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Phylogeography and Biogeography Concordance in the Marine Gastropod *Crepipatella dilatata* (Calyptraeidae) along the Southeastern Pacific Coast

Antonio Brante, Miriam Fernández, and Frédérique Viard

From the Departamento de Ecología, Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Concepción, Chile (Brante); the Estación Costera de Investigaciones Marinas of Las Cruces, Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile (Fernández); the International Associated Laboratory 'Dispersal and Adaptation in Marine Species' (Station Biologique de Roscoff (Roscoff, France) and Center for Advanced Studies in Ecology and Biodiversity (CASEB, Santiago, Chile)) (Brante, Fernández, and Viard); the Université Pierre et Marie Curie (UPMC; University Paris 06), Laboratory "Adaptation and Diversity in Marine Environment," UMR 7144, Station Biologique, Roscoff, France (Viard); and the Centre National de la Recherche Scientifique (CNRS), UMR 7144, Team Diversity and Connectivity in Coastal Marine Landscape, Station Biologique, Roscoff, France (Viard).

Address correspondence to Antonio Brante at the address above, or e-mail: abrante@ucsc.cl.

Abstract

The biogeography and phylogeography concordance hypothesis suggests that the same factors, for instance physical barriers or environmental gradients, shape both species assemblages and intraspecific genetic structure. In the marine realm, previous studies have however suggested that phylogeographic patterns are also explained by the life-history strategy of the species. However, evidence is contradictory and comes mainly from the northern hemisphere, which is characterized by specific environmental conditions and evolutionary histories of species. In this work, we evaluated the concordance hypothesis in the southern Pacific using the marine gastropod *Crepipatella dilatata* as a case study. This intertidal species with direct development exhibited a restricted dispersal potential, a feature that contrasts with previous species studied in the same area. Using the gene cytochrome oxidase I, we analyzed 253 individuals sampled at 10 locations covering 543 km of the coast of Chile. The study sites also incorporated 2 biogeographic regions separated by a well-studied biogeographic break (at 30°S). Populations of *C. dilatata* displayed a high degree of genetic structure and a perfect match between phylogeographic and biogeographic breaks at 30°S. When comparing our data with previous research over the same geographic range, life history traits related to dispersal ability seem to be a good proxy for explaining the concordance between biogeography and phylogeography along the southeastern pacific coast. In addition, in this and other marine invertebrate species, gene flow limitations across both sides of the 30°S break may act as a driver of the speciation process.

Key words: *Chilean coast, cytochrome oxidase I, dispersal potential, marine gastropod, phylogeographic break*

Biogeographic breaks are areas where a large number of species find their range limits (Cox and Moore 2000). At the intraspecific level, these areas are also characterized by changes in abundance, population and recruitment dynamics, and physiological traits (Broitman et al. 2001; Rivadeneira et al. 2002; Sanford et al. 2003; Ragionieri et al. 2009). At the genetic level, some research has reported that intraspecific phylogenies are featuring biogeographic boundaries in species with wide distribution ranges, supporting the concordance between biogeographic and phylogeographic boundaries (Avise et al. 1987). This concordance suggests that the same factors (physical and environmental like mountains, main oceanographic currents, etc.) that define species distribution may also delineate genetic boundaries within species (Avise et al. 1987).

In marine systems, oceanographic conditions like eddies and oceanographic fronts can determine biogeographic breaks and phylogeographic patterns (e.g., Collin 2001; Sanford et al. 2003; Cárdenas et al. 2009; Tellier et al. 2009; Zakas et al. 2009). However, many studies of marine species do not provide conclusive evidence supporting the hypothesis of a concordance between biogeographic and

phylogeographic boundaries (i.e., concordance: Lacson and Clark 1995; McMillan and Palumbi 1995; Benzie 1999; Collin 2001; no concordance: Burton 1998; Dawson 2001; Dawson et al. 2002). Intraspecific genetic structure cannot be predicted from only oceanographic patterns. Studies carried out to examine the influence of other characteristics, in particular life-history traits, on genetic structure have mainly been carried out in the northern hemisphere (e.g., Kelly and Palumbi 2010 for a recent study in North Pacific) with only a few in the southern hemisphere (see Cárdenas et al. 2009 for an example along the southeastern Pacific coast), where physical characteristics, geological histories, and evolutionary context are clearly different.

In the southeastern Pacific, a major topological discontinuity occurs on the oceanic shelf at 30°S: It affects the eddy kinetic energy and defines 2 discrete units in terms of ocean currents (Hormazabal et al. 2004). In addition, the northern area is strongly impacted by the El Niño Southern Oscillation (ENSO), favoring invasion by tropical species and negatively affecting the abundance of some native marine species (Glynn 1988; Castilla and Camus 1992). This environmental discontinuity delimits 2 biogeographic provinces: the Peruvian Province and the Intermediate Province (Lancellotti and Vásquez 1999; Camus 2001). This major oceanographic feature affects species distributions and population dynamics. For instance, Broitman et al. (2001) reported a gradual increase of abundance of the brown seaweed *Lessonia nigrescens* between 29° and 36°S and a sharp change in the abundance of other coastal species (e.g., *Perumytilus purpuratus*). At the population genetic level, the few studies carried out taking into account the 30°S oceanographic discontinuity did not show a consistent pattern. The seaweed *L. nigrescens* and the intertidal barnacle *Notochthamalus scabrosus* exhibited an exact match between the genetic and biogeographic breaks at 30°S, but the phylogeographic structure of the marine gastropod *Concholepas concholepas*, the barnacle *Jehlius cirratus*, and the giant kelp *Macrocystis pyrifera* did not match with this biogeographic break (Cárdenas et al. 2009; Tellier et al. 2009; Zakas et al. 2009; Macaya and Zuccarello 2010). The differing dispersal abilities of the species crossing this transition zone could be one of the main factors explaining the different effects that oceanographic conditions have on ecological traits and phylogeography. Recent papers have shown that a clear difference in genetic structure is expected for species exhibiting a direct compared with indirect development (Weersing and Toonen 2009; Kelly and Palumbi 2010). However, all the biological models used analyzed up to now in the southeastern Pacific coast have a dispersal stage in their life cycle (spores and drifting thalli for seaweeds and larvae for the invertebrates). Thus, no direct inferences can be made with respect to the relative importance of life-history strategies and oceanographic discontinuities in the understanding of population genetic structure along this region.

Crepipatella dilatata (Callyptraediae) is a protandrous gastropod distributed between 12° and 55° S, along the southeastern Pacific coast. Many populations with high densities have been observed in subtidal and intertidal habitats within this range. Adults exhibit internal fertilization, with sedentary females that brood capsules. Embryos develop inside the capsules by consuming nurse eggs until the juveniles hatch. *C. dilatata* is thus a typical direct developer (i.e., lacking a free floating larva). These characteristics make *C. dilatata* a good biological model for testing for the correspondence between biogeographic and phylogeographic boundaries in a species with a wide distribution, particular life-history strategy and restricted dispersal ability. In this work, we used the mitochondrial marker cytochrome oxidase I (COI) to test for the concordance of the 30°S biogeographic break with patterns of genetic diversity and structure in *C. dilatata*.

Materials and Methods

Sampling

The genus *Crepipatella* exhibits cryptic speciation which complicates the taxonomic identification to the species level. *C. fecunda* and *C. dilatata* share the same habitats and have similar distributional ranges. There are no external morphological characteristics that allow the 2 species to be differentiated. However, the embryonic development strategy is a good taxonomic character to differentiate between sibling species in the Callyptraeidae group (Collin 2003a). *C. dilatata*, the target species, has direct development, whereas *C. fecunda* has indirect development and the absence of nurse eggs. Only females incubating capsules containing juvenile individuals were here collected to ensure that *C. dilatata* was correctly identified.

In 2005, a total of 253 brooding females were collected from 10 locations on the coast of Chile between 28°29′S and 33°23′S (Table 1; Figure 1C), including the 30°S biogeographic break. Approximately 543 km of coastline were covered. Sites at Huasco, Punta de Choros, Temblador, and Caleta Hornos were assigned to the Peruvian biogeographic province and the 6 other sites to the Intermediate Province. The cephalic region of each individual was fixed in 95% ethanol, and the samples were stored individually in Eppendorf tubes prior to DNA analyses.

Molecular Data

For each individual, a 588 bp fragment of COI was obtained and sequenced directly from PCR products at the sequencing platform of the Biological Station of Roscoff. DNA was extracted with a Nucleospin kit (Macherey–Nagel) following manufacturers' protocol and amplified with primers and PCR protocols as detailed in Folmer et al. (1994). Double-stranded PCR products were purified using MultiScreen-PCR MANU03010 plates (Millipore) and cycle sequenced using ABI PRISM BigDye Terminators v3.0 Cycle Sequencing Kit following the manufacturer's protocol (Applied Biosystems). Unincorporated BigDye were removed using MultiScreen MAHVN4510 plates (Millipore).

Table 1 Geographic location of the 10 localities sampled along the northern-central coast of Chile

	Locality	Lat/Long	*N*	*S*	N_{hap}	H_d	Tajima's *D*	*P*-value
PP	1. Huasco	28°29′S/71°14′W	26	1	2	0.08	−1.15	0.15
	2. Punta de Choros	29°15′S/71°28′W	28	4	3	0.20	−1.56	0.03
	3. Temblador	29°28′S/71°18′W	25	1	2	0.08	−1.15	0.14
	4.Caleta Hornos	29°28′S/71°18′W	24	1	2	0.46	1.23	0.9
	Mean (±SE)		25.8 (0.9)	1.8 (0.8)	2.3 (0.3)	0.2 (0.1)		
IP	5. Herradura	29°58S′/71°21′W	23	16	7	0.67	−0.11	0.49
	6. Punta Talca	30°55′S/71°40′W	24	9	4	0.61	1.47	0.95
	7. Puerto Oscuro	31°25′S/71°36′W	30	2	3	0.13	−1.51	0.04
	8. Los Molles	32°14′S/71°30′W	24	9	7	0.47	−0.59	0.32
	9. Montemar	32°57′S/71°32′W	25	9	8	0.72	−0.85	0.22
	10. El Quisco	33°23′S/71°42′W	24	3	2	0.51	2.35	0.99
	Mean (±SE)		25 (1)	8 (2.1)	5.2 (1)	0.5 (0.1)		
	Mean (±SE)		25.3 (0.7)	5 (1.59)	4 (0.8)	0.4 (0.1)		
	Total		253	55	40	0.86	−1.29	0.08

The number of individuals sequenced (*N*) is indicated together with genetic diversity indices (segregating sites [*S*], number of haplotypes [N_{hap}], and haplotype diversity [H_d]) observed in each location. Means for each biogoegraphic province and the overall mean are also presented. Tajima *D* values are given with their associated *P*-values. PP and IP stand for Peruvian Province and Intermediate Province, respectively.

DNA was sequenced with an ABI PRISM 3100 Automated DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA). Sequences were aligned by eye using PROSEQ v2.91 (Filatov 2002).

Statistical Analyses

Standard genetic diversity indices such as the number of haplotypes (N_{hap}), the number of segregating sites (*S*), and the haplotype diversity (H_d) were estimated for each population using ARLEQUIN version 3.1 (Excoffier et al. 2005). Differences in genetic diversity indices (N_{hap}, *S*, and H_d) between both biogeographic provinces were tested using Mann–Whitney *U*-nonparametric tests in the Statistica 6.0 software (StatSoft Inc., Tulsa, OK).

To test the concordance hypothesis, an F_{st}-based hierarchical analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was carried out to test for the regional structure of populations using ARLEQUIN version 3.1 (Excoffier et al. 2005). Sites (here after called "populations") were grouped into 2 regions corresponding to the 2 biogeographic provinces. The genetic differentiation was investigated using a hierarchical analysis of the genetic variance by partitioning F_{st} into F_{sc} and F_{ct} indicating the genetic differentiation of populations within regions and between regions, respectively. These fixation indices, computed with sequence data, take into account both the haplotype frequencies and the molecular distances between haplotypes (Excoffier et al. 1992). The level of significance of F_{sc} and F_{ct} was computed by nonparametric permutational procedures (1000 permutations) of genotypes among populations within groups and among populations between groups, respectively. Given that Los Molles showed a striking genetic pattern (see Results), the analyses were also run excluding this locality in order to test for robustness of the results when excluding this population. Additionally, we applied a spatial analysis of molecular variance (SAMOVA) test (Dupanloup et al. 2002) to explore the possibility of alternative patterns of population genetic structure. SAMOVA analyses were run in the software SAMOVA 1.0 (Dupanloup et al. 2002).

We tested for genetic isolation by geographical distance (isolation by distance model; Wright 1943; Slatkin 1993): coastline geographical distances (kilometer), measured from a map, were plotted against $F_{st}/(1 - F_{st})$ to compute a linear relationship and to test for the null hypothesis of independence between genetic and geographic distances. Mantel procedure was used to test for an association between the 2 distance matrices using a randomization procedure. Analyses were run at 3 different scenarios: 1) all localities, 2) all localities excepting Los Molles (see above), and 3) making separate analyses for localities north and south of the 30°S biogeographic break without Los Molles. Population structure analyses and Mantel tests were conducted with the program ARLEQUIN v3.11 (Excoffier et al. 2005).

To picture the spatial distribution of the haplotypes, we constructed a haplotype network using the Median Joining algorithm, implemented in the NETWORK 4.5.1 software (Bandelt et al. 1999). This method is based on a maximum parsimony algorithm to simplify the complex branching pattern and to represent the most parsimonious intraspecific phylogenies (Polzin and Daneshmand 2003). In addition, to evaluate the genealogical relationships among haplotypes, we constructed a haplotype tree by using a maximum likelihood approach (1000 permutations) in MEGA 5 (Tamura et al. 2011). MODELTEST 3.6 (Posada and Crandall 1998) showed that the simplest and best model for molecular evolution was a TrN + I + G model, which was implemented in MEGA 5 for genealogical analysis. The congeneric species *C. fecunda* was used as outgroup to root the tree. Finally, demographic and/or selective history at mitochondrial DNA locus was

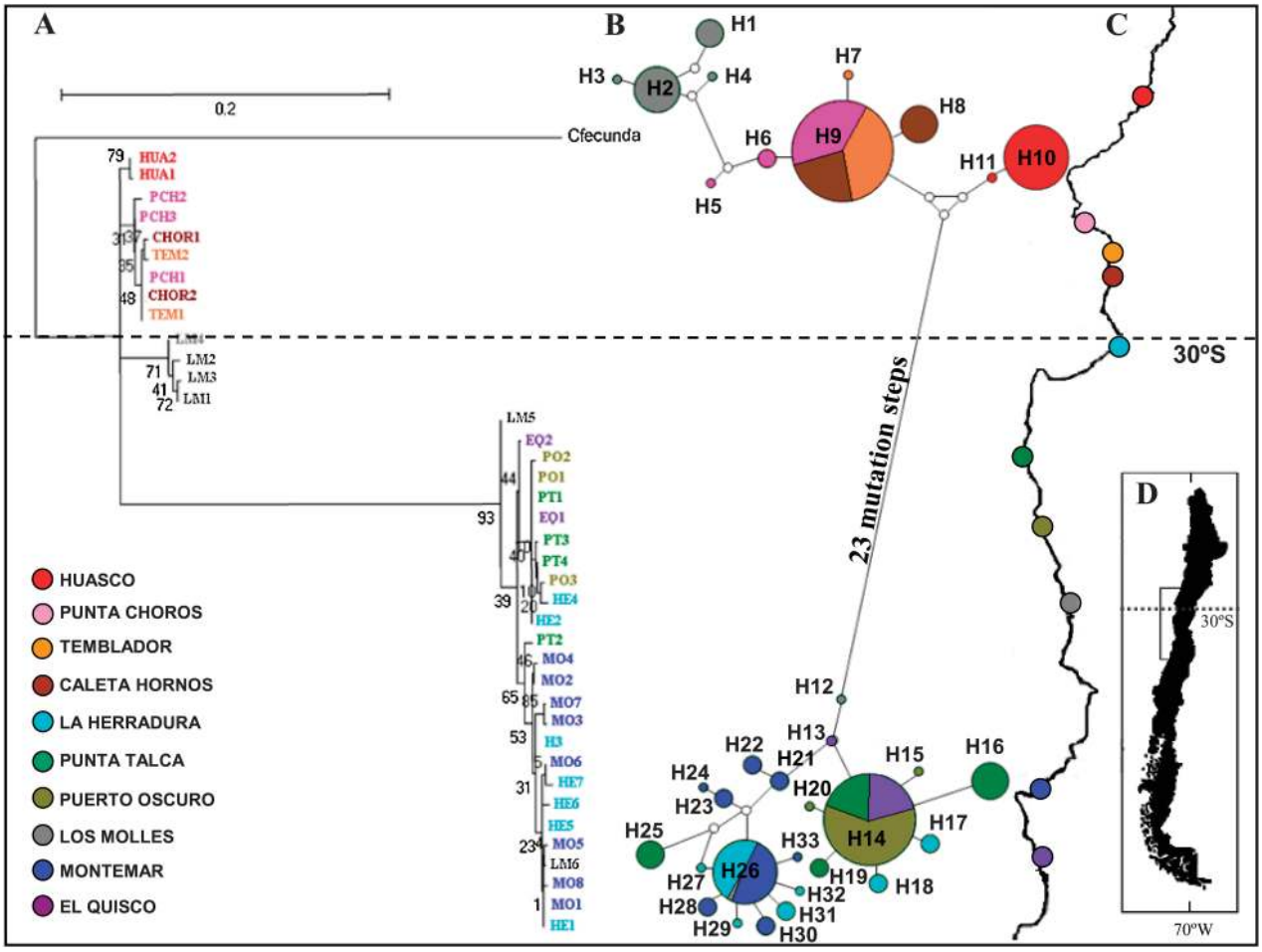

Figure 1. (**A**) Maximum likelihood tree representing the genealogic relationships between haplotypes observed in the 10 sites sampled for *Crepipatella dilatata*. Numbers above the branches represent bootstrap support. Los Molles was located in the north clade with respect to the 30°S biogeographic break, despite its southern location. HUA = Huasco, PCH = Punta Choros, CHOR = Caleta Hornos, TEM = Temblador, HE = La Herradura, PT = Punta Talca, PO = Puerto Oscuro, LM = Los Molles, MO = Montemar, EQ = El Quisco. (**B**) Haplotype network of the 33 mitochondrial DNA haplotypes detected in 253 individuals from 10 different sampling locations along the north-central Chilean coast. Haplotype circle sizes are proportional to the number of individuals. Northern and southern populations were defined with respect to the 30°S biogeographic break (segmented line). (**C**) Sampling locations in the 2 biogeographic provinces (Peruvian and Intermediate provinces) of the north-central coast of Chile. (**D**) Map of Chile showing the extension of the sampling in the Chilean coast.

examined by calculating the Tajima's *D* (Tajima 1989) in ARLEQUIN software.

Results

We sequenced and analyzed the COI gene of 253 individuals from 10 locations on both sides of the 30°S biogeographic break along the northern-central coast of Chile. Over the whole data set, we identified a total of 55 polymorphic sites leading to the definition of 33 different haplotypes (Figure 1; Supplementary Table S1). Haplotype diversity varied between 0.08 and 0.72 (Table 1) and was the only diversity index positively correlated with latitude (Spearman correlation: $R = 0.65$, $t = 2.42$, $P = 0.041$; Supplementary Figure 1). However, mean values of N_{hap}, S, and H_d all differed significantly between the 2 biogeographic provinces (Mann–Whitney *U*-test. N_{hap}: $Z = 1.99$, $P = 0.04$; S: $Z = -2.18$, $P = 0.02$; and H_d: $Z = -2.13$, $P = 0.03$; Supplementary Figure 1). Sites in the Peruvian biogeographic province exhibited lower mean values (Table 1).

The global F_{st} value was very high with an estimate of 0.97, significantly different from 0. Population pairwise F_{st} values were variable ranging from 0.01 to 0.92 (see Supplementary Table S2). Genetic distance did not correlate with geographic distance when all localities were considered (Mantel's test: $r = 0.13$, $P = 0.19$; Supplementary Figure 2). However, a significant correlation between genetic and geographic distance was observed 1) over the whole data set

Table 2 AMOVA to test for the 30°S phylogeographic break and SAMOVA for 2, 3, 4, and 5 groups of locations (locations numbered 1–10 as in Table 1)

Number of groups	Spatial structure tested	*F*-statistic	*P*-value
AMOVA			
2	(1-2-3-4) (5-6-7-8-9-10)	$F_{ct} = 0.67$	<0.001
		$F_{sc} = 0.92$	<0.001
		$F_{st} = 0.97$	< 0.001
2 (without Los Molles)	(1-2-3-4) (5-6-7-9-10)	$F_{ct} = 0.90$	<0.001
		$F_{sc} = 0.77$	<0.001
		$F_{st} = 0.97$	<0.001
SAMOVA			
2	(1-2-3-4-8) (5-6-7-9-10)	$F_{ct} = 0.83$	<0.001
		$F_{sc} = 0.71$	<0.001
		$F_{st} = 0.95$	<0.01
3	(1-2-3-4) (5-6-7-9-10) (8)	$F_{ct} = 0.87$	<0.001
		$F_{sc} = 0.62$	<0.001
		$F_{st} = 0.95$	<0.001
4	**(1) (2-3-4) (5-6-7-9-10) (8)**	$\mathbf{F_{ct} = 0.89}$	**<0.001**
		$\mathbf{F_{sc} = 0.50}$	**<0.001**
		$\mathbf{F_{st} = 0.94}$	**<0.001**
5	(1) (2-3-4) (6-7-10) (5-9) (8)	$F_{ct} = 0.78$	<0.001
		$F_{sc} = 0.12$	<0.001
		$F_{st} = 0.93$	<0.001

Grouping showing the highest values for F_{ct} is in bold. See 'Statistical Analyses' for definition of F_{st}, F_{sc}, and F_{ct}.

when Los Molles was excluded from the Mantel's test analysis ($r = 0.31$, $P = 0.042$) and 2) between populations from the northern group (Mantel's test: $r = 0.63$, $P = 0.015$). No correlation was observed for the southern localities with or without Los Molles (Mantel's test: including Los Molles: $r = -0.38$, $P = 0.93$; without Los Molles: $r = 0.28$, $P = 0.77$).

Genetic variation was primarily distributed between biogeographic provinces (67.9%) and in less proportion among localities within provinces (29.6%) and within localities (2.46%; Table 2). Similar general pattern was observed when the AMOVA was run without Los Molles with an increase in F_{ct} and a decrease in F_{sc} pointing out the particular status of this population (Table 2). The SAMOVA analysis detected 4 different groups: 2 clusters picturing a partition around the 30°S biogeographic break plus Huasco which is the most northern population studied and Los Molles (Table 2).

The network of COI haplotypes revealed 2 clades clearly differentiated and well explained by the geographical locations of the haplotypes (Figure 1B): one clade was composed exclusively of haplotypes found for individuals sampled south of the 30°S biogeographic break; the other clade was mainly composed (76%) of haplotypes found in individuals from the northern province (Figure 1B). Twenty-three mutational steps separated the northern and southern clades. The haplotype tree supported this deep bifurcation, separating the populations into 2 different clades coinciding with the limit of the 2 biogeographic provinces at the 30°S: 1) from Huasco (28°29′S) to Caleta Hornos (29°28′S) corresponding to the northern clade and 2) from La Herradura (29°58′S) to El Quisco (33°23′S) forming the southern clade (Figure 1A). The sample from Los Molles was however closely related to the northern sites despite being located south of the biogeographic break (Figure 1A). The neutrality test for the whole population revealed negative but nonsignificant Tajima *D* values ($D = -1.29$, $P = 0.08$). At the locality level, only Punta de Choros and Punta Talca exhibited negative and significant values in the Tajima's test (Table 1).

Discussion

The absence of a planktonic larva and the sedentary behavior of adults make *C. dilatata* a species with a restricted dispersal potential likely to display strong genetic structure. The population genetic analyses presented here unambiguously supported this prediction at 2 different levels. First, a high level of genetic structure between sites was observed within the 2 biogeographic provinces examined, that is, the Magellanic and Intermediate Provinces. In addition, a deep molecular divergence (i.e., a major phylogeographic discontinuity) was observed between the 2 provinces around 30°S, coincident with the biogeographic break reported for this latitude. These results suggest that the combination of particular life-history characteristics and physical oceanographic patterns at large scales determine the spatial genetic diversity pattern of this species along the southeastern Pacific coast.

Genetic diversity of the COI gene of *C. dilatata* populations exhibited low to intermediate values ($H_d = 0.08 - 0.72$; $N_{hap} = 2–8$) in comparison with marine gastropods with larval dispersal from the same region. For example, haplotype diversity in the indirect developer *C. concholepas* oscillated between 0.88 and 0.95 using similar

sampling effort (Cárdenas et al. 2009). For 2 species of intertidal barnacles with planktonic larval stages, the number of haplotypes ranged between 5 and 25 in *J. cirratus* and between 21 and 31 in *N. scabrosusosus* (Zakas et al. 2009). Higher levels of genetic diversity have been observed in marine species with indirect development and high dispersal potential in comparison to species with direct development, such as *C. dilatata* (e.g., Uthicke and Benzie, 2003; Zane et al. 2006; Lee and Boulding, 2007; Cárdenas et al. 2009). In direct developing species, limited connectivity between populations may determine smaller local effective population sizes and strong genetic drift effects which negatively affect population genetic diversity and enhance genetic structure.

The very high level of genetic structure in *C. dilatata* in the study area is exemplified by the overall F_{st} value (0.97) which contrasts with the low values found in species with planktonic larvae, such as *J. cirratus*, *N. scabrosus*, and *C. concholepas*, in the same geographical area (average F_{st} = 0.039 ±0.038; Cárdenas et al. 2009; Zakas et al. 2009). Several studies on marine species from different taxonomic groups have found that species with low dispersal potential exhibit strong population genetic structure (e.g., see a meta-analysis by Kelly and Palumbi 2010). The absence of a larva in *C. dilatata* and the sedentary behavior of adults would limit gene flow between localities causing the high genetic structure observed in this species. In fact, similar patterns of genetic structure have been observed in other Callyptraeidae species with similar life-history strategies in the Western North Atlantic (Collin 2001). For example, in *Crepidula convexa* and *C. atrasolea*, 2 species with direct development, F_{st} values fluctuated between 0.76 and 0.54, respectively.

Isolation by distance was detected only when Los Molles was not considered in the Mantel test analysis. This result together with the network and phylogenetic tree evidence an anomalous behavior of Los Molles in comparison with the general pattern observed through the 30°S biogeographic break. The haplotype network and tree and also the AMOVA and SAMOVA indeed clearly indicated the presence of a major genetic break within *C. dilatata* distinguishing the Peruvian province from the Intermediate province. Furthermore, mean genetic diversity differed between both areas. However, the sampling strategy did not allow determining if this pattern disruption effectively corresponds to an abrupt break or if it corresponds to an area of transition, more than a straight line, between both biogeographic regions (there is 40 km of distance between the border localities, Caleta Hornos, and La Herradura). A more intensive sampling on this area is needed to determine the specific location of the break (or area). In addition, SAMOVA grouped Huasco, the most northern population studied, in a different additional group. Interestingly, a similar pattern for Huasco was observed in a phylogeographic study carried out through the 30°S biogeobraphic break in *Acanthina mondon*, another marine gastropod species (Sánchez et al. 2011).

Our results together with recent studies carried out in the same geographic area suggest that the occurrence of biogeographic and phylogeographic concordance depends on the presence versus absence of a pelagic dispersal stage and its duration. Species with a medium to long-lived free floating stage (i.e., larva, spore, or vegetative structures), such as the marine gastropod *C. concholepas*, the barnacle *J. cirratus*, and the giant kelp *M. pyrifera*, did not provide support for the 30°S biogeographic break (Cárdenas et al. 2009; Zakas et al. 2009; Macaya and Zuccarello 2010). In contrast, the kelp *L. nigrescens* and the intertidal barnacle *N. scabrosus*, 2 species with a short intermediate stage, exhibited an exact match between the genetic and biogeographic breaks at 30°S (Tellier et al. 2009; Zakas et al. 2009). Recently, Sánchez et al. (2011) found that the population genetic structure of *A. monodon*, a species with direct development (absence of larva), is highly associated to the 2 major biogeographic breaks observed along the Chilean coast (the 30°S and 40°S biogeographic breaks). Limits between the Peruvian and the Intermediate Provinces are characterized by changes in oceanographic conditions, which affect ecological patterns and processes along the southeastern Pacific coast (Hormazabal et al. 2004). For example, a shift in the upwelling regimes may have profound effects on the distribution and local dynamics of coastal marine populations and entire communities (Navarrete et al. 2008). This shift in oceanographic conditions is likely to influence gene flow between populations around 30°S in *C. dilatata*, *L. nigrescens*, and *N. scabrosus*. A similar effect has been documented for Cape Blanco (42°N) in Oregon, a region affected by similar shifts in upwelling regimes favoring a strong reduction in recruitment and genetic clines in invertebrate species (Connolly et al. 2001; Sotka et al. 2004).

With one exception (Los Molles), our results indicate a major phylogeographic split between populations north and south of a marine biogeographic boundary located around 30°S along the coast of Chile. Similar pattern have been observed in other species across the 30°S biogeographic break. Two different clades, characteristics of the north and south of the 30°S break, were observed in *N. scabrosus* and *A. mondon* (Zakas et al. 2009; Sánchez et al. 2011). For the intertidal kelp *L. nigrescens*, the high genetic divergence observed between northern and southern locations indicated the occurrence of a budding speciation (Tellier et al. 2009). The large divergence observed in *C. dilatata*, with 23 mutational steps of differences (i.e., 4.5% of divergence), and the overall geographical concordance of the 2 clades suggests the existence of sibling or cryptic species or the existence of a species complex. Speciation is a dynamic process and the barrier among what can be called "species" is complex to define (e.g., Hey 2006; Pinho and Hey 2010) as shown in many marine taxa, for instance, in the *Mytilus* complex (Gérard et al. 2008). However, as a starting point, study of phylogeographic breaks may help to reveal sibling species and or cryptic species (e.g., Jolly et al. 2005, 2006). From our results only, 2 alternative hypotheses may explain population genetic patterns observed in *C. dilatata* in lack of additional data: 1) in the absence of reproductive isolation: an ancient divergence at

mitochondrial gene between northern and southern populations maintained by combination of drift and low gene flow; such a pattern is particularly noticeable at mitochondrial genes because of their reduced effective size as compared to nuclear markers (Birky et al. 1989); this process will eventually results in speciation (Pinho and Hey 2010). The absence of a larva in *C. dilatata* would facilitate this process. The strong genetic structure within northern and southern lineages suggests low levels of migration. 2) With reproductive isolation: In this case, the different clades observed in *C. dilatata* would correspond to sibling species. Under this scenario, the phylogeny/taxonomy has to be revisited again. However, according to Collin (2003b), *C. dilatata* and the outgroup species used in this study, *C. fecunda*, are the genetically least divergent sister-species studied within the Calypreaidae group. Moreover, our analyses showed limited divergence between the 2 clades (4.1%) as compared to the divergence between *C. fecunda* and the 2 clades of *C. dilatata* (7.7% and 8.3% of divergence with the northern and southern clades, respectively) supporting the hypothesis of closely related taxa. Further studies based on 1) additional markers, in particular nuclear DNA to test for the amount of hybridization and/or introgression between the 2 clades, if any, 2) experimental crosses between individuals from different origins, and 3) in-depth morphologically analyses are needed to try to elucidate the taxonomic status of the *C. dilatata* complex.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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