Restricted gene flow in Chilean barnacles reflects an oceanographic and biogeographic transition zone

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ABSTRACT: Broad scale patterns of genetic structure in coastal communities are strongly affected by both ecological transitions and larval dispersal. Along the Chilean coast, we examined 2 species of co-distributed barnacles, *Jehlius cirratus* and *Notochthamalus scabrosus*, which span an ecological transition associated with a sharp increase in larval recruitment. A distinct break in haplotype frequencies in the mitochondrial cytochrome oxidase I (mtCOI) gene was detected in *N. scabrosus*, with only marginal genetic structure observed in *J. cirratus,* suggesting significant differences in either selective pressures or dispersal ability between the species. The nuclear gene elongation factor 1α was also analyzed in *N. scabrosus*, and similarly suggested limited gene flow. The sharp genetic transition is downstream of the described ecological transition, effectively mirroring a genetic cline described in a different barnacle species along the North American Pacific coast.

KEY WORDS: Phylogeography · Oceanography · Intertidal · Chile · Barnacle · Upwelling · Recruitment

INTRODUCTION

A combination of historical processes and nearshore oceanographic conditions strongly influences the population structure of coastal species. These forces can either change species abundance over short geographic ranges (Connolly et al. 2001, Byers & Pringle 2006), which may be manifested as strong gradients in population dynamics that can be decoupled from abundance (Hughes et al. 1999), or cause unsuspected distributions of intraspecific genetic diversity (Hoskin 2000, Waters & Roy 2004, Pringle & Wares 2007). For many intertidal invertebrate and fish species, larvae may be capable of traversing hundreds of kilometers during their development. Such species generally exhibit a largely panmictic (genetically homogeneous) structure throughout their geographic range (Palumbi 1994, Thorrold et al. 2002, Kinlan & Gaines 2003); however, the interactions among oceanographic forces (e.g. upwelling fronts, eddies, tidal currents), larval be-

havior and ecology, as well as the effects of paleoclimate, may generate or maintain patterns of genetic structure that deviate from this expectation. These influences can be subtle over short timescales, yet have profound effects on the population over longer timescales (Wares et al. 2001, Wares 2002, Marko 2004).

Separating the effects of these processes on local or regional coastal population dynamics requires comparison of species that share some developmental, distributional, or ecological traits (Marko 2004). Such studies of coastal communities with relatively simple and well-studied oceanography and paleoclimate (e.g. those along the northeastern Pacific coast) have identified the importance of (1) larval behavior and/or adaptation to microhabitat conditions (Wares & Castañeda 2005), (2) population stability through climate change (Arndt & Smith 1998, Marko 2004, Hickerson & Cunningham 2005), and (3) the persistence of clines or diversity gradients in the heritable traits or genetic markers of different intertidal species (Wares et al.

2001, Sotka et al. 2004). A broad examination of how these components shape coastal diversity in the northeastern Pacific coast requires a comparison of this area with other communities with similar characteristics.

The southeastern Pacific coast (i.e. Chile and Peru) shares many broad characteristics with the northeastern Pacific (Strub et al. 1987, Halpin et al. 2004, Thiel et al. 2007). A dominant equatorward offshore circulation (Humboldt Current) sets the stage for the seasonally variable wind-driven upwelling, which is modulated by coastal topography and represents the main source of variation in oceanographic conditions in nearshore waters (Narváez et al. 2006, Aiken et al. 2007, Thiel et al. 2007, Wieters et al. 2009). The biogeography of coastal taxa is relatively well studied (Brattström & Johanssen 1983, Camus 2001), and major transitions in faunal distribution and recruitment that are associated with both paleoglacial environments (up to ~42° S; Hulton et al. 2002) and shifting oceanographic regimes have been described (Navarrete et al. 2008). Particularly along the Chilean coastline, there is a demonstrated shift in oceanographic conditions near 31° S that is mostly manifested as changes in wind patterns, upwelling regimes and associated variability in sea surface temperature (Hormazabal et al. 2004, Navarrete et al. 2005, Wieters et al. 2009). These oceanographic changes appear to control the concentration and temporal variability of surface phytoplankton in coastal waters (Thomas 1999, Navarrete et al. 2005), as well as the density and dynamics of several invertebrate species in intertidal communities (Broitman et al. 2001, Navarrete et al. 2005, Wieters et al. 2009). Specifically, Navarrete et al. (2002, 2005) demonstrated a significant decline in recruitment and adult intertidal abundance in mussel and barnacle species downstream (equatorward) of this break.

To determine whether the oceanographic and ecological processes of this coastline generate predictable interactions between larval dispersal and the potential for local adaptation, we compared patterns of spatial genetic diversity and apparent gene flow in 2 dominant intertidal barnacle species, *Jehlius cirratus* (Darwin, 1854) and *Notochthamalus scabrosus* (Darwin, 1854). We focus on an 800 km segment of the Chilean coastline where a transition in recruitment strength has been discovered for a number of species (including these barnacles) with drifting planktonic larvae. These confamilial species were chosen because they compete for space and other resources (López & González 2003), and like other sessile intertidal species, their sole opportunity for dispersal is the larval phase. Differences in their intertidal distribution (*N. scabrosus* is more abundant in the upper part of the mid-intertidal, while *J. cirratus* is most abundant throughout the high intertidal zone; Stephenson & Stephenson 1972,

Navarrete & Castilla 1990) and slight differences in their larval dispersal phase (~8 to 10 d additional development time in *N. scabrosus*; Venegas et al. 2000) may influence the connectivity of coastal populations across the recruitment break noted above.

Marko (2004) suggested that what appear to be slight differences in the ecology and microhabitat of a species may predict large differences in the historical response of a species to climate change. Given what we know of dispersal, recruitment, and competition in these 2 barnacles, we might predict greater genetic structure in *Jehlius cirratus* due to its high intertidal distribution: *J. cirratus* has a somewhat reduced larval period relative to *Notochthamalus scabrosus*, is outcompeted by *N. scabrosus* in the lower intertidal, and thus could demonstrate greater structure through postsettlement mortality among populations. However, if successful recruitment is partly governed by postsettlement mortality, we may find genetic patterns that are more closely associated with the transition in recruitment strength since this transition alters interspecific competition patterns. Here, we provide evidence for restricted gene flow in both species and a more significant and interesting pattern in *N. scabrosus*, and discuss the potential implications for coastal ecology and biodiversity along the South American Pacific (SAP) coast.

MATERIALS AND METHODS

Collection, amplification and sequencing of DNA. Specimens of *Notochthamalus scabrosus* and *Jehlius cirratus* were collected from quadrats in the mid and upper intertidal zones of sites along the Chilean coast (Tables 1 & 2). Adults were stored in 95 % ethanol prior to DNA isolation. DNA was extracted from the soma by dissection of each individual using a DNA purification kit (Puregene, Gentra Systems) and quantified using a spectrophotometer (Nanodrop). The cirri and penis,

Table 1. Collection locations, years, and diversity indices for *Jehlius cirratus* mitochondrial cytochrome oxidase I (mtCOI). Horizontal line: separation of sites based on a reported recruitment break

Site	Latitude	Collection year	No. of ind. mtCOI	Haplotype no.
Huasco	28° 23′ 41″ S	2004	21	15
Temblador	29° 30′ 45″ S	2004	22	11
Punta Talca	30° 58′ 18″ S	2004	24	14
Los Molles	32° 14′ 58″ S	2004	28	18
Las Cruces	33° 30′ 53″ S	2004	29	20
Pichilemu	34° 24′ 20″ S	2004	31	19

Table 2. Collection locations, years, and diversity indices for *Notochthamalus scabrosus* mitochondrial cytochrome oxidase I (mtCOI) and nuclear elongation factor 1α (EF1α). Horizontal line: separation of sites based on a reported recruitment break. EF1α haplotype data includes those that occur in only 1 ind.

Site	Latitude	Collection year	No. of ind. mtCOI	Haplotype no.	No. of ind. EF1α	Haplotype no.
Huasco	28° 23′ 41″ S	2004	22	16	6	4
Arrayan	29° 27′ 04″ S	2006	6	6	16	10
Temblador	29° 30′ 45″ S	2004, 2006	36	25	22	12
La Pampilla	29° 57′ 00″ S	2006	8	8	17	10
Guanaqueros	30° 11′ 58″ S	2006	7	5	23	13
Punta Talca	30° 58′ 18″ S	2004, 2006	21	17	17	11
Los Molles	32° 14′ 58″ S	2004	25	13	6	6
Las Cruces	33° 30′ 53″ S	2004	29	12	5	6
Pichilemu	34° 24′ 20″ S	2004	25	12	6	5

which are the most identifiable taxonomic features of a barnacle, were retained as voucher tissues.

Polymerase chain reaction (PCR) amplifications were performed on the mitochondrial cytochrome *c* oxidase I (mtCOI) locus using primers from Folmer et al. (1994). Both *Jehlius cirratus* and *Notochthamalus scabrosus* were analyzed at this locus. After showing genetic differentiation in mtCOI (see 'Results'), a coding portion of the nuclear elongation factor 1α (EF1α) gene region was analyzed on individuals of *N. scabrosus*, using taxon-specific primers (ChtEF-F 5′CAGACGCAG GGGCTTGTC; ChtEF-R 5′GCCACAGGGATTTCATC AAG). PCR and cycle sequencing conditions were based on Wares & Cunningham (2005), with PCR annealing temperatures of 40 and 50°C for each locus, respectively. Reactions were sequenced in both directions to reliably confirm heterozygotes (Hare & Palumbi 1999).

Analysis of DNA sequences. DNA sequence data were edited in CodonCode Aligner v.2.0.4. Sites with a PHRED score (Ewing & Green 1998) <20 were considered ambiguous and coded as such. Data were trimmed to eliminate individuals with >20% ambiguous base calls. The haplotypic phase of each EF1α genotype was determined using a Bayesian analysis implemented by PHASE (Stephens et al. 2001) in DnaSP v. 5.0.0 (Librado & Rozas 2009) under default conditions based on Sotka et al. (2004). Singleton haplotypes (those occurring only once in the PHASE-constructed data set) were considered potentially spurious reconstructions (i.e. due to sequencing error or other forms of uncertainty, rather than heterozygosity) and were not analyzed further. Sequence data at both loci were used to generate maximum parsimony trees using an unweighted heuristic search with tree bisection-reconnection branch swapping in PAUP 4.0b10 (Swofford 2002). Bootstrap resampling was performed for 1000 full heuristic replicates of each data set (and 10 000 fast stepwise addition replicates), and majority-

rule consensus values for the 1000 most parsimonious topologies were calculated. A median-joining haplotype network was calculated using NETWORK 4.5.1 (Fluxus Engineering).

Analyses of molecular variance (AMOVA) and corrected pairwise genetic differences between sites (F_{ST}) and regions (F_{CT}), as well as among sites within regions (F_{SC}) were generated using the software Arlequin 3.01 (Schneider et al. 1997). The best-fit model of molecular evolution for each data set was determined using likelihood ratio comparison of likelihood scores in MODELTEST 3.7 (Posada & Crandall 1998). A series of all geographic comparisons was arranged based on the relative location to the proposed ecological break (i.e. spatial AMOVA). In each species and locus dataset, all possible, spatially contiguous AMOVA groupings were considered as suggested by Dupanloup et al. (2002), although our *a priori* spatial reference involved sites around the 32° S demographic break. The comparison with the strongest F_{CT} value was reported. To assess the fit of all data to equilibrium models of gene flow, a test for genetic isolation by distance was performed for each dataset using a Mantel test implemented in GENALEX (Peakall & Smouse 2006). Sample sites on the Chilean coast are effectively linear, thus a 1-dimensional model of $F_{ST}/(1-F_{ST})$ versus distance (km) was applied (Rousset 1997). Basic population parameters (π) and Tajima's D (Tajima 1989) were calculated for each locus per population to examine the demographic and/or selective history at each locus. The significance of Tajima's D was estimated using a beta distribution in Arlequin.

Additionally, mtCOI data were used to estimate migration rates (M) among all populations using the coalescent analysis program MIGRATE-n (Beerli & Felsenstein 1999, 2001, Beerli 2006). Four runs of MIGRATE-n were conducted using the entire data set (2 runs of 750 000 total length and 2 longer runs of 900 000 length were conducted using the methodology of Turner et al. (2002); 4 additional runs (same analyti-

cal setup) were conducted using only sequences that belonged to the A clade (see 'Results'). The mean M toward the north (M_N) and that toward the south (M_S) were calculated for each population (i.e. no M_N for Huasco, nor M_S for Pichilemu, was calculated).

RESULTS

Jehlius cirratus

Table 1 shows the number of *Jehlius cirratus* individuals from 6 sites that were sequenced at mtCOI (Genbank accession numbers GU126073–GU126226). The completed dataset was 583 bp, with 32 parsimony informative sites. No substitutions resulted in amino acid replacement. The best-fit model based on either likelihood ratio test (LRT) or Akaike's information criterion (AIC) is a 3-parameter model (TrN) with gamma-distributed rate variation ($\alpha = 0.13$).

A parsimony network of mtCOI haplotypes is shown in Fig. 1, with the haplotype frequencies per sampled

site being indicated. A maximum parsimony tree (of 1000 equally parsimonious trees) for mtCOI shows little statistical (bootstrap) support for clade structure in *Jehlius cirratus* (not shown). However, strict consensus of these trees supports some clades that differ in relative frequency of geographic representation; 1 monophyletic clade (group A1 in Fig. 1) is 53% comprised of individuals from the 4 southern populations, while the other monophyletic clade (group B in Fig. 1) is 79% comprised of individuals from these populations. However, Mantel testing of these data does not show a significant pattern of isolation by distance ($R^2 = 0.106$, $p = 0.1$).

Analysis of molecular variance considering Punta Talca as part of the southern region showed no significant structure (but still the highest F_{CT} of all possible contiguous groupings) among regions (Table 3), with $F_{ST} = 0.032$ ($p = 0.06$) and $F_{CT} = 0.027$ ($p = 0.06$). Similar insignificant results are obtained when Punta Talca is analyzed with the northern populations (not shown) and all other possible groupings. Within-population θ (π) ranged from 5.154 to 8.959 in these 6 populations (Table 4); Tajima's D was negative (–0.771 to –1.486) but insignificant for each sampled population (Table 4).

Notochthamalus scabrosus

Table 2 shows the number of *Notochthamalus scabrosus* individuals from 9 sites that were sequenced at mtCOI (Genbank accession numbers GU125776–GU125954). The finished dataset was 626 bp, with 33
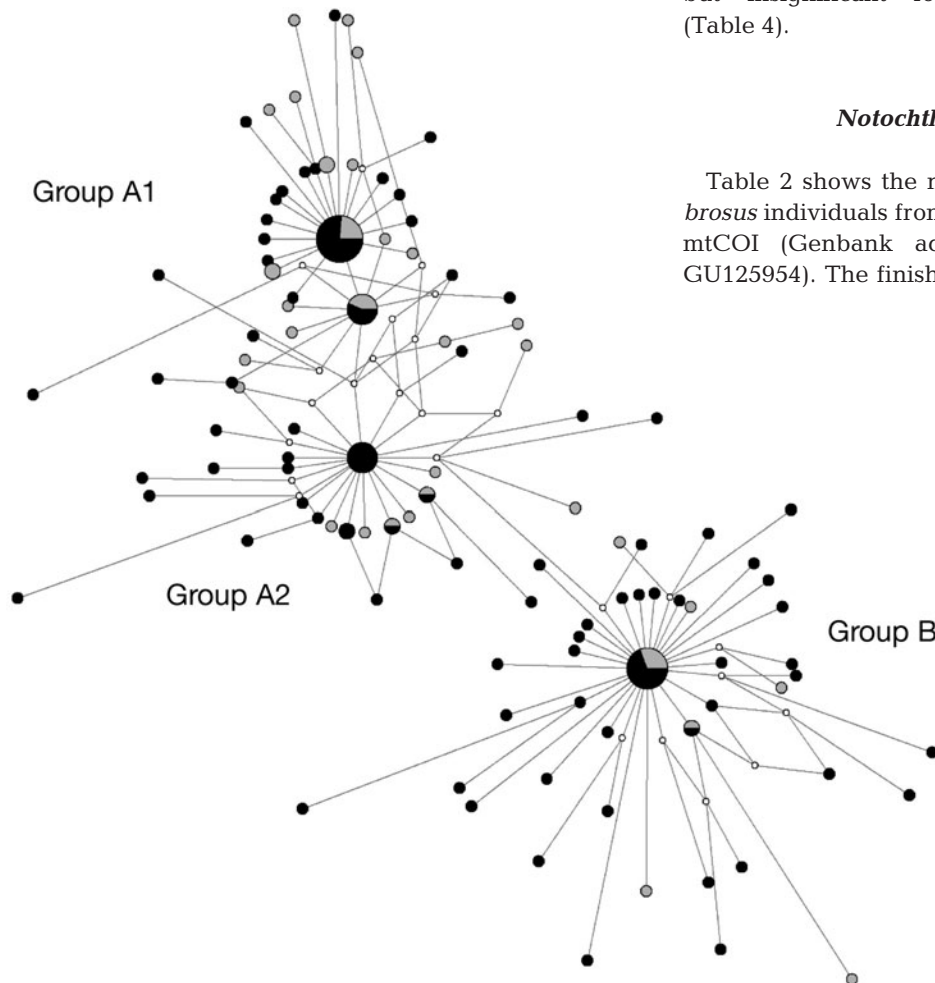

Group A1

Group A2

Group B

Fig. 1. *Jehlius cirratus.* Mitochondrial cytochrome oxidase I (MtCOI) gene network. Northern (grey) and southern (black) populations were defined based on the strongest corrected pairwise genetic difference between regions (F_{CT}) in *Notochthamalus scabrosus*, as in Fig. 2 (i.e. 'southern' is from Punta Talca southward). Note that the relative frequency of individuals from the northernmost populations is higher in phylogroup A1 at the top of the figure (phylogroups do not have strong bootstrap support, but A1 and A2 are defined by >90% consensus of maximum parsimony (MP) trees; the B group is recovered with 100% consensus of MP trees)

Table 3. Analysis of molecular variance (AMOVA) for *Jehlius cirratus* comparing sites north and south of 32°S and considering Punta Talca as part of the southern region (no site groupings were significant for F_{CT}). Fixation indices (probabilities) are F_{SC}: 0.006 (p = 0.06), F_{ST}: 0.032 (p = 0.06), F_{CT}: 0.026 (p = 0.06)

Locus	Source of variation	df	Sum of squares	Variance components	% of variation
mtCOI	Among groups	1	9.939	0.09627	2.66
	Among populations within groups	4	16.058	0.01967	0.54
	Within populations	149	521.011	3.49672	96.79

Table 4. Tajima's D statistic and θ(π) for all sites. Significant deviations were estimated in Arlequin using a beta distribution; no Tajima's D tests were significant. CO1: mitochondrial cytochrome oxidase I, EF1α: nuclear elongation factor 1α

Population	CO1 in *N. scabrosus*		CO1 in *J. cirratus*		EF1α in *N. scabrosus*		Observed heterozygosity	Expected heterozygosity
	Taj D	θ (π)	Taj D	θ (π)	Taj D	θ (π)		
Huasco	−1.023	9.144	−1.032	7.150	−0.579	0.82211	0.667	0.636
Arrayan	−0.426	10.219			−0.719	1.31394	0.714	0.857
Temblador	−1.47	9.659	−1.137	5.154	−1.440	1.45395	1.000	0.530
La Pampilla	−0.977	16.525			−0.602	1.36061	0.733	0.752
Guanaqueros	−0.663	8.655			−0.659	1.39292	0.500	0.623
Punta Talca	0.408	15.572	−0.771	7.638	−1.367	1.52575	1.000	0.656
Los Molles	0.499	11.544	−1.486	7.097	−0.690	1.92424	1.000	0.866
Las Cruces	1.168	14.378	−1.278	7.492	0.449	1.58678	1.000	0.733
Pichilemu	0.789	14.344	−1.372	8.959	−0.818	1.56883	1.000	0.681

parsimony informative sites. No substitutions resulted in amino acid replacement. The best-fit model based on either a LRT or an AIC is a 3-parameter model (TrN) with invariant/gamma-distributed rate variation (α = 1.1).

A parsimony network of mtCOI haplotypes is shown in Fig. 2, with the haplotype frequencies per sampled site being indicated. A maximum parsimony tree (of 1000 equally parsimonious trees) for mtCOI shows strong statistical support for clade structure in *Notochthamalus scabrosus* (Fig. 2, bootstrap values shown on network). Two clades with 100% consensus and 99% bootstrap support are identifiable; these are called clades A and B (Fig. 2). The A clade can be further separated into 2 subclades, A_1 and A_2, that are defined by 100% consensus but low (55%) bootstrap support. The geographic representation of these clades varies strongly: although all populations were dominated by clade A, clade B was found within ~40% of southern individuals and almost never found (<5%) among northern populations. This geographic shift in haplotype frequency appears to be strongest at ~31°S (Fig. 3). There is no significant fit to an isolation by distance model (R^2 = 0.07, p = 0.09).

In *Notochthamalus scabrosus*, AMOVA indicates significant structure among sites and regions (). The strongest F_{CT} value is obtained when all populations

from Punta Talca southward are grouped together. An overall F_{ST} of 0.08 (p < 0.01) and F_{CT} of 0.092 (p = 0.01) suggest stronger limits to gene flow than in *Jehlius cirratus*. Similar but insignificant results are obtained when data are analyzed with Punta Talca in the northern group (F_{CT} = 0.03, p = 0.4). Additional AMOVA testing with alternative regional structures (e.g. placing the break further north or south) generated no significant regional genetic structure (always a lower F_{CT}). Within-population θ (π) ranged from 8.655 to 16.525 in these 9 populations (Table 4). Diversity was generally higher in the southernmost populations, and Tajima's D values dramatically shifted from being generally negative and insignificant in northern populations to being positive in the 4 southern populations.

Estimated migration rates (M) from coalescent analysis of sequence data at the mitochondrial mtCOI locus suggest biased dispersal, with the B clade being an important indicator of restricted dispersal. When all sequence data are analyzed without regard to their phylogenetic status, results suggest stronger mean dispersal from north to south (against prevailing currents). Across 4 independent runs of MIGRATE on the entire data set, the ratio of M_N to M_S ranged from 0.66 to 0.85. A majority of M_N-M_S comparisons for each population indicated higher southward than northward emigration. However, when sequences from the
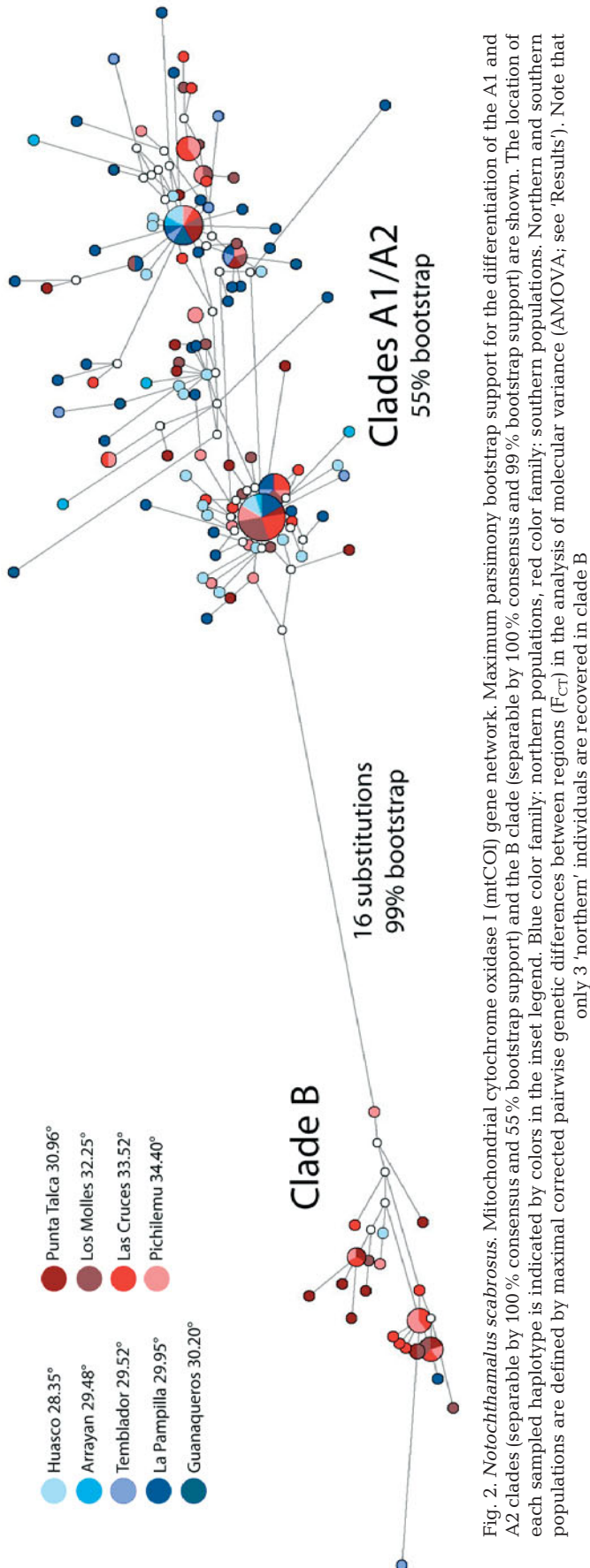
Fig. 2. *Notochthamalus scabrosus*. Mitochondrial cytochrome oxidase I (mtCOI) gene network. Maximum parsimony bootstrap support for the differentiation of the A1 and A2 clades (separable by 100% consensus and 55% bootstrap support) and the B clade (separable by 100% consensus and 99% bootstrap support) are shown. The location of each sampled haplotype is indicated by colors in the inset legend. Blue color family: northern populations, red color family: southern populations. Northern and southern populations are defined by maximal corrected pairwise genetic differences between regions (F_{CT}) in the analysis of molecular variance (AMOVA; see 'Results'). Note that only 3 'northern' individuals are recovered in clade B

B clade are removed from the data set, the ratio of northward to southward emigration rates shifts to between 1.05 and 1.33 (biased with the prevailing currents). Two (of 72) population comparisons (Los Molles to Pichilemu and Las Cruces to Punta Talca) indicated substantially higher migration than all other values in the pairwise matrix in 2 of the 4 MIGRATE runs and were removed from analysis. Inclusion of these values slightly changed the overall picture but significantly increased the variance across pairwise migration estimates.

These results at the mtCOI locus (deep clade structure, strong geographic shift in haplotype frequency) suggest that further genomic analysis was warranted. Sequence data at EF1α were collected from individuals as indicated in Tables 1 & 2 (Genbank accessions GU125955–GU126072). The aligned sequence data were 359 bp in length, with 23 variable sites, 12 of which were parsimony informative. These data BLASTed to a fragment of EF1α sequence data from *Balanus perforatus* (E score of 1.31e-145); the entire fragment sequenced for this project is the coding sequence. Only 3 parsimony informative sites resulted in amino acid replacement; 2 of these substitutions were at low frequency (≤2%), while 1 polymorphism (Val-Met) was found in 32% of all individuals but with no spatial pattern (see haplotype 4, Fig. 3; it represents 90% of all individuals carrying this allele). We determined that there were 34 haplotypes of EF1α in *Notochthamalus scabrosus* based on PHASE analysis. Of these haplotypes, 5 were common (over 98% of sampled alleles), while the other 30 were found in only 1 to 3 individuals. A parsimony network of these haplotypes is shown in Fig. 4. No significant structure is obtained through phylogenetic analysis (results not shown). The frequencies of the 5 common haplotypes are shown in Fig. 3 as a representation of the genetic clines from northern to southern populations that are demonstrated in each haplotype class. The Mantel test for isolation by distance at the EF1α locus in *N. scabrosus* indicates a significant correlation of pairwise genetic and geographic distances ($R^2 = 0.17$, p = 0.04). MIGRATE analyses of the nuclear EF1α locus are less informative regarding biased migration, with the overall ratio of inferred northward to southward migration being 0.94.

Unlike the mtCOI results, AMOVA of EF1α in *Notochthamalus scabrosus* indicates insignificant regional structure ($F_{CT} = -0.01$, ns) when Punta Talca is included in the southern region (Table 5). Additional AMOVA testing with all alternative regional structures (e.g. placing the break further north or south along the coast in all possible combinations) generates no significant genetic structure at the regional level. To test for a potential Wahlund effect (a reduction in hetero-
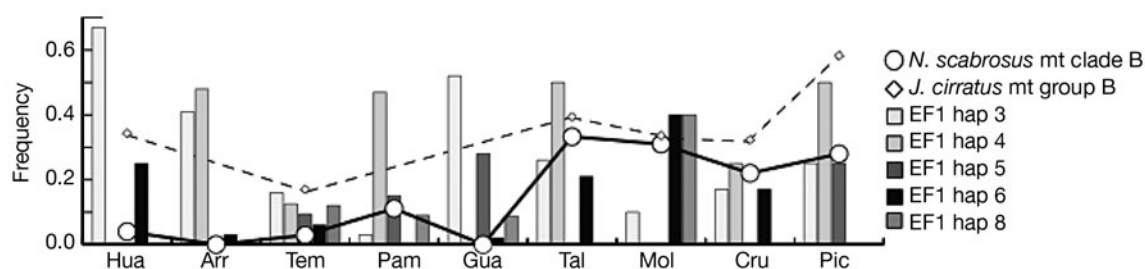
Fig. 3. Haplotype frequencies of the B clade in *Notochthamalus scabrosus* (O) and of the B phylogroup in *Jehlius cirratus* (◇) along the Chilean coast from the northernmost (Huasco) to the southernmost site (Pichilemu) (for list of sites see Table 2). The frequency shift of the mitochondrial B clade in *N. scabrosus* between Guanaqueros (30° 11′ S) and Punta Talca (30° 58′ S) is significant (*t*-test, p < 0.001). Frequency changes of haplotypes at the nuclear elongation factor 1α (EF1α) locus are consistent with isolation by distance; only the 5 most frequent haplotypes are shown

Table 5. Analysis of molecular variance (AMOVA) for *Notochthamalus scabrosus* mitochondrial cytochrome oxidase I (mtCOI) and nuclear elongation factor 1α (EF1α) comparing localities north and south of the proposed break at 32° S. All possible groupings were performed but only the grouping that has the largest corrected pairwise genetic difference between regions (F_{CT}) value (Punta Talca as part of the southern grouping) is shown. For the EF1α AMOVA, only haplotypes that occurred in >1 ind. were used. When Punta Talca is included in the southern region, fixation indices (probabilities) are F_{IS}: −0.23393 (p = 0.99), F_{SC}: 0.17078 (p = 0.0), F_{CT}: −0.01004 (p = 0.50), F_{IT}: −0.03347 (p = 0.80). F_{IS}: corrected pairwise difference for alleles within individuals within subpopulations; F_{IT}: corrected pairwise difference for alleles within individuals in the total population

Locus	Source of variation	df	Sum of squares	Variance components	% of variation
mtCOI	Among groups	1	58.263	0.61360	9.21
	Among populations within groups	7	32.345	−0.08181	−1.23
	Within populations	170	1042.338	6.13140	92.02
EF1α	Among groups	1	1.436	−0.00332	−1.00
	Among populations within groups	7	11.394	0.05706	17.25
	Among individuals within populations	108	22.923	−0.06481	−19.59
	Within individuals	117	40.0	0.34188	103.35

zygosity due to the inadvertent combination of 2 populations that are not randomly mating), inbreeding coefficients were also measured at the EF1α locus; in almost all populations, there was a slight excess of heterozygosity (Table 4) leading to low and insignificant F_{IS} and F_{IT} values. Tajima's D values at EF1α were generally negative (Table 4) and insignificant.

DISCUSSION

The available data for *Notochthamalus scabrosus* along the SAP coast suggest that, of 2 mitochondrial clades, one (clade B, Fig. 2) has extremely limited success in recruiting to northern populations. In this case, the evidence of limited migration occurs despite a presumed northward movement of larvae due to net SAP coastal currents (Aiken et al. 2007); this contrasts with the relative lack of genetic structure in *Jehlius cirratus*, suggesting that nearshore currents and their interaction with larval behavior are not the only factors responsible for the genetic cline. This is further supported by the MIGRATE-n results, where the expected oceanographic pattern of downstream (northward) migration is not recovered unless the mtCOI clade B is removed from the analyses. The overall pattern in *N. scabrosus* is supported by strong regional AMOVA (Table 5), a marginal signal of isolation by distance for the mitochondrial data, as well as a significant (p < 0.05) pattern of genetic isolation by distance at the nuclear EF1α locus. The limited gene flow is therefore not necessarily limited by migration, but could reflect significant historical and geographic differentiation of the 2 clades (Fig. 2, Table 5) (Peterson & Denno 1998, O'Brien & Freshwater 1999, Wares & Cunningham 2005, Moyle 2006).

A number of mechanisms may interact to maintain such a phylogeographic pattern. There are effectively 2 mitochondrial lineages with different geographic distributions — one occurring throughout the range of our study, and one limited near the ecological transition caused by shifting coastal upwelling regimes. Tajima's (1989) D statistic allows us to consider intrapopulation diversity for its fit to a model of neutral evolution. While the expectation for this statistic approaches 0, it is typical for mitochondrial sequence
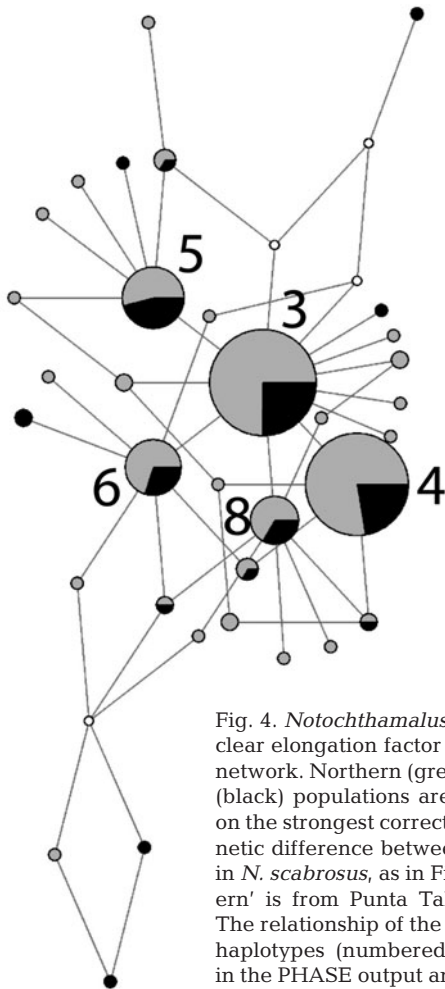
Fig. 4. *Notochthamalus scabrosus.* Nuclear elongation factor 1α (EF1α) gene network. Northern (grey) and southern (black) populations are defined based on the strongest corrected pairwise genetic difference between regions (F_{CT}) in *N. scabrosus*, as in Fig. 2 (i.e. 'southern' is from Punta Talca southward). The relationship of the 5 most common haplotypes (numbered by their order in the PHASE output and matching the haplotypes in Fig. 3) is also constant in the maximum parsimony (MP) gene trees, with 100% consensus

data to be negative due to background selection or demographic effects (e.g. *Jehlius cirratus* in Table 4; average published D for arthropods –0.52 ± 0.97, J. P. Wares in review). This same pattern is observed in northern populations of *Notochthamalus scabrosus* (Table 4), but southern populations harbor strongly positive D values that could suggest balancing selection, population decline, or other demographic factors. The shift in D from north to south may indicate a change in the selective pressures acting on these populations. Available data suggest that selection plays a role in maintaining the cline in *Balanus glandula* on the North American Pacific (NAP) coast (Sotka et al. 2004), but we can only speculate on how selection could also be involved in the diversity patterns of *N. scabrosus*. Unfortunately, no published studies have yet examined patterns of growth, survival or reproduction of *N. scabrosus* across this geographic region that could suggest the existence of varying selection pressures.

A similar pattern of Tajima's D may also indicate the presence of cryptic species. If there is simply a strongly divergent lineage of *Notochthamalus scabrosus*, we may have only sampled the geographic overlap between the ranges of these lineages. However, in such a case we would expect a Wahlund effect in the nuclear EF1α genotypic data. Nevertheless, there is no evidence of this effect in our data (Table 4). In fact, inbreeding coefficients illustrate an insignificant excess of heterozygosity (Table 4) with no clear pattern across populations (Table 5), while Tajima's D at this locus is generally slightly negative. Despite the lack of evidence for cryptic speciation in our data, there is the potential for partial reproductive isolation between southern and northern individuals. If partial hybridization is occurring, it would decrease our power to detect a Wahlund effect and only additional markers could fully resolve this possibility. Artifacts of computational haplotype reconstruction could influence these data (although singleton haplotypes were excluded in our presented results, the results are similar when all haplotypes are included), but the overall signal of the nuclear data suggests a single randomly mating species. In both the mtCOI and nEF1α data, there is a trend toward increased genetic diversity in the southern region (Table 4) that may be associated with the higher adult abundance at these sites (Broitman et al. 2001, Navarrete et al. 2005). We attempted to confirm these patterns with additional genetic data (2 nuclear gene regions; C. Zakas unpubl. data) but found insufficient intraspecific diversity in our screening.

The distinction between the A and B clades of *Notochthamalus scabrosus* is unlikely to be driven by introgression from another similar species; the genetic distance between *N. scabrosus* and the partially co-distributed confamilial *Jehlius cirratus* is more than an order of magnitude greater ($d_A = 0.16$) than the differentiation within *N. scabrosus*. The significant divergence of these intraspecific clades is unlikely to be due to purely stochastic (coalescent) mechanisms (Hein et al. 2005). It is possible that historic coastal glaciation may have been responsible for the formation of allopatric species that have since come into secondary contact (Dawson 2001). However, the extent of the last glacial maximum (LGM, 19 000 to 23 000 yr ago) covering the Chilean coast remained at latitudes south of 43°S (Hulton et al. 2002), which is outside the range examined in this study, and the net nucleotide divergence between the 2 primary lineages is 0.0180; assuming an approximate divergence rate of 3% per million years (Wares 2001), this divergence could predate the LGM by a few hundred thousand years (Wares & Cunningham 2005). Whether our study has simply recovered the transition zone between 2 de facto species, or an adaptive cline associated with fit-

ness of mitochondrial backgrounds requires further evaluation.

In recent years, it has been shown that the Chilean coast is a remarkable global reflection of the same broad processes observed along the California and Oregon coasts (Lagos et al. 2005, Navarrete et al. 2005, Navarrete et al. 2008). Both coasts are subject to strong coastal upwelling and equatorward offshore currents (Strub et al. 1998, Sotka et al. 2004). The similarities between coastal ecological transitions along the Pacific coasts of North and South America, and the associated genetic patterns in barnacle species with planktonic larvae, are intriguing. One case to consider is the 'recruitment break' at Cape Blanco, Oregon (42° N; Connolly et al. 2001). Nearshore oceanography and latitudinal shifts in the strength of upwelling leads to dramatic shifts in the density of larval recruits across Cape Blanco, with populations to the north experiencing orders of magnitude higher recruitment and higher overall population density. This, in turn, leads to distinct sets of ecological interactions: from a predator-driven 'top down' system described by Paine (1966, 2002) and Menge et al. (1994) to the nutrient-driven system further south (Menge et al. 1997a,b). The nearshore currents surrounding Cape Blanco are likely responsible for the genetic structure recovered across this break in the estuarine crab *Hemigrapsus oregonensis* (Petersen 2007). For this species, mtCOI haplotypes persisting from the mid-Pleistocene are present in all alongshore populations; however, a more recent and predominant upstream haplotype is absent in populations downstream of Cape Blanco, which is a pattern that Petersen (2007) argued was indicative of historical isolation and population expansion. Our data differ from those of *H. oregonensis* that show a significantly deeper phylogeographic split between the 2 lineages, and suggest that coastal upwelling or selection (or a combination of both) is more likely to determine current haplotype distributions.

Of the barnacle species considered to show a strong recruitment shift around Cape Blanco, the barnacle *Balanus glandula* exhibits a well-characterized genetic cline (Sotka et al. 2004) that is centered ~400 km downstream, given the general equatorward flow of coastal currents from the break in intertidal abundance. Other species that exhibit strong recruitment shifts at Cape Blanco (e.g. the barnacle *Chthamalus dalli*) show no apparent phylogeographic signal at all (Dawson 2001, Wares & Castañeda 2005). Coastal upwelling and currents drive spatial patterns of density and persistence (Byers & Pringle 2006) and can generate or maintain effective isolation of otherwise contiguous populations (Rocha-Olivares & Vetter 1999, Muss et al. 2001, Hare et al. 2005). However, because of complex interactions between coastal advection and

the potential for local adaptation (Pringle & Wares 2007), such genetic transitions (i.e. 'clines'; Endler 1977, Sotka & Palumbi 2006) may not be found uniformly across species and are not necessarily concordant with inferred ecological transitions.

While the 2 systems (NE Pacific and SE Pacific coasts) are only analogous in pattern, comparative inference of population history and gene flow among species may help identify the deterministic role that physical oceanography may play in the maintenance of coastal biodiversity. The 2 NAP barnacle species with similar adult distribution and general larval biology (*Balanus glandula* and *Cthamalus dalli*) provide contrasting evidence of effective larval dispersal: there are apparent limits to gene flow for *B. glandula* in northern California (Sotka et al. 2004, Wares & Cunningham 2005), while there is no apparent restriction in gene flow nor any sign of isolation by distance for *C. dalli* (Wares & Castañeda 2005). Similarly, in this study we show 2 SAP barnacle species respond differentially to the coastal environment. Although these species have a more recent phylogenetic relationship than those in the above studies and much greater biological similarity, they may also differ in larval development time in the field (Venegas et al. 2000), and perhaps in vertical swimming behavior during development. However, there remains a striking signal for restricted gene flow between southern and northern populations of *Notochthamalus scabrosus*, and only a marginal signal for isolation by distance in the confamilial *Jehlius cirratus* (a relative uniformity that holds up even when some additional samples from ~600 km to the south of Pichilemu are included; J. P. Wares unpubl. data).

Marko (2004) suggested that a potential explanation for different phylogeographic patterns in codistributed species with similar larval dispersal mode could be ecological or physiological. Both the high intertidal NAP and high intertidal SAP species under consideration show little to no genetic differences among sites, while the mid-intertidal NAP *Balanus glandula* and SAP *Notochthamalus scabrosus* exhibit strong differentiation. However, there are abundant cases of effectively panmictic gene flow in species distributed even lower in the intertidal (e.g. Harley et al. 2006, Ort & Pogson 2007). The physiological environment along both the NAP and SAP coasts could also drive patterns of local adaptation in species like *B. glandula* and *N. scabrosus*; while the recognized clines (Fig. 4; Sotka et al. 2004) are not obviously associated with perceived physiological transitions such as those caused by water temperature (Helmuth et al. 2006), air temperature may provide a useful explanation for *B. glandula* (E. Sotka pers. comm.). Although pelagic larval duration is a good correlate for larval dispersal capabilities and gene flow among distant populations (Kinlan & Gaines

2003), many studies have shown that effective dispersal within the same advective environment depends on larval behavior (e.g. diel vertical migration) and position in the water column throughout the dispersal phase (Poulin et al. 2002, Largier 2003, Shanks & Brink 2005, Guizien et al. 2006). Cyprids of NAP barnacle species tend to stratify in the water column as they do at settlement (Grosberg 1982); however, this stratification remains unexplored in SAP barnacles. Thus, to understand differences in dispersal and population connectivity among species with similar larval duration, it can be critical to have information on larval distribution and behavior in the water column during the dispersal phase.

Regardless of mechanisms, the relative concordance of the genealogical pattern with the recruitment transition around 31 to 32° S (Navarrete et al. 2005, Navarrete et al. 2008) is worth noting. Although the clines are not exactly coincident with the upwelling-driven ecological transitions, they are downstream of this region by only a generation or so of dispersal by an idealized passive larva (Kinlan & Gaines 2003, Shanks & Brink 2005, Pringle & Wares 2007). The multilocus genetic cline in *Balanus glandula* (Sotka et al. 2004) is downstream of the Cape Blanco (NAP) recruitment transition by a similar amount, and abiotic forces can certainly displace such patterns from the environmental gradients that cause them (Endler 1977). These signals may be coincident, and we can only speculate as to the interaction between abiotic and biotic mechanisms in maintaining this diversity, but they suggest change in coastal transport associated with change along an adaptive gradient. These mechanisms have been integrated to quantify the strength of selection necessary to maintain intraspecific diversity such as that which we find in *B. glandula* and *Notochthamalus scabrosus* (Pringle & Wares 2007). With further analysis of coastal advection and diffusion, experimental study of selection (e.g. reciprocal transplants), and spatially intensive recruitment plate studies, it may be possible to more closely predict the position of the cline (by relative input from upstream populations) and the selective advantage of downstream populations (Nagylaki 1978, Kawecki & Holt 2002, Pringle & Wares 2007), or to better understand these coastal processes in the absence of detectable selection.

Examination of the frequency of B clade individuals in recent larval recruits at northern sites will be of particular interest, as this may indicate whether there is differential mortality among individuals either as larvae or as post-settlement juveniles that is associated with their genetic background. Because B clade individuals are predominantly distributed upstream given prevailing currents (Aiken et al. 2007), we would expect them to have a dispersal advantage in reaching downstream (northern) sites, but they may not be able to survive and recruit. Preliminary recruitment data have been collected (Nov–Jan 2008-09); from a small sample size of 1 to 2 mm diameter recruits, it appears that the settlement of B clade *Notochthamalus scabrosus* is similar (χ^2 test, p > 0.10) in populations to the north and south of 31° S, suggesting that post-settlement mortality may be more important than limits to transport. A gene tree transitional analysis based on Wares et al. (2001) indicates no significant bias in movement of individuals from north to south or vice-versa (results not shown); however, coalescent analyses suggest a northward bias in movement of individuals as long as B clade individuals are excluded from analysis (see 'Results').

While the phylogeographic transition in *Notochthamalus scabrosus* is also concordant with a biogeographic transition between 30 and 32° S (Fernandez et al. 2000, Camus 2001), there does not appear to be extensive asymmetry of species with northern and southern boundaries in this region (Brattström & Johanssen 1983), as might be expected if oceanographic forces were maintaining the transition (Wares et al. 2001). Extension of geographic sampling will be necessary to fully characterize this transition, as both *N. scabrosus* and *Jehlius cirratus* are distributed along most of the South American coast from Ecuador to Chile (Brattström & Johanssen 1983, Venegas et al. 2000). Microhabitat analysis (Power et al. 1999, Wares & Castañeda 2005) may indicate the roles of environment and gregariousness among species and lineages in maintaining this pattern. Finally, further work on the effect of competition between *N. scabrosus* and *J. cirratus* on recruitment success under varying environmental conditions (e.g. López & González 2003) may be important in understanding both historical and ecological elements (Endler 1982) involved in maintaining this phylogeographic pattern.

The comparison of ecological and evolutionary patterns along the NAP and SAP coasts represents a unique opportunity for replication in phylogeography. Comparative and integrated analysis of ecological transitions (Connolly et al. 2001, Navarrete et al. 2008), changes in behavior associated with these transitions (Sanford et al. 2003), genetic patterns concordant with these transitions (Petersen 2007), and experimental evaluation of fitness (Sanford et al. 2006) at all life stages of coastal invertebrates will ultimately provide significant insights into the function of coastal communities and the diversity they harbor. Currents and their effect on dispersal are clearly responsible for some of these observed patterns, but the environmental changes in climate and topographic conditions, which usually modulate the changes in coastal hydrography, might exert unsuspected and poorly studied selective pressures. We hope these comparisons will allow the

transformation of such research toward predictions of regions that are most likely to retain larval production and novel diversity (Wares & Pringle 2008), for application in marine reserve design and coastal fisheries management.

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