



Scorched mussels (BIVALVIA: MYTILIDAE: BRACHIDONTINAE) from the temperate coasts of South America: Phylogenetic relationships, trans-Pacific connections and the footprints of Quaternary glaciations



Berenice Trovant^{a,*}, J.M. (Lobo) Orensanz^a, Daniel E. Ruzzante^b, Wolfgang Stotz^c, Néstor G. Basso^a

^a Centro Nacional Patagónico (CONICET), Boulevard Brown 2915, U9120ACF Puerto Madryn, Chubut, Argentina

^b Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada

^c Department of Marine Biology, Universidad Católica del Norte, Chile

ARTICLE INFO

Article history:

Received 1 April 2014

Revised 26 August 2014

Accepted 3 October 2014

Available online 14 October 2014

Keywords:

Phylogeography

Mussels

Dispersal

Glaciations

Southern South America

ABSTRACT

This study addresses aspects of the phylogeny and phylogeography of scorched mussels (BIVALVIA: MYTILIDAE: BRACHIDONTINAE) from southern South America (Argentina and Chile), as well as their ecophylogenetic implications. Relationships were inferred from sequences of two nuclear (28S and 18S) and one mitochondrial (COI) genes, using Bayesian and maximum likelihood analyses. Our results indicate that the monophyletic BRACHIDONTINAE include three well supported clades: [i] *Brachidontes Swainson* (= *Hormomya* Mörch), [ii] *Ischadium* Jukes-Browne + *Geukensia* van de Poel, and [iii] *Austromytilus* Laseron + *Mytilisepta* Habe (usually considered a member of the SEPTIFERINAE) + *Perumytilus* Olsson. Species of clade [iii] are distributed along the temperate coasts of the Pacific Ocean. Available evidence supports divergence between *Austromytilus* (Australia) and *Perumytilus* (South American) following the breakup of Australian, Antarctic and South American shelves. Four brachidontins occur in southern South America: *Brachidontes rodriguezii* (d'Orbigny), *B. granulatus* (Hanley), and two genetically distinct clades of *Perumytilus*. The latter are confined to the Chile-Peru (North Clade) and Magellanic (South Clade) Biogeographic Provinces, respectively warm- and cold-temperate. The South Clade is the only brachidontin restricted to cold-temperate waters. Biogeographic considerations and the fossil record prompted the hypothesis that the South Clade originated from the North Clade by incipient peripatric differentiation, followed by isolation during the Quaternary glaciations, genetic differentiation in the non-glaciated coasts of eastern Patagonia, back-expansion over southern Chile following post-LGM de-glaciation, and development of a secondary contact zone between the two clades in south-central Chile. Evidence of upper Pleistocene expansion of the South Clade parallels similar results on other organisms that have colonized coastal ecosystems from eastern Patagonia since the LGM, apparently occupying free ecological space. We emphasize that the assembly of communities cannot be explained solely in terms of environmental drivers, as history also matters.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

A fundamental question in evolutionary and conservation biology concerns the degree to which extant patterns of biological diversity and species abundance reflect the action of contemporary ecological processes vs. historical contingencies. This is the realm of the emerging field of ecophylogenetics, which incorporates the historical dimension into the ecological research agenda through

* Corresponding author.

E-mail addresses: trovant@cenpat.edu.ar (B. Trovant), lobo@uw.edu (J.M. (Lobo) Orensanz), Daniel.Ruzzante@dal.ca (D.E. Ruzzante), wstotz@ucn.cl (W. Stotz), nbasso@cenpat.edu.ar (N.G. Basso).

the widespread use of phylogenetic data (Mouquet et al., 2012). Here we investigate phylogenetic diversity in intertidal beds of “scorched mussels”, small-sized mytilid bivalves belonging to *Brachidontes* and related genera (BIVALVIA: MYTILIDAE: BRACHIDONTINAE; Supplementary Materials SM1), which are the dominant species in the mid-intertidal zone of rocky shores along extended stretches of coastline on both sides of temperate South America (Silliman et al., 2011; Tanaka and Magalhães, 2002; Thiel and Ullrich, 2002). While similar in appearance, these scorched mussel-dominated communities vary significantly in structure and organization. Predation by benthic invertebrates (e.g. starfish, snails), has been shown to be a main structuring factor on the Pacific (Navarrete and Castilla, 2003), but appears to be

of negligible significance on the Atlantic (Bertness et al., 2006; Olivier et al., 1966a), where the default hypothesis to explain weak predation invokes climate harshness. Also intriguing is the fact that scorched mussel beds are composed of different species that, when mixed, can be difficult to separate on the basis of phenotypic characters alone (Adami et al., 2013; van der Mollen et al., 2012), even if they belong to lineages with a long history of divergence (Trovant et al., 2013). Uniformity in the appearance of these communities may lead to a deceptive appearance of “community convergence” (Paine and Suchanek, 1983; Smith and Wilson, 2002), concealing a diversity of interactions between structuring ecological factors and historical drivers.

The three species of scorched mussels known to occur along the coasts of southern South America (Argentina and Chile; Fig. 1; Supplementary Materials SM2) belong to two genera: *Brachidontes* Swainson (two species) and the nominally monotypic *Perumytilus* Olsson. The monophyletic *Brachidontes* includes many species, all distributed in warm and warm-temperate seas world-wide (Lee and Ó Foighil, 2005; Trovant et al., 2013). *Perumytilus*, originally erected on the basis of ill-defined conchological characters (Adami et al., 2013), has been either retained as valid (e.g. Coan and Valentich-Scott, 2012, and many ecological studies) or considered as a junior synonym of *Brachidontes* s.l. (e.g. Huber, 2010; Adami et al., 2013).

The two species of *Brachidontes* s.s. that occur in southern South America are restricted to warm-temperate regions (Fig. 1): *B. rodriguezii* (d’Orbigny) on the Atlantic (32° to 43°S; Scarabino et al., 2006; Trovant et al., 2013) and *B. granulatus* (Hanley) on the Pacific (9° to 43°S; Bernard, 1983). The range of the third scorched mussel, *Perumytilus purpuratus* (Lamarck), spans the warm-temperate sector of the southeast Pacific (south of 3°S) and cold-temperate southern South America (north to 41°S on the Atlantic), respectively known as the Chile-Peru and Magellanic biogeographic provinces (Briggs and Bowen, 2013; Fig. 1). This is the only brachidontin known to inhabit cold-temperate waters. *Brachidontes rodriguezii* and *P. purpuratus* occur sympatrically between 41° and 43°S in the southwestern Atlantic, with one or the other dominating extensive intertidal beds respectively north and south of that latitudinal range. South of 50°S, as average monthly sea surface temperature drops below 12 °C and 5 °C during the warmest and coldest month, respectively, *Perumytilus* is replaced by *Mytilus* as the dominant component of intertidal mussel beds (M. Adami and S. Gordillo, pers. comm.). *Brachidontes granulatus* and *P. purpuratus* co-occur along the coasts of the Perú-Chile Province, but occupy different microhabitats. While *P. purpuratus* forms dense monocultures in the mid-intertidal zone over much of its latitudinal range, except in sectors where it is presumably controlled by predators, *B. granulatus* typically occurs in the intertidal and sub-

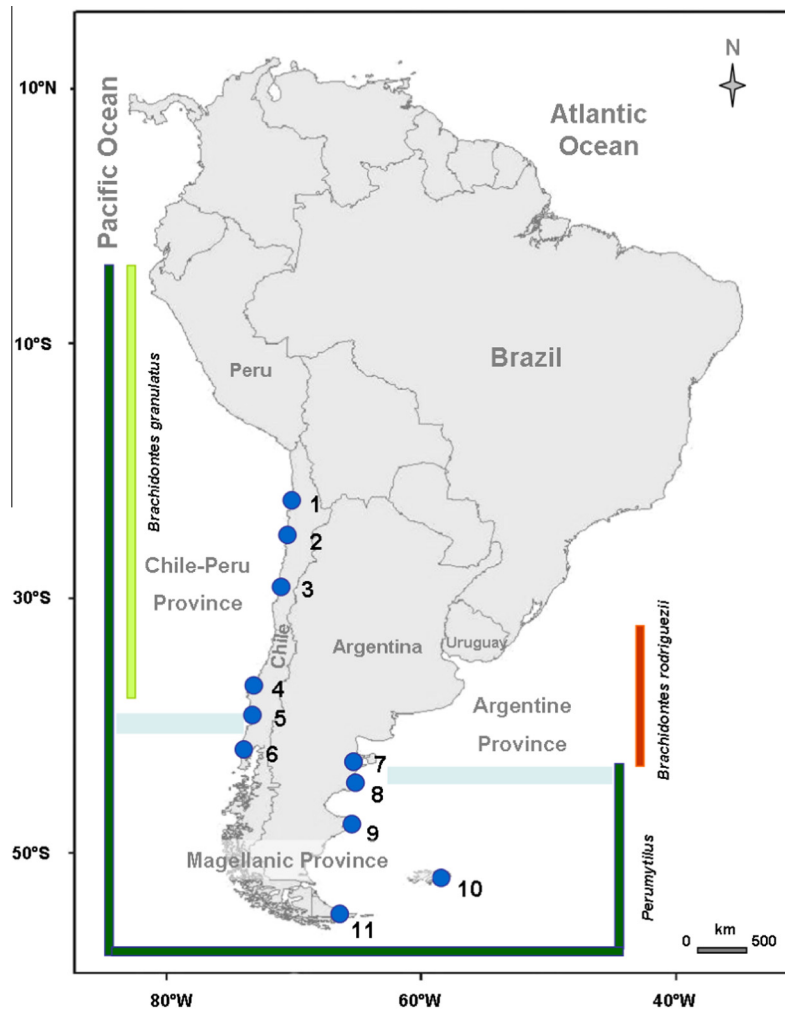


Fig. 1. Latitudinal range of scorched mussels present along the coasts of Chile and Argentina. Sampled locations: [1] San Marcos (Iquique), [2] La Chimba (Antofagasta), [3] Coquimbo, [4] Coliumo (Concepción), [5] La Misión, [6] Huinay, [7] Puerto Madryn, [8] Caleta Carolina, [9] Puerto Deseado, [10] Surfer Bay, [11] Bahía Ensenada (see Table 1 for more information).

tidal zones (down to 15 m), mostly in cryptic microhabitats: under rocks, in crevices, or among algal holdfasts.

While the distribution pattern of *Brachidontes* is consistent with hypothetical diversification in tropical marine regions (Briggs and Bowen, 2013), the phylogeographic relations of *Perumytilus* remain uncertain. The ecological literature indicates a remarkable similarity between intertidal mussel beds dominated by the monotypic South American *Perumytilus* and Australian *Austromytilus* Laseron, prompting the hypothesis of a phylogenetic relation between the two lineages. The relationships of *Austromytilus*, however, have not been investigated with molecular information. Connections of the southern South American biota have been influenced by major historical contingencies: the break-up of Gondwanaland (Sanmartín and Ronquist, 2004), the opening of the Drake Passage during the middle Eocene (Scher and Martin, 2006), the late Eocene opening of the Tasman gateway (Nelson and Cooke, 2001) and the subsequent establishment of a full circum-Antarctic circulation during the Oligocene, the consequent cooling of the Southern Ocean, and the Quaternary glaciations (Fraser et al., 2012). This suite of historical events defines the scenarios in which the phylogenetic and phylogeographic relations of *Perumytilus* are to be considered. An eventual relationship between *Perumytilus* and *Austromytilus* could be explained by early dispersion of a common ancestor followed by the breakup of southern shelves and their concomitant differentiation, or late dispersal by rafting once the circum-Antarctic circulation was established.

The geographical distribution of *P. purpuratus* around southern South America is puzzling for two reasons. First, *P. purpuratus* is the only species among the BRACHIDONTINAE that ranges extensively into cold-temperate waters. Second, while it ranges over the warm-temperate Pacific coasts of South America, this is not the case for the Atlantic coast, where it does not extend into the warm-temperate Argentine Biogeographic Province. At the time of the Last Glacial Maximum (LGM, ca. 23–25 cal. ka) the coasts of Chile south of approximately 43°S were covered by ice (Ruzzante et al., 2008, their Fig. 1). Glacial ice probably extended westward to the edge of the continental shelf, and likely extirpated many populations of rocky shore organisms. At the same time the coastline of the southwest Atlantic was located 200–400 km east of its current position depending on latitude (Ponce et al., 2011). Such contrasts in geological history between the coast of Chile and Argentina appear to be reflected in the phylogeography of taxa occurring across both regions (Ceballos et al., 2012; de Aranzamendi et al., 2011; González-Wevar et al., 2011, 2010; Ruzzante et al., 2011), including *P. purpuratus* (Trovant et al., 2013). We hypothesize that occurrence of *Perumytilus* in the cold-temperate Magellanic Province is indicative of a lineage adapted to a cold-temperate regime, followed by expansion into eastern Patagonia and incipient speciation. Trovant et al. (2013) found low genetic differentiation in *P. purpuratus* in the southwestern Atlantic, perhaps indicating late Quaternary population expansion, while the possibility of *P. purpuratus* actually including cryptic species was implied by Briones et al. (2012).

In this study we focus on the historical drivers underlying the apparent uniformity of scorched mussel-dominated intertidal assemblages around southern South America. Using one mitochondrial and two nuclear markers, we first evaluate a hypothetical sister relationship between *Austromytilus* and *Perumytilus*, which in turn demanded the clarification of phylogenetic relations among the BRACHIDONTINAE. Next we consider alternative phylogeographic hypotheses plausibly explaining the historical origin of *Perumytilus*. In third place we investigate the paradox of a species (*P. purpuratus*) spreading over two biogeographic provinces along the Pacific coast of South America, and only one on the Atlantic side. Evidence supporting the hypothesis of the existence of two genetically distinct clades and their recent demographic expansion

is presented, and a hypothesis is introduced to explain their historical origination and current distribution. Finally, using our study case as an illustrative example, we discuss the relevance of attending to historical contingencies when interpreting ecological processes.

2. Materials and methods

2.1. Specimens

Perumytilus individuals were collected from eleven locations ($N = 89$, Fig. 1, Table 1), six spread along the southeast Pacific from northern Chile (San Marcos, 20°12'S) to the Chiloé area (Huinay, 42°01'S), and five in the southwestern Atlantic, from the Beagle Channel (Bahía Ensenada, 54°49'S) and the Malvinas/Falkland Islands (Surfer Bay, 51°41'S) to Puerto Madryn (42°46'S, Argentine Patagonia). The samples from the Atlantic were used in an earlier study (Trovant et al., 2013). Specimens of the following species were also obtained for sequencing and observation of shell phenotypic characters (Supplementary Materials SM3): *Austromytilus rostratus* (W.R. Dunker) (Tasmania), *Brachidontes darwinianus* (d'Orbigny) (Uruguay), *B. erosus* (Lamarck) (Tasmania), *B. "exustus II" sensu Lee and Ó Foighil (2005)* (Venezuela), *B. granulatus* (Hanley) (Chile), *B. rodriguezii* (d'Orbigny) (Argentina), *Mytilisepta virgata* (Wiegmann) (Japan and Hong Kong), and *M. bifurcata* (Conrad) (California). All samples were fixed in 95% ethanol. In addition, sequences from related taxa were downloaded from GenBank (Table 2).

2.2. DNA extraction, amplification, sequencing and alignment

Doubly uniparental inheritance (DUI), whereby paternally inherited mitochondrial genome is present in the male germ line, has been shown to occur in several mytilids (Skibinski et al., 1994a, 1994b; Zouros et al., 1992; Rawson and Hilbish, 1995) but not in *Perumytilus* (Vargas Millacheo, 2009). Following Lee and Ó Foighil, 2004, we targeted DNA from the posterior adductor muscle to minimize the risk of confounding in the case of species where DUI cannot be disregarded. DNA was isolated using the phenol-chloroform protocol (modified from Sambrook et al., 1989, no final washing with chloroform). We used LCO1490/HCO2198 (Folmer et al., 1994) and CO1aF/CO1aR (Trovant et al., 2013), D23F/D6R (Park and Ó Foighil, 2000) and 22F/1789R (Medlin et al., 1988) to amplify one mitochondrial gene, cytochrome oxidase subunit I (COI), of 559 bp (aligned length), and two nuclear genes: the large ribosomal subunit (28S), of 813 bp (aligned length), and the small subunit rRNA (18S), of 1627 bp (aligned length). Additional primers -18S-1F, 18S-2F, 18S-3F, 18S-4F, 18S-1R, 18S-2R and 18S-3R (Goto et al., 2011)- were used for sequencing the 18S rRNA gene. When possible we sequenced ten specimens per locality for COI and two specimens per locality for 28S and 18S. In total we obtained 88 sequences (55 for COI, 21 for 28S and 12 for 18S). To amplify the genes we used Tsg polymerase (Bio Basic Inc., Canada). The protocol used included an initial denaturing temperature of 95 °C for 5 min; followed by 30 cycles of 95 °C for 45 s; an annealing temperature of 45 °C for COI, and 52 °C for 28S and 18S for 1 min; 72 °C for 1 min; and a final extension at 72 °C for 10 min. After extraction and amplification the DNA was visualized by UV transillumination in 1% agarose gels stained with green gel (BIOTUM). Extractions and amplifications of DNA samples were performed in the Gene Probe Laboratory of Dalhousie University (Nova Scotia, Canada) and in the Laboratory of Molecular Biology (CENPAT, Argentina), while the purification of PCR products and sequencing of both strands of DNA were done at CENPAT or by MacroGen Inc. (Maryland, USA), using for COI and 28S the same

Table 1

Locations where samples were collected for this study, including those of Trovant et al. (2013).

Species	Sample location	Latitude/Longitude
<i>Brachidontes exustus</i>	Laguna Chacopata, Peninsula de Araya, Venezuela	10°39'N, 63°49'W
<i>Brachidontes erosus</i>	Taroona, Tasmania, Australia	42°56'S, 147°20'E
<i>Brachidontes granulatus</i>	Coquimbo, Region IV, Chile	29°57'S, 71°20'W
<i>Brachidontes rodriguezii</i>	Santa Clara del Mar, Buenos Aires, Argentina	37°50'S, 57°30'W
	Bahia San Blas, Buenos Aires, Argentina	40°32'S, 62°15'W
	Bahia Rosas, Río Negro, Argentina	41°01'S, 64°06'W
	Puerto Madryn, Chubut, Argentina	42°46'S, 65°00'W
<i>Perumytilus purpuratus</i>	San Marcos, Iquique, Region I, Chile	20°12'S, 70°09'W
	La Chimba, Antofagasta, Region II, Chile	23°33'S, 70°23'W
	Coquimbo, Region IV, Chile	29°57'S, 71°20'W
	Coliumo, Concepción, Region VIII, Chile	36° 49'S, 73°03'W
	La Misión, Valdivia, Región X, Chile	39°47'S, 73°23'W
	Huinay, Chiloé, Region X, Chile	42°01'S, 72°42'W
	Puerto Madryn, Chubut, Argentina	42°46'S, 65°00'W
	Caleta Carolina, Camarones, Chubut, Argentina	44°47'S, 65°43'W
	Puerto Deseado, Santa Cruz, Argentina	47°44'S, 65°53'W
	Surfer Bay, Malvinas/Falkland I.	51°41'S, 57°46'W
	Bahía Ensenada, Tierra del Fuego, Argentina	54°49'S, 68°15'W
<i>Austromytilus rostratus</i>	Taroona, Tasmania, Australia	42°56'S, 147°20'E
<i>Mytilisepta virgata</i>	Shek O, Hong Kong	22°13'N, 114°15'E
	Okinawa Prefecture, Japan	26°38'N, 128°01'E
<i>Mytilisepta bifurcata</i>	Alegria Beach, Hollister Ranch, California, United States	34°28'N, 120°17'W

primers as in the amplification. DNA sequence data were edited in CodonCode Aligner v 2.0.4 and aligned using default parameters with Clustal W (Thompson et al., 1994). All DNA sequences were deposited in GenBank under the Accession Numbers KJ453810–KJ453888 and KJ598044–KJ598052.

2.3. Phylogenetic analyses and divergence time estimation

Prior to analyses, we tested for third codon saturation of mitochondrial sequences; a test of substitution saturation (Xia and Lemey, 2009) was performed in DAMBE v5 (Xia, 2013). Subsequently, tree phylogenies of the BRACHIDONTINAE based on a mitochondrial (COI) and two nuclear (18S and 28S rDNA) markers were constructed. Non-mytilid pteriomorphs (*Atrina*, PINNIDAE, and *Crassostrea*, OSTREIDAE) and *Mytilus* spp. (MYTILINAE) were selected as outgroups (Table 2). Also included in our analyses were two species of *Mytilaster* (Monterosato, 1884), *Ischadium recurvum* (as MYTILINAE in Huber, 2010) and *Geukensia* spp. (BRACHIDONTINAE), the latter two used as outgroups by Lee and Ó Foighil (2005) in their study of the *Brachidontes* 'exustus' complex.

Two methods were utilized for phylogenetic reconstruction: Maximum Likelihood (ML) and Bayesian Inference (BI). The Akaike Information Criterion (AIC), implemented in jModelTest v 2.1.5 (Darrriba et al., 2012), was applied to find the models of evolution that best fit the data. The selected models were used in ML analyses of nuclear and mitochondrial datasets, conducted with RAXML 7.4.2 (Stamatakis, 2006), implemented in raxmlGUI 1.3 (Silvestro and Michalak, 2012); a rapid bootstrapping analysis with 1000 replicates was conducted. Phylogenies reconstructed with BI were estimated with different substitution (HKY+G+I, Hasegawa et al., 1985, and GTR+G+I, Tavaré, 1985) and clock (strict and relaxed) models. The marginal-likelihood scores of the posterior distributions were compared using Bayes Factors (BFs, Kass and Raftery, 1995) with two different methods: harmonic mean estimation (HME, Newton and Raftery, 1994) and a posterior simulation-based analog of the Akaike information criterion through Markov chain Monte Carlo analysis (AICM, Raftery et al., 2007) implemented in Tracer v1.6 (Rambaut et al., 2014). Bayesian reconstructions were conducted under two tree priors (Yule and birth–death processes) using BEAST v. 1.8.0 (Drummond et al., 2012) with a Markov Chain Monte Carlo (MCMC) simulation for 20 million of generations for

the two nuclear phylogenies and 80 million generations for the mitochondrial dataset, sampling trees every 1000 generations with a burn-in of 25%. Convergence diagnostics were conducted in Tracer and reliable ESS values (>200) were ensured. Then, the maximum credibility tree was generated from the combined trees in TreeAnnotator v 1.6.1 (Drummond et al., 2012). Finally, the edition of the trees was carried out in Figtree v 1.4 (Morariu et al., 2008).

The substitution rate and divergence times among *Austromytilus*, *Perumytilus* and *Mytilisepta*, and between *Perumytilus* clades were estimated from the COI dataset using BEAST, together with the phylogenetic reconstruction. We used two calibrations points, the closure time of the Panama Isthmus (Lessios, 2008) and the divergence time between the *M. edulis* species complex and *M. californianus* (Ort and Pogson, 2007). Isthmian calibrations assume that morphologically similar 'geminant' species living now on either side of the isthmus were isolated geographically by the latest stages of seaway closure (Marko, 2002). This calibration point was applied by setting the time of the split between *Brachidontes* 'exustus II' (Lee and Ó Foighil, 2005) and *B. semilaevis* geminate lineages at 3.1–3.5 Mya [prior distribution = normal, mean = 3.3 Mya, log (SD) = 0.2]. Ort and Pogson (2007) estimated the divergence time between the *M. edulis* complex and *M. californianus* at 7.61 Mya for COX3. To estimate the node age for our mitochondrial gene (COI) we followed the same steps, using the COI sequences available in Genbank. Assuming a divergence time of 3.5 million years between *M. trossulus* and *M. edulis*/*M. galloprovincialis* (Wares and Cunningham, 2001), we estimate a first and second positions substitution rate of 1.78E–8 for COI ($\mu = \frac{1}{2} d/3.5E6$ Mya, $d = 0.125$, genetic distance). Using the genetic distance between the *M. edulis* complex and *M. californianus* ($d = 0.333$) and the calculated μ , we estimated a divergence time between the *M. edulis* complex and *M. californianus* of 9.35 Mya for COI. This calibration point was applied by setting the time of the split between *M. edulis* and *M. californianus* lineages [prior distribution = normal, mean = 9.35 Mya, Log (SD) = 1].

2.4. Genetic structure and diversity of *Perumytilus*

A maximum-parsimony COI haplotype network was constructed to represent the spatial distribution of haplotypes, using the median joining algorithm (Bandelt et al., 1999) with the MP

Table 2
Sequences used in this study.

Species	COI	28S rRNA	18S rRNA	References
<i>Atrina pectinata</i>	HM015792.1	HM015782.1	EF613241.1	Xue et al. (2012) (COI and 28S) and Wang and Gao (unpublished)
<i>Crossostrea gigas</i>	DQ659371.1	AB105362.1	AB064942.1	Cardoso et al. (unpublished), Hosoi et al. (2004), and Itoh et al. (unpublished)
<i>Austromytilus rostratus</i>	KJ453834.1, KJ453835.1	KJ453828.1–KJ453829.1	KJ453811.1–KJ453812.1	This study
<i>Brachidontes adamsianus I</i>	AY825164.1	AY825086.1	–	Lee and Ó Foighil (2005)
<i>Brachidontes adamsianus II</i>	–	AY825100.1	–	Lee and Ó Foighil (2005)
<i>Brachidontes darwinianus</i>	KC844414.1	KC844370.1	–	Trovant et al. (2013)
<i>Brachidontes dominguensis</i>	–	–	AF117736.1	Distel (2000)
<i>Brachidontes erosus</i>	–	KJ453827.1	KJ453810.1	This study
<i>Brachidontes exustus I</i>	AY825216.1	AY825104.1	–	Lee and Ó Foighil (2005)
<i>Brachidontes exustus II</i>	AY621900.1	AY825104.1	AF229623.1	Lee and Ó Foighil (2004) and Campbell (2000)
<i>Brachidontes exustus III</i>	AY621913.1	AY621993.1	KJ453813.1	Lee and Ó Foighil (2004); This study
<i>Brachidontes granulatus</i>	KJ453888.1	KJ453823.1	DQ640532.1	This study, Santaclara et al., (2006)
<i>Brachidontes modiolus</i>	AY621916.1	AY622002.1	–	Lee and Ó Foighil (2004)
<i>Brachidontes mutabilis</i>	GQ480310.1	AB103124.1	AB201233.1	Liu and Li, unpublished; Hashimoto and Matsumoto, unpublished; Owada (2007)
<i>Brachidontes pharaonis</i>	DQ836013.1	–	–	Terranova et al. (2006)
<i>Brachidontes rodriguezii</i>	KC844478.1, KC844455.1, KC844463.1, KC844474.1	KC844460.1	DQ640530.1	Trovant et al. (2013) and Santaclara et al. (2006)
<i>Brachidontes semilaevis</i>	AY825117.1	AY825089.1	–	Lee and Ó Foighil (2005)
<i>Brachidontes variabilis</i>	DQ836021.1	AY825102.1	AJ389643.1	Terranova et al. (2006), Lee and Ó Foighil (2005), and Steiner and Hammer (2000)
<i>Geukensia granossissima</i>	AY621927.1	AY622006.1	–	Lee and Ó Foighil (2004)
<i>Geukensia demissa</i>	–	–	L33450.1	Lee and Ó Foighil (2004)
<i>Ischadium recurvum</i>	AY621928.1	AY622008.1	–	Lee and Ó Foighil (2004)
<i>Mytilaster minimus</i>	DQ836022.1	–	–	Terranova et al. (2006)
<i>Mytilaster solidus</i>	JF496771.1	–	–	Plazzi et al. (2011)
<i>Mytilisepta bifurcata</i>	–	KJ453830.1–KJ453831.1	KJ453814.1–KJ453815.1	This study
<i>Mytilisepta virgata</i>	AB076941.1	KJ453832.1–KJ453833.1	KJ453816.1–KJ453817.1	Matsumoto (2003); This study
<i>Mytilus edulis</i>	AY484747.1	Z29550.1	L33448.1	Boore et al. (2004), Littlewood (1994), and Kenchington et al. (1995)
<i>Mytilus californianus</i>	U68777.1	–	–	Hoeh et al. (1997)
<i>Mytilus galloprovincialis</i>	–	AB105357.1	L33451.1	Hosoi et al. (2004) and Kenchington et al. (1995)
<i>Perumytilus purpuratus</i>	KC844415.1–KC844453.1; KJ453836.1–KJ453887.1	KC844374.1–KC844388.1; KJ453824.1–KJ453826.1, KJ598044.1–KJ598052.1	KJ453818.1–KJ453821.1	Trovant et al. (2013); This study
<i>Septifer bilocularis</i>	–	AJ307535.1	AF229622.1	Steiner and Hammer (2000) and Campbell (2000)

calculation to reduce unnecessary median vectors and links, and a ratio of transversions/transitions of 3:1 (recommended for mitochondrial DNA); default values in Network v 4.6.1 (Polzin and Daneschmand, 2003) were kept for the rest of the parameters. In addition, to determine the partition that maximizes the differences among groups an analysis of molecular variation (AMOVA; Excoffier et al., 1992) was implemented in Arlequin v 3.5 (Excoffier and Lischer, 2010). The AMOVA was performed based on a distance matrix of pairwise differences. The significance of Φ_{ST} , Φ_{SC} and Φ_{CT} , the covariance components associated with the different possible levels of genetic structure (within individuals, among populations, among groups), was tested using non-parametric permutation procedures with 10,000 iterations.

After identifying lineages using phylogenetic methods, standard diversity indices such as the number of polymorphic sites (S), number of haplotypes (k), haplotype diversity (H_d), nucleotide diversity (π) and mean number of pairwise differences (Π) were estimated for each population and clade using Arlequin. Since sampling sites are far enough apart from each other, each sampling site was taken as a distinct population. In addition, to test levels of among-population genetic differentiation, pairwise Φ_{ST} for mtDNA COI based on 10,000 permutations was estimated within each clade with Arlequin. The significance of pairwise comparisons between populations was tested applying Holm's (1979) sequential Bonferroni correction. Estimates of evolutionary divergence over sequence

pairs within and between groups were calculated using p -distance in MEGA v5 (Tamura et al., 2011).

2.5. Demographic history of *Perumytilus*

The hypothesis of neutrality, assuming constant population size and neutral variation, was examined with three tests: Fu's F_s (Fu, 1997), Tajima's D (Tajima, 1989) and R_2 (Ramos-Onsins and Rozas, 2002), performed on COI sequences using DnaSP v 5.10 (Librado and Rozas, 2009). Although only little structure was found within groups, indexes were calculated by both population and clade. Selective sweeps and selection against slightly deleterious mutations can result in a pattern of haplotype diversity similar to that produced by population expansion (Bertorelle and Slatkin, 1995). Since these analyses cannot differentiate between the effects of demographic processes and natural selection, a Mc Donald-Kreitman test (Mc Donald and Kreitman, 1991) was performed. This test is based on a contingency table of counts of polymorphic (within species) and fixed (between species) sites involving both non-synonymous and synonymous sites. By directly comparing the number of detected differences, the test indirectly compares the rate of evolution on the between-species tree with the rate on the within-species trees. If the nonsynonymous ratio is much higher than the synonymous one, then there is an excess of nonsynonymous fixed differences, indicative of positive selection,

while a deficit indicates negative selection. In addition, mismatch distribution analysis (Rogers and Harpending, 1992) was used to visualize the signature of the expansion, and the Harpending's raggedness index (r) (Harpending et al., 1993) was calculated to quantify the smoothness of the observed distribution. In a population that has been stationary for a long time these distributions from non-recombinant DNA sequences become ragged and erratic, whereas a population that has been growing generates mismatch distributions that are smooth and unimodal (Harpending, 1994). In expanding populations the raggedness value is low and non-significant, while it is usually high and significant in stationary populations. Furthermore, past population dynamics through time was estimated in *Perumytilus* using a Bayesian skyline plot method implemented in BEAST. The number of discrete changes allowed in the population history ("groups") was 7 and 9 for North and South clades respectively, and the skyline model chosen was piecewise-linear. The length of the chain was 80 million of generations and was sampled every 8000. A relaxed clock model using the estimated mutation rate for the *Perumytilus* clade was computed for each clade with a burn-in of 10%. The convergence of runs and reliable ESS values (>200) were confirmed in Tracer. Additionally, in order to test the potential pattern of isolation by distance (Slatkin, 1993), a Mantel test (Mantel, 1967) was conducted for each clade using the software XLSTAT v 2013.4 (Addinsoft, 2013).

2.6. Latitudinal gradients of sea-surface temperature

Latitudinal profiles of climatological sea-surface temperature (SST) were compiled for both coasts of southern South America, from 56°S (Cape Horn) to 20°S using the online PO.DAAC Ocean ESIP Tool (POET). Monthly average SST was calculated for the warmest and coldest months (respectively February and August) of 2008–2010. The two extreme monthly means are denoted, respectively, as SST_{max} and SST_{min} .

3. Results

3.1. Phylogeny of the BRACHIDONTINAE

The General Time Reversible model (GTR+G+I) was selected for ML estimation with the mitochondrial dataset based on the AIC criterion. The AIC weight of the selected model was 0.99. Comparing the first two models of substitutions in the rank generated by jModeltest (GTR+G+I and TVM+G+I) with the decision table of Kass and Raftery (1995), we obtained decisive evidence against TVM+G+I ($\Delta AIC = 16$). For the 18S dataset, the model with the lowest AIC in the rank was the GTR+GGTR+G+I with a weight of 0.35. Comparing the first two models of substitutions in the rank (GTR+G+I and SYM+G+I) we obtained a ΔAIC of 1.45, implying strong evidence against SYM+G+I. In the case of the 28S dataset, the GTR+G+I (third position in the AIC rank produced by jModeltest) was chosen; the two models ranking at the top are not available in raxmlGUI. In the case of Bayesian inference, Bayes factors based on HME and AICM favored the GTR+G+I and the relaxed clock models over the HKY+G+I and the strict clock models for the three genes (Supplementary Materials SM4). Divergence times were estimated using the COI dataset for the selected models, GTR+G+I and relaxed clock, under two different tree priors (Yule and birth-death processes).

The trees based on maximum likelihood and Bayesian analyses of the 18S and 28S sequences (Fig. 2) and the mitochondrial COI gene (Supplementary Materials SM5) show three well supported clades: [i] *Austromytilus* + *Mytilisepta* + *Perumytilus* ("AMP clade"), [ii] *Geukensia* + *Ischadium*, and [iii] *Brachidontes* s.l. (including *Hormomya*). While the separation of the three genera in the AMP clade is well supported, no clear genus-level groups are supported within the *Brachidontes* s.l. clade. The divergence time estimated for the AMP clade was relatively insensitive to the prior of choice: 13.35 Mya (95% HPD: 4.07–27.97 Mya) under the Yule prior, and

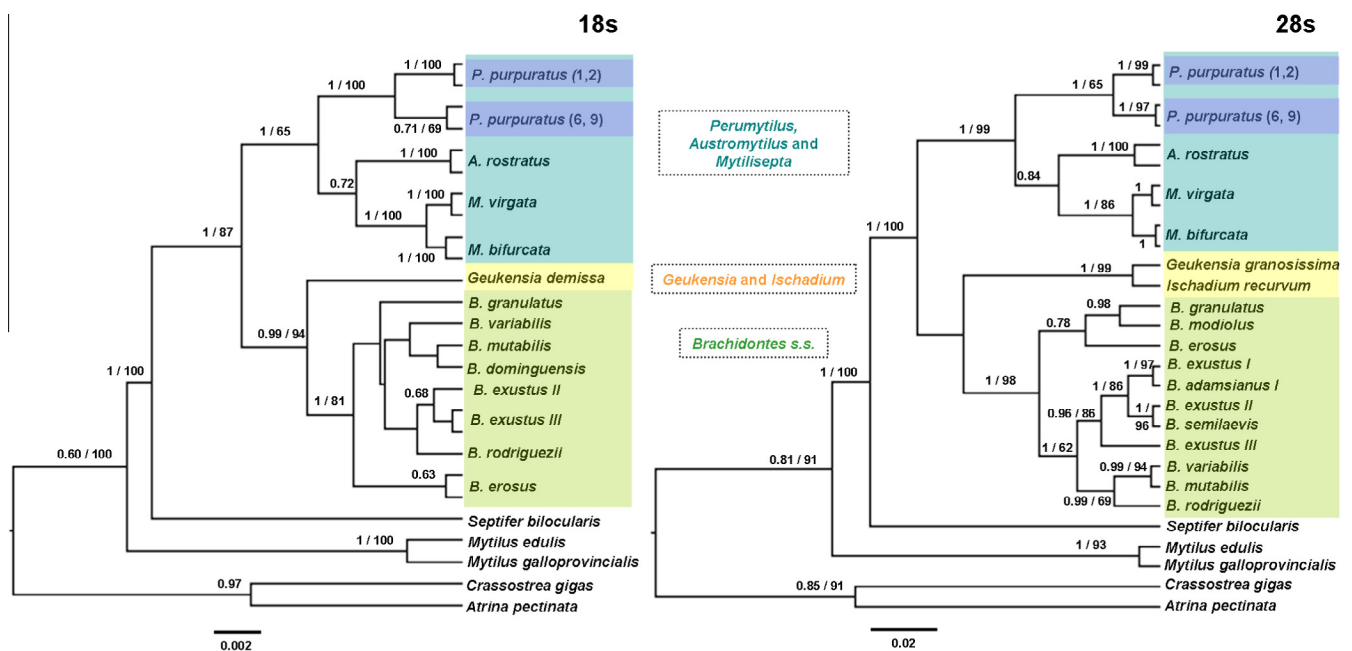


Fig. 2. Bayesian trees for the BRACHIDONTINAE based on nuclear genes 18S and 28S rDNA, using *Atrina pectinata*, *Crassostrea gigas*, *Mytilus edulis* and *M. galloprovincialis* as outgroups. Numbers above the branches represent the Bayesian posterior probabilities/ML bootstrap values (>60 only) for the supported nodes. In the case of *Perumytilus*, numbers in parentheses following species name indicate the code of the locality of the sequences (see Fig. 1, Table 1). Three well supported clades are highlighted: *Austromytilus*-*Mytilisepta*-*Perumytilus* (AMP), *Geukensia*-*Ischadium* and *Brachidontes* s.s.

11.10 Mya (95% HPD: 1.23–27.73 Mya) under a birth–death tree prior. These estimates place the expected divergence among *Austroromytilus*, *Mytilisepta* and *Perumytilus* in the Middle Miocene.

3.2. Genetic structure and diversity of *Perumytilus*

We used a total of 89 sequences of the COI mitochondrial gene (52 sequences newly generated for this study and 37 previously used in Trovant et al., 2013) and 27 sequences of the 28S rRNA gene (12 sequences newly generated for this study and 15 from Trovant et al., 2013). These samples originated from eleven localities spread over the species' distribution range. Among the 559 bp of the alignment length of COI we found 81 polymorphic sites (14.5%); of those, 30 were singleton variable sites (37%) and 47 were parsimony informative sites (63%). A total of 66 haplotypes were obtained from all the sequences. Concerning the saturation test, the observed saturation indices (I_{ss_o}) were significantly lower than the expected indices (I_{ss_e}) (I_{ss_o} : 0.18–0.20 < I_{ss_e} : 0.68–0.78; $p < 0.05$) suggesting that saturation on the mitochondrial dataset was negligible. Based on this result we proceeded with analyses using the complete alignments. No indels or stop codons were detected in the complete data set. The sampled haplotypes clustered in two groups separated by 19 substitutions (Fig. 3). From here on, we will refer to these two groups as the North and South clades. The North Clade includes sequences from San Marcos (20°12'S) to Coliumo (36°49'S) (Chile), and the South Clade includes sequences from La Misión (39°47'S) (Chile) to Puerto Madryn (42°46'S) (Argentine Patagonia). The genealogies of the two clades were star-like (Fig. 3), with one very frequent central haplotype (number 3) in the case of the North Clade, and more expanded, with two very frequent haplotypes (number 26 and 39) in the South Clade.

Results of the AMOVA conducted with mitochondrial (COI) and nuclear (28S) markers were consistent with each other; the null hypothesis of panmixia was rejected in both cases. AMOVA

analyses indicated that 82.48% (COI) and 98.22% (28S) of *Perumytilus* genetic variation was due to the partition into North and South clades, suggesting genetic structuring within *Perumytilus*. The fraction of genetic variation explained by partition among populations was only 0.61% for COI and 1.78% for 28S rDNA; partition within populations explained 16.91% of the genetic variation for COI, and 0.00% for 28S rDNA. The geographic groups recovered in this analysis (i.e. North and South clades) were considered in all the demographic analyses described below.

Although haplotype diversity was comparable between clades, the number of polymorphic sites and haplotypes, nucleotide diversity and mean number of pairwise differences were slightly higher in the South Clade (Table 3). Only the most isolated populations in the North Clade showed some genetic differentiation suggested by the significant Φ_{ST} values (San Marcos and Coliumo, Table 4). Within the South Clade, populations showed low and non-significant pairwise Φ_{ST} values (Table 5). The North and South clades showed an interlineage divergence of 5.8% (S.E.= 0.010) and an intralineage divergence of 0.8% (S.E.= 0.001) and 1.5% (S.E.= 0.002), respectively.

3.3. Demographic history of *Perumytilus*

Neutrality (constant population size and neutral variation) was tested in each clade at the population level. However, due to lack of structuring within groups, and given the low number of specimens sequenced per population, only the results calculated at the clade level were further considered (Table 6). The indices agree in the rejection of the neutrality hypothesis for both clades. Fu's F_s values were negative and significant, evidence for an excess number of allele classes, as would be expected from a recent population expansion or from gene hitchhiking. In the case of Tajima's D test, the observed negative values suggest either population expansion or purifying selection. In addition, low values of R_2 also suggest recent population expansion. A mismatch distribution analysis

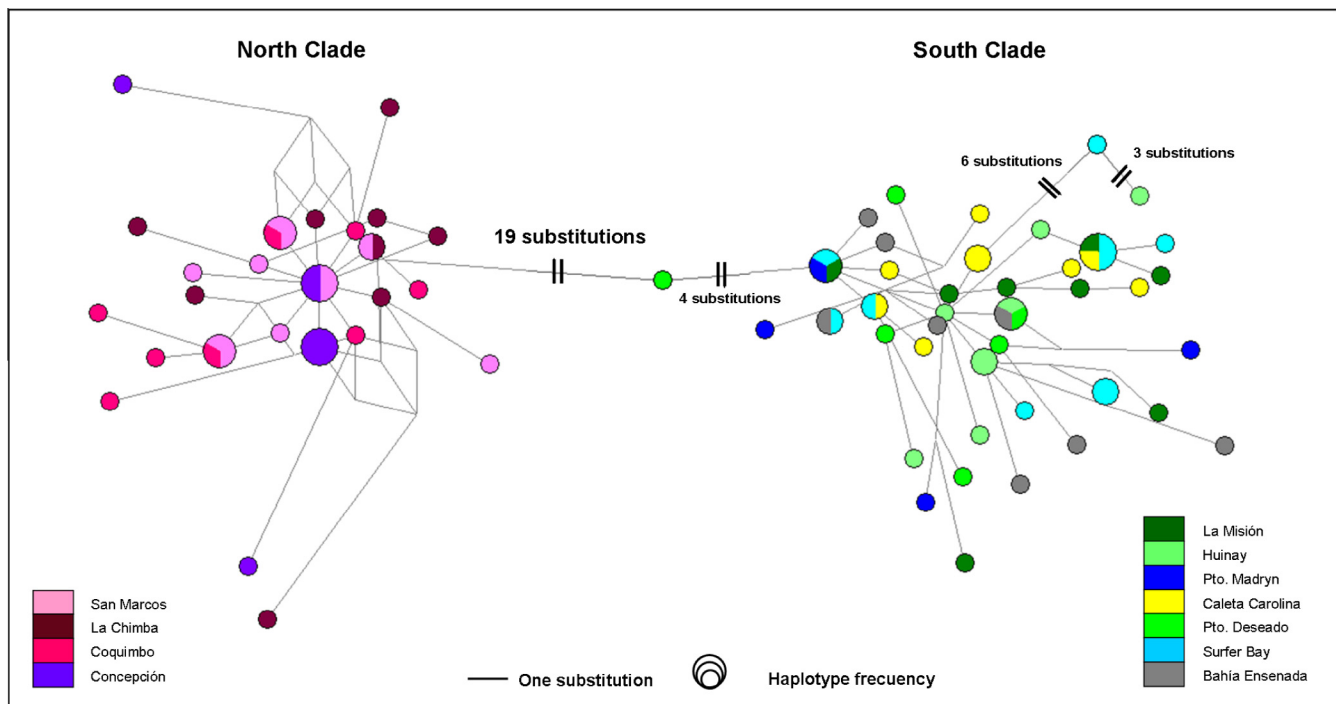


Fig. 3. Median joining haplotype network of *Perumytilus* based on COI mitochondrial gene. Each haplotype is represented by a circle whose size is proportional to its frequency; colors indicate locality of origin. See Fig. 1 and Table 1 for information about locations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Genetic diversity indices of *Perumytilus* based on mtDNA (COI) sequences. *N*: number of specimens analyzed; *S*: number of polymorphic sites; *k*: number of haplotypes; Hd: haplotype diversity; π : nucleotide diversity; II: mean number of pairwise differences; SD: standard deviation.

Locality	<i>N</i>	<i>S</i>	<i>k</i>	Hd \pm SD	$\pi \pm$ SD	II \pm SD
1- San Marcos	11	9	8	0.94 \pm 0.05	0.004 \pm 0.003	2.32 \pm 1.37
2- La Chimba	9	18	9	1.00 \pm 0.04	0.010 \pm 0.006	4.83 \pm 2.60
3- Coquimbo	8	15	8	1.00 \pm 0.06	0.009 \pm 0.005	4.53 \pm 2.49
4- Coliumo	8	13	4	0.89 \pm 0.11	0.007 \pm 0.004	3.89 \pm 2.18
North Clade	36	35	25	0.97 \pm 0.01	0.008 \pm 0.001	3.69 \pm 1.30
5- La Misi3n	8	17	8	0.98 \pm 0.007	0.010 \pm 0.006	5.57 \pm 2.99
6- Huinay	8	16	7	0.96 \pm 0.07	0.009 \pm 0.005	4.35 \pm 2.40
7- Pto. Madryn	4	10	4	1.00 \pm 0.17	0.010 \pm 0.007	5.33 \pm 3.25
8- Caleta Carolina	9	14	8	0.97 \pm 0.06	0.008 \pm 0.005	4.44 \pm 2.41
9- Pto. Deseado	6	15	6	1.00 \pm 0.09	0.010 \pm 0.006	5.40 \pm 3.02
10- Surfer Bay	10	22	8	0.97 \pm 0.05	0.011 \pm 0.007	6.35 \pm 3.29
11- Bah3a Ensenada	8	17	8	1.00 \pm 0.06	0.009 \pm 0.006	4.92 \pm 2.68
South Clade	53	56	41	0.98 \pm 0.007	0.011 \pm 0.0008	5.16 \pm 1.78

Table 4

Perumytilus, North Clade. Pairwise Φ_{ST} comparisons (below diagonal) and approximate distance among populations, in km (above diagonal). In the case of the pairwise Φ_{ST} comparisons, *p*-values uncorrected and after Holm–Bonferroni corrections are shown to the left and right of the slashbar, respectively. (*): Statistically significant differences (*p*-value lower than its corrected value).

North Clade	1	2	3	4
1- San Marcos	–	383.12	1090.59	1859.40
2- La Chimba	0.037 (0.10/0.01)	–	707.91	1477.72
3- Coquimbo	0.000 (0.41/0.05)	0.056 (0.05/0.03)	–	770.79
4- Coliumo	0.097 * (0.006/0.008)	0.028 (0.16/0.02)	0.108 (0.013/0.010)	–

was used to visualize evidence of the population expansion suggested by the other indices. The two clades showed smooth unimodal distributions (Fig. 4), a frequent pattern under a population expansion model. These outcomes were also supported by low and non-significant Harpending's Raggedness indices. The estimated mutation rate of the *Perumytilus* clade was 0.19 substitutions/Myr. Assuming this rate, the population expansion of the North Clade would have started 15 ka (end of the Pleistocene), and continued through Holocene and Recent times (Fig. 4). In the case of the South Clade, the largest change in population size would have occurred during the Holocene, 11.5 to 3.5 ka before present (Fig. 4).

The MacDonald Kreitman (MK) test could not be conducted among *Perumytilus* clades (North Clade, *n* = 36, and South Clade, *n* = 53) due to the low number of non-synonymous fixed and polymorphic sites (0 and 5, respectively). A MK test was instead conducted between each clade and another brachiopod, *B. rodriguezii* (*n* = 14, Trovant et al., 2013). The ratio of non-synonymous to synonymous changes in the between species comparison (fixed differences) was higher than the same ratio in the within species comparison (polymorphic sites) (North Clade – *B. rodriguezii*: 21/54 > 5/59; South Clade – *B. rodriguezii*: 21/52 > 2/61) suggesting strong directional selection acting at a deepest scale

in the phylogeny. Notice, though, that the MK test is biased towards the detection of ancient selection (Parsch et al., 2009).

The Mantel's test suggested no correlation among genetic and geographic distance for any of the clades (North Clade, *r* = 0.15, *p* > 0.05; South Clade, *r* = 0.06, *p* > 0.05).

3.4. Distribution of the two clades of *Perumytilus* relative to latitudinal thermal gradients

Latitudinal profiles of climatological sea-surface temperature were used to characterize the latitudinal distribution of *Perumytilus* clades along both coasts of southern South America. The latitudinal range of distribution of the two clades along the Pacific coast is clearly associated with SST (Fig. 5): individuals of the North Clade were not found where SST_{min} (August) drops below 10 °C, whereas individuals of the South Clade can be found at a SST_{min} as low as 2.82 °C (Surfer Bay, Malvinas/Falkland I.). The distribution of the two *Perumytilus* clades also appears to correlate with temperature during the warmest month. Individuals of the South Clade were found only where SST_{max} ≤ 14.2 °C on the Pacific side and SST_{max} ≤ 17.6 °C on the Atlantic side (Puerto Madryn, 43°S), while those of the North Clade can be found in waters as warm as 21.7 °C on the Pacific side (San Marcos, 20°S).

Table 5

Perumytilus, South Clade. Pairwise Φ_{ST} comparisons (below diagonal) and approximate distance among populations, in km (above diagonal). In the case of the pairwise Φ_{ST} comparisons, *p*-values uncorrected and after Holm–Bonferroni corrections are shown to the left and right of the slashbar, respectively. None of the differences is statistically significant.

South Clade	5	6	7	8	9	10	11
5- La Misi3n	–	290.19	3107.51	2874.89	2548.49	2418.13	1745.70
6- Huinay	0.043 (0.19/0.0036)	–	2820.20	2587.58	2261.18	2130.82	1458.39
7- Puerto Madryn	0.016 (0.34/0.0063)	0.080 (0.07/0.0025)	–	233.41	559.88	1088.84	1361.81
8- Caleta Carolina	0.000 (0.65/0.012)	0.138 (0.01/0.0024)	0.068 (0.11/0.0028)	–	329.21	1914.16	1129.19
9- Puerto Deseado	0.001 (0.45/0.0071)	0.021 (0.22/0.0038)	0.000 (0.90/0.050)	0.025 (0.22/0.0042)	–	–	802.79
10- Surfer Bay	0.000 (0.74/0.016)	0.000 (0.48/0.0083)	0.049 (0.19/0.0033)	0.039 (0.17/0.0031)	0.017 (0.31/0.0056)	–	672.43
11- Bah3a Ensenada	0.023 (0.28/0.0050)	0.038 (0.12/0.0029)	0.000 (0.63/0.010)	0.042 (0.101/0.0026)	0.000 (0.77/0.025)	0.021 (0.27/0.0045)	–

Table 6

Perumytilus: neutrality tests by location and clade. (*) Statistically significant differences. F_s is considered significant when $p < 0.02$, while the other tests are considered significant when $p < 0.05$.

Locality	F_s	Tajima's D	R^2
1- San Marcos	-3.89* ($p < 0.01$)	-1.01 ($p = 0.18$)	0.10* ($p = 0.01$)
2- La Chimba	-5.00* ($p < 0.01$)	-1.32 ($p = 0.10$)	0.10* ($p = 0.01$)
3- Coquimbo	-4.20* ($p < 0.01$)	-1.10 ($p = 0.14$)	0.09* ($p < 0.01$)
4- Coliumo	1.58 ($p = 0.80$)	-1.13 ($p = 0.14$)	0.17 ($p = 0.34$)
North Clade	-20.22* ($p < 0.01$)	-1.99* ($p = 0.01$)	0.04* ($p < 0.01$)
5- La Misión	-3.60* ($p = 0.01$)	-0.77 ($p = 0.21$)	0.12* ($p = 0.04$)
6- Huinay	-2.24 ($p = 0.05$)	-1.51 ($p = 0.06$)	0.15 ($p = 0.21$)
7- Pto. Madryn	-0.43 ($p = 0.21$)	-0.22 ($p = 0.55$)	0.09* ($p = 0.01$)
8- Caleta Carolina	-3.06 ($p = 0.03$)	-0.65 ($p = 0.28$)	0.11* ($p = 0.03$)
9- Pto. Deseado	-2.01 ($p = 0.06$)	-1.09 ($p = 0.14$)	0.09* ($p < 0.01$)
10- Surfer Bay	-1.36 ($p = 0.18$)	-0.86 ($p = 0.20$)	0.12 ($p = 0.11$)
11- Bahía Ensenada	-3.95* ($p = 0.01$)	-1.28 ($p = 0.11$)	0.16 ($p = 0.53$)
South Clade	-41.40* ($p < 0.01$)	-2.03* ($p < 0.05$)	0.05* ($p < 0.01$)

4. Discussion

4.1. Phylogenetic and phylogeographic relations of the brachidontins from southern South America

Our analyses recovered three well-supported clades within the BRACHIDONTINAE: [i] *Austromytilus* + *Mytilisepta* + *Perumytilus* (the AMP clade), [ii] *Ischadium* + *Geukensia*, and [iii] *Brachidontes* s.l. *Mytilisepta* has usually been considered a member of the SEPTIFERINAE by non-Japanese authors (including Huber, 2010), and even a junior synonym of *Septifer* Récluz. A phylogeny based on the mitochondrial gene COI (Matsumoto, 2003) early indicated that *Mytilisepta virgata* and *Septifer excisus* belong to different clades, supporting the retention of *Mytilisepta* as a distinct genus

placed among the BRACHIDONTINAE, as confirmed by our results. Nesting of *Mytilisepta* among the BRACHIDONTINAE implies that the septum serving for the insertion of the adductor muscle in *Septifer* and *Mytilisepta* (Supplementary Materials SM3) is a homoplasy that evolved independently in the two lineages.

Members of the AMP clade are distributed in temperate regions around the Pacific Ocean: *Austromytilus* in the southwest, *Perumytilus* in the southeast and *Mytilisepta* in the north. According to our results *Perumytilus* branched off first, and *Austromytilus* and the phenotypically apomorphic *Mytilisepta* split later. Given that scorched mussels are absent from New Zealand and are not represented there in the fossil record either (Fleming, 1959; Beu, 2006), brachidontins are likely to have radiated in the Southern Hemisphere after New Zealand broke away from Gondwana during the late Cretaceous (80 Mya; Sanmartín and Ronquist, 2004). Estimated divergence time places the radiation of the AMP clade in the Middle Miocene. Divergence times were estimated with two calibration points, one of them corresponding to the time of closure of the Panama Isthmus. The timing of this event and its biogeographic implications have been a subject of recent debate (Stone, 2013). While the commonly held Pliocene closure time hypothesis (3.1–3.5 Mya, used in our calibration) is supported by a substantial body of evidence (e.g. Jackson and O' Dea, 2013), recent studies have advocated a much earlier closure (Montes et al., 2012). However, even advocates of an early closure admit that shallow inter-oceanic links may have persisted during Pliocene times. *Brachidontes* species occur in the intertidal zone and mangrove swamps, habitats to be expected in a scenario of shallow inter-oceanic connections (Lee and Ó Foighil, 2005).

Although there is much uncertainty associated with our estimates of divergence times based on molecular data, those estimates are consistent with biogeographic patterns and ecological

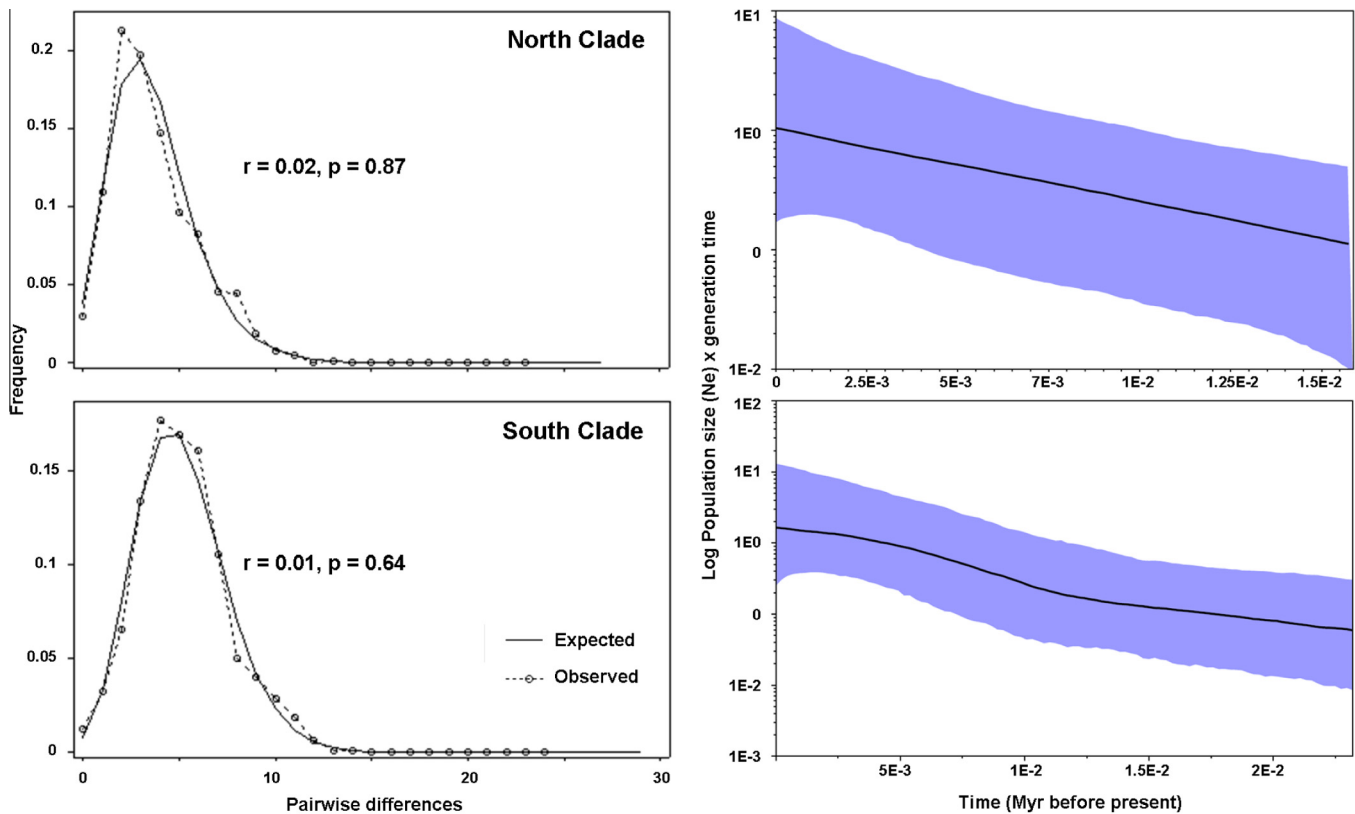


Fig. 4. *Perumytilus* clades. Mismatch distribution analysis (left) and Bayesian skyline plots (right). Mismatch distributions: observed frequencies (dotted line) and values expected under a sudden expansion model (continuous line); Harpending's raggedness index (r) and p -values shown. Bayesian skyline plots show estimated log effective population size per generation time, through time (Mya); dotted line: median values, shaded band: 95% confidence interval. Top: North Clade, Bottom: South Clade.

information in supporting the hypothesis of a split between the two lineages before middle Miocene times, and after the opening of the Tasman Gateway during the Oligocene (ca. 31 Mya; Nelson and Cooke, 2001), once circum-Antarctic circulation was established. There is a growing body of evidence demonstrating the role of the West Wind Drift (WDD), the surface current driven by strong westerly winds circling Antarctica, in the trans-Pacific dispersal of marine organisms (Waters, 2008). Dispersal of benthic organisms along the WDD is largely facilitated by rafting on kelp adrift (Fraser et al., 2013; Nikula et al., 2010; Zuccarello et al., 2008). *Perumytilus* and *Austromytilus*, however, do not appear to be prime candidates for rafting, in part because they are commonly found in the mid-intertidal zone of rocky shores, above the tide level where kelp usually attach. Thiel and Gutow (2005) compiled a list of 1205 species for which rafting was confirmed or inferred. *Perumytilus* was not recorded, even when the authors examined rafting in detail along the coasts of Chile. Also indicative of limited potential for medium to long range dispersal is the absence of *Austromytilus* from New Zealand and of *Perumytilus* from the Juan Fernandez Archipelago, 580–720 km west of the central Chilean coast. The latter has been colonized by other invertebrates originating in nearby South America, has plenty of rocky intertidal habitat, and the thermal regime is well within the bounds suitable to *Perumytilus* (Ramírez and Osorio, 2000). In the case of New Zealand, the ecological space typically occupied by members of the AMP clade in the intertidal zone of rocky shores is occupied by *Limnoperna pulex* (Lamarck) (Subfamily ARCUATULINAE), also present in the Antipode Islands, Tasmania and around the south coasts of Australia (but see Colgan and da Costa, 2013); *Limnoperna* has been well represented in New Zealand since the Miocene (Beu, 2006). With the exception of the Malvinas/Falkland Islands (relatively close to southern South America), brachidontins are absent as well from all the subantarctic islands under the influence of the WDD.

Vicariance, the default hypothesis for animals showing a southern Gondwanic pattern (SGP, Sanmartín and Ronquist, 2004), provides an alternative to dispersal. Vicariance would invoke a common ancestor distributed along the coasts of southern Australia, West Antarctica and southwest South America prior to the loss of shallow connection between the shelves of those continents and

following the incipient opening of the Drake Passage during the middle Eocene (ca. 41 Mya, Scher and Martin, 2006). Such a hypothesis would assume that brachidontins once existed in Antarctica, but got extinct later. Two fossil brachidontins have been reported from the Eocene of Antarctica (Beu, 2009), but based on conchological characters they do not appear to be related to the AMP clade.

While the hypothesis of trans-Pacific dispersal of *Austromytilus* or *Perumytilus* (or a putative common ancestor) is not supported in present scenarios, the fossil record does not offer support for a Gondwanic connection involving Antarctica either. A third alternative would be dispersal in an early WDD scenario during the late Oligocene or early Miocene, post-dating Gondwanan fragmentation events (Burrige et al., 2012).

4.2. North and South clades of *Perumytilus*

The analysis of mitochondrial (COI) and nuclear (28S and 18S) sequences showed that the monotypic genus *Perumytilus* includes two divergent lineages designated here as North and South clades. Samples from the Chilean coast between 20°S and 36°S correspond to the North Clade, while those from locations south of 39°S on the Pacific (Chile) and of 42°S on the Atlantic (Argentina) correspond to the South Clade. These results were supported by haplotype network and genetic distance analyses (COI), and AMOVA (COI and 28S). Our findings lend support to the hypothesis that *P. "purpuratus"* actually consists of two genetically distinct clades, implied by Briones et al. (2012) as a possible explanation for their discovery of "intraspecific" sperm polymorphism. Based on samples from three locations on the Chilean coast, they showed geographic coherence in variation of sperm morphology and one molecular marker (16S). Their northern (Antofagasta and Valparaiso) and southern (Valdivia) groups fit into the latitudinal range for the two clades identified in our study, which are supported by three additional markers and samples from an extensive geographic range. Solving the taxonomic problem introduced by the two clades of *P. "purpuratus"* (Supplementary Materials SM6) is beyond the scope of this study.

Considering that the BRACHIDONTINAE, with the exception of the South Clade of *Perumytilus*, are distributed in warm and

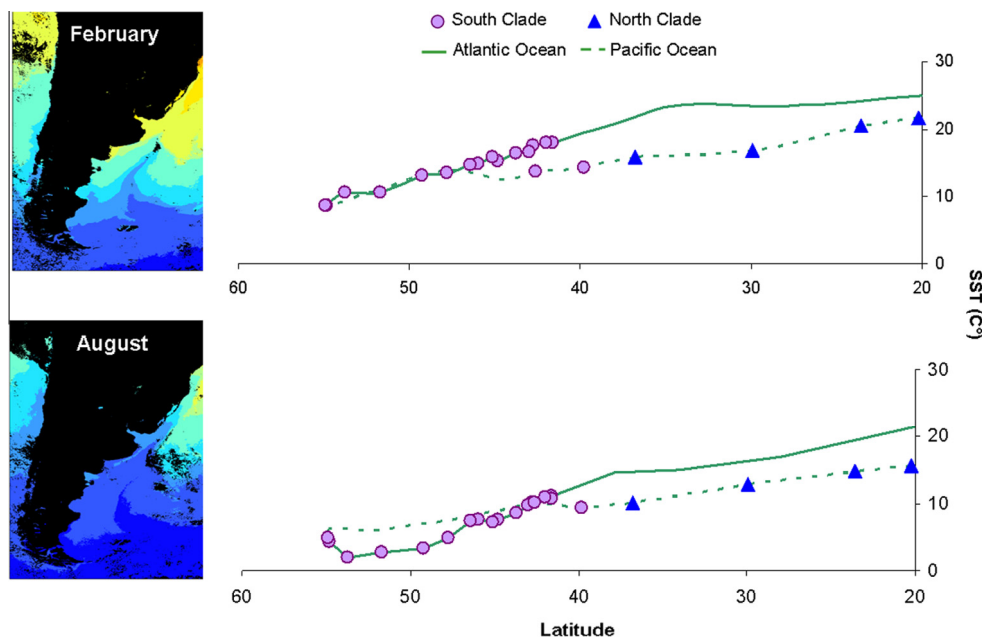


Fig. 5. Latitudinal gradients of climatological sea-surface temperature (SST, 2008–2010) for the warmest (February, top) and coldest (August, bottom) months, with indication of locations where *Perumytilus* was sampled.

warm-temperate seas, we hypothesize that *Perumytilus* originated in the warm-temperate region of the southeast Pacific, matching the latitudinal range of the extant North Clade. This hypothesis is consistent with the paleontological record; the earliest fossil records of *Perumytilus* correspond to the late Pliocene–early Pleistocene of North-Central Chile, from Coquimbo to Antofagasta (Herm, 1969; Kiel and Nielsen, 2010; Ortlieb et al., 1996; Fig. 6A and Supplementary Materials SM7). Differentiation of the South Clade may have been initiated by a gradual range expansion into southern South America (the so called Magellanic Biogeographic Province) and concurrent adaptation of marginal populations to a cold-temperate thermal regime, leading to incipient peripatric differentiation (Fig. 6B). The molecular clock estimation of divergence time between the two clades at the Pliocene–Pleistocene transition is compatible with that hypothesis. Isolation of the two clades during Quaternary times is likely to have occurred as a result of extirpation of *Perumytilus* from much of southern Chile, when that region was repeatedly glaciated (Rabassa et al., 2005), resulting in a split between populations from non-glaciated coastlines in Central Chile and Atlantic Patagonia (Fig. 6C and Supplementary Materials SM7). Besides being vulnerable to ice scouring, like other epibenthic intertidal invertebrates, its thermal preferendum would limit occurrence even in non-glaciated pockets within glaciated areas. The South Clade presently thrives in regions where SST_{min} is above 4 °C. Along colder coastlines, *Perumytilus* is replaced by *Mytilus* sp. and *Aulacomya ater* as the dominant species in intertidal mussel beds (M. Adami, unpublished results), and is not ubiquitous in Holocene units around the Fuegian region (Gordillo, 1999; Gordillo et al., 2005). Furthermore, it is absent from a cold-temperate assemblage attributable to the last interglacial of the Fuegian region (Navarino Island, 54°56'S), which has been well documented by Gordillo et al. (2010). A refugium in the coasts of Atlantic Patagonia, which were never glaciated (Rostami et al., 2000), is consistent with the earliest reliably dated records of *Perumytilus* from that region, which correspond to the last interglacial (Sangamon, Upper Pleistocene, ca. 110–130 Kya) from many sites between Cabo Raso (44°20'S) and San Julián (49°20'S); the mollusks of those sites are still living in the cold-temperate Magellanic Province (Aguirre et al., 2009, 2006, 2005; Feruglio, 1950).

We hypothesize that the South Clade expanded into southeastern Chile from the non-glaciated coasts of the southeast Atlantic as ice receded during interglacial periods (Fig. 6D), the latest one following the LGM, ~18–20 Kya (Rabassa et al., 2000). This should have resulted in a secondary contact zone between the two clades

in the relatively narrow latitudinal range where replacement between the clades takes place (Fig. 6D). That putative contact zone has not been investigated, but differences in sperm morphology described by Briones et al. (2012) may be indicative of reproductive isolation. Development of secondary contact or hybrid zones following range contraction into refugia during glacial maxima, and subsequent expansion during interglacials, is a well documented phenomenon, mostly in the Northern Hemisphere (Waters, 2011; Waters et al., 2013, p. 79). The contact region between the two *Perumytilus* clades is coincidental with two other well documented cases, both rocky shore algae. Two species-level lineages of *Durvillea* “*antarctica*” have a phylogeographic break between 36°36'S and 39°49'S (Fraser et al., 2009). One of the lineages ranges along the warm-temperate coasts of central Chile, while the other has a wide circum-subantarctic distribution, including southern Chile and most subantarctic islands (Fraser et al., 2010). The boundary between two putative sibling species of the red alga *Mazzaella laminarioides* is located within the range 37°S to 38°S (Montecinos et al., 2012). The south lineage is hypothesized to have originated in a glacial refugium area during the Pleistocene, from where it colonized the coasts of South Chile during the last post-glacial; unlike *Perumytilus*, the extant range of *Mazzaella* does not extend over the southwestern Atlantic (Boraso de Zaiexo, 2013). These three cases illustrate a suite of plausible paths leading to a contact zone between cold and warm-temperate lineages in the southeast Pacific, following deglaciation of southern South America. Cases differ in the putative location of refugia for the cold-temperate lineages during glacial maxima: the southwestern Atlantic (*Perumytilus*, this study), pockets within glaciated southern Chile (*Mazzaella*; Montecinos et al., 2012), and non-glaciated areas outside South America, with propagules arriving via the West Wind Drift (*Durvillea*; Fraser et al., 2009). No single model is likely to explain the phylogeographic patterns resulting from the Quaternary glaciations of southern South America.

The finding that the supposedly monotypic *Perumytilus* actually consists of two clades adapted to different thermal regimes solves the apparent paradox of a species ranging over the warm-temperate region of the southeast Pacific but not along its Atlantic counterpart. The latitudinal/thermal range of *Perumytilus*' South Clade is consistent along both coasts of southern South America, with its northern limit defined by a median SST of approximately 13 °C. It must be emphasized, however, that SST, usually treated as a surrogate of the thermal regimes experienced by marine organisms, is a

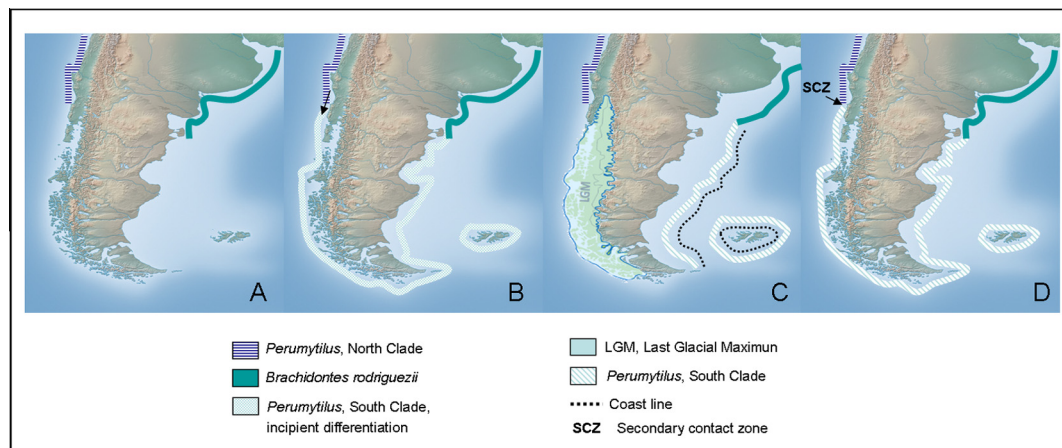


Fig. 6. Hypothetical events leading to speciation in *Perumytilus*. (A) Late Pliocene/early Pleistocene: North Clade confined to the warm-temperate region of the southeast Pacific. (B) initiation of peripatric differentiation of the South Clade following expansion into the cold-temperate Magellanic Province. (C) Pleistocene glaciations of southern Chile isolate the two clades, leading to speciation of the South Clade in the non-glaciated coasts of eastern Patagonia. (D) following de-glaciation of southern Chile the South Clade expands back to southern Chile; a secondary contact zone (SCZ) is established between the two clades.

coarse indicator in the case of intertidal organisms (Helmuth, 2009). North of its approximate thermal boundaries, the South Clade is replaced by the North Clade on the Pacific and by the warm-temperate *Brachidontes rodriguezii* on the Atlantic (Trovant et al., 2013), where the two species intermix in the same beds within the range 41°–43°S (Trovant et al., 2013). The latter replacement may prove a case of “high density blocking” (Waters, 2011; Waters et al., 2013), given that the lineage leading to the extant *B. rodriguezii* seems to have been present in that region since at least the upper Miocene (Trovant et al., 2013). This could explain why *Perumytilus* did not expand its range northwards on the Atlantic, even when there are no barriers to dispersal and it appears to be adaptable to a broad range of temperate thermal regimes.

4.3. Intra-clade genetic diversity in *Perumytilus*

Whereas the South Clade did not exhibit genetic structure across a wide geographical area, there was some indication of genetic differentiation between the northernmost (San Marcos) and southernmost sampled location (Coliumo) of the North Clade, as suggested by the significant Φ_{ST} value. Expanded sampling, including the Peruvian coasts, will be required to fully understand geographic differentiation in the North Clade. Populations from warm-temperate regions have greater phylogeographical structure due to survival and divergence at times when populations at higher latitudes were exposed to glaciation. In many cases the low latitude end of a species range functioned as a refugium, from where populations expanded back during postglacial recolonization (Provan and Bennett, 2008). In general, temperate species are less genetically diverse at the higher latitudes into which they expanded during deglaciation, but this varies with niche and geographical setting (Hewitt, 2004; Provan and Bennett, 2008). There is growing evidence indicating that during the last glacial maximum the central/northern coasts of Chile provided a refuge for several marine taxa (Fraser et al., 2010), possibly including the North Clade of *Perumytilus*, as discussed earlier.

Lack of structure, as observed in the South Clade, could be also explained as the result of gene flow, even in the absence of recent expansion and separation into sub-populations. While advective environments and pelagic larvae are expected to facilitate extensive gene flow (Kelly and Palumbi, 2010; Selkoe and Toonen, 2011), recent studies suggest that larval retention is more frequent than previously thought (Levin, 2006). Furthermore, low and non-significant Φ_{ST} values, low nucleotide diversity coupled with high haplotype diversity, lack of correlation between genetic and geographic distance, and the rejection of null hypotheses by three tests of neutrality (or population expansion) with no evidence of selection in the COI marker, all suggest that the lack of structure of the South Clade of *Perumytilus* is due to a recent population expansion.

The hypothesis of rapid expansion of populations in *Perumytilus* in the southwestern Atlantic after the LGM is well supported in two other cases: the rocky-shore limpet *Nacella magellanica* (de Aranzamendi et al., 2011; González-Wevar et al., 2011) and *Eleginops maclovinus*, a fish common in the coastal zone (Ceballos et al., 2012). At the time of the LGM the coasts of the southwestern Atlantic, which were never glaciated during the Quaternary, run far to the east of their present location (Rostami et al., 2000); most of the Patagonian Shelf was exposed, forming extensive plains (Ponce et al., 2011). As the latter were gradually flooded in post-LGM times, coastscapes were dominated by low coastlines and depositional environments (Fray and Ewing, 1963). Geographically restricted rocky shores were novel habitats, presumably populated by unsaturated assemblages. This was the likely ecological scenario encountered by expanding populations of rocky shore organisms, like *Perumytilus* and *Nacella*, during post-LGM times. Unsaturation

appears to persist into present days, as attested by conspicuous absences noticed by community ecologists (Olivier et al., 1966a, 1966b). A flurry of recent, ecologically significant invasions of rocky shore habitats (Hidalgo et al., 2005; Orensanz et al., 2002; Schwindt et al., 2008), may be the most recent chapter of the rapid post-LGM population expansions discussed above.

5. Conclusions

Phylogenies based on two nuclear (28S and 18S) and one mitochondrial (COI) genes revealed that the monophyletic BRACHIDONTINAE consist of three well supported clades: [i] *Brachidontes* Swainson (including *Hormomya* Mörch) + *Mytilaster* Monterosato, [ii] *Ischadium* Jukes-Brown + *Geukensia* van de Poel, and [iii] *Austromytilus* Lasseron + *Mytilisepta* Habe (often considered a member of the SEPTIFERINAE) + *Perumytilus* Olsson. Members of the latter clade are distributed along the temperate coasts of the Pacific Ocean. Split between *Perumytilus* (South American) and *Austromytilus* (Australian) could possibly have involve [i] vicariance following the breakdown of shelf connections between southern Australia, Antarctica and southern South America during the Eocene, and [ii] dispersal (most likely by algal rafting) after circum-Antarctic circulation was established during the Oligocene, although the two genera appear to be poor rafters. *Perumytilus* is shown to include two genetically distinct clades, one confined to the warm-temperate Chile-Peru Province and the other to the cold-temperate Magellanic Biogeographic Province; the latter is the only brachidontin restricted to cold-temperate waters. Consistent with the fossil record, it is hypothesized that differentiation of the cold-temperate clade originated from budding-off from range-edge populations of the warm-temperate clade, adaptation to a colder thermal regime, and gradual expansion along the coasts of Patagonia. During Quaternary glacial maxima populations from southern Chile were likely extirpated, and populations from central Chile and the southwest Atlantic remained separated by long periods (the last culminating in the LGM) from those in non-glaciated eastern Patagonia. After ice receded, the range of the South Clade presumably expanded back, a secondary contact zone being established between the two clades within a relatively narrow latitudinal range between Valdivia (39°48'S) and Concepcion (36°49'S). Northwards expansion into the warm-temperate region of the southwestern Atlantic may have been constrained by high-density blocking involving interaction with *Brachidontes rodriguezii*.

Acknowledgments

We are grateful to Billy Ernst, Gustavo Lovrich, Simon Grove, Steve Gaines, Carolle Blanchette, Gray Williams, Craig Mundy and Hiroshi Wada for kindly assisting with the provision of samples for molecular analysis, and to Claudia del Río for access to the collections of the Invertebrate Paleontology Division, Museo Argentino de Ciencias Naturales, Buenos Aires. Noela Sanchez assisted with the retrieval of SST information. We deeply appreciate enlightening discussion with Enrique Lessa, Christian Ibañez and Ana Parma, the two latter in relation with Bayesian inference. We also thank Gregory McCracken and Ian Paterson (Marine Gene Probe Laboratory, Dalhousie University) for assistance in the laboratory. This study was funded by Project PICT/Raices 2012-0057, FONCYT, Argentina. This contribution is part of the doctoral thesis of BT. Finally we want to express our gratitude to two anonymous reviewers for thorough reading and thoughtful suggestions, which contributed to substantial improvement of the original MS.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2014.10.002>.

References

- Adami, M.L., Pastorino, G., Orensanz, J.M., 2013. On the conchological differences between *Brachidontes rodriguezii* and *B. purpuratus* (Mollusca: Mytilidae) from the intertidal of the Argentine littoral. *Malacologia* 56, 1–9.
- Addinsoft, T.M., 2013. XLSTAT 2013, Data Analysis and Statistics Software for Microsoft Excel. Paris, France. <<http://www.xlstat.com>>.
- Aguirre, M.L., Negro-Sirch, Y., Richiano, S., 2005. Late Quaternary molluscan assemblages from the coastal area of Bahía Bustamante (Patagonia, Argentina): paleoecology and paleoenvironments. *J. S. Am. Earth Sci.* 20, 13–32.
- Aguirre, M.L., Richiano, S., Negro-Sirch, Y., 2006. Paleoenvironments and paleoclimates of the Quaternary molluscan faunas from the coastal area of Bahía Vera-Camarones (Chubut, Patagonia). *Palaeogeogr. Palaeoclimatol.* 229, 251–286.
- Aguirre, M.L., Richiano, S., Álvarez, M.F., Eastoe, C., 2009. Quaternary molluscan fauna from the littoral area of northern Santa Cruz (Patagonia, Argentina). *Geobios* 42, 411–434.
- Bandelt, H.J., Forster, P.L., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16, 37–48.
- Bernard, F.R., 1983. Catalogue of the Living Bivalvia of the Eastern Pacific Ocean: Bering Strait to Cape Horn. Canadian Special Publication of Fisheries and Aquatics Sciences, p. 102.
- Bertness, M.D., Crain, C.M., Silliman, B.R., Bazterrica, M.C., Reyna, M.V., Hidalgo, F., Farina, J.K., 2006. The communities structure of western Atlantic Patagonian Rocky Shores. *Ecol. Monogr.* 76, 439–460.
- Bertorelle, G., Slatkin, M., 1995. The number of segregating sites in expanding human populations, with implications for estimates of demographic parameters. *Mol. Biol. Evol.* 12, 887–892.
- Beu, A.G., 2006. Marine Mollusca of oxygen isotope stages of the last 2 million years in New Zealand. Part 2. Biostratigraphically useful and new Pliocene to recent bivalves. *J. Roy. Soc. N. Zeal.* 36, 151–338.
- Beu, A.G., 2009. Before the ice: biogeography of Antarctic Paleogene molluscan faunas. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 284, 191–226.
- Boore, J.L., Medina, M., Rosenberg, L.A., 2004. Complete sequences of the highly rearranged molluscan mitochondrial genomes of the Scaphopod *Graptacme eborea* and the bivalve *Mytilus edulis*. *Mol. Biol. Evol.* 21, 1492–1503.
- Boraso de Zaixso, A., 2013. Elementos para el estudio de las macroalgas de Argentina. Universidad Nacional de la Patagonia, Comodoro Rivadavia, Argentina.
- Briggs, J.C., Bowen, B.W., 2013. Marine shelf habitat: biogeography and evolution. *J. Biogeogr.* 29, 12–30.
- Briones, C., Guíñez, R., Garrido, O., Oyarzún, P.A., Toro, J.E., Pérez, M., 2012. Sperm polymorphism and genetic divergence in the mussel *Perumytilus purpuratus*. *Mar. Biol.* 159, 1865–1870.
- Burridge, C.P., McDowall, R.M., Craw, D., Wilson, M.V.H., Waters, J.M., 2012. Marine dispersal as a pre-requisite for Gondwanan vicariance among elements of the galaxiid fish fauna. *J. Biogeogr.* 39, 306–321.
- Campbell, D.C., 2000. Molecular evidence on the evolution of the Bivalvia. In: Harper, E.M., Taylor, J.D., Crame, J.A. (Eds.), *The Evolutionary Biology of the Bivalvia*. Geological Society of London, Special Publications, pp. 31–46.
- Ceballos, S.G., Lessa, E.P., Victorio, M.F., Fernández, D.A., 2012. Phylogeography of the sub-Antarctic nototheniid fish *Eleginops maclovinus*: evidence of population expansion. *Mar. Biol.* 159, 499–505.
- Coan, E.V., Valentich-Scott, P., 2012. Bivalve Seashells of Tropical West America. Marine Bivalve Mollusks from Baja California to Northern Perú. Part 1. Santa Barbara Museum of Natural History, Santa Barbara.
- Colgan, D.J., da Costa, P., 2013. Invasive and non-invasive lineages in *Xenostrobus* (Bivalvia: Mytilidae). *Molluscan Res.* 33, 272–280.
- Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9, 772.
- de Aranzamendi, M.C., Bastida, R., Gardenal, C.N., 2011. Different evolutionary histories in two sympatric limpets of the genus *Nacella* (Patellogastropoda) in the South-western Atlantic coast. *Mar. Biol.* 158, 2405–2418.
- Distel, D., 2000. Phylogenetic Relationships among Mytilidae (Bivalvia): 18S rRNA Data Suggest Convergence in Mytilid Body Plans. *Mol. Phylogenet. Evol.* 15, 25–33.
- Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973.
- Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131, 479–491.
- Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10, 564–567.
- Feruglio, E., 1950. Descripción Geológica de la Patagonia. Dirección General de Yacimientos Petrolíferos Fiscales, Buenos Aires, Argentina.
- Fleming, C.A., 1959. Notes on New Zealand Recent and Tertiary mussels (Mytilidae). *Trans. Roy. Soc. N. Zeal.* 87, 165–178.
- 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.
- Fraser, C.I., Nikula, R., Spencer, H.G., Waters, J.M., 2009. Kelp genes reveal effects of subantarctic sea ice during the Last Glacial Maximum. *Proc. Natl. Acad. Sci. Biol.* 106, 3249–3253.
- Fraser, C.I., Thiel, M., Spencer, H.G., Waters, J.M., 2010. Contemporary habitat discontinuity and historic glacial ice drive genetic divergence in Chilean kelp. *BMC Evol. Biol.* 10, 203. <http://dx.doi.org/10.1186/1471-2148-10-203>.
- Fraser, C.I., Nikula, R., Ruzzante, D.E., Waters, J.M., 2012. Poleward bound: biological impacts of Southern Hemisphere glaciation. *Trends Ecol. Evol.* 27, 462–471.
- Fraser, C.I., Zuccarello, G.C., Spencer, H.G., Salvatore, L.C., Garcia, G.R., Waters, J.M., 2013. Genetic affinities between trans-oceanic populations of non-buoyant macroalgae in the high latitudes of the Southern Hemisphere. *PLoS One* 8, e69138. <http://dx.doi.org/10.1371/journal.pone.0069138>.
- Fray, C., Ewing, M., 1963. Pleistocene sedimentation and fauna of the argentine shelf. I. Wisconsin sea level as indicated in argentine continental shelf sediments. *Proc. Acad. Nat. Sci. Philadelphia* 115, 113–152.
- Fu, Y., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147, 915–925.
- González-Wevar, C.A., Nakano, T., Cañete, J.L., Poulin, E., 2010. Molecular phylogeny and historical biogeography of *Nacella* (Patellogastropoda: Nacellidae) in the Southern Ocean. *Mol. Phylogenet. Evol.* 56, 115–124.
- González-Wevar, C.A., Nakano, T., Cañete, J.L., Poulin, E., 2011. Concerted genetic, morphological and ecological diversification in *Nacella* limpets in the Magellanic Province. *Mol. Ecol.* 20, 1936–1951.
- Gordillo, S., 1999. Holocene molluscan assemblages in the Magellan region. *Sci. Mar.* 63, 15–22.
- Gordillo, S., Coronato, A.M.J., Rabassa, J.O., 2005. Quaternary molluscan faunas from the island of Tierra del Fuego after the Last Glacial Maximum. *Sci. Mar.* 69, 337–348.
- Gordillo, S., Cusminsky, G., Bernasconi, E., Ponce, J.F., Rabassa, J.O., Pino, M., 2010. Pleistocene marine calcareous macro- and micro-fossils of Navarino Island (Chile) as environmental proxies during the last interglacial in southern South America. *Quatern. Inter.* 221, 159–174.
- Goto, T.V., Tamate, H.B., Hanzawa, N., 2011. Phylogenetic characterization of three morphs of mussels (Bivalvia, Mytilidae) inhabiting isolated marine environments in Palau Islands. *Zool. Sci.* 28, 568–579.
- Harpending, H.C., 1994. Signature of ancient population-growth in a low-resolution mitochondrial-DNA mismatch distribution. *Hum. Biol.* 66, 591–600.
- Harpending, H.C., Sherry, S.T., Rogers, A.R., Stoneking, M., 1993. The genetic structure of ancient human populations. *Curr. Anthropol.* 34, 483–496.
- Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 22, 160–174.
- Helmuth, B., 2009. From cells to coastlines: how can we use physiology to forecast the impacts of climate change? *J. Exp. Biol.* 212, 753–760.
- Herm, D., 1969. Marines Pliozän und Pleistozän in Nord- und Mittel-Chile unter besonderer Berücksichtigung der Entwicklung der Mollusken-Faunen. *Zitteliana* 2, 1–159.
- Hewitt, G.M., 2004. Genetic consequences of climatic oscillations in the quaternary. *Philos. Trans. Roy. Soc. B* 359, 183–195.
- Hidalgo, F.J., Barón, P.J., Orensanz, J.M., 2005. A prediction comes true: *Carcinus maenas*, a new invasive species in the Patagonian coast. *Biol. Invasions* 7, 547–552.
- Hoeh, W.R., Stewart, D.T., Saavedra, C., Sutherland, B.W., Zouros, E., 1997. Phylogenetic evidence for role-reversals of gender-associated mitochondrial DNA in *Mytilus* (Bivalvia: Mytilidae). *Mol. Biol. Evol.* 14, 959–967.
- Holm, S., 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* 6, 65–70.
- Hosoi, M., Hosoi-Tanabe, S., Sawada, H., Ueno, M., Toyohara, H., Hayashi, I., 2004. Sequence and polymerase chain reaction-restriction fragment length polymorphism analysis of the large subunit rRNA gene of bivalve: Simple and widely applicable technique for multiple species identification of bivalve larva. *Fish. Sci.* 70, 629–637.
- Huber, M., 2010. Compendium of Bivalves. A Full-color Guide to 3300 of the World's Marine Bivalves. A Status on Bivalvia after 250 years of Research. ConchBooks, Hackenheim.
- Jackson, J.B.C., O'Dea, A., 2013. Timing of the oceanographic and biological isolation of the Caribbean Sea from the tropical eastern Pacific Ocean. *Bull. Mar. Sci.* 89, 779–800.
- Kass, R.E., Raftery, A.E., 1995. Bayes factors. *J. Am. Stat. Assoc.* 90, 773–795.
- Kelly, R.P., Palumbi, S.R., 2010. Genetic structure among 50 species of the northeastern Pacific rocky intertidal community. *PLoS One* 5, 8594. <http://dx.doi.org/10.1371/journal.pone.0008594>.
- Kennington, E.L.R., Landry, D., Bird, C.J., 1995. Comparison of taxa of the mussel *Mytilus* (Bivalvia) by analysis of the nuclear small-subunit rRNA gene sequence. *Can. J. Fish. Aquat. Sci.* 52, 2613–2620.
- Kiel, S., Nielsen, S.N., 2010. Quaternary origin of the inverse latitudinal diversity gradient among southern Chilean mollusks. *Geology* 38, 955–958.
- Lee, T., Ó Foighil, D., 2004. Hidden Floridian biodiversity: mitochondrial and nuclear gene trees reveal four cryptic species within the scorched mussel, *Brachidontes exustus*, species complex. *Mol. Ecol.* 13, 27–42.
- Lee, T., Ó Foighil, D., 2005. Placing the floridian marine genetic disjunction into regional evolutionary context using the scorched mussel, *Brachidontes exustus*, species complex. *Evolution* 59, 39–58.

- Lessios, H.A., 2008. The Great American Schism: divergence of marine organisms after the rise of the Central American Isthmus. *Annu. Rev. Ecol. Evol. Syst.* 39, 63–91.
- Levin, L.A., 2006. Recent progress in understanding larval dispersal: new directions and digressions. *Integr. Comp. Biol.* 46, 282–297.
- Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452.
- Littlewood, D.T.J., 1994. Molecular phylogenetics of cupped oysters based on partial 28S rRNA gene sequences. *Mol. Phylogenet. Evol.* 3, 221–229.
- Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27, 209–220.
- Marko, P.B., 2002. Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Mol. Biol. Evol.* 19, 2005–2021.
- Matsumoto, M., 2003. Phylogenetic analysis of the subclass Pteriomorpha (Bivalvia) from mtDNA COI sequences. *Mol. Phylogenet. Evol.* 27, 429–440.
- McDonald, J.H., Kreitman, M., 1991. Adaptive protein evolution at the *Adh* locus in *Drosophila*. *Nature* 351, 652–654.
- Medlin, L., Elwood, H.J., Stickel, S., Sogin, M.L., 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Genetics* 71, 491–499.
- Montecinos, A., Broitman, B.R., Faugeron, S., Haye, P.A., Tellier, F., Guillemin, M.-L., 2012. Species replacement along a linear coastal habitat: phylogeography and speciation in the red alga *Mazzaella laminarioides* along the south east pacific. *BMC Evol. Biol.* 12, 97. <http://dx.doi.org/10.1186/1471-2148-12-97>.
- Montes, C., Cardona, A., McFadden, R., Moron, S.E., Silva, C.A., Restrepo-Moreno, S., Ramirez, D.A., Hoyos, N., Wilson, J., Farris, D., Bayona, G.A., Jaramillo, C.A., Valencia, V., Bryan, J., Flores, J.A., 2012. Evidence for middle Eocene and younger land emergence in central Panama: implications for Isthmus closure. *Geol. Soc. Am. Bull.* 124, 780–799.
- Morariu, V.I., Srinivasan, B.V., Raykar, V.C., Duraiswami, R., Davis, L.S., 2008. Automatic Online Tuning for Fast Gaussian Summation <<http://tree.bio.ed.ac.uk/software/figtree/>>.
- Mouquet, N., Devictor, V., Meynard, C.N., Munoz, F., Bersier, L.F., Chave, J., Couteron, P., Dalecky, A., Fontaine, C., Gravel, D., Hardy, O.J., Jabot, F., Lavergne, S., Leibold, M., Mouillot, D., Münkemüller, T., Pavoine, S., Prinzing, A., Rodrigues, A.S.L., Rohr, R.P., Thébaud, E., Thuiller, W., 2012. Ecophylogenetics: advances and perspectives. *Biol. Rev.* 87, 769–785.
- Navarrete, S.A., Castilla, J.C., 2003. Experimental determination of predation intensity in an intertidal predator guild: dominant versus subordinate prey. *Oikos* 100, 251–262.
- Nelson, C.S., Cooke, P.J., 2001. History of oceanic front development in the New Zealand sector of the Southern Ocean during the Cenozoic—a synthesis. *N. Zeal. J. Geol. Geop.* 44, 535–553.
- Newton, M.A., Raftery, A.E., 1994. Approximate Bayesian inference by the weighted likelihood bootstrap. *J. Roy. Stat. Soc. Series B* 56, 3–48.
- Nikula, R., Fraser, C.I., Spencer, H.G., Waters, J.M., 2010. Circumpolar dispersal by rafting in two subantarctic kelp-dwelling crustaceans. *Mar. Ecol. Prog. Ser.* 405, 221–230.
- Olivier, S.R., Escofet, A., Orensanz, J.M., Pezzani, S.E., Turro, A.M., Turro, M.E., 1966. Contribución al conocimiento de las comunidades bénticas de Mar del Plata. I. El litoral rocoso entre Playa Grande y Playa Chica. *Anales Comisión Investigaciones Científicas* 7, 185–206.
- Olivier, S.R., Paternoster, I.K. de, Bastida R., 1966b. Estudios biocenóticos en las costas de Chubut (Argentina) I. Zonación biocenológica de Puerto Pardelas (Golfo Nuevo). Instituto de Biología Marina (Mar del Plata, Argentina), Boletín, vol. 10, 71 pp.
- Orensanz, J.M., Schwindt, E., Pastorino, G., Bortolus, A., Casas, G., Darrigran, G., Elías, R., López-Gappa, J.J., Obenat, S., Pascual, M., Penchaszadeh, P., Piriz, M.L., Scarabino, F., Spivak, E.D., Vallarino, E., 2002. No longer the pristine confines of the world ocean – a survey of exotic marine species in the Southwestern Atlantic. *Biol. Invasions* 4, 115–143.
- Ort, B.S., Pogson, G.H., 2007. Molecular population genetics of the male and female mitochondrial DNA molecules of the California sea mussel, *Mytilus californianus*. *Genetics* 177, 1087–1099.
- Ortlieb, L., Díaz, A., Guzmán, N., 1996. A warm interglacial episode during oxygen isotope stage 11 in northern Chile. *Quat. Sci. Rev.* 15, 857–871.
- Owada, M., 2007. Functional morphology and phylogeny of the rock-boring bivalves *Leiosolenus* and *Lithophaga* (Bivalvia: Mytilidae): a third functional clade. *Mar. Biol.* 150, 853–860.
- Paine, T., Suchanek, T., 1983. Convergence of ecological processes between independently evolved competitive dominants: a tunicate-mussel comparison. *Evolution* 37, 821–831.
- Park, J., Ó Foighil, D., 2000. Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. *Mol. Phylogenet. Evol.* 14, 75–88.
- Parsch, J., Zhang, Z., Baines, J.F., 2009. The influence of demography and weak selection on the McDonald-Kreitman test: an empirical study in *Drosophila*. *Mol. Biol. Evol.*, 691–698.
- Plazzi, F., Ceregato, A., Taviani, M., Passamonti, M., 2011. A molecular phylogeny of bivalve mollusks: ancient radiations and divergences as revealed by mitochondrial genes. *PLoS One* 6, e27147. <http://dx.doi.org/10.1371/journal.pone.0027147>.
- Polzin, T., Daneschmand, S.V., 2003. On Steiner trees and minimum spanning trees in hypergraphs. *Oper. Res. Lett.* 31, 12–20.
- Ponce, J.F., Rabassa, J., Coronato, A., Borronei, A.M., 2011. Palaeogeographical evolution of the Atlantic coast of Pampa and Patagonia from the last glacial maximum to the Middle Holocene. *Biol. J. Linn. Soc.* 103, 363–379.
- Provan, J., Bennett, K., 2008. Phylogeographic insights into cryptic glacial refugia. *Trends Ecol. Evol.* 23, 564–571.
- Rabassa, J., Coronato, A., Bujalesky, G., Salemme, M., Roig, C., Meglioli, A., Heusser, C., Gordillo, S., Roig, F., Borronei, A., Quattrocchio, M., 2000. Quaternary of Tierra del Fuego, southernmost South America: an updated review. *Quatern. Int.* 68–71, 217–240.
- Rabassa, J., Coronato, A.M., Salemme, M., 2005. Chronology of the late Cenozoic Patagonian glaciations and their correlation with biostratigraphic units of the Pampean region (Argentina). *J. South Am. Earth Sci.* 20, 81–103.
- Raftery, A.E., Newton, M.A., Satogopan, J.M., Krivitsky, P.K., 2007. Estimating the integrated likelihood via posterior simulation using the harmonic mean identity. In: Bernardo, J.M., Bayarri, M.J., Berger, J.O., Dawid, A.P., Heckerman, D., Smith, A.F.M., West, M. (Eds.), *Bayesian Statistics*. Oxford University Press, Oxford, pp. 1–45.
- Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6. <<http://beast.bio.ed.ac.uk/Tracer>>.
- Ramírez, M.E., Osorio, C., 2000. Patrones de distribución de macroalgas y macroinvertebrados intermareales de la isla Robinson Crusoe, archipiélago de Juan Fernández, Chile. *Invest. Mar. (Valparaíso)* 28, 1–13.
- Ramos-Onsins, S.E., Rozas, J., 2002. Statistical properties of new neutrality tests against population growth. *Mol. Biol. Evol.* 19, 2092–2100.
- Rawson, P.D., Hilbish, T.J., 1995. Evolutionary relationships among the male and female mitochondrial dna lineages in the *Mytilus edulis* species complex. *Mol. Biol. Evol.* 12, 893–901.
- Rogers, A., Harpending, H.C., 1992. Population growth makes waves in the distribution of pairwise differences. *Mol. Biol. Evol.* 9, 552–569.
- Rostami, K., Peltier, W.R., Mangini, A., 2000. Quaternary marine terraces, sea-level changes and uplift history of Patagonia, Argentina: comparisons with predictions of the ICE-4G (VM2) model of the global process of glacial isostatic adjustment. *Quat. Sci. Rev.* 19, 1495–1525.
- Ruzzante, D.E., Walde, S.J., Gosse, J.C., Cussac, V.E., Habit, E., Zemlak, T.S., Adams, E.D.M., 2008. Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. *Mol. Ecol.* 17, 2234–2244.
- Ruzzante, D.E., Walde, S.J., Macchi, P.J., Alonso, M., Barriga, J.P., 2011. Phylogeography and phenotypic diversification in the Patagonian fish *Percichthys trucha*: the roles of Quaternary glacial cycles and natural selection. *Biol. J. Linnean Soc.* 103, 514–529.
- Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York.
- Sanmartín, I., Ronquist, F., 2004. Southern Hemisphere biogeography inferred by event-based models: plant animal patterns. *Syst. Biol.* 53, 216–243.
- Santaclara, F.J., Espineira, M., Cabado, A.G., Aldaroso, A., González-Laván, N., Vieites, J.M., 2006. Development of a method for the genetic identification of mussel species belonging to *Mytilus*, *Perna*, *Aulacomya*, and other genera. *J. Agri. Food Chem.* 54, 8461–8470.
- Scarabino, F., Zaffaroni, J.C., Clavijo, C., Carranza, A., Nin, M., 2006. Bivalvos marinos y estuarinos de la costa uruguaya: faunística, distribución, taxonomía y conservación. In: Menafra, R., Rodríguez-Gallego, L., Scarabino, F., Conde, D. (Eds.), *Bases para la Conservación y el Manejo de la Costa Uruguaya*. Vida Silvestre Publicaciones, Montevideo, pp. 157–170.
- Scher, H.D., Martin, E.E., 2006. Timing and climatic consequences of the opening of Drake Passage. *Science* 312, 428–430.
- Schwindt, E., Bortolus, A., Idaszkin, Y.L., Savoya, V., Méndez, M.M., 2008. Salt marsh colonization by a rocky shore invader: *Balanus glandula* Darwin (1854) spreads along the Patagonian coast. *Biol. Invasions* 11, 1259–1265.
- Selkoe, K.A., Toonen, R.J., 2011. Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. *Mar. Ecol. Prog. Ser.* 436, 291–305.
- Silliman, B.R., Bertness, M.D., Altieri, A.H., Griffin, J.N., Bazterrica, M.C., Hidalgo, F.J., Crain, C.M., Reyna, M.V., 2011. Whole-community facilitation regulates biodiversity on Patagonian rocky shores. *PLoS One* 6, e24502. <http://dx.doi.org/10.1371/journal.pone.0024502>.
- Silvestro, D., Michalak, I., 2012. RaxMLGUI: a graphical front-end for RAXML. *Organ. Div. Evol.* 12, 335–337.
- Skibinski, D.O.F., Gallagher, C., Beynon, C.M., 1994a. Mitochondrial DNA inheritance. *Nature* 368, 817–818.
- Skibinski, D.O.F., Gallagher, C., Beynon, C.M., 1994b. Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis*. *Genetics* 138, 801–809.
- Slatkin, M., 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47, 264–279.
- Smith, B., Wilson, J.B., 2002. Community convergence: Ecological and evolutionary. *Folia Geobot.* 37, 171–183.
- Stamatakis, A., 2006. RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Steiner, G., Hammer, S.E., 2000. Molecular phylogeny of the Bivalvia inferred from 18S rDNA sequences, with particular reference to the Pteriomorpha. In: Harper, E.M., Taylor, J.D., Crame, J.A. (Eds.), *The Evolutionary Biology of the Bivalvia*. Geological Society, Special Publications, London, pp. 11–29.
- Stone, R., 2013. Battle of the Americas. *Science* 341, 230–233.
- Tajima, F., 1989. Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. *Genetics* 123, 585–595.

- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–2739.
- Tanaka, M.O., Magalhães, C.A., 2002. Edge effects and succession dynamics in *Brachidontes* mussel beds. *Mar. Ecol. Prog. Ser.* 237, 151–158.
- Tavaré, S., 1985. Some probabilistic and statistical problems in the analysis of DNA Sequences. *Lect. Math. Life Sci.* 17, 57–86.
- Terranova, M.S., Lo Brutto, S., Arculeo, M., Mitton, J.B., 2006. Population structure of *Brachidontes pharaonis* (P. Fisher, 1870) (Bivalvia, Mytilidae) in the Mediterranean Sea, and evolution of a novel mtDNA polymorphism. *Mar. Biol.* 150, 89–101.
- Thiel, M., Ullrich, N., 2002. Hard rock versus soft bottom: the fauna associated with intertidal mussel beds on hard bottoms along the coast of Chile, and considerations on the functional role of mussel beds. *Helgol. Mar. Res.* 56, 21–30.
- Thiel, M., Gutow, L., 2005. The ecology of rafting in the marine environment. II. The rafting organisms and community. *Oceanogr. Mar. Biol. Annu. Rev.* 43, 279–418.
- Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22, 4673–4680.
- Trovant, B., Ruzzante, D.E., Basso, N.G., Orensanz, J.M., 2013. Distinctness, phylogenetic relations and biogeography of intertidal mussels (*Brachidontes*, Mytilidae) from the south-western Atlantic. *J. Mar. Biol. Assoc. UK* 93, 1843–1855.
- van der Mollen, S., Márquez, F., Idaszkin, Y.L., Adami, M., 2012. Use of Shell-shape to discriminate between *Brachidontes rodriguezii* and *Brachidontes purpuratus* species (Mytilidae) in the transition zone of their distributions (South-western Atlantic). *J. Mar. Biol. Assoc. UK* 93, 803–808.
- Vargas Millacheo, J.A., 2009. Determinación del modo de herencia del ADN mitocondrial en larvas de *Perumytilus purpuratus*, a partir de la formación de familias genéticas. Escuela de acuicultura y pesquerías. Universidad Austral De Chile, Puerto Montt, pp. 48.
- Wares, J.P., Cunningham, C.W., 2001. Phylogeography and historical ecology of the north Atlantic intertidal. *Evolution* 55, 2455–2469.
- Waters, J.M., 2008. Driven by the West Wind Drift? A synthesis of southern temperate marine biogeography, with new directions for dispersalism. *J. Biogeogr.* 35, 417–427.
- Waters, J.M., 2011. Competitive exclusion: phylogeography's 'elephant in the room'? *Mol. Ecol.* 20, 4388–4394.
- Waters, J.M., Fraser, C.I., Hewitt, G.M., 2013. Founder takes all. Density-dependent processes structure biodiversity. *Trends Ecol. Evol.* 28, 78–85.
- Xia, X., 2013. DAMBE 5: a comprehensive software package for data analysis in molecular biology and evolution. *Mol. Biol. Evol.* 30, 1720–1728.
- Xia, X., Lemey, P., 2009. Assessing substitution saturation with DAMBE. In: Lemey, P., Salemi, M., Vandamme, A.-M. (Eds.), *The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny*, second ed. Cambridge University Press, pp. 615–630.
- Xue, D., Wang, H., Zhang, T., Gao, Y., Zhang, S., Xu, F., 2012. Morphological and genetic identification of the validity of the species *Atrina chinensis* (Bivalvia: Pinnidae). *J. Shellfish Res.* 31, 739–747.
- Zouros, E., Freeman, K.R., Ball, A.O., Pogson, G.H., 1992. Direct evidence for extensive paternal mitochondrial DNA inheritance in the marine mussel *Mytilus*. *Nature* 359, 412–414.
- Zuccarello, G.C., West, J.A., Kikuchi, N., 2008. Phylogenetic relationships within the Stylonematales (Stylonematophyceae, Rhodophyta): biogeographic patterns do not apply to *Stylonema alsidii*. *J. Phycol.* 44, 384–393.