Extinction and recolonization of maritime Antarctica in the limpet *Nacella concinna* (Strebel, 1908) during the last glacial cycle: toward a model of Quaternary biogeography in shallow Antarctic invertebrates

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Abstract

Quaternary glaciations in Antarctica drastically modified geographical ranges and population sizes of marine benthic invertebrates and thus affected the amount and distribution of intraspecific genetic variation. Here, we present new genetic information in the Antarctic limpet *Nacella concinna*, a dominant Antarctic benthic species along shallow ice-free rocky ecosystems. We examined the patterns of genetic diversity and structure in this broadcast spawner along maritime Antarctica and from the peri-Antarctic island of South Georgia. Genetic analyses showed that *N. concinna* represents a single panmictic unit in maritime Antarctic. Low levels of genetic diversity characterized this population; its median-joining haplotype network revealed a typical star-like topology with a short genealogy and a dominant haplotype broadly distributed. As previously reported with nuclear markers, we detected significant genetic differentiation between South Georgia Island and maritime Antarctica populations. Higher levels of genetic diversity, a more expanded genealogy and the presence of more private haplotypes support the hypothesis of glacial persistence in this peri-Antarctic island. Bayesian Skyline plot and mismatch distribution analyses recognized an older demographic history in South Georgia. Approximate Bayesian computations did not support the persistence of *N. concinna* along maritime Antarctica during the last glacial period, but indicated the resilience of the species in peri-Antarctic refugia (South Georgia Island). We proposed a model of Quaternary Biogeography for Antarctic marine benthic invertebrates with shallow and narrow bathymetric ranges including (i) extinction of maritime Antarctic populations during glacial periods; (ii) persistence of populations in peri-Antarctic refugia; and (iii) recolonization of maritime Antarctica following the deglaciation process.

Keywords: approximate Bayesian computations, glacial refugia, maritime Antarctica, Mollusca, mtDNA, peri-Antarctic areas, private haplotype, South Georgia Island

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Introduction

The evolution of benthic invertebrates of the Southern Ocean, and especially in Antarctica, is of considerable interest given both the rapid contemporary climatic changes in the region (Tin et al. 2009; Aronson et al. 2011; Chown et al. 2012), and the complex biogeographical patterns described for this fauna, which have arisen from the interaction of geological, oceanographic, climatic and biological processes in space and time (Clarke & Crame 1989; Aronson et al. 2007; Rogers 2007;
Griffiths et al. 2009). The origin of the Antarctic shelf benthos has been explained by several, alternative hypotheses including colonization from the deep sea, colonization from northward areas via the Scotia Arc region and in situ evolution or differentiation (Knox & Lowry 1977; Clarke & Crame 1989). During the Cenozoic, the prevalence of increasingly cold conditions shaped the evolution of the Antarctic marine life and taxa that did not adapt, became extinct (Aronson & Blake 2001; Thatje et al. 2005; Aronson et al. 2007); as documented in several marine groups during the cooling period that followed the middle Miocene at c.14 Ma (Zachos et al. 2001; Shevenell et al. 2004; Verducci et al. 2009). More recently, during the Quaternary glaciations, and particular the last one, which ended ~21 ka ago, the development of large ice sheets and lower global temperatures generated major changes in the spatial distribution of species, especially at higher latitudes (Hewitt 2004). Glacial processes radically altered the geographical range of the species and the size of populations, thereby impacting the distribution of intraspecific genetic variation (Maggs et al. 2008; Alcock & Strugnell 2012). Successive ice advances and retreats had a significant impact on the marine diversity of polar and subpolar areas (Clarke & Crame 2010), but the relationships between physical and biological processes are not well understood (Gutt et al. 1996; Gutt 2001). During glacial maxima near-shore Antarctic benthic communities would have been especially vulnerable to grounded ice sheets as they extended over most of the continental shelf, from intertidal areas to about 500 m depth (Thatje et al. 2005; Dambach et al. 2012). However, glacial and interglacial cycles are also thought to be responsible for the diversification and speciation of several Antarctic groups (Thornhill et al. 2008; Pearse et al. 2009; Wilson et al. 2009; Alcock et al. 2011; Baird et al. 2012), a process termed the Antarctic diversity pump (Clarke & Crame 1989, 1992). According to Pearse et al. (2009), the Antarctic Circumpolar Current (ACC) passing through the Drake Passage could have an important role in this process as for over 30 Ma it could have transported organisms to new habitats where they diverged. Similarly, during Pliocene/Pleistocene glaciations over the Antarctic Continental Shelf, speciation processes could also have been enhanced in crustaceans and echinoderms, with nonpelagic development, when populations became fragmented into small isolated units (Pearse et al. 2009).

Several hypotheses have been proposed to explain how the Antarctic benthos could have endured the glacial events of the Pleistocene. They rely upon the Expansion-Contraction (E-C) model proposed by Provan & Bennett (2008) that describes the response of populations and species to climate oscillations (Hewitt 2004; Maggs et al. 2008; Marko et al. 2010). According to the basic E-C model, cool-temperate species survived the Last Glacial Maximum (LGM) in low-latitude refugia, contracting their distribution range to less ice-impacted areas, and then recolonized higher latitudes through range expansion after ice retreat (Provan & Bennett 2008). Genetic studies have been pivotal in improving current knowledge of the demographic response of species to major climatic changes of the Pleistocene (Hewitt 2000; Marko et al. 2010), and to identify potential glacial refugia and recolonization routes (Hewitt 2004; Pearson 2006).

Three variants of the E-C model can be proposed to account for the biogeographical history of the Antarctic benthos during the Pleistocene. (1) In contrast to the Arctic benthos that experienced latitudinal range shifts during the last glacial cycles (Marko 2004), near-shore Antarctic species cannot retreat toward lower latitudes, as they are confined to the continental shelf by deep-sea basins. According to the `deep-sea refugia’ model, the Antarctic shelf species responded to ice advances during glacial maxima by shifting their bathymetric range toward deep-sea less ice-disturbed areas and recolonized shelf areas following the deglaciation process. This model is supported by the unusual levels of eurybathy described in several Antarctic marine groups, with species distributed on the whole continental shelf and the upper part of the slope, down to a thousand meter depth (Brey et al. 1996; Brandt et al. 2009). For instance, Wilson et al. (2009) detected significant levels of genetic structure within lineages of the Antarctic sea slug Doris kerguelensis that could result from the existence of remote deep-sea refugia during the LGM. Similarly, several glacial refugia have been proposed for the circum-polar deep-sea crinoid Promachirus kerguelensis (Hemery et al. 2012). However, the `deep-sea refugia’ model fails to explain how the Antarctic shallow benthos survived the LGM. For instance, the limpet Nautilina concinna and the echinoid Sterechinus neumayeri exhibit narrow depth ranges, which does not seem to have impeded their evolutionary success. (2) The second E-C model is the `shelf in situ refugia’, which is supported by recent genetic studies showing that some marine invertebrates might have survived in situ, in one or several refugia on the Antarctic shelf (Alcock & Strugnell 2012). There is geological evidence of the dyachrony of ice-sheet extensions around Antarctica, and during the LGM, not all shelf areas were fully covered by grounded ice at the same time (Anderson et al. 2002). This was the case in the Weddell Sea, where areas with reduced sea-ice cover occurred (Smith et al. 2010), which may have enabled bryozoans to persist during the LGM (Barnes & Kuklinski 2010). Thatje et al. (2008) suggested that Antarctic shelf populations could have
permitted during the LGM at singular areas of local marine productivity known as polynyas. (3) A last alternative model is the ‘island refuge’ model, according to which shallow marine species survived out of the Antarctic continental shelf, either at adjacent Antarctic islands such as the South Shetland Islands (SSI), the Palmer Archipelago, or in geographically distant islands of the Scotia Arc, including the South Sandwich Islands, and South Georgia. Considering that the Scotia Arc islands, and particularly the South Georgia Island represent the northern boundary for many Antarctic shelf species (Barnes et al. 2006), this area constitutes a potential refugium for these species during glacial maxima.

Patterns of population genetic diversity and structure can be used to infer historical and contemporary demographic processes including extinction of lineages, distributional shifts through range contractions—expansions and to identify recolonization routes (Hewitt 2000, 2004; Maggs et al. 2008). In a recent review of molecular studies devoted to benthic organisms of the Southern Ocean, Alcocock & Strugnell (2012) examined the genetic signals that should be expected under the ‘deep-sea’ version the ‘in situ’ shelf refugia’ scenarios. Under the deep-sea model, many eurybathic species should exhibit a diffuse or parochial haplotype network with high genetic diversity, complex genetic structure and potential cryptic speciation events. Such diffuse patterns of genetic diversity have been recorded in the deep-sea shrimp Nematocarcinus lanceopes (Raupach et al. 2010), in the brittle star Astrotoma agassizi (Hunter & Halanych 2008) and in the sea star Odontaster validus (Janosik et al. 2011), while the typical parochial pattern has been identified in the sea spider Nymphon australe (Arango et al. 2011). Under the ‘in situ shelf refugia’ model, taxa should be characterized by a ‘star-like’ haplotype network as a consequence of bottleneck processes, associated with glacial resilience in small refugia followed by rapid population expansion during deglaciation. Such star-like patterns of genetic diversity have been recognized in Sterechinus neumayeri (Diaz et al. 2011), Parbolasia corrugatus (Thornhill et al. 2008) and Chorismus antarcticus (Raupach et al. 2010). Under the ‘island refuge’ model, we expect to detect high levels of genetic diversity in putative refugial areas located at lower latitude oceanic islands, while population at former glaciated areas along the Antarctic Peninsula (AP) and adjoining islands should exhibit comparatively lower genetic diversity levels. Moreover, such population should exhibit strong signals of recent population expansion as well as founder effect associated with a postglacial recolonization. Nevertheless, both bottleneck and founder effect should leave a similar genetic signature in AP populations, challenging the possibility to discriminate between scenarios. However, in the case of the ‘in situ shelf refugia’, continental Antarctic populations should have separated from peri-Antarctic oceanic islands ones at the beginning of the last glacial period (~110 ka) while in the ‘island refuge’ model, continental Antarctic populations would have originated from peri-Antarctic refuge together with the deglaciation process (~17.5 ka). According to this, it is expected that differences in divergence time between continental Antarctica and peri-Antarctic populations would be reflected in the coalescent process. Under the ‘in situ shelf refugia’ scenario, continental Antarctica and peri-Antarctic populations should exhibit deeper coalescent time than under the ‘island refuge’.

The Antarctic limpet Nacella concinna (Strebel, 1908) constitutes a good model to examine the effects of past climate events on Antarctic shallow benthos and to contrast among the competing scenarios. This species is one of most conspicuous and dominant macroinvertebrates currently restricted to ice-free rocky ecosystems of the Antarctic maritime zone (AP and adjoining islands), as well as in peri-Antarctic areas including scattered islands that are free of pack ice in winter (i.e. South Georgia, Gough, South Sandwich and Bouvet islands; Bölter et al. 2002). In contrast to many Antarctic benthic invertebrates, N. concinna exhibits a narrow bathymetric range from the upper intertidal zone down to 110 m depth (with its highest density in depths between 6 and 10 m), where it grazes on microphytobenthos, bacterial films and microalgae (Brethes et al. 1994). This limpet is dioecious, with external fertilization and a free-swimming planktotrophic larval stage that can survive for one to 2 months (Bowden et al. 2006). Molecular surveys in the species using AFLPs detected an absence of genetic differentiation between inter- and subtidal morphotypes of the species (Hoffman et al. 2010a), and a single genetic entity along the Western Antarctic Peninsula (WAP) (Hoffman et al. 2010b). In the same study area, Gonzucodep>/ucodep>lez-Wevar et al. (2011a) recognized low levels of mtDNA genetic diversity in the species and a marked signal of recent demographic expansion dating to the last glacial-interglacial period. Such a pattern of genetic diversity and structure in the species is congruent with the hypothesis of a strong impact of the last glacial period on population sizes. Considering the narrow bathymetric range of N. concinna, if this species persisted during the LGM along the coast of the AP, the extension of ice sheets may have drastically reduced its habitat to small isolated refugia of ice-free shelf areas. However, such marine refugia have not been described along AP or in the SSI. Alternatively, N. concinna could have retreated to less ice-impacted areas during the LGM, in islands of the Scotia Arc that represents the northern limit of its current distribution, and then recolonized the AP following the
deglaciation process (González-Wevar et al. 2011a). In this study, new molecular mtDNA genetic analyses of *N. concinna* were performed in populations collected from different areas along the species distribution including the AP (West and East), the South Shetlands Islands, South Orkney Island (SOI) and South Georgia islands to examine these alternative hypotheses.

**Material and methods**

**Sampling, DNA preparation, PCR amplification and alignment**

Specimens were collected between 2006 and 2012 in the intertidal zone at nine localities distributed in four main areas of interest: the AP, the SSI, and South Georgia (SGI; Fig. 1). Sampling sites were as follows: Rothera Station, Adelaide Island, West AP (67°32’S; 68°06’W; n = 24), South Bay, Anvers Island, West AP (64°54’S; 63°32’W; n = 31), Covadonga Bay, West AP (63°22’S; 58°09’W; n = 29), James Ross Island, East AP (63°55’S; 57°15’W; n = 28), Fildes Bay, King George Island, SSI (62°12’S; 58°56’W; n = 39), Admiralty Bay, King George Island, SSI (62°05’S; 58°27’W; n = 33), Elephant Island, SSI (61°07’S; 54°53’W; n = 29), SOI (60°38’S; 44°41’W; n = 26) and South Georgia Island (54°14’S; 36°23’W; n = 30; Fig. 1). Whole specimens were fixed in ethanol (95%), and DNA was extracted from the mantle using a salting-out method described by Aljanabi & Martinez (1997). A partial fragment of the mitochondrial gene cytochrome c oxidase subunit I (COI) was amplified with specific primers and following PCR conditions described by González-Wevar et al. (2011a). PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN) and sequenced in both directions using an Automatic Sequencer 3730 (QIAGEN) and sequenced in both directions using an automated sequencer 3730 x 1 at Macrogen Inc. (Seoul, Korea). Chromatograms were edited using ProSeq, version 2.91 (Filatov 2002), and the resulting sequences were aligned with ClustalW (Thompson et al. 1994). Sequences were translated to amino acids to check for the presence of pseudogenes and/or sequencing errors with MEGA 5.0 (Kumar et al. 2008). We performed a DNA saturation analysis following Roe & Sperling (2007) to evaluate how saturation of transitions accumulates in relation to nucleotide divergence in the whole COI data set. Finally, COI sequences of *Nacella concinna* were deposited in GenBank under the following Accession nos: KF261314—KF261341.

**Genetic diversity and population structure in the Antarctic limpet**

Levels of genetic polymorphism were determined using the following standard diversity indices: the number of haplotypes (*k*), the number of segregating sites (*S*), haplotype diversity (*H*), the average number of pairwise differences (*I*) and nucleotide diversity (*π*) for each locality, for each main area, and for the whole COI data set using DNASP, version 5.00.07 (Librado & Rozas 2009). To assess the potential existence of past glacial refugia, we estimated the number of private alleles per locality and sampling area following Maggs et al. (2008). We performed neutrality statistical tests (Tajima’s *D* and Fu’s *F*ₜ) for each locality and for the whole data set to measure whether data deviate from expectations under a neutral model.

We estimated the levels of genetic differentiation between the analysed localities following Pons & Petit (1996) through mean pairwise differences (*Nₛₜ*) and through their haplotype frequencies (Gₛₜ) in ARLEQUIN, version 3.5 (Excoffier et al. 2005). The statistical significance of genetic differences between localities was estimated using permutation tests (20 000) of haplotype identities.

Two different clustering methods were used to infer the spatial genetic structure of *N. concinna*. First, we estimated the number and the composition of panmictic groups, as well as the spatial boundaries among them using a Bayesian model computed with the GENELAND package, version 2.0.0 (Guillot et al. 2005) in the R environment (R, version 2.4.1; Ihaka & Gentleman 1996). This software implements a Markov chain Monte Carlo (MCMC) procedure to determine the best clustering of samples with regard to genetic and geographical information. Geographical information is taken into account at the Bayesian prior level, so that clusters corresponding to spatially structured groups are considered to be more likely than clusters that are randomly distributed in space. 5 000 000 MCMC iterations sampled each 1000 steps with a 50 000 burn-in period, and a maximum number of clusters K = 10 were run to estimate the model parameters and posterior probabilities of group membership. Second, we estimated the number and composition of groups that were the most differentiated based on sequence data with SAMOVA (Spatial Analysis of MOlecular VAriance; Dupanloup et al. 2002). SAMOVA is a popular method that uses multiple spatial scales in statistical methods for characterizing spatial genetic structure based on pairwise genetic differences. However, recent studies have noted that the spatial correlations at different spatial scales are highly correlated in isolation-by-distance processes and bear complex interactions among dispersal, spatial scale and spatial lag between distance classes (Anderson et al. 2010; Epper-son 2010). The main problem is that the size of the positive correlation makes the matrix of genetic relationship even more stochastic than would be predicted from the variances (Epperson 2010). Researchers should be
cautious of estimates based on a single genetic locus, but the use of more than one loci likely represents a good solution (Epperson 2010). In order to avoid such biases, mtDNA SAMOVA results in N. concinna were compared with those obtained by Hoffman et al. (2010a,b, 2011) in the same species and study area using AFLPs and with those obtained by Beaumont & Wei (1991) using allozymes.

Demographic inference in the Antarctic limpet

We characterized genealogical relationships in N. concinna using median-joining haplotype networks computed with Network, version 4.6 (http://fluxus-engineering.com). First, we performed a unique reconstruction including the complete COI data set. Second, to identify potential refugia for N. concinna during the last glacial period, we computed four distinct networks, one for each main investigated area (AP, SSI, SOI and SGI). To avoid the potential biases due to differences in sample sizes, we include a similar number of randomly selected individuals (between 26 and 30 specimens) for each area. To assess the patterns of demographic history, we plotted the distribution of pairwise differences between haplotypes (mismatch distributions) for each area. Then, we compared the deviation of mismatch distributions from a model of sudden expansion, using a nonlinear least squares method implemented in DnaSP.

We estimated past population dynamics through time in N. concinna using a Bayesian skyline plot method implemented in BEAST, version 1.7 (Drummond & Rambaut 2007). As a preliminary step, three models (strict clock, uncorrelated lognormal and uncorrelated relaxed clock) were computed for the groups (K) identified by the SAMOVA and GENELAND analysis, and compared statistically using a Bayes factor test (Suchard et al. 2001) run with TRACER, version 1.5 (http://beast.bio.ed.ac.uk/Tracer). The analysis showed that the uncorrelated lognormal model was the most appropriate for the COI data set in the species. We conducted three independent Bayesian MCMC runs using the GTR+G+I model, previously estimated with MRMODELTEST, version 2.3 (http://www.abc.se/~nylander/), and a tenfold evolutionary rate for nacellids at population level (10% per million years; González-Wevar et al. 2011a), following the correction for time dependence of molecular rate proposed by Ho et al. (2005, 2007, 2011). For each group (K), three independent runs were made for 250 \times 10^6 generations (sampled every 1000 iterations), discarding 10% of the trees as burn-in. The convergence of runs was confirmed with TRACER, version 1.5, ensuring a minimum of 1000 effective samplings for each statistics. The results of the multiple runs were combined using LOGCOMBINER.

Fig. 1 Sampling localities of Nacella concinna in maritime Antarctica and at South Georgia.
version 1.4.7 (Drummond & Rambaut 2007). The median and corresponding credibility intervals of the Bayesian skyline plot were depicted with Tracer.

To assess the influence of glacial cycles on the demographic history and genetic structure of *N. concinna*, we used the approximate Bayesian computation (ABC) method (Beaumont et al. 2002). We compared the posterior probabilities of two competing scenarios of genetic differentiation that are characterized by contrasting population divergence times and demographic histories. According to the first scenario or the ‘in situ shelf refugia’ scenario, *N. concinna* was supposed to have persisted along maritime Antarctica during the LGM in restricted areas and then it expanded during the deglaciation. In the second scenario or the ‘island refugia’ scenario, *N. concinna* became extinct during the last glacial period along maritime Antarctica and survived in peri-Antarctic islands. Thereby, present populations along the Antarctic continental shelf would have originated through a postglacial colonization from peri-Antarctic areas, characterized by a strong founder effect followed by rapid population expansion. Both scenarios were compared using the data sets associated with the clusters identified with the SAMOVA and Geneland analyses. Posterior probabilities were estimated with DIY ABC (Cornuet et al. 2008) using historical, demographic and mutational parameters drawn from the prior distributions. For each scenario, $1 \times 10^6$ data sets were simulated and the relative likelihoods of both scenarios were compared using a logistic regression on 1% of simulated data closest to the observed data set (Cornuet et al. 2008). As recommended by Cornuet et al. (2010), we used the model-checking function of DIY ABC to assess the goodness of fit between each model parameter-posterior combination and the observed data set in the species by using different summary statistics for parameter estimation and model discrimination.

**Results**

**Genetic diversity and population structure in the Antarctic limpet**

We included 269 individuals of *Nacella concinna* in the analyses, from which we amplified a fragment of 663 base pairs coding 221 amino acids of the mtDNA COI gene. No insertion/deletion or stop codons were detected in the whole data set. Sequences were not saturated at any position, and we recognized three amino acid changes (positions 147, 155 and 179), two transversions (T to G) and one transition (T to C). As previously estimated in the Antarctic limpet (González-Wevar et al. 2011a) and in Patagonian species (González-Wevar et al. 2011b, 2012a) sequences were A-T rich (60.6 %) compared with the mean G-C content (39.4 %). We detected low levels of genetic polymorphism in *N. concinna*, 4.2% of the nucleotides were variable and only 2.1% were parsimoniously informative. The number of polymorphic sites (s) varied between 12 in SGI and 5 at Rothera Station and South Bay in AP (Table 1). The number of haplotypes (k) varied between 11 in SGI and 5 at South Bay in AP (Table 1). A relatively high coefficient of determination ($R^2 = 0.69$, $P < 0.005$) was computed between the number of haplotypes and the degrees of latitude with the fewest haplotypes at the highest latitudes. The average number of nucleotide differences ($\Pi$) and mean nucleotide diversity ($\tau$) were low in most localities of maritime Antarctica, while levels of genetic diversity were comparatively higher in SGI (Table 1). The number of private haplotypes was much higher in SGI than in any of the other analysed localities. Similarly, the proportion of private haplotypes (8/11) found in SGI was twice that detected in AP, SSI and SOI, even though the number of analysed individuals in AP and SSI was three times higher than in SGI (Table 1).

Mean general values of differentiation, as measured with $G_{ST}$ and $N_{ST}$, were low ($G_{ST} = 0.039$ and $N_{ST} = 0.045$), but highly significant. However, when depicting pairwise comparisons among samples, only those between SGI and the rest of the localities were statistically significant after Bonferroni correction. In contrast, no significant genetic structure was detected among maritime Antarctic localities (Table S1, Supporting information).

The model based Bayesian clustering algorithm implemented in Geneland detected two main clusters ($K = 2$), the first one including maritime Antarctic localities from AP, SSI and SOI (Fig. 2a) and the second one SGI (Fig. 2b). Values of cluster membership are high for all localities (c.a. $P = 0.9$). The mean probability value ($P = 0.5$) corresponding to the boundary between the two clusters runs across the Scotia Ridge, between SOI and SGI (Fig. 2). The existence of these two clusters is supported by the results of the SAMOVA that detected the same two groups with a maximal difference accounting for 23.38% of the total variation, and only a 0.37% was due to within-group variations among localities.

**Demographic inference and scenario comparison**

The median-joining haplotype network of *N. concinna* comprised 28 different haplotypes and showed a typical star-like topology and a short genealogy (Fig. 3). The central haplotype (H1) was the most frequent one (52.4%) and distributed at all localities (Fig. S1, Supporting information), from Rothera Station in AP to SGI (Fig. 3). Despite its high frequency values (>$50\%$)
in AP, SSI and SOI, H1 was present in only a 13.3% of the sampled individuals from SGI. The second and third most dominant haplotypes (H2 and H7) showed intermediate frequency values (14.1 and 5.9%, respectively) were also widely distributed in all the analysed areas (AP, SSI, SOI, SGI) and are related to H1 through a branch length of two mutational steps (Fig. 3). In contrast to most localities (Fig. S1, Supporting information) and areas (Figs 3 and 4) that are characterized by a single dominant haplotype, in SGI, we detected at least four haplotypes with intermediate frequency values (H1, H21, H22 and H23) and three of them (H21, H22 and H23) were endemic to this peri-Antarctic island. As expected for star-like topologies, general Tajima’s and Fu’s neutrality tests were both negative and significant for the whole COI data set of the species (Table 1).

The haplotype networks constructed for each area in maritime Antarctic (AP, SSI and SOI) separately showed similarities according to their star-like topologies, short genealogies and haplotype structure (Fig. 4). In contrast, the network of SGI showed a more expanded genealogy and the presence of several private haplotypes of intermediate and low frequency values. SGI also differed from the three other investigated areas in the distribution of pairwise differences between haplotypes. As expected for star-like networks, mismatch distributions are positively skewed and unimodal for AP, SSI, SOI,

<table>
<thead>
<tr>
<th>Locality</th>
<th>n</th>
<th>k</th>
<th>p.a.</th>
<th>H</th>
<th>S</th>
<th>H</th>
<th>π</th>
<th>Tajima’s D</th>
<th>Fu’s F&lt;sub&gt;S&lt;/sub&gt;</th>
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<td>1</td>
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<td>0.00149</td>
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<td>0</td>
<td>0.74</td>
<td>7</td>
<td>1.079</td>
<td>0.00163</td>
<td>−1.17</td>
<td>−5.058**</td>
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<td>0.00164</td>
<td>−0.85</td>
<td>−3.70*</td>
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<td>Antarctic Peninsula (AP)</td>
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<td>12</td>
<td>3</td>
<td>0.69</td>
<td>11</td>
<td>1.015</td>
<td>0.00153</td>
<td>−1.31</td>
<td>−5.98**</td>
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<tr>
<td>Admiralty Bay</td>
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<td>0.47</td>
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<td>0.587</td>
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<td>−4.80**</td>
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<td>−1.34</td>
<td>−4.13*</td>
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<td>−10.19**</td>
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<td>11</td>
<td>8</td>
<td>0.88</td>
<td>12</td>
<td>2.340</td>
<td>0.00353</td>
<td>−0.74</td>
<td>−3.44*</td>
</tr>
<tr>
<td><em>N. concinna total</em></td>
<td>269</td>
<td>28</td>
<td>n.a.</td>
<td>0.68</td>
<td>28</td>
<td>1.045</td>
<td>0.00158</td>
<td>−2.10*</td>
<td>−28.91***</td>
</tr>
</tbody>
</table>

n: number of sampled individuals; k: number of haplotypes detected; p.a.: number of private alleles; S: polymorphic sites; H: haplotype diversity; H: average number of nucleotide difference; π: nucleotide diversity *P < 0.05, **P < 0.01, ***P < 0.001.

![Fig. 2](image-url) Spatial output from Geneland using all nine *Nacella concinna* populations. Black circles indicate the relative position of the sampled populations. Darker and lighter shading are proportional to posterior probabilities of membership in clusters, with lighter (yellow) areas showing the highest probabilities of clusters.

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while the distribution of pairwise differences for SGI was almost unskewed (Fig. 4).

Sudden growth model analyses based on a 10% mutational rate detected signals of an older population expansion in the South Georgia Island population. Time of expansion for the SGI population (17.5 ka) was two to three times older than the expansion times estimated within maritime Antarctic areas (AP = 7.5 ka, SSI = 5.6 ka and SOI = 8.6 ka).

Similarly, Bayesian Skyline plot analysis recognized differences in the times of the most recent common ancestor (tmca) and population expansions between SGI and maritime Antarctica populations. Based on these analyses, South Georgia Island population appears to be older with the most recent common ancestor (tmca) occurring about 24 ka against about 6.5 ka determined for maritime Antarctica (vFig. 5). Accordingly, the onset of the population expansion in SGI is dated approximately to 12 ka against the 5 ka estimated for maritime Antarctica.

The approximate Bayesian computation approach discriminated between the two competing scenarios and the posterior probability did not support the ‘in situ shelf refugia’ scenario of *N. concinna* during the LGM (Fig. S2, Supporting information). On the contrary, the ‘island refugia’ scenario received the highest probability value ($P = 0.993$), which supports the persistence of the species in peri-Antarctic areas and the subsequent colonization of maritime Antarctic through a strong founder effect followed by a rapid expansion. Moreover, for this scenario none of the test quantities used to assess model misfit had low tail probabilities, which indicates a good fit between the scenario-posterior combination and pseudo-observed data set of *N. concinna*.

**Discussion**

Major climate changes of the Quaternary are considered to have strongly impacted the abundance, structure and spatial distribution of species (Hewitt 2000; Maggs *et al.* 2008; Provan & Bennett 2008). At high latitudes, and particular in Antarctica, the successive ice-sheet advances and retreats have constrained much of the phylogeographic structure of populations (Lomolino *et al.* 2006; Lesbarrères 2009; Marko *et al.* 2010). Genetic data have played a pivotal role for understanding the impact of glacial cycles on the evolution of the biota in the Southern Ocean benthos (for review see Alcock & Strugnell 2012). Biogeographical affinities of Antarctic terrestrial fauna provide evidence demonstrating that many terrestrial taxa survived glaciations in the Antarctic continent and the sub-Antarctic islands (Convey
Fig. 4 Median joining haplotype networks and the distribution of pairwise differences between haplotypes (mismatch distribution) analyses for each area included in the analyses. A similar number (between 26 and 30) of randomly selected individuals per area where included in these analyses.
influence of Quaternary glaciations on species demography in the Southern Ocean (Clarke & Crame 2010; Allcock & Strugnell 2012; González-Wevar et al. 2012b). Accordingly, in N. concinna, the strong correspondence ($R^2 = 0.69$) between latitude and genetic diversity suggests a lesser impact of Quaternary glacial cycles on the demography of the species at lower latitudes. The starlike genealogy of N. concinna, with most haplotypes occurring at low frequency and little differentiated from a widely distributed dominant one, is congruent with the pattern of genetic diversity proposed by Allcock & Strugnell (2012) for highly dispersive species that survived the LGM in glacial refugia in the Antarctic continental shelf. According to this model, the massive reduction in population sizes generated a severe decline in haplotype diversity, such as those found in several other Antarctic marine invertebrates. More generally, such a pattern suggests a bottleneck or founder event followed by population expansion (Slatkin & Hudson 1991).

**Genetic differentiation in Nacella concinna**

Genetic structure analyses in the Antarctic limpet suggest the existence of a single panmictic unit along maritime Antarctica, from Rothera Station to SOI. This agrees with previous molecular results obtained for this species in the WAP using AFLPs (Hoffman et al. 2010b) and mtDNA sequences (González-Wevar et al. 2011a). Such a pattern of genetic homogeneity over a broad geographical range can be explained by the existence of particular life history traits including a larval stage with high dispersive potential (Bowden et al. 2006). The distance over which larvae can disperse is partly correlated with the duration of the pelagic stage which strongly conditions the geographical range and genetic structure of populations (Todd 1998). The pelagic stage in N. concinna is long enough to limit the impact of genetic differentiation due to inbreeding and/or genetic drift among the maritime Antarctica populations included here.

Ocean waters off the WAP are characterized by a complex oceanography, being influenced by both circumpolar- and shelf-water regional currents. Along the continental shelf break, ocean waters are under the influence of the northward Antarctic Circumpolar Current (ACC; Martinson et al. 2008), while near-shore areas are washed by the southward Antarctic Coastal Current (Moffat et al. 2008) that generates a clockwise circulation of waters including several mesoscale gyres (Hofmann et al. 1996). These oceanographic features might play an important role in facilitating the genetic homogeneity of Antarctic broadcasters like N. concinna (Bowden et al. 2006). Examples of larval-mediated
postglacial recolonization have been invoked for marine organisms of the Northern Hemisphere (Marko 2004; Flight et al. 2012) as well as Patagonia, southern South America (Macaya & Zuccarello 2010; Ceballos et al. 2011). This is also the case for Patagonian relatives of the Antarctic limpet (de Aranzamendi et al. 2011; González-Wevar et al. 2012a).

In contrast to the homogeneous spatial pattern observed within maritime Antarctic populations of _N. concinna_, traditional _F_{ST}-based_ methods (pairwise _N_{ST}/_G_{ST} comparisons and _SAMOVA_ analyses) together with the Bayesian analysis detected a marked genetic differentiation between SGI and maritime Antarctica localities. Despite the occurrence of shared haplotypes between maritime Antarctica and SGI (H1, H2 and H7), the dominant haplotype of SGI (H22) was not recorded in maritime Antarctica, suggesting a restricted gene flow between these two areas. This result is in agreement with previous studies that showed a significant allozymic differentiation in _N. concinna_ between populations of SOI and SGI (Beaumont & Wei 1991) and a marked _nuDNA_ (AFLPs) genetic differentiation between populations of AP and SGI (Hoffman et al. 2011).

Few biogeographical barriers to gene flow of shallow-water invertebrates with dispersal stages have been described in marine ecosystems, and most of them are associated with wide ocean stretches such as the East Pacific Barrier (Scheltema 1986) or with major oceanographic barriers such as the Antarctic Polar Front (APF; Thornhill et al. 2008). The APF plays an important role as a barrier to gene flow in several taxa among Southern Ocean provinces (Shaw et al. 2004; Krabbe et al. 2009; Wilson et al. 2009) and especially for _Nacella_ (González-Wevar et al. 2010, 2012b). However, SGI and maritime Antarctic areas have been located south of the APF during the Quaternary glacial/interglacial cycles (Gersonde et al. 2005) so that the APF can be excluded as a potential barrier to gene flow between populations of the Antarctic limpet from both areas. Drifter-based data indicate that the ACC flows from maritime Antarctica to SGI with a velocity that would allow a propagule to drift between the two areas in at least three and a half months (Matschiner et al. 2009), or 150 to 250 days from sea surface current model estimations (Thorpe et al. 2007). This is however, too long a time for the 2-month duration of _N. concinna_ larvae (Bowden et al. 2006). As stated by Todd (1998), indirect development _per se_ does not necessarily preclude genetic differentiation because genetic structuring occurs as a result of larval behaviour and ecology and is not just dependent on local hydrographic conditions. In this respect, the absence of genetic structuring between maritime Antarctica and SGI has been recorded in very few species with pelagic stages; the notothenioid fish _Gobionotothen gibberifrons_ (Matschiner et al. 2009) and the Antarctic krill _Euphausia superba_ (Bortolotto et al. 2011). In broadcasting invertebrates including _N. concinna_ (Hoffman et al. 2011; this study), the nemertean _Parbolasia corrugatus_ (Thornhill et al. 2008), the ophiuroid _Astartoma agassizii_ (Hunter & Halanych 2008), the crinoid _Promachocrinus kerguelenensis_ (Wilson et al. 2007) and the pycnogonid _Colossendeis megalonyx_ (Krabbe et al. 2009), there is evidence that major currents and deep-water considerably hinder gene flow between Antarctica and SGI. As stated by Hoffman et al. (2011), contrasting patterns of genetic structure in broadcasting Antarctic marine invertebrates could be due to differences in larval duration and ecology. In contrast to species with high dispersal capacities (i.e. pelagic/planktonic forms), limpets represent organisms with a limited autonomous dispersal potential. This might account for the restricted gene flow between SGI and maritime Antarctica detected in _N. concinna_ compared with fishes and to krill.

**Single or multiple refugia in the Antarctic limpet**

The presence of exclusive haplotypes in certain populations (Slatkin’s ‘private alleles’) has been used as proxy to identify areas of potential refugium (Maggs et al. 2008; Hemery et al. 2012; Strugnell et al. 2012). In _N. concinna_, the significant differentiation of populations between SGI and maritime Antarctica localities, together with the higher proportion of private haplotypes in the former, supports the hypothesis of SGI playing a role of a refugium for the Antarctic limpet during the LGM. Moreover, patterns of mismatch distributions and Bayesian Skyline plot analyses indicate an older demographic history in SGI than in the investigated maritime Antarctic areas, thereby confirming the island refugia hypothesis. Geological evidence also indicates that glacial conditions were less extreme at SGI (Claperton et al. 1989; Bentley et al. 2007) than in maritime Antarctic areas such as the AP (Sugden et al. 2006), SSI (Hall 2003) and SOIs (Herron & Anderson 1990) during the LGM, allowing the persistence of populations of the species. Currently, there are clear differences in seasonality and species composition between maritime Antarctica and SGI even when both ecosystems are part of a continuum, from more ice-covered regions in the south to open water regions in the north (Murphy et al. 2013).

In contrast to the SGI populations, the low levels of genetic polymorphism, the negative significant neutrality tests and the lower proportion of private haplotypes support the hypothesis of the recent postglacial expansion of populations from maritime Antarctica. Expansion time estimates of Antarctic populations are
consistent with previous results in N. concinna (González-Wevar et al. 2011a) and are supported by geological and geochemical evidence indicating that warmer water conditions (Anderson et al. 2002; Bentley et al. 2006; Convey et al. 2009) and ice retreat commenced in the AP area 17 ka ago and were achieved by 9.5 ka (Suggen et al. 2006).

Compared with postglacial populations that are expanding, populations from glacial refugia show a longer demographic history with higher levels of genetic diversity (Provan & Bennett 2008). The long-term isolation of populations in distinct remote refugia leads to genetic differentiation by genetic drift and therefore to distinct genetic lineages (Hewitt 2000; Maggs et al. 2008). Approximate Bayesian computation analyses strongly support the scenario of a postglacial recolonization of maritime Antarctica from peri-Antarctic areas and subsequent population expansion following a founder effect over the competing scenario, the in situ persistence of populations in Antarctic shelf refugia associated with a bottleneck effect. Analyses of offshore sediments along AP revealed that an extensive and deep (500 m depth) ice sheet probably extended over most of the continental shelf during the LGM (Suggen et al. 2006). Similarly, piston cores from the South Orkney Plateau recognized a widespread surface of glacial erosion providing evidence of an ice cap grounded to a depth of 250 m (Herron & Anderson 1990). Such a grounded ice mass likely represented a severe limiting factor for the survival of benthos in these areas (Brey et al. 1996; Thatje et al. 2005, 2008), especially for shallow-water species with a narrow depth range such as N. concinna.

Main conclusions
Climatic and oceanographic processes together with life history traits are the major underpinning factors explaining the phylogeographic patterns observed in Nacella concinna. Supported by the dispersal potential of the species and the observed patterns of genetic diversity and structure in the Antarctic limpet, we propose a scenario of rapid postglacial recolonization of maritime Antarctic near-shore areas from less ice-impacted peri-Antarctic areas (including SGI), associated with a strong founder effect. This scenario could also prevail for other marine groups so that the following model of Quaternary biogeography could be proposed for shallow-water invertebrates with narrow depth ranges and dispersal capabilities. The model includes (i) the eradication of near-shore Antarctic populations during glacial maxima of the Pleistocene; (ii) the persistence of populations in peri-Antarctic refugia during that time and (iii) the recolonization of Antarctic near-shore areas after ice retreat, associated with rapid population expansion through larval dispersal. At the same time, this scenario permits us to further understand why one of the most common and dominant Antarctic marine invertebrates is currently restricted to recently deglaciated areas of maritime Antarctica and does not exhibit a circumpolar distribution like other Antarctic taxa. Future studies should include a new sampling effort of the species, especially in geographically isolated peri-Antarctic areas (Bouvet, Gough and South Sandwich islands) to test these ideas. Studies of other shallow benthic invertebrates would prove similarly useful to examine the extent to which this model and others (see Allcock & Strugnell 2012) are general.

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C.A.G-W’s research interests are Evolutionary Biology, Biogeography and population genetics in near-shore marine benthic organisms in the Southern Ocean. T.S. research interests are the evolution and diversification of Austral echinoids, Jurassic and Cretaceous ones and Deep-Sea holasteroid groups. S.M. research interests are how species physiologies are correlated with their environment and the mechanisms underlying these correlations. S.L.C research interests are biogeographical and macroecological studies, macrophysiology, spatial ecology, invasion biology and how these fields are integrated, especially in the Southern Ocean. E.P.’s main research interests are the origin and evolution of the marine benthic fauna in the Southern Ocean, with special emphasis in Antarctica. He also is interested in Evolutionary Biology, biogeography and population genetics.

References

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PERI-ANTARCTIC GLACIAL REFUGIA IN THE ANTARCTIC LIMPET


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C.A.G-W and E.P. conceived the study. C.A.G-W performed the experiments. C.A.G-W and E.P. analysed the data. C.A.G-W, E.P., S.M., S.L.C. and T.S. contributed with specimens and wrote the manuscript.

**Data accessibility**

COI haplotypes sequences in the Antarctic limpet are available in GenBank under the Accession Nos: KF261314—KF261341.


**Supporting information**

Additional supporting information may be found in the online version of this article.

**Table S1** Pairwise GST values, based on haplotypic frequencies, (below diagonal) and average number of nucleotide differences between localities NST (above diagonal) between *Nacella concina* localities.

**Fig. S1** Haplotype network including 269 *Nacella concinna* mtDNA COI sequences.

**Fig. S2** Graphical representation of the two competing scenarios (the ‘island refugia’ and the ‘in situ shelf refugia’ scenarios) tested using the ABC analyses on controlled simulated data sets.