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Different evolutionary histories in two sympatric limpets of the genus *Nacella* (Patellogastropoda) in the South-western Atlantic coast

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Abstract The phylogeography of two sympatric Magellanic limpets of the genus *Nacella* was studied. The historical and contemporary processes underlying their intraspecific patterns in Argentina were inferred using the mitochondrial cytochrome oxidase I gene. Results indicate different evolutionary histories in the two species. A recent geographic and demographic expansion ∼11,000 years ago, with absence of current genetic structuring, is proposed for *Nacella magellanica*. In contrast, the phylogeographic pattern showed noticeable phylogenetic and geographic discontinuities in *Nacella deaurata*; slow continuous population size growth with current low levels of gene flow among its populations was also observed, indicating demographic equilibrium. The beginning of divergence between these closely related limpets was estimated at about 270,000 years ago with very little or none gene flow occurring after their splitting. These contrasting historic patterns could be related to distinctive responses to climate changes associated with Pleistocene glaciations, as a result of differences in their ecological traits.

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Introduction

Environmental changes associated with Pleistocene glacial cycles are known to be one of the main historical process influencing the genetic structure of aquatic species both in the northern and in the southern hemispheres (Avise 2000; Muellner et al. 2005; Ruzzante et al. 2006; Túnez et al. 2010). However, these variations have been not only related to climate changes, but also to how differing ecological traits of species can lead to distinctive responses (Ruzzante et al. 2008; Deng and Hazel 2010). In several benthic marine invertebrates showing high levels of gene flow, it has been demonstrated the influence of pelagic larval duration (Kindal and Gaines 2003; Paulay and Meyer 2006) and of marine currents in their dispersal (Murray-Jones and Ayre 1997; Sotka et al. 2004), since adults typically have low mobility. For instance, some invertebrate species living in rocky intertidal could survive the dramatic environmental and climatic change from Pleistocene glaciations in eastern North America due to their life-history traits such as mechanisms of dispersal (Wares and Cunningham 2001). Therefore, genetic variation is structured not only by the historical processes, but also by contemporary forces of genetic exchange.

Nacella species inhabiting the Magellanic Province are a suitable model to explore the impact of historical events in shaping population structure in marine coastal invertebrates with larval development and low mobile adults, since this area has been influenced by glacial processes and interglacial intervals during the last 800,000 years (McCulloch and Bentley 1998; Rabassa et al. 1992). This biogeographical province that extends along Atlantic and Pacific coasts in the southern tip of South America represents the area with the highest diversity of the genus (Valdovinos and Rüth 2005). González-Wevar et al. (2010)

recognized an event of recent diversification of the genus *Nacella* that led to an important radiation in the Magellanic Province during the late Pleistocene. In that extended region, about eight morphological species of *Nacella* have been described (Pilsbry 1891; Carcelles 1947, 1950; Powell 1973; Otaegui 1974; Castellanos and Landoni 1988; Valdovinos and Rüth 2005), exhibiting very low levels of genetic divergence among them, both in the Atlantic and in the Pacific coasts (de Aranzamendi et al. 2009; González-Wevar et al. 2010). The low genetic differentiation among these species suggests the existence of a recent radiation process accompanied by rapid morphological and ecological diversification (de Aranzamendi et al. 2009; González-Wevar et al. 2010). These results are in line with the fossils records of some species of *Nacella* that correspond to late Pleistocene—Holocene (Aguirre et al. 2005). The environmental conditions of the littoral ecosystems and its benthic communities in these regions during the periods referred were similar to those prevailing at present (Olivier et al. 1966; Gordillo 1999; Aguirre et al. 2005; Cuevas et al. 2006). These stable environmental characteristics could have led to low levels of genetic differentiation among modern *Nacella* species (de Aranzamendi et al. 2009).

Nacella magellanica (Gmelin 1791) and *Nacella deaurata* (Gmelin 1791) are distributed in the biogeographic Magellanic Province of Argentina and Chile, as well as in Malvinas (Falkland) Islands (Castellanos and Landoni 1988; Morriconi and Calvo 1993; Valdovinos and Rüth 2005). *Nacella magellanica* is the most abundant limpet in the Argentinean sector of that Province; it is highly variable in shell morphology and color patterns. *Nacella deaurata* is morphologically stable, presenting slight variations among individuals. Although this limpet has been reported all along the coasts of the southern tip of South America, in a previous study, it was demonstrated that the species is restricted to Tierra del Fuego and was absent in Patagonia (de Aranzamendi et al. 2009). The similar morphological characteristics that present some morphotypes of *N. magellanica* with *N. deaurata* could have generated the errors in the classification of the specimens from Patagonia in the literature and in the collections of several museums. These morphotypes have a displacement of the apex toward the front of the valve, a diagnostic character of *N. deaurata*; however, de Aranzamendi et al. (2009) identified these two nominal species using arbitrarily primed nuclear markers (68 loci of ISSR, Inter Simple Sequence Repeats) and recognized them as two different genetic units. In a recent study, González-Wevar et al. (2011b), using COI sequence data and elliptic Fourier analyses, reported significant genetic and morphological differences between individuals of these two species sampled in the same locality in the Magellan Strait, Southern Chile. In addition, these species differ in their vertical location on wave-exposed shorelines. *Nacella magellanica* is able to colonize all intertidal levels from the high shore to the low shore and also shallow subtidal bottoms, whereas *N. deaurata* inhabits lower levels of the intertidal zone and also shallow subtidal areas.

Phylogeography has greatly contributed to understand the genetic population structure providing a means of examining the history of genetic exchange among populations, with the potential to infer the contribution of historical and contemporary processes in the biogeographic patterns of genetic variation (Avise 2000). In the recent years, a few studies have resolved successfully questions concerning phylogeography in species of the two genera included in the family Nacellidae (Patellogastropoda), *Cellana* and *Nacella*. A comparative phylogeographic study of Hawaiian sympatric sister species of *Cellana* showed that these limpets have a contrasting pattern of population structure, in spite of their similar life histories (Bird et al. 2007). An intraspecific phylogeographic analysis of three *Cellana* species of New Zealand demonstrated genetic discontinuity among North and South Island populations as a result of allopatric fragmentation (Goldstien et al. 2006). Another study in the Antarctic limpet *Nacella concinna* suggested the existence of a recent demographic expansion with lack of genetic structure among populations, which could reflect a dramatic effect of glacial periods on population size (González-Wevar et al. 2011a).

The aim of this paper is to study the phylogeographic patterns in two closely related limpet species, *N. magellanica* and *N. deaurata*, along the South-western Atlantic coast. Specifically, we used these data to infer the contribution of historical and contemporary processes to shape the genetic structure of *Nacella* species. Since *N. magellanica* and *N. deaurata* present overlapping geographical distribution in an area highly affected by recurrent growth and retreat of Pleistocene glaciers, their phylogeographic patterns should show signals of genetic bottlenecks and subsequent postglacial expansions. Regarding contemporary processes, marine currents plus pelagic larval duration could maintain low levels of genetic subdivision among populations. Our second objective was to determine whether gene flow has been occurring between these limpet species after their divergence. Closely related species could share common haplotypes in spite of having interrupted genetic exchange among them, due to not having reached reciprocally monophyly. Alternatively, low level of gene flow might exist between two species as a consequence of incomplete reproductive isolation (Avise 2004). To fulfill these objectives, we utilized a partial sequence of the mitochondrial cytochrome oxidase I gene (COI) as molecular marker. This widely used marker has proved to be useful in population genetic and phylogeographic studies in closely related molluscs species (Collin 2001;

Kirkendale and Meyer 2004; Bird et al. 2007; Sá-Pinto et al. 2008).

Materials and methods

Sample collection

A total of 171 specimens of *N. magellanica* and 36 individuals of *N. deaurata* were collected from 13 and 3 localities, respectively, along the Patagonian coast (Río Negro, Chubut, Santa Cruz) and the Argentine sector of Tierra del Fuego (Fig. 1; Table 1). During our intensive sampling, *N. deaurata* was only found in three of the sampling localities corresponding to Tierra del Fuego and was completely absent in the Patagonian continental coast. Besides, it was not as abundant as *N. magellanica* in the intertidal zone. These explain the few number of localities and individuals of *N. deaurata* used in this analysis. Species identification was based on shell morphology following several authors (Powell 1973; Otaegui 1974; Castellanos and Landoni 1988; Morriconi and Calvo 1993; Valdovinos and Rüth 2005). In particular, the most important diagnostic elements for differentiation between *N. magellanica* and *N. deaurata* were the position and curvature of the apex. *Nacella deaurata* presents the apex situated at the anterior part of the shell's length and a slight curvature at the tip. No individual of *N. magellanica* has that curvature; the apex can present a central-subcentral (most commonly observed) position or can be displaced to the anterior part of the shell (Powell 1973; Castellanos and Landoni 1988; de Aranzamendi et al. 2009). Samples were preserved in 80% ethanol.

DNA extraction, amplification and sequencing

Total genomic DNA was obtained from foot muscle tissue following phenol–chloroform extraction (Maniatis et al. 1982). Once extracted, DNA was stored in double-distilled water at 4°C until PCR amplification. A fragment of the mitochondrial cytochrome oxidase I gene (COI) was amplified using universal primers: LCO1490 (5′-GGTCAA CAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TA AACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). The PCR amplification was performed as described in a previous work (de Aranzamendi et al. 2009). Double-stranded PCR products were purified and sequenced by the commercial service Macrogen Inc (USA). Sequencing results were analyzed using the program CHROMAS version 2.23 (McCarthy 1998) and manually edited. Some sequences of COI were available from a previous work (de Aranzamendi et al. 2009), and the new ones were submitted to GenBank database (accessing numbers:
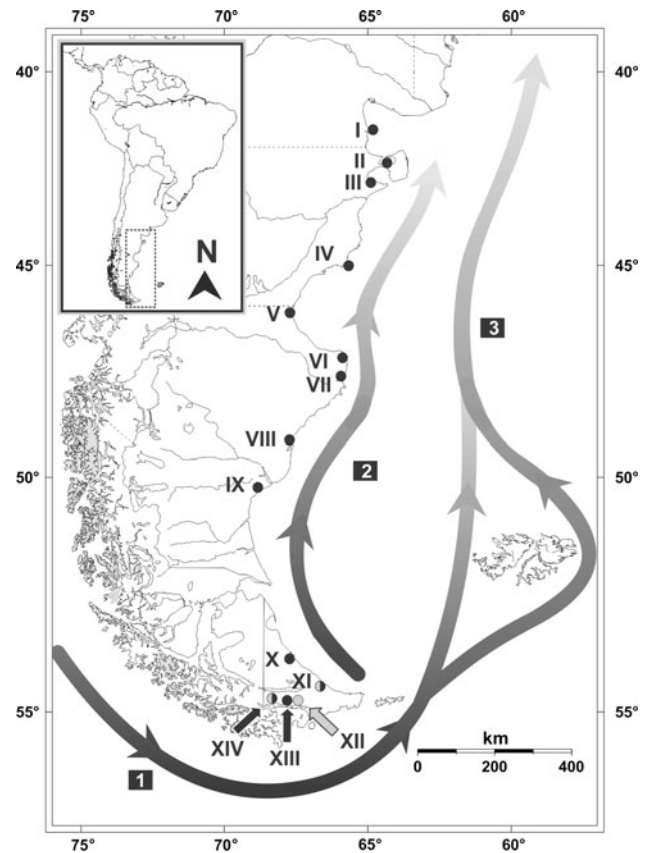

Fig. 1 Sampling sites of *Nacella* species along the study area in Patagonia and Tierra del Fuego (Argentina). Key to localities labels is given in Table 1. *Black dots* represent *N. magellanica* and *gray dots* show *N. deaurata* sample sites. *Arrows* represent principal sea surface currents affecting the study area coasts: *1* Cape Horn Current, *2* Patagonian Current, *3* Malvinas Current

EU870927-EU870985, HQ880439-HQ880550 for *N. magellanica*; EU870986-EU870999, HQ880551-HQ880573 for *N. deaurata*).

Data analyses

Sequences were aligned by eye, then alignments were corroborated using the MUSCLE program (Edgar 2004), and haplotypes were indentified using the program DnaSP 4.10 (Rozas et al. 2003). The average genetic diversity for each species was estimated using nucleotide (π) and haplotype (*h*) diversity estimates. Both estimates were calculated according to Nei's (1987) method using the program DnaSP version 4.10 (Rozas et al. 2003). For each species, statistical parsimony networks were constructed in TCS 1.21 (Clement et al. 2000) to determine the relationships among haplotypes. Since according to our sampling, *N. deaurata* is distributed only in Tierra del Fuego, a network including the specimens of this region was constructed for *N. magellanica* for comparisons purpose.

Table 1 Collection sites and summary of molecular analyses for *Nacella magellanica* and *N. deaurata*. *n*: sample size; *nh* number of haplotypes, *h* haplotype diversity, *π* nucleotide diversity

Locality	Code	Coordinates	*Nacella magellanica*				*Nacella deaurata*			
			n	*nh*	*h* (±SD)	*π* (±SD)	*n*	*nh*	*h* (±SD)	*π* (±SD)
Islote de los Pájaros	I	41°26′59″ S, 65°02′3″ W	11	6	0.891 (±0.063)	0.00339 (±0.00075)	–	–	–	–
Golfo San José	II	42°24′18″ S, 64°18′34″ W	12	5	0.727 (±0.113)	0.00408 (±0.0009)	–	–	–	–
Golfo Nuevo	III	42°49′35″ S, 64°52′58″ W	8	4	0.75 (±0.139)	0.00258 (±0.00058)	–	–	–	–
Isla Leones	IV	45°03′18″ S, 65°36′43″ W	11	8	0.927 (±0.066)	0.00369 (±0.0008)	–	–	–	–
Intertidal reef between Comodoro Rivadavia & Caleta Olivia	V	46°4′2″ S, 67°36′54″ W	12	10	0.97 (±0.044)	0.00478 (±0.00089)	–	–	–	–
Cabo Blanco	VI	47°12′30″ S, 65°44′28″ W	13	8	0.808 (±0.113)	0.00312 (±0.00083)	–	–	–	–
Puerto Deseado	VII	47°45′12″ S, 65°52′04″ W	23	10	0.826 (±0.059)	0.00274 (±0.00047)	–	–	–	–
San Julián	VIII	49°14′50″ S, 67°40′12″ W	11	8	0.891 (±0.092)	0.00369 (±0.00076)	–	–	–	–
Monte León	IX	50°15′52″ S, 68°40′31″ W	14	6	0.791 (±0.089)	0.00345 (±0.00073)	–	–	–	–
Cabo Peñas	X	53°50′43″ S, 67°32′32″ W	15	9	0.848 (±0.088)	0.00466 (±0.00106)	–	–	–	–
San Pablo	XI	54°26′59″ S, 66°28′52″ W	14	9	0.879 (±0.079)	0.00375 (±0.00073)	8	3	0.75 (±0.096)	0.00447 (±0.00069)
Estancia Harberton	XII	54°52′40″ S, 67°19′41″ W	–	–	–	–	8	3	0.464 (±0.2)	0.00288 (±0.00157)
Puerto Almanza	XIII	54°53′13″ S, 67° 40′59″ W	7	2	0.286 (±0.196)	0.00044 (±0.0003)	–	–	–	–
South of Ushuaia	XIV	54°47′53″ S, 68°14′5″ W	20	15	0.921 (±0.055)	0.0041 (±0.00074)	20	7	0.8 (±0.068)	0.00461 (±0.00058)
Total	–	–	171	51	0.868 (±0.02)	0.00356 (±0.00024)	36	10	0.8 (±0.05)	0.00467 (±0.00043)

Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) were used to test for selective neutrality of the mutations using ARLEQUIN 3.11 (Excoffier et al. 2005). To evaluate a possible historical population expansion event, the distribution of the frequencies of nucleotide pairs between individuals were compared (mismatch distribution, Rogers and Harpending 1992) with the expected distribution of a model of population expansion as implemented in ARLEQUIN. The validity of the estimated demographic model was tested, obtaining the statistical distribution of SSD (sum of squared differences) with parametric bootstrap (Excoffier and Schneider 1999). In addition, we used the raggedness index (Harpending 1994) to test the deviation from the unimodal mismatch distribution. The mismatch distribution analyses were conducted for each species, but also for Patagonian and Tierra del Fuego localities separately to compare the situation of *N. deaurata* (only found in Tierra del Fuego) with *N. magellanica* (found in Patagonia and Tierra del Fuego).

The Bayesian skyline plot, a method for estimating past population dynamics through time without dependence on a pre-specified parametric model of demographic history, was used for each species implementing BEAST version 1.6 (Drummond et al. 2005). The analyses were performed under an uncorrelated lognormal relaxed molecular clock model with a 1.0% per million years evolutionary rate (González-Wevar et al. 2011a: substitution rate estimated for the sister genus *Cellana*), using a HKY + γ (selected with Modeltest 3.5; Posada and Crandall 1998) and a piecewise constant Bayesian skyline model with 5 groups. For *N. magellanica* and *N. deaurata* data set analyses, 180 and 40 million generations were performed, respectively, making sure that the ESS values of each statistic was at least 1,000. Convergence was examined in TRACER 1.5 (Rambaut and Drummond 2007).

The relation between genetic divergence [Phi$_{ST}$/(1-Phi$_{ST}$)] and geographical distance (log of geographical distance) was assessed using a Mantel (1967) test. Pairwise values of Phi$_{ST}$ were calculated using ARLEQUIN and associated probability values were calculated using 100,000 permutations. Coastal distances between pairs of samples sites were calculated. Since only three sampling sites were obtained for *N. deaurata*, this test was not performed in this species but pairwise values of Phi$_{ST}$ were calculated to analyse the genetic structure of the species.

Finally, the isolation with the migration model implemented in the Bayesian-based IM program was used to assess possibly continuing gene flow between these closely related species (Hey and Nielsen 2004; Hey 2005). This program assumes that closely related taxa might still experience limited gene flow (Hey and Nielsen 2004). To fit the IM model to the data, a Bayesian coalescent method that integrates over all possible genealogies with a Markov chain Monte Carlo approach was used and posterior probability distributions for demographic parameters including migration (m) rate, effective population size (θ) and divergence time (t) were estimated. The IM program was run under the Hasegawa Kishino Yano (Hasegawa et al. 1985) substitution model; symmetrical gene flow ($m_1 = m_2$) and equal effective population size ($\theta_1 = \theta_2 = \theta_A$) were forced, because by reducing the number of parameters estimated, the rate of parameter updates improved substantially. Preliminary runs were done to determine the appropriate priors for subsequent runs (Won and Hey 2005). Multiple independent runs with different random seed numbers were conducted starting with a burn-in period of 200,000 steps and running for 40 million steps. Runs were monitored by using estimates of the effective sample size based on the measured autocorrelation of parameter values over the course of the run (greater than 80) and by inspection of the plots of parameter trends (ideally the posterior distribution should fall completely within the prior distribution). For credibility intervals, the 90% highest posterior density (HPD) interval for each parameter was recorded, i.e., the shortest span that includes 90% of the probability density of a parameter. To convert the parameter estimates scaled by the mutation rate to calendar years, the divergence rates available for COI of *Cellana* was used (1.0% per million years evolutionary rate; González-Wevar et al. 2011a). These divergence rates equate to 3.25×10^{-7} mutations per year for the gene region studied (650 base pairs). The migration parameters m was converted to an estimation of gene flow between species ($\theta m/2 = 2N_e m$; Hey and Nielsen 2004).

Results

On the basis of a fragment of 650–695 bp from the mitochondrial COI gene, a total of 60 haplotypes were identified (51 for *N. magellanica* and 10 for *N. deaurata*) (Table 1). Only one haplotype (h51) was shared between the two species that of major frequency in *N. magellanica* and the third in frequency in *N. deaurata*. Haplotype diversity (h) varied from 0.286 to 0.927 in *N. magellanica* and from 0.464 to 0.8 in *N. deaurata*. The nucleotide diversity (π) was low in all localities, for both species ($\pi_{(N. \, magellanica)} = 0.00044$–$0.00478$; $\pi_{(N. \, deaurata)} = 0.00288$–$0.00461$). In the total pooled samples, both species showed similar values of haplotype diversity ($h_{(N. \, magellanica)} = 0.868$; $h_{(N. \, deaurata)} = 0.8$) but *N. deaurata* presents higher nucleotide diversity ($\pi_{(N. \, magellanica)} = 0.00356$; $\pi_{(N. \, deaurata)} = 0.00467$).

The statistical parsimony network obtained for *N. magellanica* showed a star-like topology. The highest frequency haplotypes were observed both in nearby and in

geographically separated localities; there is a remarkable abundance of singletons since the 70.6% of the haplotypes are represented by a single member in the dataset (Fig. 2a; Online Resource 1). The central haplotype in the network (h51) is the most frequent (observed in 30.4% of the total individuals) and broadly distributed, occurring at most localities along the studied area. Most haplotypes diverge from haplotype 51 by only one to three mutations. There is no evident association between haplotype identity and geographical location in *N. magellanica*. Since, according

to our sampling, *N. deaurata* is distributed only in Tierra del Fuego, we constructed a network including the specimens of this region for *N. magellanica*. These networks were used to compare populations from the two species that inhabit sympatrically in the same geographical area. The network that includes only specimens of Tierra del Fuego showed also a star-like topology with the same most frequent haplotype (Fig. 2b).

Unlike *N. magellanica*, *N. deaurata* does not show a star-like network, but presented noticeable phylogenetic

Fig. 2 Haplotype network of COI mitochondrial DNA sequence data. **a** *N. magellanica* (whole data set); **b** *N. magellanica* (only samples of Tierra del Fuego); **c** *N. deaurata* (whole data set). *Circles* represent haplotypes and circle surface corresponds to haplotype frequency. The *numbers* in each circle represent the number of the haplotype. *Black circles* show intermediate haplotypes not found in the study. The colors of the network represent the haplotype frequency of the each locality group marked in the map. The groups were formed including nearby localities with similar water temperature to reduce the number of colors for an easily understanding. The h51 is the only haplotype shared between the two species
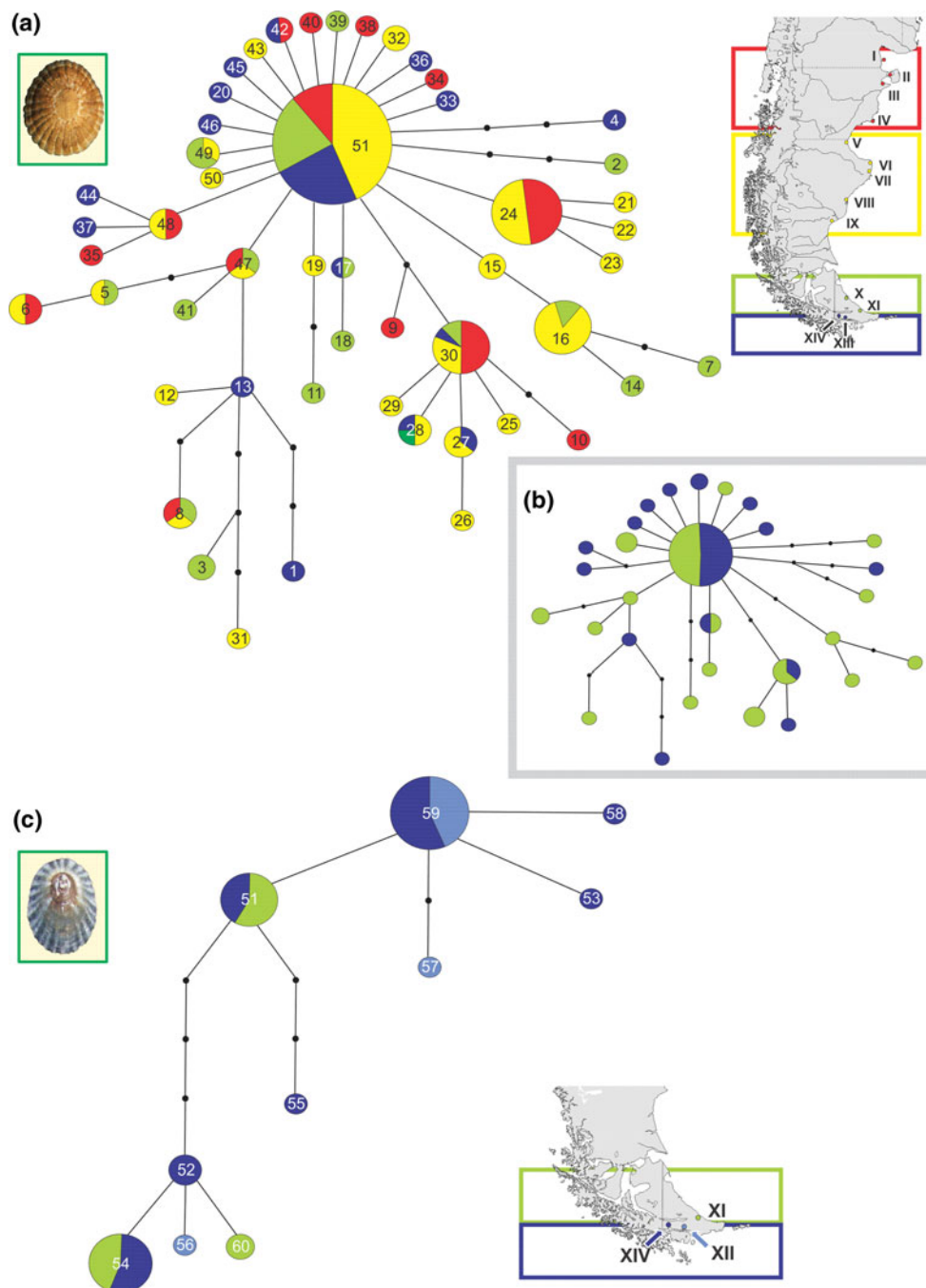
and geographic discontinuities (Fig. 2c). The main haplotype (h59) is restricted to both localities of the Beagle Channel and the second (h54) and third (h51, shared with *N. magellanica*) more abundant haplotypes are the only ones shared between San Pablo and South of Ushuaia (Beagle Channel). Seven of the ten haplotypes were restricted to only one locality, but five of these seven were singletons (Online Resource 1). The proportion number of haplotypes/sample size and the number of singletons were smaller in *N. deaurata*.

Fu's *Fs* and Tajima's *D* tests were both non-significant for *N. deaurata*, but gave significant and negative estimates for *N. magellanica*, also when considering Patagonia and Tierra del Fuego separately, which is in accordance with the excess of rare alleles found in the latter (Table 2). For all cases, a demographic expansion model was used ($\tau > 0$ and $\theta_0 < \theta_1$; Excoffier and Schneider 1999). When considering all localities for *N. magellanica*, the mismatch distribution was unimodal with short time ($\tau \approx 2$) since the last demographic expansion event, and non-significant values of raggedness index and SSD (Fig. 3a). When populations from Patagonia and Tierra del Fuego were separately considered, both showed non-significant values of raggedness index and SSD, supporting the hypothesis of demographic expansion (Fig. 3c, d). On the contrary, *N. deaurata* showed a significant deviation in the observed distribution from the simulated one under a recent expansion model (Fig. 3b). The raggedness value is one order of magnitude higher than the one of *N. magellanica* and the presence of a bimodal distribution in haplotype differences is not consistent with a model of recent range expansion. The peaks of the bimodal distribution remained if one locality at a time was removed from the analysis (data not shown), indicating the presence of relatively stable populations. The Bayesian skyline plots for *N. magellanica* showed a pattern of population expansion, initiating around

110,000 years BP (Fig. 4a). The plot for *N. deaurata* is consistent with populations at demographic equilibrium (Fig. 4b). Both plots exhibit a continuous increase in the population size without evidence of bottlenecks, but *N. magellanica* showed a higher increase in the effective population size.

A small but significant correlation between geographic and genetic distances was observed when all localities of *N. magellanica* were considered ($r = 0.38$, $P < 0.01$). The pairwise Phi$_{ST}$ values reveal that 89% of the significant values (8/9) involve two localities, Golfo San José (II) and Golfo Nuevo (III) (Table 3a). When we removed these samples from the analyses, the Mantel test did not show significant correlation ($r = 0.17$, $P = 0.23$). Nevertheless, no Phi$_{ST}$ values were significant when using a Bonferroni correction for multiple comparisons. In *N. deaurata*, there are significant values of Phi$_{ST}$ among the sample sites located on the Beagle Channel and the one on the Atlantic coast (Table 3b).

Figure 5 shows the estimated posterior probability distribution of effective population size, divergence time and migration rate between the two species obtained from the isolation with migration (IM) model. The IM results provided strongly unimodal posterior distributions of all parameter estimates and bounds fall within the prior distribution. Given the position of the peak at $t = 0.875$, time of species splitting corresponds to 269,231 years (90% interval: 162,769–442,153 years). The migration parameter *m* revealed a peak at 0.003, and its conversion to an estimation of $2N_em$ shows that after the two species began to diverge, very little or none gene flow occurred between them ($2N_em = 0.0879$).

Discussion

Distribution ranges of the species

In this work, we covered the entire range of the geographical distribution in Argentina of each of the two sympatric species, *N. magellanica* and *N. deaurata*. Although *N. deaurata* is mentioned by some authors for Patagonian coasts (Otaegui 1974; Castellanos and Landoni 1988; Valdovinos and Rüth 2005), during our intensive sampling, this species was only found in some localities in Tierra del Fuego, confirming that individuals of *N. deaurata* reported for Patagonia (both current and fossils) could correspond to specimens of *N. magellanica* that were misclassified. A previous work using a dominant nuclear marker with high mutation rate (ISSR-PCR) showed that the individuals of these two species were clearly separated into two groups in all the analyses. Specimens of *N. magellanica* sampled in Patagonia presenting a similar

Table 2 Fu's *Fs* and Tajima's *D* test statistic for selective neutrality

	Statistic test	
	Fu's *Fs*	Tajima's *D*
N. magellanica		
Patagonia ($n = 115$)	−26.39* ($P < 0.01$)	−1.77** ($P = 0.011$)
Tierra del Fuego ($n = 56$)	−26.69* ($P < 0.01$)	−2.39** ($P < 0.01$)
Total samples ($n = 171$)	−26.93* ($P < 0.01$)	−2.22** ($P < 0.01$)
N. deaurata		
Total samples ($n = 36$)	−0.40 ($P = 0.47$)	0.14 ($P = 0.61$)

P and *n* values are in parenthesis

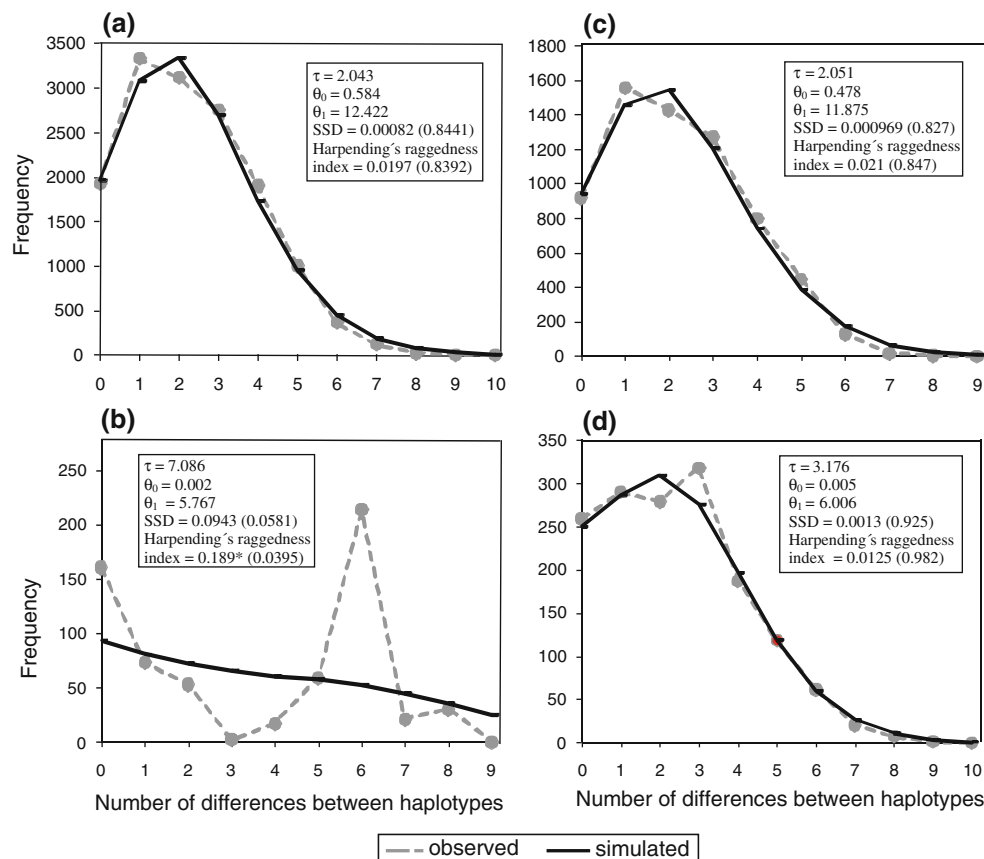* $P < 0.02$ for Fu's *Fs*; ** $P < 0.05$ for Tajima's *D*

Fig. 3 Predicted patterns of the mismatch distribution, the frequency of pairwise differences between haplotypes. **a** *N. magellanica*; **b** *N. deaurata* (Tierra del Fuego); **c** Specimens from Patagonia of *N. magellanica*; **d** Specimens from Tierra del Fuego of *N. magellanica*. τ: time in generations since the last demographic expansion; θ_0: initial population size; θ_1: final population size; SSD: sum of *squared* differences. *P* values are in parenthesis. *$P < 0.05$

N. deaurata morphotype were not grouped together with these latter individuals but with the ones of their own species (de Aranzamendi et al. 2009). As it is characteristic for *N. deaurata*, some individuals of *N. magellanica* may be flatter and have the apex located toward the anterior part of the shell. This might lead easily to a misclassification of these two species, as it was observed when consulting malacological collections of local museums (R. Bastida, personal communication). These observations support the idea of possible misclassification in the literature of individuals belonging to these two species in Patagonia. Not only water and air temperature, but also the high level of spatial competition with *N. magellanica* in the intertidal zone (R. Bastida, personal communication) could be proposed as limiting factors for the distribution range of *N. deaurata* in Argentina. In three endemic *Cellana* species that exhibit a pattern of nested sympatric distribution, the high shore species has the broadest distribution and the low shore-shallow subtidal species is the most localized one; it was proposed that colonization from high to middle to subtidal habitats over evolutionary time occurred (Bird

et al. 2011). These authors argued that a high shore species could more readily survive and adapt to the mid-low littoral shore than the subtidal environment, since the high intertidal zone requires adaptations to thermal stress and desiccation (Somero 2002). A similar scenario could be proposed for the *Nacella* species here considered, as *N. magellanica* exhibits a wide distribution on the intertidal and shallow subtidal zones throughout the Magellanic Province, while *N. deaurata* is limited to less air exposed shore levels in Tierra del Fuego. Furthermore, their Antarctic sister species *N. concinna* exhibit strong physiological differences between intertidal and subtidal morphotypes (Weihe et al. 2010) and is under discussion if these differences are indicating the beginning of a speciation process (de Aranzamendi et al. 2008; Hoffman et al. 2010). The Antarctic morphotypes of *N. concinna* could resemble the past history of *N. magellanica* and *N. deaurata* before they diverged, since those morphs present morphological differences and vertical stratification but there are important levels of gene flow between them (de Aranzamendi et al. 2008; Hoffman et al. 2010).
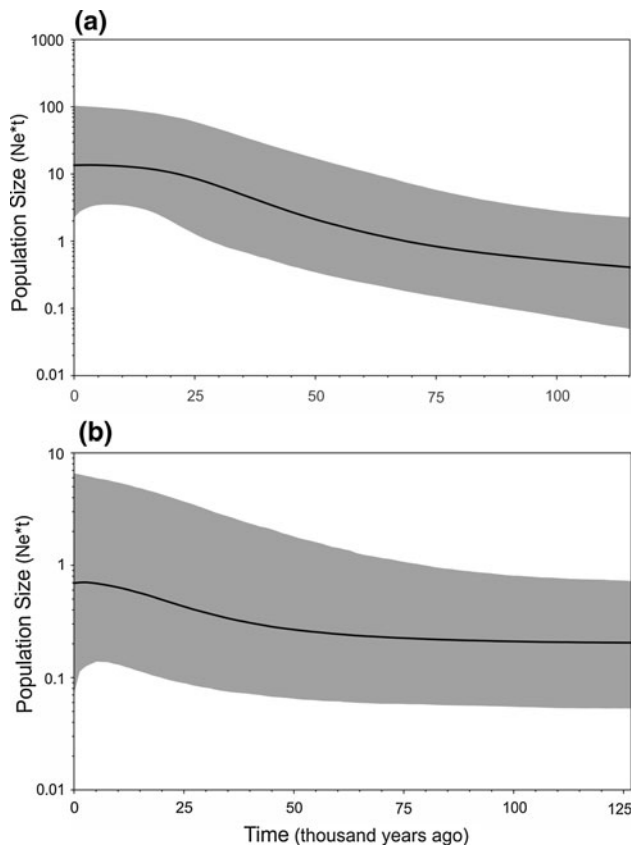
Fig. 4 Bayesian skyline plot for two sister species of the genus *Nacella*. The median estimates demographic histories are shown as *thick solid lines* and the 95% highest probability density intervals are represented by the *gray areas*. **a** *N. magellanica* plot; **b** *N. deaurata* plot

Is there ongoing gene flow between *N. magellanica* and *N. deaurata*?

The presence of one shared haplotype in the COI sequence analyses between *N. magellanica* and *N. deaurata* is consistent with the recent estimated radiation for the Magellanic species (González-Wevar et al. 2010), which is in line with the failure of mitochondrial genes to differentiate them (de Aranzamendi et al. 2009; González-Wevar et al. 2010). The existence of common haplotypes among species has been observed in other groups and was attributed to recent hybridization (Berthier et al. 2006) or to incomplete lineage sorting (García-Moreno et al. 1999; Kemppainen et al. 2009). According to the IM analysis, after *N. magellanica* and *N. deaurata* diverged, very little or none gene flow occurred among them. Besides, the hypothesis of ongoing gene flow as the cause of the appearance of a shared haplotype would be unlikely by the presence of a significant number of exclusive bands observed in each of these species in the ISSR-PCR analysis (de Aranzamendi et al. 2009). Although mitochondrial introgression cannot

be ruled out, the presence of incomplete lineage sorting would be the most suitable explanation for the genetic similarity between these recognized species of *Nacella*, since the estimation of their separation indicates that these species diverged very recently (between 162,769 and 442,153 years ago), during the Pleistocene. This estimation indicates that *N. magellanica* and *N. deaurata* would have splitted more recently than the radiation time suggested by González-Wevar et al. (2010) for most of the Magellanic species (between 2.0 and 0.4 Ma). There is paleontological evidence of *Nacella* limpets in molluscan assemblages from the coastal area of Patagonia (Aguirre et al. 2005, 2009) and Tierra del Fuego (Gordillo 1999; Gordillo et al. 2005) in the late Pleistocene. This geological epoch has been characterized by repeated glaciations accompanying climatic change, which affected South of Patagonia and Tierra del Fuego (Coronato et al. 2004; Rabassa et al. 2005). The presence of multiple refugia along the Magellanic Province during the last glacial cycles may have allowed the survival of these limpets during repeated advances and retreats of the ice. In addition, fragmentation and isolation of these areas could have favored the recent speciation in this region (Valdovinos and Rüth 2005).

Population genetic structure

In the total pooled samples, both limpets showed similar values of haplotype diversity but *N. deaurata* presented higher nucleotide diversity, suggesting a higher genetic divergence among individuals of *N. deaurata* than those of *N. magellanica*. Nevertheless, it could not be ruled out that these differences are due to the smaller sample size obtained for *N. deaurata*. For a better comparison, these parameters were recalculated only for individuals of *N. magellanica* from Tierra del Fuego, and the relation did not change ($n = 56$, $h = 0.827$, $\pi = 0.00368$). These indices in both species were higher than the ones obtained for the Antarctic limpet *N. concinna* ($h = 0.630$, $\pi = 0.00128$; González-Wevar et al. 2011a). The haplotype networks obtained for both species clearly indicate an expansion event in *N. magellanica* but not in *N. deaurata*. In *N. magellanica*, haplotype 51 would be ancestral given its internal position, its high frequency and its occurrence in most localities along the studied area. Besides, this being the only haplotype shared with *N. deaurata* supports the idea of its existence before the two species diverged.

Significant negative Tajima's *D* and Fu's *Fs* indices, the unimodal mismatch distribution and the non-significant values of raggedness index and SSD in *N. magellanica* suggest an excess of low-frequency haplotypes arising from population growth (Fu 1997; Ramos-Onsins and Rozas 2002); these results are also evidences of a recent demographic expansion from one ancestral population in

Table 3 Values of pairwise Phi$_{ST}$ among localities of *N. magellancia* and *N. deaurata*. (a) data for *N. magellanica*; (b) data for *N. deaurata*

(a)

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XIII	XIV
I	–	0.2752	0.4438	0.9999	0.8929	0.9190	0.8185	0.6075	0.5132	0.3905	0.3399	0.2028	0.6494
II	0.0128	–	0.9423	0.2601	0.8178	0.1868	0.2042	0.6247	0.0837	0.0094	0.0252	0.0242	0.0181
III	0	0	–	0.4596	0.6902	0.4329	0.6232	0.7758	0.1834	0.0302	0.0496	0.0369	0.0451
IV	0	0.0161	0	–	0.8121	0.9702	0.7786	0.7725	0.5336	0.7142	0.5491	0.3907	0.7128
V	0	0	0	0	–	0.8145	0.6210	0.5654	0.4464	0.1769	0.2507	0.1532	0.4537
VI	0	0.0281	0	0	0	–	0.9549	0.4968	0.8771	0.7240	0.8753	0.4428	0.6367
VII	0	0.0215	0	0	0	0	–	0.3793	0.3204	0.1306	0.2035	0.2513	0.1591
VIII	0	0	0	0	0	0	0.0004	–	0.1297	0.0809	0.0585	0.0452	0.0509
IX	0	0.0671	0.0525	0	0	0	0.0076	0.0502	–	0.3615	0.7215	0.1582	0.1804
X	0.0029	0.0903*	0.0709*	0	0.0230	0	0.0223	0.0416	0.0039	–	0.7894	0.9531	0.5115
XI	0.0069	0.0946*	0.0793*	0	0.0146	0	0.0179	0.0634	0	0	–	0.5338	0.6222
XIII	0.0383	0.1614*	0.2081*	0.0088	0.0487	0.0024	0.0226	0.1088*	0.0532	0	0	–	0.7203
XIV	0	0.0790*	0.0624*	0	0	0	0.0168	0.0527	0.0189	0	0	0	–

* $P < 0.05$; ** considering Bonferroni correction ($P < 0.000641$)

(b)

	XI	XII	XIV
XI	–	0.0001	0.00426
XII	0.75155**	–	0.05811
XIV	0.34323**	0.13460	–

* $P < 0.05$; ** considering Bonferroni correction ($P < 0.0166$)

Below diagonal: Phi$_{ST}$ values; above diagonal: *P* value. Key to localities labels is given in Table 1

this species. On the contrary, the mismatch distribution analysis in *N. deaurata* showed a significant deviation of the observed values from the simulated ones under a recent expansion model. Multimodal patterns are known to be the result of populations at demographic equilibrium (Slatkin and Hudson 1991; Rogers and Harpending 1992). *Nacella deaurata* presented a bimodal distribution that is consistent with the values obtained for the historical demographic parameters (Tajima's *D* and Fu's *Fs*). Besides, the results of a recent study in one population of *N. deaurata* from the Magellan Strait in Southern Chile also showed a bimodal pairwise difference distribution (González-Wevar et al. 2011b). Moreover, this species shows significant genetic structuring between the localities from Beagle Channel and the Atlantic coast. The geographic separation of these sample sites could be responsible of current low levels of gene flow among them. However, the significant Phi$_{ST}$ values between populations of these two coasts (estimated using a coding gene and therefore, slow evolving) could be also the result of historical restricted gene flow and supports the hypothesis of demographic equilibrium in samples of *N. deaurata*. During glaciations, the species were able to persist by moving into deeper water or perhaps surviving in unglaciated pockets on continental shelves (Gordillo et al. 2005), isolating populations and prompting the increase of divergence among them.

In the case of *N. magellanica*, the non-significant values of Phi$_{ST}$ support the idea of a recent event of expansion and absence of genetic structuring along the Argentinean coast. This result agrees with the non-significant correlation between geographic and genetic distances observed for *N. magellanica* when excluding two localities, Golfo San José (II) and Golfo Nuevo (III). The lack of isolation by distance could be maintained by the action of the Patagonian and Malvinas Currents that affects part of the South-western Atlantic coast and the Argentinean continental shelf. Both of them are cold water currents of subantarctic origin that flow northward and could help to increase gene flow among populations of this species, dispersing the larvae northward. Furthermore, the presence of *N. magellanica* throughout its distribution is nearly continuous in the intertidal zone due to the high homogeneity of the substrate, only interrupted by small areas of sandy or pebble substrate, facilitating the exchange of larvae among populations. The capacity of major ocean current systems to favor larval dispersal was generally supported by the high levels of gene flow estimated in other marine species (Murray-Jones and Ayre 1997; Sotka et al. 2004). The apparent high genetic exchange among localities of *N. magellanica* could support the existence of planktotrophic larvae in this species, which have a higher potential ability for dispersal compared with other modes of
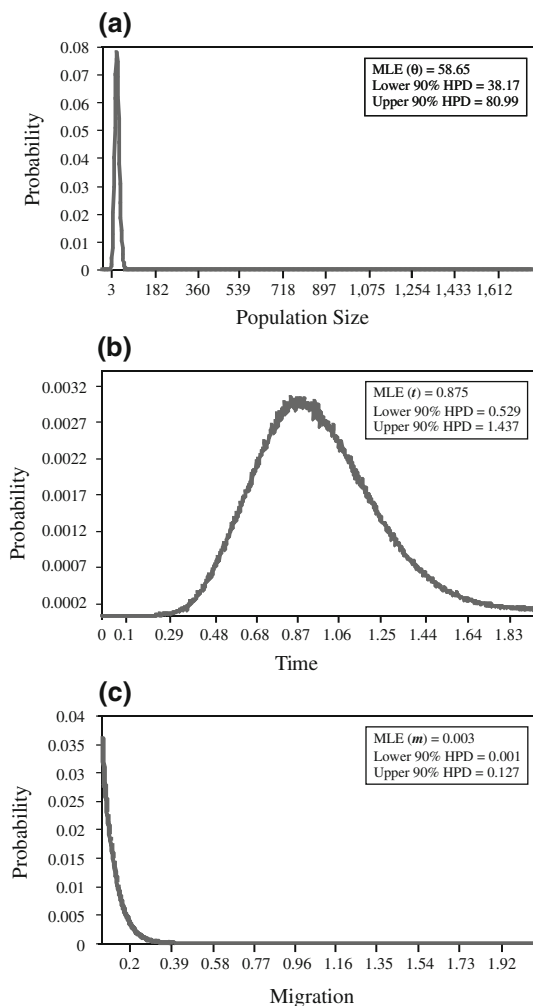
Fig. 5 The marginal posterior probability distributions for model parameters (scaled by the neutral mutation rate). *Curves* are shown for the analysis between species: **a** Effective population sizes; **b** Divergence time; **c** Migration rates. Maximum-Likelihood Estimates (MLE) and the 90% Highest Posterior Density (HPD) Intervals of demographic parameters are shown

the extent of migration among populations (Becker et al. 2007; Goldson et al. 2001; Sherman et al. 2008; Severance and Karl 2006). Bird et al. (2007), by investigating three closely related limpets of the sister genus *Cellana* from Hawaii with similar larval life histories, demonstrated that these have contrasting patterns of population structure that were attributed to differences in their biogeographical ranges and habitat preference. Since *N. magellanica* and *N. deaurata* are closely related species and probably have similar pelagic larval duration, similar phylogeographic patterns would be expected. However, our data clearly do not support this idea and reinforces the importance of historical events and possible differences in ecological traits of the species in shaping their population structure. Nevertheless, new studies employing faster-evolving markers, such as ISSR-PCR or microsatellites, would be useful to better understand the contribution of contemporary processes in population genetic differentiation.

Although in *N. magellanica* Phi_{ST} values are not significant using the correction for multiple comparisons, the high genetic differentiation between Golfo San José and Golfo Nuevo with all the localities from Tierra del Fuego is remarkable. Since the sampling sites are placed inside gulfs, where the renewal of deep waters is partial (mainly restricted to areas near their mouth) and are governed primarily by tidal currents, restricted genetic flow between those two localities and the rest could be favored. A study carried out in the scallop *Aequipecten tehuelchus* (Mollusca: Bivalvia) including localities inside Golfo San Matías (Patagonia, Argentina) attributed the genetic differences detected between northern and southern populations to the much higher residence time of water in the gulf compared with the life time for larvae of the species (Real et al. 2004).

Estimation of historical population dynamics and a possible past scenario

Using a Bayesian skyline plot approach, we estimated that the timing of the onset of expansion of *N. magellanica* is around 110,000 years ago, which corresponds to late Pleistocene. Ho et al. (2005) suggested that molecular studies at population level estimate much higher mutation rates than the substitution rates inferred from phylogenetic (species-level) analyses. Besides, it was demonstrated that short term (1–2 million years) mutation rates could be tenfold higher than long-term substitution rates in different taxa (Ho et al. 2005, 2007). Following these authors, González-Wevar et al. (2011a) incorporated a simple tenfold correction to their time estimation of the demographic expansion of *N. concinna* in Antarctica using COI partial sequence, obtaining a range of 22,000 to 7,500 years ago. The time for the onset of the expansion in *N. magellanica*

development. Larvae could be dispersed by marine currents for long distances since they remain in the plankton for longer time, promoting gene flow between geographically separated populations (Pannacciulli et al. 2009). For instance, its development could concur with the one of the Antarctic limpet of the genus, *Nacella concinna,* which has free-swimming planktotrophic larvae that can survive for 1–2 months in the water column (Bowden et al. 2006). Nevertheless, growing evidence from recent studies suggests that dispersal potential of larvae is not a good predictor of marine population connectivity and that other factors such as hydrographic conditions, discontinuous distribution of suitable substrata, stochastic fluctuations in larval supply and settlement success, or factors intrinsic to the species like spawning season and biological characteristics of the organism play a crucial role in modulating

corrected according to the same assumption could be around 11,000 years ago. At the beginning of the Holocene, Patagonia and Tierra del Fuego suffered a rapid warming ending the glacial processes that influenced their landscape in the past 800,000 years (Rabassa et al. 1992; McCulloch and Bentley 1998). There is evidence of the disappearance of ice by 12,000 years ago in different localities of Tierra del Fuego (Heusser 1989, 1998), freeing the intertidal zone in these areas. A major expansion of the fauna with further diversification of taxa took place during the mid-Holocene (5,000–4,000 years ago) under relatively warmer temperatures, indicating an evolution toward modern faunal diversity and environmental conditions in the area (Gordillo et al. 2005).

Unlike *N. magellanica*, *N. deaurata* exhibits a historical demographic equilibrium with slow continuous increase in population size and no evidence of recent demographic expansions or bottlenecks. There are several reports of species whose populations seem not to have suffered sudden decreases and/or increases in their population size, being less affected by recent glacial advances as in the gastropod *Mexacanthina lugubris angelica* (Deng and Hazel 2010) and in the fish *Percichthys trucha* (Ruzzante et al. 2008). Although extrinsic factors such as oceanic currents and isolation induced by sea level maxima during Pleistocene glacial cycles are often suggested as principal determinants of marine phylogeography, intrinsic factors such as habitat preference or reproductive mode can have significant influences (Colgan and Schreiter 2011). In a study in three closely endemic species of Hawaiian *Cellana*, habitat preference was one of the most suitable factors explaining their differences in population structures (Bird et al. 2007). Contrasting demographic history and phylogeographic patterns in two Indo-Pacific ecologically similar, co-distributed gastropods was attributed to distinct responses to shared environmental processes due to relatively minor differences in traits such as pelagic larval duration or microhabitat association (Crandall et al. 2008). Little is known about reproductive cycle and physiological characteristics in *N. magellanica* and *N. deaurata* (Morriconi 1999; Malanga et al. 2007); however, different capacities in antioxidant defense systems have been demonstrated between them (Malanga et al. 2004). Since *N. magellanica* and *N. deaurata* differ in their vertical location on wave-exposed shorelines, they could present other different physiological responses to environment characteristics. Comparative physiological and larval cycle experimentation in these *Nacella* species may contribute to a better understanding of their distinct phylogeographic patterns in the South-western Atlantic coast.

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