Journal of Biogeography (J. Biogeogr.) (2012) 39, 1041–1057

ORIGINAL ARTICLE

Phylogeography and palaeodistribution modelling in the Patagonian steppe: the case of *Mulinum spinosum* (Apiaceae)

Silvana M. Sede[1]*, Marcela V. Nicola[1], Raúl Pozner[1] and Leigh A. Johnson[2]

[1]*Instituto de Botánica Darwinion, C.C. 22, B1642HYD San Isidro, Provincia de Buenos Aires, Argentina,* [2]*Department of Biology and Bean Life Science Museum, 401 WIDB, Brigham Young University, Provo, UT 84602, USA*

ABSTRACT

Aim An integrative study of the endemic, yet ubiquitous, Patagonian shrub *Mulinum spinosum* (Apiaceae) was performed: (1) to assess the historical processes that influenced its geographical pattern of genetic variation; (2) to test hypotheses of its survival *in situ* or in glacial refugia during glacial cycles; and (3) to model its extant and palaeoclimatic distributions to assess support for the phylogeographical patterns recovered.

Location Chilean and Argentinian Andean region and Patagonian steppe.

Methods Chloroplast DNA sequences, *trn*H–*psb*A, *trn*S–*trn*G and 3′*trn*V–*ndh*C, were obtained for 314 individuals of *M. spinosum* from 71 populations. The haplotype data matrix was analysed using nested clade analysis (NCA) to construct a network. Analysis of molecular variance (AMOVA), spatial analysis of molecular variance (SAMOVA) and neutrality tests were also used to test for genetic structure and range expansion in the species. The present potential geographical distribution of *M. spinosum* was modelled and projected onto a Last Glacial Maximum (LGM) model.

Results Amongst the 29 haplotypes observed, one was widely distributed, but most were restricted to either northern or southern regions. The populations with highest haplotype diversity were found in southern Patagonia, the high Andean region, and northern Patagonia. AMOVA and SAMOVA showed latitudinal structure for Argentinian populations. NCA implied patterns of restricted gene flow or dispersal but with some long-distance dispersal and also long-distance colonization and/or past fragmentation. Neutrality tests did not support range expansions. The current distribution model was a fairly good representation of the extant geographical distribution of the species, and the distribution model for the LGM did not show important shifts of the extant range to lower latitudes, except for a shift towards the palaeoseashore.

Main conclusions Based on agreement amongst phylogeographical patterns, distribution of genetic variability, equivocal evidence of putative refugia and palaeodistribution modelling, it is probable that glaciations did not greatly affect the distribution of *Mulinum spinosum*. Our results are consistent with the *in situ* survival hypothesis, and not with the latitudinal migration of plant communities to avoid adverse climate conditions during Pleistocene glaciations. It is possible that populations of northern Patagonia may have been isolated from the southern ones by the Chubut and Deseado basins.

Keywords

Distribution modelling, Last Glacial Maximum, *Mulinum spinosum*, neneo, Patagonian steppe, phylogeography, southern South America.

*Correspondence: Silvana M. Sede, Instituto de Botánica Darwinion, C.C. 22, B1642HYD San Isidro, Provincia de Buenos Aires, Argentina. E-mail: ssede@darwin.edu.ar

Journal of Biogeography

INTRODUCTION

The present Patagonian biota has been shaped by two major geological and climatic events: the Andean orogeny during the middle–late Miocene (15–10 Ma), and the Pleistocene glaciations. The Andean orogeny introduced an important rain shadow to the east, producing a cool, dry climate across the Patagonian steppe, together with the contraction of southern South American forests and the expansion of xerophytic-adapted taxa (Barreda & Palazzesi, 2007). Although Patagonian glaciations initiated in the late Miocene, it was during the early Pleistocene when the Patagonian ice sheet reached its full development (Rabassa, 2008). The most important glacial advances in southern South America were the Greatest Patagonian Glaciation (GPG; 1–1.2 Ma) and the Last Glacial Maximum (LGM; 21 ka). The GPG resulted in the maximum expansion of ice sheets in the Patagonian steppe, compared with the LGM and other Pleistocene glaciations (Rabassa, 2008). However, substantial areas remained free of ice during the glacial episodes and climatic conditions were less severe than those in North America (Markgraf *et al.*, 1995; Rabassa, 2008).

Fluctuating Pleistocene climates and their associated glacial cycles caused great changes in species distributions (Hewitt, 2004). In Patagonia, changes in sea level produced by glaciations partially exposed the Argentinian submarine platform to the east, substantially increasing the space for animal and plant colonization (Rabassa, 2008). Vegetation changes occurred rapidly during these events in South America (Markgraf *et al.*, 1995), with large latitudinal displacements of the major ecosystems during glaciations (Rabassa & Coronato, 2009). Increased aridization of the surrounding areas, with rising extreme temperatures, decreased precipitation and lack of the moderating effect of the sea as the coastline moved eastwards (Rabassa & Coronato, 2009) influenced vegetation at higher latitudes in the Southern Hemisphere even though glaciers did not encroach into these areas (Jakob *et al.*, 2009). Despite this climatic and orogenetic knowledge of the recent past, Patagonia is amongst the least phylogeographically studied areas of southern South America (Sérsic *et al.*, 2011).

Patagonia is currently partitioned into two main ecoregions. The Andean–Patagonian forest ecoregion is mountainous, cold, humid and bordered on the east by the Patagonian steppe ecoregion. The Patagonian steppe extends eastward to the Atlantic Ocean and is mostly low-lying, cold, dry and characterized by scattered herbs and shrubs (Cabrera & Willink, 1973; León *et al.*, 1998). On the eastern side of the Andes (Argentina), Patagonia extends from Neuquén and Río Negro provinces (delimited to the north by the Neuquén and Colorado rivers, *c.* 36–37° S) to Cape Horn (56° S). On the western side of the Andes, Chilean Patagonia extends from 43° S (Palena province) to the XI (Aysén) and XII (Magallanes) administrative regions (Coronato *et al.*, 2008). While Patagonian phylogeographical studies have increased in recent years, such publications have focused mainly on the Andean region (e.g. Premoli *et al.*, 2000; Pastorino & Gallo, 2002; Cussac *et al.*, 2004; Muellner *et al.*, 2005; Marchelli & Gallo, 2006; Ruzzante *et al.*, 2006; Himes *et al.*, 2008; Tremetsberger *et al.*, 2009; Xu *et al.*, 2009; Acosta & Premoli, 2010; Mathiasen & Premoli, 2010), with only a few studies addressing patterns in the vast area of the Patagonian steppe (Kim *et al.*,1998; Morando *et al.*, 2004, 2007; Ávila *et al.*, 2006; Jakob *et al.*, 2009; Cosacov *et al.*, 2010; Lessa *et al.*, 2010). As part of a large, inclusive phylogeographical investigation of both the Andean forests and the steppe ecoregions of Patagonia, we selected an endemic shrub of the Patagonian steppe and Andean Patagonian region of Argentina and Chile: *Mulinum spinosum* (Cav.) Persoon (Apiaceae). *Mulinum spinosum* is the most widespread *Mulinum* species and one of the typical components of the Patagonian flora, being both abundant and easy to recognize in the field. It is distributed in Chile from Coquimbo (region III, *c.* 31° S) to Magallanes (region XII, *c.* 51° S), and in Argentina from San Juan Province (31° S) to southern Santa Cruz Province (52° S), mainly in the steppe but also in the ecotone with Andean zones.

Commonly known as 'neneo', *M. spinosum*, is a hemispheric cushion shrub up to 1 m high with spiny trisect leaves. Although Arroyo & Uslar (1993) considered *M. spinosum* to be a dioecious species, Constance (1988) described the species with all perfect flowers or with the external ones staminate, which could be related to functional dioecy being variable amongst individuals. Our field observations during sample collections showed that all plants have fruits. Constance *et al.* (1971) reported chromosome numbers in *M. spinosum* of $2n = 16$, 32 and possibly 48. Polyploidy is common within the genus, and some preliminary data from nuclear genes suggest the chromosome series in *M. spinosum* is autopolyploid rather than allopolyploid in origin. Although only a few populations of *M. spinosum* were studied, there is no obvious association between geographical distribution and ploidy level (Constance *et al.*, 1971), and we have observed no evidence of correlations with breeding system or haplotype diversity.

Mulinum spinosum is frequently a dominant species and replaces palatable grasses in regions with intense grazing; it also invades disturbed areas in nearby temperate forests. This plant resprouts vigorously after fires and recovers its original aerial biomass in a short time (Damascos *et al.*, 2008). Palaeobotanical data are scanty for the family Apiaceae in Patagonia and are restricted to records from the early to middle Miocene of southern Patagonia and late Miocene of eastern Patagonia (Zamaloa, 1999; Barreda *et al.*, 2007). There were plentiful *Mulinum* steppes after 10,000 yr BP (Barreda *et al.*, 2007) and pollen grains reached maximum abundance during 570–60 cal. yr BP in south-west Patagonia (Moreno *et al.*, 2009).

In the absence of palaeoecological data for the genus (especially pollen records) previous to those cited above, the use of distribution modelling (Phillips *et al.*, 2004) provides the basis for investigating the extent to which palaeoclimatic conditions influenced the distribution of this species. The projection of a species distribution during past climates can aid in the interpretation of current patterns of distribution and genetic diversity (Hijmans & Graham, 2006).

Here we report the phylogeographical patterns of *M. spinosum* in Patagonia using DNA sequences of three chloroplast regions to examine the relative importance of historical factors in the configuration of genetic variation at a geographical scale. Additionally, to assess support for the phylogeographical patterns proposed in the study, we also modelled extant and palaeoclimatic distributions. This integrative approach will contribute to a better understanding of the spatial and temporal processes that occurred in the past in the Patagonian steppe.

MATERIALS AND METHODS

Sampling

A total of 314 individuals of *M. spinosum* from 71 populations were sampled, covering most of the species' distribution range (Table 1, Fig. 1). Sampled individuals were separated by at least 10 m along a linear transect to minimize the potential of sibling relationships among samples and better represent local diversity.

DNA isolation, amplification and sequencing

Genomic DNA was isolated from silica dried leaf tissue following a modified cetyl trimethyl ammonium bromide (CTAB) protocol (Cullings, 1992). The chloroplast regions *trn*H–*psb*A (primers *trn*H^{GUG} and *psb*A; Shaw *et al.*, 2005), *trn*S–*trn*G (primers *trn*S^{GCU} and *trn*G^{UUC}; Hamilton, 1999) and 3′*trn*V–*ndh*C (primers *trn*V^{UAC} and *ndh*C; Shaw *et al.*, 2007) were chosen because they showed the highest sequence variability amongst several surveyed regions.

DNA was amplified and sequenced with a profile consisting of 94 °C for 3 min followed by 30 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min. Amplification products were purified using PCR$_{96}$ clean up plates (Millipore Corp, Billerica, MA, USA), BigDye v.3 (Applied Biosystems, Foster City, CA, USA) and purified with Sephadex (GE Healthcare, Piscataway, NJ, USA) before electrophoreses on an AB 3730xl automated sequencer housed in Brigham Young University's DNA Sequencing Centre. Electropherograms were edited and assembled using Sequencher 4.6 (Gene Codes, Ann Arbor, MI, USA). Sequences were aligned with the alignment program ClustalX 1.81 (Thompson *et al.*, 1997) with subsequent manual adjustments using BioEdit 5.0.9 (Hall, 1999). Indels were coded as binary characters using simple indel coding (Simmons & Ochoterena, 2000) as implemented in SeqState 1.4 (Müller, 2005). The three chloroplast regions were concatenated into a single matrix with no further considerations given their shared ancestry, maternal inheritance and extremely rare instances of recombination within the chloroplast genome. Unique sequences from this combined matrix were identified as haplotypes. All sequences were deposited in GenBank (*trn*H–*psb*A JN888643–JN888956; *trn*S–*trn*G JN888016–JN888328; and 3′*trn*V–*ndh*C JN888329–JN888642).

Haplotype network and geographical distribution

Nested clade analysis (NCA) was performed following the approach of Templeton *et al.* (1995) and Templeton (2004) in the program ANeCA 1.2 (Panchal, 2007). Although NCA has been criticized (Petit, 2007; Beaumont & Panchal, 2008; Knowles, 2008), it has been validated extensively (Templeton, 2008, 2009) and is used in conjunction with other population genetic parameters to draw inferences here.

A haplotype statistical parsimony network was constructed using tcs 1.21 (Clement *et al.*, 2000). Ambiguous connections (loops) were resolved using predictions from coalescent theory and information about the sampling, according to three criteria: frequency, network location and geography (Crandall & Templeton, 1993).

The resolved haplotype network was converted into a hierarchical nested design following Templeton *et al.* (1987) and Templeton & Sing (1993). Clade (D_c) and nested clades (D_n) distances were estimated to assess association between the nested cladogram and geographical distances amongst sampled localities (Templeton *et al.*, 1995) using the program Geodis 2.6 (Posada *et al.*, 2000). Null distributions (i.e. under a hypothesis of no geographical association of clades and nested clades) for permutational contingency table test comparisons were generated from 10,000 Monte Carlo replications, with a 95% confidence level. For significant associations, the inference key of Templeton *et al.* (2005) was used to recognize probable demographic processes and/or historical events of the clades.

Population genetic analyses

Haplotype diversity (*h*; Nei, 1987), nucleotide diversity (π; mean number of pairwise differences per site; Nei, 1987) and mean number of pairwise differences (*p*; Tajima, 1983) were calculated for the species as a whole, for each sampling location, and for every significant haploclade derived from NCA, using the programs Arlequin 3.1 and DnaSP 5 (Excoffier *et al.*, 2005; Librado & Rozas, 2009).

To investigate hierarchical levels of population structure, four analyses of molecular variance (AMOVA) were performed that consider genetic distances between haplotypes and their frequencies using Arlequin (Weir & Cockerham, 1984; Excoffier *et al.*, 2005). First, sampling localities were defined as Chilean and Argentinian, to test the effect of the Andes. The second analysis was applied to Chilean sampling localities: HACH, high Andean Chile (*c.* 33° S); NCH, northern Chile (36–40° S) and SCH, southern Chile (44–47° S). In the third analysis, Argentinian populations were grouped according to different latitudinal regions, defined by major rivers: (1) HARG, high Andean Argentina and NP, northern Patagonia (north of Chubut River, *c.* 35–43° S); (2) CP, central Patagonia (between Chubut and Deseado rivers, 44–46° S); and (3) SP, southern Patagonia (south of the Deseado River, 47–51° S). The aim of the fourth analysis was to test if the genetic

Table 1 Geographical location, voucher numbers, coordinates, haplotype number (N_{hap}) and sample size (N_{ind}), and molecular diversity indices of the *Mulinum spinosum* populations studied: haplotype diversity (h), nucleotide diversity (π) and mean number of pairwise differences (p). Localities (N_{loc}) are numbered consecutively, as shown on the map in Fig. 1.

N_{loc}	Geographical location/ region–province	Sample location	Voucher number	Latitude (°S)	Longitude (°W)	N_{hap} (N_{ind})	h ± SD	π ± SD	p ± SD
High Andean Chile									
1	V	Portillo	M2001	−32.8833	−70.1667	24 (1), 25 (7)	0.2500 ± 0.1800	0.0001 ± 0.0001	0.2500 ± 0.3112
2	V	San Gabriel	M2002	−33.8333	−70.0667	4 (7), 26 (1)	0.2500 ± 0.1800	0.0008 ± 0.0006	1.5000 ± 1.0057
Northern Chile									
3	VIII	Termas de Chillán	M2031	−36.9000	−71.4000	12 (8)	0.0000	0.0000	0.0000
4	VIII	Laguna de La Laja	M2033	−37.4667	−71.3167	29 (1)	–	–	–
5	VIII	Canteras	M2032	−37.4000	−71.8833	28 (8)	0.0000	0.0000	0.0000
6	VIII	Laguna El Barco	M2012	−37.9833	−71.2666	27 (8)	0.0000	0.0000	0.0000
7	IX	Curarrehue	M2023	−39.5833	−71.5167	20 (8)	0.0000	0.0000	0.0000
Southern Chile									
8	XI	Río Cisnes	M15701	−44.6282	−71.6370	18 (8)	0.0000	0.0000	0.0000
9	XI	Villa La Tapera	M15702	−44.6467	−71.7303	26 (1)	–	–	–
10	XI	Villa Ortega	M15709	−45.3670	−71.9210	17 (8)	0.0000	0.0000	0.0000
11	XI	Cerro Castillo	M15713	−46.1219	−72.1625	18 (8)	0.0000	0.0000	0.0000
12	XI	Chile Chico	M15719	−46.5471	−71.8048	2 (1)	–	–	–
13	XI	Puerto Guadal	M15715	−46.7397	−72.5117	26 (8)	0.0000	0.0000	0.0000
14	XI	Puerto Bertrand	M15729	−47.1205	−72.7667	18 (1)	–	–	–
15	XI	Cochrane	M15730	−47.2322	−72.6097	15 (1), 18 (7)	0.2500 ± 0.1800	0.0007 ± 0.0005	1.2500 ± 0.8781
Argentina – southern Patagonia									
16	Santa Cruz	Paso Roballos	CZ124	−47.0575	−71.8189	2 (7)	0.0000	0.0000	0.0000
17	Santa Cruz	Lago Ghio	CZ123	−47.2250	−71.7697	16 (2)	–	–	–
18	Santa Cruz	Laguna Klementek	MULP35	−47.9936	−71.6803	26 (2)	–	–	–
19	Santa Cruz	Lago San Martín	CZ103	−49.0442	−72.2264	3 (3)	–	–	–
20	Santa Cruz	Lago Viedma	CZ95	−49.4050	−72.7736	3 (8)	0.0000	0.0000	0.0000
21	Santa Cruz	El Calafate	MULZ87	−50.3400	−72.4733	26 (1)	–	–	–
22	Santa Cruz	Lago Argentino	MULZ86	−50.3986	−72.7303	17 (1)	–	–	–
23	Santa Cruz	Estancia Tapi Aike	CZ81	−50.9222	−71.7372	1 (1), 2 (4), 22 (3)	0.6790 ± 0.1220	0.0018 ± 0.0008	3.2857 ± 1.8871
24	Santa Cruz	Tamel Aike	MULZ114	−48.3019	−70.9725	1 (1)	–	–	–
25	Santa Cruz	Lago Cardiel	CZ112	−48.9542	−71.0325	2 (6), 3 (2)	0.4286 ± 0.1690	0.0002 ± 0.0000	0.4286 ± 0.4286
26	Santa Cruz	Cerro Moro	MULP45	−49.0969	−71.0064	2 (3)	–	–	–
27	Santa Cruz	Cerro Man Aike	MULP28	−49.7714	−70.7300	3 (2)	–	–	–
28	Santa Cruz	Coy Aike	CZ73	−51.1100	−69.5308	2 (2), 3 (3)	0.4000 ± 0.2370	0.0002 ± 0.0001	0.4000 ± 0.4350
29	Santa Cruz	Laguna del Puesto	MULP23	−50.2680	−69.6800	3 (8)	0.0000	0.0000	0.0000
30	Santa Cruz	Monte León	MULP25	−50.2430	−69.0080	3 (2)	–	–	–
31	Santa Cruz	Cerro Vanguardia	MULN45	−48.6008	−68.7597	2 (2)	–	–	–
32	Santa Cruz	Ea. Sol de Mayo	MULP39	−47.3619	−69.8203	23 (2)	–	–	–
33	Santa Cruz	Arroyo Pirámides	MULP41	−46.8908	−69.6817	2 (5), 23 (3)	0.5357 ± 0.1230	0.0021 ± 0.0005	3.7500 ± 2.1127
34	Santa Cruz	Meseta del Pedrero	MULN54	−47.4347	−68.5830	26 (2)	–	–	–
35	Santa Cruz	Puerto Deseado	MULN42	−47.8203	−66.5461	2 (2)	–	–	–
36	Santa Cruz	Ea. El Polvorín	MULN38	−47.1225	−66.4630	2 (1)	–	–	–

Table 1 Continued

N_{loc}	Geographical location/ region–province	Sample location	Voucher number	Latitude (°S)	Longitude (°W)	N_{hap} (N_{ind})	$h \pm$ SD	$\pi \pm$ SD	$p \pm$ SD
Argentina – central Patagonia									
37	Santa Cruz	Laguna Guadal	MULN25	−46.0561	−69.3419	26 (8)	0.0000	0.0000	0.0000
38	Chubut	Ex Estación Holdich	MULN57	−45.9667	−68.1997	26 (8)	0.0000	0.0000	0.0000
39	Chubut	Pastos Blancos	MULN26	−45.2705	−70.5225	17 (1)	–	–	–
40	Chubut	Buen Pasto	MULN24	−45.0692	−69.4608	14 (8)	0.0000	0.0000	0.0000
41	Chubut	Sierra Chaira	MULN60	−45.1369	−68.1739	26 (2)	–	–	–
42	Chubut	Sierra Nevada	MULN23	−44.7394	−69.6080	26 (1)	–	–	–
43	Chubut	Cerro Shequen	MULN20	−44.5953	−69.9017	17 (8)	0.0000	0.0000	0.0000
44	Chubut	Gran Laguna Salada	MULN61	−44.5703	−67.6661	26 (7)	0.0000	0.0000	0.0000
45	Chubut	Tecka	MULN18	−43.5958	−70.1647	17 (1)	–	–	–
Argentina – northern Patagonia									
46	Chubut	Paso de Indios	MULN35	−43.9944	−69.1433	26 (1)	–	–	–
47	Chubut	Lago Cronómetro	MULN15	−43.2200	−71.0794	19 (1)	–	–	–
48	Chubut	Laguna Aleusco	MULN10	−43.0806	−70.3555	26 (1)	–	–	–
49	Chubut	Cushamen	MULC14	−42.0042	−70.6697	26 (8)	0.0000	0.0000	0.0000
50	Chubut	Gastre	MULZ057	−42.0736	−69.5150	26 (2)	–	–	–
51	Chubut	Gan Gan	MULN04	−42.9375	−68.5217	9 (2), 26 (6)	0.4286 ± 0.1690	0.0005 ± 0.0002	0.8571 ± 0.6720
52	Río Negro	Quetrequile	MULZ052	−41.7525	−69.3597	9 (2), 21 (3)	0.6000 ± 0.1750	0.0010 ± 0.0003	1.8000 ± 1.2360
53	Chubut	Laguna de la Vaca	MUL21	−42.4667	−67.3000	13 (1)	–	–	–
54	Río Negro	Comicó	MULZ029	−41.1136	−67.4630	21 (5), 26 (1)	0.3333 ± 0.2150	0.0002 ± 0.0001	0.3333 ± 0.3800
55	Río Negro	Valcheta	MUL3022	−40.9697	−66.6650	26 (1)	–	–	–
56	Río Negro	Colan-Conhue	MZ47A	−40.8897	−69.2983	26 (1)	–	–	–
57	Río Negro	Chasicó	MULZ044	−40.7367	−69.1567	26 (2)	–	–	–
58	Neuquén	Junín de los Andes	MULSb	−40.0817	−71.1247	26 (8)	0.0000	0.0000	0.0000
59	Neuquén	Piedra del Águila	MULA02	−40.3258	−70.4208	9 (4), 26 (4)	0.2500 ± 0.1800	0.0003 ± 0.0002	0.5000 ± 0.4717
60	Río Negro	Aguada Guzmán	MULC10	−40.1289	−68.9386	11 (1)	–	–	–
61	Río Negro	El Cuy	MULC4	−39.6661	−68.4100	9 (1)	–	–	–
62	Río Negro	Trica-Có	MULC3	−39.6033	−68.1147	7 (2)	–	–	–
63	Río Negro	Bajada de Bravo	MULC05	−39.4100	−68.4575	22 (1), 26 (1)	–	–	–
64	Neuquén	Zapala	MULA05	−39.1050	−70.0094	10 (5), 14 (1)	0.3333 ± 0.2150	0.0006 ± 0.0004	1.0000 ± 0.7746
65	Neuquén	Las Lajas	MULSa	−38.3686	−70.5378	9 (1), 26 (7)	0.2500 ± 0.1800	0.0003 ± 0.0002	0.5000 ± 0.4717
66	Neuquén	Chos Malal	MULS4127	−37.7025	−70.1514	8 (4), 26 (4)	0.5714 ± 0.0940	0.0010 ± 0.0002	1.7143 ± 1.1136
67	Neuquén	Buta Ranquil	MULS4131	−37.2097	−69.7900	26 (2)	–	–	–
68	Neuquén	Lagunas de Epu-Lauquen	MULS4102	−36.8383	−71.0122	26 (6), 27 (1)	0.2857 ± 0.1960	0.0002 ± 0.0001	0.2857 ± 0.3408
High Andean Argentina									
69	Mendoza	La Pasarela	MULS4133	−36.2764	−69.6203	9 (2)	–	–	–
70	Mendoza	Malargüe	MULS4134	−35.7486	−69.5853	5 (5), 6 (1), 26 (1)	0.5240 ± 0.2090	0.0007 ± 0.0004	1.3333 ± 0.9351
71	Mendoza	Loma Rayo	MUL20	−35.9278	−68.6167	21 (1)	–	–	–

–, analysis not performed.

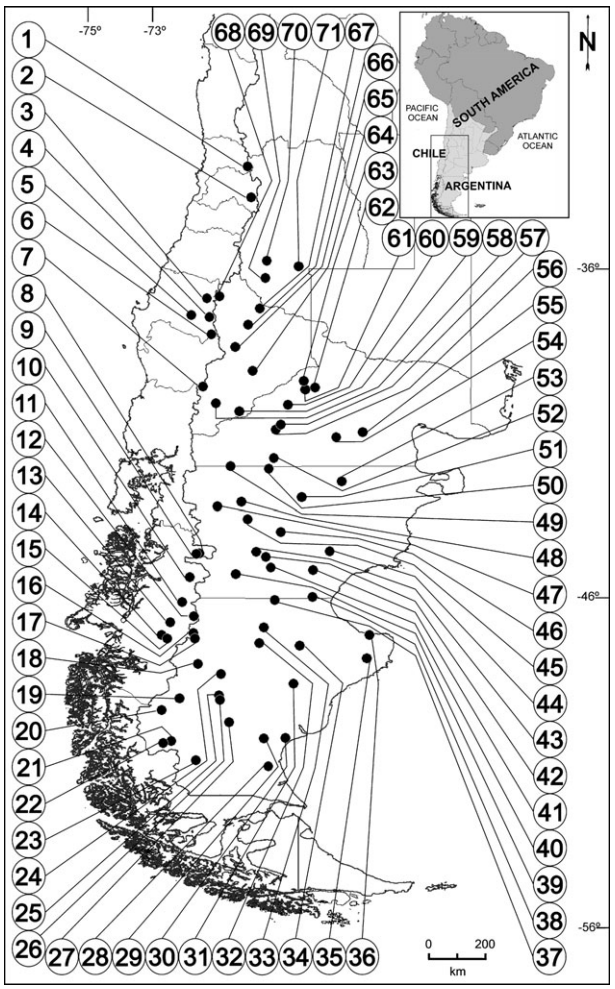

Figure 1 The inset depicts a geographical map of South America with the study area indicated in a box. The main map shows the locations of the 71 sampled populations of *Mulinum spinosum*. Locality numbers correspond to those in Table 1.

variation was significant amongst glaciated and non-glaciated sites during GPG in populations south of Deseado River in Santa Cruz province, corresponding to group 3 of the previous analysis. This region was selected given that eight of the nine populations within the ice zone were located in southern Patagonia.

To further explore the genetic structure of populations, without the prior assumption of group composition, we performed a spatial analysis of molecular variance (SAMOVA) using SAMOVA 1.0 (Dupanloup *et al.*, 2002). The program was run for 10,000 iterations for $K = 2$ to 15 from each of 200 initial conditions. Following the criteria employed by Ikeda *et al.* (2008), the number of groups (K) was inferred from the configuration with the highest value of differentiation amongst groups (F_{CT}) that did not contain any single population group.

Estimation of molecular diversity indices and AMOVA and SAMOVA tests were performed considering only populations with five or more sampled individuals.

Demographic history analyses

To analyse the demographic history of populations in each geographical area, Tajima's D (Tajima, 1989), Fu's F_S (Fu, 1997) and Ramos-Onsins and Rozas' R_2 (Ramos-Onsins & Rozas, 2002) statistics of neutrality were calculated to test for evidence of range expansions. Significant negative values of D and F_S and small positive values of R_2 indicate an excess of low-frequency mutations, relative to expectations under the standard neutral model (i.e. strict selective neutrality of variants, constant population size and lack of subdivision and gene flow). Both F_S and R_2 are the most powerful tests used to detect population growth (Ramos-Onsins & Rozas, 2002). Significance was evaluated by comparing the observed value with a null distribution generated by 10,000 replicates, using the empirical population sample size and observed number of segregating sites implemented by DNASP 5.

Species distribution modelling

To validate the phylogeographical analyses, we modelled the present potential geographical distribution of *M. spinosum* and projected it onto a LGM model *c.* 21 ka using point locality information and environmental data in DIVA-GIS 7.3 (Hijmans *et al.*, 2001) and MAXENT 3.3.3, with the maximum entropy machine-learning algorithm (Phillips *et al.*, 2004).

We recorded latitudinal and longitudinal coordinates from 134 localities covering the entire distribution range of the species using a hand-held geographical positioning system (GPS) unit. Only 12 point localities of the total were georeferenced using Google Earth (http://www.google.com/earth/index.html). Environmental data with a resolution of 2.5 arcmin (5 km) for current and past conditions were downloaded from the WorldClim database 1.4 (Hijmans *et al.*, 2005) and were represented by 19 bioclimatic variables derived from the monthly temperature and rainfall values.

Current conditions are interpolations of observed data from climate stations around the world, representing a 50-year period from 1950 to 2000. Past conditions for the LGM are calibrated and statistically downscaled reconstructions based on the WorldClim data for current conditions. We chose the prediction of one of two global climate models tested for the LGM – the Community Climate System Model (CCSM) and the Model for Interdisciplinary Research on Climate (MIROC) – by evaluating the MAXENT values of the area under the receiver operating characteristic curve (AUC) and interpreting the resulting maps of each model in DIVA-GIS.

We generated 2000 random points from across Argentina and Chile using Geo Midpoint (http://www.geomidpoint.com/random/) and extracted environmental data in DIVA-GIS. To avoid over-estimation of climatic data that can lead to misleading results (Phillips *et al.*, 2004; Peterson & Nakazawa, 2008), we used a series of correlation tests in INFOSTAT 2.0 (Di Rienzo *et al.*, 2002) following a similar procedure described by Werneck *et al.* (2011).

We used a Pearson correlation coefficient ≥ 0.75 to identify highly correlated variables and selected eight that we considered more biologically meaningful and directly relevant to *M. spinosum*. These were: mean monthly temperature range (Bio 2), isothermality (Bio 3), temperature seasonality (Bio 4), mean temperature of the wettest quarter (Bio 8), mean temperature of the driest quarter (Bio 9), precipitation seasonality (Bio 15), precipitation of the wettest quarter (Bio 16) and precipitation of the driest quarter (Bio 17). The bioclimatic layers were cropped to project from latitude 55°4′ S to 17°29′ S and longitude 75°45′ W to 56°27′ W.

Maxent was run using the following settings for current and past models: 10 replicates with auto features, response curves, jackknife tests, logistic output format, random seed, random test percentage = 25, replicate run type = crossvalidate, regularization multiplier = 1, maximum iterations = 500, convergence threshold = 0.00001 and maximum number of background points = 10,000. Variable importance was determined comparing percentage contribution values and jackknife plots.

RESULTS

Haplotype distribution and nested clade phylogeographical analysis

DNA sequence lengths ranged from 366 to 385 base pairs (bp) for *trn*H–*psb*A, 680 to 709 bp for *trn*S–*trn*G and 700 to 714 bp for 3′*trn*V–*ndh*C. Seventeen gaps, varying from 1 to 10 bp in length, were introduced during alignment. The combined matrix included 1828 characters.

A total of 29 haplotypes were identified (Table 2, Fig. 2). The most frequent and widespread haplotype (26) was found in 30.89% of the individuals and in 39.44% of the populations. It is distributed in southern Chile and in all regions of Argentina, from 35 to 50° S. The second (2) and third (3) most frequent haplotypes were found in 10.50% and 8.91% of the individuals and 14.08% and 9.85% of the populations, respectively. Both haplotypes are confined principally to the southernmost part of Argentinian Patagonia, from 46 to 52° S, with haplotype 2 also present in one population from southern

Table 2 Distribution of haplotypes of *Mulinum spinosum* amongst individuals, populations and geographical regions in Chile and Argentina.

Haplotypes	Number of individuals	Percentage of individuals	Number of populations	Percentage of populations	Geographical distribution
1	2	0.637	2	2.817	SP
2	33	10.509	10	14.084	SP and SCH
3	28	8.917	7	9.859	SP
4	7	2.229	1	1.408	NCH
5	5	1.592	1	1.408	HA-ARG
6	1	0.318	1	1.408	HA-ARG
7	2	0.636	1	1.408	NP
8	4	1.274	1	1.408	NP
9	12	3.821	6	8.450	NP and HA-ARG
10	5	1.592	1	1.408	NP and CP
11	1	0.318	1	1.408	NP
12	8	2.547	1	1.408	NCH
13	1	0.318	1	1.408	NP
14	9	2.866	2	2.817	NP and CP
15	1	0.318	1	1.408	SCH
16	2	0.636	1	1.408	SP
17	19	6.050	5	7.042	SCH, CP and SP
18	24	7.643	4	5.633	SCH
19	1	0.318	1	1.408	NP
20	8	2.547	1	1.408	NCH
21	9	2.866	3	4.225	HA-ARG and NP
22	4	1.273	2	2.816	NP and SP
23	5	1.592	2	2.816	SP
24	1	0.318	1	1.408	HA-CH
25	7	2.229	1	1.408	HA-CH
26	97	30.891	28	39.436	All regions of ARG and SCH
27	9	2.866	2	2.817	NCH and NP
28	8	2.547	1	1.408	NCH
29	1	0.318	1	1.408	NCH

HA-CH, high Andean Chile; NCH, northern Chile; SCH, southern Chile; HA-ARG, high Andean Argentina; NP, northern Patagonia; CP, central Patagonia; SP, southern Patagonia.

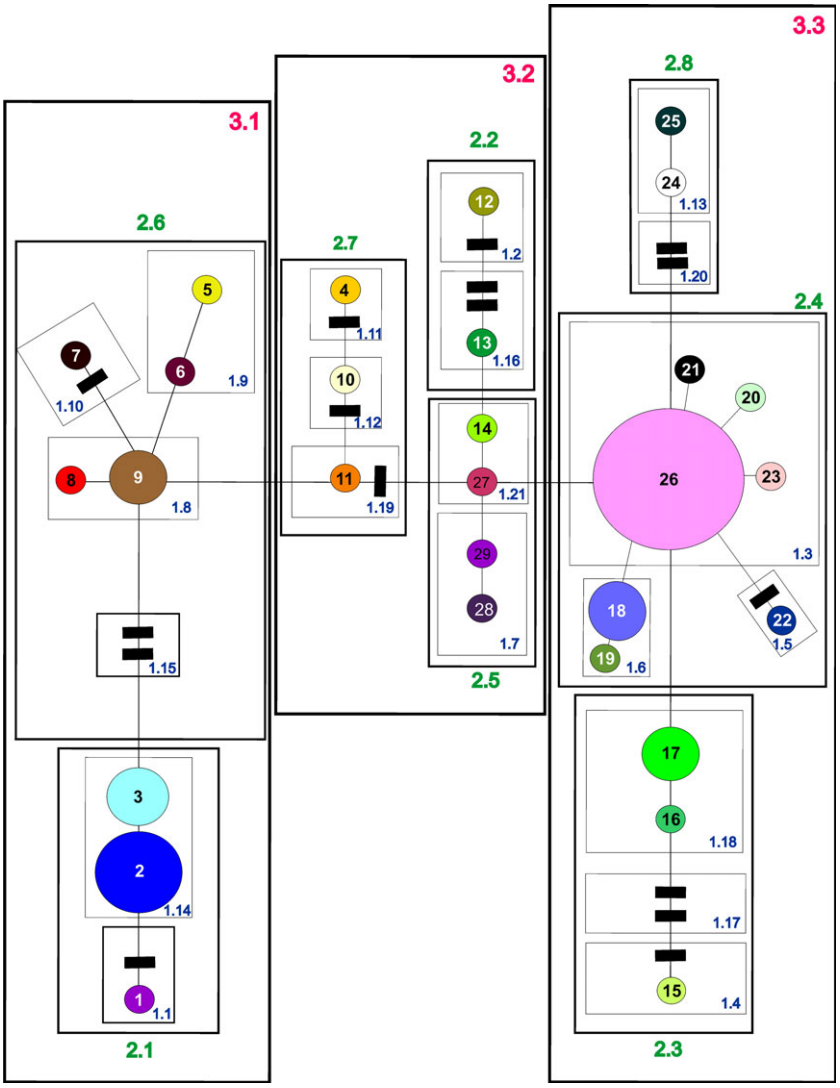

Figure 2 Statistical parsimony network and resulting set of nested clades of the 29 chloroplast DNA haplotypes found in *Mulinum spinosum*. Numbers within circles indicate sampled haplotypes. Solid bars are hypothetical haplotypes.

Chile. Haplotypes 5, 6, 7, 8 and 9 are restricted to the northern Argentinian populations, between 36 and 40° S, and except for haplotype 9, they are found in only one population each. Haplotypes 4, 12, 15, 18, 20, 24, 25, 28 and 29 are restricted to Chilean populations, most of them in the northern sites from 32 to 40° S, and, except for haplotype 18, each one is exclusive to single populations. In total, 17 of the 29 haplotypes were found in only one population, five of the 29 in two populations, and the remaining seven were found at three or more sites (see Table 2).

Most geographical regions possess exclusive haplotypes. In northern Argentina and the high Andean Argentinian region there are nine exclusive haplotypes, while in northern Chile and the high Andean Chilean region there are seven, with only one haplotype shared between east and west. In the southernmost area on both sides of the Andes, the situation is different, with four haplotypes in southern Patagonia on the Argentinian side and only two on the Chilean side, with only one haplotype shared between both regions. There are no exclusive haplotypes in central Patagonia.

Statistical parsimony resulted in a network in which five loops were resolved (Fig. 2). The network is composed of 21 one-step clades, eight two-step clades, three three-step clades, and the total cladogram (Fig. 2). Comparing the network with the geographical distribution of haplotypes, a latitudinal distribution of one- and two-step clades was observed. Haplotypes in clades 2.1 and 2.3 were located south of 43° S latitude (central and southern Patagonia) while haplotypes in clades 2.2, 2.5, 2.6, 2.7 and 2.8 (except for haplotype 14, located at one population at 45° S) were found north of 43° S (northern Patagonia and the high Andean region). Haplotypes in clade 2.4 were distributed evenly from north to south, but were absent in northern and high Andean Chile. In the three-step clades, haplotypes in clade 3.1 are located in northern and southern Patagonia, although they are absent from central Patagonia (*c.* 43 to 46° S). Haplotypes in clade 3.2 are distributed in northern Patagonia except for haplotype 14, which is also in central Patagonia. Finally, haplotypes in clade 3.3 are widely distributed.

NCA revealed significant association of clades and sampling locations at all clade levels (Table 3). Five one-step clades (1.3, 1.8, 1.14, 1.18 and 1.21) and five two-step clades (2.1, 2.4, 2.5, 2.6 and 2.7) showed significant levels of geographical association, whereas the null hypothesis of no geographical associations was rejected at all higher-level categories (three-step clades and the entire cladogram). The inferences made from Templeton's key (Templeton *et al.*, 2005) are given in Table 3. Restricted gene flow with isolation by distance was inferred for four nested clades (1.8, 2.1, 2.6 and 3.2). Also, restricted gene flow or dispersal but with some long-distance dispersal was inferred for clades 2.4 and 3.3, while both possibilities were inferred for clade 1.3. The most likely explanations for clade 3.1 and the entire cladogram were long-distance colonization and/or past fragmentation.

Haplotype diversity and population differentiation

Haplotype diversity of populations (h) ranged from 0 to 0.68. Nucleotide diversity (π) ranged from 0 to 0.002, and mean number of pairwise differences amongst haplotypes within populations (p) ranged from 0.0 to 3.75 (Table 1).

The highest values of haplotype diversity were found in Argentinian populations 23, 52, 66, 33 and 70 with 0.68, 0.6, 0.57, 0.54 and 0.53, respectively. Populations 23 and 33 are located in southern Patagonia, while 52 and 66 are located in northern Patagonia, and 70 is in the high Andean region. The highest numbers of pairwise differences were found in populations 33 and 23 (3.75 and 3.28, respectively; Table 1, Fig. 1).

In Chilean populations, the highest value of haplotype diversity (0.25) was found in three populations: 1 and 2 from high Andean Chile and 15 from southern Chile. The remaining populations did not show any variability (Table 1, Fig. 1).

The same diversity indices were calculated for each geographical area along both sides of the Andes. High Andean and northern Chile showed the highest values of h, π and p (0.65/0.788; 0.00296/0.00188 and 5.275/3.348, respectively; Table 4). On the eastern side, the high Andean region and northern Patagonia together with southern Patagonia showed the highest values (h, 0.614/0.697; π, 0.0015/0.0014, and p, 1.604/2.487).

AMOVA and SAMOVA

Analyses between Chilean and Argentinian populations showed significant between-group variation (11.98%, $P = 0.010$; Table 5), although there is more variation amongst populations within groups than between these two groups. Nevertheless, sampling on either side of the Andes is unbalanced, so results should be interpreted with caution (Table 5). The analysis amongst Chilean populations showed significant variation; differences amongst the three groups accounted for 20% of the overall variation ($P = 0.023$). SAMOVA failed to detect a meaningful break between Chilean and Argentinian groups and amongst Chilean populations.

The AMOVA analysis amongst Argentinian populations according to the regions defined by main rivers showed significant (60.02%, $P < 0.001$) variation. The inter- and intra-population differences explained 23.10% and 16.88% of the variation, respectively (Table 5). The best partitioning of the genetic diversity by SAMOVA was obtained for $K = 2$; further increases in K sequentially removed single populations rather than forming additional informative clusters. Southern

Table 3 Populational processes and/or historical events affecting genetic structure in *Mulinum spinosum*, based on nested clade analysis (NCA). Hierarchically nested clades, results of permutation contingency tests, NCA inference chain and inferred events are shown.

Clade	χ^2 statistic	*P*-value	Inference chain	Inferred pattern
1.3	408.90	< 0.01	1-2-3-5-6-7-8 YES	Restricted gene flow/dispersal but with some long-distance dispersal over intermediate areas not occupied by the species; or past gene flow followed by extinction of intermediate populations
1.8	13.00	< 0.05	1-2-3-4 NO	Restricted gene flow with isolation by distance
1.14	51.59	< 0.01	1-19-20 NO	Inadequate geographical sampling
1.18	26.00	< 0.01	1-2 IO	Tip/interior status cannot be determined – inconclusive outcome (IO)
1.21	38.00	< 0.01	1-19-20-2 IO	Tip/interior status cannot be determined – inconclusive outcome (IO)
2.1	36.97	< 0.05	1-2-3-4 NO	Restricted gene flow with isolation by distance
2.4	318.14	< 0.01	1-2-3-5-6-7 YES	Restricted gene flow/dispersal but with some long-distance dispersal
2.5	28.00	< 0.01	1-19-20-NO	Inadequate geographical sampling
2.6	42.00	< 0.01	1-19-20-2-3-4 NO	Restricted gene flow with isolation by distance
2.7	26.00	< 0.01	1-19-20-2-3-5-15-16-18 NO	Geographical sampling inadequate to discriminate between fragmentation, range expansion and isolation by distance
3.1	84.00	< 0.01	1-19-20-2-11-12-13 YES	Long-distance colonization possibly coupled with subsequent fragmentation or past fragmentation followed by range expansion
3.2	93.40	< 0.01	1-2-3-4 NO	Restricted gene flow with isolation by distance
3.3	402.30	< 0.01	1-19-20-2-3-5-6-7 YES	Restricted gene flow/dispersal but with some long-distance dispersal
Total cladogram	598.47	< 0.01	1-2-11-12-13-14 NO	Long-distance colonization and/or past fragmentation (not necessarily mutually exclusive)

Table 4 Diversity indices: haplotype (h) and nucleotide (π) diversity, and mean number of pairwise differences (p), and demographic analyses of *Mulinum spinosum*. Tajima's D, Fu's F_S and Ramos-Onsins & Rozas' R_2 statistics are shown for each geographical area in Chile and Argentina.

	Diversity indices			Demographic analyses		
Geographical area	h (± SD)	π (± SD)	p (± SD)	D	F_S	R_2
Chile						
High Andean region	0.650 ± 0.075	0.00296 ± 0.00025	5.275 ± 2.690	2.79853**	4.990	0.2638
Northern Chile	0.788 ± 0.022	0.00188 ± 0.00022	3.348 ± 1.762	2.07698*	3.537	0.2093
Southern Chile	0.623 ± 0.060	0.00073 ± 0.00019	1.302 ± 0.830	−1.61178	0.479	0.0961
Total	0.880 ± 0.017	0.00209 ± 0.00019	3.718 ± 1.895	−0.41243	−0.012	0.0839
Argentina						
High Andean region and Northern Patagonia	0.614 ± 0.051	0.00150 ± 0.00018	1.604 ± 0.959	−0.63901	−2.494	0.0734
Central Patagonia	0.579 ± 0.059	0.00045 ± 0.00005	0.799 ± 0.589	1.35958	1.368	0.1998
Southern Patagonia	0.697 ± 0.035	0.00140 ± 0.00018	2.487 ± 1.357	0.59806	1.051	0.1243
Total	0.757 ± 0.025	0.00245 ± 0.00010	2.614 ± 1.403	0.49361	−1.862	0.1003

*$P < 0.05$, **$P < 0.01$.

Patagonian populations were clustered in one group, and the remaining central and northern Patagonian populations in another group (68.02% of variation between groups). The composition of the southern Patagonian group (populations below the Deseado River in Santa Cruz province) was exactly the same as the group tested by AMOVA. In the AMOVA analyses between glaciated and non-glaciated sites, no significant effect of glaciations was found (Table 5).

Demographic analysis

Fu's F_S and Ramos-Onsins and Rozas' R_2 statistics showed no significant deviation from values expected under a basic coalescent model at all geographical areas. Tajima's D-values are not significant except for the high Andean and northern Chilean regions, which showed positive values of 2.80 and 2.08, respectively (Table 4).

Table 5 Results of the analysis of molecular variance (AMOVA) for 71 populations of *Mulinum spinosum* based on chloroplast DNA sequence data. The first analysis was performed to test the effect of the Andes. The second analysis was to test differences amongst latitudinal groups in Chile. The aim of the third analysis was to test differences amongst Argentinian latitudinal groups divided by the Chubut and Deseado rivers. The fourth analysis was performed on the Argentinian group 3 (southern Patagonia), to test the effect of the Greatest Patagonian Glaciation (GPG) on ice-covered and uncovered sites. Degrees of freedom (d.f.), sum of squared deviations (SSD), variance components (VC), percentage of total variance (% total) and significance value (P) are given for each hierarchical level.

Source of variation	d.f.	SSD	VC	% of total	P-value
1. Argentina vs. Chile					
Amongst groups	1	39.789	0.240	11.98	= 0.010
Amongst populations within groups	32	369.926	1.489	74.28	< 0.001
Within populations	224	61.715	0.275	13.74	< 0.001
Total	257	471.430	2.005		
2. Chilean populations					
Amongst groups	2	48.963	0.426	20.00	= 0.023
Amongst populations within groups	8	101.537	1.577	73.60	< 0.001
Within populations	77	10.500	0.136	6.39	< 0.001
Total	87	161.000	2.132		
3. Argentinian populations					
Amongst groups	2	142.249	1.238	60.02	< 0.001
Amongst populations within groups	20	77.177	0.476	23.10	< 0.001
Within populations	147	51.215	0.348	16.88	< 0.001
Total	169	270.641	2.063		
4. Glaciated vs. non-glaciated Argentinian populations					
Amongst groups	1	0.639	−0.108	−12.57	n.s.
Amongst populations within groups	5	16.628	0.370	43.04	< 0.001
Within populations	45	26.925	0.598	69.53	< 0.001
Total	51	44.192	0.860		

n.s., not significant.

Potential current and Pleistocene species distribution models

Present and LGM species distribution models for *M. spinosum* are presented in Fig. 3. In predicting the current distribution, the mean value of the AUC for the 10 replicates was 77%. Because the mean value of the AUC using the MIROC model was 79%, while that using the CCSM model was 77%, we selected the former model for the projection of the LGM distribution.

The current distribution model (Fig. 3a) was a fairly good representation of the extant geographical distribution of the species, although some inconsistencies between projected and realized distribution areas were found, mainly in Tierra del Fuego island and the Islas Malvinas/Falkland Islands, where *M. spinosum* has never been observed.

Despite some differences with the extant species range, the distribution model for the LGM (Fig. 3b) did not show any substantial shifts to lower latitudes, except for a probability of occurrence in northern Argentina (Jujuy province, 22° S) and also in Chile from 25 to 29° S. Additionally, there are two predicted areas on the present Atlantic submarine platform: one extending from *c.* 42 to 52° S adjacent to Chubut and Santa Cruz provinces, and the second from 37 to 40° S flanking the southern part of Buenos Aires province. The model did not predict the species in Tierra del Fuego and Islas Malvinas/Falkland Islands, and the probability of occurrence is higher in northern Patagonia and the high Andean regions during the LGM than in the current distribution, suggesting an important difference of suitability in habitat.

In creating these distribution models, the largest contribution was from variable Bio 8 (mean temperature of the wettest quarter), which had a contribution of 66 and 57.7% for current and palaeodistributional predictions, respectively. This was followed by variable Bio 4 (temperature seasonality), with a contribution of 15.7% and 25.3%, respectively.

Jackknife tests showed Bio 8 to have the most useful information independently from the other variables and also to have the most information not present in the other variables (not recovered from the other variables), followed by Bio 4.

DISCUSSION

Phylogeographical pattern and past demography

Our study detected population structure in *Mulinum spinosum*. Despite unbalanced sampling, significant variation exists between and amongst populations on both sides of the Andes. Significant latitudinal divergence was observed amongst geographical regions in Chile and in Argentina: high Andean–northern Patagonia, central Patagonia and southern Patagonia. Moreover, the geographical discontinuity in southern Patagonian populations was highly corroborated. There were no shared haplotypes amongst most of the clades between northern and southern regions.

The finding of high haplotype diversity and many unique haplotypes in populations in northern and high Andean regions, either east or west of the Andes, indicates that these regions have been inhabited for a long period of time. The great proportion of northern populations with private haplotypes suggests that there is little seed-mediated gene flow amongst populations, and that isolation over time has permitted the accumulation of differences. It has been

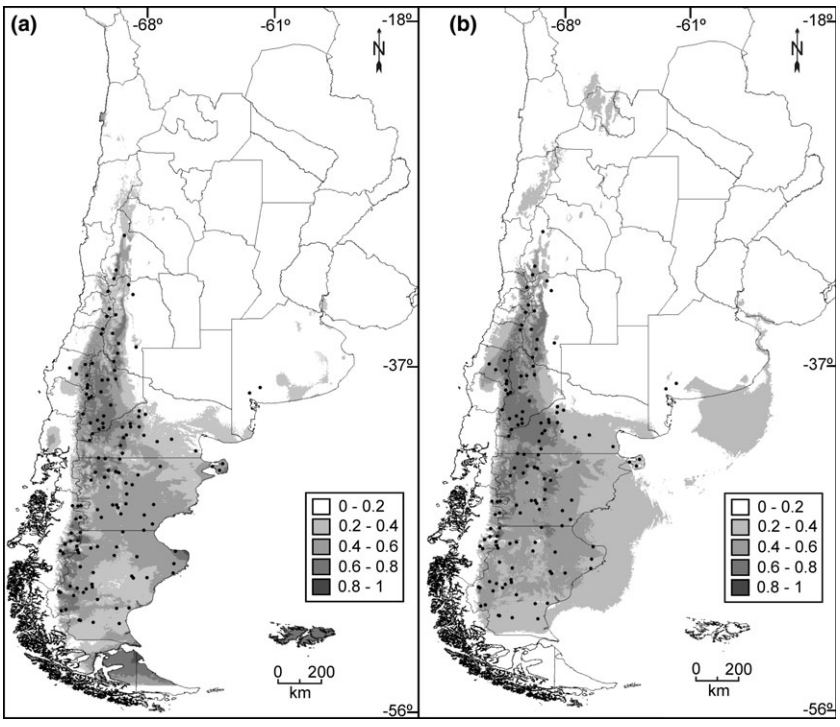

Figure 3 Predictive distribution models of *Mulinum spinosum* for (a) the present and (b) the Last Glacial Maximum, showing habitat suitability in southern South America calculated with Maxent, where darker greys indicate regions with a higher probability of species occurrence. Black dots refer to point localities on which the models are based.

hypothesized that northern Patagonia and the high Andean regions, on both sides of the Andes, harbour isolated endemic species (Hoffman *et al.*, 1997; Domínguez *et al.*, 2006; Escalante *et al.*, 2009). These warm and dry environments are the richest in species number and have elements in common with dry steppe communities at lower elevations to the east (Ferreyra *et al.*, 1998). Consequently, it is possible that this area could have favoured the persistence of small isolated populations. This scenario agrees with previous studies on both plants and animals (Morando *et al.*, 2007; Azpilicueta *et al.*, 2009; Cosacov *et al.*, 2010).

A small number of haplotypes were restricted to the southernmost regions compared with the north, and our results support the same hypothesis of long-term persistence in southern latitudes. The highest haplotype diversity was found in two *M. spinosum* populations (*c.* 47–51° S), in accordance with refugia proposed by several studies (Jakob *et al.*, 2009; Tremetsberger *et al.*, 2009; Cosacov *et al.*, 2010). Interestingly, Domínguez *et al.* (2006) suggested that this zone could be an area of endemism of the Patagonian steppe, in which many relict taxa are present.

Although it is difficult to evaluate the latitudinal genetic structure on the Chilean side (mainly because of a sampling gap), the haplotypes of northern and high Andean regions are not found in the south, and there is no common widely distributed haplotype as on the Argentinian side.

It is possible that *M. spinosum* withstood Pleistocene glacial aridity and cooling within multiple regions in the Patagonian steppe either near the Andes or towards the east. Although most populations of *M. spinosum* with higher haplotype diversity are in northern Patagonia, some also exist in southern Patagonia, with one population of high diversity within the limit of the GPG ice sheet. This suggests that survival also occurred at higher latitudes as well as in northern Patagonia, without any limitation of space during the cooling periods (Jakob *et al.*, 2009). This hypothesis is consistent with the evidence that a large portion of Patagonian steppe on the east remained unglaciated through the late Pleistocene (Rabassa, 2008).

Phylogeographical hypotheses for Patagonia are generally formulated around the Pleistocene glaciations, either the LGM or the GPG, and patterns explained by range contractions into refugia and subsequent expansion/recolonization events from the refugia. Several latitudinal breaks in other taxa seem to be associated with the range expansion of ancestral lineages from north to south (e.g. Jakob *et al.*, 2009; Cosacov *et al.*, 2010; see review of Sérsic *et al.*, 2011). Barriers such as marine introgressions, volcanic arches, ice sheets, river basins and terrain elevations, could have restricted gene flow (Sérsic *et al.*, 2011).

The general tendencies of the inferred phylogeographical patterns for *M. spinosum* are restricted gene flow (RGF) with isolation by distance, or RGF/dispersal, but with some long-distance dispersal. High haplotype diversity and a high number of haplotypes in northern Patagonia and the high Andean regions, and in southern Patagonia, suggests that *M. spinosum* persisted in each of these areas. For one of the clades, which includes no-overlapping northern and southern haplotypes, and for the total cladogram, long-distance colonization possibly coupled with subsequent fragmentation or a past fragmentation followed by range expansion is hypothesized.

The model of historical demographic expansion in Patagonia assumes that current distribution of a species at higher latitudes is the result of post-glacial colonization either from lower latitudes after climate amelioration (Zemlak *et al.*, 2008; Xu *et al.*, 2009; Lessa *et al.*, 2010; although they all suggest older dates of expansion than after the LGM), or colonization from the east to the west from periglacial refugia (Cosacov *et al.*, 2010). In our study, there is no evidence of range expansion of *M. spinosum*: the three summary statistics, by failing to reject the null hypothesis, consistently support this hypothesis and reinforce the inference of limited gene flow. Nevertheless, there is evidence of possible strong purifying selection, shrinking population size or a population bottleneck in northern Chile. Unfortunately, there is a sampling gap in this area, so results must be interpreted cautiously. Our results are consistent with the hypothesis of *in situ* differentiation within regions in which species occurred within their current distribution during long periods of time (Jakob *et al.*, 2009; Tremetsberger *et al.*, 2009).

Major genetic discontinuities in terrestrial Patagonian organisms follow a clear latitudinal pattern and several breaks are concordant with river basins that currently drain, or that drained in the past, the east Andean watershed by crossing the steppe to the Atlantic Ocean (Sérsic *et al.*, 2011). According to Kim *et al.* (1998) and Morando *et al.* (2007), the central area of Chubut province has been a historical isolating barrier for many taxa. The Chubut River leads to the east and was an important drainage as well as a latitudinal boundary (Clapperton, 1993; Iriondo & García, 1993). Thus, it is possible that populations in northern Patagonia may have been isolated from those of southern Patagonia by the Chubut and Deseado rivers, as both were important historical drainages (Martínez & Coronato, 2008). Other scenarios have been hypothesized to explain lineage divergences: pre-Quaternary processes such as tectonic/orogenic events, volcanism, and palaeobasins could have favoured diversification in middle and northern areas of Patagonia (Sérsic *et al.*, 2011).

Present and palaeodistribution modelling

The model of the present potential distribution for *M. spinosum* largely agrees with the species' realized distribution. The current distribution modelling showed suitable habitats of the species in both Tierra del Fuego island and the Islas Malvinas/Falkland Islands where the species is not known to occur, which can be explained by suitable climatic conditions in both places yet failure of the species to disperse to the islands. The predicted extant distribution in the southern part of Buenos Aires province is not surprising given that the species has been found in that region (Parodi, 1941; Politis & Tonni, 1982; Frangi & Bottino, 1995).

Climatically, the geographical distribution of *M. spinosum* appears to be mainly influenced by the mean temperature of the wettest quarter. This variable is biologically important as it is related to the beginning of the growing period. The rainfall in Patagonia is principally concentrated in winter (the coldest period), implying that rainfall precedes rather than co-occurs with the primary growing season (Paruelo *et al.*, 1998; Jobbágy *et al.*, 2002).

The Quaternary glaciations led to the formation of a continuous mountain ice sheet extending between 36 and 56° S, that almost completely covered the Patagonian Andean ranges not far beyond the mountain front on most of the Argentinian side of the Andes, and extended over the piedmont areas to the east over the present submarine platform south of 51° S (southern Santa Cruz province) and to sea level in the Pacific side (Rabassa, 2008). The Patagonian glaciations were progressively less extensive during successive glacial advances after the GPG (Rabassa, 2008). Unfortunately, GPG bioclimatic layers are not available, so we will restrict our discussion to the palaeoclimatic period of the LGM.

Pleistocene glaciations were very extensive in Tierra del Fuego, where the ice sheet reached the present submarine platform, on several occasions covering the entire island (Rabassa, 2008). Also, the entire Islas Malvinas/Falkland Islands were affected by Pleistocene glaciers (Clapperton, 1993). This concurs with the projected absence of the species in both islands.

The suitability of habitat for *M. spinosum* over the extant submarine platform also concurs with the sea level lowering during the full late Cenozoic glaciations, which dropped by up to 100–140 m, partially exposing the Argentinian submarine platform. This process occurred both in the southern Pampa region (southern Buenos Aires province) and Patagonia, with almost a doubling in size of the continental areas, leading to space becoming available for plant colonization (Rabassa, 2008). For the southern Pampa, the environment was extremely arid to semi-arid, with precipitation *c.* 100 mm lower than present (Quattrocchio *et al.*, 2008). These historically suitable climatic conditions may have allowed the Patagonian steppe to expand into the pampean grassy prairies that were spatially reduced and pushed north and north-east (Frangi & Bottino, 1995; Rabassa, 2008).

The higher probability of occurrence in northern Patagonia and the high Andean region during both current and palaeodistribution modelling reinforces the conclusion from observed patterns of high haplotype number and diversity that the species has probably persisted in that region over a long period of time. Despite the high genetic diversity in populations of southern Santa Cruz, that were very close to the ice limit sheet, the palaeodistribution modelling proposes a low probability of occurrence of *M. spinosum* in that area. Nevertheless, other studies showed that this is an area of endemism (Domínguez *et al.*, 2006) and putative refugia (Jakob *et al.*, 2009; Tremetsberger *et al.*, 2009; Cosacov *et al.*, 2010). The southern portion of the south-western Patagonian steppe deserves more detailed study of its biological, geological and climatic past.

CONCLUSIONS

The primary contribution of the present work is in formulating hypotheses of historical processes that have impacted the distribution of *M. spinosum* in the Patagonian steppe. Our data are consistent with the *in situ* survival hypothesis and not with the latitudinal migration of plant communities to avoid adverse climate conditions during Pleistocene cycles. The regions with high levels of DNA variation are inferred to represent areas that have been inhabited for a long time or have served as glacial refugia. We favour persistence rather than migration for *M. spinosum*, as persistence in both northern and southern regions was corroborated by independent analyses.

The pattern of restricted gene flow with isolation by distance concurs with a scenario of fragmentation and isolated population groups. The latitudinal breaks with the consequent structuring of populations may be explained by the influence from ancient processes that probably occurred long before the main glaciations in Patagonia or from barriers during deglaciation periods (e.g. the main river basins).

Mulinum spinosum appears to be a long-time inhabitant of the Patagonian steppe that has survived through the glacial–intergalcial cycles without significant changes in its distributional range, with neither northward shifts during cold climate cycles nor reduction in its current population distribution. This scenario is strongly corroborated by the palaeodistribution modelling. Agreement amongst the phylogeographical patterns proposed here indicates that glaciations probably did not have a great effect on the distribution of *M. spinosum* populations in Patagonia.

ACKNOWLEDGEMENTS

We thank A. Sérsic, L. Ávila, C. Zanotti, A. Cosacov, V. Paiaro, C. Baeza and E. Ruiz for assistance with fieldwork, A. Cosacov for providing valuable comments on an earlier version of this manuscript, and A.M. and C. Fernández for their help with figures. S.M.S. thanks the Fulbright Commission for the fellowship. S.M.S. and R.P. acknowledge the National Research Council of Argentina (CONICET) as researchers and M.V.N. as its doctoral fellowship holder. DNA sequencing was aided by US NSF award DBI 0520978, and fieldwork, in part, by Brigham Young University's MLBM. The overall project is part of a US NSF-PIRE award (OISE 0530267), supporting collaborative research on Patagonian biodiversity, granted to the following institutions (alphabetically): Brigham Young University, Centro Nacional Patagónico (AR), Dalhousie University, Instituto de Botánica Darwinion (AR), Universidad Austral de Chile, Universidad de Concepción, Universidad Nacional del Comahue, Universidad Nacional de Córdoba, and University of Nebraska.

REFERENCES

Acosta, M.C. & Premoli, A.C. (2010) Evidence of chloroplast capture in South American *Nothofagus* (subgenus *Nothofagus*, Nothofagaceae). *Molecular Phylogenetics and Evolution*, **54,** 235–242.

Arroyo, M.T.K. & Uslar, P. (1993) Breeding systems in a temperate mediterranean-type climate montane sclerophyllous forest in central Chile. *Botanical Journal of the Linnean Society*, **111,** 83–102.

Ávila, L.J., Morando, M. & Sites, J.W., Jr (2006) Congeneric phylogeography: hypothesizing species limits and evolutionary processes in Patagonian lizards of the *Liolaemus boulengeri* group (Squamata: Liolaemini). *Biological Journal of the Linnean Society*, **89,** 241–275.

Azpilicueta, M.M., Marchelli, P. & Gallo, L.A. (2009) The effects of Quaternary glaciations in Patagonia as evidenced by chloroplast DNA phylogeography of southern beech *Nothofagus obliqua*. *Tree Genetics and Genomes*, **5,** 561–571.

Barreda, V. & Palazzesi, L. (2007) Patagonian vegetation turnovers during the Paleogene–Early Neogene: origin of arid-adapted floras. *The Botanical Review*, **73,** 31–50.

Barreda, V., Anzótegui, L.M., Prieto, A.R., Aceñolaza, P., Bianchi, M.M., Borromei, A.M., Brea, M., Caccavari, M., Cuadrado, G.A., Garralla, S., Grill, S., Guerstein, G.R., Lutz, A.I., Mancini, M.V., Mautino, L.R., Ottone, E.G., Quattrocchio, M.E., Romero, E.J., Zamaloa, M.C. & Zucol, A. (2007) Diversificación y cambios de las angiospermas durante el Neógeno en Argentina. *Ameghiniana*, **11,** 173–191.

Beaumont, M.A. & Panchal, M. (2008) On the validity of nested clade phylogeographical analysis. *Molecular Ecology*, **17,** 2563–2565.

Cabrera, A.L. & Willink, A. (1973) *Biogeografía de América Latina*. Serie Biología, Monografía No. 13. Organización de Estados Americanos, Washington, DC.

Clapperton, C. (1993) *Quaternary geology and geomorphology of South America*. Elsevier, Amsterdam.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9,** 1657–1659.

Constance, L. (1988) Umbelliferae. *Flora Patagónica*, Vol. 8, Part 5 (ed. by N.M. Correa), pp. 310–379. Instituto Nacional de Tecnología Agropecuaria, Buenos Aires.

Constance, L., Chuang, T.-I. & Bell, C.R. (1971) Chromosome numbers in Umbelliferae. IV. *American Journal of Botany*, **58,** 577–587.

Coronato, A.M.J., Coronato, F., Mazzoni, E. & Vázquez, M. (2008) The physical geography of Patagonia and Tierra del Fuego. *Developments in Quaternary Science*, **11,** 13–55.

Cosacov, A., Sérsic, A.N., Sosa, V., Johnson, L.A. & Cocucci, A. (2010) Multiple periglacial refugia in the Patagonian steppe and post-glacial colonization of the Andes: the phylogeography of *Calceolaria polyrhiza*. *Journal of Biogeography*, **37,** 1463–1477.

Crandall, K.A. & Templeton, A.R. (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics*, **134,** 959–969.

Cullings, K.W. (1992) Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. *Molecular Ecology*, **1,** 233–240.

Cussac, V.E., Ortubay, S., Iglesias, G., Milano, D., Lattuca, M.E., Barriga, J.P., Battini, M. & Gross, M. (2004) The distribution of South American galaxiid fishes: the role of biological traits and post-glacial history. *Journal of Biogeography*, **31,** 103–121.

Damascos, M.A., Barthélémy, D., Ezcurra, C., Martínez, P. & Brion, C. (2008) Plant phenology, shoot growth, and branching pattern in *Mulinum spinosum* (Apiaceae), a cushion shrub of the arid Patagonian steppe of Argentina. *Journal of Arid Environments*, **72,** 1977–1988.

Di Rienzo, J.A., Robledo, C.W., Balzarini, M.G., Casanoves, F., Gonzalez, L. & Tablada, M. (2002) *InfoStat/Estudiantil, version 2.0*. Grupo InfoStat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Ed. Brujas, Córdoba, Argentina.

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

Dupanloup, I., Schneider, S. & Excoffier, L. (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, **11,** 2571–2581.

Escalante, T., Linaje, M., Illoldi-Rangel, P., Rivas, M., Estrada, P., Neira, F. & Morrone, J.J. (2009) Ecological niche models and patterns of richness and endemisms of the southern Andean genus *Eurymetopum* (Coleoptera, Cleridae). *Revista Brasileira de Entomologia*, **53,** 379–385.

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin version 3.01: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1,** 47–60.

Ferreyra, M., Cingolani, A., Ezcurra, C. & Bran, D. (1998) High-Andean vegetation and environmental gradients in northwestern Patagonia, Argentina. *Journal of Vegetation Science*, **9,** 307–316.

Frangi, J.L. & Bottino, O.J. (1995) Comunidades vegetales de la Sierra de la Ventana, Provincia de Buenos Aires, Argentina. *Revista de la Facultad de Agronomía, La Plata*, **71,** 93–133.

Fu, Y.X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147,** 915–925.

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

Hamilton, M.B. (1999) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology*, **8,** 521–523.

Hewitt, G.M. (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **359,** 183–195.

Hijmans, R.J. & Graham, C.H. (2006) The ability of climate envelope models to predict the effect of climate change on species distributions. *Global Change Biology*, **12,** 2272–2281.

Hijmans, R.J., Guarino, L., Cruz, M. & Rojas, E. (2001) Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. *Plant Genetic Resources Newsletter*, **127,** 15–19.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25,** 1965–1978.

Himes, C.M.T., Gallardo, M.H. & Kenagy, G.J. (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.

Hoffman, A., Liberona, F., Muñoz, M. & Watson, J. (1997) *Plantas altoandinas en la Flora Silvestre de Chile*. Ediciones Fundación Claudio Gay, Santiago.

Ikeda, H., Senni, K., Fujii, N. & Setoguchi, H. (2008) Consistent geographic structure among multiple nuclear sequences and cpDNA polymorphisms of *Cardamine nipponica* Franch. et Savat. (Brassicaceae). *Molecular Ecology*, **17,** 3178–3188.

Iriondo, M.H. & García, N.O. (1993) Climatic variation in the Argentine plains during the last 18000 years. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **101,** 209–220.

Jakob, S.S., Martinez-Meyer, E. & Blattner, F.R. (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.

Jobbágy, E.G., Sala, O.E. & Paruelo, J.M. (2002) Patterns and controls of primary production in the Patagonian steppe: a remote sensing approach. *Ecology*, **83,** 307–319.

Kim, I., Phillips, C.J., Monjeau, J.A., Birney, E.C., Noack, K., Pumo, D.E., Sikes, R.S. & Dole, J.A. (1998) Habitat islands, genetic diversity, and gene flow in a Patagonian rodent. *Molecular Ecology*, **7,** 667–678.

Knowles, L.L. (2008) Why does a method that fails continue to be used? *Evolution*, **62,** 2713–2717.

León, R.J.C., Bran, D., Collantes, M., Paruelo, J.M. & Soriano, A. (1998) Grandes unidades de vegetación de la Patagonia extra andina. *Ecología Austral*, **8,** 125–144.

Lessa, E.P., D'Elía, G. & Pardiñas, U.F.J. (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 version 5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25,** 1451–1452.

Marchelli, P. & Gallo, L.A. (2006) Multiple ice-age refugia in a southern beech of South America as evidenced by chloroplast DNA markers. *Conservation Genetics*, **7,** 591–603.

Markgraf, V., McGlone, M. & Hope, G. (1995) Neogene paleoenvironmental and paleoclimatic change in southern temperate ecosystems – a southern perspective. *Trends in Ecology and Evolution*, **10,** 143–147.

Martínez, O.A. & Coronato, A.M.J. (2008) The Late Cenozoic fluvial deposits of Argentine Patagonia. *The Late Cenozoic of Patagonia and Tierra del Fuego* (ed. by J. Rabassa), pp. 205–226. Elsevier, Oxford.

Mathiasen, P. & Premoli, A. (2010) Out in the cold: genetic variation of *Nothofagus pumilio* (Nothofagaceae) provides evidence for latitudinally distinct evolutionary histories in austral South America. *Molecular Ecology*, **19,** 371–385.

Morando, M., Ávila, L.J., Baker, J. & Sites, J.W., Jr (2004) Phylogeny and phylogeography of the *Liolaemus darwinii* complex (Squamata: Liolaemidae): evidence for introgression and incomplete lineage sorting. *Evolution*, **58,** 842–861.

Morando, M., Ávila, L.J., Turner, C.R. & Sites, J.W., Jr (2007) Molecular evidence for a species complex in the Patagonian lizard *Liolaemus bibronii* and phylogeography of the closely related *Liolaemus gracilis* (Squamata: Liolaemini). *Molecular Phylogenetics and Evolution*, **43,** 952–973.

Moreno, P.I., François, J.P., Villa-Martínez, R.P. & Moy, M.C. (2009) Millennial-scale variability in southern Hemisphere westerly wind activity over the last 5000 years in SW Patagonia. *Quaternary Science Reviews*, **28,** 25–38.

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

Müller, K. (2005) SeqState, primer design and sequence statistics for phylogenetic DNA data sets. *Applied Bioinformatics*, **4,** 65–69.

Nei, M. (1987) *Molecular evolutionary genetics*. Columbia University Press, New York.

Panchal, M. (2007) The automation of nested clade phylogeographic analysis. *Bioinformatics*, **23,** 509–510.

Parodi, L.R. (1941) Viaje a la región de Bahía Blanca. *Revista del Museo de La Plata, Nueva Serie* (Sección Oficial 1940), 69–78.

Paruelo, J.M., Beltrán, A., Jobbágy, E., Sala, O.E. & Golluscio, R.A. (1998) The climate of Patagonia: general patterns and controls on biotic processes. *Ecología Austral*, **8,** 85–101.

Pastorino, M.J. & Gallo, L.A. (2002) Quaternary evolutionary history of *Austrocedrus chilensis*, a cypress native to the Andean–Patagonian forest. *Journal of Biogeography*, **29,** 1167–1178.

Peterson, A.T. & Nakazawa, Y. (2008) Environmental data sets matter in ecological niche modelling: an example with

Solenopsis invicta and *Solenopsis richteri*. *Global Ecology and Biogeography*, **17,** 135–144.

Petit, R.J. (2007) The coup de grâce for the nested clade phylogeographic analysis? *Molecular Ecology*, **17,** 516–518.

Phillips, S.J., Dudík, M. & Schapire, R.E. (2004) A maximum entropy approach to species distribution modeling. *Proceedings of the Twenty-First International Conference on Machine Learning* (ed. by C.E. Brodley), pp. 655–662. AMC Press, New York.

Politis, G.G. & Tonni, E.P. (1982) Arqueología de la región pampeana. El sitio 2 de Zanjón Seco (Partido de Necochea, Provincia de Buenos Aires, República Argentina). *Revista de Pré-Historia (Univ. de Sao Paulo)*, **3,** 109–139.

Posada, D., Crandall, K.A. & Templeton, A.R. (2000) GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. *Molecular Ecology*, **9,** 487–488.

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

Quattrocchio, M.E., Borromei, A.M., Deschamps, C.M., Grill, S.C. & Zavala, C.A. (2008) Landscape evolution and climate changes in the Late Pleistocene–Holocene, southern pampa (Argentina): evidence from palynology, mammals and sedimentology. *Quaternary International*, **181,** 123–138.

Rabassa, J. (2008) Late Cenozoic glaciations in Patagonia and Tierra del Fuego. *The Late Cenozoic of Patagonia and Tierra del Fuego* (ed. by J. Rabassa), pp. 151–204. Elsevier, Oxford.

Rabassa, J. & Coronato, A. (2009) Glaciations in Patagonia and Tierra del Fuego during the Ensenadan Stage/Age (Early Pleistocene–earliest Middle Pleistocene). *Quaternary International*, **210,** 18–36.

Ramos-Onsins, S.E. & Rozas, J. (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, **19,** 2092–2100.

Ruzzante, D.E., Walde, S.J., Cussac, V.E., Dalebout, M.L., Seibert, J., Ortubay, S. & Habit, E. (2006) Phylogeography of the Percichthyidae (Pisces) in Patagonia: roles of orogeny, glaciations, and volcanism. *Molecular Ecology*, **15,** 2949–2968.

Sérsic, A.N., Cosacov, A., Cocucci, A.A., Johnson, L.A., Pozner, R., Ávila, L.J., Sites, J.W., Jr & Morando, M. (2011) Emerging phylogeographic patterns of plants and terrestrial vertebrates from Patagonia. *Botanical Journal of the Linnean Society*, **103,** 475–494.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W.S., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L. (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany*, **92,** 142–166.

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.A. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in Angiosperms: the tortoise and the hare III. *American Journal of Botany*, **94,** 275–288.

Simmons, M.P. & Ochoterena, H. (2000) Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology*, **49,** 369–381.

Tajima, F. (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetics*, **105,** 437–460.

Tajima, F. (1989) The effect of change in population size on DNA polymorphism. *Genetics*, **123,** 598–601.

Templeton, A.R. (2004) Statistical phylogeography: methods of evaluating and minimizing inference errors. *Molecular Ecology*, **13,** 789–809.

Templeton, A.R. (2008) Nested clade analysis: an extensively validated method for strong phylogeographic inference. *Molecular Ecology*, **17,** 1877–1880.

Templeton, A.R. (2009) Why does a method that fails continue to be used? The answer. *Evolution*, **63,** 807–812.

Templeton, A.R. & Sing, C.F. (1993) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. *Genetics*, **134,** 659–669.

Templeton, A.R., Boerwinkle, E. & Sing, C.F. (1987) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in *Drosophila*. *Genetics*, **117,** 343–351.

Templeton, A.R., Routman, E. & Phillips, C.A. (1995) Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics*, **140,** 767–782.

Templeton, A.R., Maxwell, T., Posada, D., Stengard, J.H., Boerwinkle, E. & Sing, C.F. (2005) Tree scanning: a method for using haplotype trees in phenotype/genotype association studies. *Genetics*, **169,** 441–453.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25,** 4876–4882.

Tremetsberger, K., Urtubey, E., Terrab, A., Baeza, C.M., Ortiz, M.A., Talavera, M., König, C., Temsch, E.M., Kohl, G., Talavera, S. & Stuessy, T.F. (2009) Pleistocene refugia and polytopic replacement of diploids by tetraploids in the Patagonian and Subantarctic plant *Hypochaeris incana* (Asteraceae, Cichorieae). *Molecular Ecology*, **18,** 3668–3682.

Weir, B.S. & Cockerham, C.C. (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38,** 1358–1370.

Werneck, F.P., Costa, G.C., Colli, G.R., Prado, D.E. & Sites, J.W., Jr (2011) Revisiting the historical distribution of

Seasonally Dry Tropical Forests: new insights based on palaeodistribution modelling and palynological evidence. *Global Ecology and Biogeography*, **20,** 272–288.

Xu, J.W., Pérez-Losada, M., Jara, C.G. & Crandall, K.A. (2009) Pleistocene glaciation leaves deep signature on the freshwater crab *Aegla alacalufi* in Chilean Patagonia. *Molecular Ecology*, **18,** 904–918.

Zamaloa, M.C. (1999) *Estudio palinológico de la Formación* Cullén *(Terciario Superior), Tierra del Fuego, Argentina*. PhD Thesis, Universidad de Buenos Aires, Argentina.

Zemlak, T.S., Habit, E.M., Walde, S.J., Battini, M.A., Adams, E.D.M. & Ruzzante, D.E. (2008) Across the southern Andes on fin: glacial refugia, drainage reversals and a secondary contact zone revealed by the phylogeographical signal of *Galaxias platei* in Patagonia. *Molecular Ecology*, **17,** 5049–5061.

BIOSKETCH

Silvana M. Sede holds a full time research position at the Darwinion Botanical Institute (CONICET), Argentina. Her interests are in the systematics and phylogeography of plant species in Patagonia.

Author contributions: L.A.J. and R.P. conceived the idea; M.V.N. collected the samples; L.A.J. and S.M.S. performed the molecular analysis; S.M.S. and M.V.N. performed the species distribution modelling; S.M.S. analysed the data and led the writing; L.A.J., R.P. and M.V.N. improved the final version of the manuscript.

Editor: Len N. Gillman