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Out of Antarctica: quaternary colonization of sub-Antarctic Marion Island by the limpet genus *Nacella* (Patellogastropoda: Nacellidae)

Claudio A. González-Wevar · Steven L. Chown · Simon Morley · Nestor Coria · Thomas Saucède · Elie Poulin

Abstract The distribution of the Southern Ocean near-shore marine benthic fauna is the consequence of major geologic, oceanographic, and climatic changes during the last 50 Ma. As a result, a main biogeographic principle in the Southern Ocean is the clear distinction of the Antarctic biota. The Antarctic Polar Front (APF) represents an important barrier between Antarctica and other sub-Antarctic provinces. However, the high degree of genetic affinity between populations of the Antarctic limpet *Nacella concinna* and its sub-Antarctic relative *Nacella delesserti* from Marion Island stands against this tenet. Here, we performed new phylogenetic reconstructions in *Nacella* with special emphasis on the relationship between *N. concinna* and *N. delesserti*. Similarly, we performed population-based analyses in *N. concinna* and *N. delesserti* to further understand the genetic legacy of the Quaternary glacial cycles. Phylogenetic reconstructions recognized *N. concinna* and *N. delesserti* as two closely but distinct monophyletic entities and therefore as valid evolutionary units. The cladogenetic process separating them occurred ≈ 0.35 Ma and is consistent with the origin of Marion Island (≈ 0.45 Ma). Exceptional long-distance dispersal between provinces located inside and outside the APF, rather than revealing the permeability of the Antarctic Polar Front, seems to be related to latitudinal shift in the position of the APF during coldest periods of the Quaternary. Diversity indices, neutrality tests, haplotype networks, and demographic inference analysis showed that the demography of both species exhibits a clear signal of postglacial expansion.

Keywords Biogeography · Southern Ocean · Antarctic Polar Front · Long-distance dispersal · Marion Island Bayesian skyline plot

Introduction

Waters of the Southern Ocean encircling Antarctica are moved around this continent by the strong eastward flow of the Antarctic Circumpolar Current (ACC) that is delimited by two main boundaries: the Antarctic Polar Front (APF) to the south and the sub-Antarctic Front (SAF) to the north (Rintoul et al. 2001; Barker et al. 2007; Rintoul 2011). The positions of these fronts have major biogeographic consequences for the SO biota (Lawver and Gahagan 2003; Mackensen 2004; Greve et al. 2005; Linse et al. 2006; Griffiths et al. 2009; Pierrat et al. 2013). On the one hand, the ACC constitutes a major oceanographic barrier for many invertebrate groups (Shaw et al. 2004; González-Wevar et al. 2010, 2012a; Poulin et al. 2014) and delimits Antarctic and sub-Antarctic provinces. On the other hand, the ACC can transport organisms between geographically distant sub-Antarctic areas of the Southern Ocean, especially in those species with high dispersive potential such as marine invertebrates with planktotrophic larvae (Page and Linse 2002; Díaz et al. 2011; Mortimer et al. 2011), kelps (Fraser et al. 2009; Macaya and Zuccarello 2010), and kelp-dwelling organisms (Nikula et al. 2010; Cumming et al. 2014). Recent biogeographic reviews highlighted both the high level of endemism of the Antarctic biota and the marked differentiation among sub-Antarctic, low Antarctic, and Antarctic/high Antarctic marine species (Linse et al. 2006; Griffiths et al. 2009; Terauds et al. 2012). Faunal relationships among provinces of the SO perfectly match with the flows of the two main oceanographic currents, the ACC and the Antarctic Coastal currents (Pierrat et al. 2013).

True limpets of the genus *Nacella* (Patellogastropoda: Nacellidae) are dominant organisms of inter- and sub-tidal rocky ecosystems of the Southern Ocean. *Nacella* currently includes at least 11 nominal species distributed in different provinces of the Southern Ocean: South America, sub-Antarctic Islands, and Antarctica (Powell 1973; Valdovinos and Rütth 2005; Fig. 1). Species of *Nacella* are dioecious organisms with external fertilization, and their larval life span can extend for more than 2 months (Bowden et al. 2006). At least eight nominal species were described in Patagonia (Powell 1973; Valdovinos and Rütth 2005) but recent molecular and morphometric analyses (de Aranzamendi et al. 2011; González-Wevar et al. 2011a) indicate that only four of them represent valid evolutionary units (González-Wevar et al. 2011a). One South American species, *Nacella clypeator*, expands its distribution up north to the Peruvian Province following the cold waters of the Humboldt Current System (Powell 1973; Valdovinos and Rütth 2005). Almost half of the species of the genus are endemic to one or two remote sub-Antarctic isolated islands including Kerguelen, Heard, Macquarie,

Campbell, and Marion (Fig. 1). One of them, *Nacella delesserti*, is endemic to sub-Antarctic Marion Island, an extremely isolated volcanic island located north of the APF close to South Africa (Chown and Froneman 2008; Hall 2009; Hall et al. 2011). Finally, the Antarctic limpet *Nacella concinna* is one of the dominant macroinvertebrate species of ice-free rocky ecosystems and is currently restricted to mari-time Antarctica (Antarctic Peninsula and associated islands) and peri-Antarctic islands including South Georgia, South Sandwich, and Bouvet (Bölter et al. 2002).

Phylogenetic reconstructions based on mtDNA sequences recognized marked divergences among lineages of *Nacella* from Antarctica, sub-Antarctic Heard Island, and South America (González-Wevar et al. 2010). However, a recent population-based study in the Antarctic limpet including western Antarctic Peninsula populations recognized high levels of genetic identity (99 %) with a single individual of *N. delesserti* from Marion Island (González-Wevar et al. 2011b). Levels of genetic identity recorded between these nominal species fell within the range of intraspecific variation detected in nacellids (Goldstien et al. 2006; Bird et al. 2007) and *Nacella* species from the Magellanic Province (de Aranzamendi et al. 2011; González-Wevar et al. 2012b). At the same time, some ecologic features of *N. concinna*, including reproductive strategy, predation avoidance, population density, and growth rate, are similar to the ones described in *N. delesserti* (Blankley and Branch 1985). Accordingly, the taxonomic status of *N. concinna* and *N. delesserti* is still unclear and the latter could just represent an eastward population of the Antarctic limpet. However, this statement is questionable considering that few epipelagic species, with the exception of mammals and birds, are distributed both inside and outside the APF (Dell 1972; Aronson et al. 2007).

In this study, we present new molecular reconstructions in *Nacella* using phylogenetic and phylogeographic approaches with special emphasis on the relationship between the Antarctic limpet and sub-Antarctic Marion Island *N. delesserti*. Using different DNA sources, we aim to examine the validity of these nominal species and thereby to determine whether the Antarctic Polar Front constitutes an efficient barrier between Antarctic and sub-Antarctic *Nacella* populations. At the same time, levels of genetic diversity and structure are expected to clarify the influence of contemporary climate change over benthic marine invertebrate populations of the Southern Ocean, especially in remote island endemic lineages. These results should help us to further understand the role of historic and recent processes in the distribution of the biota of the Southern Ocean.

Materials and methods

Sample collection

Specimens were collected between 2006 and 2012 from maritime Antarctica, South Georgia Island, and sub-Antarctic Marion Island. New *N. concinna* individuals were collected at Signy Island (60° 43' S; 45° 36' W; n = 16) during the summer of 2012. Additionally, we include 16 new individuals from South Georgia Island collected during the summer of 2006. New sequences from Signy and South Georgia islands were added to previous COI database in the species including populations from the South Shetland Islands (SSI), the Scotia Arc (SA), West-Antarctic (WAP), and East-Antarctic Peninsula (EAP; González-Wevar et al. 2011b, 2013). Individuals of *N. delesserti* were collected at Trypot beach (46° 53' S; 37° 52' E; n = 40) and Ships Cove (46° 51' S; 37° 50' E n = 24), Marion Island.

DNA preparation, PCR amplification, and sequence analyses

Specimens were fixed in ethanol (95 %), and DNA was extracted from the mantle using a standard salting-out method (Aljanabi and Martinez 1997). For phylogenetic purposes, we amplified a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene using specific primers (González-Wevar et al. 2010) and a fragment of the nuclear 28S rRNA gene with universal primers (Littledwood et al. 2000; Williams et al. 2003). New mitochondrial sequences of *N. delesserti* and *N. concinna* were included in previous COI data matrix for the genus (González-Wevar et al. 2010), and it was necessary to amplify the nuclear 28S rRNA gene in all the analyzed *Nacella* species. In order to test the reciprocal monophyly of *N. concinna* and *N. delesserti*, a minimum of ten randomly selected sequences of the COI and five sequences of the 28S rRNA genes were included in the reconstructions, together with sequences of other *Nacella* species from South America and Heard Island (González-Wevar et al. 2010). As previously stated for patellogastropods (Koufopanou et al. 1999; Nakano and Ozawa 2007), sequences of *Cellana* were included as sister group and sequences of other patellogastropod genera were used as outgroups. Mitochondrial and nuclear sequences were assembled independently and edited using Geneious version 5.1.7 (Drummond et al. 2010), and multiple alignments were optimized by eye with Proseq version 3.2 (Filatov 2009). Base composition and nucleotide sequences were analyzed with MEGA version 4 using a

standard invertebrate amino acid coding table (Tamura et al. 2007). Similarly, we performed a DNA saturation analysis following Roe and Sperling (2007).

Phylogenetic reconstructions

Phylogenetic relationships were estimated using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian analyses (BA). Maximum Parsimony analyses were performed with PAUP* version 4.0b (Swofford 2002) following González-Wevar et al. (2010). Nucleotide substitution models for ML and BA analyses were selected separately for each gene using jmodeltest 2 (Darriba et al. 2012) and Mrmodeltest version 2.3 (<http://www.abc.se/~nylander/>), respectively. The GTR \neq I \neq G (COI) and GTR \neq G (28S rRNA) substitution models were used for ML analyses in PhyML (Guindon and Gascuel 2003). Tree node supports for MP and ML analyses were assessed through nonparametric bootstrap (BS) analysis with a full heuristic search option and 1,000 pseudoreplicates (Felsenstein 1981). Bayesian analyses were used to estimate posterior probabilities (BPP) at each node using the Metropolis-coupled Markov Chain algorithm (MCMC) available in MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). Four chains were run twice in parallel for 5×10^7 generations, and trees were sampled every 1,000 generations until the average deviation of split frequencies was less than 0.01 (Ronquist and Huelsenbeck 2003). Bayesian posterior probabilities were estimated as the percentage of trees (after burning 5,000 trees) that showed a particular node. Posterior probabilities were summarized as a maximum clade credibility tree with TreeAnnotator version 1.6.1 (<http://beast.bio.ed.ac.uk/TreeAnnotator>) and visualized using FigTree version 1.4 (<http://tree.bio.ed.ac.uk/software/figtree>).

Divergence time estimations

Divergence time estimations analyses were performed on the COI data set using a relaxed molecular clock analyses with an uncorrelated lognormal (ucln) model of molecular evolutionary rate heterogeneity and the GTR \neq I \neq G substitution model implemented in BEAST version 1.6.2 (Drummond et al. 2006, 2012; Drummond and Rambaut 2007). For calibration purposes, we incorporated age priors within the *Nacella* analyses including the most recent common ancestor (tmrca) for the Nacellidae; the age of the fossil record of *Cellana ampla* (38 Ma, SD 3.8 Ma; Lindberg and Hickman 1986), the oldest *N. concinna* fossil from Cockburn Island (5 Ma, SD 0.5 Ma; Jonkers and Kelley 1998), and *N. clypeater*-like fossil from southern Peru (4.5 Ma, SD 0.45 Ma; DeVries 2009). Four chains were run twice for 3×10^7 generations, and trees were sampled every 1,000 generations. Majority rule consensus phylograms, as well as posterior probabilities of nodes, were estimated.

Genetic diversity and population structure in *N. concinna* and *N. delesserti*

Levels of genetic polymorphisms were estimated in *N. concinna* and *N. delesserti* using standard diversity indices including the number of haplotypes (k), the number of segregating sites (S), haplotypic diversity (H), the average number of pairwise differences (P), and nucleotide diversity (p) for each locality and for each species using DnaSP, version 5.00.07 (Librado and Rozas 2009). We performed neutrality statistical tests (Tajima's D and Fu's F_S) for each locality and species to measure whether data deviate from expectations under a neutral model. We estimated the levels of genetic differentiation between the analyzed localities following Pons and Petit (1996) through mean pairwise differences (N_{ST}) and haplotype frequencies (G_{ST}) in Arlequin version 3.5 (Excoffier et al. 2005). The statistical significance of genetic differences was estimated using permutation tests (20,000 iterations). The spatial analysis of molecular variance implemented in SAMOVA version 1 (Dupanloup et al. 2002) was performed to estimate the number and composition of groups that were the most differentiated. The statistical significance of this test was evaluated with 20,000 random permutations.

Demographic inference in *N. concinna* and *N. delesserti*

We reconstructed genealogical relationships in *N. concinna* and *N. delesserti* using median-joining haplotype networks computed with Network, version 4.6.1 (<http://fluxus-engineering.com>). For this purpose, we performed a unique reconstruction including the complete COI data set. At the same time, we estimated past population dynamics through time using a Bayesian skyline plot method implemented in BEAST, version 1.7 (Drummond and Rambaut 2007). Three independent Bayesian MCMC runs for 5×10^7 generation (sampled every 1,000 iterations) were conducted in each AMOVA detected group (maritime Antarctica, South Georgia Island, and Marion Island) using the GTR \neq G \neq I model. For these analyses, we used a rough tenfold evolutionary rate correction for population-based analyses in

nacellids (González-Wevar et al. 2011b). Such a correction was implemented considering that a time dependency of molecular evolutionary rates has been described showing that short-term mutation rates (1–2 Myr) could be tenfolds higher than long-term substitution rates used for phylogenetic reconstructions (Ho et al. 2005, 2007, 2011). Convergence between runs was confirmed with Tracer version 1.5, and the results for multiple runs were combined using LogCombiner version 1.4.7 (Drummond and Rambaut 2007). The median and corresponding credibility intervals of the Bayesian Skyline plot were depicted with Tracer.

Results

Within *Nacella*, COI sequences include 663 base pairs (bp) coding for 221 amino acids, and as expected when working with coding regions, no insertions/deletions were detected. A total of 123 positions (17.19 %) were variable, and most of them (92.1 %) were parsimoniously informative. 28S rRNA sequences include 840 bp, 49 positions were variable (5.83 %) and 85.7 % of them were parsimoniously informative. As previously estimated in *Nacella* (González-Wevar et al. 2011a, b, 2012b, 2013), COI sequences were A-T rich (65.7 %) compared to the mean G-C content (34.3 %). In contrast, 28S rRNA sequences were G-C-rich (64.1 %) compared to the mean T-A content (35.9 %).

Phylogenetic reconstructions and divergence time estimations in *Nacella*

All the reconstruction methods (MP, ML, and BA) and markers (COI and 28S rRNA) highly resolved the relationship among patellogastropods, and no topologic incongruence was detected within *Nacella* (Fig. 2). The monophyly of *Nacella* and *Cellana* within the *Nacellidae* was highly supported. Within *Nacella*, mtDNA and nu-cDNA reconstructions recognized two main clades. The first one includes species from sub-Antarctic Islands (Heard and Marion) and the Antarctic limpet *N. concinna*. The second one encompasses the South American species (Patagonia and Central Chile) of the genus. High levels of genetic divergence were recorded among lineages belonging to Antarctica, South America, and sub-Antarctic Islands. The different reconstruction methods and markers used in this study recovered the reciprocal monophyly and the sister relationship between *N. concinna* and *N. delesserti*; Fig. 2a, b). Divergence time estimations based on COI sequences suggest that the most common recent ancestor of the current lineages of *Nacella* occurred \approx 12 Ma (14.5–10 Ma), after the Middle Miocene (Fig. 2a). Following this, a diversification of *Nacella* in different provinces of the SO (Antarctica, sub-Antarctic Islands, and South America) occurred at the end of the Miocene between 8.5 and 5.5 Ma (10–4.5 Ma). A second diversification of *Nacella* took place during the Quaternary and includes its radiation in Patagonia \approx 0.8 Ma (1.3–0.6 Ma) and the colonization of Marion Island by *N. delesserti* with its subsequent separation from its Antarctic relative \approx 0.35 Ma (0.65–0.2 Ma; Fig. 2a).

Population genetic diversity and structure in *N. concinna* and *N. delesserti*

Whole COI data set includes 307 individuals of *N. concinna* and 64 individuals of *N. delesserti* (Table 1). Levels of genetic polymorphism in the Antarctic limpet were low with 32 variable positions (3.61 %). Levels of genetic diversity in *N. delesserti* were much lower showing only four variable positions (0.6 %). At species level (Table 1), the number of polymorphic sites (S) in *N. concinna* varied between 13 (South Georgia Island) and 4 (Signy Island; Table 1), while in *N. delesserti* this index was either 3 (Trypot beach) or 2 (Ships Cove). The number of haplotypes (k) in the Antarctic limpet ranged between 12 (South Georgia Island) and 5 (South Bay and Signy Island) while in *N. delesserti* it was either 4 (Trypot beach) or 3 (Ships Cove). The average number of nucleotide differences (P) and the mean nucleotide diversity (ρ) were very low in *N. delesserti*, while these indices showed comparatively higher values for populations of *N. concinna* (Table 1). As expected working with different taxonomic units, pairwise comparison based on G_{ST} and N_{ST} recognized significant levels of genetic differentiation between *N. concinna* and *N. delesserti* populations. Within *N. concinna*, these analyses also detected significant genetic differentiation between South Georgia Island population and the rest of the analyzed localities from maritime Antarctica. No significant genetic structure was detected among maritime Antarctica populations, from Adelaide Island to Laurie Island, South Orkneys (Table 2). Similarly, no genetic differentiation was detected between the two populations of *N. delesserti* (Trypot beach and Ships Cove; Table 2). Population genetic structure based on G_{ST} and N_{ST} analyses was strongly supported by SAMOVA analyses recognizing three main groups: maritime Antarctica, South Georgia Island, and Marion Island. SAMOVA analysis recognized a maximal difference accounting for 68.01 % of the total variance, while only 0.22 % was due to within-group variations among localities.

Demographic inference

Median-joining network comprises 29 haplotypes in *N. concinna* with a typical star-like topology and a short genealogy (Fig. 3). Only five haplotypes were recorded in *N. delesserti* showing again a star-like topology but a much shorter genealogy. Even when more than 350 individuals were included in the analyses, no shared haplotype was detected between *N. concinna* and *N. delesserti*. The dominant haplotype in *N. concinna* (H01) was present in 51.41 % of the individuals and was broadly distributed along the sampling sites, from Adelaide Island to South Georgia Island (Fig. 3). Despite its high frequency, H01 was only present in a 15.5 % of the sampled individuals from South Georgia Island. In fact, in contrast to the rest of

Table 1 Diversity indices and neutrality tests for *Nacella concinna* and *Nacella delesserti*

Locality	N	K	H	S	P	p	Tajima's D	Fu's F _S
Rothera station, WAP	24	6	0.645	5	0.844	0.00127	-1.07	-2.494*
South Bay, WAP	31	5	0.688	5	0.985	0.00149	-0.576	-0.634
Covadonga Bay, WAP	29	9	0.741	7	1.079	0.00163	-1.17	-5.058**
James Ross Island, EAP	28	8	0.735	6	1.087	0.00164	-0.85	-3.70*
Admiralty Bay, SSI	33	7	0.470	6	0.587	0.00089	-1.68	-4.80**
Fildes Bay, SSI	39	6	0.555	6	0.688	0.00104	-1.38	-2.483
Elephant Island, SSI	29	8	0.729	7	0.980	0.00148	-1.34	-4.13*
Laurie Island, SOI	26	9	0.625	8	0.757	0.00114	-2.02*	-6.535*
Signy Island, SOI	23	5	0.605	4	0.711	0.00107	-0.95	-1.83
South Georgia Island (SGI)	45	12	0.871	13	2.101	0.00449	-0.89	-3.74*
<i>N. concinna</i> total	307	29	0.708	29	1.103	0.00167	-2.06*	-28.76***
Trypot Beach, MA	40	4	0.345	3	0.362	0.00055	-1.06	1.736*
Ships Cove, MA	24	3	0.359	2	0.373	0.00056	-0.66	-0.633
<i>N. delesserti</i> total	64	5	0.345	4	0.362	0.00055	-1.23*	-2.723**

n number of sampled individuals, k number of haplotypes, S polymorphic sites, H haplotype diversity, P average number of nucleotide difference, p nucleotide diversity * p < 0.05, ** p < 0.01, *** p < 0.001.

Table 2 G_{ST} (below diagonal and N_{ST} (above diagonal) pairwise comparisons for populations of *Nacella concinna* and *Nacella delesserti*

Localities	R.S.	B.S.	A.B.	F.B.	C.B.	E.I.	J.R.I.	S.O.I.	S.I.	S.G.I.	T.B.	S.C.
R.S.	****	0.011	0.020	0.007	0.000	0.014	0.033	0.024	0.047	0.125	0.849	0.833
B.S.	0.000	****	0.011	0.001	0.006	0.000	0.050	0.007	0.031	0.120	0.829	0.810
A.B.	0.007	0.029	****	0.014	0.013	0.017	0.048	0.000	0.025	0.126	0.867	0.858
F.B.	0.000	0.003	0.002	****	0.000	0.000	0.040	0.013	0.031	0.142	0.854	0.843
C.B.	0.000	0.000	0.035	0.001	****	0.000	0.000	0.002	0.025	0.123	0.822	0.801
E.I.	0.000	0.000	0.038	0.002	0.000	****	0.034	0.010	0.027	0.120	0.832	0.813
J.R.I.	0.000	0.000	0.031	0.009	0.000	0.000	****	0.017	0.052	0.135	0.824	0.802
L.I.	0.000	0.001	0.000	0.000	0.000	0.000	0.000	****	0.000	0.111	0.854	0.840
S.I.	0.005	0.012	0.012	0.006	0.001	0.001	0.009	0.000	****	0.121	0.862	0.850
S.G.I.	0.148	0.121	0.223	0.189	0.108	0.105	0.114	0.152	0.160	****	0.683	0.642
T.B.	0.526	0.495	0.596	0.551	0.473	0.479	0.477	0.532	0.546	0.385	****	0.000
S.C.	0.498	0.465	0.580	0.529	0.440	0.447	0.445	0.505	0.519	0.353	0.000	****

B.S. Rothera Station; S.B. South Bay; A.B. Admiralty Bay; F.B. Fildes Bay; C.B. Covadonga Bay; E.I. Elephant Island; J.R.I. James Ross Island; L.I. Laurie Island; S.I. Signy Island; S.I.G. South Georgia Island; T.B. Trypot Beach, Marion Island; S.C. Ships Cove, Marion Island. Statistical significant differences 100,000 iterations (p < 0.05) are marked in bold circle indicating the site of collection. The size of each haplotype is proportional to its frequency in the whole sampling effort

maritime Antarctica populations, not one dominant, but four medium frequency haplotypes were detected in South Georgia Island. The dominant haplotype (H31) in *N. delesserti* was present in 76.98 % of the analyzed individuals and was found at both localities in Marion Island.

As expected for star-like topologies, both general Tajima's and Fu's neutrality tests were negative and significant for the whole COI data set of each nominal species (Table 1). Sudden growth model analyses detected an older population expansion at South Georgia Island (~18 ka), while maritime Antarctica and Marion Island populations exhibited similar population expansions, 7.0 and 5.0 ka, respectively. Bayesian Skyline plot analyses recognized differences in the age of the

trmca and in the timing of population expansion among South Georgia Island, maritime Antarctica, and Marion Island populations (Fig. 4). The trmca for South Georgia Island was estimated as 25 Ka, while in maritime Antarctica and Marion Island populations, the trmca occurred about 6.5 and 4.5 ka, respectively. Similarly, the onset of population expansion in South Georgia occurred 14 ka while in maritime Antarctica and Marion Island, they took place ~5 and ~2 ka, respectively.

Discussion

The evolution of the marine benthic fauna in the Southern Ocean is the result of historic and contemporary processes operating at different temporal and geographic scales since the Cenozoic (Crame 1999; Linse et al. 2006; Aronson et al. 2007; Fraser et al. 2009; Griffiths et al. 2009; González-Wevar et al. 2012a; Pierrat et al. 2013; Strugnell et al. 2012; Poulin et al. 2014). Major geologic, climatic, and oceanographic changes explain the current distribution of families, genera, and species in different provinces of this region (Clarke and Crame 1989; Clarke et al. 1992; Crame 1999). At the same time, major landscape changes associated with continental ice sheet advances and retreats during the Quaternary have shaped patterns of genetic diversity and structure of populations, particularly in those species restricted to continental shelves (Allcock and Strugnell 2012; Strugnell et al. 2012; González-Wevar et al. 2013).

Biogeography in *Nacella*

In this study, new phylogenetic reconstructions based on mtDNA and nucDNA sequences recognized two main clades currently distributed at (1) South America and (2) Antarctica & Sub-Antarctic Islands. The South American clade includes species from Central Chile (*N. clypeator*) and from Patagonia (*Nacella deaurata*, *Nacella flammea*, *Nacella magellanica*, and *Nacella mytilina*). A second clade includes species from Heard Island (*Nacella kerguelensis* and *Nacella* cf. *macquariensis*), the Antarctic limpet (*N. concinna*), and its closest relative *N. delesserti* from sub-Antarctic Marion Island. In this context, the reciprocal monophyly of both *N. delesserti* (Marion Island) and *N. concinna* (Antarctica), and their close affinity, was supported by different reconstruction methods and sources of DNA, and consequently, these nominal species unequivocally constitute different evolutionary units.

The origin and diversification of *Nacella* took place after the Middle Miocene, long after the physical separation of the continental landmasses or to the formation of the Antarctic Polar Front estimated between 41 Ma (Scher and Martin 2006) and 24 Ma (Pfuhl and McCave 2005). This period represents a phase of major climatic and oceanographic changes in the Southern Ocean that triggered the initiation of polar subzero conditions in the region. Such changes are supported by a number of data sources, including the strengthening of the westerly winds and the intensification of the ACC, atmospheric $\delta^{18}\text{O}$ signatures (Flower and Kennett 1994), and by faunal composition changes at the Kerguelen Plateau (Verducci et al. 2009) and at continental Antarctica (Lewis et al. 2008). A Late Miocene diversification of *Nacella* in Antarctic and sub-Antarctic is consistent with recent molecular studies in Southern Ocean fishes and marine invertebrates. For instance, molecular divergence between Antarctic and sub-Antarctic notothenioid genera indicate a separation that ranges between 9 Ma (Bargelloni et al. 2000) and 6.1 Ma (Stankovic et al. 2002). Similarly, levels of genetic divergence between Antarctic and South American congeneric echinoderms (Hunter and Halanych 2008; Janosik et al. 2010; Díaz et al. 2011), mollusks (Page and Linse 2002; Linse et al. 2006; Wilson et al. 2009; González-Wevar et al. 2012a, Poulin et al. 2014), pycnogonids (Krabbe et al. 2009), and nemertean (Thornhill et al. 2008) indicate that their respective separations occurred during the Late Miocene and Pliocene, no more than 8 Ma. The effective separation between Antarctic and sub-Antarctic lineages in different groups of marine benthic invertebrates during the Late Miocene may be a consequence of major oceanographic changes associated with the geodynamics of the Scotia Ridge. A recent study in the Central Scotia Sea recognized a remnant, now-submerged, volcanic arc that may have formed a barrier to deep eastward circulation until after the Mid-Miocene, approximately 11.6 Ma (Dalziel et al. 2013). The development of a full ACC seems to have played an important role in the subsequent cryosphere expansion (Lear et al. 2000; Zachos et al. 2001), as well as in the intensification of the ACC (Flower and Kennett 1994; Shevenell et al. 2004). It is possible to envision that such changes generated the characteristic polar conditions of the Southern Ocean, with an Antarctic Polar Front acting as an effective barrier among provinces of the Southern Ocean.

Marion Island colonization

The close relationship between the Antarctic limpet and Marion Island *N. delesserti* recorded in this study contests one

important paradigm of the Southern Ocean biogeography, the historic isolation of the Antarctic continent, and therefore points toward a potential permeability of the APF during the Quaternary. According to Barnes et al. (2006) on short ecological timescales, many natural dispersal processes including airborne, oceanic eddy, rafting, and hitchhiking on migrants permit the passage to and from Antarctica. Similarly, humans have become influential by directly transporting organisms and indirectly by increasing the survival and establishment of exogenous taxa via climate change (Barnes et al. 2006). Therefore, at macro- and mesoscales, the flow of the ACC is complex in direction and velocity and rapid turbulent flow create abundant eddies that make the APF porous to transport plankton both northwards or southwards (Froneman et al. 1997). In this context, the finding of Antarctic krill in Chilean fjords (Antezana 1999) and the presence of non-Antarctic benthic crustaceans larvae at King George Island, South Shetland Islands, Antarctic Peninsula (Thatje and Fuentes 2003; Thatje and Arntz 2004; Thatje et al. 2005) are evidence of oceanographic connectivity across the APF probably mediated by eddies (Clarke et al. 2005). However, in the particular case of *Nacella*, the absence of shared haplotypes between Antarctica and Marion Island constitutes strong evidence toward a lack of contemporary gene flow across the APF. Accordingly, it seems like the APF presently constitutes an impassable barrier for *Nacella* larvae and/or adults between these areas of the Southern Ocean. Moreover, reciprocal monophyly of *N. concinna* and *N. delesserti* recorded with different sources of DNA (COI and 28S rRNA) suggests a prolonged time of independent evolution (Hudson and Coyne 2002; de Queiroz 2007). In this context, the cladogenetic process separating both species agrees with the known geologic age of Marion Island (~0.45 Ma; Chown et al. 1998). Hence, our results could be explained by a transient permeability of the APF during the Quaternary, allowing the colonization of this newly formed island. After this, APF became again an efficient barrier that prevented further gene flow between populations from Antarctica and Marion Island, a process that marked the onset of their genetic divergence. As an alternative explanation for these results, the colonization of Marion Island by *Nacella*, rather than be related to the permeability of the APF, could be associated with latitudinal shifts of this oceanographic front. The boundaries of the APF are dynamic in space and time, and its position has varied during Quaternary glacial cycles (Barnes et al. 2006; Fraser et al. 2009). Mapping of diatom mat deposits in the Atlantic provides evidence of a glacial–interglacial front migration of up 6° of latitude during the Early/Mid-Pleistocene. The Mid-Pleistocene transition marks a stepwise minimum 7° northward migration sustained for about 450 ka until an abrupt southward return to its modern position (Kemp et al. 2010). Similarly, Gersonde et al. (2005) recorded a northward expansion of Antarctic cold waters by 5°–10° in latitude and a relatively small displacement of the subtropical front during the Last Glacial Maximum. Under this scenario, it is possible to envision that Marion Island could have been located within the APF at times during the Quaternary, allowing long-distance dispersal events from Antarctic to islands that are currently located in the sub-Antarctic realm. For this, Southern Ocean islands including South Georgia, South Sandwich, and Bouvet may have played a key role as bridges between Antarctica and Marion Island through a stepping-stone model of colonization.

Population-based analyses in *N. concinna* and *N. delesserti*

In general terms, levels of genetic diversity in Antarctic and sub-Antarctic populations of *Nacella* are lower than those found in temperate patellogastropods (Nakano and Ozawa 2005) and in *N. magellanica* from Patagonia (de Aranzamendi et al. 2009, 2011; González-Wevar et al. 2011a, 2012b). Similar patterns of genetic diversity have been recorded in several Antarctic marine invertebrates including *Chorismus antarcticus* (Raupach et al. 2010), *Sterechinus neumayeri* (Díaz et al. 2011), and *Parbolasia corrugatus* (Thornhill et al. 2008). These studies evidence the strong influence of Quaternary glaciations over the demographic dynamics in different phyla of the Southern Ocean (Convey et al. 2009; Fraser et al. 2009; Clarke and Crame 2010; Allcock and Strugnell 2012; González-Wevar et al. 2012a). Major reduction in shallow rocky habitats during glacial periods must have generated considerable reduction in population sizes, especially in shallow benthic invertebrates, and thus generated a marked decline in haplotype diversity; a process that has been usually explained by bottleneck effects or by founder events followed by population expansion (Slatkin and Hudson 1991). Patterns of mismatch distribution and Bayesian Skyline plot in *N. concinna* recognized an older demographic history in South Georgia Island populations. As previously stated (González-Wevar et al. 2013), the high proportion of private haplotypes detected in South Georgia and its high genetic diversity compared to maritime Antarctic populations supports the hypothesis that this island acted as a glacial refugia for the species during the Last Glacial Maximum. After this, the Antarctic limpet would have recolonized maritime Antarctica through a strong founder effect following the deglaciation process (González-Wevar et al. 2013).

In Marion Island, *N. delesserti* exhibited extremely low level of genetic diversity compared to *N. concinna* from South Georgia and maritime Antarctica, even compared to the southernmost populations from western Antarctic Peninsula. Haplotype diversity, star-like topologies, short genealogies, significant negative neutrality indices, and demographic inference analyses recorded in Marion Island populations suggest the occurrence of a dramatic bottleneck event during the last glacial period followed by a postglacial population expansion during the last 5 ka. Such decrease in population size may be the result of strong glacial impact on this small, steep, and isolated island characterized by short and narrow seashore. In

fact, glacial reconstructions in Marion Island suggest that this island was strongly impacted and almost entirely covered by ice during the Last Glacial Maximum (Hall 1982, 2009; Hall and Meiklejohn 2011; Hall et al. 2011). This study provides evidence of Quaternary (≈ 0.35 ka) colonization of Marion Island, a newly formed sub-Antarctic island, by an Antarctic benthic species and its subsequent differentiation. It also highlights the importance of historic and contemporary climatic/oceanographic processes in explaining the current distribution of genealogical lineages in different provinces of the Southern Ocean. Alternation of glacial and interglacial periods of the Quaternary is responsible for the current patterns of genetic diversity and structure observed in Antarctic and Sub-Antarctic species of *Nacella*. Similar demographic trajectories detected in *N. concinna* from maritime Antarctica and *N. delesserti* from Marion Island are evidence of the strong effect of past glacial events, and especially of the LGM on the demography and distribution of polar benthic invertebrates with shallow and narrow bathymetric ranges.

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Fig. 1 Distribution of *Nacella* species in different provinces of the Southern Ocean (South America, Antarctica, and sub-Antarctic islands). *Dashed lines* along the coast indicate the approximate

distribution of the different lineages. *White rectangles* contain the analyzed species of *Nacella*

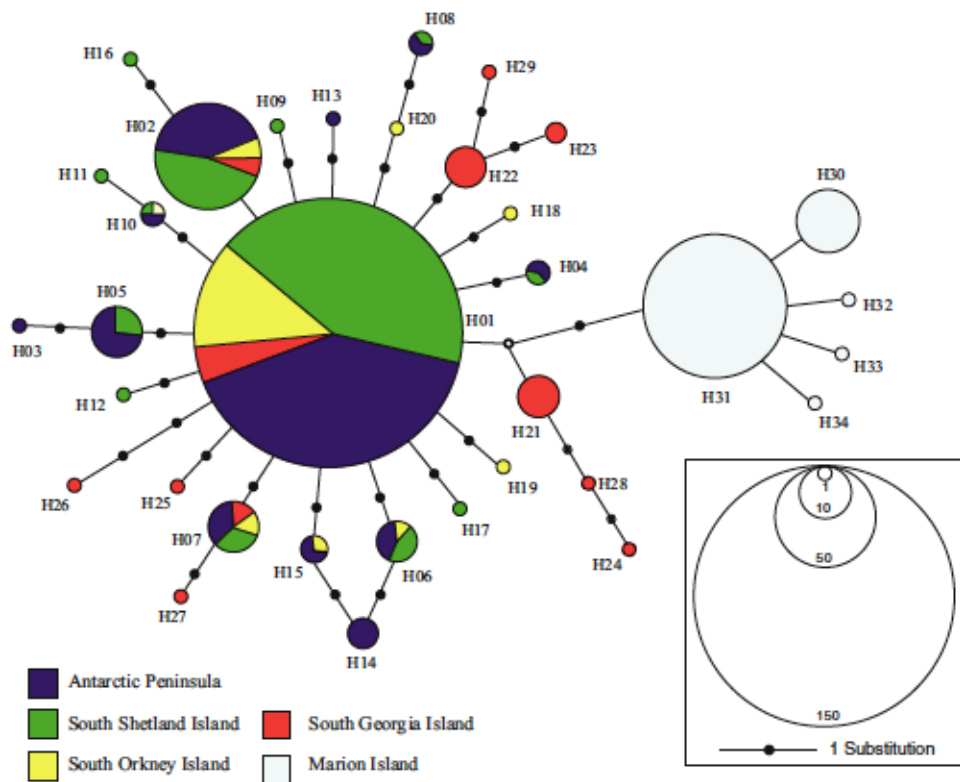


Fig. 3 Median-joining haplotype network based on mtDNA COI sequences obtained from 307 *Nacella concinna* and 64 *Nacella delesserti* individuals. Each haplotype is represented by a colored

circle indicating the site of collection. The size of each haplotype is proportional to its frequency in the whole sampling effort