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# Present-day connectivity of historical stocks of the ecosystem engineer *Perumytilus purpuratus* along 4500 km of the Chilean Coast

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ABSTRACT

Historical processes leading to the emergence of marine stocks, the mechanisms maintaining stock structure, and present-day connectivity patterns are elementary pieces of information to design wiser and more sustainable management of marine resources, and to understand speciation processes in the sea. Over geographical scales, biological and genetic continuity in the Southern Pacific coast is determined by historical geological-climate events (e.g., LGM), as well as by present oceanographic processes (Humboldt Current, ENSO and others) that can affect both, local adaptation and dispersal. The relative influence of such processes on the current distribution of species can be comprehensibly inferred from a detailed population sampling comprising the whole species range. In this work, we aimed to provide insights into historical and modern patterns of genetic connectivity between stocks of the intertidal mussel *Perumytilus purpuratus*, a species reported to have moderate pelagic development times of about 14–18 days. To this end, we analyzed the geographical pattern of variation of microsatellite polymorphisms across 17 localities scattered along ca. 4500 km off the Chilean Coast and encompassing four biogeographic regions, previously defined based on compositional changes in marine flora and fauna, and which coincide with large changes in topography, the historical influence of glaciation, circulation patterns and upwelling variability around 26°S, 30°S and 40°S. Genetic structural data suggest that historical geological and climatic events have shaped the actual range of this species in the south-eastern Pacific, creating biogeographical limits, i.e., the north-central stock and the southern stock, that moderate to restricted dispersers cannot cross given present day coastal oceanographic processes. Conversely, the pattern of IBD observed within those two major biogeographical regions suggests that present-day coastal processes modulate the effective dispersal of this species.

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## 1. Introduction

Exchange of individuals among marine populations of benthic species occurs primarily during pelagic larval stages. Since these stages are quasi microscopic and are moved by ocean currents and fronts, large dispersal distances are expected in association with the pelagic larval development (PLD), which can take from hours to several months. Despite this potential for long distance dispersal, studies have shown that several species exhibit lower connectivity than previously assumed and genetic structuring has been observed over the range of many coastal marine species with moderate pelagic development times (Caley et al., 1996; Hellberg et al., 2002). Indeed, in several species dispersal of propagules can be restricted to adjacent populations and retention of larvae might be more frequent than suspected (Burgess et al., 2014; Cowen and Sponaugle, 2009). Therefore, coastal stocks of marine taxa may be more structured over spatial scales smaller than originally thought (Levin, 2006).

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There are theoretical reasons and growing empirical evidence indicating that the chance of successful dispersal decreases with increasing distance, following a general stepping-stone pattern of isolation-by-distance (IBD) among populations (Palumbi, 2001, 2003), although in most oceanic dispersive environments, such a pattern can be highly non-Euclidian, asymmetrical and temporally stochastic (Aiken and Navarrete, 2011). Therefore and despite the complexities of dispersal in a real ocean for marine benthic organisms, duration of planktonic larval stages is expected to correlate with dispersal ability (Ayre et al., 2009; Haye et al., 2014; Paulay and Meyer, 2006). Regardless the inherent dispersive characteristics of marine organisms, other factors such as abrupt environmental gradients, patchy population size distribution and life-cycle strategies can deeply modulate the structure of species (Pelc et al., 2009). The interaction of biotic and abiotic factors can help explain why within very close zoological groups some species may show a marked population structure and some others do not (e.g., Dawson et al., 2014; Marko and Barr, 2007; Zakas et al., 2009).

Despite the current technological advancement, the relative strength of factors structuring marine populations at macro-, meso- and microscale remains largely unmeasured (e.g., Cowen and Sponaugle, 2009). The fossil record (Davis and Shaw, 2001; Huntley, 1991) and reconstructions based on molecular data (Hughes, 2000; Lima et al., 2007; McCarty, 2001) indicate that climate changes generate a profound influence on the expansion-contraction range of species, as well as in their patterns of genetic diversity and structure (Hewitt, 2004; Lessa et al., 2003, 2010; Lima et al., 2007; Maggs et al., 2008; Marko, 2004; Marko et al., 2010; Pearson and Dawson, 2003; Provan and Bennett, 2008). Therefore, the dominant role that climate exerts over the distribution of species has become one of the central premises of current biogeography. Understanding how ecosystems and species respond to climate change has become a major focus of ecology and conservation biology of worldwide environmental policies (Davis et al., 2005; Marshall et al., 2008; McCarty, 2001; Parmesan, 2006).

The Chilean coast spans between 18°S and ~56°S and can be divided into northern and southern regions due to the characteristics of its coastal features. The northern portion, from 18°S to ~40°S runs almost-straight north-south, without major geological accidents (Ahumada et al., 2000; Camus, 2001; Thiel et al., 2007). This large section of the coast is characterized by rocky-shore habitats interspersed with sandy beaches with a dominant equatorward offshore circulation, the Humboldt Current. The coastal topography is dominated by strong winds running parallel to the coast that create a seasonally- and spatially-variable upwelling circulation and which are the main source of variability in oceanographic conditions in nearshore waters (Aguirre et al., 2012; Aiken et al., 2007; Narváez et al., 2006; Thiel et al., 2007; Wieters et al., 2009;). Conversely, the Southern region spanning from ~40°S/42°S to 56°S (Cape Horn) comprises ~1300 km of open coast, running mostly north-south, but including about 84,000 km of irregular coastline of islands in interior waters that cover about 240,000 km$^2$ (González-Wevar et al., 2012; Miloslavich et al., 2011; Pantoja et al., 2011; Rozzi et al., 2012). Accordingly, the southern Chilean coast can be considered as a major insular system where the Chilean Patagonian shore is broken by fjords, islands, peninsulas and channels that generate a very complex landscape, the result of the marked climatic changes that took place during the Quaternary.

Many phylogeographic breaks or intraspecific genetic discontinuities occur where past and/or present-day barriers to dispersal allow for genetic drift and prevent homogenization of populations (Avise et al., 1987; Wares, 2002). Along the Chilean coast, three biogeographic regions can be defined by two major discontinuities. The first discontinuity corresponds to an abrupt and persistent break in the dynamics of benthic and pelagic communities placed north to 30–32°S, exhibiting sharp discontinuities in upwelling regimes (Briones et al., 2014; Broitman et al., 2001; Navarrete et al., 2005), leading to sharp changes in the relative importance of synoptic, intra-seasonal and seasonal-scale thermal variability and discontinuities in dynamics and concentration of offshore surface Chlorophyll-a (Navarrete et al., 2005; Thomas, 1999; Thomas et al., 2001) and nutrient regimes in coastal waters (Tapia et al., 2009). This discontinuity corresponds to the biogeographic transition between the Peruvian Province and the so-called Intermediate Area at 30–42°S, (Camus, 2001; Thiel et al., 2007), where changes in extant invertebrate species richness (Rivadeneira et al., 2002) and macroalgae (Camus, 2001) are observed. Changes in functional group abundance (Broitman et al., 2001; Wieters et al., 2009) and breaks in genetic structure of several species are also documented at this junction (Haye et al., 2014; Laughlin et al., 2012; Montecinos et al., 2012; Tellier et al., 2009; Zakas et al., 2009). Genetic data suggest a secondary discontinuity at 26°S that may also be related to environmental heterogeneity (Briones et al., 2013; Trovant et al., 2015). As a whole, this northern region of the Chilean coast north to 40–41°S was largely unaffected by ice during the Pleistocene glaciations (Harrison, 2004), but is deeply impacted by the oceanic-climatic phenomenon known as El Niño Southern Oscillation (ENSO), as well as by the opposite phase known as La Niña Southern Oscillation (LNSO), especially in the central and northern parts of this region (Thiel et al., 2007). By contrast, the second portion of the Chilean coast distributed south to 41°S, corresponds to the Magellan Province and is dominated by a southward offshore circulation (the Antarctic Circumpolar Current and the Cape Horn Current) (Camus, 2001), strong influence of freshwater runoff from glacial melting, and complex local circulation patterns strongly influenced by large tidal variation, haline buoyancy forcing and complex topography and bathymetry (e.g., Aiken, 2012; Cáceres et al., 2006; Pérez-Santos et al., 2014; Schneider et al., 2014). At the Last Glacial Maximum (LGM), an extensive ice sheet covered most of this region (Hulton et al., 2002; McCulloch et al., 2000).

The Chilean coast represents a natural scenario to assess the relative role of historical events and habitat discontinuities on the distribution and connectivity of mollusk species with limited dispersal. One of them, the marine mussel *Perumytilus purpuratus* (Lamarck, 1819) (Bivalvia: Mytilidae) has an ample biogeographic distribution along the Southeast Pacific coast, extending from Ecuador (2°00′S 77°30′W) to 56°S in the Chilean coast, south around the Cape Horn and reaching mid-latitudes of La Lobería in the Argentinean Patagonia (41°8′S 63°10′W) (Zagal et al., 2001). This species inhabits mid-intertidal rocky shores where plays a community structuring role by forming extensive multi-layered beds where intensive intraspecific competition occurs (Acevedo et al., 2010; Caro et al., 2011; Guiñez, 1996, 2005; Guiñez and Castilla, 1999; Guiñez et al., 2005; Prado and Castilla, 2006). While most marine mussels exhibit planktotrophic larval development and larger development times (Seed, 1969), this species might possess a lecitotrophic larval development (Garrido, 1996) lasting roughly 14–18 days under laboratory conditions (Ramorino and Campos, 1979). The metapopulation genetic structure of this species can give important clues on the ecological relevance of its dispersive characteristics, the distribution and the dynamics of this and similar invertebrates in intertidal habitats of South American coasts.

In this study we aimed to provide insights into historical and modern connectivity of *P. purpuratus* stocks along its distribution ca. 4500 km off the Chilean Coast as inferred from geographical patterns of microsatellite genetic variation. We also aimed to test if previous micro-scale genetic structures reported for this species in the northern Chilean coast (Briones et al., 2013) are either detectable at the macroscale or overwhelmed by major biogeographic structure in the southern pacific. We hypothesize that the geological history of the south-east Pacific, the geographic discon-

**Table 1**
Sampling location, code, coordinates and sample size (*N*) of 17 samples of *P. purpuratus* populations collected in four latitudinal intervals (regions) of the Chilean coast.

| Sampling location | Code | Coordinates | | *N* | Region[a] |
|---|---|---|---|---|---|
| Arica (Las Pesqueras) | ARI | 18°31′S | 70°19′W | 55 | 1 |
| Iquique (Punta Cavancha) | IQU | 20°14′S | 70°09′W | 52 | 1 |
| El Way | WAY | 23°45′S | 70°27′W | 55 | 1 |
| Caleta Bolfin | BOL | 23°51′S | 70°30′W | 50 | 1 |
| Taltal (Las Bandurrias) | TAL | 25°12′S | 70°26′W | 51 | 1 |
| Genetic discontinuity 26°S (Briones et al., 2013) | | | | | |
| Temblador | TEM | 29°01′S | 71°30′W | 50 | 2 |
| Guanaqueros | GUA | 30°10′S | 71°28′W | 50 | 2 |
| Punta de Talca | PTA | 30°56′S | 71°41′W | 50 | 2 |
| Ecological discontinuity 32°S (Broitman et al., 2001) | | | | | |
| Montemar | MON | 32°57′S | 71°33′W | 53 | 3 |
| Punta de Tralca | PTR | 33°26′S | 71°42′W | 57 | 3 |
| Iloca | ILO | 34°56′S | 72°11′W | 46 | 3 |
| Caleta de Tumbes | TUM | 36°38′S | 73°05′W | 48 | 3 |
| Mehuín | MEH | 39°25′S | 73°13′W | 46 | 3 |
| Physiological discontinuity 40°S (Briones et al., 2012) | | | | | |
| Pucatrihue (Valdivia) | PUC | 40°33′S | 73°45′W | 38 | 4 |
| Mar Brava (Chiloé) | CHI | 41°47′S | 73°53′W | 50 | 4 |
| Islas Toto (Puerto Aysen) | AYS | 44°44′S | 73°32′W | 24 | 4 |
| Punta Arenas (Puerto Arturo) | PAR | 53°10′S | 70°54′W | 58 | 4 |

[a] Region 1 comprised samples from ARI (18°31′S) to TAL (25°12′S), this latter close to latitude 26°S where a genetic discontinuity has been reported for this species (Briones et al., 2013); region 2 comprised samples from TEM (29°01′S) to PTA (30°56′S) located northern to the 32°S ecological discontinuity (Broitman et al., 2001; Navarrete et al., 2002, 2005); region 3 comprised samples from MON (32°57′S), located south to the 32°S discontinuity, to MEH (39°25′S) close to the 40°S major population split reported upon morpho-physiological studies in this species (Briones et al., 2012); region 4 comprised samples from PUC (40°33′S) to PAR (53°10′S), i.e., south to the 40°S discontinuity.

tinuities described along the Chilean coastline and the circulation system in the coastal ocean would be distinctly reflected in the phylogeographic pattern of taxa with moderate dispersal, such as *Perumytilus*. Since its pelagic development times are moderately low (Ramorino and Campos, 1979; Garrido, 1996), we also hypothesize that its phylogeographical structure could likely fit a stepping-stone-like model of dispersion under IBD, and therefore a pattern shaped by both, potential larval dispersal and contemporary oceanography (Pelc et al., 2009). In contrast, genetic breaks at major ecological barriers are expected to violate IBD models and to reflect historical rather than actual oceanographic processes (Hilbish, 1996; Jacobs et al., 2004; Pelc et al., 2009; Wares, 2002).

## 2. Materials and methods

### 2.1. Population sampling and microsatellite genotyping

Adult specimens of the mussel *P. purpuratus* were sampled in 2007 at 17 mid-rocky intertidal localities scattered along 4450 km of the Chilean coastline in the south-eastern Pacific. Sampling locations were distributed between ARICA (18°31′S, 70°19′W) and PUNTA ARENAS (53°10′S, 70°54′W) in the Magellan Strait (Table 1). Mussels were hand-collected by scrapping and mantle tissues from about 50 mussels per locality were kept in pure ethanol and the GPS position registered at each sampling location. Genomic DNA was extracted and purified from ca. 100 mg of ethanol-preserved mantle using the method FENOSALT (Pérez and Presa, 2011) and DNA was finally resuspended in 100 μL of TE buffer and kept at −20 °C until PCR analysis. Individuals were genotyped with five polymorphic microsatellites described for this species (Pérez et al., 2008). PCR reactions of microsatellites loci *Pepu*1 and *Pepu*4 were multiplexed in a single PCR consisting of 5 pmol of each primer, 1.5 mM $MgCl_2$, and co-amplified at 55 °C of annealing temperature. Loci *Pepu*5, *Pepu*7 and *Pepu*2 were individually amplified using the conditions described by Pérez et al. (2008). The PCR amplification mixture consisted of 50 ng DNA template, 200 μM of each dNTPs, 1% BSA, 0.75 U Flexi-Taq DNA polymerase (Promega), the corresponding locus-dependent concentration of $MgCl_2$ and primers, and ultrapure water up to 15 μL. The forward primer of each locus was 5′-end labeled with fluorophores FAM (Locus *Pepu*1), HEX (Loci *Pepu*2 and *Pepu*7) and NED (Loci *Pepu*4 and *Pepu*5). PCR amplification conditions comprised an initial step at 95 °C for 5 min, followed by 40 cycles at 95 °C for 1 min, annealing temperature for 1 min, 72 °C for 55 s, and a final elongation step was enforced at 72 °C for 30 min. Amplified fragments of labelled PCR products were separated in an ABI PRISM 3130 automated capillary sequencer from CACTI facilities (Servicio de Genómica, University of Vigo) and allele size was calculated relative to the GeneScan™-400 ROX™ size standard. Genotypic data were analysed using GeneMarker V2.4.2 (Softgenetics). The consistency of the allelic series was calculated with MICRO-CHECKER 2.2.3 (van Oosterhout et al., 2004).

### 2.2. Mathematical and statistical analyses

Allele frequencies per locus were calculated with the program GENALEX (Peakall and Smouse, 2006). Deviations from neutral polymorphism in the five microsatellite loci were investigated with the outlier approach implemented in LOSITAN (Antao et al., 2008). This approach is based on methods originally proposed by Beaumont and Nichols (1996) and simulates a distribution of $F_{ST}$ values under neutral expectations in order to identify candidates for positive and balancing selection upon a plot of average locus heterozygosity vs. $F_{ST}$ (Beaumont and Nichols, 1996; Beaumont and Balding, 2004). Simulations were run to generate locus-independent distributions of $F_{ST}$-based on 500,000 replicates under a Stepwise allele Mutation Model (SMM) and a forced neutral mean $F_{ST}$. Neutrality tests consisted on 1,000,000 iterations under both, the Infinite Allele Model (IAM) and the SMM, as implemented in LOSITAN. The probabilistic threshold was calculated as the number of simulated $F_{ST}$ that were smaller than those observed and was corrected with the False Discovery Rate approach test (FDR) (Benjamini and Hochberg, 1995). Genetic parameters such as the number of alleles, allelic richness and fixation indices within samples ($F_{IS}$) and between samples ($F_{ST}$) were calculated with FSTAT 2.9.3.2 (Goudet, 1995, 2001). Observed ($H_O$) and expected ($H_E$) heterozygosities and exact tests for Hardy–Weinberg equilibrium were carried out with GENEPOP 4.0 (Rousset, 2008). The probability associated to the fixation index within samples ($F_{IS}$) was calculated with the Markov Chain Method implemented in GENEPOP 4.0 using 20 batches and 5000 iterations each. Statistical differences in genetic parameters between regions, i.e., $H_O$, $H_E$, $R_S$, $F_{IS}$ and $F_{ST}$ (see description of AMOVA hierarchical levels below) were assessed against their parametric distributions generated from 15,000 permutations tests of samples among the corresponding population groups, using FSTAT. Frequencies of null alleles were calculated with FREENA (Chapuis and Estoup, 2007).

The probability dependent on the microsatellite/sampling system enforced, to erroneously rejecting the null hypothesis of no population structuring when it was true (α error) as well as the statistical power, i.e., the probability of rejecting the null hypothesis when it was false, were assessed using POWSIM version 4.0 (Ryman and Palm, 2006). The genetic variance was hierarchically partitioned using the Analysis of Molecular Variance (AMOVA) (Excoffier et al., 2005) as implemented in ARLEQUIN 3.5 (Excoffier and Lischer, 2010). The AMOVA explored the distribution of the molecular variance among samples, within groups, and within samples. The n-groups considered (see Table 1) were as follows: $n = 1$, the whole dataset; $n = 2$, made upon a 40°S biogeographic discontinuity observed with GENELAND (Group 1: 13 samples from

ARICA to MEHUÍN; Group 2: four samples from PUCATRIHUE to PUNTA ARENAS); $n = 3$, built upon the biogeographic discontinuities reported around latitudes 30–32°S and 40–42°S (Broitman et al., 2001; Navarrete et al., 2002, 2005) (Group 1: eight samples from ARICA to PUNTA DE TALCA; Group 2: five samples from MONTEMAR to MEHUIN; Group 3: four samples from PUCATRIHUE to PUNTA ARENAS); $n = 4$, built upon previous discontinuities at 32°S and 40°S plus the genetic one reported around latitude 26°S (Briones et al., 2013) (Group 1: five samples from ARICA to TALTAL; Group 2: three samples from TEMBLADOR to PUNTA DE TALCA, Group 3: five samples from MONTEMAR to MEHUIN; Group 4: four samples from PUCATRIHUE to PUNTA ARENAS); $n' = 3$, built exclusively with northern stock areas (Groups 1 to 3 of the previous $n = 4$ partition). A Principal Coordinate Analysis (PCoA) was computed to calculate a dissimilarity matrix among samples upon the variance present in their molecular polymorphism using GENALEX.

GENELAND 3.3.0 (Guillot et al., 2005b) incorporates information on geographical coordinates for genotyped individuals and was used in the Bayesian exploration of the number of gene pools ($k$) present in the sample set. The Bayesian analysis was enforced for a spatial model with uncorrelated allele frequencies (Guillot et al., 2005a), 1,000,000 iterations and correction for null alleles (including missing data) (Guillot et al., 2008). The most likely number of gene pools ($k$) present in the sample set was inferred from the modal likelihood value and the spatial boundaries between pools were detected as geographical areas with the lowest posterior probability of membership to gene pools. Several independent MCMC runs were assayed with all loci as well as with four loci (excluding locus *Pepu*5 as the one putatively shaped by balanced selection under both, the IAM and the SMM mutation models). The large genetic variance observed among samples advocated for the comparison between the classic gene flow estimate ($F_{ST}$) and the genetic differentiation index $D_{EST}$ (Jost, 2008), this latter believed to better fit the interpopulation genetic differentiation estimated with highly polymorphic markers (Hedrick, 2005; Jost, 2008). Pairwise $D_{EST}$-values among samples, its confidence intervals and its statistical significances were calculated after 1000 bootstrap replicates using DEMETICS 0.8-7 (Gerlach et al., 2010) in R 2.13.1 (the R project for statistical computing). Corrections for multiple tests were performed using the false discovery rate approach (FDR). Correlation between the differentiation index ($D_{EST}$) and the fixation index ($F_{ST}$) were explored using a linear regression using package SPSS 17.0. The regression of both genetic distances ($F_{ST}/(1 - F_{ST})$) and $D_{EST}$ with geographical distances (coastal) between a given pair of samples was tested with the Mantel permutation procedure implemented in program ISOLDE from GENEPOP 4.0. The Bayesian approach implemented in BAYESASS 3.0 (Wilson and Rannala, 2003) was used to infer the migration rate m between samples, provided that no migration-drift equilibrium is a requisite for its calculation. Analytical settings consisted on 10,000,000 MCMC iterations, a 1,000,000 burn-in threshold and a 1000-iterations sampling interval. A priori mixing parameters were *delta*M (migration rates) = 0.85; *delta*A (allele frequencies) = 0.85 and *delta*F (inbreeding coefficients) = 0.95. A posteriori acceptance rates for proposed changes after convergence (ideally 0.2–0.4) were *delta*M = 0.41, *delta*A = 0.31 and *delta*F = 0.44.

## 3. Results

### 3.1. Intrapopulation variation

All microsatellites were highly polymorphic and the number of alleles per locus ranged 17 (marker *Pepu*7)–47 (marker *Pepu*2) (Appendix 1). One-hundred and thirty three alleles were observed in 833 individuals and all loci showed a unique modal allele in most samples (Appendix 2). Global expected heterozygosity ranged 0.526 (marker *Pepu*7)–0.882 (marker *Pepu*2) (Appendix 2). A significant deficit of heterozygotes was observed in all loci across samples (from two to four deviated locus per sample). Per locus average $F_{IS} \pm$ SD was $0.117 \pm 0.174$ (*Pepu*1), $0.316 \pm 0.172$ (*Pepu*2), $0.071 \pm 0.179$ (*Pepu*4), $0.299 \pm 0.260$ (*Pepu*5) and $0.439 \pm 0.270$ (*Pepu*7) (Appendix 2). No allele dropout was observed but a putative stuttering was suggested after MICROCHECKER in microsatellite *Pepu*5 for ARI, IQU, GUA, PTA, MON, and PAR, as well as in microsatellite *Pepu*2 for ILO and PAR. Allele frequencies of putative null alleles were higher than 10% in loci *Pepu*2, *Pepu*5 and *Pepu*7 (Appendix 2).

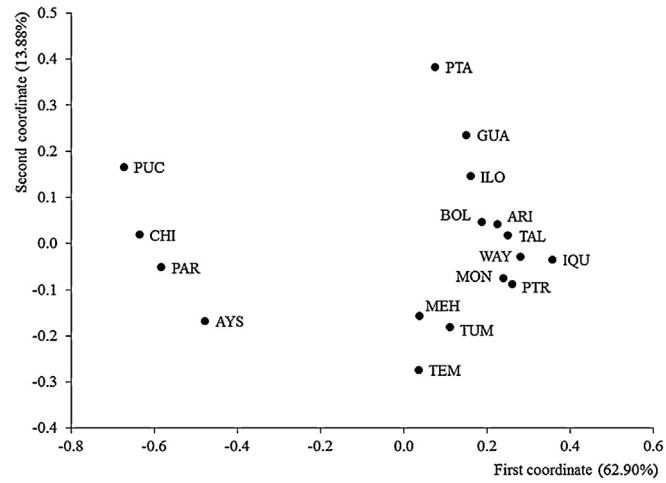


**Fig. 1.** Principal Coordinate Analysis (PCoA) based on a correlation matrix of allele frequencies showing the relative relationships between *P. purpuratus* samples from the latitude interval 18–53°S in the Chilean coast. Variation of the first coordinate (62.90%) discriminates the two gene pools flanking the 40°S population split in this species.

Global simulated $F_{ST}$ under the IAM model was 0.082, marker *Pepu*5 showed a $F_{ST}$-like outlier and was candidate for balancing selection after the FDR correction and the rest of markers showed neutral variation. Genetic differentiation among samples calculated using the four neutral loci, or the full set of five loci (including the putative balanced variation of *Pepu*5) gave equivalent inter-population scenarios (see Fig. 2A). Global simulated $F_{ST}$ under the SMM model was 0.070. Markers *Pepu*2 ($F_{ST} = 0.100$; $p = 0.989$) and *Pepu*5 ($F_{ST} = 0.035$; $p = 0.007$) showed $F_{ST}$-outliers and were candidates for both, positive selection and balancing selection, respectively; those $F_{ST}$-outlier deviations were non-significant after FDR correction.

### 3.2. Interpopulation variation

The statistical power of the sample system after 1000 iterations was 1.0 regardless of both, the effective size enforced (1000 or 5000 individuals) and the statistical method used (chi-square test or Fisher's test). Estimates of type I error using chi-square and Fisher's methods were 0.041 and 0.092, respectively, for an effective size of 1000, and 0.044 and 0.076, respectively, for an effective size of 5000. Power analyses suggest that present combination of the marker system and the sampling set are powerful enough to reject panmixia. The analysis of molecular variance (Table 1) showed a significant differentiation among samples ($F_{ST} = 0.071$, $p < 0.01$), as well as among samples within region, this latter being independent of the hierarchical grouping enforced ($F_{SC}$ range 0.026–0.037). Two population clusters ($n = 2$) comprising regions 1 + 2 + 3 (northern-central stock, NCS) vs. region 4 (southern stock, SS) was the

**Table 2**
Hierarchical AMOVA analyses on 17 samples of *P. purpuratus* grouped in four biogeographical regions delineated after multidisciplinary studies performed in this species; $^{*}p \leq 0.01$ is the probability that the observed values were equal or smaller than those expected by random; ns, non-significant.

| Hierarchical level[a] | Source of variation | Sum of squares | Variance components | Percentage of variation | Fixation indexes |
|---|---|---|---|---|---|
| Whole dataset ($n = 1$) | Among samples | 198.881 | 0.12523 | 7.05 | $F_{ST} = 0.071^{*}$ |
| | Within samples | 2409.485 | 1.65106 | 92.95 | |
| Between stocks ($n = 2$) | Among groups | 94.495 | 0.19788 | 10.36 | $F_{CT} = 0.104^{*}$ |
| | Among samples within groups | 104.386 | 0.06128 | 3.21 | $F_{SC} = 0.036^{*}$ |
| | Within samples | 2409.485 | 1.65106 | 86.43 | $F_{ST} = 0.140^{*}$ |
| Between regions ($n = 3$) | Among groups | 100.521 | 0.09565 | 5.29 | $F_{CT} = 0.053^{*}$ |
| | Among samples within groups | 98.360 | 0.06254 | 3.46 | $F_{SC} = 0.037^{*}$ |
| | Within samples | 2409.485 | 1.65106 | 91.26 | $F_{ST} = 0.090^{*}$ |
| Between Areas ($n = 4$) | Among groups | 116.949 | 0.09312 | 5.18 | $F_{CT} = 0.052^{*}$ |
| | Among samples within groups | 81.932 | 0.05339 | 2.97 | $F_{SC} = 0.031^{*}$ |
| | Within samples | 2409.485 | 1.65106 | 91.85 | $F_{ST} = 0.083^{*}$ |
| Between Northern Areas ($n' = 3$) | Among groups | 22.454 | 0.01444 | 0.84 | $F_{CT} = 0.008^{ns}$ |
| | Among samples within groups | 57.376 | 0.04435 | 2.61 | $F_{SC} = 0.026^{*}$ |
| | Within samples | 1954.899 | 1.64313 | 96.55 | $F_{ST} = 0.035^{*}$ |

[a] The distribution of variance was tested among regional groups, i.e., $n = 2$ (regions 1 + 2 + 3 vs. region 4); $n = 3$ (regions 1 + 2, region 3 and region 4); $n' = 3$ (three independent northern regions 1, 2 and 3); $n = 4$ (four independent regions 1, 2, 3 and 4), (see Table 1 for regional ascription of samples).

partition providing the highest interregional differentiation signal ($F_{CT} = 0.104$, $p < 0.01$, Table 2). That inter-stock divergence was ca. ten-fold higher that the non-significant 0.84% variation observed among regions of the northern stock (AMOVA, $n' = 3$; $F_{CT} = 0.0084$; $p = 0.013$, Table 2).

The first two PCoA coordinates explained nearly 77% of the global variation present in the dataset and showed a clear separation between, the northern-central stock (NCS) (which spans from ARICA (18°31′S) to MEHUÍN (39°25′S) and exhibited 109 alleles in 663 individuals) and the southern stock (SS) (which comprised 4 localities from PUCATRIHUE (40°33′S) to PUNTA ARENAS (53°10′S) and contained 64 alleles in 170 individuals) (Fig. 1 and Appendix 2). The Bayesian clustering analysis performed with GENELAND under the spatial model with uncorrelated allele frequencies and the null allele algorithm, also showed two most probable gene pools ($k = 2$) in the whole sample system (Fig. 2A). The map built upon the posterior probability of belonging to one of those clusters showed a clear-cut division of samples at latitude 40°S, which was coincident with the split in the PCoA ordination. None of the genetic parameters computed, e.g. number of alleles, heterozygosity or allele richness, differed significantly among the various sample clusters enforced in AMOVA or observed after PCoA or GENELAND analyses.

Pairwise $F_{ST}$ values between samples ranged 0.000–0.232 (Table 3) and most of them were significantly different from zero. All pairwise $F_{ST}$ values or $D_{EST}$ values (Appendix 3) between any sample from the NCS pool (regions 1 + 2 + 3) and any other from the SS pool (region 4) were significant after correction for multiple tests and were one order of magnitude higher than those within regions or within stocks. Regressions performed using the Mantel test resulted in significant correlations within gene pool (NCS and SS) either for $[F_{ST}/(1 - F_{ST})]$ ($r^2 = 0.164$; SC-regression = 0.008; SC-residual = 0.039; $F = 16.088$; $p < 0.0001$) (Fig. 3A) or for $D_{EST}$ ($r^2 = 0.151$; SC-regression = 0.037; SC-residual = 0.207; $F = 14.602$; $p < 0.001$) (Fig. 3B). No Mantel correlation was observed between gene pools using either $[F_{ST}/(1 - F_{ST})]$ ($r^2 = 0.007$; SC-regression = 0.001; SC-residual = 0.186; $F = 0.338$; $p = 0.563$) (Fig. 3A) or $D_{EST}$ ($r^2 = 0.003$, SC-regression = 0.001; SC-residual = 0.309; $F = 0.133$, $p = 0.717$) (Fig. 3B). A high correlation was observed between $F_{ST}$ and $D_{EST}$ pairwise values between samples ($r^2 = 0.938$, $F = 2027.15$, $p < 0.0001$) (Fig. 4). Bayesian-inferred significant migration rates were observed within gene pools but not between gene pools, being latitude 40°S the migration split point over the latitudinal interval 18–53°S off the Chilean coast (Appendix 4, Fig. 2B).

## 4. Discussion

Present population genetic data of *P. purpuratus* from the Chilean coast suggests that historical geological and climatic events have shaped the current genetic structure of this species in the SE Pacific, i.e., historical paleogeography seem to have created and enforced the sharp biogeographical limits separating a southern stock, south of 40°S, from the northern-central stock in the region between 18 and 40°S. We suggest that this sharp structure is historical in nature and is maintained in species such as *P. purpuratus,* due to its environment-related restricted larval dispersal, to its own limited potential for dispersal, or to a genetic/reproductive barrier between its historical lineages (e.g., see Briones et al., 2012) despite ongoing connectivity between each other (see Section 4.2). Migration patterns within stocks cannot be properly conjugated with present-day above-platform coastal currents, due to the interpretative limitations imposed by a high null-allele-frequency marker set. However, the lack of divergence between regions within stocks (NCS and SS) regarding various, genetic parameters, AMOVA, PCoA and bayesian pools, suggests that an IBD-like connectivity exists among regions within stocks.

### 4.1. Intrapopulation variation

The high levels of both, genetic diversity and number of alleles observed in all samples of *P. purpuratus* (e.g., $H_E = 0.526$–$0.882$) were similar to those reported for highly polymorphic microsatellites in marine bivalves characterized by large population sizes (*Mytilus galloprovincialis*, $H_E = 0.390$–$0.908$, Diz and Presa, 2008; *Placopecten magellanicus*, $H_E = 0.497$–$0.954$, Kenchington et al., 2006; *Perna viridis*, $H_E = 0.18$–$0.94$, Lin et al., 2007; *Mactra chinensis*, $H_E = 0.870$–$0.903$, Ni et al., 2011; *Crassostrea ariakensis*, $H_E = 0.787$–$0.832$, Xiao et al., 2010). The significant deficit of heterozygotes observed in all loci, averaging $F_{IS} = 0.248 \pm 0.136$ is common in marine bivalves (Gruenthal and Burton, 2008; Rose et al., 2006). This phenomenon is likely caused by null PCR-alleles from sequence variation at priming sites (Callen et al., 1993) and is a widespread background deficit of present *P. purpuratus* markers. A high frequency of null alleles impedes strong infer-

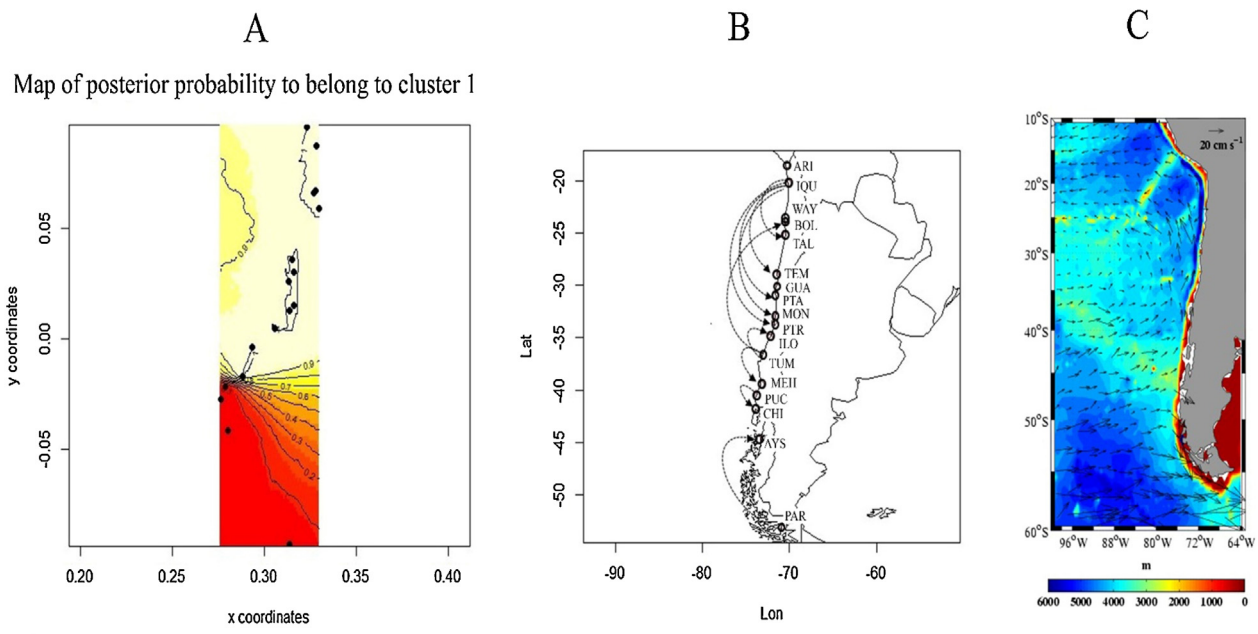


**Fig. 2.** (A) Bayesian probability isoclines identified by GENELAND under a geographically-constrained spatial-uncorrelated allele frequency model with null alleles. (B) Arrows indicate significant migration rates between samples within the latitudinal interval 18–53°S on the Chilean coast. (C) The oceanographic map shows the circulation pattern and water column depth off the Chilean coast (after Pizarro, 2004).

**Table 3**
Pairwise estimates of differentiation ($D_{EST}$, above diagonal) and fixation index ($F_{ST}$, below diagonal) among 17 samples of *P. purpuratus*. Statistical significance (indicated with an asterisk) of $D_{EST}$ was derived from the probability *P* calculated after the false discovery rate approach (*p*BH, 2100 permutations, alpha = 0.002381). The significance threshold for $F_{ST}$ was generated after 100 MC batches of 5000 iterations each for alpha = 0.01. Non-significant pairwise comparisons are bolded.

| | Region 1 | | | | | Region 2 | | | Region 3 | | | | | Region 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ARI | IQU | WAY | BOL | TAL | TEM | GUA | PTA | MON | PTR | ILO | TUM | MEH | PUC | CHI | AYS | PAR |
| ARI | – | 0.044* | 0.114* | 0.115* | 0.089* | 0.098* | 0.118* | 0.122* | 0.064* | 0.142* | 0.097* | 0.121* | 0.172* | 0.492* | 0.493* | 0.381* | 0.448* |
| IQU | 0.019* | – | **0.040** | **0.047** | **0.033** | 0.057* | 0.086* | 0.072* | **0.026** | 0.070* | 0.055* | 0.076* | 0.130* | 0.515* | 0.497* | 0.351* | 0.427* |
| WAY | 0.040* | 0.016* | – | **0.018** | **0.022** | 0.073* | 0.107* | 0.075* | **0.035** | 0.087* | **0.044** | 0.076* | 0.131* | 0.514* | 0.502* | 0.361* | 0.409* |
| BOL | 0.036* | 0.016* | **0.005** | – | 0.056* | 0.074* | 0.096* | **0.066** | 0.058* | 0.108* | **0.048** | **0.046** | 0.081* | 0.448* | 0.442* | 0.322* | 0.382* |
| TAL | 0.037* | 0.015* | **0.007** | 0.015* | – | **0.040** | 0.057* | **0.031** | **0.002** | 0.049* | **0.020** | 0.061* | 0.118* | 0.504* | 0.490* | 0.358* | 0.403* |
| TEM | 0.041* | 0.025 | **0.032** | **0.020** | **0.017** | – | **0.031** | **0.016** | **0.032** | 0.029 | 0.075* | **0.034** | **0.071** | 0.462* | 0.419* | 0.276* | 0.373* |
| GUA | 0.050* | 0.041* | 0.048* | 0.028* | 0.028* | 0.009 | – | **0.014** | **0.054** | 0.070* | 0.073* | **0.035** | 0.075* | 0.477* | 0.428* | 0.301* | 0.398* |
| PTA | 0.055* | **0.041** | 0.039* | **0.022** | **0.018** | **0.006** | **0.003** | – | **0.028** | 0.070* | **0.068** | **0.041** | **0.021** | 0.422* | 0.389* | 0.290* | 0.341* |
| MON | 0.026* | **0.010** | 0.014* | 0.015* | **0.000** | **0.013** | **0.025** | **0.015** | – | **0.047** | **0.030** | **0.055** | 0.110* | 0.517* | 0.487* | 0.350* | 0.412* |
| PTR | 0.081* | 0.043* | 0.047* | 0.048* | **0.032** | **0.019** | 0.039* | 0.042* | **0.028** | – | 0.095* | 0.065* | 0.130* | 0.554* | 0.504* | 0.317* | 0.413* |
| ILO | 0.035* | 0.021* | 0.011* | **0.009** | 0.005* | **0.027** | 0.034* | **0.032** | 0.008* | 0.051* | – | **0.039** | 0.130* | 0.524* | 0.515* | 0.396* | 0.454* |
| TUM | 0.045* | 0.030* | 0.029* | **0.008** | 0.023* | 0.005* | 0.011* | **0.015** | 0.021* | 0.032* | **0.016** | – | **0.061** | 0.436* | 0.404* | 0.274* | 0.356* |
| MEH | 0.076* | 0.068* | 0.063* | **0.032** | 0.055* | 0.027 | 0.026* | **0.009** | 0.053* | 0.069* | 0.058* | **0.023** | – | 0.368* | 0.339* | 0.237* | 0.275* |
| PUC | 0.228* | 0.231* | 0.212* | 0.158* | 0.194* | 0.155* | 0.149* | 0.128* | 0.186* | 0.232* | 0.187* | 0.135* | 0.112* | – | **0.056** | 0.326* | 0.282* |
| CHI | 0.201* | 0.200* | 0.186* | 0.135* | 0.171* | 0.124* | 0.120* | 0.107* | 0.161* | 0.186* | 0.165* | 0.107* | 0.089* | **0.002** | – | **0.214** | 0.200* |
| AYS | 0.143* | 0.136* | 0.120* | 0.085* | 0.123* | 0.078* | 0.085* | 0.081* | 0.112* | 0.114* | 0.121* | 0.064* | 0.063* | **0.111** | **0.061** | – | **0.081** |
| PAR | 0.169* | 0.171* | 0.138* | 0.111* | 0.138* | 0.121* | 0.118* | 0.095* | 0.136* | 0.160* | 0.144* | 0.103* | 0.066* | **0.094** | **0.061** | **0.025** | – |

ences on population processes within stocks regarding parameters assuming HWE or using heterozygosities. Additionally, a Wahlund effect cannot be ruled out as co-responsible of such deficit since sporadic exchange among meta-population demes provides chances for admixture of drifted genetic backgrounds (see Fig. 2B). Inbreeding facilitated by a comparatively short larval dispersal period, is an unlikely explanation because of the external fertilization and the high fecundity in this species (Guiñez, 1996; Oyarzún Cabañas et al., 2010; Ramorino and Campos, 1979). Likewise, hitchhiking of present a priori neutral microsatellites to genes remains in theoretical realm (Nielsen et al., 2006) since simulations performed herein under the Stepwise Mutation Model gave no evidence of any kind of selection enforced on present markers after FDR corrections. Noteworthy, this result contrasts with the apparent selective scenario suggested upon variation of microsatellite *Pepu*1 within the (25–28°S) ecological discontinuity and putatively associated with mesoscale processes operating in this area (Briones et al., 2013). However, no selective role can be assigned to any microsatellite assayed in the present study. A parsimonious explanation to the selective role of *Pepu*1 at local scale but not so at global scale can simply be 'statistics in nature'. Since loci exhibiting outlier $F_{ST}$ are ranked upon the global $F_{ST}$ signal retrieved from the whole marker system (Antao et al., 2008), the higher molecular variance of locus *Pepu*2 (average $F_{ST} \pm SD = 0.089 \pm 0.089$) than locus *Pepu*1 (average $F_{ST} \pm SD = 0.077 \pm 0.073$) at global Chilean scale, overwhelms mathematically the observed deviation of *Pepu*1 at this and at narrower sample systems (e.g., Briones et al., 2013).

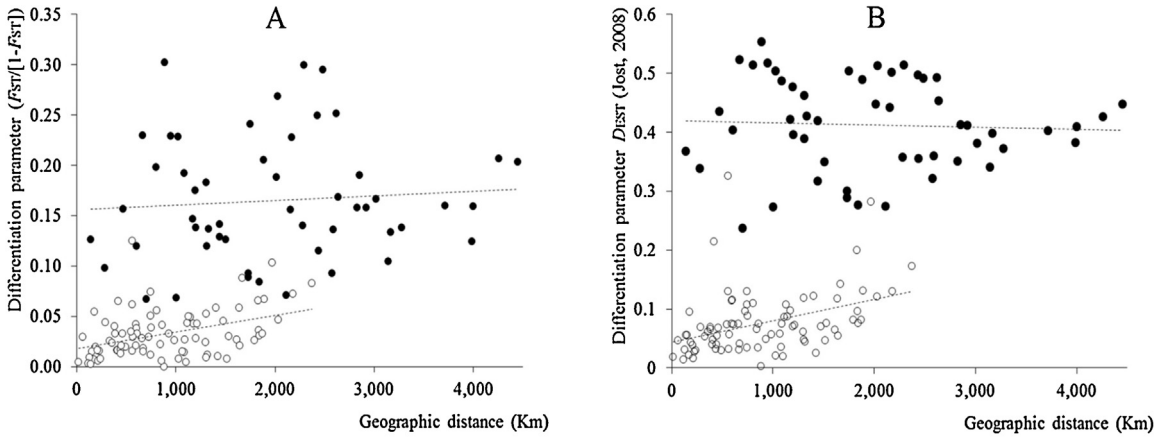


**Fig. 3.** Linear regression between the geographical distance (direct shoreline marine distance connecting sample locations) and (A) the differentiation parameter ($F_{ST}/[1-F_{ST}]$) or (B) the differentiation coefficient $D_{EST}$ (Jost, 2008), calculated upon five microsatellite loci. Closed circles represent pair-wise comparisons among samples between stocks separated by the 40°S split ($F_{ST}/[1-F_{ST}]$, $r^2 = 0.007$; SC-regression = 0.001; SC-residual = 0.186; $F = 0.338$; $p = 0.563$; $D_{EST}$, $r^2 = 0.0027$; SC-regression = 0.001; SC-residual = 0.309; $F = 0.133$; $p = 0.717$). Open circles correspond to pair-wise comparisons of samples within stocks, ($F_{ST}/[1-F_{ST}]$), $r^2 = 0.164$; SC-regression = 0.008; SC-residual = 0.039; $F = 16.088$; $p = 0.000$; $D_{EST}$; $r^2 = 0.151$; SC-regression = 0.037; SC-residual = 0.207; $F = 14.602$; $p < 0.001$).

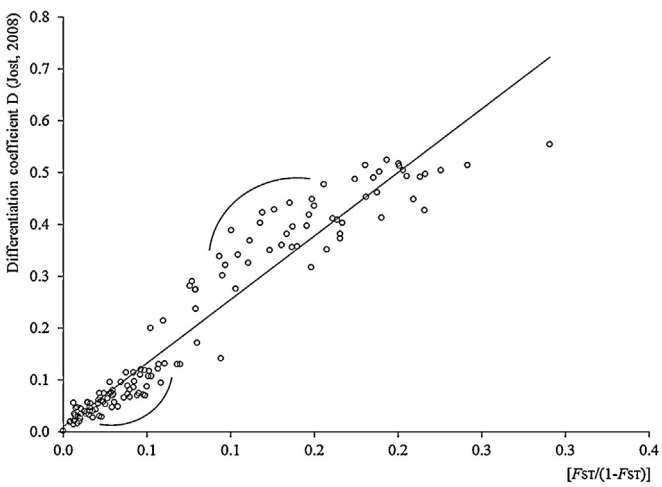


**Fig. 4.** Linear regression between the differentiation coefficient DEST and the Fixation index $F_{ST}$ between samples ($r^2 = 0.923$; $y = 2.452x + 0.0103$; $F = 2027.150$; $p < 0.001$).

### 4.2. Regional variation between stocks

The probability to rejecting panmixia among samples of *P. purpuratus* of the Chilean coast is supported by the statistical power of the sample/marker system under distinct priors and methods. Also present concordant results from mathematic and probabilistic methods are congruent with the phylogeography of other species from the southern Pacific coast of Chile (e.g., Fraser et al., 2010). The amount of molecular variation observed among samples ($F_{ST} = 0.071$) being double than that within regions ($F_{SC} = 0.026–0.037$), indicates that the component of variance between stocks adds significantly to such interpopulation divergence. Therefore, despite genetic divergence was significant among all the four regional partitions ($F_{CT} = 0.052$) most of it was due to the interstock pairwise comparisons (see $n' = 3$ from NCS in Table 2, $F_{CT} = 0.008^{ns}$) and therefore only the 40°S population breakpoint ($n = 2$, $F_{CT} = 0.104$) was genetically supported by all scenarios, (PCoA AMOVA, IBD, and GENELAND), i.e., a northern-central stock (NCS, from ARICA to MEHUÍN, ca. 2370 km of coastline) and a southern stock (SS, from PUCATRIHUE to PUNTA ARENAS, ca. 1800 km).

Congruent patterns of phylogeographic structure across taxa are particularly interesting since a given genetic break within species may arise by chance (Irwin, 2002) but concordant genetic breaks across species are a likely signal of a major barrier to gene flow (Kuo and Avise, 2005). For instance, the differentiation scenario of *P. purpuratus* in Southern Chile resembles the one of the rocky southern bull-kelp (*Durvillaea antarctica*) which shares range distribution with *P. purpuratus* along the Chilean coast (32–56°S). Two divergent population clusters have been documented using mtDNA COI and chloroplast DNA rbcl in *D. antarctica* (Fraser et al., 2010), i.e., a central Chilean clade (32–44°S) separated by a 44°S population break from a Patagonian clade (44–56°S). Although *D. antarctica* shows a genetic break at 44°S and *P. purpuratus* at 40°S, it is known that current locations of phylogeographical breaks may not reflect the sites where those breaks originated because asymmetric dispersal driven by ocean currents can shift the positions of genetic breaks or clines (Endler, 1977; Pringle and Wares, 2007). A major barrier to gene flow in *P. purpuratus* has been described using selective markers (see Briones et al., 2012; Trovant et al., 2015). In both studies, the analysis of functional mitochondrial and nuclear sequences showed that *P. purpuratus* comprises two divergent lineages termed North and South clades by Trovant et al. (2015). Present results obtained using statistically neutral microsatellites together with those reported by Briones et al. (2012) on sperm polymorphisms, strongly suggest that current stocks NCS and SS (North and South clades, sensu Trovant et al. (2015)) are in fact two sibling species.

The major effective barrier to gene flow operating at 40–44°S in several species is strongly influenced by the Humbolt current, in a similar manner as occurs in one of the sharpest thermal boundaries at Cape Hatteras (North Carolina) where 44% of the finfish species reported therein had their northern limit (McCartney et al., 2013) provoked by strong currents interacting with oceanic climates and physiographic factors (Gray and Cerame-Vivas, 1963; Pietrafesa et al., 1985). Therefore, much genetic differentiation appears to be ruled by major biogeographic breaks rather than by larval biology (Avise, 1992, 2000). However, not all biogeographic breaks are associated with strong genetic differentiation (Burton, 1998; Kelly and Palumbi, 2010). For instance, the strong biogeographic break at Point Conception (California) is well known by shifts in marine population genetics (e.g., Marko, 1998; Wares et al., 2001), but many species show little or no divergence in that region (see Burton 1998; Cassone and Boulding, 2006; Dawson, 2001). In an exten-

sive study combining mtDNA data from 34 invertebrate species and 16 previously published Kelly and Palumbi (2010) show that Cape Mendocino between Oregon and Monterrey can be considered as a barrier to genetic exchange but such divergence is stronger in species that inhabit high-intertidal areas and therefore it is a barrier dependent at least on the habitat depth.

An important question to elucidate is whether the above biogeographical break in *P. purpuratus* is a consequence of (a) historical geomorphological processes in southern Chile prompting historical speciation and reproductive incompatibility under post-speciation connectivity, (b) contemporary oceanography coupled with physiographic factors which hamper long distance dispersal (e.g., Pelc et al., 2009) or (c) a reduced species-specific dispersal potential. For instance, *D. antarctica* is a restricted disperser but it is buoyant and exhibits high dispersal potential once detached (Fraser et al., 2009), at least as much as it is expected in *P. purpuratus*. However, genetic and physiological evidence suggest that the separation between its stocks is the result of historical processes. The high genetic homogeneity observed among *D. antarctica* samples located south to 44°S strongly supports the hypothesis of postglacial recolonization after the Patagonian Ice Sheet during the Last Glacial Maximum, LGM (Fraser et al., 2009, 2010). Likewise, low levels of genetic structure have been observed in the limpet *Nacella magellanica* in the Patagonia area (Fraser et al., 2010; Hewitt, 1996, 2000), which are also observed in *P. purpuratus* from the SS stock (see Table 3), and suggest an interstock reproductive isolation after the last glacial maximum LGM. As a matter of fact, the distinct sperm morphology found between the two stocks of *P. purpuratus* and the dissimilarity of mtDNA genic sequences (16S, CO1, 18S and 28S) between them (Briones et al., 2012; Trovant et al., 2015) strongly support the hypothesis (a) outlined above.

### 4.3. Interpopulation variation

Panmixia as a reproductive model and SMM as an IBD pattern of connectivity are rejected at the macroscale (4450 km from the southern pacific coast) in *P. purpuratus*. In fact, Bayesian-inferred migration rates never crossed latitude 40°S which has been identified as a latitudinal break in all the analyses so far performed. Meanwhile, the consistent Mantel correlation observed within stocks is compatible with an IBD model of dispersal (see Fig. 3A and B). The directionally asymmetric m-rates heading south in the NCS stock cannot be explained by the large-scale northward oceanic flow of both regions (see Fig. 2C from Pizarro, 2004), which will probably have little bearing on dispersal of a species if short to moderate PLD (Pelagic Larval Duration). Instead, the pattern could be consistent with the much more complex coastal in which there can be extensive regions of the Chilean shore with southward-biased asymmetries in successful settlement, depending on PLD and depth at which larvae are found (Kelly and Palumbi, 2010; see also Fig. 6 and Fig. 7 in Aiken et al., 2011). Indeed, without more information on the ecology of the larval stages of *Perumytilus*, specially its vertical distribution through ontogeny, it is difficult to determine whether larvae could be influenced by the seasonally variable southerly flowing coastal currents such as the superficial Counter-Current of Peru (CCPC) located between HC and CCC and the Sub-superficial Current of Peru-Chile (CSPC), a pole-ward undercurrent heading south under the thermocline from 20 m depth (Aguirre et al., 2012; Strub et al., 1998), or by even more coastal processes, such as upwelling circulation and relaxation.

On the SS stock, if the opposite significant *m*-rates inferred were realistic (flowing southwards from PUC to CHI but northwards from PAR to AYS, see Fig. 2B) they cannot be explained by the same forces. Offshore southwards circulation pattern driven by the southern flow of the Humboldt Current branch or Channel Current is unlikely to affect this species dispersal. However, the complex circulation in this part of the fiords, e.g., an East-West sub-superficial current flowing around the South American tip or a northwards superficial wind-dependent current on the Chilean coast (Acha et al., 2004). Although the above currentological patterns could be made congruent with observed m-rates, the expected bias of present migration figures within stock due to a putative polymorphism underscoring and the lack of collateral support from in silico particle dispersal and from biological tracking systems do not allow to deep our knowledge on within stock dispersal beyond its fitting to an IBD-like model of connectivity.

### 4.4. Conclusion

The between-stocks historical speciation hypotheses is congruent with geological and fossil data, which suggest that *P. purpuratus* would have evolved in the Atlantic ca. 2,600,000 BP and colonized the Pacific coast round the South America tip, and expanded more recently to the Pacific coast up to Ecuador (Aguirre et al., 2006; Guzmán et al., 2000; Ortlieb et al., 1994). Geological and climatic historical events have shaped the ranges of species in the south-eastern Pacific, generating either reproductive isolation and/or physiographic phenomena that restricted dispersers cannot overcome nowadays driven by present-day coastal processes, i.e., the NCS and the SS of *P. purpuratus*, two putative LGM-issued subspecies.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fishres.2016.02.013.

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