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;

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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.*t 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; Allcock & 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; Allcock *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 Plio/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 kerguelenensis* 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 *Promachrinus kerguelenensis* (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 *Nacella 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 (Allcock & Strugnell 2012). There is geological evidence of the diachrony 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

persisted during the LGM at singular areas of local marine productivity known as polynyas. (3) A last alternative model is the 'island refugia' 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, Allcock & 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 (Díaz et al. 2011), Parbolasia corrugatus (Thornhill et al. 2008) and Chorismus antarcticus (Raupach et al. 2010). Under the 'island refugia' 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 refugia' model, continental Antarctic populations would have originated from peri-Antarctic refugia 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 refugia'.

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 (Brêthes 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, SOI 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°15W: $n = 28$), 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 × 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), haplotypic diversity (H), the average number of pairwise differences (Π) 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_S) 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 (N_{ST}) and through their haplotype frequencies (G_{ST}) 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; Epperson 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
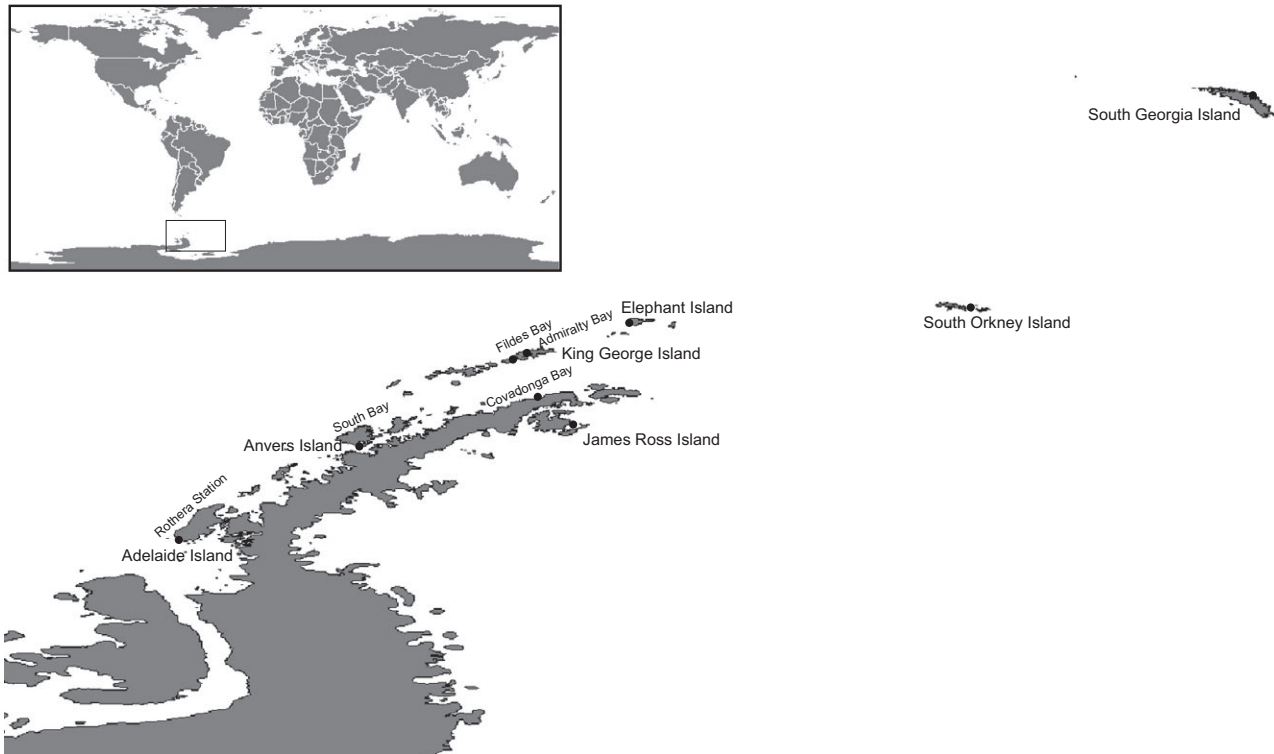
Fig. 1 Sampling localities of *Nacella concinna* in maritime Antarctica and at South Georgia.

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 250×10^6 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,

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, 1×10^6 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 (*Π*) and mean nucleotide diversity (*π*) 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%)

Table 1 Genetic diversity indices and neutrality tests in *Nacella concinna*

Locality	*n*	*k*	*p.a.*	*H*	*S*	*Π*	*π*	Tajima's D	Fu's F$_S$
Rothera Station	24	6	1	0.64	5	0.844	0.00127	−1.07	−2.494*
South Bay	31	5	1	0.68	5	0.985	0.00149	−0.576	−0.634
Covadonga Bay	29	9	0	0.74	7	1.079	0.00163	−1.17	−5.058**
James Ross Island	28	8	0	0.73	6	1.087	0.00164	−0.85	−3.70*
Antarctic Peninsula (AP)	112	12	3	0.69	11	1.015	0.00153	−1.31	−5.98**
Admiralty Bay	33	7	2	0.47	6	0.587	0.00089	−1.68	−4.80**
Fildes Bay	39	6	1	0.55	6	0.688	0.00104	−1.38	−2.483
Elephant Island	29	8	2	0.72	7	0.980	0.00148	−1.34	−4.13*
South Shetland Islands (SSI)	101	13	4	0.58	13	0.742	0.00112	−1.89*	−10.19**
South Orkney Island (SOI)	26	9	3	0.62	8	0.757	0.00114	−2.02*	−6.535*
South Georgia Island (SGI)	30	11	8	0.88	12	2.340	0.00353	−0.74	−3.44*
N. concinna total	269	28	n.a.	0.68	28	1.045	0.00158	−2.10*	−28.91***

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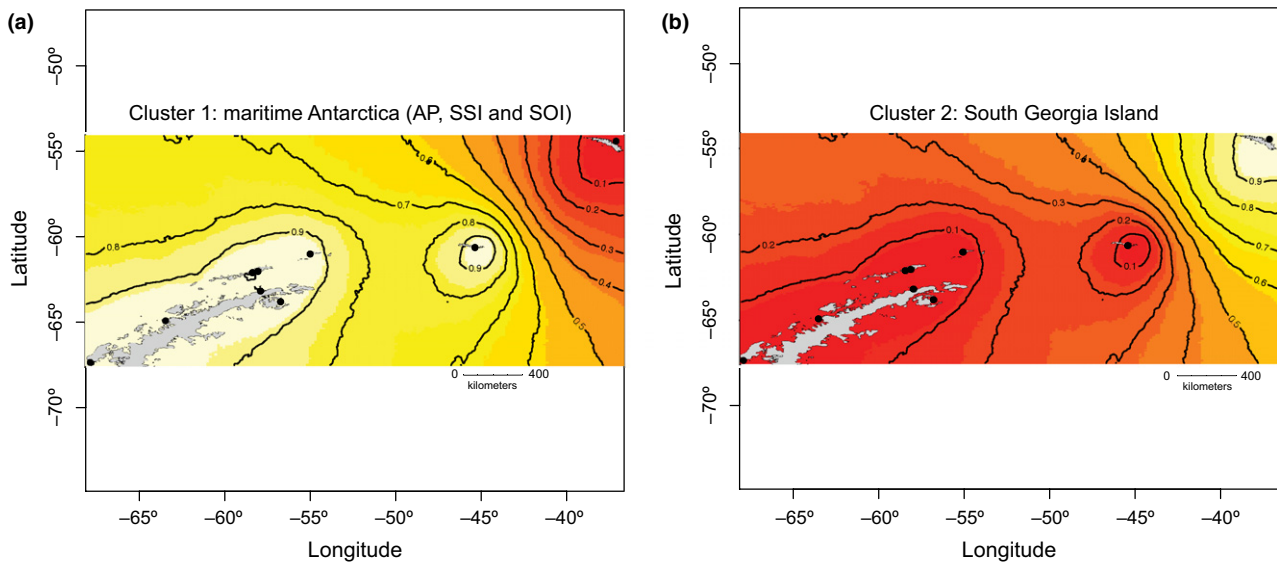

Fig. 2 Spatial output from Geneland using all nine *Nacella concinna* populations. Black circles indicate the relative position of the sampled populations. Darker and lighter shading are proportional to posterior probabilities of membership in clusters, with lighter (yellow) areas showing the highest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

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,
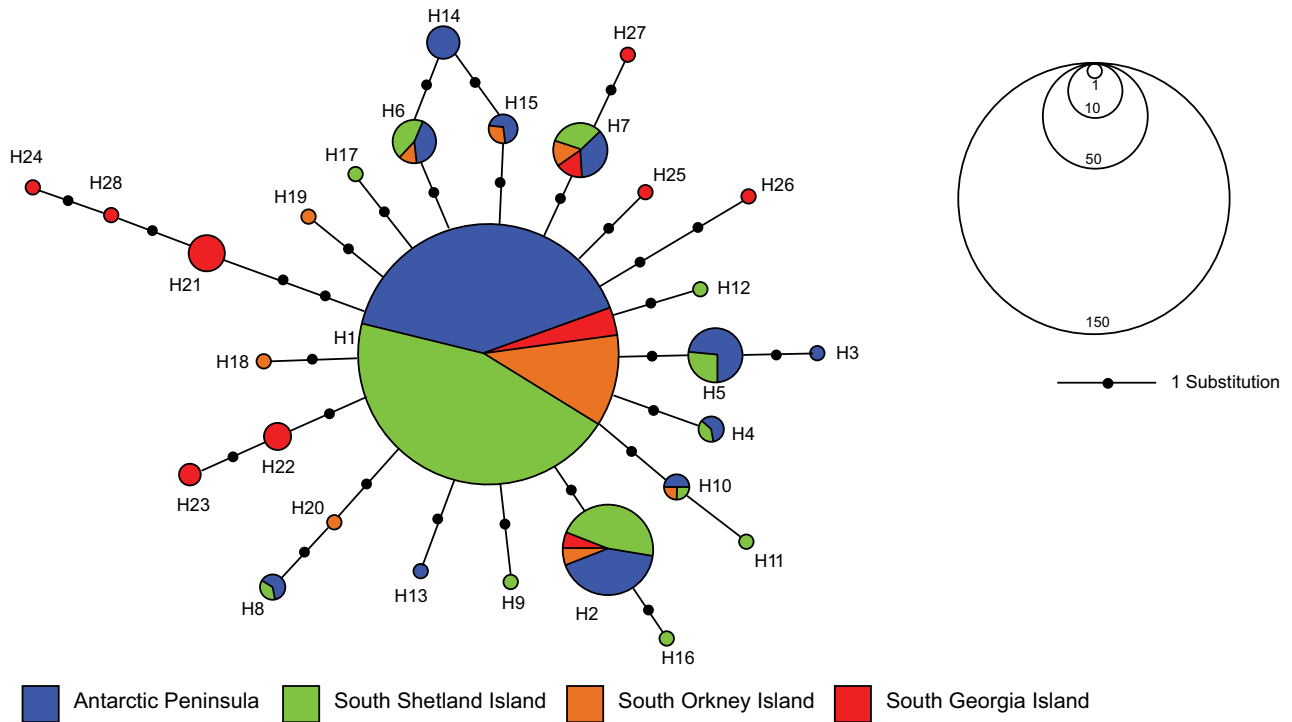
Fig. 3 General median joining haplotype network including 269 *Nacella concinna* mtDNA COI sequences. Each haplotype is represented by a colored circle indicating the main area where it was collected; the size of the circle is proportional to its frequency in the whole sampling effort. mv = median vector (theoretical haplotype that has not been collected but should exist).

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 (trmca) 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 (trmca) occurring about 24 ka against about 6.5 ka determined for maritime Antarctica (vvFig. 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 Allcock & 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
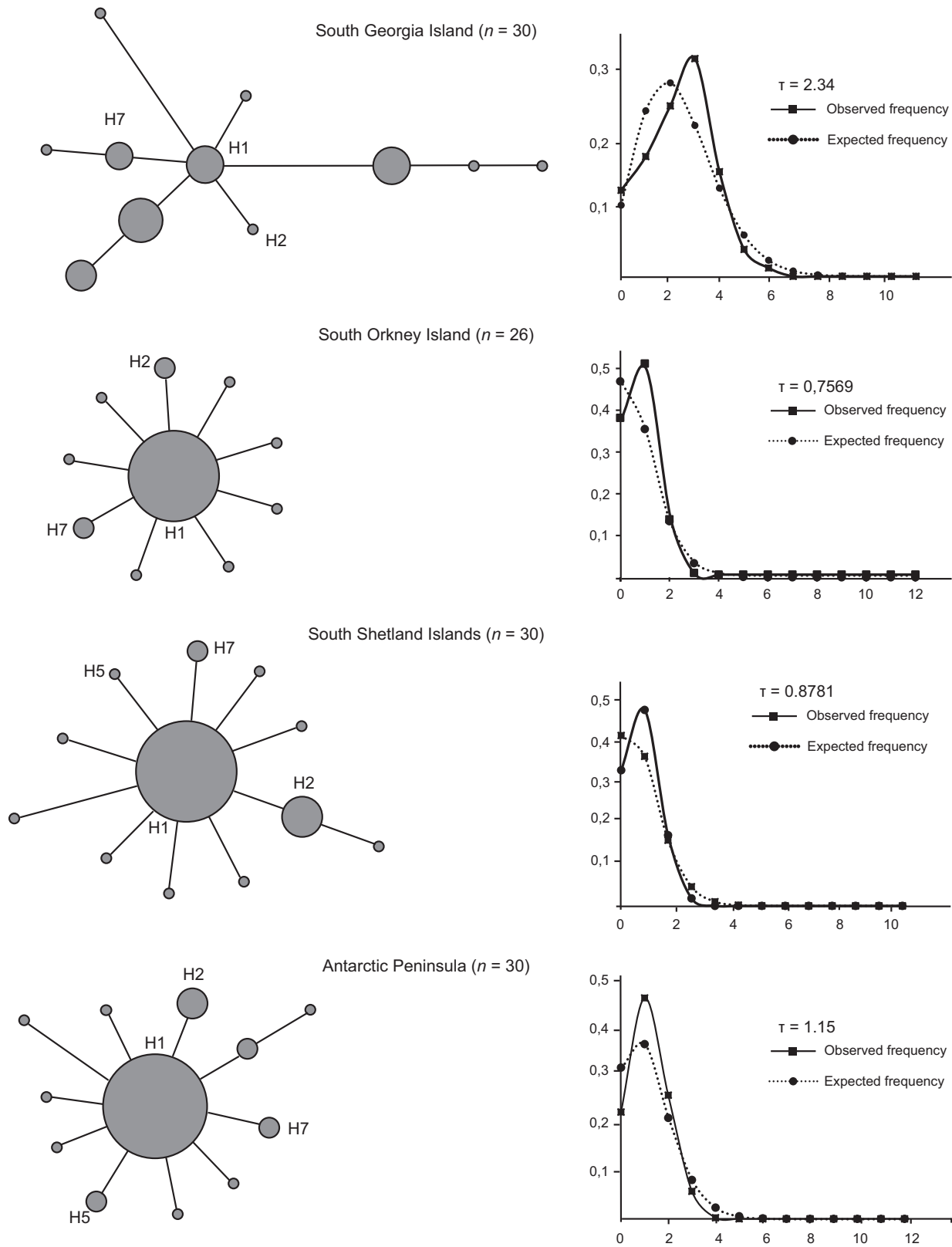
Fig. 4 Median joining haplotype networks and the distribution of pairwise differences between haplotypes (mismatch distribution) analyses for each area included in the analyses. A similar number (between 26 and 30) of randomly selected individuals per area where included in these analyses.
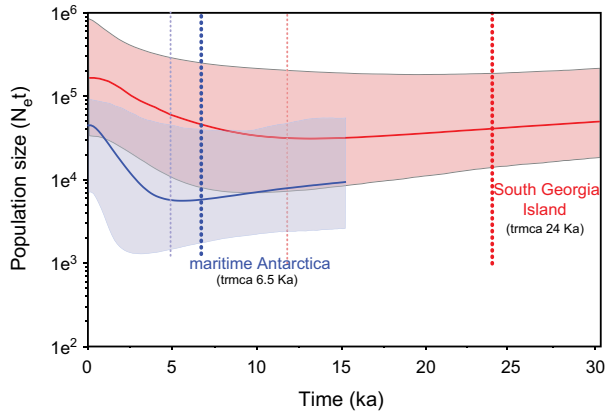
Fig. 5 Historical demographic trends of the effective population size (Ne) constructed using a Bayesian skyline plot approach based on Cytochrome oxidase subunit I (COI) haplotypes of *Nacella concinna*. The *y*-axis is the product of effective population size (Ne) and generation length in a log scale while the *x*-axis is the time in 10^3 before present. The median estimate (blue solid line for maritime Antarctica and red solid line for South Georgia) and 95% highest probability density (HPD) limits (blue for maritime Antarctica and red for South Georgia) are shown. The thick dashed line represents the time of the most recent ancestor (trcma) and the thin dashed line represents time for the expansion in the species.

et al. 2008, 2009; Mortimer *et al.* 2011). In the marine realm, several endemic Antarctic clades have undergone considerable speciation over the last 30 Ma (Pearse *et al.* 2009). High levels of endemism in several Antarctic and sub-Antarctic marine groups (Linse *et al.* 2006; Rogers 2007; Griffiths *et al.* 2009) are consistent with a scenario of long *in situ* evolution over complete eradication during the LGM. In the present study, new genetic data from the Antarctic limpet *N. concinna* challenge these proposed scenarios.

Pattern of genetic diversity in the Antarctic limpet Nacella concinna

Populations of *N. concinna* show overall low levels of nucleotide polymorphism in comparison with temperate patellogastropods (Nakano & Ozawa 2005) and to its Patagonian relatives (de Aranzamendi *et al.* 2011; González-Wevar *et al.* 2011b, 2012a). This can be explained by the recent history of the Antarctic climate and the eradication of shallow marine species from Antarctica due to glaciations and subsequent increased ice disturbance in coastal environments. Such patterns of genetic diversity have been found in other Antarctic invertebrates including the nemertean *Parbolasia corrugatus* (Thornhill *et al.* 2008), the malacostracan *Chorismus antarcticus* (Raupach *et al.* 2010) and the echinoid *Sterechinus neumayeri* (Díaz *et al.* 2011) showing the strong

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 star-like 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, selection 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 nucDNA (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 *Gobbionotothen 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 *Astrotoma agassizii* (Hunter & Halanych 2008), the crinoid *Promachocrinus kerguelensis* (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 (Clapperton *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 (Sugden *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 (Sugden *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.

References

Aljanabi S, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, **25**, 4692–4693.

Allcock AL, Strugnell JM (2012) Southern Ocean diversity: new paradigms from molecular ecology. *Trends in Ecology & Evolution*, **27**, 520–528.

Allcock A, Barratt I, Eléaume M *et al*. (2011) Cryptic speciation and the circumpolarity debate: a case study on endemic Southern Ocean octopuses using the COI barcode of life. *Deep-Sea Research Part II, Topical Studies in Oceanography*, **58**, 242–249.

Anderson J, Shipp S, Lowe A, Smith Wellner J, Mosola AB (2002) The Antarctic Ice sheet during the Last Glacial Maximum and its subsequent retreat history: a review. *Quaternary Science Reviews*, **21**, 49–70.

Anderson CD, Epperson BK, Fortin M-J *et al*. (2010) Considering spatial and temporal scale in landscape-genetic studies of gene flow. *Molecular Ecology*, **19**, 3565–3575.

Arango C, Soler-Membrives A, Miller K (2011) Genetic differentiation in the circum-Antarctic sea spider *Nymphon australe* (Pycnogonida; Nymphonidae). *Deep-Sea Research Part II, Topical Studies in Oceanography*, **58**, 212–219.

de Aranzamendi M, Bastidas R, Gardenal C (2011) Different evolutionary histories in two sympatric limpets of the genus *Nacella* (Patellogastropoda) in the South-western Atlantic coast. *Marine Biology*, **158**, 2405–2418.

Aronson R, Blake D (2001) Global climate and the origin of modern benthic communities in Antarctica. *American Zoologist*, **41**, 27–39.

Aronson R, Thatje S, Clarke A *et al*. (2007) Climate change and invasibility of the Antarctic benthos. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 129–154.

Aronson RB, Thatje S, McClintock JB, Hughes KA (2011) Anthropogenic impacts on marine ecosystems in Antarctica. *Annals of the New York Academy of Sciences*, **1223**, 82–107.

Baird H, Miller K, Stark J (2012) Genetic Population Structure in the Antarctic Benthos: insights from the Widespread Amphipod, *Orchomenella franklini*. *PLoS ONE*, **7**, e34363.

Barnes D, Kuklinski P (2010) Bryozoans of the Weddell Sea continental shelf, slope and abyss: did marine life colonize the Antarctic shelf from deep water, outlying islands or in situ refugia following glaciations? *Journal of Biogeography*, **37**, 1648–1656.

Barnes D, Linse K, Waller C *et al*. (2006) Shallow benthic fauna communities of South Georgia Island. *Polar Biology*, **29**, 223–228.

Beaumont A, Wei J (1991) Morphological and genetic variation in the Antarctic limpet *Nacella concinna* (Strebel, 1908). *The Journal of Molluscan Studies*, **57**, 443–450.

Beaumont MA, Zhang W, Balding DJ (2002) Approximate Bayesian computation in population genetics. *Genetics*, **162**, 2025–2035.

Bentley M, Fogwill C, Kubik P, Sugden D (2006) Geomorphological evidence and cosmogenic 10Be/26Al exposure ages for the Last Glacial Maximum and deglaciation of the Antarctic Peninsula Ice Sheet. *Geological Society of America Bulletin*, **118**, 1149–1159.

Bentley M, Evans D, Fogwill C, Hansom J, Sugden D, Kubik P (2007) Glacial geomorphology and chronology of deglaciation, South Georgia, sub-Antarctic. *Quaternary Science Reviews*, **26**, 644–677.

Bölter M, Beyer L, Stonehouse B (2002) Antarctic Coastal landscapes: characteristics, ecology and research. In: *Ecological Studies vol. 154: Geoecology of Antarctic Ice-Free Coastal Landscapes* (eds Beyer L, Bölter M), pp. 5–9. Springer-Verlag Berlin, Heidelberg.

Bortolotto E, Bucklin A, Mezzavilla M, Zane L, Patarnello T (2011) Gone with the currents: lack of genetic differentiation at the circum-continental scale in the Antarctic krill *Euphausia superba*. *BMC Genetics*, **12**, 32.

Bowden D, Clarke A, Peck L, Barnes D (2006) Antarctic sessile marine benthos: colonisation and growth on artificial substrata over three years. *Marine Ecology Progress Series*, **316**, 1–16.

Brandt A, Linse K, Schüller M (2009) Bathymetric distribution patterns of Southern Ocean macrofaunal taxa: Bivalvia, Gastropoda, Isopoda and Polychaeta. *Deep Sea Research Part I: Oceanographic Research Papers*, **56**, 2013–2025.

Brêthes J, Ferreyra G, de la Vega S (1994) Distribution, growth and reproduction of the limpet *Nacella* (*Patinigera*) *concinna* (Strebel 1908) in relation to potential food availability, in Esperanza Bay (Antarctic Peninsula). *Polar Biology*, **14**, 161–170.

Brey T, Dahm C, Gorny M, Klages M, Stiller M, Arntz W (1996) Do Antarctic benthic invertebrates show an extended level of eurhybathy. *Antarctic Science*, **8**, 3–6.

Ceballos S, Lessa E, Victorio M, Fernández D (2011) Phylogeography of the sub-Antarctic notothenioid fish *Eleginops maclovinus*: evidence of population expansion. *Marine Biology*, **159**, 499–505.

Chown SL, Lee JE, Hughes KA *et al*. (2012) Challenges to the future conservation of the Antarctic. *Science*, **337**, 158–159.

Clapperton CM, Sugden DE, Birnie JF, Wilson MJ (1989) Late-Glacial and Holocene glacier fluctuations and environmental change in South Georgia, Southern Ocean. *Quaternary Research*, **31**, 210–228.

Clarke A, Crame JA (1989) The origin of the Southern Ocean marine fauna. *Geological Society, London, Special Publications*, **47**, 253–268.

Clarke A, Crame JA (1992) The Southern Ocean benthic fauna and climate change: a historical perspective. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **338**, 299–309.

Clarke A, Crame J (2010) Evolutionary dynamics at high latitudes: speciation and extinction in polar marine faunas. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **365**, 3655–3666.

Convey P, Gibson J, Hillenbrand C *et al*. (2008) Antarctic terrestrial life – challenging the history of the frozen continente. *Biological Reviews*, **83**, 103–117.

Convey P, Stevens M, Hodgson D *et al*. (2009) Exploring biological constraints on the glacial history of Antarctica. *Quaternary Science Reviews*, **28**, 3035–3048.

Cornuet J-M, Santos F, Beaumont MA *et al*. (2008) Inferring population history with DIY ABC a user-friendly approach to approximate Bayesian computation. *Bioinformatics*, **24**, 2713–2719.

Cornuet JM, Ravigne V, Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). *BMC Bioinformatics*, **11**, 401.

Dambach J, Thatje S, Rödder D, Basher Z, Raupach M (2012) Effects of late-Cenozoic Glaciation on habitat availability in Antarctic Benthic Shrimps (Crustacea: Decapoda: Caridea). *PLoS ONE*, **7**, e46283.

Díaz A, Féral J, David B, Saucède T, Poulin E (2011) Evolutionary pathways among shallow and deep-sea echinoids of the genus *Sterechinus* in the Southern Ocean. *Deep-Sea Research Part II, Topical Studies in Oceanography*, **58**, 205–211.

Drummond A, Rambaut A (2007) BEAST: Bayesian Evolutionary Analysis by Sampling Trees. *BMC Evolutionary Biology*, **7**, 214.

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of population. *Molecular Ecology*, **11**, 2571–2581.

Epperson BK (2010) Spatial correlation at different spatial scales are themselves highly correlated in isolation by distance processes. *Molecular Ecology Resources*, **10**, 845–853.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 37–50.

Filatov DA (2002) PROSEQ: a software for preparation and evolutionary analysis of DNA sequence data sets. *Molecular Ecology Notes*, **2**, 621–624.

Flight P, O'Brien M, Schmidt P, Rand D (2012) Genetic structure and the North American Postglacial Expansion of the Barnacle, *Semibalanus balanoides*. *Journal of Heredity*, **103**, 153–165.

Gersonde R, Crosta X, Abelmann A, Armand L (2005) Sea-surface temperature and sea ice distribution of the Southern Ocean at the EPILOG Last Glacial Maximum: a circum-Antarctic view based on siliceous microfossil records. *Quaternary Science Reviews*, **24**, 869–896.

González-Wevar C, Nakano T, Cañete J, Poulin E (2010) Molecular phylogeny and historical biogeography of *Nacella* (Patellogastropoda: Nacellidae) in the Southern Ocean. *Molecular Phylogenetics and Evolution*, **56**, 115–124.

González-Wevar C, David B, Poulin E (2011a) Phylogeography and demographic inference in *Nacella* (*Patinigera*) *concinna* (Strebel, 1908) in the western Antarctic Peninsula. *Deep-Sea Research Part II, Topical Studies in Oceanography*, **58**, 220–229.

González-Wevar C, Nakano T, Cañete J, Poulin E (2011b) Concerted genetic, morphological and ecological diversification in *Nacella* limpets in the Magellanic Province. *Molecular Ecology*, **20**, 1936–1951.

González-Wevar C, Hüne M, Cañete J, Mansilla A, Nakano T, Poulin E (2012a) Towards a model of postglacial biogeography in shallow marine species along the Patagonian Province: lessons from the limpet *Nacella magellanica* (Gmelin, 1791). *BMC Evolutionary Biology*, **12**, 139.

González-Wevar CA, Díaz A, Gérard K, Poulin E (2012b) Divergence time estimations and contrasting patterns of genetic diversity between Antarctic and southern South America benthic invertebrates. *Revista Chilena de Historia Natural*, **85**, 445–456.

Griffiths H, Barnes D, Linse K (2009) Towards a generalized biogeography of the Southern Ocean benthos. *Journal of Biogeography*, **36**, 162–177.

Guillot G, Mortier F, Estoup A (2005) Geneland: a computer package for landscape genetics. *Molecular Ecology Notes*, **5**, 712–715.

Gutt J (2001) On the direct impact of ice on marine benthic communities, a review. *Polar Biology*, **24**, 553–564.

Gutt J, Starmans A, Dieckman G (1996) Impact of iceberg scouring on polar benthic habitats. *Marine Ecology Progress Series*, **137**, 311–316.

Hall BL (2003) An overview of Late Pleistocene glaciation in the South Shetland Islands. *Antarctic Research Series*, **79**, 103–113.

Hemery L, Eléaume M, Roussel V *et al.* (2012) Comprehensive sampling reveals circumpolarity and sympatry in seven mitochondrial lineages of the Southern Ocean crinoid species *Promachocrinus kerguelensis* (Echinodermata). *Molecular Ecology*, **21**, 2502–2518.

Herron MJ, Anderson JB (1990) Late Quaternary glacial history of the South Orkney Plateau, Antarctica. *Quaternary Research*, **33**, 265–275.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hewitt G (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **359**, 183–195.

Ho S, Phillips M, Cooper A, Drummond A (2005) Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular Biology and Evolution*, **22**, 1561–1568.

Ho S, Shapiro B, Phillips M, Cooper A, Drummond A (2007) Evidence for time dependency of molecular rates. *Systematic Biology*, **56**, 515–522.

Ho S, Lanfear R, Bromham L *et al.* (2011) Time-dependent rates of molecular evolution. *Molecular Ecology*, **20**, 3087–3101.

Hoffman J, Peck L, Hillyard G, Zieritz A, Clark M (2010a) No evidence for genetic differentiation between Antarctic limpet *Nacella concinna* morphotypes. *Marine Biology*, **157**, 765–778.

Hoffman J, Clarke A, Linse K, Peck L (2010b) Effects of brooding and broadcasting reproductive modes on the population genetic structure of two Antarctic gastropod molluscs. *Marine Biology*, **158**, 287–296.

Hoffman J, Peck L, Linse K, Clarke A (2011) Strong population genetic structure in a broadcast-spawning Antarctic marine invertebrate. *Journal of Heredity*, **102**, 55–66.

Hofmann EE, Klinck JM, Lascara CM, Smith DA (1996) Water mass distribution and circulation west of the Antarctic Peninsula and including Bransfield Strait. In: *Foundations for Ecological Research West of the Antarctic Peninsula, Antarctic Research Series vol. 70* (eds Hofmann EE, Ross RM, Quetin LB), pp. 61–80. AGU, Washington, District of Columbia.

Hunter R, Halanych K (2008) Evaluating connectivity in the brooding brittle star *Astrotoma agassizii* across the Drake Passage in the Southern Ocean. *Journal of Heredity*, **99**, 137–148.

Ihaka R, Gentleman R (1996) R: a language for data analysis and Graphics. *Journal of Computational and Graphical Statistics*, **5**, 299–314.

Janosik A, Mahon A, Halanych K (2011) Evolutionary history of Southern Ocean *Odontaster* sea star species (Odontasteridae; Asteroidea). *Polar Biology*, **34**, 575–586.

Knox GA, Lowry JK (1977) A comparison between the benthos of the Southern Ocean and the North Polar Ocean with special reference to the Amphipoda and the Polychaeta. In: *Polar oceans* (ed Dunbar MJ), pp. 423–462. Arctic Institute of North America, Calgary.

Krabbe K, Leese F, Mayer C, Tollrian R, Held C (2009) Cryptic mitochondrial lineages in the widespread pycnogonid *Colossendeis megalonyx* Hoek, 1881 from Antarctic and Subantarctic waters. *Polar Biology*, **33**, 281–292.

Kumar S, Nei M, Dudley J, Tamura K (2008) MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics*, **9**, 299–306.

Lesbarrères D (2009) Post-glacial phylogeography: new insight into an old story: the post-glacial recolonization of European biota. *Heredity*, **102**, 213.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 451–452.

Linse K, Griffiths HJ, Barnes DKA, Clarke A (2006) Biodiversity and biogeography of Antarctic and sub-Antarctic mollusca. *Deep Sea Research II: Topical Studies in Oceanography*, **53**, 985–1008.

Lomolino MV, Riddle BR, Brown JH (2006) *Biogeography*, 3rd edn. Sinauer Associates, Inc., Sunderland, MA.

Macaya E, Zuccarello G (2010) Genetic structure of the giant kelp *Macrocystis pyrifera* along the southeastern Pacific. *Marine Ecology Progress Series*, **420**, 103–112.

Maggs C, Castihlo R, Foltz D *et al.* (2008) Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. *Ecology*, **89**, S108–S122.

Marko P (2004) 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. *Molecular Ecology*, **13**, 597–611.

Marko P, Hoffman J, Emme S, McGovern T, Keever C, Cox N (2010) The "Expansion-Contraction" model of Pleistocene biogeography: rocky shores suffer a sea change. *Molecular Ecology*, **19**, 146–169.

Martinson DG, Stammerjohn SE, Iannuzzi RA, Smith RC, Vernet M (2008) Western Antarctic Peninsula physical oceanography and spatio-temporal variability. *Deep Sea Research II: Topical Studies in Oceanography*, **55**, 1964–1987.

Matschiner M, Hanel R, Salzburger W (2009) Gene flow by larval dispersal in the Antarctic notothenioid fish *Gobionotothen gibberifrons*. *Molecular Ecology*, **18**, 2574–2587.

Moffat C, Beardsley R, Owens B, van Lipzig N (2008) A first description of the Antarctic Peninsula Coastal Current. *Deep Sea Research Part II: Topical Studies in Oceanography*, **55**, 277–293.

Mortimer E, Jansen van Vuuren B, Lee JE, Marshall DJ, Convey P, Chown SL (2011) Mite dispersal among the Southern Ocean Islands and Antarctica before the last glacial maximum. *Proceedings of the Royal Society B: Biological Sciences*, **278**, 1247–1255.

Murphy EJ, Hofmann EE, Watkins JL *et al.* (2013) Comparison of the structure and function of Southern Ocean regional ecosystems: the Antarctic Peninsula and South Georgia. *Journal of Marine Systems*, **109–110**, 22–42.

Nakano T, Ozawa T (2005) Systematic revision of *Patelloida pygmaea* (Dunker, 1860) (Gastropoda: Lottiidae), with a description of a new species. *The Journal of Molluscan Studies*, **71**, 357–370.

Pearse JS, Mooi R, Lockhart SJ, Brandt A (2009) Brooding and species diversity in the Southern Ocean: selection for brooders or speciation within brooding clades? In: *Smithsonian at the Poles: Contributions to International Polar Year Science*(eds Krupnik I, Lang MA, Miller SE), pp. 181–196. Smithsonian Institution Scholarly Press, Washington.

Pearson RG (2006) Climate change and the migration capacity of species. *Trends in Ecology & Evolution*, **21**, 111–113.

Pons O, Petit R (1996) Measuring and testing genetic differentiation with ordered *Versus* unordered alleles. *Genetics*, **144**, 1237–1245.

Provan J, Bennett K (2008) Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology & Evolution*, **23**, 564–571.

Raupach M, Thatje S, Dambach J, Rehm P, Misof B, Leese F (2010) Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes*. *Marine Biology*, **157**, 1783–1797.

Roe AD, Sperling FAH (2007) Patterns of evolution of mitochondrial cytochrome c oxidase I and II DNA and implications for DNA barcoding. *Molecular Phylogenetics and Evolution*, **44**, 325–345.

Rogers A (2007) Evolution and biodiversity of Antarctic organisms: a molecular perspective. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **362**, 2191–2214.

Scheltema R (1986) On dispersal and planktonic larvae of benthic invertebrates an eclectic overview and summary of problems. *Bulletin of Marine Science*, **39**, 290–322.

Shaw P, Arkhipkin A, Al-Khairulla H (2004) Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep-water troughs as barriers to genetic exchange. *Molecular Ecology*, **13**, 3293–3303.

Shevenell A, Kennett J, Lea D (2004) Middle Miocene Southern Ocean Cooling and Antarctic Cryosphere Expansion. *Science*, **305**, 1766–1770.

Slatkin M, Hudson R (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Smith J, Hillenbrand C, Pudsey C, Allen C, Graham A (2010) The presence of polynyas in the Weddell Sea during the Last Glacial Period with implications for the reconstruction of sea-ice limits and ice sheet history. *Earth and Planetary Science Letters*, **296**, 287–298.

Strugnell J, Watts P, Smith P, Allcock A (2012) Persistent genetic signatures of historic climatic events in an Antarctic octopus. *Molecular Ecology*, **21**, 2775–2787.

Suchard M, Weiss R, Sinsheimer J (2001) Bayesian selection of continuous-time markov chain evolutionary models. *Molecular Biology and Evolution*, **18**, 1001–1013.

Sugden D, Bentley M, Cofaigh Ó, C, (2006) Geological and geomorphological insights into Antarctic ice sheet evolution. *Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences*, **364**, 1607–1625.

Thatje S, Hillenbrand C, Larter R (2005) On the origin of Antarctic marine benthic community structure. *Trends in Ecology & Evolution*, **20**, 534–540.

Thatje S, Hillenbrand C, Mackensen A, Larter R (2008) Life hung by a thread: endurance of Antarctic fauna in glacial periods. *Ecology*, **89**, 682–692.

Thompson J, Higgins D, Gibson T (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680.

Thornhill D, Mahon A, Norenburg J, Halanych K (2008) Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nemertea: Lineidae). *Molecular Ecology*, **17**, 5104–5117.

Thorpe S, Murphy E, Watkins J (2007) Circumpolar connections between Antarctic krill (*Euphausia superba* Dana) populations: investigating the roles of ocean and sea ice transport. *Deep Sea Research Part I: Oceanographic Research Papers*, **54**, 792–810.

Tin T, Fleming ZL, Hughes KA *et al.* (2009) Impacts of local human activities on the Antarctic environment. *Antarctic Science*, **21**, 3–33.

Todd CD (1998) Larval supply and recruitment of benthic invertebrates: do larvae always disperse as much as we believe. *Hydrobiologia*, **375**, 1–21.

Verducci M, Foresi L, Scott G, Sprovieri M, Lirer F, Pelosi N (2009) The Middle Miocene climatic transition in the Southern Ocean: evidence of paleoclimatic and hydrographic changes at Kerguelen plateau from planktonic foraminifers and stable isotopes. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **280**, 371–386.

Wilson N, Hunter R, Lockhart S, Halanych K (2007) Multiple lineages and absence of panmixia in the "circumpolar" crinoid *Promachocrinus kerguelensis* from the Atlantic sector of Antarctica. *Marine Biology*, **152**, 895–904.

Wilson N, Schrödl M, Halanych K (2009) Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). *Molecular Ecology*, **18**, 965–984.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations on global climate 65 Ma to present. *Science*, **292**, 686–693.

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.

COI haplotypes sequence alignment: Dryad doi:10.5061/dryad.8, version 3r7.

Supporting information

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

Table S1 Pairwise G_{ST} values, based on haplotypic frequencies, (below diagonal) and average number of nucleotide differences between localities N_{ST} (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.