Molecular Ecology (2006) **15**, 2949–2968 doi: 10.1111/j.1365-294X.2006.03010.x

Phylogeography of the Percichthyidae (Pisces) in Patagonia: roles of orogeny, glaciation, and volcanism

DANIEL E. RUZZANTE,* SANDRA J. WALDE,* VÍCTOR E. CUSSAC,† MEREL L. DALEBOUT,¶* JACOB SEIBERT,* SILVIA ORTUBAY‡ and EVELYN HABIT§

**Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1, †CRUB — Universidad Nacional del Comahue — Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina, ‡Administración de Parques Nacionales, Delegación Regional Patagonia, Argentina, §Centro de Ciencias Ambientales, EULA, Universidad de Concepción, Concepción, Chile*

Abstract

We used molecular evidence to examine the roles that vicariance mechanisms (mountain-building and drainage changes during the Pleistocene) have played in producing phylogeographical structure within and among South American fish species of the temperate perch family Percichthyidae. The percichthyids include two South American genera, *Percichthys* and *Percilia*, each containing several species, all of which are endemic to southern Argentina and Chile (Patagonia). Maximum-likelihood phylogenies constructed using mitochondrial DNA (mtDNA) control region haplotypes and nuclear GnRH3-2 intron allele sequences support the current taxonomy at the genus level (both *Percichthys* and *Percilia* form strongly supported, monophyletic clades) but indicate that species-level designations need revision. Phylogeographical patterns at the mtDNA support the hypothesis that the Andes have been a major barrier to gene flow. Most species diversity occurs in watersheds to the west of the Andes, together with some ancient divergences among conspecific populations. In contrast, only one species (*Percichthys trucha*) is found east of the Andes, and little to no phylogeographical structure occurs among populations in this region. Mismatch analyses of mtDNA sequences suggest that eastern populations last went through a major bottleneck *c.* 188 000 BP, a date consistent with the onset of the penultimate and largest Pleistocene glaciation in Patagonia. We suggest that eastern populations have undergone repeated founder-flush events as a consequence of glacial cycles, and that the shallow phylogeny is due to mixing during recolonization periods. The area of greater diversity west of the Andes lies outside the northern limit of the glaciers. mtDNA mismatch analysis of the genus *Percilia* which is restricted to this area suggests a long-established population at equilibrium. We conclude that patterns of genetic diversity in these South American genera have been primarily influenced by barriers to gene flow (Andean orogeny, and to a lesser extent, isolation in river drainages), and by glacial cycles, which have resulted in population contraction, re-arrangement of some watersheds, and the temporary breakdown of dispersal barriers among eastern river systems.

Keywords: Andes, intron, mtDNA, Percichthys, Percilia, phylogeography, Pleistocene glaciations, vicariance

Received 1 January 2006; revision received 18 April 2006; accepted 2 May 2006

Correspondence: Daniel E. Ruzzante, Fax: (902) 494 3736; E-mail: daniel.ruzzante@dal.ca.
¶Present address: School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, Australia
**Present address: IWK Health Centre, Department of Pediatrics, Division of Pediatric Hematology/Oncology, Halifax, NS, Canada B3K 6R8

Introduction

Geographical patterns of divergence can often give insight into the processes generating biological diversity. The biogeography of the largely Gondwanan fauna and flora of temperate South America appears to be explained largely by vicariance for many animal groups and by long distance dispersal for plants (SanMartín & Ronquist 2004). Little is known, however, about the roles these and other processes played in shaping phylogeographical patterns of aquatic species in southern Argentina and Chile (Patagonia). Tectonics, volcanism and the Pleistocene glaciations greatly altered the landscape of this region and influenced species distributions. These distributional shifts undoubtedly led to regional isolations, novel associations between species, and local extirpations, which have shaped patterns of species diversity in temperate regions of South America.

One of the most important geological events to occur in Patagonia was the uplift of the southern Andes starting at the beginning of the Miocene [*c.* 23 million years ago (Ma)] and continuing to the present (Ramos 1989). This uplift separated east- and west-flowing river systems and their associated biota. Periodic global cooling during the Pleistocene (1.8 Ma–10 000 BP) also produced shifts in climate, landscape, and sea level. Throughout the Holocene (10 000 BP to present), numerous volcanic eruptions influenced species distributions through landscape and drainage alterations (Clapperton 1993). During the Last Glacial Maximum (20 000–18 000 BP), an ice sheet about 1800 km long covered the Andes across 20 degrees of latitude from 35°S to almost 56°S (Clapperton 1993; McCulloch *et al*. 2000). In Europe and North America, the effects of glaciations on species diversity are well known (Hewitt 2000; Willis & Whittaker 2000), but much less is known about the effects of isolation in refugia on the evolution of temperate species in the Southern Hemisphere.

The few studies of Patagonian species conducted to date suggest that no single factor has dominated in shaping phylogeographical histories. For example, responses to glaciation differed markedly between the two best-studied groups — plants and rodents. During glacial maxima, ice covered the Andean mountains forcing many species into refugia in the north and northwest or in the east, on the arid, but unglaciated Patagonian steppes. For some plants, patterns of genetic variation reflect population divergence that occurred during such periods of isolation in refugia. Examples include the alerce, *Fitzroya cupressoides*, a threatened, long-lived conifer endemic to the southern Andes (Allnutt *et al*. 1999; Prémoli *et al*. 2000), the cypress, *Austrocedrus chilensis* (Pastorino & Gallo 2002, Pastorino *et al*. 2004), and an herbaceous alpine plant, *Hypochaeris palustris* (Muellner *et al*. 2005). For rodents, dispersal from glacial refugia was generally followed by differentiation along environmental gradients. However, the location of these refugia, and subsequent patterns of dispersal varied among species (Kim *et al*. 1998; Smith *et al*. 2001; Palma *et al*. 2002, 2005). Patterns of divergence among lizards (*Liolaemus* spp.) also appear to have been influenced by allopatric isolation, population expansion, and renewed contact and mixing during the Holocene (Morando *et al*. 2004).

Aquatic taxa are likely to show phylogeographical patterns that reflect the histories of river drainages. A number of changes have occurred in the major watersheds of Patagonia since the Pleistocene, as huge palaeolakes formed at the base of melting glaciers, and some high altitude drainages reversed direction from the Atlantic to the Pacific Ocean (McCulloch *et al*. 2000; Turner *et al*. 2005). Studies of phylogeography in aquatic organisms have been confined mostly to the Order Crustacea (e.g. the endemic crab family, Aeglidae, and the decapod family, Parastacidae) for which vicariance effects appear to have been common (Crandall *et al*. 2000; Pérez-Losada *et al*. 2002, 2004). Patterns of genetic diversity in teleost fish have been little studied, but might be expected to reflect a hierarchy of influences, with Pleistocene (and perhaps Holocene) divergence patterns due to climate change layered onto ancient divergence patterns influenced by the Andean orogeny.

Species in the temperate perch family Percichthyidae exhibit a southern Gondwanan distribution. Two genera, *Percichthys* and *Percilia* are endemic to South America and the remaining genera are found only in Australia. The phylogenetic relationship between the South American and Australian groups is uncertain. The discovery of fossil *Percichthys* spp. dating from the upper Palaeocene (65–54 Ma) indicates, however, that this family likely originated well before the separation of South America from Antarctica and Australia 40 Ma (Chang *et al*. 1978). Recent molecular analyses indicate a monophyletic and freshwater origin for at least five of the Australian genera (Jerry *et al*. 2001). Little is known of the evolutionary relationships within and among the South American species. While two species of *Percilia* (*Pa. gillissi* and *Pa. irwini*) have been described and are relatively well accepted, there is some disagreement on the number of extant species of *Percichthys* (*Pt.*) with as many as six species described from morphological data (Ringuelet *et al*. 1967; Arratia 1982; Arratia *et al*. 1983; Campos & Gavilán 1996; López-Albarello 2004).

Divergence between freshwater species can be influenced by several mechanisms, including vicariance, dispersal, and local adaptation. Biogeographical footprints for Patagonian *Percichthys* and *Percilia* indicate that vicariance has been an important evolutionary mechanism. Of the two genera, *Percilia* is restricted to the western side of the Andes while *Percichthys* spp. is found on both sides. *Pt. trucha* occurs both west of the Andes in Pacific drainages and east of the Andes in Atlantic and Pacific drainages (Pacific drainages are found east of the Andes), but *Pt. melanops* is restricted to western drainages west of the Andes. Other

described *Percichthys* species, including *Pt. colhuapiensis*, *Pt. altispinnis*, and *Pt. vinciguerrai*, have been reported only from restricted locations on the eastern side of the Andes (Ringuelet *et al*. 1967) and morphological evidence for the distinctiveness of these taxa is weak (D.E.R., personal observation).

Here, we first use sequence variation at the mitochondrial DNA (mtDNA) control region to evaluate three biogeographical hypotheses that may have contributed to the phylogeographical patterns of the Patagonian Percichthyidae: (i) Andean orogeny has affected phylogeographical patterns by restricting gene flow across the Andes. (ii) Glacial cycles influenced phylogeographical structure, resulting in (a) shallower structure for populations and species in the previously glaciated region in Patagonia compared to those in regions beyond the influence of the Pleistocene glaciers to the north and northwest of the Last Glacial Maximum, and (b) reduced molecular diversity in populations inhabiting postglacial lakes. We test these hypotheses by comparing the molecular diversity within and among populations of the most widespread species, *Pt. trucha*, and the diversity within the *Percilia* species complex. In the light of the mtDNA, results we then examine patterns of variation at a nuclear intron in an attempt to resolve some problematic issues in the systematics of these genera. We conclude by examining mtDNA-based patterns of population expansion and stasis in *Pt. trucha* and *Pa. irwini,* and interpret our results in relation to Pleistocene glacial cycles.

Materials and methods

Sample collection

A total of 355 *Percichthys* (*Pt*.) spp. were collected from 14 locations (lakes and rivers) spanning the range of distribution of the genus in southern Argentina and Chile (Fig. 1). Ten of these locations are in southern Argentina (one is a cross-border lake), six drain to the Atlantic (Colorado and Negro Rivers, Lakes Quillén, Espejo, Musters, and Argentino), three drain to the Pacific (Lakes Rivadavia, Futalaufquen-Kruger, and Pueyrredón-Cochrane) and one is endorheic (Blanca Lagoon). Four locations are west of the Andes, in Chile, and are of Pacific drainage (Mataquito, Itata, Andalien, and Bio Bio Rivers) (Table 1, Fig. 1). We obtained samples of *Pt. melanops* from the Bio Bio and Mataquito Rivers.

Samples in Argentina were taken using gillnets (stretched mesh sizes ranging from 30 to 140 mm) during the summers of 1998 (Lakes Quillén, Espejo and Rivadavia), 2000 (Lakes Rivadavia and Futalaufquen-Kruger), 2001 (Lakes Musters, Pueyrredón-Cochrane, and Argentino), and 2004 (Colorado and Negro Rivers and Blanca Lagoon). In Chile, fish were obtained by electrofishing during 2004. Our samples thus span the latitudinal and altitudinal range of distribution of *Pt. trucha* and *Pt. melanops* (Fig. 1), and they also include the locations where the more geographically restricted *Pt. altispinnis* (Colorado river), *Pt. colhuapiensis* (Musters lake), and *Pt. vinciguerrai* (lake Argentino) have been described (Ringuelet *et al*. 1967; Arratia *et al*. 1983).

Percilia (*Pa*.) spp. were collected from seven rivers in Chile: *Pa. gillissi* from the rivers Maipo, Mataquito, and Maule in the north, and from the rivers Imperial and Cruces in the south ($n = 13$), and *Pa. irwini* from the rivers Andalien and Bio Bio ($n = 5$) (Table 1, Fig. 1). Two Percichthyidae from Australia (*Nannoperca macquaria* and *Macculochella macquariensis*) were used as outgroups. Although here we treat both genera, *Percichthys* and *Percilia* as belonging to the family Percichthyidae, it is recognized that the higher-level systematics of this group are not fully resolved, with *Percilia* considered either a member of Percichthyidae (Johnson 1984), or a distinct family, Perciliidae, in its own right (Arratia 1982).

Four of the lakes sampled in Argentina (Rivadavia, Futalaufquen-Kruger, Pueyrredón, and Argentino) are within the geographical range of the Last Glacial Maximum (LGM, Mercer 1976; Clapperton 1993; see Fig. 1), though Lake Argentino is found at the western end of a wide valley that extends well into the Patagonian steppe. Two other lakes, Quillén and Espejo, are near the limit of the LGM, and may have been ice-covered. The other two Argentine lakes (Musters and Blanca Lagoon), and the rivers east and west of the Andes, are beyond the known limit of the LGM.

Generation of molecular data

DNA extraction. Total genomic DNA was isolated from fresh tissue samples using the Hot-SHOT procedure (Truett *et al*. 2000) using 5 μL of blood or 2 × 2 mm gill tissue. The blood or tissue was dried of ethanol by incubation for 20–40 min at 55 °C. Upon addition of the alkaline lysis buffer, the samples were incubated at 95 °C for ~25 min.

Mitochondrial DNA sequencing. A fragment of the mtDNA control region (*c*. 380 bp) was amplified from all samples using the polymerase chain reaction (PCR) and standard methods on a MJ PTC-225 Thermocycler in 25-μL volumes using 2 μL of DNA extract as template. The primers L19 and MT16498H (Jerry & Baverstock 1998) were used under the following conditions: an initial denaturing cycle of 94 °C for 5 min, 35 cycles with denaturation at 94 °C for 1 min, annealing at 51 °C for 1 min and extension at 72 °C for 1 min, followed by a final extension step of 5 min at 72 °C. PCR products were purified using QIAGEN MinElute 96 PCR purification plates (QIAGEN). Sequencing reactions used Beckman dye terminator cycle fluorescent chemistry (DTCS) and were run on a Beckman CEQ 8000 automated capillary sequencer. For all samples, the amplified fragment was sequenced in both directions.

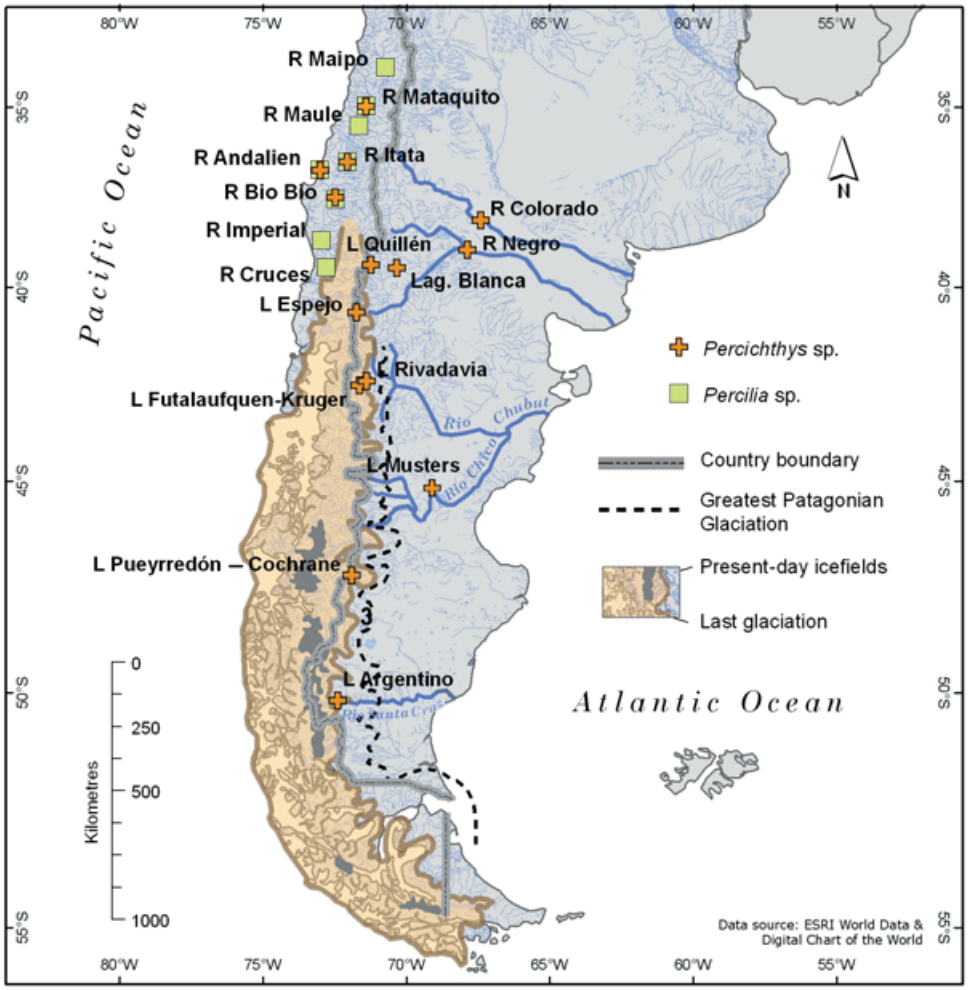

Fig. 1 Map of Patagonia indicating sampling locations for *Percichthys* sp. (✚): *Pt. trucha* in Argentina, and *Pt. trucha* and/or *Pt. melanops* in Chile) and *Percilia* sp. (▭) in Chile. Of the systems with *Pt. trucha* in Argentina some drain into the Atlantic (Rivers Colorado and Negro, lakes Quillén, Espejo, Musters, and Argentino) and some drain into the Pacific (Lakes Rivadavia, Futalaufquen-Kruger and Pueyrredón — Cochrane) and one is endorheic (Blanca Lagoon). All systems sampled in Chile are rivers of Pacific drainage and are located in nonglaciated areas north of the island of Chiloé (Rivers Maipo, Mataquito, Maule, Itata, Andalien, Bio Bio, Imperial and Cruces). Extent of Last Glacial Maximum (LGM) and contour of largest Patagonian glaciation adapted from Clapperton (1993) and Turner *et al.* (2005). The line of political (country) boundary follows the line of highest peaks.

Nuclear intron: length polymorphism analysis and sequencing. GnRH3-2 fragments were amplified from a total of 29 individuals; *Pt. trucha* ($n = 7$), *Pt. melanops* ($n = 7$), *Pa. gillissi* ($n = 7$), *Pa. irwini* ($n = 6$), and one each from the two outgroup species *N. macquaria* and *M. macquariensis*, using unlabelled GnRH2F and GnRH2R primers in 25 µL volumes with conditions as above. PCR products were purified using Montage PCRµ96 microwell filter plates (Millipore) with 0.5-µL volumes from each individual subsequently cloned using pDrive cloning vectors (QIAGEN), transformed into QIAGEN EZ competent cells, and plated on imMedia Amp Blue agar (Invitrogen). Four positive colonies from each plate were screened for suitably sized inserts by direct PCR and agarose gel electrophoresis of colony picks using M13 primers in 20 µL volumes under standard PCR conditions. The resultant vector-flanked GnRH3-2 amplicon from each individual was purified using Montage PCRµ96 microwell filter plates in preparation for sequencing using a Beckman Coulter Quick Start sequencing kit and M13 primers. Sequencing reactions were run on a Beckman CEQ™ 8000 automated capillary sequencer and all amplified fragments were sequenced in both directions.

Phylogenetic reconstruction

For mitochondrial and nuclear intron sequences, alignment, confirmation of polymorphic sites, and haplotype/allele designation was conducted using the program MEGA version 3.0 (Kumar *et al.* 2004).

Table 1 *Percichthys* sp. and *Percilia* sp. collections indicating whether location is east or west of the Andes (in Argentina or Chile, respectively) or cross-border, and whether drainage is to the Atlantic or Pacific Oceans (note that some systems east of the Andes drain to the Pacific Ocean). *N*, sample size in number of individuals assayed for mtDNA polymorphism; *h*, haplotype diversity (SD); π, nucleotide diversity (SD). The samples from Rio Colorado were collected at the location Casa de Piedra. The samples from Rio Negro originate from four locations along the river (Villa Regina, Choele Choel, Guardia Mitre, Conesa). See text for details of mitochondrial DNA clade structure among *Percilia* spp. Rio Colorado, and lakes Musters and Argentino are the locations where the putative morphological *Percichthys* species, *Pt. altispinnis*, *Pt. colhuapiensis* and *Pt. vinciguerrai*, came from, respectively. Our samples from these locations include individuals that would fit the morphological description for these putative species

Species complex	Location relative to Andes — drainage	Lake or river	*N*	*h* (SD)	π (SD)
Percichthys spp.	East — Atlantic	R Colorado	25	0.527 (0.111)	0.006 (0.004)
	East — Atlantic	R Negro	15	0.857 (0.090)	0.015 (0.008)
	East — Endorheic	Lag. Blanca	10	0.778 (0.091)	0.017 (0.010)
	East — Atlantic	L Quillén	35	0.874 (0.043)	0.015 (0.006)
	East — Atlantic	L Espejo	35	0.872 (0.042)	0.017 (0.009)
	East — Pacific	L Rivadavia	72	0.608 (0.037)	0.006 (0.004)
	East — Pacific	L Futalaufquen — Kruger	35	0.570 (0.010)	0.006 (0.004)
	Cross border — Pacific	L Pueyrredón (AR) — Cochrane (CH)	35	0.625 (0.062)	0.010 (0.006)
	East — Atlantic	L Musters	37	0.955 (0.016)	0.015 (0.008)
	East — Atlantic	L Argentino	33	0.907 (0.033)	0.016 (0.009)
	West — Pacific	R Bio Bio	10	0.956 (0.059)	0.013 (0.008)
	West — Pacific	R Andalien	2	0	0
	West — Pacific	R Itata	4	0.833 (0.222)	0.005 (0.004)
		Overall (except samples from Chile) (87 hap)	332	0.937 (0.007)	0.016 (0.008)
		Overall (+ Chile) (99 hap)	348	0.942 (0.007)	0.019 (0.008)
Percilia spp.	West – Pacific	*Percilia irwini* + *gillissi*	18	0.974 (0.025)	0.049 (0.026)
		Percilia irwini clade	13	0.962 (0.041)	0.035 (0.019)

MtDNA control region. Initial phylogenetic analyses were conducted with all individuals from all sampled species using neighbour-joining (NJ) and maximum parsimony (MP). However, establishing positional homology among all sequences throughout the alignment (conducted with CLUSTAL X and checked by eye) was difficult due to high levels of divergence between the *Percichthys* and *Percilia* species, which are not sister groups. Therefore, these analyses were used simply to identify appropriate outgroups for subsequent analyses of phylogeographical structure in *Pt. trucha* and *Percilia* spp. Alignments within each of the *Percichthys* and *Percilia* groups were, however, clear enough that appropriate outgroups could be chosen in both cases. Phylogenetic relationships among haplotypes from these species were reconstructed using maximum likelihood (ML). For *Pt. trucha*, *Pt. melanops* was used as an outgroup (total length of alignment, 369 bp). For *Percilia* spp., *N. macquaria* and *M. macquariensis* were used as outgroups (total length of alignment, 391 bp). For both groups, HKY + G models of sequence evolution were derived by MODELTEST 3.06 (Posada & Crandall 1998): *Percichthys* spp. — nucleotide frequencies, A = 0.3625, C = 0.1844, G = 0.1408, and T = 0.3123, transition-transversion ratio = 2.25, g(alpha) shape parameter = 0.57; *Percilia* spp. – nucleotide frequencies, A = 0.3848, C = 0. 1751, G = 0.1263, and T = 0.3138, transition-transversion ratio = 1.84, g(alpha) shape parameter = 0.27. Heuristic search conditions for ML used starting trees obtained by stepwise addition with 10 random sequence addition replicates and subtree pruning-reconnection (SPR) branch swapping. The robustness of phylogenetic groupings was assessed by bootstrap resampling (1000 fast stepwise replicates). All phylogenetic analyses for the mt dataset (and nuclear intron; see below) were conducted using PAUP* 4.0b10 (Swofford 1999). Minimum spanning networks based on uncorrected 'p' distances were constructed using the program MINSPNET (Excoffier 1993), as implemented in ARLEQUIN version 2.000 (Schneider *et al*. 2000).

Nuclear intron. Phylogenetic relationships among *Percichthys* and *Percilia* spp. based on GnRH3-2 intron allele sequences were reconstructed using ML and MP, with *N. macquariensis* as an outgroup. For ML, an HKY model of sequence evolution was derived by MODELTEST, with estimated nucleotide frequencies A = 0.2952, C = 0.1384, G = 0. 1726, and T = 0.3938, and transition-transversion ratio = 0.9512. ML heuristic search conditions used starting trees obtained by stepwise addition with 10 random sequence addition replicates and tree-bisection–reconnection (TBR) branch swapping. MP reconstructions used branch-and-bound searching, with insertion-deletions coded as a fifth base.

The robustness of phylogenetic groupings was assessed by bootstrap resampling (1000 fast stepwise replicates).

Mitochondrial phylogeography and patterns of population expansion

Standard indices of genetic variation (number of haplotypes, nucleotide diversity, π, and haplotype diversity, *h*) were calculated for each sampling location (lake or river) and over all *Pt. trucha* samples using ARLEQUIN. Pairwise F_{ST} values were calculated for *Pt. trucha* populations where sample sizes were relatively large (≥ 10). The relationship among populations based on these pairwise F_{ST} scores was visualized with a Multidimensional Scaling plot.

For *Pt. trucha and Percilia* spp. haplotype mismatch distributions were used to distinguish between models invoking past exponential growth vs. historical population stasis (Slatkin & Hudson 1991; Rogers & Harpending 1992). For *Pt. trucha* we looked at three different data sets: (i) all samples; (ii) all samples east of the Andes; and (iii) samples by individual lakes and rivers. We assumed a per-site mutation rate for the mtDNA control region of 5% per million years (Bowen *et al*. 2005); thus μ, the per-haplotype mutation rate is $\mu = 1.845 \times 10^{-5}$ (5×10^{-8} multiplied by 369 bp) for *Pt. trucha* and $\mu = 1.875 \times 10^{-5}$ for *Percilia* sp. (5×10^{-8} multiplied by 375 bp). An estimate of the time *t* (in generations) since the population expansion began can be obtained from τ, the crest of the mismatch distribution ($\tau = 2\ \mu t$). For *Pt. trucha*, we assumed a generation time of 2 years (50% maturity reached at 2 years of age, Milano 1996), whereas for *Percilia* spp. we assumed a generation time of 1 year.

Haplotype distributions were examined with Tajima's *D* (Tajima 1989) and Fu's F_S (Fu 1997) to detect excesses of low-frequency haplotypes that may be due to past bottlenecks in population size and population expansions or selection (Rand 1996). Significances of *D* and F_S were assessed with the distribution of random samples generated using a coalescent algorithm assuming neutrality and population equilibrium as implemented in ARLEQUIN.

Results

Mitochondrial DNA diversity

Percichthys. We found 99 polymorphic sites defining 99 haplotypes in a 369 bp fragment of the mtDNA control region among the 348 fish identified as *Percichthys trucha*. Of the polymorphic sites, 70 were transition substitutions, 20 were transversions, eight incorporated both substitution types, and one consisted of a transversion and insertion-deletion combination. The transition:transversion ratio was therefore approximately 3.5:1. Approximately 80% (79 of 99) of the haplotypes were present in only one or two individuals (Fig. 2a). Eight haplotypes were present in 10 or more individuals, and accounted for more than 50% of all individuals sampled ($n = 199$). Only 13 haplotypes occurred in more than one sampling location and included five of the eight most common haplotypes; hap 46 ($n = 62$), hap 33 ($n = 31$), hap 2 ($n = 28$), hap 14 ($n = 25$), and hap 13 ($n = 13$). Common haplotypes that occurred in single locations include haplotype 44 found only in Lake Espejo (11 of 35 fish), and haplotypes 66 and 67 found only in Lake Pueyrredón-Cochrane (10 and 19 of 35 fish, respectively).

Overall haplotype diversity (*h*) for *Pt. trucha* was 0.942 ± 0.007, and nucleotide diversity (π) was 0.019 ± 0.010 (Table 1). The lowest haplotype diversity was found in three mountain lakes with Pacific drainages that were covered by ice during the LGM (Rivadavia, Futalaufquen-Kruger, Pueyrredón-Cochrane), and the northernmost river east of the Andes (Colorado) (Table 1). East of the Andes, the highest haplotype diversity was in a lake with an Atlantic drainage (Musters) which was likely free of ice during the LGM. Within the entire sampled *Pt. trucha* complex, haplotypes differed from one another by a maximum of 44 bp (12.0%). No haplotypes were shared between locations west and east of the Andes. East of the Andes, the 87 haplotypes found differed from one another by a maximum of 16 bp (4.40%). Interestingly, there was less divergence between the nine haplotypes found west of the Andes in Bio Bio and Andalien and those to the east of the Andes (5–18 bp; 1.37–4.95%), than between Bio Bio–Andalien and the three haplotypes found in the other western population, Itata (31–44 bp) (Fig. 3a). No diagnostic sites were found to unite all haplotypes west of the Andes to the exclusion of those east of the Andes. However, the Itata haplotypes possess 20 fixed diagnostic sites (*sensu* Davis & Nixon 1992), while those from Bio Bio-Andalien possess two fixed diagnostic sites.

Percilia. We found 51 polymorphic sites defining 14 unique haplotypes in a 375-bp fragment of the mtDNA control region among 18 fish identified as *Percilia* spp. (Fig. 2b). Of the polymorphic sites, 28 were transition substitutions, nine were transversions, nine incorporated both substitution types, and five were insertion-deletions (approximate transition to tranversion ratio, 3.1:1).

Total haplotype diversity (*h*) was 0.974 ± 0.025, and nucleotide diversity (π) was 0.0495 ± 0.0257. Over all *Percilia* spp. sampled, haplotypes differed from one another by a maximum of 27 bp (7.2%). The 12 fish from Maipo, Mataquito, Maule, Cruces, and Imperial identified as *Percilla gillissi* represented nine haplotypes, which differed from one another by 1–27 bp (average, 16 bp). The six fish from Andalien and Bio Bio identified as *Percilla iriwini* represented five haplotypes which differed from one another by 1–11 bp (2.9%). All haplotypes were private and each found in only a single drainage, with the number of haplo-

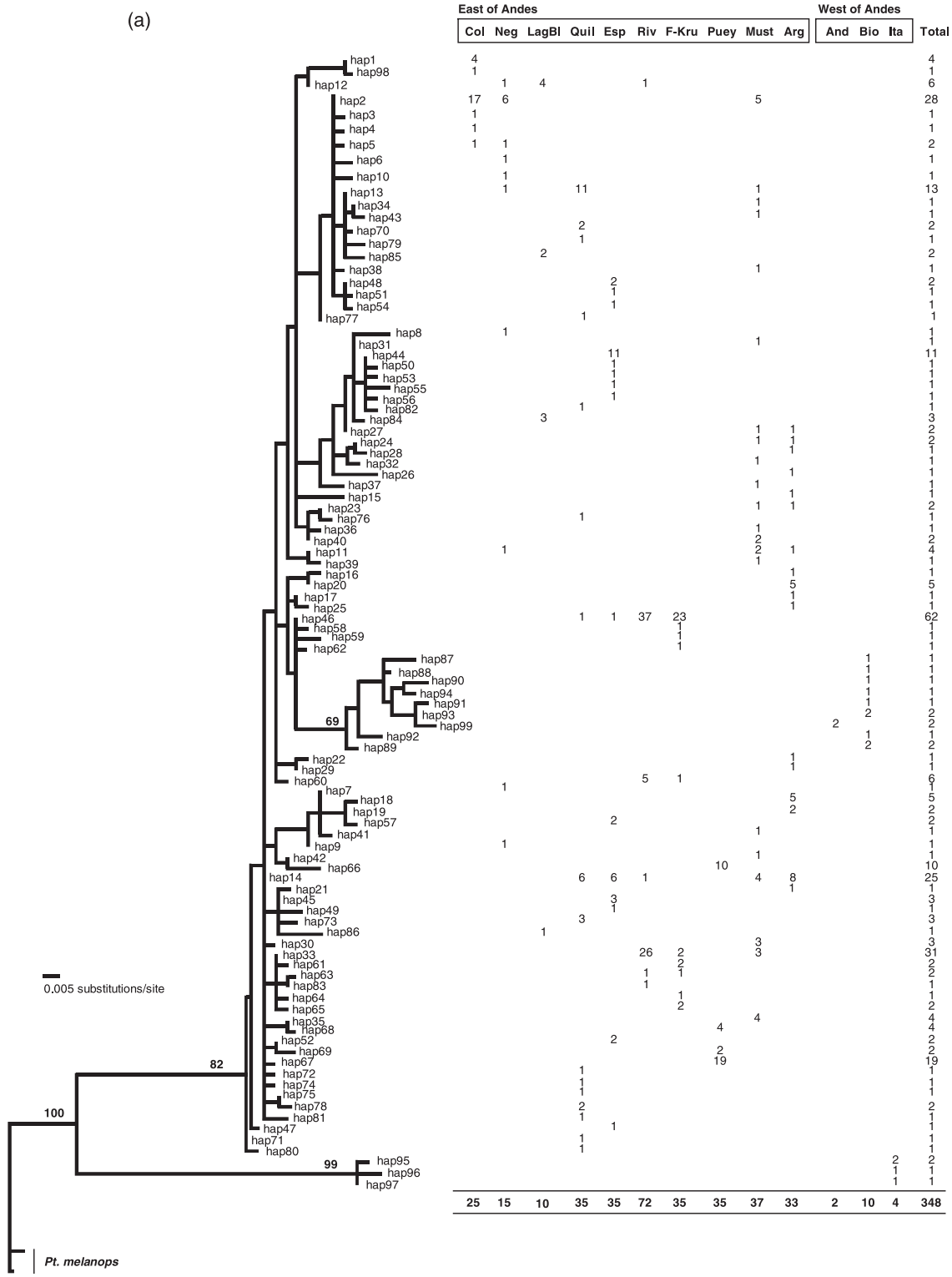

Fig. 2 Phylogenetic relationships based on mitochondrial DNA control region haplotypes reconstructed using maximum likelihood (ML) for (a) *Percichthys trucha* and (b) *Percilia* spp. For *Percilia* spp., the bootstrap consensus tree is shown. Numbers above branches represent bootstrap scores from 1000 ML resamplings of the data. For (b), numbers below branches represent Bremer support indices. Location abbreviations are as follows: Col, Rio Colorado; Neg, Rio Negro, LagBl, Laguna Blanca; Quil, Quillén; Esp, Espejo; Riv, Rivadavia; F-Kru, Futalaufquen-Kruger; Puey, Pueyrredón-Cochrane; Must, Musters; Arg, Argentino; And, Andalien; Bio, Bio Bio; Ita; Itata; Mai, Maipo; Mat, Mataquito; Mau, Maule; Imp, Imperial; Cru, Cruces.

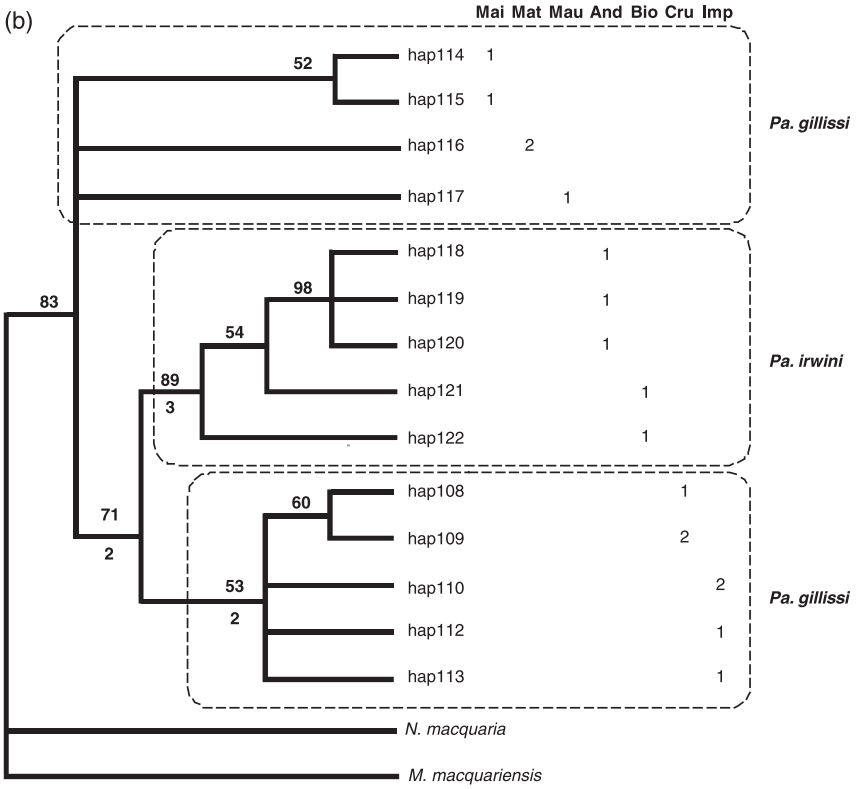

Fig. 2 *Continued*

types per river ranging from one (Mataquito and Maule) to three (Imperial and Andalien).

Sequences representing all haplotypes have been deposited in GenBank (Accession nos for *Pt. trucha*: DQ324115–DQ324213; *Pt. melanops*: DQ324214–DQ324221; *Pa. gillissi*: DQ324222–DQ324230 and *Pa. irwini*: DQ324231–DQ324235).

Mitochondrial DNA phylogeny

Percichthys. In phylogenetic reconstructions including all species, all *Percichthys* haplotypes grouped together to the exclusion of those from other genera (bootstrap score 100%; not shown). In ML reconstructions using *Percichthys melanops* as an outgroup, haplotypes from all fish identified as *Pt. trucha* grouped together in a strongly supported clade (bootstrap score 100%; Fig. 2a). Haplotypes from Andalien and Bio Bio west of the Andes formed a weakly supported clade (bootstrap 69%) nested within a clade consisting of all eastern population haplotypes (bootstrap 82%). This 'eastern plus Bio Bio–Andalien' clade was reciprocally monophyletic with a strongly supported clade comprising the three haplotypes from the western Itata River (bootstrap 99%). In comparison, little phylogeographical structure was observed among the 10 populations east of the Andes.

Similar patterns were observed in the unrooted minimum spanning network (Fig. 3a). A single connection was inferred between haplotypes from Andalien and Bio Bio and those from populations east of the Andes, via a common haplotype (46), found primarily in Rivadavia (n = 37) and Futalaufquen-Kruger (n = 23) (both Pacific drainages), as well as in Quillén and Espejo (n = 1 each) (both Atlantic drainages). Two potential connections were inferred between the highly divergent haplotypes from Itata and eastern populations via the rare haplotypes 57 and 58. Haplotype 58 was found in a single fish from Futalaufquen-Kruger, but it differs by 1 bp from the common haplotype 46. Haplotype 57 was found only in Espejo (n = 2), and differs by 1 bp from a cluster of related haplotypes found in lakes Argentino, Negro, and Musters. No connections were inferred between the Itata haplotypes and haplotypes from the neighbouring Andalien and Bio Bio Rivers. Haplotypes from populations to the east of the Andes form several star-like clusters, with common and widespread haplotypes surrounded by rare haplotypes that differ from these by one to several nucleotide substitutions. The majority of inferred alternative links connect haplotypes within these clusters, though several also provide links between clusters.

Percilia. In phylogenetic reconstructions including all species, all *Percilia* haplotypes grouped together to the exclusion of those from other genera (bootstrap score 100%; not shown). In ML reconstructions using *Nannoperca macquaria* and *Maccullochella macquariensis* as an outgroup, the five *Pa. irwini* haplotypes from Andalien and Bio Bio formed a strongly supported clade (bootstrap score, 89%)

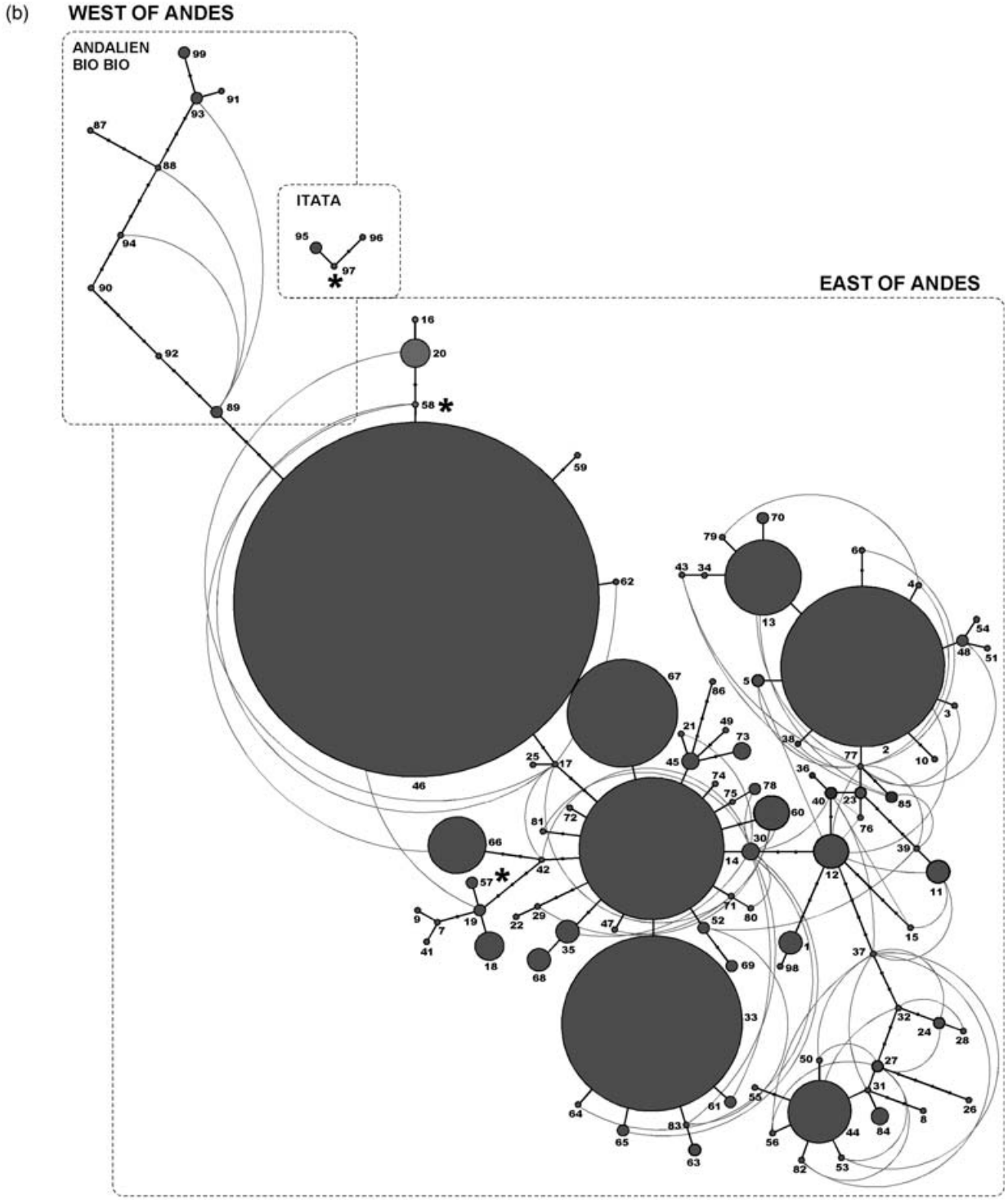

Fig. 3 Unrooted minimum spanning network of mtDNA control region haplotypes for (a) *Percichthys trucha* and (b) *Percilia* spp. Circles represent unique haplotypes scaled according to their frequency in the overall sample for each genus. Small black circles on branches connecting nodes represent inferred missing haplotypes. Grey curved branches represent alternative connections between haplotypes. For (a), asterisks next to haplotypes 57 and 58 indicate the two potential connections found between the highly divergent Itata haplotypes and all others, east and west of the Andes.

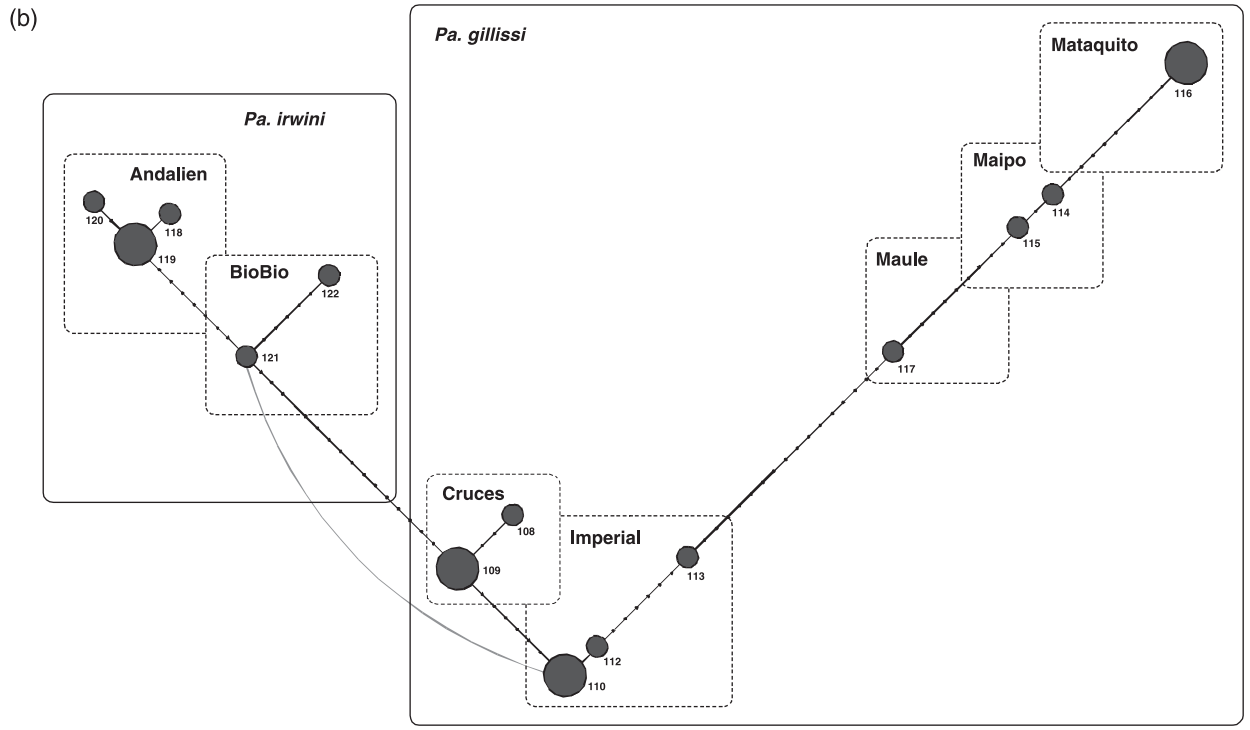

Fig. 3 *Continued*

that nested within the *Pa. gillissi* haplotypes (Fig. 2b), such that *Pa. gillissi* was paraphyletic in these reconstructions. The *Pa. irwini* clade grouped closely with a weakly supported clade of *Pa. gillissi* haplotypes from Cruces and Imperial (bootstrap score 71%). Bremer support indices (Bremer 1988) calculated using TREEROT, version 2.0 (Sorensen 1999) for one of the four best MP trees (tree length = 297, consistency index = 0.896) further supported the robustness and relationships among these clades. In the unrooted minimum spanning network, *Percilia* spp. haplotypes were linked to one another in a long, linear chain (Fig. 3b), yet formed clusters corresponding to the same clades observed in the ML tree. In this network, the Andalien-BioBio *Pa. irwini* and Mataquito-Maipo-Maule *Pa. gillissi* haplotype clusters formed the two terminal ends, with the Cruces-Imperial *Pa. gillissi* haplotypes in the middle. Only one alternative connection was found, linking one of the Imperial *Pa. gillissi* haplotypes to a *Pa. irwini* haplotype from Bio Bio.

Nuclear phylogeography and taxonomy

Apparent inconsistencies between the mtDNA results and current taxonomy prompted us to examine patterns of nuclear differentiation within and among these taxa. For *Percichthys*, the lack of phylogeographical structure among populations east of the Andes is inconsistent with the presence of multiple species in this region. This conclusion is also supported by the weak and conflicting patterns of morphological divergence observed among the widespread species, *Pt. trucha*, and the other putative species (*Pt. colhuapiensis*, *Pt. altispinnis*, and *Pt. vinciguerrai*) with more localized distributions (Ringuelet *et al*. 1967; Arratia *et al*. 1983; vs. López-Albarello 2004; Ruzzante *et al*. unpublished data). The deep divergence observed among some *Pt. trucha* populations west of the Andes suggests, however, that this region could in fact harbour additional, undocumented species. For *Percilia*, the paraphyletic relationships among *Pa. gillissi* and *Pa. irwini* haplotypes indicate that there may be great taxonomic complexity in this genus than is currently recognized. Following Hassan *et al*. (2002), we examined length polymorphism and sequence variation at a single copy nuclear (scn) locus, the Gonadotrophin-releasing hormone 3 intron 2 (GnRH3-2) to investigate these issues for *Percichthys* and *Percilia*. Sequences representing all alleles have been deposited in GenBank (Accession nos DQ328506–DQ328518).

Length and sequence polymorphism. In *Percichthys*, fish identified as *Pt. melanops* (Mataquito and Bio Bio) possessed GnRH3-2 alleles that were 222 bp in length (Table 2). Fish identified as *Pt. trucha* possessed alleles that were either 221 bp or 220 bp in length. Interestingly, neither these length polymorphisms nor the inferred relationships among the *Pt. trucha* alleles based on nucleotide substitutions and indels (Fig. 4) recapitulated the deep divergences observed among some of these populations at the mtDNA. Fish from three eastern lakes (Quillén, Argentino, and Musters) possessed alleles 221 bp in length, while fish from

Table 2 Polymorphic sites, including insertion-deletions, defining nuclear GnH3-2 intron alleles among *Percilia* (*Pa.*) and *Percichthys* (*Pt.*) species. For each genus, alleles are matched to the first row with dots indicating identity to this sequence. Dashes indicate insertion-deletions (indels). Location abbreviations are as used in Fig. 2. Numbers in brackets indicate the number of fish representing each allele

Genus and species	Location	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 2 1 1 2 2 2 2 2 3 3 3 3 3 4 4 6 7 0 0 1 2 2 2 2 2 2 2 2 3 3 3 3 3 3 4 6 7 9 1 1 1 9 2 4 1 2 4 5 9 0 3 4 6 8 0 2 9 4 2 4 9 0 1 2 3 4 5 6 9 0 1 2 3 4 5 8 0 3 1 1 0 2 7
Percilia		
Pa. gillisi	Mai (2)	G T G – – T T T G C A A A C T A A T A – – – – – – – – – A A G C C T G – T G A C G C G
Pa. gillisi	Mau (1)	. . . . – – . . . . G . . – – – – – – – – – . . . . – . . . .
Pa. gillisi	Mat (1)	. . A – – . . . . – – – – – – – – – . . . . . A . T . . . .
Pa. gillisi	Imp (1)	. . . . . – – A . . . G . . . . T – – – – – – – – – . . . . . A . T . . . .
Pa. irwini	And (1), Bio (1)	C . . . – – . . . . – – – – – – – – – . . . . . A . T . . . .
Percichthys		
Pt. trucha	Bio (1), Ita (1)	G T G – T T T – G T A A A T T T A G G A C T T G C T T T T T A C A T T T T G G C G G G
Pt. trucha	Col (1)	. . . . – . . . – . . . . C . .
Pt. trucha	Riv (1)	. . . . – . . . – . . . . A . . . .
Pt. trucha	Must (1), Arg (1)	. . . . – . . . T . . . .
Pt. trucha	Quil (1)	. . . . – . . . T . . . . A . . . . A . . . . A
Pt. melanops	Bio (1)	. . . A . . . T . . . . G . . . . . A . A . . . . A . A T . . .
Pt. melanops	Bio (1), Mat (1)	. . . A . . . T . . . . G . . . . . A . A . . . . A . A . . . .

two other eastern locations (Rivadavia and Colorado), as well as fish from the western rivers of Bio Bio and Itata possessed alleles 220 bp in length. With the exception of this 1-bp indel, no other diagnostic sites were found supporting these groupings. Overall, five alleles, defined by two transitions, two transversions, and the 1-bp indel, were found among the seven *Pt. trucha* screened for this locus.

Percilia. For *Percilia*, GnRH3-2 intron results were in agreement with those from mtDNA. Length polymorphisms and inferred relationships among alleles indicate that there are at least two species of Percilia, but these groupings do not correspond to current taxonomic divisions. Fish identified as *Pa. gillissi* from Maipo and Maule possessed alleles that were 212 bp in length, while fish identified as *Pa. gillissi* from Imperial and Mataquito, as well as those identified as *Pa. irwini* from Andalien and Bio Bio possessed alleles that were 211 bp in length. In addition to this 1 bp indel, the latter group was further distinguished by a tranversion substitution (Table 2). Overall therefore, two diagnostic sites sensu Davis & Nixon (1992) differentiate these two groups at the GnRH3-2 intron (Table 2).

Mitochondrial phylogeography and patterns of population explansion

Percichthys trucha. Pairwise F_{ST} comparisons revealed that although no deep structuring was revealed by the

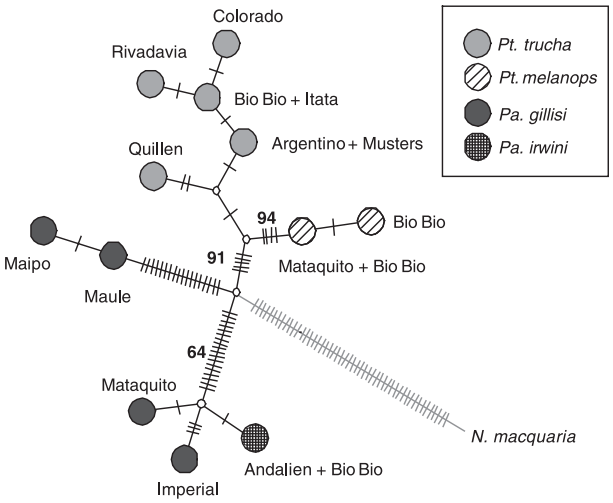

Fig. 4 Unrooted parsimony (MP) network of GnRH3-2 intron sequences. This network did not differ the rooted ML phylogeny using *Nannoperca macquaria* as an outgroup. Nodes represent unique alleles, the distribution of which is indicated by the legend. Small unfilled nodes represent inferred missing alleles. Cross bars on the branches represent nucleotide substitutions or insertion-deletions. Maximum-likelihood bootstrap values are shown next to branches with > 60% support. Six equally parsimonious networks were found which differed only in relationships among the *Percichthys trucha* alleles. Seventy-six variable sites were observed over a 226-bp alignment including *N. macquaria*, of which 28 were phylogenetically informative (consistency index, CI = 0.97).

Table 3 Pairwise F_{ST} scores based on mtDNA haplotype frequencies among *Percichthys trucha* populations for which sample sizes were ≥ 10 (See Table 1 for sample size information). Nonsignificant ($P = 0.05$) estimates are underlined and in italic bold. No Bonferroni correction

Location	Neg	LagBl	Quil	Esp	Riv	F-Kru	Must	Puey	Arg	Bio
Col	***0.0553***	0.3740	0.2896	0.2905	0.4233	0.4500	0. 1735	0.4202	0.2743	0.2972
Neg	—	0.1576	0.1152	0.1346	0.2960	0.3089	***0.0339***	0.2754	0.1141	0.0962
LagBl		—	0.1666	0.1675	0.3298	0.3525	0.1214	0.3164	0.1482	0.1333
Quil			—	0.0997	0.2598	0.2643	0.0600	0.2504	0.0709	***0.0901***
Esp				—	0.2606	0.2652	0.0690	0.2513	0.0718	0.0911
Riv					—	***0.0773***	0.2075	0.3844	0.2560	0.2648
Fut-K						—	0.2326	0.4025	0.2631	0.2788
Must							—	0.2087	0.0397	***0.0448***
Puey								—	0.2350	0.2420
Arg									—	***0.0712***

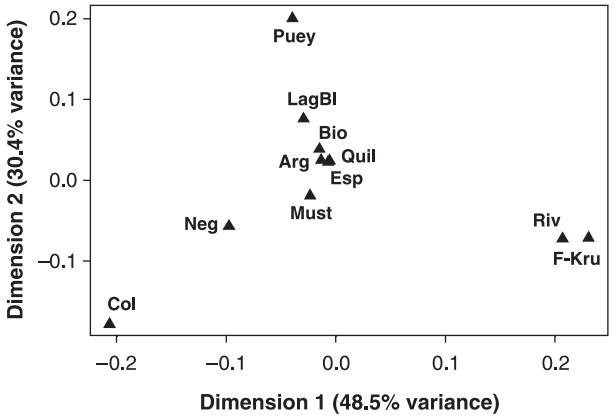

Fig. 5 Multidimensional scaling plot of pairwise F_{ST} estimates among *Percichthys trucha* populations on both sides of the Andes for which $n \geq 10$ fish were sampled (sample sizes shown in Table 1). Abbreviated population names are as in Fig. 2. The multidimensional scaling analysis was conducted considering five dimensions, with dimensions 3, 4, and 5 explaining 15.8%, 3.7%, and 1.6%, respectively.

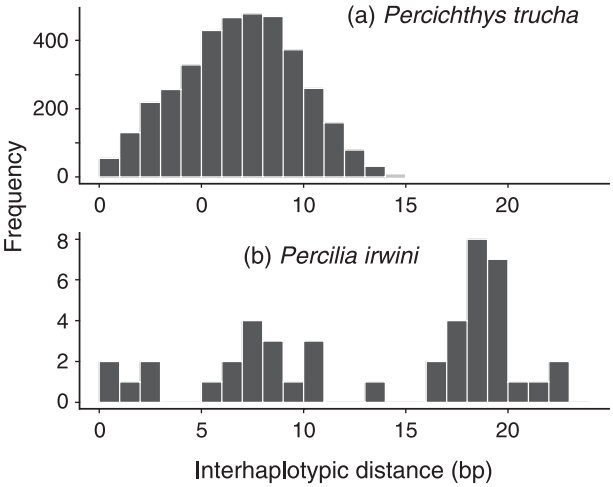

Fig. 6 Mismatch distribution for (a) *Percichthys trucha* haplotypes found in Andean Patagonia and on the Patagonian steppe (east of the Andes) ($n = 87$) and (b) *Percilia irwini* in Chile ($n = 13$). (a) The mismatch distribution for *Percichthys* is unimodal with parameters $\tau = 6.938$ and $\theta_0 = 0.006$ (See Table 1) placing the start of population expansion at *c*. 188 000 BP (95% CI = 94 000–283 000) a date consistent with the penultimate and largest glacial advance in Patagonia (see limit of this glacial maximum in Fig. 1). *Percichthys* thus appears to have experienced a huge population decline during the Pleistocene ice ages but prior to the onset of the most recent 'Llanquihue' glaciation in South America (110 000 BP). Both Tajima's *D* test and Fu's F_S test indicate population expansion (see text and Table 1). (b) The mismatch distribution for *Pa. irwini* is multimodal as expected for long established populations near equilibrium, with parameters $\tau = 20.402$ and $\theta_0 = 0$ (see Table 1) placing the beginning of population expansion at *c*. 05. Ma (95% CI: 0.300–0.700 Ma) consistent with the fact that the *Percilia* sp. populations sampled are located in areas beyond the LGM (i.e. north of it, see Fig. 1).

phylogenetic analyses, the majority of *Pt. trucha* populations east of the Andes were nonetheless significantly different from one another ($P \leq 0.05$; Table 3). The exceptions were Colorado and Negro ($F_{ST} = 0.0553$), Musters and Negro ($F_{ST} = 0.0339$), and Futalaufquen-Kruger and Rivadavia ($F_{ST} = 0.0773$). A multidimensional scaling plot (considering five dimensions) of these pairwise F_{ST} estimates indicated that populations from the rivers Colorado and Negro are markedly different along dimension 1 (explaining 48.5% of the total variance) from a cluster of samples that include lakes Quillén, Espejo, Musters and Argentino, Bio Bio, Blanca lagoon and lake Pueyrredón (Fig. 5). Interestingly, the samples from lakes Rivadavia and Futalaufquen-Kruger also differ from the central cluster of samples along dimension 1 (Fig. 5). Along dimension 2 (30.4% of variance), the most divergent samples are those from lake Pueyrredón and Colorado River, which lie at opposite extremes along this axis (Fig. 5).

The mismatch distribution for eastern *Pt. trucha* populations (87 haplotypes) was unimodal (Fig. 6a). Such a distribution fits an exponential growth model and reflects the shallow haplotype phylogeny and low nucleotide diversity found within the region (high haplotype- and low

nucleotide diversity, $h = 0.937$, $\pi = 0.016$, Table 1). When all *Pt. trucha* from both east and west of the Andes are included, the mismatch distribution becomes bimodal (data not shown) with the right hand peak reflecting the frequency distribution of interhaplotype distances between the large 'eastern plus Bio Bio–Andalien' clade and highly divergent *Pt. trucha* from the Itata River. Results from both Tajima's *D* and Fu's F_S indicate a population expansion for *Pt. trucha*, whether estimated for all 99 haplotypes from both sides of the Andes, or only on the 87 haplotypes found east of the Andes (Table 4).

From the location of the crest of the unimodal mismatch distribution (τ) we estimated that the major population expansion among eastern *Pt. trucha* began about 188 000 BP (95% CI: 94 000–283 000 BP). Similarly, from θ_0, we estimated the initial effective female population size to have been a few tens of individuals (95% CI, 0–35 000; Table 4). Expansion of the populations associated with some lakes (Rivadavia, Futalaufquen-Kruger) appears to have ocurred more recently, while others (Negro, Quillén, and Espejo) appear to be older.

Percilia irwini. The mismatch distribution for *Pa. irwini* was multimodal as would be expected for a long established population near equilibrium (Fig. 6b). Population expansion is estimated to have begun about 0.5 Ma (95% CI: 0.300–0.700 Ma; Table 4), with estimated female effective population size at this time ranging from 0 to 171 000 (founder effect). No attempts were made to interpret a distribution of interhaplotypic distances for *Pt. gillissi* given the apparent paraphyly of this species. Neither Tajima's *D* nor Fu's F_S suggest a recent expansion for the genus *Percilia*, either for the entire complex (*Pa. irwini* and *Pa. gillissi*, $n = 17$ individuals) or the *Pa. irwini* clade alone ($n = 5$ individuals).

Discussion

The patterns of genetic diversity within and among species belonging to the family Percichthyidae in southern South America reflect both vicariance mechanisms (presence of the Andes, isolation of river drainages) and the consequences of glacial cycles during the Pleistocene. No deep phylogenetic structure was detected among *Percichthys* populations east of the Andes, in contrast to patterns expected if this region harboured multiple species. The lack of phylogenetic structure is likely due to mixing and dispersal that took place during the repeated population contractions and expansions (founder-flush events) associated with Pleistocene glacial cycles, when huge proglacial palaeolakes connected present-day lakes and rivers. Much greater phylogenetic diversity was observed in the nonglaciated areas west of the Andes, both at the species and population levels. Some western populations of *Percichthys trucha* appear far more closely related to conspecifics east of the Andes than others. Below, we use the patterns of genetic diversity to discuss probable phylogeographical scenarios for the group, and discuss implications for the systematics of *Percichthys* and *Percilia* in Patagonia.

Phylogeographical scenarios

Like the Alps in Europe, the Andes have long represented a major barrier to gene flow. Among the South American Percichthyidae, species distributions clearly reflect this obstacle to dispersal, with *Percilia* spp. and *Percichthys melanops* found only to the west of the Andes. Isolation due to the Andean divide has also apparently contributed to within-species divergence in *Pt. trucha*, the only species found on both sides of the Andes. Significant mtDNA haplotype divergence was observed between *Pt. trucha* populations in the northern (38°) western drainages (Bio Bio, Andalien, Itata), and those east of the Andes. The divergence of the Itata population from other western populations, as well as from those to the east, is particularly dramatic (Figs 2a and 3a). Vicariance has, without a doubt, been an important mechanism contributing to current patterns of divergence in this family. The apparent anomaly to this general pattern is the absence of deep divergence between the populations in the previously glaciated, southeastern Andean lakes that drain westwards to the Pacific (lakes Rivadavia, Futalaufquen-Kruger, Pueyrredón) and populations in eastern lakes and rivers that drain to the Atlantic. At least two terrestrial species complexes show spatially similar patterns. Northern coastal populations of the conifer, *Fitzroya cupressoides*, west of the Andes (from ~40°S) exhibit relatively deep divergences, while southern populations are quite similar on both sides of the Andes (Allnutt *et al*. 1999). A broadly similar pattern holds for a forest dwelling rodent complex, *Abrothrix olivaceous/xanthorinus* (Smith *et al*. 2001).

Vicariance at a smaller spatial scale, among drainages, has also contributed to patterns of divergence in the Percichthyidae, but this effect has been much stronger on the western side of the Andes. Two genera, each with at least two species are present in Chile, while all the Percichthyidae from Argentina appear to belong to a single species (see below). The differentiation within *Percilia* is associated with watersheds, with the three mtDNA clades reflecting distinct drainages at different latitudes. The deepest divergence within *Pt. trucha* was also found within western watersheds. Similarly, species differentiation within *Percichthys* (*Pt. trucha* vs. *Pt. melanops*) was only found on the Chilean side. These species are not found in distinct watersheds, however, suggesting a more complex history. A more complete examination of *Percichthys* west of the Andes will be required to determine the history of species divergence in this region. Regardless, the generally higher levels of between-population divergence west of the

Table 4 Population expansion and mismatch distribution parameters for *Percichthys trucha* and *Percilia* spp. Information is provided on whether the location is east (Arg) or west (Chile) of the Andes and whether current drainage is to the Atlantic or Pacific Oceans. Population expansion: Tajima's *D* and Fu's F_S tests with *P* values and parameters of the mismatch distribution Tau, θ_0, and θ_1. Generation time for *Percichthys* sp. 2 years and mutation rate, $\mu = 3.69 * 10^{-5}$. Generation time for *Percilia* sp. 1 year and mutation rate, $\mu = 1.875 * 10^{-5}$ (see text for details)

			Population expansion		Mismatch distribution parameters		
Species complex	Location relative to Andes and drainage	Lake or river (N)	Tajima *D* (*P* value)	Fu's F_S (*P* value)	τau (time of exp · Ma) [95% CI]	θ_0 ($N_{ft=0}$) · 10^3 [95% CI]	θ_1 ($N_{ft=1}$) · 10^3
Percichthys trucha	East — Atlantic	R Colorado (25)	−0.029 (0.503)	−0.139 (0.480)	6.812 (0.185) [0.049–0.334]	0.016 (0.217) [0–36]	1.010 (13.7) [0–56.5]
	East — Atlantic	R Negro (15)	–1.157 (0.134)	–1.912 (0.155)	11.107 (0.300) [0.135–0.545]	0.002 (0.027) [0–120]	7.233 (98) [32–658]
	East — Atlantic	L Quillén (35)	−0.569 (0.304)	1.283	9.111 (0.247) [0.106–0.470]	0.001 (0.014) [0–49]	10.918 (148) [56–889]
	East — Atlantic	L Espejo (35)	0.645 (0.800)	–1.42 (0.294)	9.974 (0.270) [0.119–0.413]	0.003 (0.041) [0–77]	16.528 (224) [121–28 000]
	East — Pacific	L Rivadavia (72)	0.558 (0.755)	1.283	4.697 (0.127) [0.037–0.270]	0.000 (0) [0–37]	3.247 (44) [17.8–20 700]
	East — Pacific	L Futalaufquen — Kruger (35)	−0.882 (0.197)	–2.523 (0.107)	5.797 (0.157) [0.049–0.279]	0.004 (0.054) [0–23]	1.829 (25) [2.8–223.6]
	Cross border — Pacific	L Pueyrredón-Cochrane (35)	−0.606 (0.305)	5.417	6.895 (0.187) [0.031–0.424]	0.002 (0.027) [0–110]	5.359 (73) [10.5–1,720]
	East — Atlantic	L Musters (37)	0.970 (0.847)	–7.64 (0.009)	6.666 (0.180) [0.106–0.250]	0.000 (0) [0–32]	26.157 (355) [197–88 000]
	East — Atlantic	L Argentino (33)	−0.715 (0.278)	–3. 669 (0.080)	7.062 (0.191) [0.109–0.280]	0.000 (0) [0–33]	22.461 (304) [173–50 850]
Overall (except *Pt. trucha* from Chile) (87 hap) (332)			–1.402 (0.040)	–24.75 (0.001)	6.938 (0.188) [0.094–0.283]	0.006 (0.081) [0–32 000]	18.618 (250) [181–5,800]
Overall (+ *Pt. trucha* from Chile) (99 hap) (348)			–1.666 (0.001)	–24.46 (0.010)	6.500 (0.176) [0.095–0.289]	0.799 (10) [0–35 000]	18.836 (255) [201–925]
Percilia spp. West — Pacific		*Percilia irwini* + *gillissi* (18)	—	—	27.305 (1.480) [—]	0.742 (10) [—]	47.91 (650) [—]
		Percilia irwini clade (13)	—	−0.55 (0.269)	20.402 (0.544) [0.310–0.697]	0 (0) [0–171 000]	38.85 (1000) [403–102 500]

Andes suggest relatively stable drainage patterns with limited dispersal among individual drainages. Thus, not only have the Andes been a barrier to gene flow, but they appear to separate populations that have undergone significant divergence (on the western side) from populations that show little divergence (eastern side).

Effects of glaciation

Some of the observed among- and within-species patterns of genetic divergence appear to be linked to events during the Pleistocene. Aquatic taxa underwent major distributional shifts as the glaciers repeatedly advanced and retreated, and habitable lakes and rivers disappeared and re-appeared. Dispersal patterns were affected by shifting drainages and by the formation (and disappearance) of large proglacial palaeolakes. We suggest that these events are probably responsible for the absence of deep phylogenetic structure among populations in watersheds currently or previously draining to the east. Genetic divergence among these populations is shallow, suggesting that: (i) the populations are relatively young (recently colonized from a single source); (ii) rates of evolution have been slow; and/or (iii) there has been extensive population mixing. We favour mixing as the dominant mechanism, though the various hypotheses are not mutually exclusive.

Percichthys, as a genus, is not young in Patagonia. Fossil evidence suggests it was present *c*. 40 Ma (Chang *et al*. 1978). Existing *Pt. trucha* populations to the east of the Andes (including those in previously glaciated lakes) appear however, to be relatively young. The unimodal mismatch distribution of interhaplotype differences is suggestive of recent exponential growth, beginning 100 000–280 000 BP. These populations therefore appear to be recovering from a bottleneck that occurred during the Pleistocene ice ages, prior to the onset of the most recent Llanquihue glaciation in South America (110 000 BP) (Clapperton 1993). Northern Hemisphere fish populations from temperate regions that were glaciated during the Pleistocene also have similar genetic signatures suggesting that they have gone through founder-flush cycles associated with glacial advances and retreats (Bernatchez & Wilson 1998). The mean point estimate (188 000 BP) for the start of population expansion for *Pt. trucha* in eastern Patagonia corresponds approximately to the penultimate and largest glacial advance in the region (Kaplan *et al*. 2005). This was also a period of major canyon cutting along the eastern flank of the Patagonian Andes, establishing fluvial channels that were followed during subsequent glacial expansions (Clapperton 1993). For *Percilia*, the most recent bottleneck undergone by *Pa. irwini* populations in the northwestern drainages appears to have been somewhat earlier (300 000–700 000 BP). Given the uncertainty in the molecular clock for the mtDNA control region (reviewed in Bowen *et al*. 2005), and the fact that estimates may vary with the length of time over which rates are being estimated (Ho *et al*. 2005), estimates of time since population expansion have large variances and can only be taken as rough approximations. Despite these limitations, our estimates do strikingly suggest that: (i) a more recent bottleneck and subsequent population expansion for *Percichthys* on the east side of the Andes than for *Percilia* on the west side, and (ii) the occurrence of a major founder-flush event for the eastern *Percichthys* at a time when glacial processes dominated, following which populations began to be divided into more distinct watersheds.

Where were the refugia, and what was the major direction of population expansion? The sampling locations from currently eastern draining lakes and rivers come from four large, but unconnected watersheds, from north to south, the Rio Colorado, the Rio Negro, the Rio Senguer-Chico-Chubut and the Rio Santa Cruz. The Senguer-Chico-Chubut watershed is huge, extending north almost to Lake Rivadavia, and south almost to Lake Pueyrredón-Cochrane (Fig. 1). One possible scenario is that populations in the more southern drainages are the youngest, and recolonization originated from refugia in the northern unglaciated region. Current patterns of genetic differentiation do not show much support for this hypothesis, however. The haplotype network for western *Pt. trucha* populations (Fig. 2) shows no obvious latitudinal gradient that would indicate a predominantly southward expansion. Haplotype diversity was highest in one of the central watersheds (Lake Musters of the Senguer-Chico-Chubut watershed), closely followed by the most southerly lake (Argentino in the Santa Cruz watershed). In addition, the population in Lake Musters shares haplotypes with the two eastern drainages to the north, the eastern drainage to the south, as well as one of the southern drainages that currently flows west (Futalaufquen-Kruger). If dispersal from the north was the process by which *Pt. trucha* became established (or re-established) in the southern drainages, other processes must have largely obliterated the genetic signal.

A more plausible alternative hypothesis is that *Pt. trucha* was already established throughout eastern Patagonia long before the Pleistocene, and that populations in the eastern drainages underwent severe reductions in size and strong genetic bottlenecks as they were repeatedly restricted to waters to the east of the glaciers. As the glaciers retreated, lakes and river systems were recolonized from the remnant populations. However, substantial mixing among these populations must have occurred to produce such a shallow haplotype phylogeny for this region. In addition, the fact that Lake Musters has haplotypes in common with all other eastern draining watersheds indicates that there must have been fairly recent connections among populations. We suggest that mixing occurred during the period of glacial retreat, due to higher water levels which linked tributaries, and due to the formation of large proglacial

palaeolakes along the eastern flank of the Andes in front of the melting glaciers. Known palaeolakes include Lake *Elpalafquen* (41°S), Lake *Cari Lafquen*, on the Patagonian steppe also at ~41°S (Clapperton 1993; p. 506), a large unnamed palaeolake that covered the present-day lakes Buenos Aires-Gral Carrera and Pueyrredón-Cochrane (Turner *et al*. 2005), Lake *Caldenius*, encompassing Lakes Azara, Belgrano, Mogote, Nansen, Volcán and Burmeister (~48°S) (González 1992; Aschero *et al*. 1998; Tatur *et al*. 2002), and Lake *Fuegian*, on the island of Tierra del Fuego (Tatur *et al*. 2002). Mixing among watersheds within the latitudinal range of the glaciers, but not farther north, might explain the greater divergence (and lower haplotype diversity) of the northernmost watershed, Rio Colorado.

Populations from west-draining lakes that were covered by glaciers (Rivadavia, Futalaufquen-Kruger and Pueyrredón-Cochrane) show low haplotype diversity, and distinctive haplotype compositions, patterns usually associated with founder effects following recent colonization. However, populations in these lakes do not separate out from those in the eastern-draining watersheds of Patagonia, and are not closely related to the other western draining systems to the north. The most likely explanation is that these lakes were also recolonized from the eastern refugia during the period of glacial retreat. Western coastal refugia are unlikely south of ~42°S, as glaciers are known to have reached the edge of the continental shelf in the Pacific at these latitudes. Glacial melting was sometimes accompanied by catastrophic watershed rearrangements that included the opening of fluvial channels across the Andes, with upper watershed tributaries shifting drainage direction from the Atlantic to the Pacific Ocean (Tatur *et al*. 2002; Turner *et al*. 2005). Lake Pueyrredón-Cochrane is known to be within one of the regions that experienced a catastrophic watershed re-arrangement. For 2000 years, lake Pueyrredón-Cochrane drained to the east into Rio Deseado as the glaciers were melting, but a catastrophic breaching of an ice dam to the west at *c*. 12 800 BP changed its flow to the Pacific, where it remains today (Turner *et al*. 2005). A similar reversal in drainage direction took place *c*. 10 000 BP for some of the lakes in the palaeolake *Caldenius* to the south (Aschero *et al*. 1998). The area occupied by lakes Rivadavia and Futalaufquen-Kruger has not been studied, to our knowledge, but could well have had a similar history. Thus, relatively recent changes in watershed drainage patterns could explain both the low haplotype diversity (founder effect due to limited colonization time) in these lakes and their interspersion within the large clade of eastern drainages. In summary, we suspect that the lack of deep phylogeographical structure among *Pt. trucha* from west and east draining systems in Patagonia is due to the populations' recent expansion, mostly from refuges in the east, and due to substantial dispersal and mixing during this period.

Populations of Percichthys trucha *west of the Andes*

Inferred relationships among *Pt. trucha* populations in the west-draining rivers (Itata, Bio Bio, Andalien) west of the Andes have a number of apparent anomalies. One is the lack of a close relationship between the population in Itata and those in the neighbouring drainages of Bio Bio and Andalien. In the mtDNA phylogeny, the Itata haplotypes form strongly supported clade that is reciprocally monophyletic with all other *Pt. trucha* haplotypes west and east of the Andes. The closest links between haplotypes from Itata and other locations involve 30 + nucleotide substitutions (steps), and weakly suggest a relationship with rare eastern haplotypes found in Futalaufquen-Kruger (formerly glaciated) or Espejo (Rio Negro watershed) rather than with other western populations. These results suggest that the Itata population has been isolated for a long period of time, despite its geographical proximity to other western rivers. Molecular clock estimates based on a mutation rate of approximately 5% per million years (Bowen *et al*. 2005) put the time of divergence at about 1 Ma While very crude, this estimate is consistent with geomorphological evidence for the area indicating the Bio Bio and Itata valleys were formed and separated from one another during the uplift of the coastal mountain chain (Cordillera de la Costa) in the late Pliocene (Pliocene 5.3–1.8 Ma, Mardones 2002).

The second apparent anomaly is the close relationship among the western Bio Bio and Andalien populations and populations east of the Andes. The Bio Bio and Andalien Rivers, though currently unconnected, were part of one system until approximately 7000 BP in the early Holocene when they separated following volcanic activity. The similar haplotype composition of these populations is thus not unexpected. However, their close relationship with *Pt. trucha* populations east of the Andes is more puzzling. The answer probably lies in the fact that the upper reaches of the Bio Bio River on the western side of the Andes are very close to the upper tributaries of the Rio Negro watershed on the eastern side of the Andes (north of Quillén), such that exchange may have occurred between these systems in the past. In summary, mtDNA patterns suggest an ancient divergence (~1 Ma) between the Itata population and all others in our study, and more recent contact between Bio Bio–Andalien and the other drainages, perhaps via past connections between west and east flowing drainages in this area.

Nuclear phylogeography and taxonomy

The lack of deep phylogenetic structure among *Percichthys* in watersheds east of the Andes, coupled with the weak and conflicting evidence for morphological divergence between the widespread *Pt. trucha* and the more localized

presumed *Pt. colhuapiensis*, *Pt. altispinnis*, and *Pt. vinciguerrai* (Ringuelet *et al*. 1967; Arratia *et al*. 1983; vs. López-Albarello 2004) prompted us to examine the evidence for species-level differentiation in the nuclear genome in our samples of both *Percichthys* spp. and *Percilia* spp. In the following, we adopt the genealogical/lineage concordance species concept (GCC) of Avise & Ball (1990), which attempts to reconcile elements of the biological species concept (Mayr 1963) with the phylogenetic species concept (Cracraft 1989). Under the GCC (Avise & Ball 1990; Avise 2001), a group of organisms is considered to constitute a species if there is: (i) concordance across sequence characters within a genetic locus; (ii) concordance in these genealogical patterns across multiple loci, both mitochondrial and nuclear; (iii) concordance with biogeographical patterns; and (iv) concordance with morphological characters.

The Percichthys *complex*

Our molecular results are inconsistent with the existence of more than a single *Percichthys* species east of the Andes. Both the mtDNA and the nuclear sequence analysis provide concordant support for the lack of deep phylogenetic structure or differentiation among the *Percichthys* spp. collected in Andean Patagonia and on the Patagonian plateau. Our samples cover the group's latitudinal and altitudinal range in Argentine Patagonia, and include the localities where the various putative morphological 'species' with restricted distribution were originally described (Rio Colorado for *Pt. altispinnis*, Lake Musters for *Pt. colhuapiensis*, and Lake Argentino for *Pt. vinciguerrai*) (Ringuelet *et al*. 1967; Arratia *et al*. 1983; López-Albarello 2004). In other respects, our results on *Percichthys* support the conclusions of Ringuelet *et al*. (1967): *Pt. trucha* is found both east and west of the Andes, and it differs substantially from *Pt. melanops*, a species that is endemic to Chile.

Morphological variability has not been able to unambiguously resolve species issues for *Percichthys*. Originally, Ringuelet *et al*. (1967) described five extant species, using mouth, jaw, spine, and caudal peduncle traits but without providing reliable information on variability within or among populations belonging to the same species. Recently, using similar traits, several of these species were collapsed and another separated (López-Albarello 2004). Morphological variation in these characters, among populations (López-Albarello 2004; Ruzzante *et al*. unpublished data) and within populations (Cussac *et al*. 1998; Ruzzante *et al*. 1998, 2003; unpublished data), is now known to be substantial. Our analysis involves probably the most complete geographical coverage of this genus to date, including all its known range on the eastern side of the Andes, and more limited sampling from several drainage systems in Chile. In western drainages, *Pt. melanops* separates clearly from the remaining *Percichthys*. However, neither nuclear nor mitochondrial sequence divergences support the existence of more than one species of *Percichthys* east of the Andes.

The *Pt. trucha* from one drainage system west of the Andes (Rio Itata) were highly divergent at the mtDNA, but not at the nuclear intron, leading us to classify them as a variant of *P. trucha*. Thus, the molecular evidence to date indicates that there are two species of *Percichthys* in Chile, *Pt. melanops* and *Pt. trucha*, and only one species, *Pt. trucha* in Argentina. Eastern and western *Pt. trucha* populations form a monophyletic group, with *Pt. melanops* as a sister group.

The Percilia *complex*

Discrepancies also arose between morphological and molecular variability in *Percilia*. Morphological data appears to indicate that there are two *Percilia* species in Chile (Ringuelet *et al*. 1967). *Pa. irwini* is thought to be restricted to the Andalien–Bio Bio drainages (~37°S), and is listed as threatened by the IUCN (World Conservation Monitoring Centre 1996). *Pa. gillissi* has been found both north and south of the Bio Bio–Andalien drainages from the Aconcagua river in the north (~32°45′-S) to the lake Llanquihue in the south (41°10′) (Dyer 2000). However, our results revealed a somewhat different phylogenetic structure. *Pa. irwini* forms a monophyletic clade, based on both mtDNA and the nuclear intron sequence variation. However, *Pa. gillissi* from the southern drainages (Imperial and Cruces, ~40°S) are strongly differentiated from *Pa. gillissi* in the three northern drainages (Maipo, Mataquito and Maule, ~34 to 36°S), and appear to be more closely related to *Pa. irwini*. It is clear from these results that the current division of the genus into a restricted *Pa. irwini* and a widely distributed *Pa. gillissi* is inconsistent with the patterns of genetic variation. We thus conclude that *Pa. gillissi*, as previously defined, is not monophyletic and suggest that the taxonomic and phylogeographical patterns within the genus *Percilia* are more complex than previously thought.

Conclusion

In summary, the Andes separate the South American Percichthyidae into a western region which includes several deeply divergent lineages, and an eastern region with a very shallow phylogeography. All *Percichthys* populations in previously glaciated as well as nonglaciated lakes and rivers east of the Andes appear to belong to a single species, *Pt. trucha*. These eastern populations exhibit a phylogeographical footprint consistent with a genetic bottleneck that occurred relatively recently, probably during the penultimate Pleistocene glacial cycle (~188 000 BP), and the lack of deep phylogeographical structure is probably the consequence of repeated population contraction and expansion events (founder-flush events), accompanied by mixing when huge proglacial palaeolakes connected

present-day lakes and rivers. West-flowing drainages in previously glaciated regions were also likely recolonized from the east. In contrast, western populations north of ~42°S, to the north and northwest of the LGM show a much deeper phylogeographical structure, with two genera, four species and greater divergence within the *Pt. trucha* species. Estimates of the time of population expansion for *Pa. irwini*, endemic to this region, puts its most recent bottleneck at roughly half a million years, back in the middle Pleistocene. Thus, phylogeographical patterns within the South American Percichthyidae appear to have been shaped, first and foremost, by the uplift of the Andes resulting in limited gene flow between populations east and west of the mountains (except for drainage reversals), and second by the isolation of populations in individual western drainages north of the glacial limits, and thirdly by temporary and longer-lasting rearrangements of eastern drainages during Pleistocene glacial cycles.

Acknowledgements

We thank P. Bentzen (Dalhousie University) and S.Grant (University of Alaska, Anchorage) for discussions during the development of this paper and J.Gosse (Dalhousie, Earth Sciences) and C. Fogwill (Edinburgh, Institute of Geography) for assistance in the interpretation of geological information. The assistance during fieldwork of P. Macchi, M. Alonso, M. Battini, D. Milano, J. Aigo, J. P. Barriga, and M. A. Denegri is gratefully acknowledged. P. Unmack kindly provided the two specimens of Australian Percichthyidae. Pascal and Sacha Walde Ruzzante contributed to the fieldwork and its enjoyment. We also thank C. Chehébar and P. Martinez of the 'Delegación Regional Patagonia, Administración de Parques Nacionales' for permission to collect native fish within National Parks, the park wardens from National Parks 'Nahuel Huapi', 'Los Alerces', 'Perito Moreno', and 'Los Glaciares' for assistance, and M. Ugucioni and J. Pépe De Giusto for logistical support, without which, much of the field work would have been far more difficult. We also thank J Milne (GIS, Killam Library, Dalhousie University) for assistance with Fig. 1. M. L. Dalebout was supported by a Killam Postdoctoral Fellowship while at Dalhousie University, and currently by a University of New South Wales Vice-Chancellor's Postdoctoral Fellowship. Our work was supported by funds from Natural Sciences and Engineering Research Council (NSERC — Canada), the National Geographic Society (Washington DC, USA), Universidad Nacional del Comahue (Argentina) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET — Argentina) and the EULA Centre of the Universidad de Concepción (Chile).

References

Allnutt TR, Newton AC, Lara A *et al.* (1999) Genetic variation in Fitzroya cyppressoides (alerce), a threatened South American conifer. *Molecular Ecology*, **8**, 975–987.

Arratia G (1982) A review of the freshwater percoids from South America (Pisces, Osteichthys, Perciformes, Percithyidae and Perciliidae). *Abhandlungen der Senckenbergischen Naturforshenden Gesellshaft*, **540**, 1–52.

Arratia G, Peñafort MB, Menu Marque S (1983) Peces de la región sureste de los Andes y sus probables relaciones biogeográficas actuales. *Deserta*, **7**, 48–107.

Aschero CA, Goñi RA, Civalero MT *et al.* (1998) Holocenic park: arqueología del Parque Nacional Perito Moreno (PNPM). *Anales de la Administración de Parques Nacionales no. 17*.

Avise JC (2001) *Phylogeography: the History and Formation of Species*. Harvard University Press, Cambridge, Massachusetts, USA.

Avise JC, Ball Jr (1990) RM Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Surveys in Evolutionary Biology*, **7**, 45–67.

Bernatchez L, Wilson CC (1998) Comparative phylogeography of Neartic and Paleartic fishes. *Molecular Ecology*, **7**, 431–452.

Bowen BW, Muss A, Rocha LA, Grant WS (2005) Shallow mtDNA coalescence in Atlantic pygmy angelfishes (genus *Centropyge*) indicates a recent invasion from the Indian Ocean. *Journal of Heredity*, **97**, 1–12.

Bremer K (1988) The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution*, **42**, 795–803.

Campos H, Gavilán JF (1996) Diferenciación morfológica entre *Percichthys trucha* y *Percichthys melanops* (Perciformes: Percichthyidae) entre 36° y 41° L.S. (Chile y Argentina), a través de análisis multivariados. *Gayana Zoología*, **60** (2), 99–120.

Chang A, Arratia G, Alfaro G (1978) *Percichthys lonquimayiensis n.sp* from the Upper Paleocene of Chile (Pisces, Perciformes, Serranidae). *Journal of Paleontology*, **52**, 727–736.

Clapperton CC (1993) *Quaternary Geology and Geomorphology of South America*. Elsevier, Amsterdam.

Cracraft J (1989) Speciation and its ontology. The empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. In: *Speciation and its Consequences* (eds Otte D, Endler JA), pp. 28–59. Sinauer Associates, Sunderland, Massachusetts.

Crandall KA, Fetzner Jr JW, Jara CG, Buckup L (2000) On the phylogenetic positioning of the South American freshwater crayfish genera (Decapoda: Parastacidae). *Journal of Crustacean Biology*, **20**, 530–540.

Cussac VE, Ruzzante DE, Walde SJ *et al.* (1998) Body-shape variation of three species of *Percichthys* in relation to their coexistence in the Limay river basin, in Northern Patagonia. *Environmental Biology of Fishes*, **53**, 143–153.

Davis JI, Nixon KC (1992) Populations, genetic variation and the delimitation of phylogenetic species. *Systematic Biology*, **41**, 421–435.

Dyer B (2000) Systematic review and biogeography of the freshwater fishes of Chile. *Estudios Oceanológicos*, **19**, 77–98.

Excoffier L (1993) MINSPNET. *Genetics and Biometry Laboratory Department of Anthropology, University of Geneva, Switzerland*. Available as freeware for PC from http://cmpg.unibe.ch.ch/excoffier/.

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

González MA (1992) Paleoambientes del Pleistoceno Tardio / Holoceno Temprano en la cuenca de los lagos Belgrano y Burmeister (47°40′/48° Sur, 72°30′ Oeste, Santa Cruz). *Fundación Carl C:Zon Caldenius*. Informe Técnico n° 9, 1–7.

Hassan M, Lemaire C, Fauvelot C, Bonhomme F (2002) Seventeen new exon-primed intron-crossing polymerase chain reaction amplifiable introns in fish. *Molecular Ecology Notes*, **2**, 334–340.

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

Ho SYW, Phillips MJ, Cooper A, Drummond AJ (2005) Time dependency of molecular rate estimates and systematic

overestimation of recent divergence times. *Molecular Biology and Evolution*, **22**, 1561–1568.

Jerry DR, Baverstock PR (1998) Consequences of a catadromous life-strategy for levels of mitochondrial DNA differentiation among populations of the Australian bass, *Macquaria novemaculeata*. *Molecular Ecology*, **7**, 1003–1013.

Jerry DR, Elphinstone MS, Baverstock PR (2001) Phylogenetic relationships of Australian members of the family Percichthyidae inferred from mitochondrial 12S rRNA sequence data. *Molecular Phylogenetics and Evolution*, **18**, 335–347.

Johnson GD (1984) Percoidei: development and relationships. In: *Ontogeny and Systematics of Fishes* (eds Moser HG, Richards WJ, Cohen DM, Fahay MP, Kendall Jr AW, Richardson SL), pp. 464–498. Special Publication 1, American Society of Ichthyologists and Herpetologists.

Kaplan MR, Douglass DC, Singer BS, Ackert RP, Caffee MW (2005) Cosmogenic nuclide chronology of pre-last glaciation maximum moraines at Lago Buenos Aires, 46°S, Argentina. *Quaternary Research*, **63**, 301–315.

Kim I, Phillips CJ, Monjeau JA *et al*. (1998) Habitat islands, genetic diversity, and gene flow in a Patagonian rodent. *Molecular Ecology*, **7**, 667–678.

Kumar S, Tamura K, Nei M (2004) MEGA 3*:* integrated software for molecular evolutionary analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150–163.

López-Albarello A (2004) Taxonomy of the genus *Percichthys* (Perciformes: Percichthyidae). *Ichthyological Explor Freshwaters*, **15** (4), 331–350.

Mardones M (2002) Evolución morfogenética de la hoya del río Laja y su incidencia en la geomorfología de la Región del Biobío, Chile. *Revista Geographyráfica de Chile Terra Australis*, **49**, 97–127.

Mayr E (1963) *Animal Species and Evolution*. Harvard University Press, Cambridge, Massachusetts.

McCulloch RD, Bentley MJ, Purves RS, Hulton NRJ, Sugden DE, Clapperton CM (2000) Climatic inferences from glacial and paleoecological evidence at the last glacial termination, southern South America. *Journal of Quaternary Science*, **15**, 409–417.

Mercer JH (1976) Glacial history of southernmost South America. *Quaternary Research*, **6**, 125–166.

Milano D (1996) *Reproducción de la perca de boca chica Percichthys trucha (Cuvier & Valenciennes, 1840) del Lago Moreno*. Licentiate Thesis. Universidad Nacional del Comahue, Bariloche, Argentina.

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

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

Palma RE, Rivera-Milla E, Salazar-Bravo J *et al*. (2005) Phylogeography of Oligoryzomys longicaudatus (Rodentia: Sigmodontinae) in temperate South America. *Journal of Mammalogy*, **86**, 191–200.

Palma RE, Rivera-Milla E, Yates TL, Marquet PA, Meynard AP (2002) Phylogenetic and biogeographic relationships of the mouse oposum *Thylamus* (Didelphimorphia, Didelphidae) in southern South America. *Molecular Phylogenetics and Evolution*, **25**, 245–253.

Pastorino MJ, Gallo LA (2002) Quaternary evolutionary history of *Austrocedrus chlensis*, a cypress native to the Andean-Patagonian forest. *Journal of Biogeography*, **29**, 1167–1178.

Pastorino MJ, Gallo LA, Hattemer HH (2004) Genetic variation in natural populations of *Austrocedrus chilensis*, a cypress of the Andean Patagonian forest. *Biochemical Systematics and Ecology*, **32**, 993–1008.

Pérez-Losada M, Bond-Buckup G, Jara CG, Porter ML, Crandall KA (2004) Molecular systematics and biogeography of the southern South American freshwater 'crabs'*Aegla* (Decapoda: Nomura: Aeglidae) using multiple heuristic tree search approaches. *Systematic Biology*, **53**, 767–780.

Pérez-Losada M, Jara CJ, Bond-Buckup G, Porter ML, Crandall KA (2002) Phylogenetic position of the freshwater Anomuran family Aeglidae. *Journal of Crustacean Biology*, **22**, 670–676.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics and Applications Note*, **14**, 817–818.

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

Ramos VA (1989) The birth of southern South America. *American Scientist*, **77**, 444–450.

Rand DM (1996) Neutrality tests of molecular markers and the connection between DNA polymorphism, demography and conservation biology. *Conservation Biology*, **10**, 665–671.

Ringuelet RA, Aramburu RH, Alonso de Aramburu A (1967) *Los Peces Argentinos de Agua Dulce*. Provincia de Buenos Aires, Gobernación. Comisión de Investigación Científica. 602 pp.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise gene divergence. *Molecular Biology and Evolution*, **9**, 552–569.

Ruzzante DE, Walde SJ, Cussac VE, Macchi PJ, Alonso MF (1998) Trophic polymorphism, habitat and diet segregation in *Percichthys trucha* (Pisces: Percichthyidae) in the Andes. *Biological Journal of the Linnean Society*, **65**, 191–214.

Ruzzante DE, Walde SJ, Cussac VE, Macchi PJ, Alonso MF, Battini M (2003) Resource polymorphism in a Patagonia fish *Percichthys trucha* (Percichthyidae): phenotypic evidence for interlake pattern variation. *Biological Journal of the Linnean Society*, **78**, 497–515.

SanMartín I, Ronquist F (2004) Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology*, **53**, 216–243.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN, *(version 2.000): Software for Population Genetics Analysis*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland. Available from http:// anthropologie.unige.ch/ arlequin.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Smith MF, Kelt DA, Patton JL (2001) Testing models of diversification in mice in the *Abrothrix olivaceus/xanthorhinus* complex in Chile and Argentina. *Molecular Ecology*, **10**, 397–405.

Sorensen MD (1999) TREEROT, *version 2*. Boston University, Boston Massachussetts. Available from http://people.bu.edu/msoren/ TreeRot.html.

Swofford DL (1999) PAUP*: *Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, Massachussetts.

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

Tatur A, del Valle R, Bianchi MM *et al*. (2002) Late Pleistocene palaeolakes in the Andean and Extra-Andean Patagonia at mid latitudes of South America. *Quaternary Internation*, **89**, 135–150.

Truett GE, Heeger P, Mynatt AA, Truett JA, Walker JA, Warman ML (2000) Preparation of PCR-quality mouse genomic DNA with Hot Sodium Hydroxide and Tris (HotSHOT). *BioTechniques*, **29**, 52–54.

Turner KJ, Fogwill CJ, McCulloch RD, Sugden DE (2005) Deglaciation of the eastern flank of the North Patagonian Icefield and associated continental-scale lake diversions. *Geographyrafiska Annaler*, **87A** (2), 363–374.

Willis KJ, Whittaker RJ (2000) The refugial debate. *Science*, **287**, 1406–1407.

World Conservation Monitoring Centre (1996) *Percilia gillissi*. In. *IUCN 2004. 2004 IUCN Red List of Threatened Species*. <www.redlist.org>.

This paper is a result of an ongoing collaborative effort between DER and SJW of Dalhousie University (Canada), VEC of Universidad del Comahue (Argentina) and EH of Universidad de Concepción (Chile) to examine the evolutionary biology and phylogeography of the native fish fauna of Patagonia. MLL was a Killam postdoctoral fellow in DER's laboratory interested in the phylogeography and conservation genetics of aquatic organisms and is currently at the University of New South Wales (Australia). JS is a research technician at Dalhousie University and SO is a research scientist with the National Park Service in Argentina.