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Consequences of the last glacial maximum on *Nyctelia confusa* (Coleoptera: Tenebrionidae) in Patagonia

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The Last Glacial Maximum (LGM) has affected the population size and spatial distribution of a number of organisms in southern Patagonia. It has been hypothesized that species were able to persist in isolated refuges, which has generated processes of population expansion and genetic structure of populations after the LGM. In the present study, we evaluate these hypotheses and their association with local morphotypes in the endemic species *Nyctelia confusa*, a coleopteran that has low vagility and restricted distribution in the region. Accordingly, sixty-nine specimens were sequenced for the gene for mitochondrial cytochrome *c* oxidase I. Effective population size was estimated through time, along with population structure and the phylogenetic signal of the morphs. The results suggest the existence of recent population expansion (10 Kyr BP), although there was no evidence of population structure or the phylogenetic signal for the described morphs. We propose that, during the LGM, *N. confusa* survived in multiple refuges, probably in the oriental slopes of the Andes range. The surviving populations would have expanded once the steppe was re-established after the glaciers receded. This may have produced various secondary contact zones, homogenizing the genetic diversity, which would explain the observed pattern of panmixia. The morphological differentiation reported previously may be a result of local ecological adaptation not associated with the historical events of the LGM. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **117**, 705–715.

ADDITIONAL KEYWORDS: cytochrome *c* oxidase I – panmixia – phylogenetic signal – phylogeography – Pleistocene.

INTRODUCTION

In the southern extreme of South America (Patagonia), the landscapes have been modified by historical geological and climatic events, such as the Pleistocene glaciations that likely affected the southern part of the region. During the Last Glacial Maximum (LGM; between 20 and 14 kya), the western portion (McCulloch *et al.*, 2000) and part of the eastern portion (Glasser *et al.*, 2008) of this area were completely covered by ice, which strongly affected the local populations of terrestrial plants and animals. This determined the spatial and temporal distribution of the organisms, modulated diversity patterns by processes of genetic differentiation, and has left signals in the genetic variability of the populations (Avise, 2000; Hewitt, 2000; Sérsic *et al.*, 2011).

As a result of the environmental instability to which the austral terrestrial biota was subject during the LGM, on the western slopes, the spatial distribution of organisms was restricted to isolated refuges that were not covered by ice during this period (Sérsic *et al.*, 2011). As a result, these glacial events may have reduced the population sizes of the species, producing a decrease in their genetic diversity. The response of the biota to these historical events has been widely documented for some organisms at lower latitudes (mainly in the most northerly extent of the ice tongue during the LGM), where the

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advance and retreat of the ice determined the microevolutionary history of various population lineages (Beheregaray, 2008). For example, in *Liolaemus monticola* (Tropiduridae), the increase in river flows as a result of the retreat of the glaciers during the Pleistocene produced geographical barriers that would explain the population structuring and differentiation of lineages in the Maipo River in Chile (Torres-Pérez *et al.*, 2007; Vásquez, Torres-Pérez & Lamborot, 2007). Evidence has also been found of multiple localities in Chile that served as refuges for some plant and animal lineages during the LGM, which generated events of post-glacial recolonization (Premoli, Kitzberger & Veblen, 2000; Muellner *et al.*, 2005; Palma *et al.*, 2005; Himes, Gallardo & Kenagy, 2008; Victoriano *et al.*, 2008). Although, in Patagonia, phylogeographical studies are still scarce (Beheregaray, 2008; Sérsic *et al.*, 2011), it has been proposed that the biota currently found in this region would be the sink of a biota from lower latitudes as a result of recolonization after the LGM (Muellner *et al.*, 2005; Rodríguez-Serrano, Cancino & Palma, 2006). However, recently Jakob, Martinez-Meyer & Blattner (2009) and Tremetsberger *et al.* (2009), working with plants of the genera *Hordeum* and *Hypochaeris*, respectively, suggested that the species of the extreme south probably persisted in the zone even during the LGM and, from there, began to expand to other latitudes. On the other hand, with respect to the insects, there is no evidence from the phylogeographical perspective concerning the effects that these events have had in southern South America.

Many of the coleopterans that inhabit Patagonia are good study models because they present high degree of endemism and limited vagility. One of the most abundant groups is the family Tenebrionidae, which has a large number of genera and species with restricted distribution in the Patagonian steppes (Flores, 1997). The special and temporal magnitude of the Pleistocene glaciations in the austral region must have had a great influence on the insects in the area, modelling of microevolutionary processes and the distribution patterns of the current insect fauna.

The darkling beetle *Nyctelia confusa* Zúñiga-Reinoso is a species endemic to austral Patagonia. It distribution is limited to extreme south-western Chile in sectors around the Torres del Paine National Park (northern limit), Puerto Natales (southern limit), and Río Turbio in Argentina (eastern limit) (Zúñiga-Reinoso & Jerez, 2012). Thus, the distribution range of this species concurs with the endemism area called Meridian Sub-Andean Patagonia proposed by Domínguez *et al.* (2006). However, in this small distribution range, *N. confusa* has two morphological variants with different locations areas: (1) the northern variety, with more elevated elytra and shallow striation, found mainly in the sector of Sierra Baguales and in the Torres del Paine National Park, limited to the south by the Río de las Chinas (Fig. 1, yellow and red sampling localities), and (2) the variety with flatter elytra and deep striation, found from the Cerro Castillo to Puerto Natales and Río Turbio (Fig. 1, blue and green sampling localities). These forms are separated by the river systems of the Río de las Chinas watershed, which fragments the landscape into north–south sections in the last part of its trajectory (Fig. 1, red and green + blue zones). The disjunct distribution of these morphological variants may thus be evidence of the post-LGM consequences that structured these populations (Zúñiga-Reinoso & Jerez, 2012).

Based on the restricted distribution of *N. confusa* associated with an area that was highly unstable in the past, completely covered by ice in the LGM, and later fragmented by rivers and floods (McCulloch *et al.*, 2000; Solari *et al.*, 2012), we propose two hypotheses. (1) The persistence of *N. confusa* during the Pleistocene events is a result of the existence of isolated ice-free refuges, which would have affected its effective population size and thus the species should present signals of recent demographic expansion. (2) The two morphological variants that currently exist were generated by geographical population disjunction produced by the river, resulting in two genetically structured evolutionary lineages represented by the two existing morphs, which would be determined by ancestor-descendent relations (i.e. phylogenetic signal of morphs).

MATERIAL AND METHODS

Collection of individuals and selection of study sites

Specimens of *N. confusa* were collected manually from the entire distribution area of the species (Fig. 1). Sampling localities were chosen using the following criteria: (1) published localities (Kulzer, 1963; Peña, 1963; Vidal & Guerrero, 2007; Zúñiga-Reinoso & Jerez, 2012) and those in reference entomological collections (Instituto de la Patagonia, Museo Nacional de Historia Natural, Museo de Zoología de la Universidad de Concepción, Universidad Metropolitana de Ciencias de la Educación, Instituto Argentino de Investigaciones de Zonas Áridas) and (2) localities on both sides of the Río de las Chinas watershed. The sample units were divided using a criterion of possible geographical barriers, assigning a code to each: PAI (Torres del Paine), BAG (Sierra Baguales), NAT (Puerto Natales), and TUR (Río Turbio). Figure 1 and Table 1 provide details of the

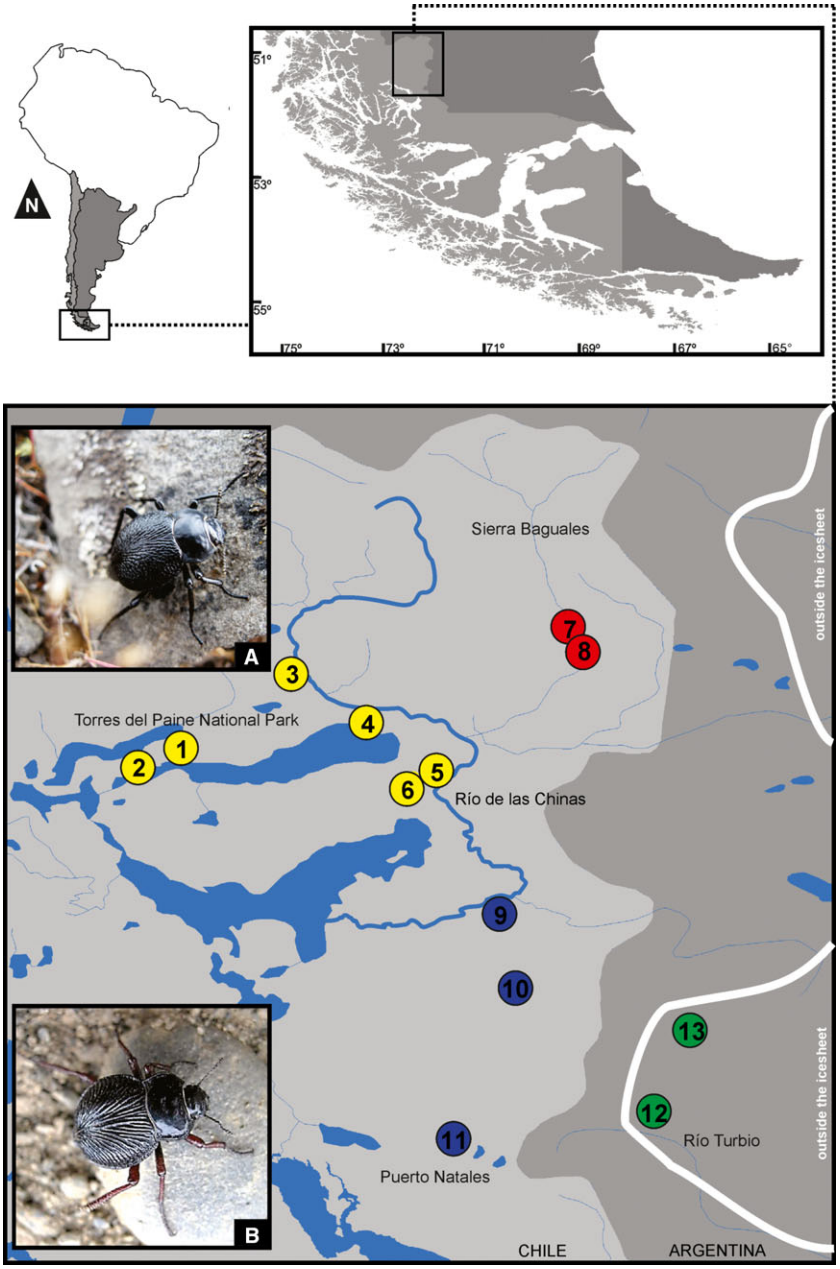

Figure 1. Map of the study zone, which included the entire distribution range of *Nyctelia confusa*. The white line indicates the limit East of ice cover during the Last Glacial Maximum (LGM) (Glasser *et al.*, 2008). Current rivers are indicated in blue; sampling localities are shown in circles with numbers, which are grouped into sampling units: 1–6, yellow: Torres del Paine unit ('PAI', north-west of the Río de las Chinas); 7–8, red: Sierra Baguales unit ('BAG', north-east of the Río de las Chinas); 9–11, blue: Puerto Natales unit ('NAT', south of the Río de las Chinas); 12–13, green: Río Turbio unit ('TUR', east of the Andes). A, North morpho, with distribution in yellow and red circles. B, South morpho, with distribution in green and blue circles.

geographical location and number of individuals collected in each of the sampling units. All material was preserved in 95% ethanol for later DNA extraction.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

DNA was extracted from muscles of the legs and thorax, using a modification of the salt extraction method of Jowett (1986). A fragment of 845 pb of the

Table 1. Sampling sites of *Nyctelia confusa* and distribution of haplotypes of the fragment of cytochrome *c* oxidase I analyzed in the present study

Locality code (number map)	GeneBank code	Country	Locality	Latitude (S)	Longitude (W)	Sample size	Haplotipe (copy number)
PAI 1 (1)	KT715551–KT715556	Chile	Parque Nacional Torres del Paine, Laguna de los Cisnes.	51°1′51.80″	72°52′21.50″	6	1 (1), 8 (4), 13 (1)
PAI 3 (2)	KT715557–KT715566	Chile	Parque Nacional Torres del Paine, western point of Lake Sarmiento.	51°3′2.20″	72°55′15.60″	10	5 (1), 8 (1), 12 (2), 14 (1), 15 (1), 16 (1), 17 (1), 18 (1), 19 (1)
PAI 5 (3)	KT715567–KT715576	Chile	Parque Nacional Torres del Paine, road to Laguna Azul.	50°55′51.70″	72°44′5.90″	10	1 (3), 5 (1), 8 (1), 12 (1), 18 (1), 19 (1), 20 (1), 21 (1)
PAI 6 (4)	KT715577–KT715586	Chile	Torres del Paine, Sarmiento–Amarga crossing.	50°59′8.70″	72°37′48.60″	10	1 (1), 2 (1), 4 (1), 5 (1), 7 (1), 8 (2), 22 (1), 23 (1), 24 (1)
PAI 7 (5)	KT715587–KT715591	Chile	Torres del Paine, Route 9 crosses Torres del Paine.	51°3′34.20″	72°32′36.50″	5	1 (1), 2 (1), 8 (2), 12 (1)
PAI 8 (6)	KT715592	Chile	Estancia El Lazo, Route Y-180, road to Estancia El Lazo.	51°4′20.90″	72°34′14.40″	1	25 (1)
BAG 2 (7)	KT715531–KT715532	Chile	Sierra Baguales, entrance to Estancia Sierra Leona.	50°53′16.30″	72°21′21.70″	2	1 (2)
BAG 3 (8)	KT715533–KT715537	Chile	Sierra Baguales, 1 km from entrance to Estancia Sierra Leona.	50°53′49.10″	72°20′56.80″	5	2 (1), 3 (1), 4 (1), 5 (1), 6 (1)
NAT 2 (9)	KT715538–KT715543	Chile	Cerro Castillo, road to Bahía El Bote.	51°14′6.90″	72°27′32.40″	6	1 (2), 2 (1), 7 (1), 8 (1), 9 (1)
NAT 4 (10)	KT715544–KT715547	Chile	Laguna Sofía, route 9 crossing to Laguna Sofía	51°32′34.20″	72°30′46.10″	4	1 (1), 4 (1), 5 (1), 7 (1)
NAT 5 (11)	KT715548–KT715550	Chile	Cueva del Milodón, close to Silla del Diablo.	51°34′25.70″	72°36′0.60″	3	10 (1), 11 (1), 12 (1)
TUR 1 (12)	KT715593–KT715596	Argentina	RíoTurbio, route 40, 11 km north of RíoTurbio	51°30′24.50″	72°15′21.00″	4	1 (1), 5 (1), 8 (1), 26 (1)
TUR 3 (13)	KT715597–KT715599	Argentina	Cancha Carrera, route 40, 15 km south of Cancha Carrera	51°23′19.30″	72°12′15.60″	3	1 (1), 2 (1), 4 (1)

PAI, north-west of the Río de las Chinas; BAG, north-east of the Río de las Chinas; NAT, south of the Río de las Chinas; TUR east of the Andes.

mitochondrial cytochrome *c* oxidase I (COI) was amplified, using the primers C1-J-2183 ('Jerry': CAA CATTTATTTTGATTTTTTGG) and TL2-N-3014 ('Pat': TCCAATGCACTAATCTGCCATATTA). The reaction mixture included 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM each primer, 1 U of Taq polymerase (Invitrogen), and 50–100 ng total DNA. The thermal polymerase chain reaction (PCR) profile was 94 °C for 2 min, followed by 36 cycles of 94 °C for 30 s, 56 °C for 45 s, and 72 °C for 90 s, with a final extension at 72 °C for 10 min. The PCR products were purified and sequenced by Macrogen Inc.. The DNA sequences of each individual were reviewed, edited, and then aligned using CLUSTAL W (Thompson *et al.* 1994) in BIOEDIT, version 7.0.5.3 (Hall, 1999). In addition, we evaluated whether the sequences were saturated and thus useful for the phylogenetic analysis, using Xia's test implemented in DAMBE, version 5.1.5 (Xia & Xie, 2001). This is an entropy-based index that estimates a substitution saturation index (Iss) and compares it with a critical substitution saturation index (Iss.c) via a randomization process with 95% confidence intervals (Xia *et al.*, 2003).

Haplotype network and population analysis

To determine phylogenetic relations and visualize geographical patterns of genetic diversity, we constructed a haplotype network using the median joining method proposed by Bandelt, Forster & Röhl (1999) implemented in NETWORK (http://www.fluxusengineering.com/sharenet).

For each population unit, we calculated the haplotype diversity (H_D), nucleotide diversity (P_I), and the haplotype richness N_H using DNASP (Rozas *et al.*, 2003). To evaluate signals of expansion in the demographic history, we performed a mismatch analysis (Rogers & Harpending, 1992) in ARLEQUIN, version 3.5 (Excoffier & Lischer, 2010). To explore the demographic history associated with the DNA sequences, we performed a skyline plot (Pybus *et al.*, 2000) as implemented in BEAST, version 1.8 (Drummond & Rambaut, 2007). This approach is based on a genealogy inferred from DNA sequences and estimates the effective population size (N_E) over time using a Bayesian approach. The phylogenetic model selected by the Bayesian information criterion using JMODELTEST (Posada, 2008) was TPM2uf + G. The TPM2uf + G model is not implemented in BEAST; thus, the model model was over-parameterized by the GTR + G model. The analysis was performed using the uncorrelated relaxed exponential clock model, based on the selection performed in Bayes factors implemented in TRACER, version 1.4 (Drummond & Rambaut, 2007). We assumed a neutral mutation rate of 3.54% per million years estimated for Pimelinae (Tenebrionidae) (Papadopoulou, Anastasiou & Vogler, 2010). For reconstruction, we selected a priori the Bayesian skyline model based on coalescence. To estimate the parameters in the convergence zone, we ran 10 million generations, sampling every 1000 generations and discarding the first million generations as burn-in. The results were visualized in TRACER, version 1.4 (Drummond & Rambaut, 2007).

Analysis of population structure

To evaluate the effects of the palaeorivers post-LGM and the current rivers on the population structure of *N. confusa*, we used GENELAND (Guillot *et al.*, 2005). This software is based on an explicit spatial model using geographical and genetic information to estimate the number of populations in the dataset and delineate their spatial organization. Thus, it allows the detection of genetic groupings delimited by isolines of probability, whose representation in a map permits defining the number of probable populations and visualizing whether these are limited by geographical barriers. Two a priori hypotheses of structure were evaluated under a real spatial model: (1) The existence of four population units (i.e. PAI, BAG, NAT and TUR) as a result of possible geographical barriers between them and (2) two population units (i.e. PAI + BAG and NAT + TUR), corresponding to the observed morphological differences. To test these hypotheses, we performed five independent runs of five million iterations, sampling every 1000 iterations, to estimate *K* (number of populations). The five runs were post-processed, eliminating the first 5% of the iterations, obtaining the a posteriori probabilities of each individual belonging to a population and to each pixel in the spatial dominion. With this, we obtained a histogram with the a posteriori probabilities, along with the estimation of *K*. Finally, we evaluated the effect of isolation by distance using the Mantel test, incorporated in the ZT (Bonnet & Van de Peer, 2002). For this analysis, 100 000 randomizations were run, where the process chooses random sets from all the orders possible, correlating the matrix of genetic distances with the matrix of geographical distances. A significant positive correlation between the matrices indicates an effect of isolation by distance, whereas the absence of correlation would indicate that the localities have panmictic behaviour.

Phylogenetic reconstruction and evolution of the morphs

We performed a phylogenetic reconstruction with all the COI sequences, using *Nyctelia newporti*

Waterhouse, 1841 as outgroup. To construct the phylogenetic hypothesis, we used the Markov chain Monte Carlo (MCMC) method in a Bayesian framework, aiming to produce an a posteriori probability of the phylogenetic trees. Because molecular markers have different patterns and rates of nucleotide substitution, we used the general mixed model (MM) of Pagel & Meade (2004), based on the general reversible time model plus gamma distribution (GTR + G) of sequence evolution. The MM model takes into account cases in which the sites in the alignment have qualitatively different modes of evolution without the need to know these patterns a priori or to partition the data. To select the number of patterns (or GTR models), we used the MCMC method with reversible jumps (Pagel & Meade, 2008). This approximation allows the exploration of a variety of possible models and their associated parameters, converging to the model which best fits the data in the sample of trees. These analyses were performed in BayesPhylogenies, version 1.1 (http://www.evolution.rdg.ac.uk/BayesPhy.html). We used 40 million iterations of phylogenetic trees, sampling every 10 000 trees to ensure that the samples were independent. The first tree was excluded because it was outside the convergence zone of the Markov chain; thus, we obtained a sample of 3999 trees. This sample was used to estimate the phylogenetic signal of the morphs of *Nyctelia confusa* (see Supporting information, Fig. S1).

Finally, to test the hypothesis that the formation of the rivers is a historical barrier that determined the described morphs, we evaluated the phylogenetic signal of both morphs (north and south varieties) of *N. confusa*, using the statistical association index based on the method proposed by Parker, Rambaut & Pybus (2008) to measure the phylogenetic signal in discrete traits. The significance of the phylogenetic signals of the morphs was evaluated using a sample of trees obtained by Bayesian inference. This analysis was performed in BATS (Parker *et al.*, 2008) (http://zoo.ox.ac.uk/evolve/software). Additionally, aiming to confirm that the two morphs are not genetically isolated, we run two Bayesian phylogenetic analyses in BEAST, version 1.8. We used 10 million iterations of phylogenetic trees, sampling every 10 000 trees to ensure that the samples were independent. The first analysis comprised an unconstrained tree and, later, we ran a constrain tree, where all individuals of a single morph were monophyletic (constrained analysis). Subsequently, we compared the fit and marginal likelihood between the 'constrained tree' and 'unconstrained tree' to select which is the model that best fit the data.

RESULTS

Sequences information, haplotype network, and demographic history

We obtained fragments of 845 bp for the COI gene and found 48 variable sites. A total of 26 haplotypes from 69 individuals were obtained from this mitochondrial gene. Additionally, the COI sequences presented low saturation because the critical index of substitution saturation values (Iss.c = 0.756) was significantly higher than the observed index of substitution saturation values (Iss = 0. 013; $P < 0.0001$); therefore, the sequences are suitable for performing phylogenetic analyses. The haplotype network showed a star-shaped distribution, with one haplotype in high frequency and a number of rare haplotypes ($N = 11$) derived from it, and other haplotypes with high frequency ($N = 7$) but separated by a greater number of mutations (Fig. 2). The haplotypes did not show strict geographical filiation, although most of the rare haplotypes were only found in Torres del Paine (11 of 17), suggesting that they are distributed randomly in the entire haplotype network and do not form a geographically structured topology (Fig. 2).

The highest value of genetic variability of N_H was found in Torres del Paine (PAI). However, the values of genetic diversity P_i and H_d in all sampling areas (PAI, BAG, NAT and TUR) were homogeneous (Table 2). Also, the graph of the mismatch distribution showed a tendency to a unimodal curve. Based on the nonsignificant value of the raggedness index (ragg. index = 0.02, $P = 0.15$), the null hypothesis of sudden demographic expansion was not rejected (Fig. 3). Also, the reconstruction of effective population sizes over time using Bayesian skyline indicated population expansion later than 10 000 years BP, reaching a stable population size only 2000 years BP (Fig. 4).

Population structure and morphological variation

The results of the GENELAND analysis did not provide evidence for structure among the pre-established population units; it indicated one large population ($K = 1$) in the entire distribution, with almost 80% a posteriori probability (Fig. 5). The Mantel test did not indicate isolation by distance ($r = -0.023$; $P = 0.29$). Finally, the difference between the observed and expected AI index was not significant ($P = 0.49$). Adicionally, the 'unconstrained tree' showed a better fit of marginal likelihood (Lh = −1584.56) vs. the 'constrained model' (Lh = −1738.94). Thus, the morphological differences observed by Zúñiga-Reinoso & Jerez (2012) are not

Table 2. Indices of genetic diversity in sequences of mtDNA of *Nyctelia confusa*

Population parameters	BAG	PAI	NAT	TUR	ALL
Number of haplotypes (N_H)	6	20	10	6	26
Nucleotide diversity (P_I)	0.008	0.01	0.011	0.01	0.01
Haplotype diversity (H_D)	0.952	0.916	0.949	0.952	0.919

PAI, north-west of the Río de las Chinas; BAG, north-east of the Río de las Chinas; NAT, south of the Río de las Chinas; TUR' east of the Andes.

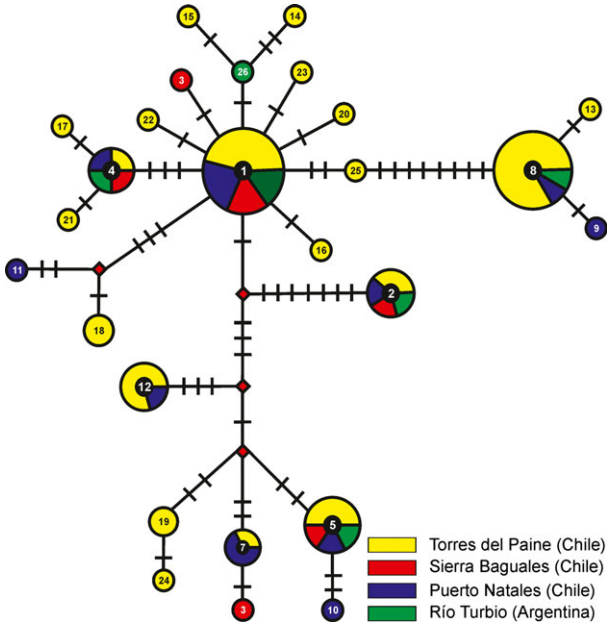

Figure 2. Median-joining haplotype network for the studied fragment of the COI gene. Each haplotype is represented by a circle, whose size is proportional to its frequency; haplotypes are identified by the numbers in the circles (Table 1). Hatches on the lines represent mutational steps. Red rhombi represent intermediate haplotypes not present in the samples; colours within the circles represent the geographical regions (sampling units) where haplotypes were found.

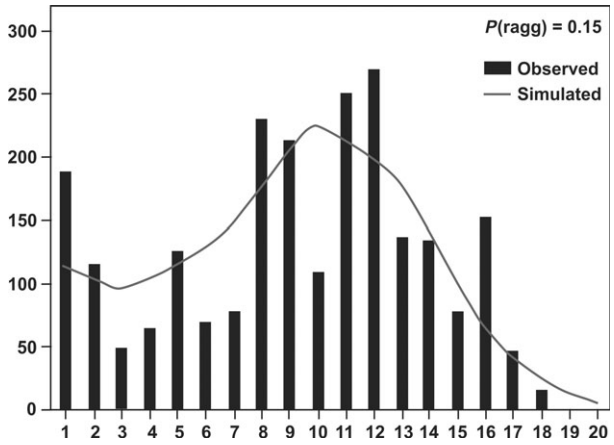

Figure 3. Frequency distribution of paired differences between all haplotypes of *Nyctelia confusa*. Black bars indicate observed frequencies and the light grey curve indicates the expected frequencies.

associated with historical processes that determined the current variability of the morphs (i.e. absence of phylogenetic signal).

DISCUSSION

The haplotype network and the diversity index did not allow the identifcation of a refuge within the current distribution range of *N. confusa*. However, because of the presence at least two haplogroups (Fig. 2), the populations of *N. confusa* survived the LGM in more than one refuge, probably to the east of the ice sheet (eastern slopes of the Andes) (Sérsic *et al.*, 2011). This makes sense if we consider the steppe-forest dynamics proposed by Villa-Martínez & Moreno (2007), according to which the last maximum expansion of the steppe would correspond temporally to the population expansion of *N. confusa*. Our results suggest that populations of this species could probably have followed the vegetation fluctuations (steppe-forest), where the last maximum expansion of the steppe (8–10 Kyr BP) gave rise to a recent population expansion of *N. confusa* (Fig. 4). The south-western portion of the province of Santa Cruz, Argentina (Fig. 1, green circles, Rio Turbio and surrounding area) appears to have been an area of greater environmental stability, free of ice during this period (McCulloch *et al.*, 2000; Glasser *et al.*, 2008). According to the proposals of Jakob *et al.* (2009) and Tremetsberger *et al.* (2009), this zone was an environmentally stable area during the LGM, where the steppe would have persisted and served as a refuge for some species plants such as *Hordeum* and *Hypochearis*. Thus, because *N. confusa* is a steppe insect, this area adjacent to the current distribution may have served as a refuge for the gene pool

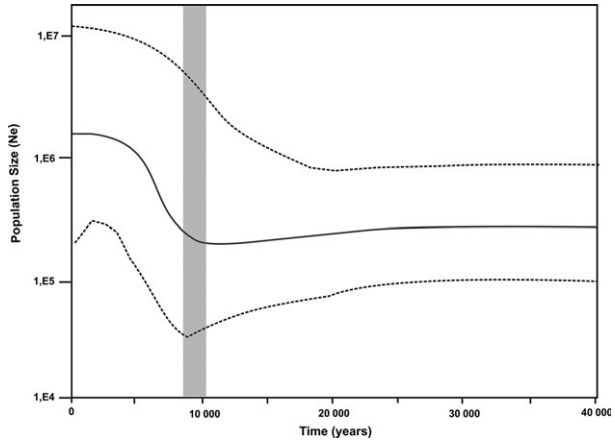

Figure 4. Reconstruction of the demographic history using the studied fragment COI gene of *Nyctelia confusa*, using Bayesian skyline. *x*-axis: reconstructed time interval in the period 0–40 thousand years BP. *y*-axis: reconstruction of population size in logarithmic scale. The solid dark grey line is the mean estimate of population size over time. The dotted lines represent the 95% highest posterior density regions. The light grey strip marks the beginning of the population expansion of *N. confusa*, approximately 8000–10 000 years BP.

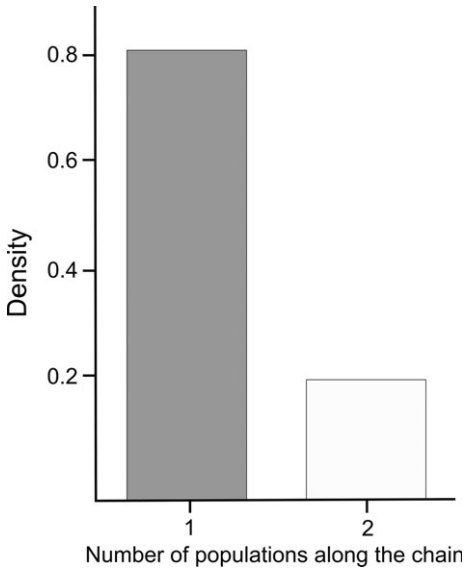

Figure 5. Histogram of probabilities for estimating the number of populations in *Nyctelia confusa* along the chains.

of the species. According to Sérsic *et al.* (2011), this zone contained low land refuges, which would have aided the colonization of new areas by these insects. For a walking insect such as *N. confusa*, a low altitude, almost flat area is ideal for the colonization of more northern sectors subsequent to the establishment of the steppe after the retreat of the ice. According to Sugden, Hulton & Purves (2002), this is a zone in which the ice retreated more rapidly as a consequence of the low altitude of the sector, retreating both in the north and in the south. This produced a separation of the two great ice masses, which gave rise to the current South Patagonia Ice Field and the Darwin Range Ice Field, between 13 000 and 8000 years BP (McCulloch *et al.*, 2000; Sugden *et al.*, 2002). This time period coincides with a period of cold climate and low humidity that favoured the establishment of pre-Andean vegetation formations and, to a lesser degree, steppe in the zone (Villa-Martínez & Moreno, 2007).

The results of GENELAND and the Mantel tests suggest that, currently, *N. confusa* behaves as a panmictic population; however, the landscape was inundated by the formation of palaeorivers and lagoons during the LGM, which divided the territory into a form different from the current situation (Glasser *et al.*, 2008; Solari *et al.*, 2012). This, when added to the rapid retreat of glaciers because of an increase in precipitation and temperature (Villa-Martínez & Moreno, 2007), may have created different fluvial systems some 10 800 years BP that would have acted as barriers to the free flow of individuals between the populations of *N. confusa*. However, once these systems disappeared, gene flow could increase; these events may have occurred repeatedly during the interglacial periods, which would have produced the current genetic homogenization of the populations. Mardulyn, Mikhailov & Pasteels (2009) proposed that a zone of secondary contact may homogenize the genetic diversity in Chrysomelidae, making it equal to the diversity of the rest of the populations. Moreover, the absence of genetic structure in mitochondrial (mt)DNA in insects could be a consequence of the maintenance of ancestral polymorphism or even the occurrence of introgressive mtDNA hybridization (Franco *et al.*, 2015), although this must be evaluated further to obtain more evidence.

Also, in contrast to species of temperate environments, species of cold climates would have had a panmictic distribution during the glacial periods and the fragmentation would occur during interglacial periods (Mardulyn *et al.*, 2009). Thus, the time of existence of the current river (Las Chinas) is probably insufficient to leave a footprint in the molecular marker used in the present study; hence, no structure was found in the distribution of the genetic diversity of *N. confusa* in a historical context. Additionally, the river could not have acted as an important physical barrier to gene flow between these populations. The dispersion capacity of the

genus *Nyctelia* may be underestimated because the observations of Peña (1963) indicate that they are good walkers on sunny days. In a number of generations (one generation per year), they could cover long distances, including the total current limited range of the species.

The panmictic population dynamics, the lack of phylogenetic signal, and the marginal likelihood of unconstrained tree of the morphological variants in *N. confusa* suggests that the morphological variability found by Zúñiga-Reinoso & Jerez (2012) would be the result of local ecological variations in the distribution range of this species, which may be a signal of phenotypic plasticity and not of historical processes associated with ancestor–descendent relations. Normally, variations at the microclimatic scales are linked to subtle morphological differences in tenebrionids (De Los Santos *et al.*, 2000); thus, the climatic gradient of the Patagonia could have modelled the morphology of *N. confusa*. According to De Los Santos *et al.* (2000), the abdomen will be larger and the elytra will be higher in the tenebrionids that inhabit more arid lands as a result of the adaptive properties that these structures confer to individuals in desert environments. Thus, the individuals of *N. confusa* that inhabit southern areas (less arid) have more compressed bodies and elytrae than individuals of the northern (more arid) areas (Zúñiga-Reinoso & Jerez, 2012). These results suggest that it is necessary to review the origin of the morphological variation of insects in desert and semi-desert environments instead of assigning a priori a taxonomic or ecological interpretation to this variation.

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REFERENCES

Avise JC. 2000. *Phylogeography. The history and formation of species*. Cambridge: Hardvard University Press.

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.

Bonnet E, Van de Peer Y. 2002. zt: a software tool for simple and partial Mantel tests. *Journal of Statistical Software* **7:** 1–12.

De Los Santos A, Gómez-González L, Alonso C, Arbelo C, De Nicolas J. 2000. Adaptive trends of darkling beetles (Col. Tenebrionidae) on environmental gradients on the island of Tenerife (Canary Islands). *Journal of Arid Environment* **45:** 85–98.

Domínguez M, Roig-Juñet S, Tassin J, Ocampo F, Flores G. 2006. Areas of endemism of the Patagonian steppe: an approach based on insect distributional patterns using endemicity analysis. *Journal of Biogeography* **33:** 1527–1537.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214–221.

Excoffier L, Lischer HEL. 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.

Flores GE. 1997. Revisión de la tribu Nycteliini (Coleoptera: Tenebrionidae). *Revista de la Sociedad Entomológica Argentina* **56:** 1–19.

Franco FF, Lavagnini TC, Sene FM, Manfrin MH. 2015. Mito-nuclear discordance with evidence of shared ancestral polymorphism and selection in cactophilic species of *Drosophila*. *Biological Journal of the Linnean Society* **116:** 1095–8312.

Glasser N, Harrison S, Jansson K, Kleman J. 2008. The glacial geomorphology and Pleistocene history of southern South America between 38°S and 56°S. *Quaternary Science Reviews* **27:** 365–390.

Guillot G, Mortier F, Estoup A, 2005. GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes* **5:** 708–711.

Himes C, Gallardo M, Kenagy G. 2008. Historical biogeography and post-glacial recolonization of South American temperate rain forest by the relictual marsupial *Dromiciops gliroides*. *Journal of Biogeography* **30:** 1415–1424.

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

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

Himes C, Gallardo M, Kenagy G. 2008. Historical biogeography and post-glacial recolonization of South American

temperate rain forest by the relictual marsupial *Dromiciops gliroides*. *Journal of Biogeography* **30:** 1415–1424.

Jakob S, Martinez-Meyer E, Blattner F. 2009. Phylogeographic analyses and paleodistribution modeling indicate Pleistocene *in situ* survival of *Hordeum* species (Poaceae) in Southern Patagonia without genetic or spatial restriction. *Molecular Biology and Evolution* **26:** 907–923.

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

Kulzer H. 1963. Revision der südamerikanischen Gattung *Nyctelia* Latr. (Col. Teneb.) (24 Beitrag zur Kenntnis der Tenebrioniden). *Entomologische Arbeiten aus dem Museum George Frey* **14:** 1–171.

Mardulyn P, Mikhailov YE, Pasteels JM. 2009. Testing phylogeographic hypotheses in a Euro-Siberian cold-adapted leaf beetle with coalescent simulations. *Evolution* **63:** 2717–2729.

McCulloch R, Bentley M, Purves R, Hulton N, Sugden D, Clapperton C. 2000. Climatic inferences from glacial and palaeoecological evidence at the last glacial termination, southern South America. *Journal of Quaternary Science* **15:** 409–417.

Muellner AN, Tremetsberger K, Stuessy T, Baeza CM. 2005. Pleistocene refugia and recolonization routes in southern Andes: insights from *Hypochaeris palustris* (Asteraceae, Lactuceae). *Molecular Ecology* **14:** 203–212.

Pagel M, Meade A. 2004. A phylogenetic mixture model for detecting pattern heterogeneity in gene sequence or character-state data. *Systematic Biology* **53:** 571–581.

Pagel M, Meade A. 2008. Modelling heterotachy in phylogenetic inference by reversible-jump Markov chain Monte Carlo. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **363:** 3955–3964.

Palma R, Rivera-Milla E, Salazar-Bravo J, Torres-Perez F, Pardinas U, Marquet P, Spotorno A, Meynard A, Yates T. 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.

Parker J, Rambaut AR, Pybus OG. 2008. Correlating viral phenotypes with phylogeny: accounting for phylogenetic uncertainty. *Infection, Genetics and Evolution* **8:** 239–246.

Peña L. 1963. Las *Nyctelia* (Coleoptera, Tenebrionidae). *Entomologische Arbeiten aus dem Museum George Frey* **9:** 72–75.

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

Premoli A, Kitzberger T, Veblen T. 2000. Isozyme variation and recent biogeographical history of the long-lived conifer *Fitzroya cupressoides*. *Journal of Biogeography* **27:** 251–260.

Pybus OG, Rambaut A, Harvey PH. 2000. An integrated framework for the inference of viral population history from reconstructed genealogies. *Genetics* **155:** 1429–1437.

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

Rogers AR, Harpending HC. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9:** 552–569.

Rozas J, Sánchez-DelBarrio JC, Messegyer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19:** 2496–2497.

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

Solari MA, Le Roux JP, Hervé JP, Airo F, Calderón M. 2012. Evolution of the Great Tehuelche Paleolake in the Torres del Paine National Park of Chilean Patagonia during the Last Glacial Maximum and Holocene *Andean*. *Geology* **39:** 1–21.

Sugden D, Hulton N, Purves R. 2002. Modelling the inception of the Patagonian icesheet. *Quaternary Internatinal* **96:** 55–64.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4673–4680.

Torres-Pérez F, Lamborot M, Boric-Bargetto D, Hernández C, Ortiz JC, Palma RE. 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.

Tremetsberger K, Urtubey E, Terrab A, Baeza CM, Ortiz MA, Talavera M, König C, Temsch EM, Kohl G, Talavera S, Stuessy TF. 2009. Pleistocene refugiaand polytopic replacement of diploids by tetraploids in the Patagonian and Subantarctic plant Hypochaerisincana (Asteraceae, Cichorieae). *Molecular Ecology* **18:** 3668–3682.

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.

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

Vidal P, Guerrero M. 2007. *Los Tenebriónidos de Chile*. Santiago: Ediciones Universidad Católica de Chile.

Villa-Martínez R, Moreno P. 2007. Pollen evidence for variations in the southern margin of the westerly winds in SW Patagonia over the last 12,600 years. *Quaternary Research* **68:** 400–409.

Xia X, Xie Z. 2001. DAMBE: 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 Phylogenetic and Evolution* **26:** 1–7.

Zúñiga-Reinoso A, Jerez V. 2012. Revisión del estado taxonómico de *Nyctelia multicristata* Blanchard, 1846 y descripción de *Nyctelia confusa* Zuñiga-Reinoso n. sp. (Coleoptera: Tenebrionidae). *Gayana* **76:** 38–45.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Phylogenetic tree used to estimate the phylogenetic signal from the morph of *Nyctelia confusa*. The coloured squares represent the geographical origin of the samples (Fig. 1).