



A phylogeographical analysis across three biogeographical provinces of the south-eastern Pacific: the case of the marine gastropod *Concholepas concholepas*

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ABSTRACT

Aim The present-day population structure of a species reflects the influence of population history as well as contemporary processes. Little is known about the mechanisms that have shaped the geographical distribution of genetic diversity in marine species present on the south-eastern Pacific (SEP) coast. Here we provide the first comprehensive phylogeographical study of a species distributed along the SEP coast by analysing the endemic and emblematic muricid gastropod *Concholepas concholepas*.

Location The study localities were distributed along the SEP coast ranging from Matarani (11° S) to Puerto Eden (49° S), crossing three major biogeographical provinces: Peruvian Province, Intermediate Area and Magellanic Province.

Methods A total of 337 specimens of *C. concholepas* were collected from 14 localities in the three biogeographical provinces/areas. Mitochondrial cytochrome oxidase I (COI) gene partial sequences (658 bp) were obtained and analysed using coalescence-based methods to infer molecular diversity and phylogeographical patterns.

Results Across the 337 individuals, we found a large diversity, with a total of 179 haplotypes at the COI gene fragment. Although a slight decrease in gene diversity was observed from north to south, an analysis of molecular variance did not reveal any significant spatial population differentiation from Peru to the tip of Chile, not even across the recognized biogeographical boundaries at 30° S and 42° S. In addition, a star-like haplotype network suggested the past occurrence of a rapid demographic and geographical expansion over the total range examined. Calculations of the onset of this expansion suggest that it might be due to climatic conditions during the period of the marine isotope stage 11 (MIS 11, 400,000 years ago), the longer and warmer interglacial episode during the Pleistocene epoch.

Main conclusions Our phylogeographical analyses indicate that in the recent past *C. concholepas* mitochondrial DNA lineages underwent a sudden population expansion event. In addition, our data do not support the hypothesis of concordance between biogeographical barriers and phylogeographical breaks along the SEP coast. These two results are in accordance with the paradigm of high larval dispersal ability in marine species with an extended pelagic larval phase.

Keywords

Biogeography, *Concholepas concholepas*, cytochrome oxidase I, phylogeography, range distribution, south-eastern Pacific Ocean.

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INTRODUCTION

Global environmental changes and geological events have repeatedly modified the distribution ranges of species over ecological and evolutionary time scales. The direction (expansion vs. contraction of species range) and magnitude of these changes depend on numerous factors, including geology and the life-history traits of the species (Hewitt, 2000). Species' range evolution over time leaves genetic signatures at population and species levels, which can be investigated using phylogeographical analyses (interpretation of the spatial distribution of gene lineages; Avise, 2000). Phylogeographical patterns have often documented the concordance between phylogeographical breaks and biogeographical boundaries. For instance, in marine coastal species, such a concordance has been reported for the limpet *Tectura depicta* (Louie, 2003) around the centre of the California coast. The identification of major phylogeographical break points has even led to the recognition of cryptic species on both sides of biogeographical boundaries (e.g. the annelids *Pectinaria koreni* and *Owenia fusiformis* in the north-eastern Atlantic; Jolly *et al.*, 2005, 2006). In most of these studies, phylogeographical break points have been related to thermal changes that occurred during glacial and interglacial periods, particularly during the Last Glacial Maximum (LGM). Therefore, along the California coast numerous taxa display patterns suggesting a post-Pleistocene range expansion (e.g. *Nucella emarginata*, Marko, 1998; *Acanthinucella spirata*, Hellberg *et al.*, 2001; *Nucella ostrina*, Marko, 2004).

During the middle-late Pliocene (3.6–1.8 Ma), severe geological and oceanographic changes affected the Eastern Pacific region, such as the closing of the Panama seaway, the expansion of coastal upwelling and the appearance of the modern Humboldt Current system (Zinmeister, 1978; Tsuchi, 2002). Periodic global cooling during the Pleistocene (1.8 Ma to 10,000 years ago) also produced changes in climate, landscape and sea level (Rohling *et al.*, 1998). During the LGM, an ice sheet c. 1800 km long covered the land at the tip of the Andes down to the sea, between 35° S and c. 56° S of latitude (Clapperton, 1993; McCulloch *et al.*, 2000). Throughout the Holocene, natural cycles such as El Niño/Southern Oscillation (ENSO) events (McPhaden, 1999) and the Pacific decadal oscillation (Chavez *et al.*, 2002) also altered the climate. These series of climatic and geological events have modified species assemblages and species ranges, thus promoting the present-day distribution of species along the south-eastern Pacific (SEP) coast (Camus, 2001; Valdovinos *et al.*, 2003; Rivadeneira, 2005; Ruzzante *et al.*, 2006). However, in contrast to the Atlantic and the north-eastern Pacific, in the SEP the influence of such historical events, and the relationship between biogeographical provinces and the genetic structure of the species, are poorly documented.

We refer to the SEP as the area that includes large regions of year-round upwelling (from the equator to c. 30° S) and seasonal upwelling (c. 30–40° S) and the Patagonian Area (south of 40° S) which is characterized by fjords, inlets, sounds and straits. Camus (2001) suggested the following bioge-

ographical classification: (1) the Peruvian Province, a northern province ranging from 4° to 30° S, which contains a warm-temperate biota; (2) the Magellanic Province, ranging from 42 to 56° S, which contains an austral related biota; and (3) the Intermediate Area between the two previously mentioned provinces. These three major biogeographical provinces led Camus (2001) to define two major biogeographical breaks. The first is around 30–32° S and corresponds to a transitional zone, where important differences in the species abundance, diversity, and recruitment of several intertidal marine invertebrate species have been documented (Broitman *et al.*, 2001; Rivadeneira *et al.*, 2002). This zone correlates with a temperature gradient of the sea surface (Broitman *et al.*, 2001), a peculiar water circulation pattern (Hormazabal, 2004) and an abrupt narrowing of the continental shelf (Strub *et al.*, 1998). The second is located at around 42° S and has been recognized as a major biogeographical break point. In this area, the divergence of the main oceanic currents (Humboldt and Cape Horn current systems) occurs, corresponding to the northern boundary of the Magellanic Province, contributing to the relative isolation of the marine fauna within the Patagonian fjords (Castilla *et al.*, 1993; Valdovinos *et al.*, 2003).

In the SEP, most of the biogeographical studies have focused on the description of the species assemblages without an assessment of the mechanisms that generated this pattern (reviewed by Camus, 2001). Although sharp contrasts exist among the three biogeographical provinces, phylogeographical studies are almost absent, and the few that have been carried out covered only a small part of the SEP coast (e.g. *Sardinops* sp., Bowen & Grant, 1997). This limits our understanding of the processes (historical and contemporary) that led to the present-day distribution of the species and the effect of such sharp transition between the biogeographical areas. To address this issue, in this study we selected a species displaying a geographical range covering the entire SEP coast (certainly including the transition zone between adjacent biogeographical provinces): the gastropod *Concholepas concholepas* (Bruguière, 1789), a muricid endemic to the SEP. *Concholepas concholepas* has exceptional characteristics, making it an interesting biological model to test for connectivity patterns across different biogeographical provinces. (1) It has an extensive range of distribution from tropical (Lobos Afuera Island, 6° S) to sub-antarctic habitats (Cape Horn, 56° S) and thus has a range that encompasses the three provinces of the SEP coast. (2) It has a planktonic larval stage, spending at least 3 months in the water column (DiSalvo, 1988). (3) It shows variations in shell morphology along its geographical distribution (Stuardo, 1979). According to Stuardo (1979), the high level of morphological variation in *C. concholepas* should have a taxonomic value, thus opening the possibility for the existence of several subspecies along its geographical range distribution. (4) The palaeontological evidence provides insight into the scenario that the Chilean coast was colonized from Peru: palaeontology studies suggested a Peruvian origin of *C. concholepas* during the Pliocene–Pleistocene transition (DeVries, 1995, 2000). Moreover, *C. concholepas* is a mollusc

heavily exploited in Chile and Peru (Leiva & Castilla, 2002), and therefore the identification of potential operational taxonomic units (OTUs), as implemented in this study, may provide an important regional context in which to frame marine management or conservation efforts.

Attempts have been made to analyse the genetic structure of populations of *C. concholepas*. Nevertheless, none of these previous studies encompassed the overall range of distribution of *C. concholepas*, and contradictory results have been obtained. Guíñez *et al.* (1992) used isozymatic variation to study the genetic structure across six localities from southern Peru (Mollendo, 17°00' S) to southern Chile (Mehuín, 39°27' S). The authors showed a strong genetic structure and suggested at least three genetic groups across the area sampled. In contrast, Gallardo & Carrasco (1996), using the same genetic markers, analysed *C. concholepas* populations from central (Quintay, 33°10' S) to southern Chile (Chiloé Island, 42°38' S), showed low levels of population subdivision, and suggested the existence of genetic cohesiveness in the species. More recently, Kinlan & Gaines (2003), using the isolation by distance (IBD) approach, re-analysed the data from Gallardo & Carrasco (1996) and showed that the scale of dispersal for *C. concholepas* larvae might be around 122 km. However, despite the apparent high larval dispersal potential of *C. concholepas*, larval retention has also been documented. For instance, Moreno *et al.* (1993) suggested that *C. concholepas* veligers might stay near the bottom during at least the first week of development, thereby decreasing their dispersal ability. Also, the existence of a transport vs. retention mechanism has been suggested, by which *C. concholepas* larvae could remain

around the coastal settling areas, thus avoiding offshore dispersal (Poulin *et al.*, 2002a,b).

Here, we document the phylogeographical pattern displayed in SEP populations of the marine gastropod *C. concholepas*, based on the analysis of a fragment of 658 base pairs (bp) of the cytochrome oxidase I (COI) mitochondrial gene. The sampling covered most of the present-day distributional range of the species, with populations sampled in each of the three biogeographical provinces described for the SEP coast. Using population genetic analyses, haplotype network computations and molecular-based methods to infer demographic changes, we provide the first comprehensive phylogeographical study of a species distributed along the SEP coast, and address the following questions. (1) Is there any concordance between the known biogeographical breaks and the distribution of the gene lineages revealed in *C. concholepas*? (2) What processes may have led to the present-day distribution of the molecular diversity in *C. concholepas*?

MATERIALS AND METHODS

Sampling and gathering of molecular data set

Fourteen localities were sampled for *C. concholepas* (Table 1; Fig. 1): seven in the Peruvian Province (PP), four in the Intermediate Area (IA) and three in the Magellanic Province (MP). Field sampling was carried out between August 2004 and August 2005, with one exception, Puerto Eden (49°08' to 74°27'), in March 2006. Immediately after collection, a 2-cm² piece of tissue was excised from the border of the foot muscle

Table 1 Mitochondrial cytochrome oxidase subunit I (COI) diversity in *Concholepas concholepas* by region and locality used in this study.

Region	Locality	Lat.–Long.	<i>N</i>	<i>N</i> _{hap}	<i>S</i>	<i>H</i> _c	π_1	π_2
PP	1. Marcona	11°56'–77°07'	24	15	22	0.89 (0.06)	2.28 (1.29)	0.003 (0.002)
	2. Pisco	13°45'–75°30'	21	14	27	0.87 (0.07)	3.16 (1.70)	0.005 (0.003)
	3. Matarani	17°00'–72°18'	29	19	22	0.86 (0.06)	2.01 (1.16)	0.003 (0.002)
	4. Ilo	17°40'–72°44'	26	21	22	0.97 (0.02)	2.44 (1.36)	0.004 (0.002)
	5. Antofagasta	23°31'–70°32'	22	12	16	0.80 (0.09)	1.77 (1.06)	0.003 (0.002)
	6. Pan de Azúcar	26°08'–70°39'	21	15	24	0.90 (0.06)	2.72 (1.50)	0.004 (0.003)
	7. Temblador	29°28'–71°18'	28	17	28	0.88 (0.06)	2.39 (1.33)	0.004 (0.002)
Overall			171	113	161	0.88 (0.04)	2.38 (1.30)	0.004 (0.002)
Mean			24.4	16.1	23	0.88 (0.06)	2.40 (1.34)	0.004 (0.002)
IA	8. El Quisco	33°23'–71°42'	19	11	15	0.83 (0.09)	1.77 (1.07)	0.003 (0.002)
	9. Las Cruces	33°31'–71°38'	27	21	32	0.95 (0.03)	2.64 (1.45)	0.004 (0.002)
	10. Lenga	36°44'–73°09'	25	16	26	0.85 (0.07)	2.37 (1.33)	0.004 (0.002)
	11. Pta Colum	40°10'–73°39'	25	23	31	0.99 (0.02)	3.34 (1.77)	0.005 (0.003)
Overall			96	71	104	0.92 (0.02)	2.58 (1.39)	0.004 (0.002)
Mean			24	17.8	26	0.91 (0.05)	2.50 (1.40)	0.004 (0.002)
MP	12. Gaviota	44°51'–73°20'	20	14	19	0.91 (0.05)	2.42 (1.36)	0.004 (0.002)
	13. Pto. Aguirre	45°15'–72°40'	29	19	25	0.86 (0.06)	2.23 (1.26)	0.003 (0.002)
	14. Pto. Eden	49°08'–74°27'	21	15	20	0.90 (0.06)	2.67 (1.47)	0.004 (0.002)
Overall			70	48	64	0.88 (0.04)	2.42 (1.33)	0.003 (0.002)
Mean			23.3	16.0	21.3	0.89 (0.02)	2.44 (1.37)	0.004 (0.000)

PP, Peruvian Province; IA, Intermediate Area; MP, Magellanic Province.

*N*_{hap}, number of haplotypes; *S*, indicates number of polymorphic sites; *H*_c, haplotype diversity; π_1 , mean number of pairwise differences; π_2 , nucleotide diversity. Standard deviations are in parentheses.

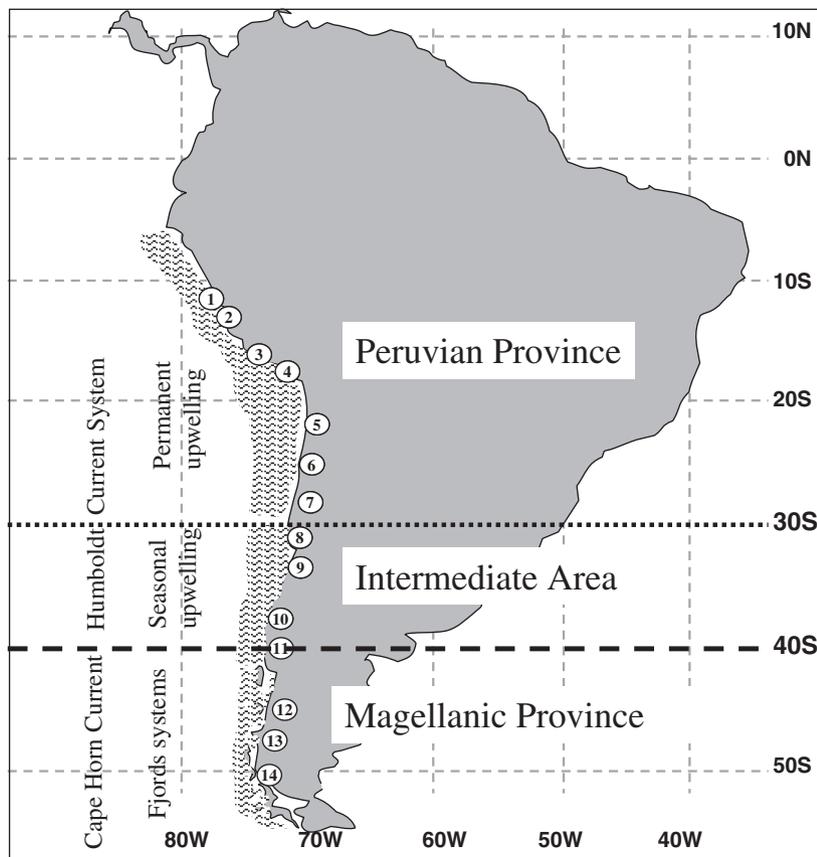


Figure 1 Map of the south-eastern Pacific Ocean showing the collection sites examined in this study. The present-day distribution of *Concholepas concholepas* is shown as wavy lines. The broken lines separate the biogeographical provinces along the Peru–Chilean coast: Peruvian Province, Intermediate Area and Magellanic Province. The code for each sampling population refers to the numbers in Table 1. Main oceanographic characteristics of this area are also described.

of each individual and was stored in 95% ethanol before DNA extraction.

Total DNA was extracted using the Nucleospin Tissue Kit according to the manufacturer's protocol (Macherey-Nagel, Düren, Germany). To amplify the COI gene, we used the universal primers defined by Folmer *et al.* (1994). A detailed methodology of polymerase chain reaction and sequencing conditions can be found in Jolly *et al.* (2006). Sequences were edited using PROSEQ ver. 2.9 beta (Filatov, 2002) and aligned with CLUSTALX (Thompson *et al.*, 1997). The sequences have been deposited in the GenBank database under accession numbers EU517122–EU517458.

Data analyses

Diversity indices

From the 658-bp COI sequences, standard diversity indices such as the number of mitochondrial haplotypes (N_{hap}), number of segregating sites (S), haplotype diversity (H_e), mean number of pairwise differences (π_1), as well as nucleotide diversity (π_2) were estimated for each population and biogeographical province using ARLEQUIN ver. 3.11 (Excoffier *et al.*, 2005). Differences in genetic diversity indices between the three biogeographical provinces were tested using a Kruskal–Wallis ANOVA by rank tests in the STATISTICA ver. 6.0 package

(StatSoft Inc., Tulsa, OK, USA). To compare the genetic diversity between localities, we plotted the distribution of shared (Hs) and private (Hu) haplotypes as compared with the total number of haplotypes (Ht) in each population. The detail of this analysis is available in Fig. S1.

Genetic structure

Conventional population genetics methods were used to describe the overall genetic structure between populations. To test for the hypothesis of genetic breaks in *C. concholepas* coincident with the recognized biogeographical boundaries in the SEP, we performed an F_{ST} -based hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) using ARLEQUIN. The genetic differentiation was investigated using a hierarchical analysis of the genetic variance by partitioning an estimator of F_{ST} into two components, F_{SC} and F_{CT} , indicating the genetic differentiation of samples within and between regions, respectively. Molecular information, the number of differences between haplotypes, was taken into account when performing the AMOVA analysis. Populations from each biogeographical province were grouped: PP ($n = 7$), IA ($n = 4$) and MP ($n = 3$). Tests of significance of F_{ST} , F_{SC} and F_{CT} estimators' values were computed by a nonparametric permutational procedure. An isolation-by-distance model was used to test the hypothesis of a step-by-step dispersal of

C. concholepas along the coastline. Geographical distances were determined in kilometres from the latitudinal and longitudinal coordinates using Haversine geodesic distances (Sinnott, 1984). Correlation between matrices of pairwise geographical and genetic distances was estimated using Mantel tests as implemented in ARLEQUIN.

Gene lineages and demographic analyses

The genealogical relationships among haplotypes were assessed with a haplotype network constructed using a median-joining algorithm as implemented in the software NETWORK 4.201 (Bandelt *et al.*, 1999). We applied a maximum parsimony algorithm to simplify the complex branching pattern and to represent all the most parsimonious intraspecific phylogenies (Polzin & Daneschmand, 2003).

We carried out a series of analyses to test for the hypothesis of a rapid expansion and to estimate the population growth rate and time since expansion. First, we calculated Tajima D (Tajima, 1989) and F_s (Fu, 1997) statistics to test for deviations from a neutral Wright–Fisher model consistent with a population expansion under neutrality hypothesis. Second, to further characterize expansion, the program ARLEQUIN was used to estimate τ ($2 \mu t$, where t = time in generations and μ = mutation rate/generation), the time since demographic expansion, as well as initial and final θ , under a model of sudden demographic expansion (Schneider & Excoffier, 1999). A generalized least-squares approach was used for parameter fitting to the pairwise mismatch distribution. Validity of the sudden expansion model was assessed using parametric bootstrap coalescent simulations, that is, a goodness-of-fit test based on the proportion of bootstrap replicates in which the sum-of-squared deviations (SSD) for the simulated data sets equalled or exceeded the observed SSD. The τ parameter is an estimate of time after expansion in mutational units. If the divergence rate per nucleotide and year ($\tau = 2 \mu$, where μ is the substitution rate per lineage) and the number of nucleotides of the fragment analysed (l) are known, it is possible to calculate the age when the expansion occurred using the expression $\tau = \mu lt$, modified from Harpending *et al.* (1993). Third, a generalized skyline plot was used to picture the historical demographic changes in *C. concholepas*. This skyline plot provides a graphical nonparametric estimate of effective population size, particularly useful with partially resolved phylogenies and data sets, which contain identical sequences (Strimmer & Pybus, 2001). The first step of this analysis is to generate a phylogenetic tree with branch lengths proportional to time. MODELTEST 3.6 (Posada & Crandall, 1998) showed that the simplest and best model for molecular evolution was a TVM+I model. Unweighted pair group method with arithmetic mean (UPGMA) trees were estimated in PAUP* software (Swofford, 2002). Next, the skyline plot was generated in GENIE 3.0 (Pybus & Rambaut, 2002), in which the trees were subjected to a smoothing algorithm. The ε parameter governing this algorithm was chosen using a corrected Akaike information criterion. Based on a maximum likelihood

approach, GENIE allows testing for the best model to describe the demographic history of the species studied. The best model here was an expansion growth model described in the following equation: $N_{(t)} = N_{(o)} [\alpha + (1 - \alpha) e^{-rt}]$, where $N_{(o)}$ corresponds to the present-day population size, r to the exponential growth rate, and α to the population size at $t = \infty$ as a proportion of $N_{(o)}$. The parameters of this model were obtained using maximum likelihood estimation (Pybus *et al.*, 2000). The last step involved dating the estimated demographic expansion: we converted the trees into a time scale using a substitution rate for COI sequences in *C. concholepas*. To round up the analyses and to interpret the graphs constructed with GENIE, a mutation rate (μ) estimate is needed. To get μ for COI in *C. concholepas*, we first used a phylogenetic approach (see Fig. S2).

RESULTS

Mitochondrial DNA diversity across populations and latitude

We analysed a 658-bp fragment of the COI gene for a total of 337 individuals from 14 localities along the Chilean and Peruvian coasts. Over the whole data set, we identified 155 polymorphic sites leading to the definition of 179 haplotypes (Table 1). One haplotype (H4) was found in every population and occurred frequently (32%) over the total data set. Except for H4, 30 haplotypes were shared by at least two localities. These shared haplotypes represented 23% of the total number of individuals. Finally, 148 were private (unique to a single population), most of them being singleton haplotypes.

The genetic diversity was large in every location and region (Table 1; e.g. mean gene diversity per population, $H_e = 0.89$). At the regional level, there was an apparent decrease in the total number of haplotypes from north to south, with 113 haplotypes for PP, 71 for IA and 48 for MP (Table 1). However, this was mainly due to sample size differences at the biogeographical region level. The other genetic diversity indices were not statistically different among regions (H_e , π_1 and π_2 , all $P > 0.5$). This is also true at the population level, with no significant relationship between any of the genetic diversity indices (H_e , π_1 and π_2) and the latitude ($R^2 = 0.03$, 0.02, 0.26, respectively, all $P > 0.1$). However, the genetic diversity indices used above are strongly influenced by the large frequency of H4, the most common haplotype. Interestingly, the analysis of the latitudinal variation of Hu/Ht and Hs/Ht showed a pattern more consistent with a regional effect, an increase of Hu/Ht towards the south while the inverse pattern occurred with Hs/Ht (for details see Fig. S1).

Geographical structure

The AMOVA did not reveal any regional genetic concordance with the biogeographical regions described along the 4200 km of coastline analysed. The AMOVA revealed a non-significant level of genetic structure among regions ($F_{CT} = 0.000$,

$P = 0.34$), while the variation among populations within a region was low but significant ($F_{SC} = 0.005$, $P = 0.02$). The majority of variation was accounted for by the variation among populations regardless of biogeographical region ($F_{ST} = 0.005$; $P = 0.01$). In the same way, the Mantel test was not significant ($P = 0.31$), indicating a lack of genetic isolation by geographical distance.

Gene genealogy and demographic analysis

The median-joining network revealed a 'star-like' haplotype network (Fig. 2) featuring a rapid expansion (Slatkin & Hudson, 1991). The central haplotype (H4) is the most common, and being the more broadly distributed geographically, H4 is the most likely ancestral haplotype (Posada & Crandall, 2001). The majority of the derived haplotypes are linked to H4 with a maximum divergent branch length of nine mutational steps. A second haplotype (H8), just one mutational step away from H4, is also internal and is present in five populations (Marcona, Ilo, Antofagasta, Colun and Gaviota) from the three biogeographical provinces. At the regional level, no pattern is observed with respect to the biogeographical zones (PP, IA and MP), suggesting that *C. concholepas* had a recent rapid geographical and demographical expansion along its distributional range.

As expected from the star-like network, Tajima's D and F_u 's tests were both significantly negative for each population and for the whole data set (Tajima's $D = -2.72$, $P < 0.000$; F_u 's test = -26.46 , $P < 0.000$) indicating, under a neutral model,

that this species experienced a demographic expansion event. To characterize the expansion pattern further, a model of sudden demographic growth was fitted to the pairwise sequence mismatch distribution. A model of simple demographic expansion was applied to *C. concholepas* in each population and a mean value for each parameter was also calculated. In each case, there is a fit of the model of sudden expansion (parametric bootstrap goodness-of-fit tests did not reject the model). The calculation of its parameters per population is shown in Table S1. The distribution of lineages over time was also used to infer demographic trends from the gene genealogy perspective using the skyline plot approach. The skyline plot suggests a growth expansion in the distant past and a constant and reduced population size in the more recent past (Fig. 3). However, detailed analysis of contemporary demography must be treated with caution, given that the phylogenetic signal in the data does not permit accurate estimation of the short internode intervals near the tips in the genealogy. Based on a mutation rate of 8.6×10^{-9} per site (Fig. S2) and assuming a generation time of 1 year (Manríquez *et al.*, 2008), the onset of the most recent demographic expansion in *C. concholepas* was estimated from τ and the skyline plot. The mismatch analysis for *C. concholepas* showed a unique and prominent peak near to two mismatches (Fig. 4); the estimate of τ for the whole data set would correspond to an onset of expansion *c.* 427,000 (95% confidence interval = 206,800–606,100) years before present (BP). In the case of the skyline plot, this analysis showed an onset of expansion at around 352,000–404,000 yr BP. These estimates suggest a

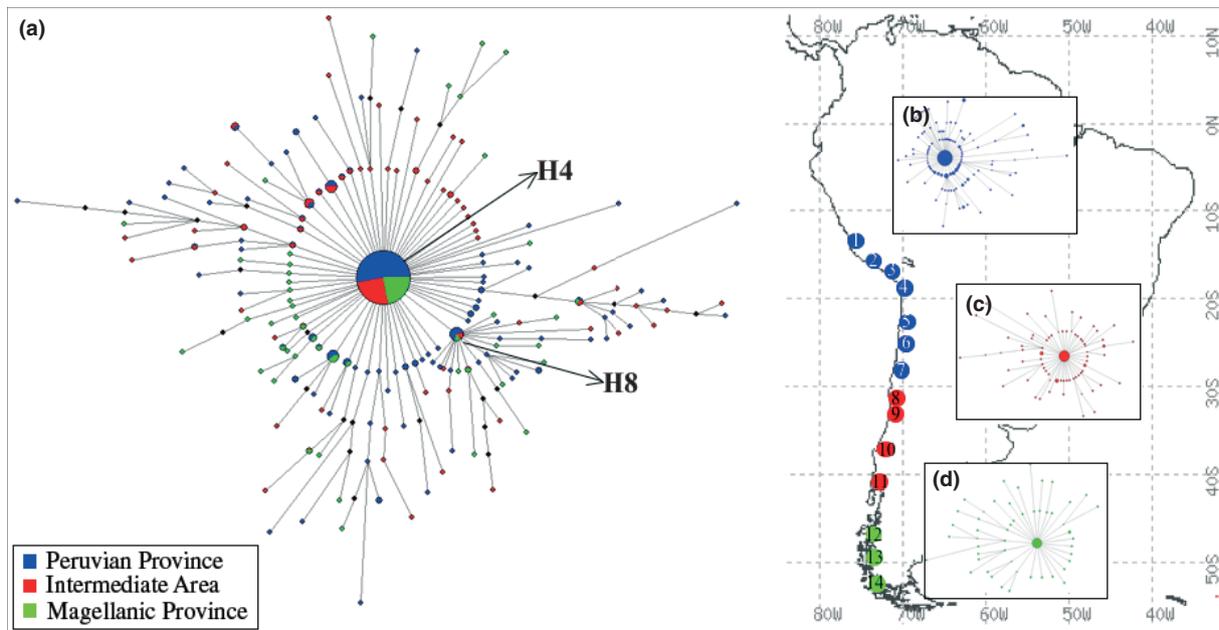


Figure 2 Haplotype network of the 179 *Concholepas concholepas* mtDNA haplotypes (a). Each haplotype is represented by a circle with its size proportional to the number of individuals bearing the haplotype over the whole data set. Colours indicate the location of the haplotype: blue, red and green correspond to the Peruvian (b), Intermediate (c) and Magellanic (d) provinces, respectively.

Figure 3 Generalized skyline plot for *Concholepas concholepas*. The *x*-axis represents time per substitution per site; the *y*-axis represents estimated ancestral population size. The onset of the expansion in the present lineages of *C. concholepas* corresponds to a genetic distance of 0.0035–0.004. The parameter ϵ determines how much temporal structure in the data is retained and hence controls the degree to which the skyline plot is smoothed.

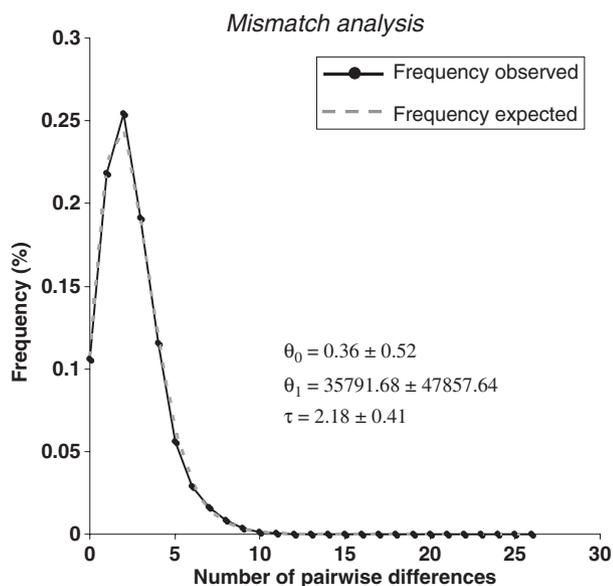
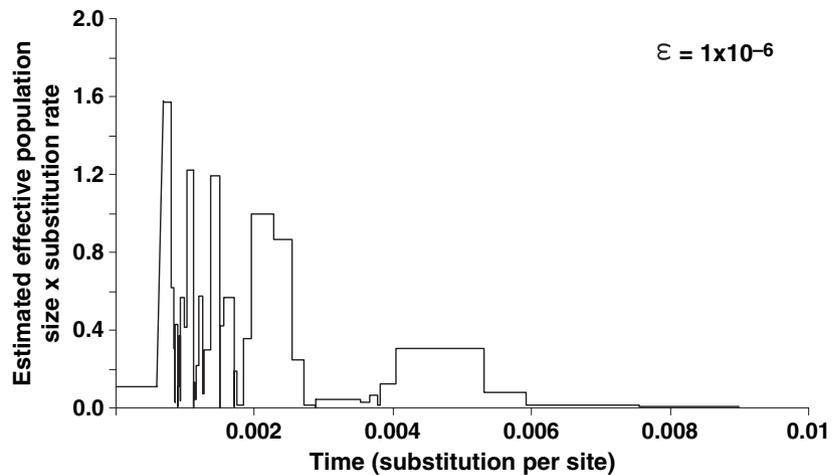


Figure 4 Mismatch analysis. Frequency distribution of pairwise differences among cytochrome oxidase subunit I (COI) haplotypes in *Concholepas concholepas*.

demographic expansion of *C. concholepas* related to the marine isotope stage (MIS) 11, the longer and warmer interglacial episode during the Pleistocene.

DISCUSSION

Distribution of the genetic diversity of *C. concholepas* along the SEP coast: the present-day situation

We analysed populations of the marine gastropod *C. concholepas* that were sampled over three biogeographical provinces corresponding to c. 4200 km of the SEP coastline. Several environmental features typify these biogeographical provinces. For example, the Magellanic Province, composed of an austral biota, is characterized by a complex coastal morphology with

numerous fjords, inlets and channels, a wide shelf platform, and relative isolation due to the presence of the major current divergence at around 42° S (Arntz *et al.*, 1991; Valdovinos *et al.*, 2003), whereas the Peruvian Province, with a warm-temperate biota, is characterized by a more linear coast with a narrower shelf platform and is strongly influenced by upwelling processes and oceanographic disturbances such as ENSO, a phenomenon that provokes a series of alterations in the structure of the current system with regional scale influences up to 30–36° S (Camus, 1990). Finally, a mixture of climatic and oceanographic effects that emphasize its intermediate nature characterizes the Intermediate Area, which includes mixed biota components. This area has a linear coast with many high-flow rivers (e.g. Bio-Bio, Cautín, Toltén, Calle-Calle) reaching the sea (Fernández *et al.*, 2000), and an oceanographic regime modulated by the Humboldt Current System (Camus, 2001). Within this area, major shifts in species assemblages have been observed (e.g. mollusc species; Valdovinos *et al.*, 2003; rocky intertidal herbivores, Rivadeneira *et al.*, 2002; benthic polychaetes, Hernández *et al.*, 2005), and even at the species level, shifts in the abundance of mussels have been documented (Broitman *et al.*, 2001). One important outcome of our analyses is that, despite such large-scale and spatial features, we did not find any evidence of genetic structure related to the spatial/regional distribution of the populations. This was shown by the output of the AMOVA analysis, the low level of divergence among populations (pairwise θ divergence < 1%), and the presence of one haplotype in every population (H4) as pictured in the haplotype network. Also, the gene and haplotypic diversity estimates were high and of the same order whatever the population or region.

Mitochondrial markers are the most popular markers for phylogeography studies: they were shown to be reliable because the lack of recombination facilitates the analyses as compared with nuclear genes (Hare, 2001). Moreover, the effective population size for mitochondrial genes is four times lower than for nuclear markers, so any genetic drift effects are emphasized and isolation by distance patterns should be easier to detect (Díaz-Almela *et al.*, 2004). Additionally, as

mitochondrial DNA (mtDNA) is often used, comparative analyses can be carried out (e.g. Maggs *et al.*, 2008). However, Bazin *et al.* (2006) recently argued that the use of mtDNA could be questionable for phylogeographical analyses of invertebrates because of possible selection effects due to the non-recombinant nature of the mitochondrial genome. This issue is still debated (see replies by Berry, 2006; Wares *et al.*, 2006), in particular in relation to the time scale relevant for strong selective effects. Moreover, outputs of many recent phylogeographical studies obtained with mtDNA markers fit with expectations based on the life-history traits of the species analysed (e.g. comparison of gastropod species with benthopelagic and direct development; Collin, 2001). More specifically concerning *C. concholepas*, it is noteworthy that results similar to those observed here were observed with nuclear microsatellite markers in a preliminary study of two populations also examined in the present study (Matarani and Puerto Aguirre; Cárdenas *et al.*, 2007). Both populations were indeed found to be highly polymorphic (mean allelic richness of 16.3 (± 7.1) and 17.4 (± 8.4) in Puerto Aguirre and Matarani, respectively) with the same level of expected heterozygosity. Additionally, they did not show significant genetic differences even though they were separated by 3000 km. Thus, in the following discussion, we will assume a neutral model of evolution of the mtDNA marker used in this study.

Previous studies of genetic diversity in *C. concholepas* based on enzymatic loci showed low levels of genetic diversity ($H_o = 0.065$, Guíñez *et al.*, 1992) as compared with the genetic diversity detected in other gastropods using the same molecular markers (e.g. *Crepidula fornicata* $H_o = 0.211$, Dupont *et al.*, 2003). Thus, the level of genetic diversity detected in our study was higher than expected based on previous work. This discrepancy is easily explained by the mutation rates of enzymatic loci, which are much less than in mtDNA (Avisé, 2000). Over the spatial range investigated (from central Peru to southern Chile), 179 haplotypes were observed in 337 study specimens, and *c.* 80% of the haplotypes occurred only once (unique sequences). However, in comparison we observed low nucleotide diversity (< 0.005 per population; Table 1), a pattern due to the close similarities in nucleotide sequences among haplotypes (average $\pi_1 = 2.44$; Table 1). Several marine species with high dispersal potential display such a pattern of large gene diversity coupled with low nucleotide diversity (e.g. the sea cucumber *Holothuria nobilis*, Uthicke & Benzie, 2003; the silverfish *Pleuragramma antarcticum*, Zane *et al.*, 2006; the gastropod *Litorinna keenae*, Lee & Boulding, 2007). The occurrence of numerous rare variants has been regarded as an evolutionary signature of population expansion (Uthicke & Benzie, 2003; Lee & Boulding, 2007), and thus deviation to mutation-drift equilibrium. In *C. concholepas*, the occurrence of significant negative values of Tajima's *D* and Fu's statistics (Aris-Brosou & Excoffier, 1996), as well as the smooth and unimodal shape displayed in the mismatch distribution (Fig. 4; Rogers & Harpending, 1992), support this hypothesis.

The lack of significant structure at population and regional levels reflects historical, and probably contemporary, levels of

gene flow. In addition, no IBD pattern was detected. Clearly, these results are not in agreement with the classical and often documented scenario of a concordance between biogeographical barriers and phylogeographical breaks (Avisé, 2000; Maggs *et al.*, 2008). However, this result is in accordance with the paradigm of high larval dispersal ability in marine species with an extended pelagic larval phase (Palumbi, 1995). Following internal fertilization, females lay egg capsules on low intertidal and shallow subtidal rocky surfaces (Manríquez & Castilla, 2001). After *c.* 1 month of intracapsular development, small planktotrophic veliger larvae are released and spend at least 3 months in the water column (DiSalvo, 1988). The observed genetic homogeneity at the level of the species range distribution has been documented in other marine species with a long larval phase. For example, in the slipper limpet *Crepidula fornicata* distributed in the North-Western Atlantic, Collin (2001) observed two mitochondrial clades with no regional signature: individuals from New England and individuals from Florida were found to occur in the same clade. Other examples can be found in other oceans (e.g. Tasman Sea: *Jasus edwardsii*, Booth & Ovenden, 2000; north-eastern Pacific Coast: *Littorina scutulata*, Kyle & Boulding, 2000; Australia–New Zealand coasts: *Nerita atramentosa*, Waters *et al.*, 2005). Our study is therefore consistent with many studies that positively correlate dispersal ability with gene flow (e.g. Palumbi, 1995; Kinlan & Gaines, 2003; Siegel *et al.*, 2003) and with geographical range (e.g. Scheltema, 1968; Victor & Wellington, 2000). However, to ascertain this relationship, comparative phylogeographical studies between groups of closely related sympatric species with different pelagic larval duration have to be carried out in the SEP.

A historical perspective: when did the sudden and rapid expansion of *C. concholepas* occur?

The current distributional range of *C. concholepas* extends from Lobos Afuera Island to Cape Horn, with disjoint populations in the Juan Fernández Archipelago. The fossil record of *C. concholepas* shows that this gastropod appeared during the Pliocene–Pleistocene transition in the northern part of its present-day geographical range (DeVries, 1995, 2000), coincident with the establishment of the contemporary upwelling system and the consolidation of the present oceanographic systems in this area (Rivadeneira, 2005). From these observations and data, the hypothesis of the north-to-south expansion appears to be reasonable. The large frequency of haplotype H4, likely to be an ancestral one (see below), in the northern part of our study area, supports this hypothesis. We nevertheless observed only a slight cline in the number of shared and unique haplotypes (Fig. S1). With a southern expansion, a decrease in the genetic diversity towards the south would have been expected (Hewitt, 2000). More recent demographic expansion events in combination with contemporary gene flow have probably decreased the imprinting of the original southern expansion. The genealogical relationships among the *C. concholepas* haplotypes revealed a star-like network in which the most common and central, thus

presumably an ancestral haplotype (Avice, 2000; Hewitt, 2000), is connected by a few mutation steps to many rare haplotypes. In light of the coalescence theory (Slatkin & Hudson, 1991), this pattern suggests a population size expansion from a limited number of founders. The coalescence-based demographic analysis fits with an expansion growth model, and calculations of the onset of the expansion of *C. concholepas* suggest a demographic expansion related to the favourable climatic conditions at around 400,000 yr BP during MIS 11, the longer and warmer interglacial episode that occurred in the Pleistocene.

Palaeontological and palaeoecological particularities of MIS11 coastal deposits along the SEP coast show that for thousands of years, warm-water species from the present Panamic Province (north of 6° S) lived as far south as 30° S (Ortlieb *et al.*, 2003). Thus, these warm faunal elements co-existed with the cool-water species of the Peruvian Province, forming 'thermally anomalous molluscan assemblages' (TAMA; Valentine, 1955; Roy *et al.*, 1995). Unique climatic conditions were necessary to allow the warm-water species to reach the south. The large and dense populations of MIS 11 fossil shells pertaining to species of TAMA suggests that strong ENSO-like events frequently were able to bring stocks of larvae and refurbish the area (Ortlieb *et al.*, 2003). Species with a pelagic larval phase are highly represented in the suite of species that have shifted their distribution associated with short-term pulses of increased temperatures, such as those during ENSO events (e.g. the gastropod *Kelletia kelletia*; Zacherl *et al.*, 2003). Thus, we propose that the demographic expansion detected in *C. concholepas* was related to these particular climatic conditions present at this time (MIS 11). In the same way, the episodic occurrence has been documented, after contemporary El Niño events, of Panamic molluscan fauna (*Bulla punctulata*, *Janthina janthina*, *Donax peruvianus*, *Pteria sterna*; Gúzman *et al.*, 1998; Paredes *et al.*, 2004) in protected areas of Antofagasta Bay (at around 23° S), supporting the previous proposed mechanism of the expansion of *C. concholepas* populations. Additionally, given that the genetic homogeneity covers a tremendous spatial scale (more than 4000 km), we can hypothesize that this demographic expansion occurred simultaneously along the complete range of distribution and was thus concomitant with a geographical expansion with high levels of gene flow between distant localities.

Apart from the surprising pattern of genetic homogeneity, a second main feature of our data set was the large genetic diversity observed at the population and regional levels. However, conventional diversity indices (such as H_e or π) have limited power when there is a high number of unique or singleton haplotypes (Neilson & Wilson, 2005), as is the case here. A significant correlation of the proportion of shared and private haplotypes with latitude was observed: the number of shared haplotypes was lower towards the southern part of the range of *C. concholepas*, whereas the number of unique haplotype was higher. This suggests a rapid exponential growth of the southern populations that might have occurred after a primary geographical expansion (the expansion from the

northern centre of origin towards the south). With a rapid demographic expansion, genetic drift could have been weak, favouring the upsurge of new neutral alleles, a founder-flush demographic process (Slatkin, 1996). Given the recent founding of these southern populations, these new mutants would have had only a limited time to spread.

The climatic cycle of the Pleistocene had a major impact on marine biodiversity patterns. This has been documented in many species and many oceans (e.g. North Atlantic, Maggs *et al.*, 2008). Yet there is an enormous gap in our understanding of how historical and contemporary events were/are acting to modify the present-day pattern of biodiversity along the SEP coast, although some regions in the SEP are among the richest in terms of marine resources in the world (Arntz *et al.*, 1991). To address this issue, we carried out pioneering work by reporting a high level of genetic cohesiveness among populations of the muricid *C. concholepas*, across three biogeographical provinces in the SEP. This pattern is probably due to the combined effects of historical events (e.g. MIS11 and ENSO-like events), life-history characteristics of the species (dispersal ability), and the contemporary oceanographic characteristics in the area. Interestingly, the effect of the LGM on the phylogeographical pattern of many marine species has been reported regularly along the entire North-Eastern Pacific coast (Hewitt, 2000, 2004), but the results presented here suggest that this glacial event did not have the strongest influence on the present-day genetic structure of *C. concholepas*. However, it is clear that new research is necessary to examine other species with different life-history traits, habitat preferences and distributions along the SEP coast, in order to understand better the mechanisms that generate the regional biogeographical patterns in the SEP.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Proportion of shared (Hs/Ht) and private (Hu/Ht) haplotypes.

Figure S2 Maximum likelihood tree of Rapaninae taxa.

Table S1 Mismatch distribution analysis of *Concholepas concholepas*.

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BIOSKETCHES

Leyla Cárdenas investigates the historical and contemporary processes that affect the genetic architecture in marine species along the south-eastern Pacific Coast. The present work is part of her doctoral thesis, conducted through the association between the Pontificia Universidad Católica (PUC), Chile and the Laboratory 'Adaptation & Diversité en Milieu Marin' (AD2M) in the Station Biologique de Roscoff (SBR), France.

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Frédérique Viard is a population geneticist at the SBR. The main focus of her research concerns the understanding of dispersal processes in marine coastal species, with a special emphasis on introduced and invasive marine algae and molluscs.

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