Genetics, Gene Flow, and Glaciation: The Case of the South American Limpet *Nacella mytilina*

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Abstract

Glacial episodes of the Quaternary, and particularly the Last Glacial Maximum (LGM) drastically altered the distribution of the Southern-Hemisphere biota, principally at higher latitudes. The irregular coastline of Patagonia expanding for more than 84.000 km constitutes a remarkable area to evaluate the effect of Quaternary landscape and seascape shifts over the demography of near-shore marine benthic organisms. Few studies describing the biogeographic responses of marine species to the LGM have been conducted in Patagonia, but existing data from coastal marine species have demonstrated marked genetic signatures of post-LGM recolonization and expansion. The kelp-dweller limpet *Nacella mytilina* is broadly distributed along the southern tip of South America and at the Falkland/Malvinas Islands. Considering its distribution, abundance, and narrow bathymetry, *N. mytilina* represents an appropriate model to infer how historical and contemporary processes affected the distribution of intraspecific genetic diversity and structure along the southern tip of South America. At the same time, it will be possible to determine how life history traits and the ecology of the species are responsible for the current pattern of gene flow and connectivity across the study area. We conducted phylogeographic and demographic inference analyses in *N. mytilina* from 12 localities along Pacific Patagonia (PP) and one population from the Falkland/Malvinas Islands (FI). Analyses of the mitochondrial gene COI in 300 individuals of *N. mytilina* revealed low levels of genetic polymorphism and the absence of genetic differentiation along PP. In contrast, FI showed a strong and significant differentiation from Pacific Patagonian populations. Higher levels of genetic diversity were also recorded in the FI population, together with a more expanded genealogy supporting the hypothesis of glacial persistence of the species in these islands. Haplotype genealogy, and mismatch analyses in the FI population recognized an older and more complex demographic history than in PP. Demographic reconstructions along PP suggest a post-LGM expansion process (7.5

ka), also supported by neutrality tests, mismatch distribution and maximum parsimony haplotype genealogies. Migration rate estimations showed evidence of asymmetrical gene flow from PP to FI. The absence of genetic differentiation, the presence of a single dominant haplotype, high estimated migration rates, and marked signal of recent demographic growth, support the hypothesis of rapid post-glacial expansion in *N. mytilina* along PP. This expansion could have been sustained by larval and rafting-mediated dispersal of adults from northernmost populations following the Cape Horn Current System. Marked genetic differentiation between PP and FI could be explained through differences in their respective glacial histories. During the LGM, Pacific Patagonia (PP) was almost fully covered by the Patagonian Ice Sheet, while sheet coverage in the FI ice was restricted to small cirques and valleys. As previously recorded in the sister-species *N. magellanica*, the FI rather than represent a classical glacial refugium for *N. mytilina*, seems to represent a sink area and/or a secondary contact zone. Accordingly, historical and contemporary processes, contrasting glacial histories between the analyzed sectors, as well as life history traits constitute the main factors explaining the current biogeographical patterns of most shallow Patagonian marine benthic organisms.

Introduction

The development of large continental ice sheets during glacial episodes of the Quaternary, most recently the last glacial maximum (LGM), between 23 and 18 ka, generated major climate and environmental changes that deeply affected the distribution of the present day biota [1–3]. Several studies have demonstrated how these episodes radically altered the demography and the geographical range of species and populations and therefore also affected the distribution of intraspecific genetic variation [1,3–7]. A vast array of records from the Northern Hemisphere provided the empirical basis for the Expansion-Contraction (E-C) model of Pleistocene biogeography [1], describing the response of populations and species to climatic change [2–4,8–11]. Under a simple E-C model, cool-temperate species survived ice advances at lower latitude refugia, only re-populating higher latitudes through range expansion [1,2,5,12]. This model constitutes a fundamental paradigm of Quaternary biogeography and also provides a potentially useful explanation to understand how species assemblages will respond to future climate change [3]. The development of molecular-based techniques for population genetic and phylogeographic studies has revolutionized our understanding of the consequences of past glacial processes concerning patterns of diversity, connectivity and structure at different geographical and temporal scales [2,4,5,10,13]. The Quaternary history of South America is fairly well understood with glacial events generating major changes in sea level, climate, and landscape [14–17]. During the LGM most of the Pacific Patagonian (PP) fjords and channels from Chiloé Island (42°S) to Cape Horn (56°S) were covered by the Patagonian Ice Sheet, expanding more than 480.000 km^2 with an ice volume of around 500.000 km^3 [14,15,18,19].

During the last three decades more and more genetic data have been accumulated for different groups of Patagonian taxa. They include population-based and phylogeographic studies in aquatic invertebrates [20–25], freshwater [26–31] and marine [32–35] fishes, lizards [36,37], amphibians [38], mammals [39–43], and plants [44–47]. Most of these studies evidenced clear patterns of postglacial recolonization and recognized the presence of Quaternary glacial refugia on the East side of the Andes, along the Patagonian Steppe [26,29], with refugia on the West

side of Andes both within [23,29,38] and outside of the glacier limits [26,34]. Absence of genetic structure and a strong signal of recent demographic growth recorded in shallow Patagonian marine benthic invertebrates support the hypothesis of rapid postglacial expansion [20–22,25]. Species with high dispersive potential (i.e., *Durvillaea antarctica* and *Macrocystis pyrifera*) recolonized Patagonia since the LGM from geographically distant regions [46,48] evidence of the major role of long-distance dispersal in the biogeography of Subantarctic nearshore marine benthic organisms [49–51].

True limpets of the genus *Nacella* (Patellogastropoda: Nacellidae) are dominant organisms of Antarctic and Subantarctic inter- and sub-tidal rocky ecosystems. As typical patellogastropods, the members of *Nacella* are dioecious organisms with external fertilization and in the Antarctic limpet *Nacella concinna* the larval lifespan can extend for more than two months [52–54]. The genus includes at least 11 nominal species currently distributed in different provinces of the Southern Ocean including maritime Antarctica, South America, and Subantarctic islands (South Georgia, South Sandwich, Marion, Kerguelen, Heard, Macquarie, and Campbell) [55]. Recent molecular studies have helped to further understand the biogeography of the genus across its distribution in the Southern Ocean [56], and particularly in Patagonia [20,24,25]. Geographically distant species from the Antarctic Peninsula (*Nacella concinna*), central Chile (*Nacella clypeater*), Patagonia (*Nacella magellanica*, *Nacella deaurata*, *Nacella flammea*, and *Nacella mytilina*) and Heard Island (*Nacella kerguelenensis* and *Nacella* cf. *macquariensis*) showed high levels of genetic divergence indicating the existence of transoceanic discontinuities in the genus [56]. At the same time, divergence time estimations indicate that the origin and diversification of *Nacella* is relatively modern and not associated with continental drift processes but with climatic and oceanographic processes associated to the middle Miocene Climatic Transition (< 15 Ma) [56]. South America, and particularly Patagonia exhibits the highest taxonomic richness, with at least eight nominal species [55,57]. Hence, this region was considered as the center of origin of the genus from where seaweed-associated species spread eastward to other Subantarctic areas [55]. Nonetheless, recent molecular studies and divergence time estimations indicate that number of Patagonian species of *Nacella* was overestimated and that they constitute a recently derived South-American lineage [20,24,56]. Moreover, recent molecular and morphological analyses indicate that the recent radiation of *Nacella* in Patagonia includes only four evolutionary significant units: *N. deaurata*, *N. flammea*, *N. magellanica* and *N. mytilina* [20,24].

One of the Patagonian *Nacella* species, the kelp-dweller *Nacella mytilina* (Helbling, 1779), exhibits the broadest distribution of the genus including the southern tip of South America, the Falkland/Malvinas Islands, and Kerguelen Island, located more than 7000 kilometers away [55]. Nevertheless, recent molecular studies indicate that *N. mytilina*-like individuals from Kerguelen Island constitute a particular kelp-associated morphotype of *N. kerguelenensis* (González-Wevar, under review). Kelp-associated individuals from Kerguelen Island and Patagonian *N. mytilina* showed more than 8.04% of genetic divergence, similar to the degree of divergence detected between South American and Antarctic species of the genus [56]. Accordingly, *N. mytilina* is restricted to the southern tip of South America and exhibits a narrower distribution than other Patagonian relatives (*N. magellanica* and *N. deaurata*). The species is currently found between Guarelo Island (50° S) in the Pacific to the Santa Cruz Province (45°–52°S) in the Atlantic, including the Strait of Magellan, Cape Horn, Tierra del Fuego, and the Falkland/Malvinas Islands [25,55–57]. The species is relatively abundant over macroalgae such as *Macrocystis pyrifera*, *Gigartina skottsbergii*, and *Lessonia* [58,59]. Considering its habitat preferences, *N. mytilina* is morphologically well distinguished from other Patagonian limpets in terms of shell shape, sculpture and thickness. Recent comparative dietary composition analyses in Patagonian *Nacella* species indicate that *N. mytilina*'s diet is highly specialized compared to

its relatives *N. magellanica* and *N. deaurata*. *Nacella mytilina* feeds over *M. pyrifera* and particular kelp-associated diatoms [60]. At the same time, all Patagonian *Nacella* species, with the exception of *N. mytilina*, showed changes in their dietary composition between winter and summer [60]. Recent phylogeographic studies of its relatives *N. magellanica* and *N. deaurata*, along the Atlantic coast recorded medium levels of genetic diversity, an absence of genetic structure, and evidence of recent demographic expansions [20]. Similarly, a study conducted in *N. magellanica* along PP also registered an absence of genetic structure, the presence of a single dominant haplotype, high levels of gene flow, and strong signals of recent demographic expansion [25]. Nevertheless, in the same study González-Wevar et al. [25] recorded significant differences between Pacific Patagonia (PP) and Falkland/Malvinas Islands (FI) populations that could be a consequence of their distinct glacial histories. The current knowledge about the pattern of genetic diversity in *N. mytilina* is restricted to a single locality in the Strait of Magellan [24]. Therefore, phylogeographic analyses are required in order to further understand the legacy of the glacial Quaternary cycles over this kelp-associated species, as well as the role of rafting in the patterns of genetic connectivity.

Methods

Ethics Statement

This work was conducted using "mauchos" (*Nacella mytilina*) as study model, a common limpet species from the southern tip of South America. The species is not protected by the Chilean Fishery Subsecretary and has not been included in the Chilean fishery statistics. Permission to undertake field studies and to collect specimens was issued by the Chilean Fishery Service Director (Carlos Orellana Céspedes), under the technical memorandum (249/2015). Permits to undertake field studies and collect specimens in Falkland Islands were issued by the Environmental Planning Department, the Falkland Islands Government. The Instituto de Ecología y Biodiversidad (IEB/15-2015) and Chilean Fishery Service (SERNAPESCA 429/2015) ethic committees approved sampling protocols and experiments. For this, we complied with local legislation and the Convention on Biological Diversity.

Samplings, DNA preparation, PCR amplification and sequencing

Individuals were sampled from 13 localities along Pacific Patagonia (PP) and the Falkland/Malvinas Islands (FI; Fig 1). Samples from PP populations (n = 12) were collected along the southern Pacific coast of Patagonia, the Strait of Magellan and Cape Horn and included: 1) Tamar Island (52° 54' S; 73° 48' W), 2) Duntze Sound (54°19'S; 73°48'W), 3) London Island (54°40'S; 72°00'W), 4) Carlos III Island (53°39'S; 72°23'W), 5) Santa Ana Point (53°37'S; 70°54'W), 6) Carrera Point (53°35'S; 70°55'W), 7) Otway Sound (53°06'S; 71°20'W), 8) Chabunco (52°59'S; 70°48'W), 9) Laredo Bay (52°56'S; 70°47'W), 10) Possession Bay (52°13'S; 69°17'W), 11) Virginia Bay (54°54'S; 67°36'W) and 12) Paula Bay (54°56'S; 67°39'W; Fig 1). Individuals from the Falkland/Malvinas Islands (FI) were collected at Hookers Point (51°42'S; 57°46'W; Fig 1).

Whole specimens were fixed in ethanol (99%) and DNA was prepared from the mantle using a salting-out methodology [61]. A partial fragment of the mitochondrial gene Cytochrome c Oxidase Subunit I (COI) was amplified using universal primers [62]. PCR amplifications were performed in a 25 µl reaction containing 2.5 µl 10X Buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.0), 1.0 µl of 50 mM $MgCl_2$, 200 mM dNTPs, 0.5 µl of each primer (10 pg/µl), 1 U Taq polymerase (Invitrogen), 16.7 µl of double-distilled water and 50 ng of DNA. Thermal cycling parameters included an initial denaturation step at 94°C for 5 min, followed by 35 cycles at 94°C (60 sec), 48°C (60 sec), 72°C (90 sec), and a final 5 min extension at 72°C. PCR
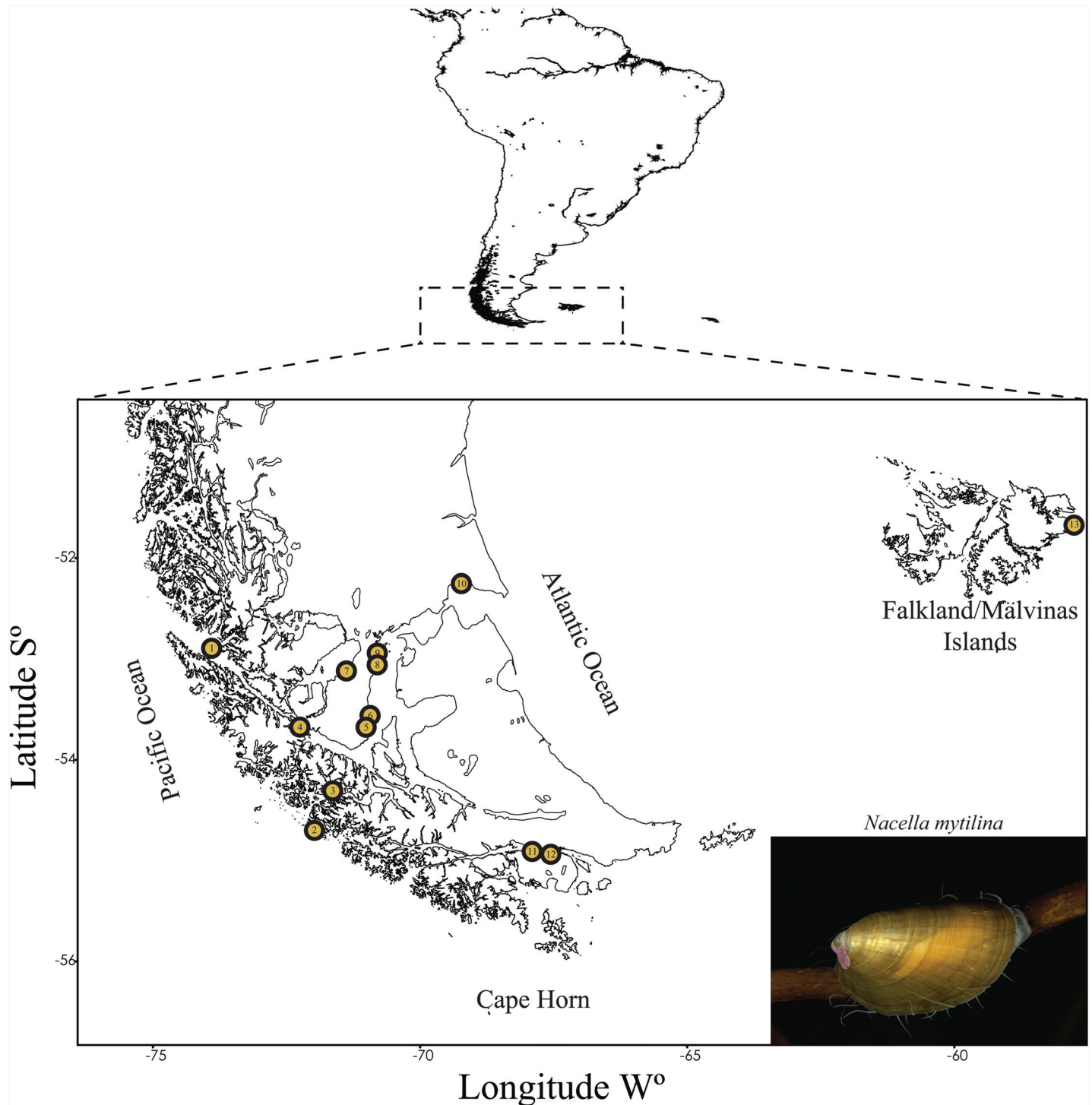
Fig 1. Sampling localities of *N. mytilina* along Pacific Patagonia (PP) and the Falkland/Malvinas Islands (FI). Shapefiles of the Patagonian coastlines available in the database of GEOdas (NOAA) and were filtered using GEOdas Coastline Extractor v. 1.1.3.1 (https://www.ngdc.noaa.gov/mgg/geodas/geodas.html). 1) Tamar Island (PP); 2) London Island (PP); 3) Duntze Sound (SM); 4) Carlos III Island (SM), 5) Santa Ana Point (SM), 6) Carrera Point (SM), 7) Otway Sound (SM), 8) Chabunco (SM), 9) Laredo Bay (SM), 10) Possession Bay (SM), 11) Virginia Bay (CH), 12) Paula Bay (CH) and 13) Hookers Point. SM = Strait of Magellan, PP = Pacific Patagonia; CH = Cape Horn; FI = Falkland/Malvinas Islands. Photograph of *N. mytilina* over *Macrocytis pyrifera* courtesy of César Cárdenas (ccardenas@inach.cl).

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amplicons were purified using QIAquick Gel Extraction Kit (QIAGEN) and sequenced in both directions with an Automatic Sequencer ABI3730 x 1 at Macrogen Inc. (Seoul, South Korea).

Genetic diversity and population structure in *N. mytilina*

The COI sequences of *Nacella mytilina* were edited using Proseq v. 3.5 [63], and aligned with ClustalW [64]. New COI sequences in *N. mytilina* were added to previous data for the species [24] and have been deposited in Genbank under the Accession Numbers KX600474—KX600492. A DNA saturation analysis following Xia and Xie [65] was performed to evaluate how saturation of transitions accumulates in relation to nucleotide divergence in the complete *N. mytilina* COI data set. Levels of genetic polymorphism were estimated using standard diversity indices, such as the number of haplotypes (k), the number of segregating sites (S), haplotypic diversity (H), the average number of pairwise differences (II), and nucleotide diversity (π) for each locality, and for the complete COI data set using DnaSP v.5.00.07 [66]. Levels of genetic differentiation between the analyzed localities were estimated following Pons & Petit [67] through main pairwise differences (N_{ST}) and haplotype frequencies (G_{ST}) in Arlequin v. 3.5 [68]. The statistical significance of genetic pairwise differences was determined using permutation tests (20 000 iterations) of haplotype identities. Similarly, we estimated the levels of genetic differentiation using the nearest-neighbor statistic (S_{nn}) that measure how often nearest neighbor's (in sequence space) sequences are from the same locality in geographic space [69]. The statistical significance of S_{nn} analyses was determined through a permutation test using 20 000 iterations.

Two different clustering methods were used to determine the spatial genetic structure of *N. mytilina*. First, we estimated the number and the composition of panmictic groups and the spatial boundaries among them using a Bayesian model computed in GENELAND v. 2.0.0 [70] in the R environment (R, version 2.4.1; [71]). This R package implements a Markov Chain Monte Carlo (MCMC) procedure to determine the best clustering of samples with regard to genetic and geographic information. The geographic information is considered at the Bayesian prior level, so that clusters corresponding to spatially structured groups are considered to be more likely than clusters that are randomly distributed in space. Analyses were run using 50 x 10^6 MCMC iterations sampled each 1000 steps. Assembled MCMC scores were graphed against generations using Tracer v.1.5 (http://beast.bio.ed.ac.uk/Tracer) to identify stationarity, and therefore determine the number of generations that must be discarded as burn-in. In the particular case of *N. mytilina* a 10% of the trees were discarded as burn-in. A maximum number of clusters (K = 14) were run to estimate the model parameters and posterior probabilities of group membership. Second, we determined the spatial genetic structure in *N. mytilina* using Arlequin [68] by estimating the number and composition of groups that were most differentiated based on sequence data with Analysis of Molecular Variance (AMOVA). This analysis uses multiple spatial scales in statistical methods to characterize spatial genetic structure by partitioning the variance: within populations, among populations within groups, and among groups. Finally, we performed a test for isolation by distance using a Mantel test with 10 000 permutations in Arlequin to determine the correlation between Slatkin's linearized localities genetic differentiation [72] and the linear geographic distance (km) between populations measured using FOSSIL [73].

Demographic inference in *N. mytilina*

Genealogical relationships in *N. mytilina* were constructed using parsimony networks in Hapview (http://www.cibiv.at/~greg/haploviewer). At the same time, we performed neutrality statistical tests (Tajima's D and Fu's F_S) using DnaSP for the whole COI data set and for each

recognized group to estimate whether sequences deviate from mutation-drift equilibrium. At the same time, population demographic histories were estimated comparing the distribution of pairwise differences between haplotypes (mismatch distribution) for each recognized group to the expected distribution under the sudden expansion growth model of Rogers & Harpending [74]. This analysis is a common phylogeographic method since the amount of nucleotide differences between haplotypes depends on the length of time since they diverged. This analysis rests in the estimation of three parameters: i) τ = the date of growth/decline measured in units of mutational time ($\tau = 2\mu t$) where t = time in years and μ = mutational rate per sequence per year, ii) initial theta $\theta_i = 2 N_i\mu$ before the population growth/decline) and iii) final theta $\theta_t = 2 N_t\mu$ after population growth/decline. The demographic expansion parameters were estimated using the nonlinear least square approach described by Schneider & Excoffier [75] implemented in Arlequin. The goodness of fit between the observed and expected mismatch distributions was tested using a parametric bootstrap approach that uses the sum of squared deviations as a statistic test implemented in Arlequin.

Gene flow and connectivity

Different models of gene flow were compared between the recorded groups in *N. mytilina* (PP and FI) to test for different scenarios using the software MIGRATE v.3.5 [76]. For this purpose, we defined four candidate models constraining the existence of directionality of gene flow between the recorded genetic groups. The first model allowed bidirectional gene flow between PP and FI (full island model). Asymmetric levels of gene flow between the recorded groups defined models 2 (from PP to FI) and 3 (from FI to PP). The last model assumed PP and FI as the same panmictic population. All the analyses were run using a HKY + I substitution model and transition-transversion ratio of 9.9401 as previously estimated by jModeltest v2 [77]. The specific substitution rate for the selected marker was set to constant, as suggested by the developer. Analysis consisted of one long chain with 500 000 recorded parameter steps, a sampling interval of 100 and a burn-in of 10%, running multiplate replicates (10 independent chains). A heated scheme (1.0, 1.5, 3.0. and 1000000.0) was used to calculate the marginal likelihoods for model comparisons. We used a thermodynamic integration approximation (T.I.) for the log-equivalent Bayes Factor (LBF) because this analysis results in LBFs with high repeatability and little variance [78]. Higher T.I. values indicate a better fit of the model than lower ones. Finally, the associated probability of each model in relation to other was estimated following Kaas and Rafery [79].

Finally, we estimated different demographic indices and evaluated two asymmetric isolation-with-migration models between Pacific Patagonia (PP) and the Falkland/Malvinas Islands (FI) using IMa2 software [80,81]. The first model: from Pacific Patagonia to the Falkland/Malvinas Island (m PP > FI) and the second one: from the Falkland/Malvinas Islands to Patagonia (m FI > PP). We carried out separately several preliminary runs in the M-mode (Markov Chain Monte Carlo; MCMC mode) of the software to determine the best set of priors that ensure mixing and convergence. Uniform priors were used to determine the effective population size (Θ1, Θ2, and ancestral Θa, Θ = 600) and splitting time (t = 30), whereas an exponential prior (mean = 20) for gene flow (m) was adopted. We performed 80 x 10^6 MCMC steps sampling every 100 generations, with a burn-in period of 10%. COI sequences in *N. mytilina* were assumed to mutate under the HKY mutation model following Hey and Nielsen [81]. Once convergence was achieved under the M-mode, we used the same simulated genealogies under the L-Mode (Load Tree mode) to determine the log maximum-likelihood and credibility intervals (95% under HPD) estimates for migration parameters using a likelihood ratio test. Under the L-Mode we compared our data with a null model of no migration [80,81].

Results

Genetic diversity and structure in *N. mytilina*

The complete COI data set in *N. mytilina* included 300 individuals and consisted of 687 nucleotide positions coding for 229 aminoacids. As expected analyzing coding regions, no insertion/deletion or stop codons were detected in the whole data set. Sequences were not saturated at any position and a single amino acid substitution (from Alanine to Valine at amino acid 182) was detected using the invertebrate mitochondrial table [82]. Low levels of genetic variability characterized *N. mytilina*, with 26 polymorphic characters (3.78%); 16 of them (61.53%) were parsimoniously informative. As previously estimated in other species of *Nacella* [24,25,56], sequences were A-T rich (60.8%) compared to the mean G-C content. Haplotype diversity (*H*) varied between 0.284 (Carlos III Island) and 0.822 (Hookers Point, FI; Table 1). The number of polymorphic sites (*S*) varied between 3 (several PP localities; Table 1) and 19 (Hookers Point, FI). Similarly, the number of haplotypes (*k*) varied between 4 (several PP localities) and 8 (Hookers Point and Laredo Bay; Table 1). The average number of nucleotide differences (*II*) and mean nucleotide diversity (*π*) were low in most localities of PP, while the diversity of these indices was comparatively higher in FI (Table 1). In fact, permutation test analyses (100,000 iterations) detected significant higher values for *II* (P = 0.00053) and *π* (0.00048) in the FI population compared to those from PP (Table 1). Finally, the number of private haplotypes (6/8) was higher in FI than in any PP locality (Table 1).

Mean general values of differentiation measured over 13 populations of *N. mytilina* were low, especially considering average $G_{ST} = 0.025$ and $N_{ST} = 0.032$. At the same time, pairwise comparisons based on N_{ST} and G_{ST} did not recognize significant structure among PP populations of *N. mytilina* from the Strait of Magellan to Cape Horn (Table 2). In contrast, G_{ST} and N_{ST} pairwise comparisons detected significant differences between the FI population and the

Table 1. Diversity indices and neutrality tests in *Nacella mytilina* along Pacific Patagonia and the Falkland/Malvinas Islands.

Locality	*n*	*k*	*H*	*S*	*II*	*π*	p.a.	Tajima´s D	Fu´s FS
Tamar Island	21	4	0419	3	0.457	0.0006	0	-1.18	-1.72
Duntze Sound	18	4	0.608	3	0.843	0.0012	0	-0.09	-0.50
London Island	25	4	0.357	4	0.600	0.0008	0	-1.17	-0.95
Carlos III Island	20	4	0.284	4	0.489	0.0007	0	-1.63*	-1.61
Carrera Bay	18	5	0.405	5	0.654	0.0009	0	-1.74*	-2.38
Santa Ana	30	4	0.409	4	0.549	0.0008	0	-1.17	-2.24
Otway Sound	18	4	0.314	3	0.314	0.0004	2	-1.71	-2.60
Chabunco	22	5	0.528	3	0.667	0.0009	1	-0.49	-2.60
Laredo Bay	28	8	0.542	8	0.765	0.0011	2	-1.93*	-5.37*
Possession Bay	23	5	0.391	5	0.585	0.0008	0	-1.66	-2.36
Virginia Bay	26	4	0.345	3	0.425	0.0006	0	-1.12	-1.70
Paula Bay	27	7	0.627	6	0.838	0.0012	2	-1.35	-3.59*
PP Total	276	13	0.435	12	0.599	0.0008	-	-1.62*	-9.64*
Hookers Point	24	8	0.822	19	4.18	0.0060	6	-0.64	0.68
Total	300	19	0.481	26	0.966	0.0014		-2.11**	-13.49***

Where: *n* = number of analyzed individuals; *k* = number of haplotypes; *S* = polymorphic sites; *H* = haplotype diversity; *II* = average number of pairwise differences; *π* = nucleotide diversity; p.a. = private haplotypes.

* p<0.05,

** p<0.01,

*** p<0.001.

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Table 2. Pairwise G_{ST} (below the diagonal) and N_{ST} (above the diagonal) values calculated among the analyzed populations of *Nacella mytilina*. 20 000 iterations. Statistical significant differences are marked in bold.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	----	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	**0.193**
2	0.000	----	0.000	0.026	0.000	0.000	0.074	0.000	0.008	0.013	0.000	0.000	**0.131**
3	0.000	0.027	----	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	**0.202**
4	0.000	0.047	0.000	----	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	**0.192**
5	0.000	0.000	0.000	0.000	----	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.175**
6	0.000	0.000	0.000	0.000	0.000	----	0.009	0.000	0.000	0.000	0.000	0.000	**0.204**
7	0.000	0.000	0.000	0.000	0.000	0.000	----	0.025	0.000	0.000	0.001	0.043	**0.201**
8	0.000	0.000	0.003	0.016	0.000	0.000	0.010	----	0.000	0.000	0.000	0.000	**0.186**
9	0.000	0.000	0.000	0.021	0.000	0.000	0.004	0.000	----	0.000	0.000	0.005	**0.206**
10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	----	0.000	0.000	**0.191**
11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	----	0.000	**0.200**
12	0.000	0.000	0.040	0.050	0.008	0.004	0.043	0.000	0.000	0.010	0.019	----	**0.159**
13	**0.141**	**0.084**	**0.197**	**0.206**	**0.148**	**0.156**	**0.186**	**0.102**	**0.103**	**0.156**	**0.177**	**0.052**	----

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rest of the PP ones ([Table 2](#)). Considering the pattern of genetic structure recorded in the species, we examined the significance of the correlation between genetic divergence measured as Slatkin's linearized F_{ST} [$Phi_{ST}/(1- Phi_{ST})$] and geographical distance among PP localities using a Mantel test implemented in Arlequin. The associated probabilities of these analyses were estimated using 25 000 permutations. We didn't detect any significant correlation between genetic and geographic distance along PP (r = 0.001; P = 0.6).

The nearest-neighbor statistic (S_{nn}) in *N. mytilina* (S_{nn} = 0.09) showed low but significant levels of phylogeographic signal (P < 0.00001) for the whole COI data set. However, when this analysis was performed considering the pattern of genetic structure recorded in the species (PP vs FI) S_{nn} became very high (Snn = 0.9) showing the elevated degree of phylogeographic signal recorded between these two areas. This pattern of genetic structure was further supported by the model based on Bayesian clustering algorithm, which detected two main clusters (K = 2). The first cluster includes all the PP localities from the Strait of Magellan to Cape Horn ([Fig 2A](#)) while the second one included the individuals from FI ([Fig 2B](#)). Values of cluster membership were high for all localities (c.a. P = 0.9). The mean probability value (P = 0.5) corresponds to the boundary between the clusters run between Pacific Patagonia and the Falkland/Malvinas Islands ([Fig 2](#)). Similarly, AMOVA analyses detected two maximally differentiated groups accounting for 43.09% of the total variance, and only 1.17% was due to within-group variation among PP localities ([Table 3](#)). These groups were 1) Hookers Point, FI, and 2) the rest of the localities from Pacific Patagonia.

Demographic inference in *N. mytilina*

Parsimony network of *N. mytilina* recorded a total of 19 different haplotypes and showed a typical star-like topology and a very short genealogy ([Fig 3A](#)). A central haplotype (H01) was the most frequent one (71.33%) and distributed at all localities ([Fig 3A](#)). Following Posada and Crandall [83], this dominant haplotype should represent the most ancestral one, whereas the most derived one is related to it with a maximum branch length of 8 mutational steps (H19 from FI). A second haplotype (H02) of intermediate frequency (10%), separated by a single mutational step from H01, was also recorded in all the analyzed localities along PP and FI ([Fig 3A](#)). Four haplotypes (H03, H04, H05, and H06) were recorded in more than three individuals belonging to different PP localities ([Fig 3A](#)). A total of 10 singletons were recorded in the
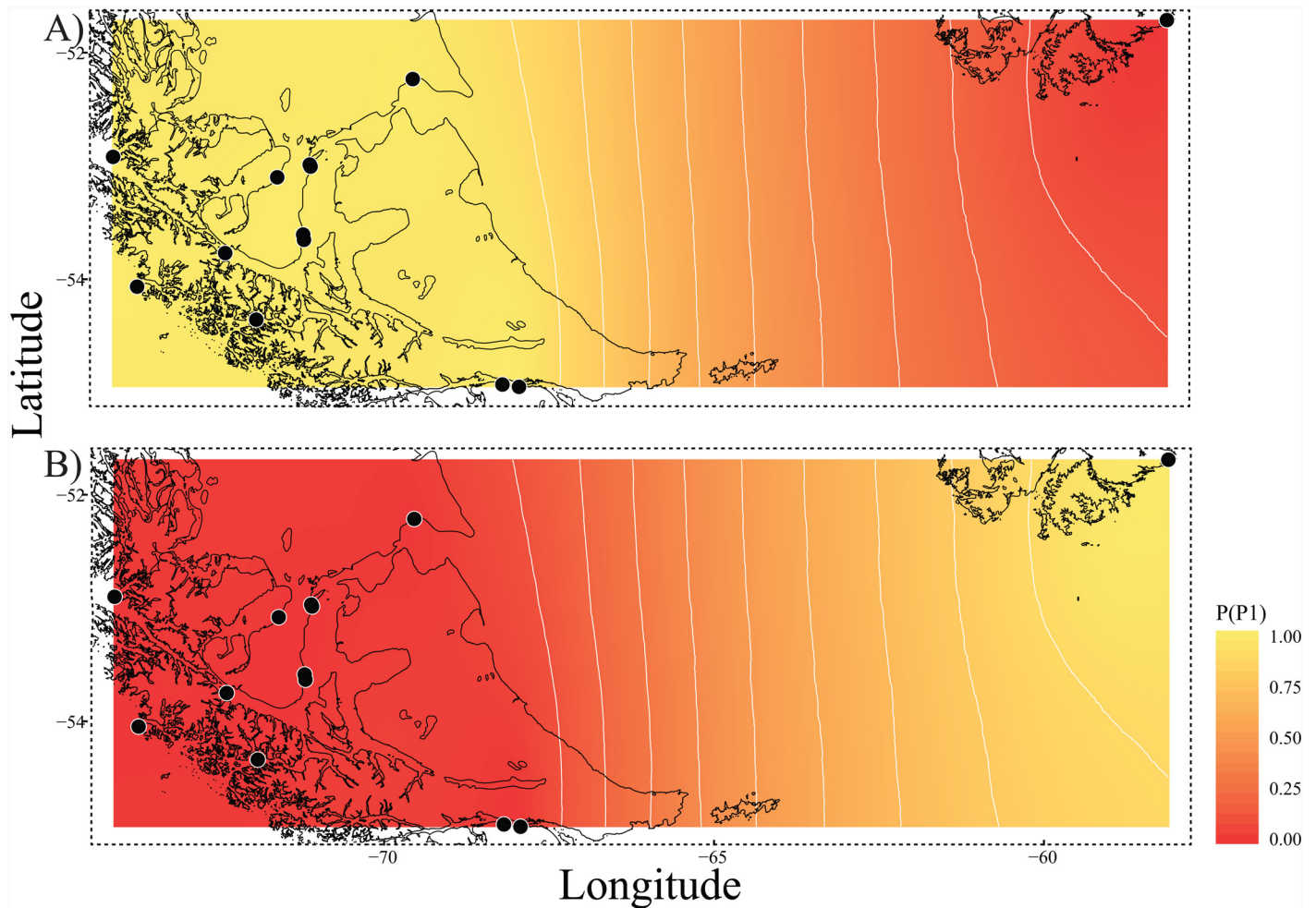
Fig 2. Spatial output from Geneland using all 13 *N. mytilina* populations. Black circles indicate the relative position of the sampling localities. Darker and lighter shading are proportional to posterior probabilities of membership to clusters, with lighter (yellow) areas showing the highest probabilities of clusters. Posterior probabilities of membership were plotted to the *shapefiles* of the Patagonian coastline available in the database of GEOdas (NOAA) and filtered using GEOdas Coastline Extractor v 1.1.3.1 (https://www.ngdc.noaa.gov/mgg/geodas/geodas.html).

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Table 3. Analysis of Molecular Variance (AMOVA) depicting the percentage of variation explained among groups (Pacific Patagonia and Falkland/Malvinas Islands), among populations within groups, and within populations. F_{SC}: differentiation within populations among groups; F_{CT}: differentiation among groups (** p<0.01, *** p<0.001).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	14.645	0.32492 Va	43.09
Among populations within groups	11	2.455	0.00881 Vb	1.17
Within populations	305	133.567	0.43792 Vc	58.08
Total	317	150.667	0.75404	

Fixation Index

F_{SC}: 0.02052 ***

F_{CT}: 0.43091 ***

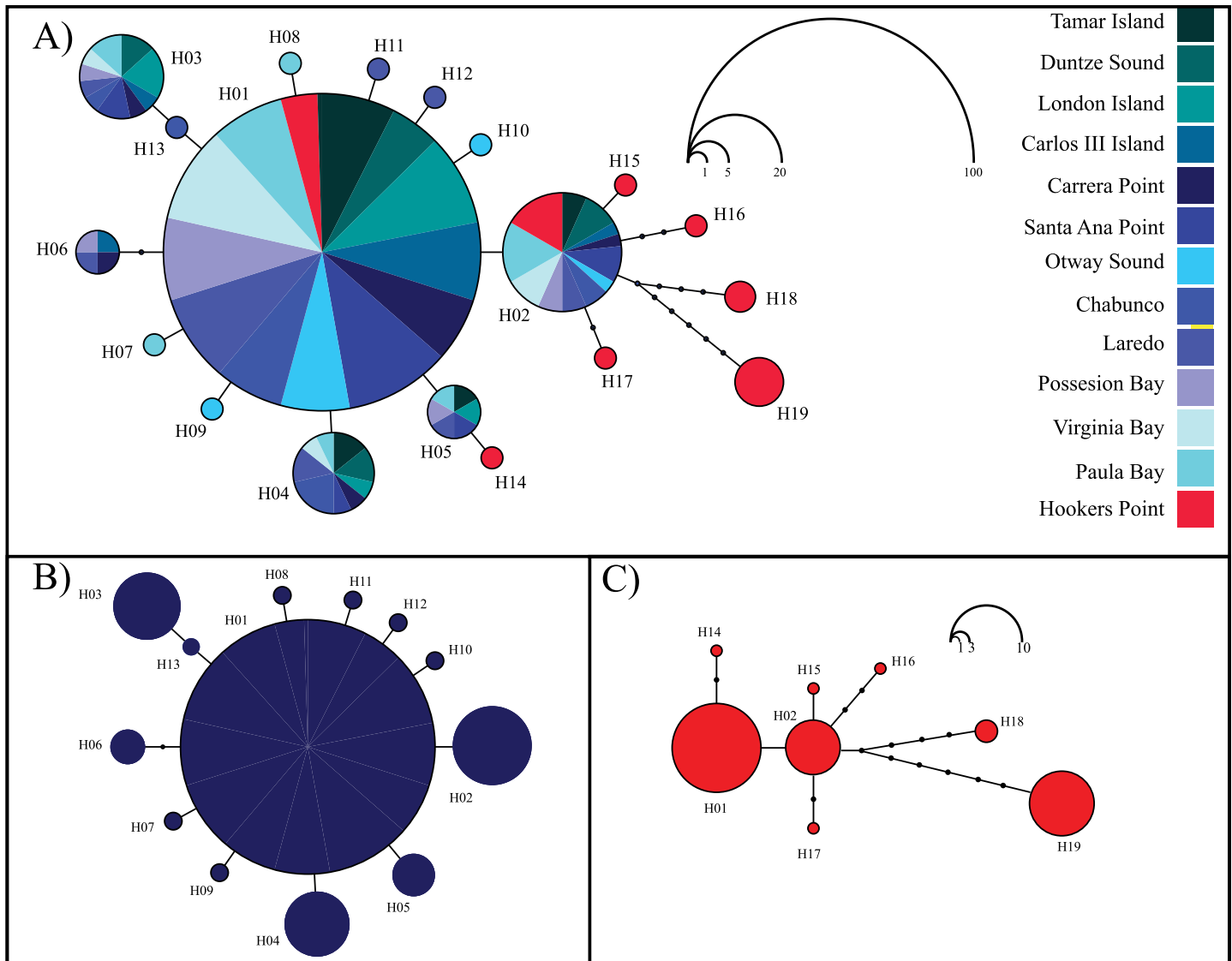doi:10.1371/journal.pone.0161963.t003

Fig 3. A) Parsimony haplotype network including 300 individuals of *Nacella mytilina* mtDNA COI sequences from 13 localities along PP and FI. Each haplotype is represented by a colored circle indicating the locality where it was collected. Circles sizes are proportional to the frequency of the haplotype in the whole sampling effort. B) General maximum parsimony haplotype network including PP populations. C) General maximum parsimony network in Hookers Point, FI. The size of the haplotypes circles (B and C) is proportional to the their frequency.

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whole data set, six of them were recorded in PP and the rest in FI ([Fig 3](#)). Despite the high frequency of H01 in PP (>70%), that haplotype was present in only 33% of the sampled individuals from FI. Several haplotypes from FI (H14, H15, H16, and H17) were closely related to and even shared (H01 and H02) with the Patagonian diversity. Finally, two haplotypes of medium frequency recorded in FI (H18 and H19) are endemic to these islands and separated from H01 by five (H18) and seven (H19) mutational steps ([Fig 3A](#)). As expected for star-like genealogies, global Tajima's *D* and Fu's *F* neutrality tests were both negative and significant for the whole COI data set ([Table 1](#)). However, these neutrality tests showed contrasting results between the recorded genetic groups (PP and FI). Across PP, Tajima's *D* and Fu's *F* were negative and significant while both indices were not significant in FI ([Table 1](#)). Similarly, the distribution of pairwise differences varied considerable among the recognized genetic groups in *N. mytilina*.

The mismatch distribution in PP was L-shaped ([Fig 4A]) and in FI it had a multimodal distribution ([Fig 4B]).

Sudden growth model analyses based on a 10% mutational rate detected that population expansion in PP occurred approximately 7.5 ka. The presence of a multimodal mismatch distribution in FI ([Fig 4B]), evidenced by the haplotype genealogy of this population and the absence of good-fit between the observed and expected distribution precluded the analysis of population expansion in this locality.

Gene flow and connectivity

Gene flow analyses using MIGRATE and different migration models detected evidence of asymmetrical gene flow from PP to FI. Among the tested models the one from PP to FI received the highest probability ([Table 4]). Similarly, the coalescent approach of isolation-with-migration implemented in IMa2 detected overall medium levels of gene flow between PP and FI ([Table 5]). The model predicted a most likely pattern of asymmetric migration with highly significant migration from PP to FI (LRT, 40.723, [Fig 5A]), and no migration between FI to PP (LTR, 0.000, [Fig 5B]).

Discussion

Climate during the Quaternary is characterized by the alternation between glacial and interglacial periods commonly known as the Ice Ages [1,9]. Near-shore marine benthic communities at higher latitudes would have been particularly vulnerable to the advances and retreats of continental ice sheets [84,85]. Radical glacial landscape and seascape shifts along the Magellan fjords resulted in the periodic local and temporal elimination of the associated fauna of these ecosystems [86,87] allowing the later colonization of vacant niches, as well as creating opportunities for geographical isolation and speciation [3,7,24,88]. The availability of discrete refugia may have enabled taxa to survive repeated glacial advances. Consequently, fragmentation and isolation into discrete refugia could have favored the diversification of species in the Magellan fjords [86,89]. There is a higher proportion of congeneric species south of 42°S (3.3 species per genus), than along the Central coast of Chile (1.6 species per genus). Such results suggest that the increased Patagonian diversity was generated by local radiation rather than by recent colonization from other areas as observed in New Zealand and Antarctica [55,86,89]. In this context, molecular-based genetic approaches have become fundamental to further understand and unravel the role of Quaternary glacial episodes over the distribution and demography of populations, species, and communities [1–3,5,90–93]. Mitochondrial DNA has been especially popular and represents a marker of choice in numerous phylogeographic, population genetic, and molecular taxonomy studies. Main advantages in the use these markers are: i) easy of amplification, ii) the haploid nature of the mitochondrion makes the identification of haplotypes straightforward, and iii) generally exhibit higher mutation rates compared with nuclear sequences. However, mitochondrial history does not always reflect the true history of species being studied and is often incongruent with nuclear data. Difference between the observed patterns of mitochondrial and nuclear gene variation are the consequence of introgression and incomplete lineage sorting. In spite of these caveats, and as previously stated mitochondrial DNA markers remained the marker of choice for reconstructing historical patterns of population demography, admixture, biogeography and speciation.

Main patterns of genetic diversity and structure in *N. mytilina*

Levels of mtDNA genetic diversity in *N. mytilina* are lower than those recorded in tropical and temperate patellogastropods [94,95] and in other Patagonian nacellid limpets [20,24,25]. In
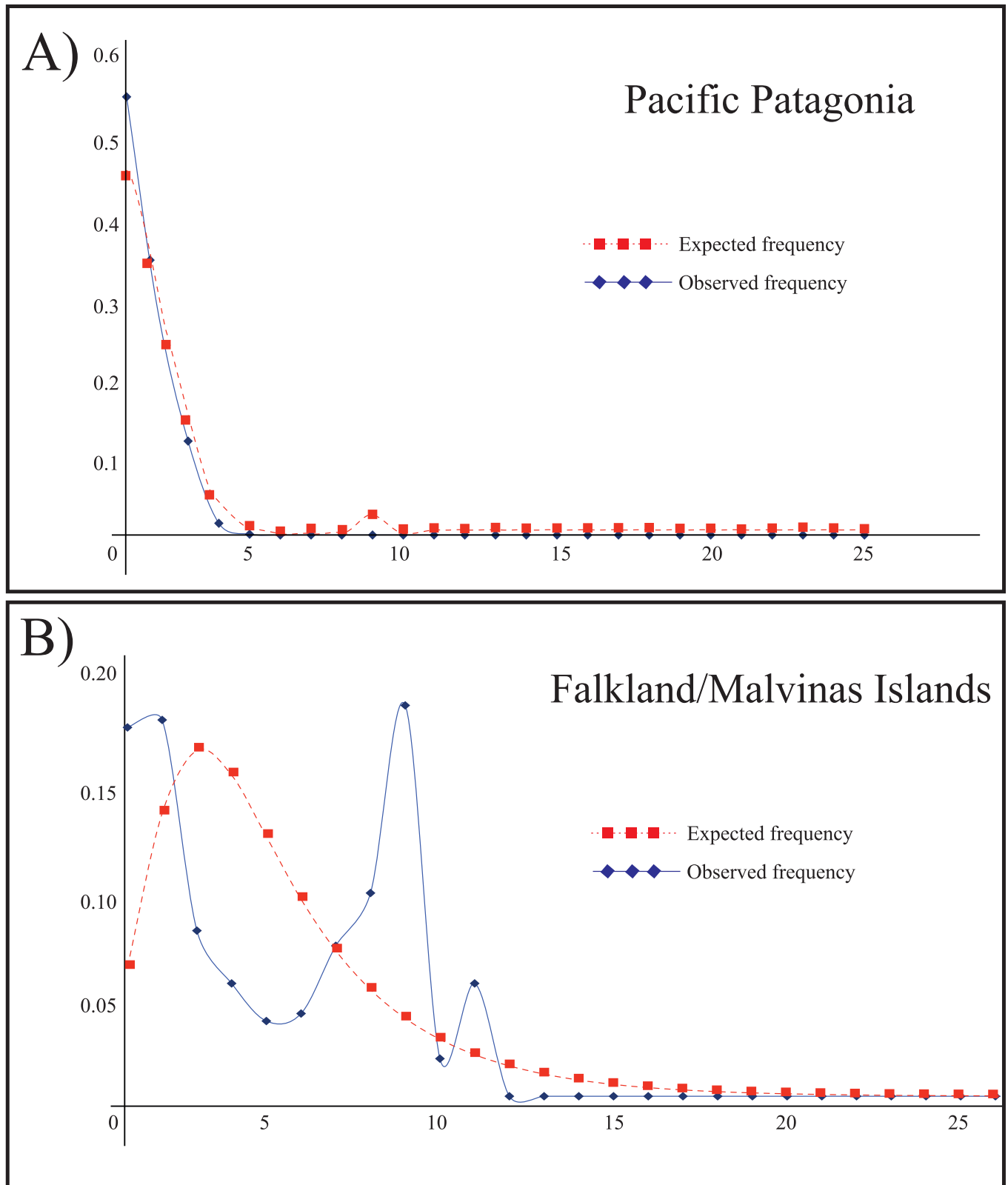
Fig 4. Pairwise difference distribution for the COI gene in *N. mytilina* populations from A) Pacific Patagonia (PP) and B) Falkland/Malvinas Islands (FI). X-axis = Pairwise differences and y-axis = frequency.

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Table 4. Thermodynamic integration (T.I.) and log Bayes factor (LBF) comparisons for different migration models between Pacific Patagonia (PP) and the Falkland/Malvinas Islands (FI) in *Nacella mytilina*.

Model	T.I.	LBF	Model prob	Model rank
1. full migration	-1514.834	-30.194	<0.001	2
2. PP to FI	-1499.737	0	1	1
3. FI to PP	-1515.015	-30.555	<0.001	3
4. panmixia	-1545.644	-91.813	<0.001	4

fact, levels of genetic polymorphism in *N. mytilina* are lower than those registered in the Antarctic limpet *N. concinna* [96,97]. In spite of these results, main patterns of genetic diversity in *N. mytilina* are similar to those found in other Subantarctic nacellid limpets from New Zealand [95,98] and Marion Island [99]. Extremely low levels of genetic diversity in *N. mytilina* along PP could be a consequence of the drastic demographic effects during Quaternary glacial processes on the instraspecific variation of the species, as well as particular characteristics of the species, including its strong habitat dependency on macroalgae. Considering that post-glacial establishment of *N. mytilina* populations required the previous colonization of macroalgae (i.e. *Macrocystis*, *Lessonia*, *Gigartina*), the expansion process in *N. mytilina* would have been hampered or delayed. As observed in *N. mytilina*, molecular phylogeographic analyses in Patagonian populations of macroalgae including *Macrocystis pyrifera* (n = 144) [48] and *G. skottsbergii* (n = 166) [100] recorded very low levels of genetic diversity and shallow genealogies (compared to other macroalgae species). Similarly, and as observed across *N. mytilina*'s distribution in PP, both *Macrocystis* and *Gigartina*, exhibited the presence of a broadly distributed dominant haplotype and very low levels of genetic diversity suggesting a recent expansion processes in Patagonia. In the case of *M. pyrifera* the single Patagonian haplotype was also detected in several geographically distant subantarctic islands (i.e. South Georgia, Marion and Macquarie) suggesting a very recent colonization of the subantarctic region. However, in the Falkland/Malvinas Islands (n = 4), *M. pyrifera* individuals carried a different haplotype separated by a single substitution from the Patagonian one. In the particular case of *S. skottsbergi*, even when the dominant PP haplotype was also highly abundant (> 70%) in FI, no population level analyses were performed in this species in order to evaluate population differentiation [100]. Considering these results in Patagonian kelps, there is a degree of resemblence between the

Table 5. Estimates of migrations rates (m) in each direction and genetic diversities (Θ), based on the isolation-with-migration model implemented in IMa2.

Value	Θ_A	Θ_{PP}	Θ_{FI}	$m_{FI \to PP}$	$m_{PP \to FI}$
HiPt	5.1	36.9	16.5	0.01	1.67
Mean	5.743	171.5	295.6	1.152	2.655
95%Lo	1.5	20.1	13.5	0.01	0.79
95%Hi	11.1	558.3	584.7	6.55	6.65
HPD95Lo	1.5	9.3	0		
HPD95Hi	12.9	519.3	568.5		
LRT				0.000ns	40.723***

For each parameter, the high point (HP), mean and 95% highest posterior density (95% HPD) of the marginal posterior probabilities are shown. Significant m values of the LRT are marked with asterisks:
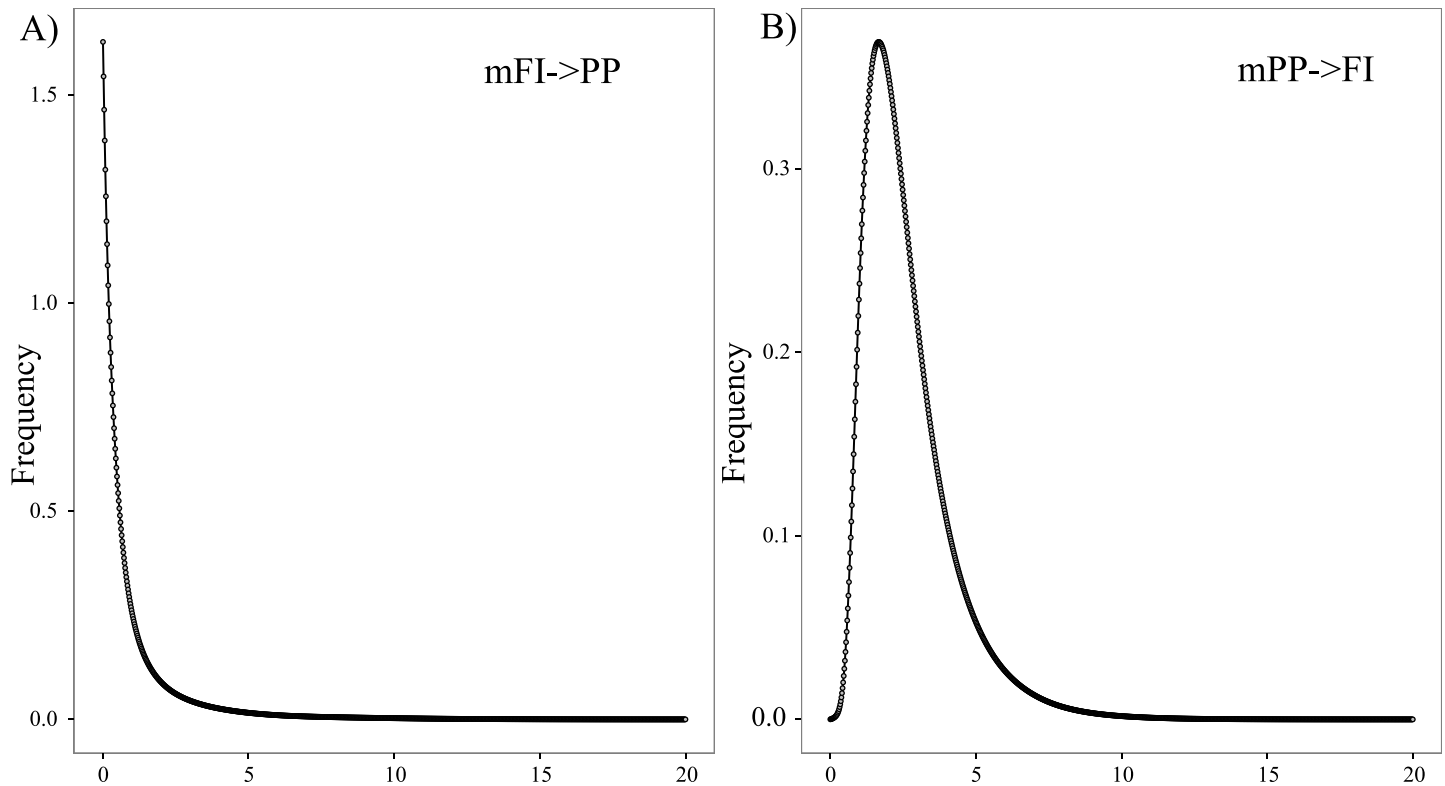
* p<0.05;

** p<0.01;

*** p<0.001.

Fig 5. Marginal posterior probability distribution (frequency) of migration rate estimates in each direction for *N. mytilina* using IMa2. a) Migration pattern estimations from Falkland/Malvinas Islands (FI) to Pacific Patagonia (PP). B) Migration pattern estimations from PP to FI.

phylogeography of the limpet and the macroalgae where it occurs. Following this, and according to Macaya and Zuccarello [48] the high dispersal potential of kelps rafts may help to explain the absence of genetic differentiation across PP and the low genetic diversity observed in *N. mytilina*. At the same time, several studies have demonstrated that kelp-specialized Subantarctic invertebrates show very low levels of genetic diversity and structure along their distribution and also across geographically distant areas [101,102]. Such an hypothesis is supported by the comparative higher levels of COI genetic diversity recorded in other rocky Patagonian species of *Nacella* [20,21,24,25]. General molecular diversity indices in *N. mytilina* along PP ($\Theta k = 0.89$; $\Theta S = 1.15$; $\Theta H = 0.90$; $\Theta \pi = 1.00$) would be sustained by effective population sizes (N_e) between 73.500 and 148.000 individuals. These estimates are lower than those recorded for other Patagonian *Nacella* species [25] and by direct observations and estimates for the species [60]. Low levels of genetic diversity together with the presence of a dominant haplotype broadly distributed are consistent with the hypothesis of a recent range expansion along PP [74,103] and high levels of migration along its distribution range [3,92]. Furthermore, significant negative Tajima's D and Fu's F_S indices and the presence of L-shaped mismatch distribution in PP are the result of an excess of low frequency haplotypes commonly explained by a very recent demographic expansion process. Population expansion along PP would have occurred ~ 7.5 ka according to the sudden growth model and this estimate is consistent with analyses of other Patagonian species of *Nacella* [20,25], as well as with thermal records of warmer conditions in Patagonia. According to Hein et al. [17] the timing of the LGM extent and the onset of deglaciation occurred almost synchronously across Patagonia. In northern areas of Patagonia the warming process started at 17.5 ka [104]. Major and rapid warming in

the Strait of Magellan occurred between 14–10 ka [14,15,17,18,105]. Pollen starigraphic analyses in the Beagle Channel and Tierra del Fuego indicate that the disappearance of ice in the southern tip of South America occurred ~ 11 ka [14,15,17,106]. A postglacial molluscan faunal expansion in the Beagle Channel occurred after the full recession of glaciers around 10 ka. In fact, fossil assemblages records the diversification of different taxa, including *Nacella*, beginning at this time [107–110]. In contrast, the FI population of *N. mytilina* showed higher levels of genetic diversity comparable to those recorded for *N. magellanica* and *N. deaurata* in the Atlantic [20,21] and Pacific [24,25] Patagonia.

In spite of the geographical and oceanographical complexity along the southern tip of South America we recorded a single genetic unit in *N. mytilina* along PP from the Strait of Magellan to Cape Horn. For benthic species the primary dispersal phase is associated with the earliest life history stages (spore, egg, or larva) and therefore considerable focus is placed on the processes that influence developmental modes, type of larvae, larval development and lifespan [92,111]. Hence, the duration of these dispersal stages is expected to correlate with the dispersal ability and with the genetic structure of the organism [112–114]. Absence of genetic structure along PP has been registered in several Patagonian marine organisms with high dispersal potential including invertebrates [25,115–117] fishes [32,35,118], and macroalgae [46,48,100,119]. Regretfully, no direct information about larval duration in Patagonian species of *Nacella* exist but it is expected that their development should be similar to the Antarctic relative *N. concinna* [52–54]. Considering the effect of temperature on development and metabolism [120,121] it would be expected that *N. mytilina* larval lifespan could extend for at least six weeks. Gene flow mediated by larval dispersal and/or rafting in this kelp-dweller species may have been enhanced by oceanographic conditions in southern South America. Off southern Chile, the West Wind Drift divides in two major branches: i) the Peru or Humboldt Current that flows northward and transports subantarctic water of relatively low temperature and salinity towards lower latitudes and ii) the Cape Horn Current that flows southward around Cape Horn [122,123]. As expected under the general circulation pattern in Patagonia we recorded an absence of genetic differentiation among *N. mytilina* populations across Pacific Patagonia. Strong genetic differentiation between PP and FI recorded in *N. mytilina* supports a recent phylogeographic study in the sister-species *N. magellanica* [25] and in isopods of the genus *Serolis* [124] recognizing both areas as different genetic units. At the same time, FI population of *N. mytilina* showed higher levels of genetic diversity than PP ones that are comparable to those recorded for *N. magellanica* and *N. deaurata* populations from the Atlantic [20,21] and the Pacific [24,25] coasts of Patagonia. As stated by Leese et al. [124], while the fauna of the FI has often been accepted to share most of their faunal inventory with Patagonia, molecular analyses are indicating that shallow benthic species may in fact be strongly differentiated populations or even reproductively isolated species. Similarly, in the case of *M. pyrifera* even when the single Patagonian haplotype was also detected in different geographically distant subantarctic islands (i.e. South Georgia, Marion and Macquarie) it was absent in the Falkland/Malvinas Islands (n = 4). *Macrocystis pyrifera* individuals from FI carried a different haplotype separated by a single substitution from the Patagonian one. In the particular case of *S. skottsbergi*, even when the dominant PP haplotype is highly abundant (> 70%) in FI, no population level analyses were performed in this species in order to evaluate population differentiation [100]. Marked differences in terms of diversity, structure, and demography between PP and FI populations may be the consequence of their distinct glaciological histories during the coldest glacial periods. On the one hand, PP was almost completely covered by ice during the LGM and thus shallow marine habitats should have been severely reduced throughout this area. Accordingly, a marked decline in genetic diversity through bottleneck processes or founder effects followed by population expansion is expected [103]. Evidence of such demographic processes has been

recorded in several Patagonian near-shore marine species [20,24,25,34,111–116,125,126]. In contrast, little evidence of LGM ice apart from the small cirques and short (max. 2.7 km) glacially eroded valleys are recorded in the Falkland/Malvinas Islands [14,127,128]. Moreover, an absence of widespread LGM glaciation at altitude in these islands is supported by cosmogenic isotope (^{10}Be and ^{26}Al) surface exposure dates [129]. Studies of Quaternary environments [130,131] provide no evidence of LGM glaciation beyond the cirques and small glaciers and there is no study showing evidence for LGM glaciers extending offshore [131]. Following this scenario, near-shore marine benthic habitats along PP would have been more hampered during LGM than in the FI, as suggested by levels of genetic diversity, haplotype genealogies, significant negative neutrality tests and L-shaped mismatch distribution. In contrast, the FI population showed deviation from the mutation-drift equilibrium model and exhibited multimodal mismatch distribution, in conformity with the expectation for more stable populations. Major differences between PP and FI in terms of habitat availability has been recognized in comparative biodiversity analyses [132].

Source-sink model as consequence of asymmetrical gene flow

Traditional genetic models of postglacial recolonization and refugia include the prediction that formerly ice-covered areas should exhibit low levels of genetic diversity and a small number of haplotypes dominating large areas [1]. Simultaneously, recolonized areas should also exhibit low divergence among haplotypes and lower levels of genetic structure than refugial ones. Following our results, and considering this general Quaternary genetic model [1–3], FI could be then considered as a glacial refugium for *N. mytilina*. In fact, these islands have been previously proposed as refugial areas for several plant species during the LGM [133] and as an important area for conservation [134]. MtDNA data in *N. concinna*, as well as in its sister-species *N. magellanica*, support the resiliency of some near-shore marine benthic invertebrates in FI during the LGM. At the same time, our migration analyses in *N. mytilina* do not support an scenario of posterior colonization from FI to PP.

An asymmetrical dispersal pattern from PP to FI recorded in *N. mytilina* is strongly supported by the general oceanographic circulation in the study area. After rounding the southern tip of South America, the Cape Horn Current is divided into two minor branches. The first one flows eastward to the South Georgia [122,123] while the second one flows northwards on both sides of the Falkland/Malvinas Islands up north to Rio de la Plata Province (30°S) following the continental shelf margin [135,136]. As previously proposed for another Patagonian nacellid limpet [25], FI seems to represent a sink area, as well as a secondary contact zone where endemic haplotypes that survived the LGM are mixed together with recently arrived PP ones. Future studies in Patagonian near-shore marine benthic invertebrates will require to integrate mtDNA/nucDNA genes together with fast-evolving markers (microsatellites, RADseq, SNPs) to further understand the role of the Quaternary cycles over the current patterns of genetic diversity, structure and connectivity. At the same time, it is also required to expand the sampling effort toward areas of difficult access that have been neglected in previous studies.

Main Conclusions

Molecular-based phylogeographic analyses in *N. mytilina* indicate that historical and contemporary processes, as well as life-history traits played a major role in the current pattern of genetic diversity, structure, and connectivity. This study corroborates previous analyses in the genus showing the important role of oceanography in explaining the asymmetry in gene flow across the southern tip of South America. Even in the presence of gene flow from PP to FI, the migration between both areas is not enough to homogenize populations and they constitute

different genetic units. According to the general pattern of genetic diversity, neutrality tests and demographic inference analyses, *N. mytilina* populations in FI are older than those from PP. However, haplotype identities and gene flow analyses indicate that FI did not participate in the postglacial recolonization of PP. In this context, more studies in other shallow marine benthic organisms are required to determine if the pattern recorded in two species of *Nacella* constitute a general rule of the Quaternary biogeography of southern South America.

Acknowledgments

Author Contributions

Conceived and designed the experiments: CAG-W EP.

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Analyzed the data: CAG-W EP MH NIS.

Contributed reagents/materials/analysis tools: CAG-W EP AM KG SR AD PB MH.

Wrote the paper: CAG-W NIS MH EP.

References

1. Provan J, Bennett KD (2008) Phylogeographic insights into cryptic glacial refugia. Trends Ecol Evol 23: 564–571. doi: 10.1016/j.tree.2008.06.010 PMID: 18722689

2. Maggs CA, Castihlo R, Foltz D, Henzler C, Jolly MT, Kelly J, et al. (2008) Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. Ecology 89: S108–S122. PMID: 19097488

3. Marko PB, Hoffman JM, Emme SA, McGovern TM, Keever CC, Cox LN (2010) The "Expansion-Contraction" model of Pleistocene biogeography: rocky shores suffer a sea change? Mol Ecol 19: 146–169. PMID: 20092033

4. Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature 405: 907–913. PMID: 10879524

5. Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Philos T R Soc B 359: 183–195.

6. Wares JP, Cunningham CW (2001) Phylogeography and historical ecology of the North Atlantic intertidal. Ecology 55: 2455–2469.

7. Allcock AL, Strugnell JM (2012) Southern Ocean diversity: new paradigms from molecular ecology. Trends Ecol Evol 27: 520–528. doi: 10.1016/j.tree.2012.05.009 PMID: 22727016

8. Bennett KD, Tzedakis PC, Willis KJ (1991) Quaternary refugia of North European trees. J Biogeogr 18: 103–115.

9. Webb T III, Bartlein PJ (1992) Global changes during the last 3 million years—climatic controls and biotic responses. Ann Rev Ecol Evol S 23: 141–173.

10. Taberlet P, Fumagalli L, Wust-Saucy A-G, Cosson J-F (1998) Comparative phylogeography and post-glacial colonization routes in Europe. Mol Ecol 7: 453–464. PMID: 9628000

11. Hewitt GM (1999) Post-glacial re-colonization of European biota. Biol J Linn Soc 68: 87–112.

12. Excoffier L, Foll M, Petit RJ (2009) Genetic consequences of range expansions. Annu Rev Ecol Evol S 40: 481–501.

13. Fraser CI, Nikula R, Ruzzante DE, Waters JM (2012) Poleward bound: biological impacts of Southern Hemisphere glaciation. Trends Ecol Evol 27: 462–471. doi: 10.1016/j.tree.2012.04.011 PMID: 22658874

14. Clapperton CM (1994) The quaternary glaciation of Chile: a review. Rev Chil Hist Nat 67: 369–383.

15. Hulton NRJ, Purves RS, McCulloch RD, Sugden DE, Bentley MJ (2002) The last glacial maximum and deglaciation in southern South America. Quat Sci Rev 21: 233–241.

16. Rabassa J, Coronato AM, Salemme M (2005) Chronology of the Late Cenozoic Patagonian glaciations and their correlation with biostratigraphic units of the Pampean region (Argentina). J S Am Earth Sci 20: 81–103.

17. Hein AS, Hulton NRJ, Dunai TJ, Sugden DE, Kaplan MR, Xu S (2010) The chronology of the Last Glacial Maximum and deglacial events in central Argentine Patagonia. Quat Sci Rev 29: 1212–1227.

18. 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. J Quat Sci 15: 409–417.

19. Denton GH, Anderson RF, Toggweiler JR, Edwards RL, Schaefer JM, Putnam AR (2010) The Last Glacial Termination. Science 328: 1652–1656. doi: 10.1126/science.1184119 PMID: 20576882

20. De Aranzamendi MC, Gardenal CN, Martin JP, Bastida R (2009) Limpets of the genus *Nacella* (Patellogastropoda) from the Southwestern Atlantic: species identification based on molecular data. J Mollus Stud 75: 241–251.

21. De Aranzamendi MC, Bastidas R, Gardenal C (2011) Different evolutionary histories in two sympatric limpets of the genus *Nacella* (Patellogastropoda) in the South-western Atlantic coast. Mar Biol 158: 2405–2418.

22. De Aranzamendi M, Bastida R, Gardenal CN (2014) Genetic population structure in *Nacella magellanica*: Evidence of rapid range expansion throughout the entire species distribution on the Atlantic coast. J Exp Mar Biol Ecol 460: 53–61.

23. Xu J, Pérez-Lozada M, Jara CG, Crandall KA (2009) Pleistocene glaciation leaves deep signature on the freshwater crab *Aegla alacalufiin* Chilean Patagonia. Mol Ecol 18: 904–918. doi: 10.1111/j.1365-294X.2008.04070.x PMID: 19207249

24. González-Wevar CA, Nakano T, Cañete JI, Poulin E (2011) Concerted genetic, morphological and ecological diversification in *Nacella* limpets in the Magellanic Province. Mol Ecol 20: 1936–1951. doi: 10.1111/j.1365-294X.2011.05065.x PMID: 21418364

25. González-Wevar CA, Hüne M, Cañete JI, Mansilla A, Nakano T, Poulin E (2012) Towards a model of postglacial biogeography in shallow marine species along the Patagonian Province: lessons from the limpet *Nacella magellanica* (Gmelin, 1791). BMC Evol Biol 12: 139. doi: 10.1186/1471-2148-12-139 PMID: 22871029

26. Ruzzante DE, Walde SJ, Cussac VE, Dalebout ML, Seibert J, Ortubay S, et al. (2006) Phylogeography of the Percichthyidae (Pisces) in Patagonia: roles of orogeny, glaciation, and volcanism. Mol Ecol 15: 2949–2968. PMID: 16911213

27. Ruzzante DE, Walde SJ, Gosse JC, Cussac VE, Habit EM, Zemlak TS, et al. (2008) Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. Mol Ecol 17: 2234–2244. doi: 10.1111/j.1365-294X.2008.03738.x PMID: 18363661

28. 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. Biol J Linn Soc 103: 514–529.

29. Zemlak TS, Habit EM, Walde SJ, Battini MA, Adams EDM, Ruzzante DE (2008) Across the southern Andes on fin: glacial refugia, drainage reversals and a secondary contact zone revealed by the phylogeographical signal of *Galaxias platei* in Patagonia. Mol Ecol 17: 5049–5061. doi: 10.1111/j.1365-294X.2008.03987.x PMID: 19017262

30. Zemlak TS, Habit EM, Walde SJ, Carrea C, Ruzzante DE (2010) Surviving historical Patagonian landscapes and climate: molecular insights from *Galaxias maculatus*. BMC Evol Biol 10: 67. doi: 10.1186/1471-2148-10-67 PMID: 20211014

31. Zemlak TS, Walde SJ, Habit EM, Ruzzante DE (2011) Climate-induced changes to the ancestral population size of two Patagonian galaxiids: the influence of glacial cycling. Mol Ecol 20: 5280–5294. doi: 10.1111/j.1365-294X.2011.05352.x PMID: 22077139

32. Ceballos SG, Lessa EP, Victorio MF, Fernández DA (2012) Phylogeography of the sub-Antarctic notothenioid fish *Eleginops maclovinus*: evidence of population expansion. Mar Biol 159: 499–505.

33. Ceballos SG, Lessa EP, Licandeo R, Fernández DA (2016) Genetic relationships between Atlantic and Pacific populations of the notothenioid fish *Eleginops maclovinus*: the footprints of Quaternary glaciations in Patagonia. Heredity 116: 372–377. doi: 10.1038/hdy.2015.106 PMID: 26696136

34. González-Wevar CA, Salinas P, Hüne M, Segovia NI, Vargas-Chacoff L, Astorga M, et al. (2015) Phylogeography in *Galaxias maculatus* (Jenyns, 1848) along two biogeographical provinces in the Chilean coast. PLoS ONE 10: e0131289. doi: 10.1371/journal.pone.0131289 PMID: 26161896

35. Hüne M, González-Wevar CA, Poulin E, Mansilla A, Fernández DA, Barrera-Oro E (2015) Low levels of genetic divergence between *Harpagifer* fish species (Perciformes: Notothenioidei) suggests a Quaternary colonization of Patagonia from the Antarctic Peninsula. Polar Biol 38: 607–617.

36. Victoriano PF, Ortiz JC, Benavides E, Adams BJ, Sites JW Jr (2008) Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata: Tropiduridae) from the central-southern Andean range. Mol Ecol 17: 2397–2416. doi: 10.1111/j.1365-294X.2008.03741.x PMID: 18430148

37. Vidal MA, Moreno PI, Poulin E (2011) Genetic diversity and insular colonization of *Liolaemus pictus* (Squamata, Liolaeminae) in north-western Patagonia. Austral Ecol 37: 67–77.

38. Nuñez JJ, Wood NK, Rabanal FE, Fontanella FM, Sites JW Jr (2011) Amphibian phylogeography in the Antipodes: Refugia and postglacial colonization explain mitochondrial haplotype distribution in the Patagonian frog *Eupsophus calcaratus* (Cycloramphidae). Mol Phylogenet Evol 58: 343–352. doi: 10.1016/j.ympev.2010.11.026 PMID: 21145400

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

40. Lessa EP, D'Elía G, Pardiñas UFJ (2010) Genetic footprints of late Quaternary climate change in the diversity of Patagonian-Fueguian rodents. Mol Ecol 19: 3031–3037. doi: 10.1111/j.1365-294X.2010.04734.x PMID: 20618900

41. Pardiñas UFJ, Teta P, D'Elía G, Lessa EP (2011) The evolutionary history of sigmodontine rodents in Patagonia and Tierra del Fuego. Biol J Linn Soc 103: 495–513.

42. Palma RE, Boric-Bargetto D, Torres-Pérez F, Hernández CE, Yates TL (2012) Glaciation effects on the phylogeographic structure of *Oligoryzomys longicaudatus* (Rodentia: Sigmodontinae) in the Southern Andes. PLoS ONE 7: e32206. doi: 10.1371/journal.pone.0032206 PMID: 22396751

43. Marin JC, González BA, Poulin E, Casey CS, Johnson WE (2012) The influence of the arid Andean high plateau on the phylogeography and population genetics of guanaco (*Lama guanicoe*) in South America. Mol Ecol 22: 463–482. doi: 10.1111/mec.12111 PMID: 23206254

44. Pastorino MJ, Gallo LA (2002) Quaternary evolutionary history of *Austrocedrus chilensis*, a cypress native to the Andean-Patagonian forest. J Biogeogr 29: 1167–1178.

45. Jakob SS, Martinez-Meyer E, Blattner FR (2009) Phylogeographic analyses and paleodistribution modeling indicate Pleistocene In Situ survival of *Hordeum* species (Poaceae) in Southern Patagonia without genetic or spatial restriction. Mol Biol Evol 26: 907–923. doi: 10.1093/molbev/msp012 PMID: 19168565

46. Fraser CI, Thiel M, Spencer HG, Waters JM (2010) Contemporary habitat discontinuity and historic glacial ice drive genetic divergence in Chilean kelp. BMC Evol Biol 10: 203. doi: 10.1186/1471-2148-10-203 PMID: 20594354

47. Sérsic AN, Cosacov A, Cocucci AA, Johnson LA, Pozner R, Avila LJ, et al. (2011) Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. Biol J Linn Soc 103: 475–494.

48. Macaya EC, Zuccarello GC (2010) Genetic structure of the giant kelp *Macrocystis pyrifera* along the southeastern Pacific. Mar Ecol-Prog Ser 420: 103–112.

49. Waters JM (2008) Driven by the West Wind Drift? A synthesis of southern temperate marine biogeography, with new directions for dispersalism. J Biogeogr 35: 417–427.

50. Leese F, Agrawal S, Held C (2010) Long-distance island hopping without dispersal stages: transportation across major zoogeographic barriers in a Southern Ocean isopod. Naturwissenschaften 97: 583–594. doi: 10.1007/s00114-010-0674-y PMID: 20454771

51.   Gillespie RG, Baldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK (2012) Long-distance dispersal: a framework for hypothesis testing. Trends Ecol Evol 27: 47–56. doi: 10.1016/j.tree.2011.08.009 PMID: 22014977

52.   Bowden DA, Clarke A, Peck LS, Barnes DKA (2006) Antarctic sessile marine benthos: colonisation and growth on artificial substrata over three years. Mar Ecol-Prog Ser 316: 1–16.

53.   Bowden DA, Clarke A, Peck LS (2009) Seasonal variation in the diversity and abundance of pelagic larvae of Antarctic marine invertebrates. Mar Biol 156: 2033–2047.

54.   Peck L, Heiser S, Clark M (in press) Very slow embryonic and larval development in the Antarctic limpet *Nacella polaris*. Polar Biol. doi: 10.1007/s00300-016-1894-1

55.   Powell AWB (1973) The patellid limpets of the World (Patellidae). Auckland Institute and Museum Auckland, New Zealand. pp. 1–132.

56.   González-Wevar CA, Nakano T, Cañete JI, Poulin E (2010) Molecular phylogeny and historical biogeography of *Nacella* (Patellogastropoda: Nacellidae) in the Southern Ocean. Mol Phylogenet Evol 56: 115–124. doi: 10.1016/j.ympev.2010.02.001 PMID: 20139020

57.   Valdovinos C, Rüth M (2005) Nacellidae limpets of southern South America: taxonomy and distribution. Rev Chil Hist Nat 78: 497–517.

58.   Reid DG, Osorio C (2000) The shallow-water marine mollusca of the Estero Elefantes and Laguna San Rafael, southern Chile. Bull Br Mus Nat Hist Zool 66: 109–146.

59.   Rosenfeld S, Aldea C, Mansilla A, Marambio J, Ojeda J (2015) Richness, systematics, and distributions of molluscs associated with the macroalga *Gigartina skottsbergii* in the Strait of Magellan: a biogeographic affinity study. Zookeys 519: 49–100. doi: 10.3897/zookeys.519.9676 PMID: 26448707

60.   Rosenfeld S (2016) Variación espacio-temporal en la composición dietaria de especies del género Nacella Schumacher, 1817, en la Ecoregión Subantártica de Magallanes. M. Sc. Thesis, Universidad de Magallanes. 145 pp.

61.   Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res 25: 4692–4693. PMID: 9358185

62.   Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3: 294–299.

63.   Filatov DA (2009) Processing and population genetic analysis of multigenic datasets with ProSeq3 software. Bioinformatics 25: 3189–3190. doi: 10.1093/bioinformatics/btp572 PMID: 19797407

64.   Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics. 23: 2947–2948. PMID: 17846036

65.   Xia X, Xie Z (2001) DAMBE: Software package for data analysis in molecular biology and evolution. J Hered 92: 371–373. PMID: 11535656

66.   Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452. doi: 10.1093/bioinformatics/btp187 PMID: 19346325

67.   Pons O, Petit RJ (1996) Measuring and testing genetic differentiation with ordered *versus* unordered alleles. Genetics 144: 1237–1245. PMID: 8913764

68.   Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol Bioinf Online 1: 37–50.

69.   Hudson RR (2001) A new statistic for detecting genetic differentiation. Genetics 155: 2011–2014.

70.   Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape genetics. Mol Ecol Notes 5: 712–715.

71.   Ihaka R, Gentleman R (1996) R: A language for data analysis and graphics. J Comp Graph Stat. 5: 299–314.

72.   Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457–462. PMID: 7705646

73.   Vavrek MJ (2011) Fossil: palaeoecological and palaeogeographical analysis tools. Palaeontologia Electronica 14:1T.

74.   Rogers AR, Harpending H (1992) Population growth makes waves. Mol Biol Evol 9: 552–569. PMID: 1316531

75.   Schneider S, Excoffier L (1999) Estimation of past demographic parameters from a distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152: 1879–1889.

76. Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proc Natl Acad Sci USA 98: 4563–4568. PMID: 11287657

77. Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9: 772–772.

78. Beerli P, Palczewski M (2010) Unified framework to evaluate panmixia and migration direction among multiple sampling locations. Genetics 185: 313–326. doi: 10.1534/genetics.109.112532 PMID: 20176979

79. Kaas RE, Raftery AE (1995) Bayes factors. J Am Stat Assoc 90: 773–795.

80. Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158: 885–896. PMID: 11404349

81. Hey J, Nielsen R (2007) Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. Proc Natl Acad Sci USA 104: 2785–2790. PMID: 17301231

82. Thomson JD, Higgins DG, Gibson TJ (1994) Clustal W: improving the sensititivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680. PMID: 7984417

83. Posada D, Crandall KA (2001) Intraspecific gene genealogies: trees grafting into networks. Trends Ecol Evol 16: 37–45. PMID: 11146143

84. Thatje S, Hillenbrand C-D, Larter R (2005) On the origin of Antarctic marine benthic community structure. Trends Ecol Evol 20: 534–540. PMID: 16701431

85. Dambach J, Thatje S, Rödder D, Basher Z, Raupach MJ (2012) Effects of late-Cenozoic glaciation on habitat availability in Antarctic benthic shrimp (Crustacea: Decapoda: Caridea). PLoS ONE 7: e46283. doi: 10.1371/journal.pone.0046283 PMID: 23029463

86. Valdovinos C, Navarrete SA, Marquet PA (2003) Mollusk species diversity in the Southern Pacific: why are there more species towards the pole? Ecography 26: 139–144.

87. Zattara EE, Premoli AC (2005) Genetic structuring in Andean landlocked populations of *Galaxias maculatus*: effects of biogeographic history. J Biogeogr 32: 5–14.

88. Near TJ, Dornburg A, Kuhn KL, Eastman JT, Pennington JN, Patarnello T, et al. (2012) Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. Proc Natl Acad Sci USA 109: 3434–3439. doi: 10.1073/pnas.1115169109 PMID: 22331888

89. Crame JA (1997) An evolutionary framework for the polar regions. J Biogeogr 24: 1–9.

90. Hewitt GM (1999) Some genetic consequences of ice ages, and their role, in divergence and speciation. Biol J Linn Soc 58: 247–276.

91. Marko PB (2004) 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. Mol Ecol 13: 597–611. PMID: 14871364

92. Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Ann Rev Ecol Evol S 37: 1–35.

93. McMahon SM, Harrison SP, Armbruster WS, Bartlein PJ, Beale CM, Edwards ME, et al. (2011) Improving assessment and modelling of climate change impacts on global terrestrial biodiversity. Trends Ecol Evol 26: 249–259. doi: 10.1016/j.tree.2011.02.012 PMID: 21474198

94. Bird CE, Holland BS, Bowen BW, Toonen RJ (2007) Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories. Mol Ecol 16: 3173–3186. PMID: 17651195

95. Goldstien SJ, Schiel DR, Gemmel NJ (2006) Comparative phylogeography of coastal limpets across a marine disjunction in New Zealand. Mol Ecol 15: 3259–3268. PMID: 16968269

96. González-Wevar CA, David B, Poulin E (2011) Phylogeography and demographic inference in *Nacella* (*Patinigera*) *concinna* (Strebel, 1908) in the western Antarctic Peninsula. Deep-Sea Res Pt II. 58: 220–229.

97. González-Wevar CA, Saucède T, Morley SA, Chown SL, Poulin E (2013) Extinction and recolonization of maritime Antarctica in the limpet *Nacella concinna* (Strebel, 1908) during the last glacial cycle: toward a model of Quaternary biogeography in shallow Antarctic invertebrates. Mol Ecol 22: 5221–5236. doi: 10.1111/mec.12465 PMID: 24102937

98. Reisser CMO, Wood AR, Bell JJ, Gardner JPA (2011) Connectivity, small islands and large distances: the *Cellana strigilis* limpet complex in the Southern Ocean. Mol Ecol 20: 3399–3413. doi: 10.1111/j.1365-294X.2011.05185.x PMID: 21771140

99. González-Wevar CA, Chown SL, Morley S, Coria N, Saucède T, Poulin E (2016) Out of Antarctica: quaternary colonization of sub-Antarctic Marion Island by the limpet genus *Nacella* (Patellogastropoda: Nacellidae). Polar Biol 39: 77–89.

100. Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin M-L (2015) Deep genetic divergence between austral populations of the red alga *Gigartina skottsbergii* reveals a cryptic species endemic to the Antarctic continent. Polar Biol 38: 2021–2034.

101. Nikula R, Fraser CI, Spencer HG, Waters JM (2010) Circumpolar dispersal by rafting in two subantarctic kelp-dwelling crustaceans. Mar Ecol-Prog Ser 405: 221–230.

102. Cumming RA, Nikula R, Spencer HG, Waters JM (2014) Transoceanic genetic similarities of kelp-associated sea slug populations: long-distance dispersal via rafting? J Biogeogr 41: 2357–2370.

103. Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129: 555–562. PMID: 1743491

104. Moreno PI, León AL (2003) Abrupt vegetation changes during the last glacial to Holocene transition in mid-latitude South America. J Quat Sci 18: 787–800.

105. McCulloch RD, Fogwill CJ, Sugden DE, Bentley MJ, Kubik PW (2005) Chronology of the last glaciation in Central Strait of Magellan and Bahía Inútil, Southernmost South America. Geogr Ann 87:1–24.

106. Heusser CJ (1998) Deglacial paleoclimate of the American sector of the Southern Ocean: Late Glacial—Holocene records from the latitude of Canal Beagle (55°S), Argentine Tierra del Fuego. Palaeogeogr Palaeoclimatol 141:277–301.

107. Gordillo S, Coronato AMJ, Rabassa JO (2005) Quaternary molluscan faunas from the islands of Tierra del Fuego after the Last Glacial Maximum. Sci Mar 69: 337–348.

108. Gordillo S, Rabassa J, Coronato A (2008) Paleoecology and paleobiogeographic pattern of mid-Holocene mollusks from the Beagle Channel (southern Tierra del Fuego, Argentina). Rev Geol Chile 35: 321–333.

109. Gordillo S (2010) Quaternary marine mollusca in Tierra del Fuego: Insights from integrated taphonomic and paleoecologic analysis of shell assemblages in raised deposits. An Inst Patagon (Chile) 37: 5–16.

110. Cárdenas J, Gordillo S (2009) Paleoenvironmental interpretation of late Quaternary molluscan assemblages from southern South America: A taphonomic comparison between the Strait of Magellan and the Beagle Channel. Andean Geol 36: 81–93.

111. Cowen RK, Sponaugle S (2009). Larval dispersal and marine population connectivity. Ann Rev Marine Sci 1: 443–466.

112. Paulay G, Meyer C (2006) Dispersal and divergence across the greatest ocean region: Do larvae matter? Integr Comp Biol 46: 269–281. doi: 10.1093/icb/icj027 PMID: 21672741

113. Miller KJ, Ayre DJ (2008) Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. J Anim Ecol 77: 713–724. doi: 10.1111/j.1365-2656.2008.01387.x PMID: 18422556

114. Ayre DJ, Minchinton TE, Perrin C (2009) Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? Mol Ecol 18: 1887–1903. PMID: 19434808

115. Toro JE, Ojeda JA, Vergara AM (2004) The genetic structure of *Mytilus chilensis* (Hupe 1854) populations along the Chilean coast based on RAPDs analysis. Aquat Res 35: 1466–1471.

116. Güller M, Zelaya DG, Ituarte C (in press) How many *Siphonaria* species (Gastropoda: Euthyneura) live in southern South America? J Mollus Stud doi: 10.1093/mollus/eyv036

117. Nuñez JD, Fernández Iriarte PJ, Ocampo EH, Iudica C, Cledón M (2015) Deep phylogeographic divergence among populations of limpet *Siphonaria lessoni* on the east and west coasts of South America. Marine Biology. 162: 595–605.

118. Nuñez JJ, González MT, Pérez-Losada M (2010) Testing species boundaries between Atlantic and Pacific lineages of the Patagonian rockfish *Sebastes oculatus* (Teleostei: Scorpaenidae) through mitochondrial DNA sequences. Rev Biol Mar Oceanogr 45: 565–573.

119. Fraser CI, Zuccarello GC, Spencer HG, Salvatore LC, Garcia GR, Waters JM (2013) Genetic affinities between trans-oceanic populations of non-buoyant macroalgae in the high latitudes of the Southern Hemisphere. PLoS ONE 8: e69138. doi: 10.1371/journal.pone.0069138 PMID: 23894421

120. Green BS, Fisher R (2004) Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. J Exp Mar Biol Ecol 299: 115–132.

121. O'Connor MIO, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP, et al. (2007) Temperature control larval dispersal and the implications for marine ecology, evolution, and conservation. Proc Natl Acad Sci USA 104: 1266–1271. PMID: 17213327

122. Knox GA (1960) Littoral ecology and biogeography of the Southern Ocean. Proc Roy Soc Lond B 152: 577–624.

123. Brattström H, Johanssen A (1983) Ecological and regional zoogeography of the marine benthic fauna of Chile. Sarsia: 68: 289–339.

124. Leese F, Kop A, Wägele J-W, Held C (2008) Cryptic speciation in a benthic isopod from Patagonian and Falkland Island waters and the impact of glaciations on its population structure. Front Zool 5: 1–15.

125. González-Wevar CA, Salinas P, Hüne M, Segovia NI, Vargas-Chacoff L, Oda E, et al. (2015) Contrasting genetic structure and diversity of *Galaxias maculatus* (Jenyns, 1848) along the Chilean Coast: stock identification for fishery management. J Hered 106: 439–447. doi: 10.1093/jhered/esv005 PMID: 26245779

126. Sánchez R, Sepúlveda RD, Brante A, Cárdenas L (2011) Spatial pattern of genetic and morphological diversity in the direct developer *Acanthina monodon* (Gastropoda: Mollusca). Mar Ecol-Prog Ser 434: 121–131.

127. Clapperton CM, Sugden DE (1976) The maximum extent of glaciers in part of west Falkland. Journal of Glaciology. 1976; 17: 73–77.

128. Hodgson DA, Graham AGC, Roberts SJ, Bentley MJ, Cofaigh CÓ, Verleyen E, et al. (2014) Terrestrial and submarine evidence for the extent and timing of the Last Glacial Maximum and the onset of deglaciation on the maritime-Antarctic and sub-Antarctic islands. Quat Sci Rev 100: 137–158.

129. Wilson P, Bentley MJ, Schnabel C, Clark R, Xu S (2008) Stone run (block stream) formation in the Falkland Islands over several cold stages, deduced from cosmogenic isotope (^{10}Be and ^{26}Al) surface exposure dating. J Quat Sci 23: 461–473.

130. Clark R, Huber UM, Wilson P (1998) Late Pleistocene sediments and environmental change at Plaza Creek, Falkland Islands, South Atlantic. J Quat Sci 13: 95–105.

131. Wilson P, Clark R, Birnie J, Moore DM (2002) Late Pleistocene and Holocene landscape evolution and environmental change in the Lake Sulivan area, Falkland Islands, South Atlantic. Quat Sci Rev 21: 1821–1840.

132. Miloslavich P, Klein E, Díaz JM, Hernández CE, Bigatti G, Campos L, et al. (2011) Marine biodiversity in the Atlantic and Pacific Coasts of South America: Knowledge and gaps. PLoS ONE 6: e14631. doi: 10.1371/journal.pone.0014631.t008 PMID: 21304960

133. Morrone JJ (2011) Island evolutionary biogeography: analysis of the weevils (Coleoptera: Curculionidae) of the Falkland Islands (Islas Malvinas). J Biogeogr 38: 2078–2090.

134. Papadopoulou A, Jones AG, Hammond PM, Vogler AP (2009) DNA taxonomy and phylogeography of beetles of the Falkland Islands (Islas Malvinas). Mol Phylogenet Evol 53: 935–947. doi: 10.1016/j.ympev.2009.08.027 PMID: 19723584

135. Bastida R, Roux A, Martínez DE (1992) Benthic communities of the Argentine continental shelf. Oceanol Acta 15: 687–698.

136. Acha EM, Mianzan HW, Guerrero RA, Favero M, Bava J (2004) Marine fronts at the continental shelves of austral South America. J Mar Syst 44: 83–105.