to identify different morphological species, our data suggest the existence of an elevated cryptic diversity within the *C. armatus* complex that warrants and encourages more research into the processes that have promoted this diversity. Understanding the taxonomic limits of this cryptic diversity is important to make correct sense of biological processes. For instance, morphological and behavioural studies conducted on *C. armatus* from Concepcion (Angulo, 2001) suggested that this species has a large variance in morphology and behaviour. However, based on our study two species can be found in this area; therefore, these previous analyses might inadvertently be reporting variation of two different species instead of one, overestimating intraspecific variation. Additional data and the incorporation of the two other *Cratomelus* species will strengthen our understanding of species limits within the genus and help to test evolutionary processes underlying the origin of this diversity.

The impact of glaciations on *C. armatus*

Although still limited in number, phylogeographical studies generally agree with the notion of a severe impact of glacial cycles on species' intraspecific diversity in southern South America (e.g. Victoriano *et al.*, 2008; Sérsic *et al.*, 2011). Therefore, molecular patterns reflecting a gradient of increasing impact from North to South are to be expected.

Our results supported this general view. Lower lineage diversity and signals of population expansion in the southern range of *C. armatus* suggest a stronger impact of glaciations in southern Chile. In contrast, high levels of lineage diversity and signals of greater demographic stability in the northern range of the species suggest a lower impact of glaciations in central Chile. These results are consistent with previous studies, supporting the hypothesis that this area has been a general refugium for several taxa (e.g. Rodríguez-Serrano *et al.*, 2006; Victoriano *et al.*, 2008; Azpilicueta, Marchelli & Gallo, 2009; Palma *et al.*, 2012). Likewise, related orthopterans at similar latitudes in New Zealand show higher diversity in the north compared with the south of their ranges (Morgan-Richards, Trewick & Wallis, 2001; Bulgarella *et al.*, 2014), and the phylogeographical structure of some species, such as the alpine scree weta *Deinacrida connectens*, has been affected by Pliocene mountain building and maintained by the Pleistocene glacial and interglacial periods (Trewick, Wallis & Morgan-Richards, 2000).

Some of our results, however, did not follow the expected pattern of a stronger impact at higher latitudes. Genetic diversity (nucleotide and haplotype diversity estimated for each sampling site) did not decrease with latitude. Similar results have previously been reported for freshwater fish (Unmack *et al.*, 2009; Muñoz-Ramírez *et al.*, 2014). These mixed patterns could suggest that *C. armatus* has recovered rapidly from the inferred bottlenecks or that its populations have not been impacted by the most recent glaciations; at least, not to a sufficient degree to decrease genetic diversity severely. Perhaps only older (previous to the LGM) and more intense glaciations have had a detectable impact on the demography of *C. armatus*, providing enough time for populations to recover genetic diversity to the present observed levels. Indeed, our BSP analysis (Supporting Information, Fig. S5) estimates the start of the demographic expansion for the southern lineage at ~150 kya, coinciding with the end of one of the most extensive glaciations in the past 400 kyr (the OIS 6 event; Mortyn *et al.*, 2003) and not during the LGM (~23 kya) as has been often found for other taxa (e.g. Ruzzante *et al.*, 2006; Vera-Escalona *et al.*, 2012). *Cratomelus armatus* may have persisted in large numbers during less severe glaciation events (insect population sizes are often several orders of magnitude larger than those of vertebrates), favoured perhaps by its fossorial habits (Angulo, 2001), water-efficient physiology (Nespolo, Artacho & Castañeda, 2007) and generalist feeding behaviour (Angulo, 2001). Our results, along with other reported cases of demographic expansion pre-dating the LGM (e.g. Zemlak *et al.*, 2010; Vianna *et al.*, 2011), support a scenario in which the LGM was not equally detrimental for all taxa.

Although our demographic results demonstrate a stronger impact of glaciations in southern areas of Chile, they do not necessarily contradict a role of glaciations in central Chile. It has been recently suggested that glaciations may have promoted diversification in several taxa distributed along mountain ranges (Wallis *et al.*, 2016). These authors suggest that latitudinal phylogeographical breaks in several southern Andes taxa are evidence of isolation in multiple glacial refugia resulting from transverse glacial barriers. This hypothesis is consistent with our finding of several lineages in the northern range (central Chile) of *C. armatus*. Although central Chile has often been assumed to be less impacted by glaciations compared with southern Chile, some glaciations, particularly the most severe ones, could have contributed to the diversification of *C. armatus* in areas where the presumably lesser glacier development may have fragmented

rather than displacing its habitat, facilitating population isolation and divergence. In contrast, more continuous and widespread ice coverage in southern areas may have prevented isolation in multiple isolated refugia; hence, it prevented the diversification of the southern lineage. Interestingly, our dated genealogy indicates that divergence times for all major clades of the northern lineage (clades A–D; Fig. 2) date back to 1.1–0.84 Mya (Table 2), matching the period of the most extensive Andean glaciation (Largest Patagonian Glaciation; Singer, Ackert & Guillou, 2004). These results support a role of glaciations in the diversification of the *C. armatus* complex in the northern range of its distribution and add evidence to a growing body of knowledge about the synergistic role of glaciations and mountain ranges on the evolution of biodiversity during the Pleistocene (Wallis *et al.*, 2016).

It is also possible that other factors could have contributed to the diversification of the northern lineage. The broad match between clade limits and several Andean river basins (see Fig. 3) may suggest a role of river basins in the phylogeographical structure of *C. armatus* in Central Chile, similar to that observed in some terrestrial vertebrates (Lamborot & Eaton, 1997; Lamborot, Eaton & Carrasco, 2003; Torres-Pérez *et al.*, 2007; Vásquez, Torres-Pérez & Lamborot, 2007; Sallaberry-Pincheira *et al.*, 2011) and aquatic taxa (Ruzzante *et al.*, 2006; Unmack *et al.*, 2009; Muñoz-Ramírez *et al.*, 2014; Muñoz-Ramírez, Victoriano & Habit, 2015). However, it is difficult to postulate rivers as the main factor responsible for diversification in *Cratomelus* for several reasons. First, most of the largest river basins of Chile are located in the south, where the southern lineage is present. However, the southern lineage does not show internal phylogeographical breaks as would be expected if rivers played a role in diversification. Second, central and southern Chile are longitudinally dissected by many rivers, so it is expected that any latitudinal phylogeographical break will match a river to some extent, even if rivers were not responsible for the breaks. Therefore, evidence for the impact of rivers on the diversification of the north lineage remains elusive. We hypothesize that both rivers and multiple glacial refugia could have shaped patterns of diversity seen today in the north lineage of *C. armatus*. However, glacial refugia could have played a greater role in the origin of the diversity of the north lineage, whereas rivers may have played a role in their maintenance.

ACKNOWLEDGEMENTS

We are grateful to CONAF (Corporación Nacional Forestal, Chile) for allowing us to work in the National System of Wild Protected Areas (SNASPE). This study was funded by FONDECYT project 1140540 (M.A.M.) and CONICYT fellowships no. 2013-22130123 (F.M.A.) and no. 21110367 (A.Z.-R.). We thank three anonymous reviewers for their helpful comments that helped to improve the manuscript and Lafayette Eaton for proofreading the English version. We are especially indebted to Luis Pastenes, Paola Sáez, Pamela Morales, Franco Cruz, Dusan Boric, Einer Sepúlveda, Daniela Mardones, Jaime Pizarro-Araya, Andrés Ojanguren-Affilastro, Ricardo Botero-Trujillo, Hernán Iuri, Jaime Zuñiga and Fermín Alfaro Contreras for their invaluable help in the laboratory and during the collecting trips.

REFERENCES

Alfaro FM, Zuñiga-Reinoso A, Muñoz-Ramírez C, Elgueta M. 2015. New records reveal the actual distribution of *Cratomelus meritus* Gorochov (Orthoptera: Anostostomatidae), a giant red cricket from Chile. *Neotropical Entomology* **44:** 194–196.

Allegrucci G, Massa B, Trasatti A, Sbordoni V. 2013. A taxonomic revision of western *Eupholidoptera* bush crickets (Orthoptera: Tettigoniidae): testing the discrimination power of DNA barcode. *Systematic Entomology* **39:** 7–23.

Allegrucci G, Trucchi E, Sbordoni V. 2011. Tempo and mode of species diversification in *Dolichopoda* cave crickets (Orthoptera, Rhaphidophoridae). *Molecular Phylogenetics and Evolution* **60:** 108–121.

Angulo AO. 2001. Anatomy, development and behaviour of the Chilean red cricket, *Cratomelus armatus*. In: Field LH, ed. *The biology of the wetas, king crickets and their allies*. Wallingford: CABI Publishing, 205–222.

Arif S, Adams DC, Wicknick JA. 2007. Bioclimatic modelling, morphology, and behaviour reveal alternative mechanisms regulating the distributions of two parapatric salamander species. *Evolutionary Ecology Research* **9:** 843–854.

Avise JC. 2000. *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.

Azpilicueta MM, Marchelli P, Gallo LA. 2009. The effects of Quaternary glaciations in Patagonia as evidenced by chloroplast DNA phylogeography of Southern beech *Nothofagus obliqua*. *Tree Genetics & Genomes* **5:** 561–571.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16:** 37–48.

Beheregaray LB. 2008. Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology* **17:** 3754–3774.

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10:** e1003537.

Brettschneider H, Chimimba CT, Scholtz CH, Bastos ADS, Bateman PWN. 2009. The tusked king cricket, *Libanasidus vittatus* (Kirby, 1899) (Anostostomatidae), from

South Africa: morphological and molecular evidence suggest two cryptic species. *Insect Systematics & Evolution* **40:** 85–103.

Bulgarella M, Trewick SA, Minards NA, Jacobson MJ, Morgan-Richards M. 2014. Shifting ranges of two tree weta species (*Hemideina* spp.): competitive exclusion and changing climate. *Journal of Biogeography* **41:** 524–535.

Chappell EM, Trewick SA, Morgan-Richards M. 2012. Shape and sound reveal genetic cohesion not speciation in the New Zealand orthopteran, *Hemiandrus pallitarsis*, despite high mitochondrial DNA divergence. *Biological Journal of the Linnean Society* **105:** 169–186.

Clapperton CM. 1993. *Quaternary geology and geomorphology of South America*. Amsterdam: Elsevier.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29:** 1969–1973.

Elgueta M, Cammouseight A, Carbonnel CS. 1999. Catálogo de Orthoptera (Insecta) de Chile. *Publicación Ocasional, Museo Nacional de Historia Natural (Chile)* **54:** 1–60.

Excoffier L, Lischer HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10:** 564–567.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147:** 915–925.

Gorochov AV. 1999. A new species of *Cratomelus* from Chile (Orthoptera: Anostostomatidae: Cratomelinae). *Zoosystematica Rossica* **8:** 242.

Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 968–971.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95–98.

Hewitt GM. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405:** 907–913.

Hewitt GM. 2004. The structure of biodiversity – insights from molecular phylogeography. *Frontiers in Zoology* **1:** 4.

Himes CMT, Gallardo MH, Kenagy GJ. 2008. Historical biogeography and post-glacial recolonization of South American temperate rain forest by the relictual marsupial *Dromiciops gliroides*. *Journal of Biogeography* **35:** 1415–1424.

Jaksić FM, Jiménez JE, Medel RG, Marquet PA. 1990. Habitat and diet of Darwin's fox (*Pseudalopex fulvipes*) on the Chilean mainland. *Journal of Mammalogy* **71:** 246–248.

Jankowski JE, Robinson SK, Levey DJ. 2010. Squeezed at the top: interspecific aggression may constrain elevational ranges in tropical birds. *Ecology* **91:** 1877–1884.

Jimenez JE, Marquet PA, Medel RG, Jaksić FM. 1990. Comparative ecology of Darwin's fox (*Pseudalopex fulvipes*) in mainland and island settings of southern Chile. *Revista Chilena de Historia Natural* **63:** 177–186.

Jowett T. 1986. Preparation of nucleic acids. In: Roberts DB, ed. *Drosophila: a practical approach*. Oxford: IRL Press, 275–286.

Kaya S, Çiplak B. 2016. Budding speciation via peripheral isolation: the *Psorodonotus venosus* (Orthoptera, Tettigoniidae) species group example. *Zoologica Scripta* **45:** 521–537.

Lamborot M, Eaton L. 1997. The Maipo River as a biogeographical barrier to *Liolaemus monticola* (Tropiduridae) in the mountain ranges of central Chile. *Journal of Zoological Systematics and Evolutionary Research* **35:** 105–111.

Lamborot M, Eaton L, Carrasco BA. 2003. The Aconcagua River as another barrier to *Liolaemus monticola* (Sauria: Iguanidae) chromosomal races of central Chile. *Revista Chilena de Historia Natural* **76:** 23–34.

Lefébure T, Douady CJ, Gouy M, Gibert J. 2006. Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimitation. *Molecular Phylogenetics and Evolution* **40:** 435–447.

Leigh JW, Bryant D. 2015. POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6:** 1110–1116.

Lessa EP, D'Elía G, Pardiñas UF. 2010. Genetic footprints of late Quaternary climate change in the diversity of Patagonian-Fueguian rodents. *Molecular Ecology* **19:** 3031–3037.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25:** 1451–1452.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27:** 209–220.

Miller MP. 2005. Alleles In Space (AIS): computer software for the joint analysis of interindividual spatial and genetic information. *Journal of Heredity* **96:** 722–724.

Morgan-Richards M, Trewick SA, Wallis GP. 2001. Chromosome races with Pliocene origins: evidence from mtDNA. *Heredity* **86:** 303–312.

Morrone JJ. 2006. Biogeographic areas and transition zones of Latin America and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna. *Annual Review of Entomology* **51:** 467–494.

Mortyn P, Charles C, Ninnemann U, Ludwig K, Hodell D. 2003. Deep sea sedimentary analogs for the Vostok ice core. *Geochemistry, Geophysics, Geosystems* **4:** 1525–2027.

Muñoz-Ramírez CP, Unmack PJ, Habit E, Johnson JB, Cussac VE, Victoriano P. 2014. Phylogeography of the ancient catfish family Diplomystidae: biogeographic, systematic, and conservation implications. *Molecular Phylogenetics and Evolution* **73:** 146–160.

Muñoz-Ramírez C, Victoriano P, Habit E. 2015. Inter-basin dispersal through irrigation canals explains low genetic structure in *Diplomystes cf. chilensis*, an endangered freshwater catfish from Central Chile. *Limnologica* **53:** 10–16.

Nei M, Kumar S. 2000. *Molecular evolution and phylogenetics*. Oxford: Oxford University Press.

Nespolo RF, Artacho P, Castañeda LE. 2007. Cyclic gas-exchange in the Chilean red cricket: inter-individual variation

and thermal dependence. *The Journal of Experimental Biology* **210:** 668–675.

Nevo E, Naftali G, Guttman R. 1975. Aggression patterns and speciation. *Proceedings of the National Academy of Sciences of the United States of America* **72:** 3250–3254.

Palma RE, Boric-Bargetto D, Torres-Pérez F, Hernández CE, Yates TL. 2012. Glaciation effects on the phylogeographic structure of *Oligoryzomys longicaudatus* (Rodentia: Sigmodontinae) in the southern Andes. *PLoS One* **7:** e32206.

Palma RE, Rivera Milla E, Salazar Bravo J, Torres Pérez F, Pardiñas UFJ, Marquet PA, Spotorno Oyarzún ÁE, Meynard AP, Yates TL. 2005. Phylogeography of *Oligoryzomys longicaudatus* (Rodentia: Sigmodontinae) in temperate South America. *Journal of Mammalogy* **86:** 191–200.

Papadopoulou A, Anastasiou I, Vogler AP. 2010. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution* **27:** 1659–1672.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

Pratt RC, Morgan-Richards M, Trewick SA. 2008. Diversification of New Zealand weta (Orthoptera: Ensifera: Anostostomatidae) and their relationships in Australasia. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363:** 3427–3437.

Puillandre N, Lambert A, Brouillet S, Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* **21:** 1864–1877.

Rambaut A. 2008. *FigTree v112*. Available at: http://treebioedacuk/software/figtree

Rambaut A, Drummond AJ. 2009. *Tracer v1.5.0*. Available at: http://beast.bio.ed.ac.uk/Tracer

Rodríguez-Serrano E, Cancino R, Palma RE. 2006. Molecular phylogeography of *Abrothrix olivaceus* (Rodentia: Sigmodontinae) in Chile. *Journal of Mammalogy* **87:** 971–980.

Ruzzante DE, Walde SJ, Cussac VE, Dalebout ML, Seibert J, Ortubay S, Habit E. 2006. Phylogeography of the Percichthyidae (Pisces) in Patagonia: roles of orogeny, glaciation, and volcanism. *Molecular Ecology* **15:** 2949–2968.

Sallaberry-Pincheira N, Garin CF, González-Acuña D, Sallaberry MA, Vianna JA. 2011. Genetic divergence of Chilean long-tailed snake (*Philodryas chamissonis*) across latitudes: conservation threats for different lineages. *Diversity and Distributions* **17:** 152–162.

Sérsic AN, Cosacov A, Cocucci AA, Johnson L, Pozner R, Avila LJ, Sites JW, Morando M. 2011. Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. *Biological Journal of the Linnean Society* **103:** 475–494.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87:** 651–701.

Singer BS, Ackert RP, Guillou H. 2004. $^{40}Ar/^{39}Ar$ and K–Ar chronology of Pleistocene glaciations in Patagonia. *Geological Society of America Bulletin* **116:** 434–450.

Song H, Moulton MJ, Whiting MF. 2014. Rampant nuclear insertion of mtDNA across diverse lineages within Orthoptera (Insecta). *PLoS One* **9:** e110508.

Sunnucks P, Hales DF. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution* **13:** 510–524.

Torres-Pérez F, Lamborot M, Boric-Bargetto D, Hernández C, Ortiz JC, Palma R. 2007. Phylogeography of a mountain lizard species: an ancient fragmentation process mediated by riverine barriers in the *Liolaemus monticola* complex (Sauria: Liolaemidae). *Journal of Zoological Systematics and Evolutionary Research* **45:** 72–81.

Trewick SA. 2008. DNA barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). *Cladistics* **24:** 240–254.

Trewick SA, Morgan-Richards M. 2004. Phylogenetics of New Zealand's tree, giant and tusked weta (Orthoptera: Anostostomatidae): evidence from mitochondrial DNA. *Journal of Orthoptera Research* **13:** 185–196.

Trewick SA, Wallis GP, Morgan-Richards M. 2000. Phylogeographical pattern correlates with Pliocene mountain building in the alpine scree weta (Orthoptera, anostostomatidae). *Molecular Ecology* **9:** 657–666.

Turchetto-Zolet AC, Pinheiro F, Salgueiro F, Palma-Silva C. 2013. Phylogeographical patterns shed light on evolutionary process in South America. *Molecular Ecology* **22:** 1193–1213.

Unmack PJ, Bennin AP, Habit EM, Victoriano PF, Johnson JB. 2009. Impact of ocean barriers, topography, and glaciation on the phylogeography of the catfish *Trichomycterus areolatus* (Teleostei: Trichomycteridae) in Chile. *Biological Journal of the Linnean Society* **97:** 876–892.

Vásquez M, Torres-Pérez F, Lamborot M. 2007. Genetic variation within and between four chromosomal races of *Liolaemus monticola* (Tropiduridae) in Chile. *Herpetological Journal* **17:** 149–160.

Vera-Escalona I, D'Elia G, Gouin N, Fontanella F, Muñoz C, Sites J, Victoriano P. 2012. Lizards on ice: evidence for multiple refugia in *Liolaemus pictus* (Liolaemidae) during the last glacial maximum in the Southern Andean beech forests. *PLoS One* **7:** e48358.

Vianna JA, Medina-Vogel G, Chehébar C, Sielfeld W, Olavarría C, Faugeron S. 2011. Phylogeography of the Patagonian otter *Lontra provocax*: adaptive divergence to marine habitat or signature of southern glacial refugia? *BMC Evolutionary Biology* **11:** 53.

Victoriano PF, Ortiz JC, Benavides E, Adams BJ, Sites JW Jr. 2008. Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata: Tropiduridae) from the central-southern Andean range. *Molecular Ecology* **17:** 2397–2416.

Victoriano PF, Vera I, Olmos V, Dib M, Inzunza B, Muñoz-Ramírez C, Montoya R, Jara A, Habit E. 2012. Patrones idiosincráticos de diversidad genética de peces nativos del Río San Pedro (Cuenca del Río Valdivia), un sistema de la región glaciada del sur de Chile. *Gayana* **76:** 71–85.

Wallis GP, Waters JM, Upton P, Craw D. 2016. Transverse Alpine speciation driven by glaciation. *Trends in Ecology & Evolution* **31:** 916–926.

Xia X, Xie Z. 2001. DAMBE: software package for data analysis in molecular biology and evolution. *Journal of Heredity* **92:** 371–373.

Xia X, Xie Z, Salemi M, Chen L, Wang Y. 2003. An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* **26:** 1–7.

Xu J, Pérez-Losada M, Jara CG, Crandall KA. 2009. Pleistocene glaciation leaves deep signature on the freshwater crab *Aegla alacalufi* in Chilean Patagonia. *Molecular Ecology* **18:** 904–918.

Zemlak TS, Habit EM, Walde SJ, Carrea C, Ruzzante DE. 2010. Surviving historical Patagonian landscapes and climate: molecular insights from *Galaxias maculatus*. *BMC Evolutionary Biology* **10:** 67.

Zúñiga-Reinoso A, Jerez V, Avaria-Llautureo J, Hernández CE. 2016. Consequences of the Last Glacial Maximum on *Nyctelia confusa* (Coleoptera: Tenebrionidae) in Patagonia. *Biological Journal of the Linnean Society* **117:** 705–715.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Histograms resulting from the species delineation analyses depicting pairwise genetic distance between individuals. Note that the histograms are bimodal for all three types of genetic distances, and the gap between modes would indicate the gap between intraspecific (left mode) and interspecific (right mode) pairwise comparisons.

Figure S2. Relationship between genetic diversity (π and *Hd*) and latitude, calculated for each locality of *Cratomelus armatus*. A, B, results using all localities. C, D, results excluding localities with admixed lineages. All these results included only localities with at least five individuals.

Figure S3. Outlier analyses for genetic diversity (π) for populations of *Cratomelus armatus*.

Figure S4. Relationship between genetic distance and latitude, calculated for southern areas of *Cratomelus armatus*.

Figure S5. Bayesian skyline plot of estimated effective population size (logarithmic scale) across time (years) for the north (A) and south (B) lineages of *Cratomelus armatus*. The black continuous lines represent the median effective population size. The grey shaded areas are the 95% high posterior density (HPD) intervals. The dashed vertical lines represent the approximated time span of the OIS 6 event, one of the most extensive glaciations in the past 400 kya (Mortyn *et al.*, 2003).