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Genetic and phenotypic differentiation among *Galaxias maculatus* populations in a Patagonian postglacial lake system

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Understanding the influence of landscape features on population differentiation is fundamental to evolutionary biology studies. We examined spatial patterns of genetic and phenotypic variability among *Galaxias maculatus* populations in a complex of four postglacial lakes in northwestern Patagonia differing in size and connectivity among them. A hierarchical Bayesian analysis grouped the individuals collected from eleven localities into three genetic clusters, first defining the populations of the two large lakes and separating the two small lakes in subsequent analysis. Genetic structuring was restricted within large lakes. It is known that the larval stage of *Galaxias maculatus* migrate to the limnetic zone of Patagonian lakes, possibly exerting an homogenizing effect on gene flow within lakes. Gene flow asymmetry and divergences among lakes can be explained by a combination of landscape characteristics and the presence of predators in the short streams that connect them. Individuals from the small lakes are the most divergent morphologically and genetically. The population in the isolated Redonda Lake, exhibits meristic differences as well, suggesting strong drift and environmental effects. This population is likely to have been isolated following the decline in water level of a paleolake that existed in this region approximately 13.2 kya BP. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, ••, ••–••.

ADDITIONAL KEYWORDS: morphometrics – meristic counts – structuring.

INTRODUCTION

A fundamental goal in evolutionary biology is to understand what processes are responsible for the genetic and phenotypic differentiation among populations during intraspecific diversification. The roles of microevolutionary processes (e.g. selection, genetic drift and gene flow) and their interactions with landscape features can be examined with the tools of Landscape Genetics, a discipline which focuses on shorter temporal and smaller geographical scales than other genetic approaches (e.g. phylogeography; Manel *et al*., 2003). Freshwater fish offer a good opportunity for landscape genetics studies because they are confined to lakes and streams where the combined effects of geography, life history, and habitat fragmentation in limiting dispersal can be assessed (Gomez-Uchida, Knight & Ruzzante, 2009).

Several studies of northern hemisphere postglacial lakes indicate that these environments promote divergence and intraspecific ecotypic diversity within freshwater fish populations (Taylor, 1999; McPhee, Noakes & Allendorf, 2012). Similar evidence for Southern Hemisphere fishes is scarce. In South America, recent studies on Patagonian fishes have addressed broad scale phylogeographical patterns (Ruzzante *et al*., 2006, 2008, 2011; Zemlak *et al*., 2008, 2010), although very few have thus far focused on their small-scale structuring patterns. In the present study, we examined the genetic and phenotypic structure among populations of the small puyen,

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Galaxias maculatus (Jenyns, 1842), in a complex of postglacial lakes in northwestern Patagonia.

Galaxias maculatus is widely distributed throughout the southern hemisphere (Australia, New Zealand, and southern South America). In South America, it is found poleward of 32° S, reaching the southernmost limit of its distribution range at 55° S (Cussac *et al*., 2004). The species has a remarkable plasticity in its life history, in part reflected by the existence of migratory (diadromous) and freshwater resident populations (McDowall, 1968). Although a few populations in southern Patagonia (54° S) have been reported as diadromous (Boy, Morriconi & Calvo, 2007; Boy *et al*., 2009), most *G. maculatus* populations in Patagonia exhibit a resident life history (Cussac *et al*., 2004; Zattara & Premoli, 2004). These populations have shown evidence of an annual generation time (Barriga *et al*., 2002) as has been reported for Australian and New Zealand populations (Pollard, 1971; Chapman *et al*., 2006). Adult females in resident populations attain smaller sizes than their diadromous counterparts in Patagonia (32–80 mm versus 48–160 mm, respectively), nevertheless retaining a relative high fecundity (107–2825 eggs/female; Cussac *et al*., 2004).

The larvae of resident *G. maculatus* populations are known to migrate from the shallow vegetated areas in the littoral zone of lakes to the deeper waters in the limnetic zone (Cussac, Cervellini & Battini, 1992; Rowe & Chisnall, 1996; Barriga *et al*., 2002; Rechencq *et al*., 2011). Under this scenario, the detection of a pattern of isolation by distance (IBD; a positive correlation of geographical versus neutral genetic distances) among shoreline sampling locations within lakes would suggest the existence of homing behaviour of juveniles returning to their natal sites in the littoral zones. An IBD pattern could also result from the influence of breaks (e.g. geographic, physical, chemical) in the landscape, providing barriers to gene flow (Guillot *et al*., 2009). Alternatively, the absence of an IBD pattern among sampling locations within lakes would suggest that movement of the adults within the lake is not restricted and/or that the limnetic *G. maculatus* larvae experience significant mixing. Larvae could be transported away by currents resulting in the settling on locations along the littoral zone different from their natal site, thus exerting a homogenizing effect on gene flow within lakes.

Gene flow has a constraining effect on local adaptation (Moore *et al*., 2007), and therefore reductions on gene flow can allow adaptive phenotypic divergences among populations. Larvae of *G. maculatus* have been shown to exhibit morphological differentiation involving body slenderness and the length of the caudal peduncle as a function of differences in food availability and predation risk (Barriga *et al*., 2012). In addition, variation in vertebral counts within *G. maculatus* is influenced by diverse factors such as life history and latitude (McDowall, 2003a). These variations could be adaptive, the result of phenotypic plasticity, or likely a mixture of both (McDowall, 2007). In theory, populations with a high degree of isolation have a high potential for divergence.

In the present study, we focused on a system of four postglacial lakes in north-western Patagonia differing in size and connectivity: Lake Nahuel Huapi, which is a large lake (583 km^2) with a winding shoreline, many bays, and peninsulas that favour the existence of different microhabitats, as well as three smaller lakes that connect to it: Correntoso, Redonda, and Larga lakes. *Galaxias maculatus* within the Nahuel Huapi Lake have been inferred to exhibit fine scale spatial structure on the basis of qualitative (taxonomic) and quantitative differences in the parasite content among adult specimens collected from sites at most 40 km apart (Revenga & Scheinert, 1999). The present study aimed to examine how *G. maculatus* genetic and phenotypic variability is distributed geographically within and among the lakes in this system. In addition, we estimated migration patterns among populations. We hypothesized that restrictions in gene flow could lead to population divergence and an IBD pattern. We predicted that populations in smaller lakes and with a higher degree of isolation would exhibit higher phenotypic divergences than those in larger lakes.

MATERIAL AND METHODS

Study area

We focused on a system of four lakes in the Nahuel Huapi National Park in North-western Patagonia (Fig. 1). A large central lake, Nahuel Huapi Lake (583 km^2) and three smaller lakes: Correntoso Lake (20 km^2), Larga Lake (0.5 km^2), and Redonda Lake (0.01 km^2). Larga and Redonda lakes are located on Victoria Island within the Nahuel Huapi Lake itself. Victoria Island is the largest (37 km^2) of a number of islands dotting Nahuel Huapi Lake. The Nahuel Huapi Lake has a long and irregular shoreline (approximately 400 km) resulting from its seven arms of different sizes, bays, peninsulas, and isthmuses (Diaz, Pedrozo & Temporetti, 1998). Correntoso Lake drains into the Nahuel Huapi Lake through a short river (approximately 120 m long). Larga Lake drains into Nahuel Huapi Lake via a short intermittent stream (M. Nuñez, pers. comm.) and Redonda Lake has no present day connection to other lakes. The Nahuel Huapi system drains into the Atlantic Ocean through the Limay River. These lakes are

likely remnants of a paleolake in the area (Tatur *et al*., 2002) formed by the melting of the ice after the last glaciation, which reached its maximum approximately 25 kya BP (Rabassa, Coronato & Martinez, 2011). As the glaciers receded and the water level decreased, the paleolake became fragmented, approximately 13.2 kya BP (Tatur *et al*., 2002).

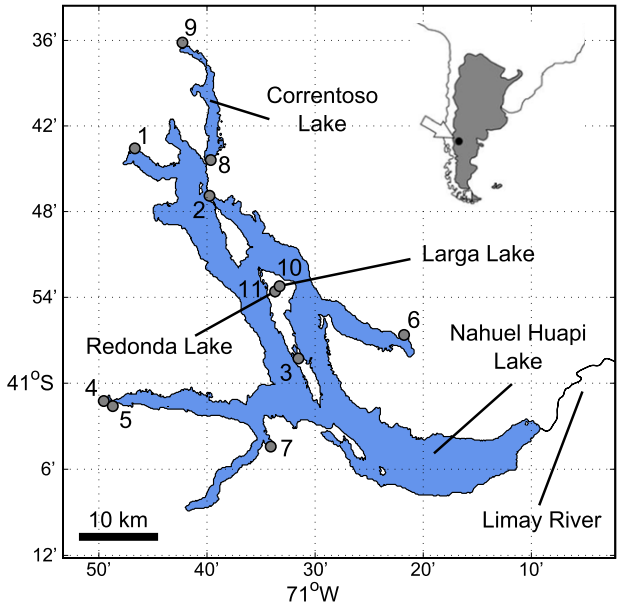

Figure 1. Map of the studied system depicting sampling locations for *Galaxias maculatus*. Locations 1–7 in Nahuel Huapi Lake: 1-Rincón Arm, 2-Quetrihue, 3-Anchorena, 4-Blest arm close to tributary draining Cántaros Lake, 5-Blest arm close to tributary draining Frías Lake, 6-Huemul Arm, and 7-Bahía Lopez. Locations 8 and 9 are in Correntoso Lake. Locations 10 and 11 correspond to two small lakes within Victoria Island: 10-Larga Lake and 11-Redonda Lake.

We collected individuals from eleven sites (Fig. 1). Seven were located within Nahuel Huapi Lake, and two within Correntoso Lake. The remaining two samples originated from each of the two small lakes on Victoria Island. Samples of adult individuals were collected from a mixture of several seine nets-catches between March and April 2008. Fish were sacrificed with an overdose of benzocaine upon collection. Tissue samples were conserved in 96% ethanol. Information on sample size per location is provided in Table 1.

Data collection

Genomic DNA was extracted from 704 samples of tissue following the glass milk method described by Elphinstone *et al*. (2003). Approximately 5–10 mg of each sample tissue was first digested in 300 µl of buffer (100 mM NaCl, 50 mM Tris–HCl pH 8, 10 mM ethylenediaminetetraacetic acid, pH 8, 0.5 mM sodium dodecyl sulphate) and 2 µl of proteinase K overnight at 55 °C and 200 r.p.m. The extractions were performed in 96-well filter plates using a liquid handling robot (Multiprobe II; Perkin-Elmer).

Ten species-specific microsatellite markers (Carrea *et al*., 2009) were amplified by polymerase chain reactions (PCR) in 384-well plates. PCR mixture (5 µl volume) contained 10–50 ng of DNA, 20 mM Tris–HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 0.1% Triton X-100, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 U of Tsg DNA polymerase (BioBasic D0081) and 0.1–0.2 µM of each primer. Microsatellite fragment lengths were visualized in polyacrylamide gels using a LICOR

Table 1. Allelic patterns across populations overall loci (N = individuals sampled for each location)

Sampling location	N	Mean ± SE H_E	Mean ± SE Ho	AR	Mean ± SE number of alleles	Polymorphic loci	Number of private alleles
NHL Huemul	88	0.492 ± 0.22	0.495 ± 0.078	5.026	6.5 ± 1.18	100%	2
NHL Blest_Frías	38	0.475 ± 0.25	0.504 ± 0.068	4.637	5.375 ± 0.86	100%	0
NHL Rincón	50	0.490 ± 0.19	0.527 ± 0.058	4.576	5.5 ± 1.04	100%	1
NHL Anchorena	62	0.522 ± 0.24	0.509 ± 0.065	5.021	6.5 ± 1.32	100%	3
NHL Blest_Cántaros	44	0.464 ± 0.23	0.506 ± 0.065	4.444	5.5 ± 1.02	100%	0
NHL Bahía Lopez	88	0.485 ± 0.22	0.487 ± 0.075	4.778	6.875 ± 1.37	100%	1
NHL Quetrihue	47	0.509 ± 0.25	0.536 ± 0.69	4.704	5.375 ± 1.15	100%	0
Redonda Lake	88	0.191 ± 0.21	0.242 ± 0.077	2.243	3.25 ± 0.59	75%	0
Larga Lake	47	0.238 ± 0.25	0.261 ± 0.082	2.789	3.125 ± 0.69	75%	3
Correntoso Lake 1	88	0.351 ± 0.25	0.385 ± 0.077	3.941	5.625 ± 1.03	100%	0
Correntoso Lake 2	64	0.322 ± 0.21	0.303 ± 0.083	3.875	5 ± 0.85	100%	1

H_E, expected heterozygosity; H_O, observed heterozygosity; AR, Allelic richness (based on a minimum sample size of 21 diploid individuals); NHL, localities in Nahuel Hupapi Lake.

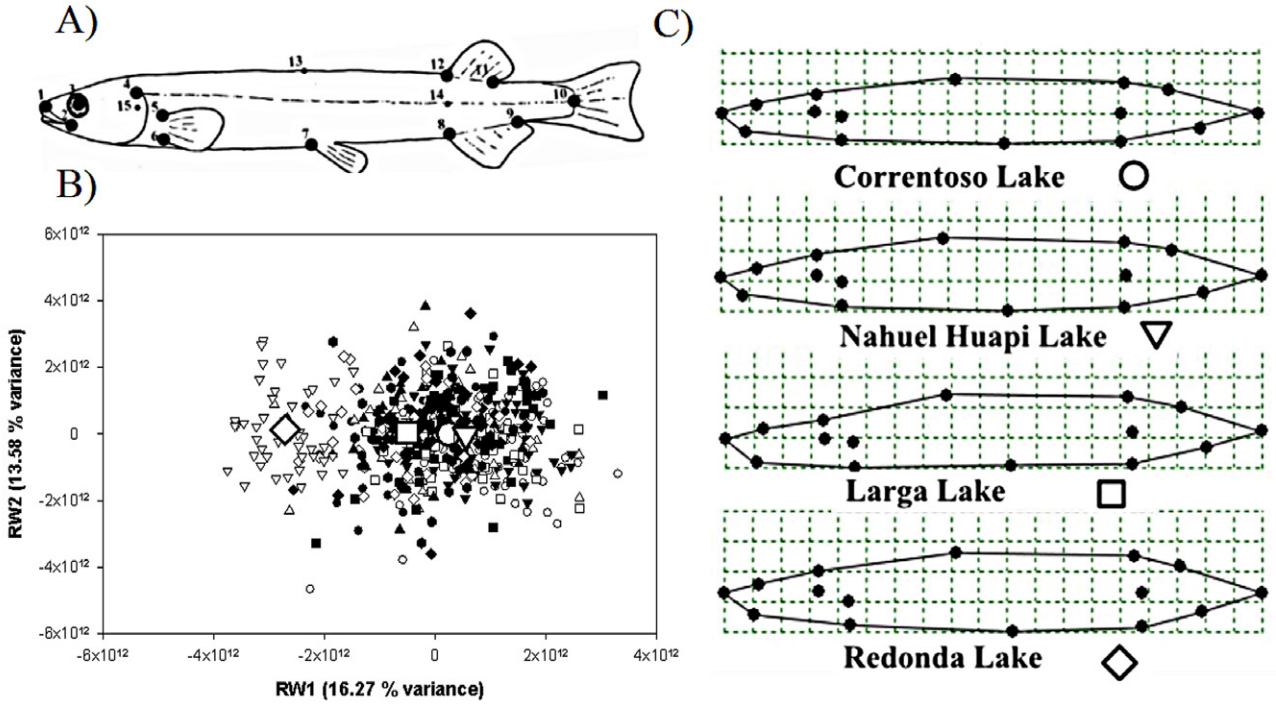

Figure 2. (A) Landmark configuration used to study body shape of *Galaxias maculatus* populations. Large circles (1–12) represent landmarks, whereas small circles are semilandmarks placed to detect body depth (13) and for the unbending of individuals (14 and 15). (B) Relative warp (RW)1 versus RW2. Close symbols represent the 11 localities. Open symbols represent the means for each of the four genetic groups. (C) The panels on the right show the consensus body shape for each of the four genetic groups.

sequencer (Biosciences). Genotypes were examined with MICROCHECKER (van Oosterhout *et al*., 2004) to detect potential genotyping errors or technical artefacts, including null alleles, large allele drop out and stuttering. Two loci (Gmac2 and Gmac10) out of the ten examined showed evidence of potential null alleles for multiple samples and were therefore omitted from further analysis. All genotypes included in the analysis can be downloaded from the Dryad database (Carrea *et al*., 2012).

Body shape variation was examined using geometric morphometrics (Bookstein, 1991; Dryden & Mardia, 1998). Images of the lateral view (left side) of a subset of 514 individuals were captured using a digital camera and a ruler for later scaling. On the images, fifteen landmarks were digitalized using TPSDIG (Rohlf, 2005), two of which were semilandmarks used for the unbending of specimens and one of which was a slider used to detect body depth (Fig. 2A) using TPSUTIL (Rohlf, 2004). Vertebral number, and number of anal and dorsal fin rays were counted from a subset of 125 individuals over X-ray images *sensu* McDowall (1971).

Genetic data analysis

Linkage disequilibrium and Hardy–Weinberg exact tests were performed per locus and population using the Markov chain randomization test (Guo & Thompson, 1992) as implemented in GENEPOP, version 1.2 (Raymond & Rousset, 1995). A sequential Bonferroni correction for 88 tests was applied *sensu* Rice (1989). Mean observed and expected heterozygosities, mean number of alleles, number of private alleles, and polymorphism percentage were calculated using GENEALEX, version 6 (Peakall & Smouse, 2006). Allelic richness per locus and population were calculated using FSTAT, version 2.9.3 (Goudet, 2001), which implements a rarefaction method to correct the estimation for sample size effects *sensu* Petit, Mousadik & Pons (1998).

Population structure

To estimate the number of genetic groups we used the Bayesian clustering approach implemented in STRUCTURE, version 2.2 (Pritchard, Stephens & Donnelly, 2000). The analysis was set to a burn-in

length of 50 000 replicates and a Markov chain Monte Carlo (MCMC) run of 250 000 replicates. We assumed a model of admixture and correlated allele frequencies (Falush, Stephens & Pritchard, 2003). This analysis was conducted hierarchically. We first examined the entire data set involving collections from all 11 sites. Ten iterations were run for each *K*-value in the range 1–11. The second round of STRUCTURE analysis was carried out separately on each of the groups identified in the first run. To determine the most appropriate *K*, we used the highest posterior probability method (Pritchard *et al*., 2000) and examined the individual membership coefficients (Q plots) for the different values of *K*.

Analysis of molecular variance (AMOVA) was performed using a locus by locus procedure with ARLEQUIN, version 3.1 (Excoffier, Laval & Schneider, 2005). This procedure adjusts for sample size differences when there are missing data. A total of 16 000 permutations was used to assess the significance of the genetic variation components. The AMOVA was first conducted using all data and grouping individuals according to the three populations inferred in the first round of STRUCTURE analysis. We then repeated this analysis but grouping individuals into four pools, each corresponding to a lake as inferred in the second round of STRUCTURE analysis. The third AMOVA was conducted on the subset of individuals from lakes Nahuel Huapi and Correntoso (i.e. after excluding individuals from the small lakes). The final AMOVA used only the individuals from Nahuel Huapi Lake.

Bayesian inference of recent migration events among inferred populations was estimated using a MCMC method based on multilocus genotypes implemented in BIMr, version 1.0 (Faubet & Gaggiotti, 2008). To minimize convergence problems, ten runs of MCMC were carried out and results are shown for the run with the lowest Bayesian deviance *sensu* Faubet, Waples & Gaggiotti (2007). For each run, 100 000 iterations were used for sampling, 50 000 were burn-in, and the sampling frequency was 50 iterations (thinning interval).

IBD

To test for a statistical correlation between geographical and genetic distances, a Mantel test was performed between pairwise genetic distances, using the linearized Slatkin's F_{ST} transformation $[F_{ST}/(1 - F_{ST})]$ (Rousset, 1997) and geographical distances (in km), as implemented in GENEALEX, version 6 (Peakall & Smouse, 2006). The geographical distances between localities were measured using Google Earth to obtain 'real distances' for fish that move along the shoreline. To explore the relative importance of IBD-related ecological processes versus the effect of geographical barriers to gene flow, genetic versus geographical distances were plotted distinguishing pairs of populations coming from the same/different genetic clusters (Guillot *et al*., 2009) inferred by STRUCTURE. A resulting cline of genetic differentiation among sites across space would indicate an IBD pattern, whereas the clustering of genetic distances according to sites across space indicates the presence of barriers to gene flow (Fontaine *et al*., 2007). The same procedure of matrix correspondence was applied between morphological (Mahalanobis distances) and geographical distances, as well as between genetic and morphological distances.

Testing an overall IBD pattern by the correlation of genetic and geographical distances in complex population systems can mask individual collection sites that differ from the rest by some important environmental or geographical barrier (Koizumi, Yamamoto & Maekawa, 2006). To examine this possibility, we performed a decomposed pairwise regression analysis (Koizumi *et al*., 2006). This analysis involves examining the mean and 95% confidence intervals of the residuals obtained from the regression of all pairwise genetic and geographical distances to identify outlier populations (i.e. those for which the 95% confidence intervals do not include 0).

Morphological and meristic data analysis

The first step in the analysis of landmark data is to perform a superimposition method to remove non-shape variation by a general Procrustes analysis, which essentially calculates an average shape and aligns specimens to this average (Rohlf & Slice, 1990). To examine the distribution of shape variation, a relative warp (RW) analysis, which is equivalent to a principal component analysis, was then performed with the TPSRELW (Rohlf, 2003). A thin-plate spline technique was then conducted to visualize graphically the statistical results in terms of the configuration of landmarks. Body shape differences are presented in transformation grids where the mean shape is deformed depending on the specimens (Bookstein, 1991).

To compare morphological divergences within and between genetic populations, two classification criteria were applied: sampling localities within Nahuel Huapi Lake (seven groups) and genetic clusters obtained using STRUCTURE (four groups). To test for the existence of significant shape differences between groups, a canonical variate analysis (CVA) was performed on the Procrustes coordinates using MORPHOJ (Klingenberg, 2008). In addition, a matrix of pairwise Mahalanobis distances between the 11 sampling localities was obtained with this

procedure for comparison of morphological distances with genetic and geographical distances. Polynomial regressions of the CVs on centroid size were performed to assess allometry. Subsequently, the unstandardized residuals obtained from these regressions were utilized to visualize shape differences (corrected for size effects) among groups.

Meristic counts were compared using a nonparametric test after normality and homocedasticity tests failed. Under these conditions, we used Kruskal–Wallis one-way analysis of variance on ranks and all pairwise multiple comparisons by Dunn's method applied using SIGMASTAT, version 3.5 (Systat Software). The total 123 individuals corresponded to Redonda ($N = 25$) and Larga ($N = 20$) from Victoria Island, and to Anchorena ($N = 19$), Bahia Lopez ($N = 19$), Blest ($N = 20$), and Quetrihue ($N = 20$) from Nahuel Huapi Lake.

RESULTS

Genetic diversity

No evidence of linkage disequilibrium was detected between loci ($P > 0.05$ for all comparisons). The null hypothesis of Hardy–Weinberg equilibrium could not be rejected for 78 out of 88 exact tests, although only two tests remained significant after sequential Bonferroni correction. These results, as well as details on sample size and allele frequencies by locus and sampling location, are shown in the Supporting information (Table S1).

The two populations on Victoria Island exhibited the lowest genetic diversity values (heterozygosity, allelic richness, percentage of polymorphic loci, and mean number of alleles). The samples from Correntoso and Nahuel Huapi lakes exhibited intermediate and the highest diversity values, respectively (Table 1).

Population genetic structure

Posterior probability values obtained in the first round of STRUCTURE analysis (involving the complete data set) indicated $K = 3$ as the most likely estimate for the number of gene pools: P_r ($K = 1$) = 3.0. 10^{-73}, P_r ($K = 2$) = 2.5. 10^{-3}, and P_r ($K = 3$) = 0.9. These clusters correspond to the group of locations in: (1) Nahuel Huapi Lake, (2) Correntoso Lake, and (3) the two small lakes on Victoria Island (Fig. 3). The second round of finer-scale STRUCTURE analysis, using each of the clusters identified in the first round, failed to identify further subdivision within the Nahuel Huapi and Correntoso lakes. Two groups ($K = 2$) were instead identified separating the two small lake populations from Victoria Island (Fig. 3).

A hierarchical AMOVA involving the three clusters identified in the first round of STRUCTURE analysis indicated 9.9% of the total genetic variation is a result nof differences among clusters and 2% to the variation within clusters (Table 2). When samples were grouped into the four clusters inferred in the second round of STRUCTURE analysis, the percentage of the genetic variance explained by differences among clusters increased to 11.3% and the variation within clusters decreased to 0.85% (Table 2). When the two lake populations from Victoria Island were excluded from the AMOVA, the fraction of the total variation explained by differences between the remaining two groups (Nahuel Huapi and Correntoso lake populations) dropped to 6.8% (Table 2). Finally, when only the seven locations within Nahuel Huapi Lake were considered, only 0.8% of the total variation was explained by differences among locations (Table 2). In all cases, the results were significant ($P < 0.001$). In addition, the pairwise F_{ST} between the 11 sample localities is shown in Table 3.

Migration rates among inferred populations

The population with highest proportion of migrant genes is the one in Nahuel Huapi Lake, with most of the genes originating from Correntoso Lake (Table 4). Both small lakes on Victoria Island showed a 100% of resident genes and no migration into these lakes was detected. This was expected in the case of Redonda Lake, which is not currently connected to the other water bodies, and is consistent with the existence of gene flow barriers observed for Larga Lake (see below). On the other hand, migration was detected from Redonda Lake towards Correntoso and Nahuel Huapi Lakes, which might be explained by the region's glacial history. A great proglacial lake in the area (Tatur *et al*., 2002) connecting these water bodies likely harboured an ancestral population that became fragmented after the water level descended.

IBD

Genetic ($F_{ST}/1 - F_{ST}$) and geographical distances were not correlated (Mantel test, $r = 0.239$, $P = 0.09$, $N = 10$ localities; i.e. the isolated Redonda lake is not included in this analysis) indicating the dispersal process is not affected by geographical distances. Geographical barriers to gene flow (rather than an IBD) is suggested by the fact that, for a given distance class, genetic distances are much larger for pairs of sites from different lakes than for pairs of sites in the same lake (Fig. 4). The gene flow barrier between lakes Larga and Nahuel Huapi appears to be more effective than the one between lakes Correntoso and Nahuel Huapi (Fig. 4).

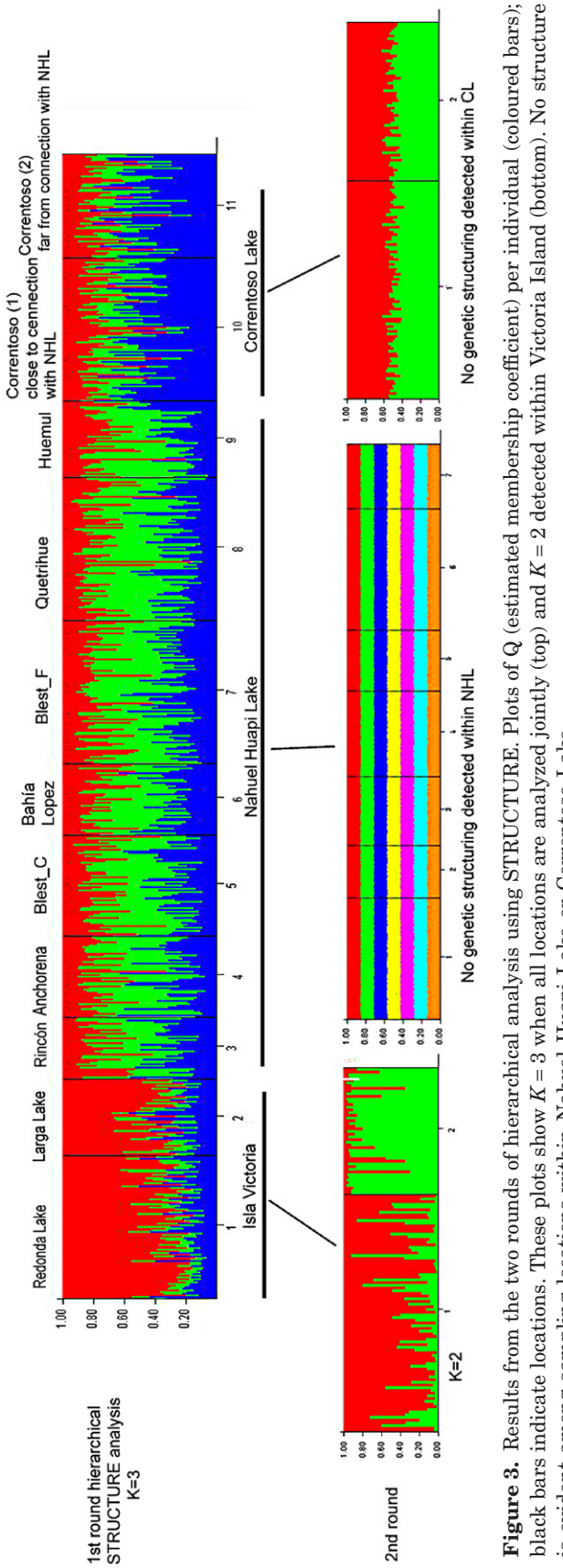

Figure 3. Results from the two rounds of hierarchical analysis using STRUCTURE. Plots of Q (estimated membership coefficient) per individual (coloured bars); black bars indicate locations. These plots show $K = 3$ when all locations are analyzed jointly (top) and $K = 2$ detected within Victoria Island (bottom). No structure is evident among sampling locations within Nahuel Huapi Lake or Correntoso Lake.

Table 2. Analysis of molecular variance results as a weighted average over all loci, including the three genetic groups inferred in the first round of STRUCTURE analysis (complete data set, $N = 704$ individuals); the four genetic groups inferred in the second round of STRUCTURE analysis (complete data set, $N = 704$ individuals); a subset of 569 individuals from Nahuel Huapi and Correntoso lakes; and a subset of 417 individuals from the seven localities within Nahuel Huapi Lake

Source of variation	Percentage variation	*F*-statistic (*P*-value)*
Three genetic groups inferred in the first round of STRUCTURE analysis		
Among the three genetic clusters	9.91	$F_{ST} = 0.12$ (< 0.001)
Among locations within clusters	1.96	$F_{SC} = 0.02$ (< 0.001)
Within locations	88.13	$F_{CT} = 0.09$ (< 0.001)
Four genetic groups inferred in the second round of STRUCTURE analysis		
Among the four genetic clusters	11.28	$F_{ST} = 0.12$ (< 0.001)
Among locations within clusters	0.85	$F_{SC} = 0.01$ (< 0.001)
Within locations	87.86	$F_{CT} = 0.11$ (< 0.001)
Subset of 569 individuals from Nahuel Huapi and Correntoso lakes		
Among Nahuel Huapi and Correntoso Lakes	6.784	$F_{ST} = 0.07$ (< 0.001)
Among locations within Lakes	0.703	$F_{SC} = 0.01$ (< 0.001)
Within locations	92.511	$F_{CT} = 0.07$ (< 0.001)
417 individuals from the seven localities within Nahuel Huapi Lake		
Among locations within Nahuel Huapi Lake	0.772	$F_{ST} = 0.01$(< 0.001)
Within locations	99.227	

*The significance of fixation indices is tested using a nonparametric permutation approach (Excoffier *et al.*, 1992), consisting of permuting individual genotypes among localities and among groups (F_{ST}), individual genotypes among locations but within groups (F_{SC}) and whole locations among groups (F_{CT}).

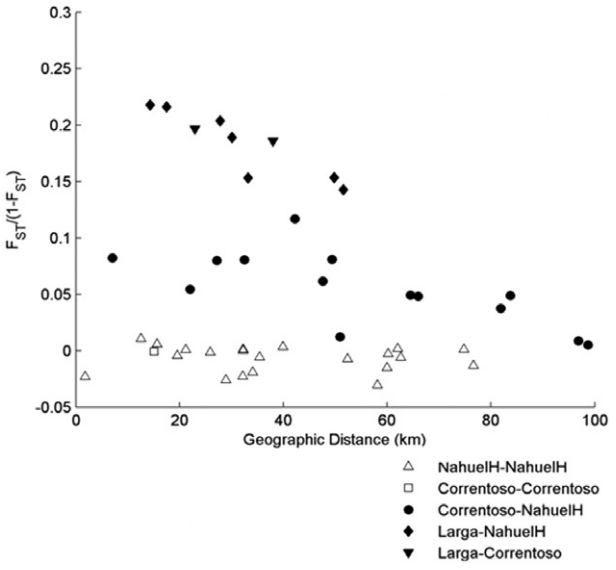

Figure 4. Plot of genetic versus geographical distances for pairs of sampled localities indicating gene flow barriers between lakes. White coloured symbols indicate localities within the same lake and black coloured symbols indicate pairs of localities from different lakes. The symbols' shapes indicate group membership.

No outlier localities were detected within Nahuel Huapi Lake by decomposed pairwise regression analysis, indicating that no local divergences are being masked (see Supporting information, Fig. S1). Consistent with the absence of an IBD pattern obtained from the genetic analysis, morphological differences and geographical distances were also not correlated ($r = -0.042$, $P = 0.370$, seven localities within Nahuel Huapi Lake).

Morphological and meristic variation

The small and isolated Redonda Lake on Victoria Island harbours the morphologically most divergent *G. maculatus* population in this system; its mean along the RW1 axis (16.27% variance) differs most from those of the other populations (Fig. 2B). To a lesser extent, this is also true for *G. maculatus* from Larga Lake, as well as on Victoria Island (Fig. 2B). A relatively short caudal peduncle for individuals in Redonda Lake and a higher body depth for both Larga and Redonda lakes can be observed on the transformation grids obtained by TPS (Fig. 2C). However, the relatively low percentage of variance explained (RW1, 16.27%; RW2, 13.58%; RW3, 12.52%) suggests that the morphological differences are only moderate.

Table 3. Pairwise $F_{ST}/(1 - F_{ST})$ between 11 sample localities in the Nahuel Huapi system

Location	Huemul (NH)	Blest_m (NH)	Rincón (NH)	Anchorena (NH)	Blest_c (NH)	B Lopez (NH)	Quetrihue (NH)	Larga (IV)	Redonda (IV)	Correntoso_F	Correntoso_N
Huemul (NH)	0.000										
Blest_m (NH)	–0.002	0.00000									
Rincón (NH)	–0.006	0.001	0.000								
Anchorena (NH)	0.006*	0.001	0.003	0.000							
Blest_c (NH)	0.002	–0.024	–0.014	–0.019	0.000						
B Lopez (NH)	–0.006	–0.004	–0.007	0.010*	0.001	0.000					
Quetrihue (NH)	–0.027	–0.031	–0.023	–0.007	–0.015	0.001	0.000				
Larga Lake	0.133*	0.190*	0.169*	0.178*	0.124*	0.159*	0.179*	0.000			
Redonda Lake	0.161*	0.234*	0.205*	0.220*	0.138*	0.201*	0.234*	0.190*	0.000		
Correntoso_F	0.012*	0.008*	0.051*	0.058*	0.050*	0.047*	0.074*	0.164*	0.242*	0.000	
Correntoso_N	0.046*	0.036*	0.076*	0.074*	0.046*	0.075*	0.105*	0.157*	0.262*	–0.001	0.000

*$P < 0.05$, permutation test. Negative values can be obtained when gene frequencies are almost identical.
NH, Nahuel Huapi Lake.

Body shape differences could be set apart between the four genetic groups ($P < 0.001$) by the CVA. CV1 explained 63.8% of the total morphological variation. The percentage of variation explained by CV1 was reduced to a 40.5% of the total morphological variation when locations within Nahuel Huapi Lake were consider, rather than genetic clusters (or different lakes). The previous three functions depended significantly on centroid size indicating allometry (see Supporting information, Fig. S2A). However, an appreciable fraction of the differences among groups remained unexplained within a common size range (see Supporting information, Fig. S2A), as could also be observed through the plotting of nonstandardized regression residuals (correction for size effects; see Supporting information, Fig. S2B).

Meristic counts were lower for individuals in Redonda Lake (KW and Dunn tests, $P < 0{,}001$) than for those in any of the other sampling locations, including vertebral number, fin ray number and dorsal fin ray number (Table 5).

Genetic ($F_{ST}/1 - F_{ST}$) and morphological distances (calculated from body shape variables) were correlated (Mantel test, $r = 0.847$, $P < 0.01$). Pairs of localities within the same genetic cluster are more similar morphologically than pairs of localities from different genetic clusters. Redonda Lake stands out as the most divergent in both genetic and body shape structure compared to localities in the three other genetic groups (Fig. 5). The correlation coefficient dropped to $r = 0.739$ ($P < 0.01$) when the Redonda Lake population was removed from the analysis, although it remained significant. Removing also Larga Lake virtually eliminated the correlation (Mantel test, $r = 0.329$, $P = 0.09$), suggesting that the genetic–morphological correlation holds mostly when individuals from these two small lakes (one isolated the other nearly so) are included in the analysis.

DISCUSSION

In the present study, we examined the genetic and phenotypic patterns of differentiation among *G. maculatus* populations in a complex of Patagonian postglacial lakes. Gene flow restrictions among populations were expected to lead to divergences and an IBD pattern. Our results show that populations within the larger lakes (i.e. Nahuel Huapi and Correntoso) exhibit restricted genetic structuring. However, populations appear to be structured by reductions in gene flow among lakes. Genetic distances between localities are correlated with morphological differences and the populations in the smaller lakes are the most divergent both genetically and morphologically. It is worth noting that the divergences found for these populations on Victoria Island make them biologically inter-

Table 4. Estimated migration rates between genetic populations

	Migration rate into population: mean/mode/95% HPDI			
Population	Redonda	Larga	Nahuel Huapi	Correntoso
Redonda ($N = 88$)	1 1 (1–1)	0.000 0.000 (10^{-05}–0.0001)	0.0747 0.0704 (0.0173–0.153)	0.193 0.192 (0.052–0.373)
Larga ($N = 47$)	0.000 0.000 (10^{-12}–10^{-06})	1 1 (0.994–1)	0.0103 0.0028 (0.0021–0.071)	0.046 0.038 (0.009–0.19)
Nahuel Huapi ($N = 417$)	0.000 0.000 (10^{-07}–10^{-06})	0.000 0.000 (10^{-05}–0.001)	0.585 0.581 (0.478–0.69)	0.0369 0.0213 (0.0069–0.159)
Correntoso ($N = 152$)	0.000 0.000 (10^{-08}–10^{-06})	0.000 0.000 (0.0001–0.003)	0.33 0.333 (0.192–0.452)	0.724 0.727 (0.551–0.858)

The posterior mean, mode, and the highest posterior density interval (HPDI) at the 95% level are presented.

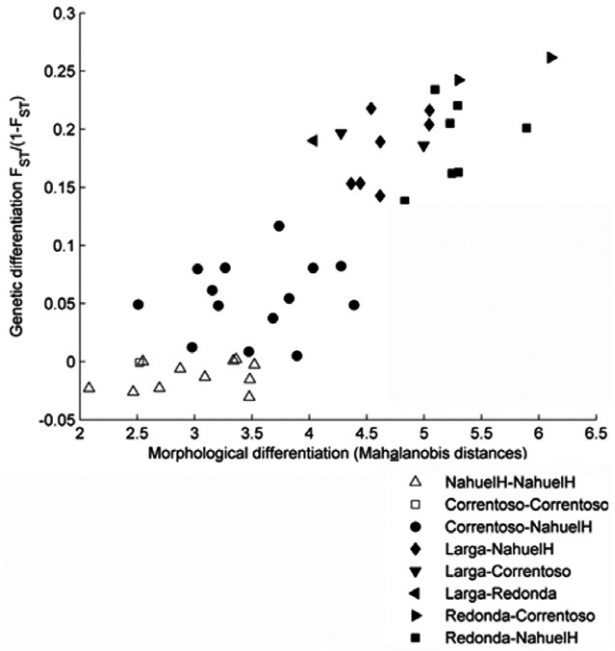

Figure 5. Genetic versus morphological distances for pairs of sampled localities. Comparisons between two sampling sites belonging to the same genetic group are shown in white, and those belonging to different genetics groups are shown in black. The shape of the symbols indicates the genetic group identity of the pair.

esting. Efforts towards ensuring their persistence are therefore recommended, especially considering that their low genetic diversity and lack of connectivity indicate they are highly vulnerable.

The lack of IBD pattern within Nahuel Huapi Lake suggests that larvae coming from different spawning sites mix in the limnetic zone and no homing behaviour occurs among the juveniles. The movement of *G. maculatus* adult individuals within this lake does not appear to be restricted, as previously suggested by parasitological evidence (Revenga & Scheinert, 1999; Revenga, 2003). Interestingly, if adults in Nahuel Huapi Lake are assumed sedentary (Revenga & Scheinert, 1999), our results suggest that the limnetic larvae might have an important dispersive role for resident *G. maculatus* populations. Under this scenario, larval movements thus exert a homogenizing effect on the distribution of genetic variance in *G. maculatus* within Nahuel Huapi Lake that, ultimately, can constrain the potential for local adaptation. Accordingly, a remarkably high density of galaxiid larvae performing diel movements was observed using hydroacoustic methods in the limnetic zone of a similar lake in the region (Rechencq *et al*., 2011). In addition, picocyanobacteria in the Nahuel Huapi Lake also exhibit very limited spatial structuring (Caravati *et al*., 2010), suggesting this lake can be characterized as having a well mixed limnetic zone where larvae can mix.

The short rivers connecting lakes Larga and Correntoso respectively with Nahuel Huapi Lake were, a priori, not expected to reduce gene flow between them because upstream migration is known for diadromous and freshwater resident *G. maculatus* populations (Barriga, Battini & Cussac, 2007). However, the genetic exchange between Larga and Nahuel Huapi lakes is reduced and the exchange between Correntoso and Nahuel Huapi lakes was found to be asym-

Table 5. *Galaxias maculatus* individuals from Redonda Lake showed lower meristic counts, including vertebral counts, anal fin ray counts, and dorsal fin ray counts

		Number of individuals with the following vertebral count:									Dunn's
Locality	*N*	50	51	52	53	54	55	Median	25%	75%	method
Redonda Lake	25	25						50	50	50	A
Anchorena (NH)	19			3	12	3	1	53.1	0.7	53	B
Bahía Lopez (NH)	19			4	10	5		53	0.7	53	B
Larga Lake	20			2	12	6		53.2	0.5	53	B
Quetrihue (NH)	20			6	10	4		52.9	0.7	53	B
Blest (NH)	20			6	9	5		53	0.7	53	B

		Anal fin ray count:								Dunn's
Locality		12	13	14	15	16	Median	25%	75%	method
Redonda Lake	25	11	13	1			13	12	13	A
Anchorena (NH)	19		2	10	6	1	14	14	15	B
Bahía Lopez (NH)	19		2	11	6		14	14	15	B
Larga Lake	20		1	10	9		14	14	15	B
Quetrihue (NH)	20		2	15	3		14	14	14	B
Blest (NH)	20		2	10	5	3	14	14	15	B

		Dorsal fin ray count:							Dunn's
Locality		9	10	11		Median	25%	75%	method
Redonda Lake	25	18	7			9	9	10	A
Anchorena (NH)	19		13	6		10	10	11	B
Bahía Lopez (NH)	19		14	5		10	10	11	B
Larga Lake	20	1	12	7		10	10	11	B
Quetrihue (NH)	20	2	15	3		10	10	10	B
Blest (NH)	20	1	12	7		10	10	11	B

NH, Nahuel Huapi Lake.

metric: it is one order of magnitude higher in the downstream than in the upstream direction (Table 4). This asymmetry could be explained by ecological barriers counteracted by the transportation of larvae by the water current in the downstream direction. Native (*Percichthys trucha*) and introduced (salmonid fishes) predators are especially abundant in the short river connecting Correntoso and Nahuel Huapi lakes (L. Buria pers. comm.). Both are known to prey voraciously on *G. maculatus* (Macchi *et al*., 1999; Macchi, Pascual & Vigliano, 2007; Vigliano *et al*., 2009). Moreover, introduced salmonid fishes have been shown to exert a relatively large impact on the native species in rivers in Patagonia (Soto *et al*., 2006; Habit *et al*., 2010), as well as in New Zealand (Townsend & Crowl, 1991).

The genetic, morphological, and meristic divergence observed for the Redonda Lake population suggest that this population has long been isolated and is likely under the strong influence of drift and of differential environmental effects. The absence of private alleles in Redonda Lake (Table 1) is not expected after long isolation; however, rare alleles would be rapidly lost under the strong effects of genetic drift affecting small-sized populations. The first level of genetic structure detected by the Bayesian analysis (Fig. 3) separated the small lakes in Isla Victoria from the larger lakes. The implication could be that these two populations originated from repeated founder events from Nahuel Huapi Lake. Alternatively, any connection between the Redonda and Nahuel Huapi lakes was probably lost following the post-glacial drop in the water level of the paleolake originating these water bodies over 10 kya BP (Tatur *et al*., 2002). The pairwise F_{st} (Table 3) show that the two small lakes in Victoria Island are currently highly differentiated.

The morphological differences exhibited by the two small populations on the Victoria Island lakes can be explained by a lower predatory pressure on these populations. Predation is known to be a source of evolutionary divergence in fishes by generating selection favoring escape performance (DeWitt & Langerhans, 2003). For example, caudal morphology in another galaxiid fish endemic to Patagonia (*Galaxias platei*) has been correlated with the ability to avoid predators (Milano *et al*., 2002, 2006). In addition, Barriga *et al*. (2012) found *G. maculatus* larvae have deeper bodies and shorter caudal peduncles in lakes with lower predation risk and higher food availability compared to lakes where this relation is reversed. Similarly, no predator species have been reported in Redonda Lake where individuals have shorter caudal peduncles, and individuals from Nahuel Huapi and Correntoso have larger peduncles and more elongate bodies, where predators such as *P. trucha* or introduced salmonids exert a notably high predation pressure on the species (Vigliano *et al*., 2009). Alternatively, the different body shapes could be the result of phenotypic plasticity in response to environmental differences among lakes (e.g. small lakes in Victoria Island are shallower than Nahuel Huapi and Correntoso lakes).

Finally, the mean vertebral count observed for the Redonda Lake population (50) is the lowest recorded for freshwater resident populations of *G. maculatus* in Patagonia (Table 5). The mean vertebral count reported by McDowall (2003a) for lacustrine populations in South America was in the range 51.5–59.9, and a count of 50 was reported only in one locality (Australia) out of 28 sampled lakes across Australia, New Zealand and South America. It has been proposed that the complex variation patterns of vertebral number in Galaxiidae fishes are influenced by an amalgam of factors such as fish size, life history, and other environmental components (e.g. temperature or salinity) (McDowall, 2003a). However, unravelling environmental and inherited causalities is not simple and the adaptive and functional value of vertebral variation in fishes is not well elucidated (McDowall, 2003b, 2007). The system investigateed in the present study, where vertebral variation is observed among populations in postglacial lakes likely sharing an ancestral population, offers a good opportunity for investigations aiming to disentangle the causes of meristic variation at the intraspecific level. For example, future studies could explore developmental rate heterogeneity because it is a mechanism proposed to facilitate intraspecific ecotypic divergence (including body shape and meristic counts) in postglacial fishes (McPhee *et al*., 2012).

In conclusion, we suggest that a number of processes could be involved in the differentiation among *G. maculatus* populations in this lacustrine system. A significant genetic exchange takes place among locations within lakes (i.e. within Nahuel Huapi Lake and within Correntoso Lake), suggesting an important dispersive role of the limnetic larvae. Geographical isolation has allowed the genetic and phenotypic divergences observed in Redonda Lake population. Potential ecological factors could be causing gene flow reductions/assymetries between Larga/Correntoso and Nahuel Huapi lakes, respectively.

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REFERENCES

Barriga JP, Battini MA, Cussac VE. 2007. Annual dynamics variation of landlocked *Galaxias maculatus* (Jenyns 1842) population in a river of Northern Patagonia: occurrence of juvenile upstream migration. *Journal of Applied Ichthyology* **23:** 128–135.

Barriga JP, Battini MA, Garcia-Asorey M, Carrea C, Macchi P, Cussac VE. 2012. Intraspecific variation in

diet, growth, and morphology of landlocked *Galaxias maculatus* during its larval period: the role of food availability and predation risk. *Hydrobiologia* **679:** 27–41.

Barriga JP, Battini MA, Macchi PJ, Milano D, Cussac VE. 2002. Spatial and temporal distribution of landlocked *Galaxias maculatus* and *Galaxias platei* (Pisces, Galaxiidae) in a lake in the South American Andes. *New Zealand Journal of Marine and Freshwater Research* **36:** 349–363.

Bookstein FL. 1991. *Morphometric tools for landmark data: geometry and biology*. Cambridge: Cambridge University Press, 435.

Boy CC, Morriconi E, Calvo J. 2007. Reproduction in puyen, *Galaxias maculatus* (Pisces: Galaxiidae), in the southernmost extreme of distribution. *Journal of Applied Ichthyology* **23:** 547–554.

Boy CC, Perez AF, Lattuca ME, Calvo J, Morriconi E. 2009. Reproductive biology of *Galaxias maculatus* (Jenyns 1842) in the Rio Ovando, a high-latitude environment in southernmost Patagonia, Argentina. *Journal of Applied Ichthyology* **25:** 661–668.

Caravati E, Callieri C, Modenutti B, Corno G, Balseiro E, Bertoni R, Michaud L. 2010. Picocyanobacterial assemblages in ultraoligotrophic Andean lakes reveal high regional microdiversity. *Journal of Plankton Research* **32:** 357–366.

Carrea C, Barriga JP, Cussac VE, Ruzzante DE. 2012. Data from: genetic and phenotypic differentiation among *Galaxias maculatus* populations in a Patagonian postglacial lake system. *Dryad Digital Repository* doi: 10.5061/dryad.0578v

Carrea C, Paterson I, Cussac V, Ruzzante DE. 2009. Ten novel microsatellite loci characterized for a remarkably widespread fish: *Galaxias maculatus* (Galaxiidae). *Molecular Ecology Resources* **9:** 1503–1505.

Chapman A, Morgan DL, Beatty SJ, Gill HS. 2006. Variation in life history of land-locked lacustrine and riverine populations of *Galaxias maculatus* (Jenyns 1842) in Western Australia. *Environmental Biology of Fishes* **77:** 21–37.

Cussac V, Ortubay S, Iglesias G, Milano D, Lattuca ME, Barriga JP, Battini MA, 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.

Cussac VE, Cervellini PM, Battini MA. 1992. Intralacustrine movements of *Galaxias maculatus* (Galaxiidae) and *Odonthestes microlepidotus* (Atherinidae) during their early life history. *Enviromental Biology of Fishes* **35:** 141–148.

DeWitt TJ, Langerhans RB. 2003. Multiple prey traits, multiple predators: keys to understanding complex community dynamics. *Journal of Sea Research* **49:** 143–155.

Diaz MM, Pedrozo FL, Temporetti PF. 1998. Phytoplankton of two Araucanian lakes of differing trophic status (Argentina). *Hydrobiologia* **369–370:** 45–57.

Dryden IL, Mardia KV. 1998. *Statistical shape analysis*. Chichester: Wiley, 347.

Elphinstone MS, Hinten GN, Anderson MJ, Nock CJ. 2003. An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. *Molecular Ecology Notes* **3:** 317–320.

Excoffier L, Laval G, Schneider S. 2005. Arlequin 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1:** 47–50.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131:** 479–491.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: extensions to linked loci and correlated allele frequencies. *Genetics* **164:** 1567–1587.

Faubet P, Gaggiotti O. 2008. A new Bayesian method to identify the environmental factors that influence recent migration. *Genetics* **178:** 1491–1504.

Faubet P, Waples RS, Gaggiotti OE. 2007. Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. *Molecular Ecology* **16:** 1149–1166.

Fontaine M, Baird S, Piry S, Ray N, Tolley KA, Duke S, Birkun JA, Ferreira M, Jauniaux T, Llavona A, Öztürk B, Öztürk AA, Ridoux V, Rogan E, Sequeira M, Siebert U, Vikingsson GA, Bouquegneau JM, Michaux JR. 2007. Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in old world waters. *BMC Biology* **5:** 30.

Gomez-Uchida D, Knight TW, Ruzzante DE. 2009. Interaction of landscape and life history attributes on genetic diversity, neutral divergence and gene flow in a pristine community of salmonids. *Molecular Ecology* **18:** 4854–4869.

Goudet J. 2001. FSTAT 2.9.3: a program to estimate and test gene diversities and fixation indices. Available at: http://www2.unil.ch/popgen/softwares/fstat.htm

Guillot G, Leblois R, Coulon A, Frantz AC. 2009. Statistical methods in spatial genetics. *Molecular Ecology* **18:** 4743–4756.

Guo SW, Thompson EA. 1992. Performing the exact test for Hardy–Weinberg proportion for multiple alleles. *Biometrics* **48:** 361–372.

Habit E, Piedra P, Ruzzante DE, Walde SJ, Belk MC, Cussac VE, Gonzales J, Colin N. 2010. Changes in the distribution of native fishes in response to introduced species and other anthropogenic effects. *Global Ecology and Biogeography* **19:** 697–710.

Jenyns L. 1842. Fish. In: Darwin C, ed. *The zoology of the voyage of HMS Beagle, under the command of Captain Fitzroy, R.N., during the years 1832–1836*. London: Smith, Elder & Co.

Klingenberg CP. 2008. MorphoJ. Faculty of Life Sciences, University of Manchester. Available at: http://www.flywings.org.uk/MorphoJ_page.htm

Koizumi I, Yamamoto S, Maekawa K. 2006. Decomposed pairwise regression analysis of genetic and geographic

distances reveals a metapopulation structure of stream-dwelling dolly vardencharr. *Molecular Ecology* **15:** 3175–3189.

Macchi PJ, Cussac VE, Alonso MF, Denegri MA. 1999. Predation relationships between introduced salmonids and the native fish fauna in lakes and reservoirs in Northern Patagonia. *Ecology of Freswater fishes* **8:** 227–236.

Macchi PJ, Pascual MA, Vigliano PH. 2007. Differential piscivory of the native *Percichthys trucha* and exotic salmonids upon the native forage fish *Galaxias maculatus* in Patagonian Andean lakes. *Limnologica* **37:** 76–87.

Manel S, Schwartz MK, Luikart G, Taberlet P. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution* **18:** 189–197.

McDowall RM. 1968. *Galaxias maculatus* (Jenyns), the New Zealand whitebait. *Fisheries Research Bulletin No. 2*. Wellington: New Zealand Marine Department.

McDowall RM. 1971. The galaxiid fishes of South America. *Zoological Journal of the Linnean Society* **50:** 33–73.

McDowall RM. 2003a. Variation in vertebral number in galaxiid fishes (Teleostei: Galaxiidae): a legacy of life history, latitude and length. *Environmental Biology of Fishes* **66:** 362–381.

McDowall RM. 2003b. Variation in vertebral number in galaxiid fishes, how fishes swim and a possible reason for pleomerism. *Reviews in Fish Biology and Fisheries* **13:** 247–263.

McDowall RM. 2007. Jordan's and other ecogeographical rules, and the vertebral number in fishes. *Journal of Biogeography* **35:** 501–508.

McPhee MV, Noakes DLG, Allendorf FW. 2012. Developmental rate: a unifying mechanism for sympatric divergence in postglacial fishes? *Current Zoology* **58:** 21–34.

Milano D, Cussac VE, Macchi PJ, Ruzzante DE, Alonso MF, Vigliano PH, Denegri MA. 2002. Predator associated morphology in *Galaxias platei* in Patagonian lakes. *Journal of Fish Biology* **61:** 138–156.

Milano D, Ruzzante DE, Cussac VE, Macchi P, Ferriz R, Barriga J, Aigo J, Lattuca ME, Walde S. 2006. Latitudinal and ecological correlates of morphological variation in *Galaxias platei* (Pisces, Galaxiidae) in Patagonia. *Biological Journal of the Linnean Society* **87:** 69–82.

Moore JS, Glow JL, Taylor EB, Hendry AP. 2007. Quantifying the constraining influence of gene flow on adaptive divergence in the lake-stream three spine stickleback system. *Evolution* **6:** 2015–2026.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel population genetic software for teaching and research. *Molecular Ecology Notes* **6:** 288–295.

Petit RJ, Mousadik AE, Pons O. 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* **12:** 844–855.

Pollard DA. 1971. The biology of a landlocked form the normally catadromous salmoniform fish *Galaxias maculatus* (Jenyns). 1. Life cycle and origin. *Australian Journal of Marine and Freshwater Research* **22:** 91–123.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155:** 945–959.

Rabassa J, Coronato AM, Martinez O. 2011. Late Cenozoic glaciations in Patagonia and Tierra del Fuego: an updated review. *Biological Journal of the Linnean Society* **103:** 316–335.

Raymond M, Rousset F. 1995. GENEPOP 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86:** 248–249.

Rechencq M, Sosnovsky A, Macchi P, Alvear P, Vigliano P. 2011. Extensive diel fish migrations in a deep ultraoligotrophic lake of Patagonia Argentina. *Hydrobiologia* **658:** 147–161.

Revenga J. 2003. Polución asociada a la piscicultura de trucha arcoiris en jaulas. Efectos parasitológicos sobre peces autóctonos de la Patagonia. Magister Thesis, Universidad de Buenos Aires.

Revenga J, Scheinert P. 1999. Infections by helminth parasites in 'puyenes', *Galaxias maculatus* (Galaxiidae, Salmoniformes), from Southern Argentina with special reference to Tylodelphysbarilochensis (Digenea, Platyhelminthes). *Memorias Do Instituto Oswaldo Cruz* **94:** 605–609.

Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* **43:** 223–225.

Rohlf FJ. 2003. *tpsRelw, relative warps analysis*, Version 1.36. Department of Ecology and Evolution, State University of New York at Stony Brook. Available at: http://life.bio.sunysb.edu/morph

Rohlf FJ. 2004. *tpsUtil, file utility program*, Version 1.26. Department of Ecology and Evolution, State University of New York at Stony Brook. Available at: http://life.bio.sunysb.edu/morph

Rohlf FJ. 2005. *tpsDig, digitize landmarks and outlines*, Version 2.05. Department of Ecology and Evolution, State University of New York at Stony Brook. Available at: http://life.bio.sunysb.edu/morph

Rohlf FJ, Slice D. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39:** 40–59.

Rousset F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145:** 1219–1228.

Rowe DK, Chisnall BL. 1996. Ontogenetic habitat shifts by *Galaxias gracilis* (Galaxiidae) between the littoral and limneticzones of Lake Kanono, New Zealand. *Environmental Biology of Fishes* **46:** 255–264.

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

Ruzzante DE, Walde SJ, Gosse JC, Cussac VE, Habit E, Zemlak TS, Adams EDM. 2008. Climate control on ancestral population dynamics: insight from patagonian fish phylogeography. *Molecular Ecology* **17:** 2234–2244.

Ruzzante DE, Walde SJ, Macchi PJ, Alonso M, Barriga JP. 2011. Phylogeography and phenotypic

diversification in the Patagonian fish *Percichthys trucha*: the roles of Quaternary glacial cycles and natural selection. *Biological Journal of the Linnean Society* **103:** 514–529.

Soto D, Arismendi I, Gonzales J, Sanzana J, Jara F, Jara C, Guzmán E, Lara A. 2006. Southern Chile, trout and salmon country: invasion patterns and threats for native species. *Revista Chilena de Historia Natural* **79:** 97–117.

Tatur A, del Valle R, Bianchi MM, Outes V, Villarosa G, Niegodzizs J, Debaene G. 2002. Late Pleistocene paleolakes in the Andes and Extra-Andean Patgonia at mid-latitudes of South America. *Quaternary International* **89:** 135–150.

Taylor EB. 1999. Species pairs of north temperate freshwater fishes: evolution, taxonomy and conservation. *Reviews in Fish Biology and Fisheries* **9:** 299–324.

Townsend CR, Crowl TA. 1991. Fragmented population structure in a native New Zealand fish: an effect of introduced brown trout? *Oikos* **61:** 348–354.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4:** 535–538.

Vigliano PH, Beauchamp DA, Milano D, Macchi PJ, Alonso MF, GarciaAsorey MI, Denegri MA, Ciancio JE. 2009. Predation on Galaxiids and other native organisms by introduced rainbow trout in an ultraoligotrophic lake in Northern Patagonia, Argentina: a bioenergetics modeling approach. *Transactions of the American Fisheries Society* **138:** 1405–1419.

Zattara EE, Premoli AC. 2004. Genetic structuring in Andean landlocked populations of *Galaxias maculatus*: effects of biogeographic history. *Journal of Biogeography* **31:** 1–10.

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

Zemlak TS, Habit EM, Walde SJ, Battini MA, Adams E, Ruzzante DE. 2008. Across the southern Andes on fin: glacial refugia, drainage reversals & a secondary contact zone revealed by the phylogeographic signal of *Galaxias platei* in Patagonia. *Molecular Ecology* **17:** 5049–5061.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. No outlier locality was detected by decomposed pairwise regression method within Nahuel Huapi Lake. Mean and 95% confidence interval for the residuals obtained from the regression of all pairs of localities within are shown, all confidence intervals include 0.

Figure S2. (A) Regression and 95% confidence intervals of canonical variates (CVs) versus centroid size. CV1 adjusted to a quadratic function (left panel), whereas CV2 and CV3 (middle and right panel, respectively) adjusted to a cubic function. Each genetic group is shown with media and 95% confidence interval. Centroid size range of each genetic group is shown to the lower right of the left panel. (B) Mean canonical variates after removing size effect from shape variables.

Table S1. Allele frequencies and sample size by locus and location. Private alleles are bold. *P*-values for the Hardy–Weinberg equilibrium exact tests are shown.

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