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Impact of glaciations on genetic diversity of pelagic mollusks: Antarctic *Limacina antarctica* and Arctic *Limacina helicina*

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ABSTRACT: Contemporary genetic constitution of marine species carries signatures of Pliocene-Pleistocene glacial cycles. Molecular studies of polar organisms show that isolation in refugia during glaciation often results in loss of genetic diversity. However, recent studies of marine organisms from the Southern Ocean have highlighted their remarkably high level of infraspecific genetic differentiation and the presence of cryptic species. Thus, demographic responses to climate change vary substantially with geography and life history. To elucidate the relative role of glacial period in driving the evolution of Antarctic and Arctic fauna we examined the genetic diversity and historical demography of the pelagic marine gastropods *Limacina antarctica* from Drake Passage in the Antarctic and *Limacina helicina* from Spitsbergen fjords in the Arctic. Diversity was assessed by comparing nucleotide sequences from part of the mitochondrial gene encoding the cytochrome *c* oxidase subunit I (COI). Sequences from 60 individuals of *L. antarctica* collected at 7 stations along Drake Passage were compared with those of 67 individuals of *L. helicina* from the fjords Hornsund and Isfjorden. We identified 47 different haplotypes for *L. antarctica* and 25 for *L. helicina*. No spatial genetic structure was found in either species, indicating that studied populations in each species belong to a single evolutionary unit. Demographic analyses of haplotype networks and significant negative Tajima's *D* and Fu's F_S indices suggest recent rapid population expansion in both species. However, *L. antarctica* populations displayed a higher level of haplotype and nucleotide diversity than *L. helicina* populations, which suggests that the impact of glaciations was less prominent in *L. antarctica*.

KEY WORDS: Polar regions · Glaciations · *Limacina antarctica* · *Limacina helicina* · Cytochrome *c* oxidase subunit I (COI) · Genetic diversity · Median-joining network · Bottleneck effect

INTRODUCTION

The present genetic structure of many polar species was formed by the Quaternary ice ages; during this period, polar ice sheets expanded considerably and caused species extinctions over large parts of their distribution ranges or displacement to refugial areas (Hewitt 2000). Reductions in the number and size of habitats, as well as decreases in effective population size over several thousands of years caused a loss of genetic diversity. Nonetheless, independent evolution in glacial refugia and secondary contact of differentiated genetic lineages could have increased genetic polymorphism (Wilson et al. 2007). Cryptic species or restricted gene flow have been demonstrated in a variety of taxa e.g. the brittle star *Astrotoma agassizii* (Hunter & Halanych 2008), the crinoid *Promachocrinus kerguelensis* (Wilson et al. 2007), and the sea slug *Doris kerguelensis* (Wilson et al. 2009). The fragmentation of ranges and subsequent recolonization is thought to have been an important mechanism of allopatric speciation in Antarctic taxa,

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and is termed the 'diversity pump' (Clarke et al. 1992).

The study of biological diversity in Antarctic fauna is especially interesting, not only because of the extreme environmental conditions they face, but also due to the unique geological history and prolonged isolation of this habitat. This region has been isolated for millions of years by geographic distance, strong circumpolar currents and specific environmental conditions (Clarke 2000). Sea-water cooling and the destruction of habitats, driven by variability in ice sheet extent, led to a drastic reduction in the number of species in several key groups of animals, such as brachyuran crabs, lobsters and teleost fishes. The current biodiversity in Antarctica is the result of different evolutionary and biogeographical processes occurring since the Mesozoic (Scher & Martin 2006). On the other hand, many other groups like sponges, sea spiders, echinoderms and amphipods are abundant and diverse, indicating that isolation and climatic change have not restricted their success (Boltovskoy et al. 2005). During the Cenozoic glaciations, the advance and retreat of the ice sheet promoted speciation by separating populations for long periods of time. Many species are found only in Southern Ocean waters, where endemism may reach over 90% (Boltovskoy et al. 2005).

Antarctica is currently experiencing one of the faster rates of regional climate change. Over the past half century, the mean annual air temperature on the Antarctic Peninsula has increased by 3°C (King et al. 2013). This has caused the retreat of the ice sheet and the exposure of new habitats. Many aspects of the biogeography, phylogeny and genetic diversity of organisms from this region remain unexplained. To better understand patterns of Antarctic biodiversity and the factors influencing population connectivity, it is essential to collect genetic data from a variety of taxa inhabiting this polar region.

Glaciations have also significantly influenced the genetic structure of northern polar species. Comparative analysis of phylogeographic data for 42 North American fish species clearly demonstrated latitudinal shifts in intraspecific genetic diversity. Relative nucleotide diversity showed significant breakpoints matching the southern limit of the glaciations (Bernatchez & Wilson 1998). In the case of the red king crab *Paralithodes camtschaticus*, low heterozygosity values and a lack of differentiation among populations has been connected with ice age population bottlenecks (Grant et al. 2011). The absence of migration-drift equilibrium after post-glacial expansion has also been described in species with high levels of gene flow, such as the Pacific cod *Gadus macrocephalus* (Canino et al. 2010).

Suitable study systems are necessary to elucidate the relative roles of unique processes and environmental factors in driving the evolution of Antarctic and Arctic fauna. The genus *Limacina* has representatives in both polar regions: *Limacina helicina* in the Arctic and *Limacina antarctica* in the Antarctic, and is therefore ideal for this purpose.

Members of the genus *Limacina* represent a major component of polar zooplankton. They can constitute >50% of total zooplankton abundance and play a significant ecological role, both as phytoplankton grazers and as prey for larger zooplankton species and for pelagic and demersal fish (Hunt et al. 2008). *Limacina* spp. belong to the group of aragonite-shelled (thecosome) pteropods, which contribute substantially to carbonate and organic carbon fluxes through the production of fast sinking aragonite shells, fecal pellets and mucus flocs (Hunt et al. 2008). Their aragonite shell makes pteropods particularly sensitive to ocean acidification (Comeau et al. 2010). Other invertebrates show significant variation in their response to acidification, not only between species, but also between populations of the same species (Fabry et al. 2008). Consequently, populations from different regions are not necessarily equally sensitive to changing environmental conditions. This highlights the importance of knowledge on the population genetic structure of these organisms.

Despite performing important functions in polar ecosystems, little is known about the Pteropoda as a group, particularly their genetics. *L. helicina* has been recognized as a species complex comprising 2 sub-species: *Limacina helicina helicina* in the Arctic and *Limacina helicina antarctica* in the Antarctic. However, Hunt et al. (2010) found 33.56% difference at the cytochrome *c* oxidase subunit I (COI) locus and suggested at least species level separation. Hunt et al. (2010) concluded that species divergence time, estimated at 31 million years ago, indicated independent evolution since the early Oligocene. For these reasons separate names are used: *Limacina helicina* for species from the Arctic and *Limacina antarctica* for species from the Antarctic. Jennings et al. (2010) presented a molecular dataset of holoplanktonic gastropods, which indicated significant genetic variation among conspecific specimens of some pteropod species from different geographic regions. Nevertheless, there remains a dearth of significant research on the population genetics of these organisms.

In this study, we analyzed patterns of haplotypic diversity in *Limacina helicina* and *L. antarctica*, with special emphasis on the influence of historical processes on their genetic diversity and evolutionary history. This analysis was based on the examination of DNA sequences from the COI gene. This marker was chosen because its widespread use permitted comparison with many other species. Furthermore, mitochondrial genetic markers, especially COI, provide insights into the demographic history of populations (Wilson et al. 2007, González-Wevar et al. 2011).

MATERIAL AND METHODS

Sampling, DNA extraction, PCR amplification and sequence alignments

Limacina antarctica was collected from 7 different stations along Drake Passage during an expedition on the R/V Akademik Ioffe in 2010. *Limacina helicina* was collected from Spitsbergen (Arctic) during expeditions on the R/V Oceania in 2010 (Hornsund fjord) and in 2011 (Isfjorden fjord). Collections were made using plankton nets and samples were fixed in 96% ethanol. Detailed information on sampling is given in Table 1 and Fig. 1.

Genomic DNA was extracted from up to 15 mm^3 of tissue per individual, using a QIAGEN DNeasy Tissue Kit, in accordance with manufacturer's instructions. A 660 bp fragment of the COI gene was amplified by PCR using the universal primers described by Folmer et al. (1994). Reaction mixtures (50 µl) had the following composition: 1× GoTaq Flexi buffer (Promega), 2.5 mM $MgCl_2$,, 2 pmol dNTPs, 0.4 µl of each primer, 1 U of Taq polymerase (Promega) and approximately 50 ng of extracted genomic DNA as template (substituted by water for negative controls). Amplification was achieved using the following program: initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 45 s, extension at 72°C for 1 min; plus a final extension at 72°C for 5 min. PCR products were analyzed by electrophoresis on 2.5% TBE agarose gels. The amplicons were purified using a QIAGEN QIAquick PCR Purification Kit and then bidirectionally sequenced with an ABI PRISM 3500 sequencer (DNA Research Centre, Poznań) using the PCR primers. Sequences were assembled in DNA Baser v.3 (Heracle BioSoft) and manually edited, then exported into MEGA 5.05 (Tamura et al. 2007). Following manual alignment, the COI sequences were translated to check for premature stop codons or frameshifts indicative of sequencing errors. Sequences were trimmed to 559 bp for subsequent analysis.

Table 1. Location, sampling dates and number of individuals collected during Antarctic and Arctic expeditions

Collection station	Collection date (mo/yr)	Latitude	Longitude	Number of individuals
Antarctic				
Drake Passage 1	Jan 10	62° 52′ S	62° 41′ W	12
Drake Passage 2	Jan 10	62° 33′ S	63° 29′ W	5
Drake Passage 3	Jan 10	61° 39′ S	64° 01′ W	5
Drake Passage 4	Jan 10	61° 00′ S	64° 16′ W	5
Drake Passage 5	Jan 10	60° 50′ S	64° 19′ W	16
Drake Passage 6	Jan 10	60° 20′ S	64° 30′ W	9
Drake Passage 7	Jan 10	59° 21′ S	64° 51′ W	8
Arctic				
Hornsund	Jul 10	77° 02′ N	13° 34′ E	14
Isfjorden 1	Jul 11	78° 39′ N	16° 36′ E	21
Isfjorden 2	Jul 11	78° 33′ N	16° 32′ E	18
Isfjorden 3	Jul 11	78° 31′ N	16° 23′ E	14

Population genetic structure and historical inference

The level of genetic polymorphism in *L. antarctica* and *L. helicina* was determined using DnaSP 5 software (Librado & Rozas 2009), by standard diversity indices, such as the number of haplotypes (k), number of segregating sites (S), haplotype diversity (H), sequence differences (Π), and nucleotide diversity (π). Genetic differentiation between populations was estimated by pairwise Φ_{ST} using Arlequin 3.5.1.2 (Excoffier & Lischer 2010); this parameter is analogous to F_{ST} and represents the correlation between alleles within individuals, relative to the combined population. Differentiation indices Φ_{ST} were tested through haplotype permutation implemented in Arlequin 3.5.1.2.

Genealogical relationships among *Limacina* individuals were determined from haplotype networks constructed using the median-joining algorithm in Network 4.6.1.0 (Bandelt et al. 1999). The network was optimized using the maximum parsimony criterion. To determine past demographic changes, Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) tests were performed using Arlequin 3.5.1.2. Significant negative values for these tests are evidence of an excess of low frequency haplotypes relative to neutral mutation-drift equilibrium, indicating either recent demo-

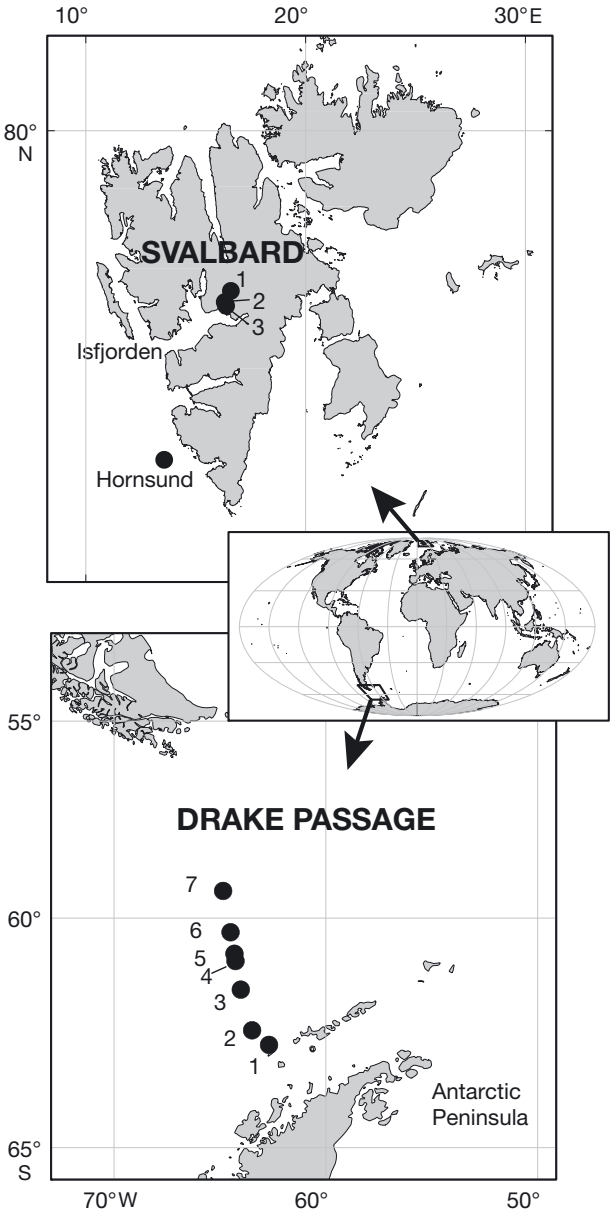

Fig. 1. Sampling locations for *Limacina helicina* and *Limacina antarctica* (see Table 1 for collection station names and coordinates)

graphic expansion or positive selection. Significance for these tests was obtained by simulating 10 000 samples in accordance with the assumptions of selective neutrality and population equilibrium, using a coalescent approach. We also calculated mismatch distribution of pairwise differences between the specimens using Arlequin 3.5.1.2. For each species, the shape of the distribution was compared with the distribution of a simulated data set under a sudden population expansion model. The fit between the observed and estimated distribution under this model was subjected to tests for goodness-of-fit: standardized squared differences (SSD) and raggedness index tests. These tests provide information on how well the simulated model of population expansion fits the observed mismatch distribution. Parameters Tau (τ), Theta 0, and Theta 1 were inferred from the model of demographic expansion in Arlequin 3.5.1.2.

The approximate date of population expansion was estimated using the formula $T = \tau/2u$ (Rogers & Harpending 1992), where T is time since expansion, τ is calculated from the mismatch distribution, and 2u equals the mutation rate × generation time × number of bases sequenced. The 2.4 % per 1 Myr COI molecular clock for gastropods was applied (Hellberg & Vacquier 1999). Ho et al. (2005) showed that rates of microevolutionary change (measured between successive generations) could be 10-fold higher than rates of macroevolutionary change (inferred from the fossil records). Thus, we also showed results with the simple 10-fold correction of mutation rate as done by González-Wevar et al. (2011).

The demographic histories of *L. helicina* and *L. antarctica* were additionally inferred from Bayesian skyline analyses implemented in BEAST v. 2.1.3 (Drummond et al. 2005). Bayesian skyline analyses were run under the GTR+I model of nucleotide substitution that best fit to our data using Akaike Information Criterion, as indicated by the analysis with jModelTest v. 2.1.3 (Darriba et al. 2012). We employed the 2.4 % per 1 Myr molecular clock, 10 million iterations of the Markov Chain Monte Carlo (MCMC) chains and sampled every 1000 iterations. The Bayesian skyline plots were generated with the program Tracer v. 1.6 (Drummond et al. 2005).

RESULTS

COI gene sequences were obtained from 60 individuals of *Limacina antarctica* and 67 individuals of *L. helicina*, and comprised 559 nucleotide positions. As expected for coding regions, no indels or stop codons were found. No amino acid substitutions were detected within species.

Limacina antarctica

Variability of the COI gene fragment

From the total number of nucleotide sites considered, 57 (10.2 %) were variable and 21 of them (3.8 %) were parsimoniously informative. Overall haplotype

diversity H was 0.967, while nucleotide diversity π was 0.0061 (Table 2).

Application of the median-joining algorithm resulted in a star-like haplotype network for *L. antarctica* (Fig. 2). The central haplotype (A1) was the most frequent (18.3%) and occurred at 4 out of 7 sampling locations in Drake Passage. Most of the numerous low frequency haplotypes had a branch length of 2 or more mutational steps from A1. Haplotypes A2 and A3 exhibited respective frequencies of 5 and 3.3%, while the remaining 44 haplotypes occurred in only one specimen each (singletons). Pairwise Φ_{ST} comparisons between collection stations showed no significant differences at $p < 0.05$, except for the comparison between Stns 4 and 6 ($p = 0.041$) (Table A1 in the Appendix). General Φ_{ST} comparisons indicated the absence of genetic structure in *L. antarctica* along the 400 km course of Drake Passage.

Population history based on COI

For analysis of the population history of *L. antarctica*, sequences of all specimens were pooled into a single group. Tajima's D and Fu's F_S neutrality tests were significantly negative for the population as a whole, indicating an excess of low frequency polymorphism, which is contrary to the expected value under a neutral model of evolution (Table 2). The model of demographic expansion could not be rejected at the 5% confidence level. Molecular clock estimates dated the onset of the expansion at 260 000 yr BP (CI = 192 000–303 000 yr BP), while after 10-fold correction of mutation rate at 26 000 yr BP (CI = 19 200–30 300 yr BP). The conclusion that *L. antarctica* has experienced population expansion was also supported by Bayesian skyline analysis (Fig. 3a).

Table 2. Genetic diversity and demographic parameters for the complete COI gene sequence data sets for *Limacina antarctica* (N = 60) and *L. helicina* (N = 67). $^{**}p < 0.01$, $^{***}p < 0.001$, ^{ns} = not significant

	Limacina antarctica	*Limacina helicina*
Diversity indices		
Number of haplotypes (k)	47	25
Polymorphic sites (S)	57	29
Haplotype diversity (H)	0.967 (±0.017)[a]	0.778 (±0.053)[a]
Nucleotide diversity (π)	0.0061 (±0.0005)[a]	0.0031 (±0.0005)[a]
Average number of nucleotide differences (Π)	1.749	3.389
Neutrality tests		
Tajima's D	−2.454***	−2.268**
Fu's F_S	−26.198***	−23.916***
Mismatch analysis		
Tau	3.486 (2.580–4.070)[b]	1.758 (0.586–3.016)[b]
Theta 0	0.009 (0–0.541)[b]	0.011 (0–0.295)[b]
Theta 1	inf (44.469-inf)[b]	8.423 (2.786-inf)[b]
SSD	0.001[ns]	0.001[ns]
Raggedness index	0.025[ns]	0.028[ns]

[a] SD in parentheses, [b] 95% CI in parentheses

Limacina helicina

Variability of the COI gene fragment

A total of 29 variable sites (5.2%) were detected from the analyzed *L. helicina* COI gene sequences, of which, 11 (2%) were parsimoniously informative. Genetic diversity indices, like haplotype diversity, polymorphic sites and nucleotide diversity, were all lower than in *L. antarctica* (Table 2).

Application of the median-joining algorithm for *L. helicina* resulted in a typical star-like haplotype network and short genealogy (Fig. 2). The central haplotype (S1) was the most frequent (46.3%) and the most broadly distributed, occurring at all Arctic sampling locations. S1 represented the ancestral haplotype, which radiated low frequency haplotypes, most of which had a branch length of one mutational step. Five haplotypes: S2, S3, S4, S5 and S6, each located one or 2 mutational steps away from S1, exhibited intermediate frequency (9, 6, 5, 3 and 3%, respectively). The remaining 19 haplotypes occurred in only one specimen each (singletons). Pairwise Φ_{ST} comparisons between collection stations showed no significant differences between *L. helicina* populations in the 2 fjords (Table A2 in the Appendix).

Population history based on COI

Due to the lack of significant genetic differentiations among locations, the sequences of all specimens were pooled into a single group. The results of Tajima's D and Fu's F_S neutrality tests were significantly negative (Table 2). Goodness of fit tests did not reject the model of demographic expansion at the 5% confidence level. The start of the expansion was dated

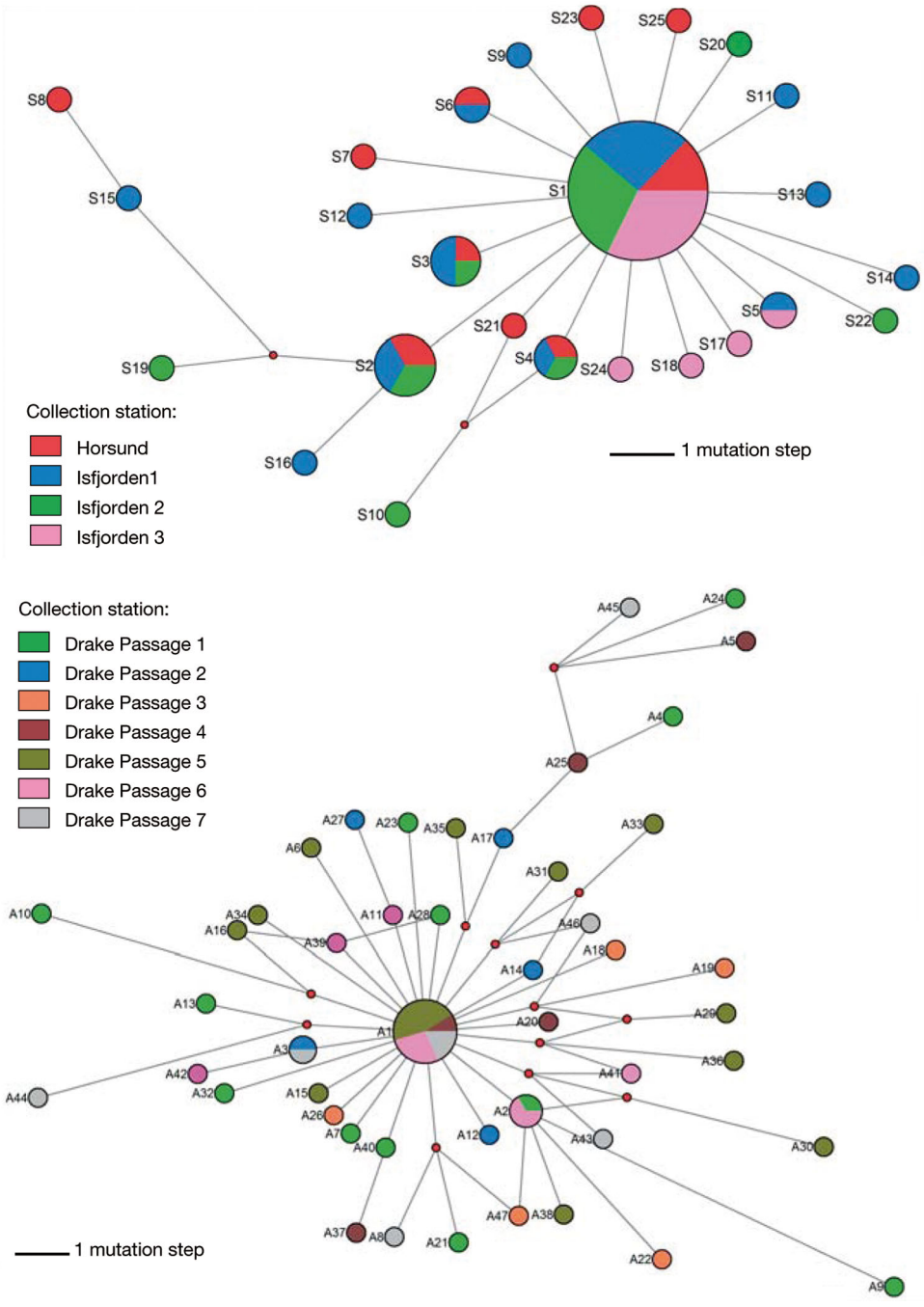

Fig. 2. Median-joining haplotype networks for *Limacina helicina* (top) and *Limacina antarctica* (bottom) based on mitochondrial COI gene sequence data. Each haplotype is depicted by a circle colored according to the station where it was collected. Areas are proportional to haplotype frequency. Median vectors are represented by small red circles

at 131 000 yr BP (CI = 44 000–225 000 BP), while after 10-fold correction of mutation rate at 13 100 yr BP (CI = 4400–22 500 yr BP). Demographic expansion of *L. helicina* was also supported by the Bayesian skyline analysis (Fig. 3b).

DISCUSSION

Various hypotheses have been proposed to describe how the Southern Ocean fauna persisted in face of the disturbance caused by Pliocene-Pleistocene glacial cycles: (1) recolonization of Antarctic waters from sub-Antarctic regions after glacial period (e.g. Dell 1972); (2) recolonization of the Antarctic shelf from deep sea, as many Antarctic organisms can prosper at a wide range of depths (e.g. Brey et al. 1996); (3) fauna could have survived in small-scale refugia at different locations in the Southern Ocean (Anderson et al. 2002); (4) some organisms could have survived by moving from one shelter to another, as ice-free areas of local marine productivity (poly-

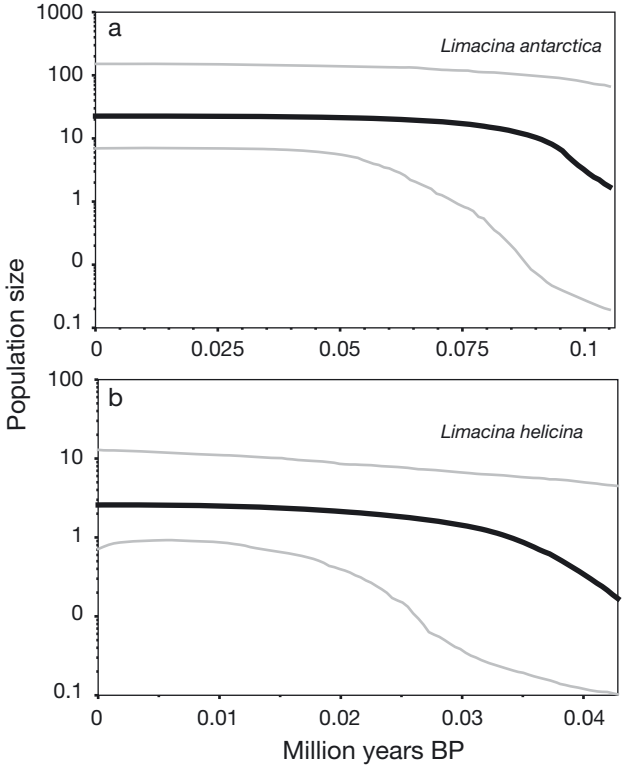

Fig. 3. Bayesian skyline plots showing changes in effective population sizes for (a) *Limacina antarctica* and (b) *Limacina helicina* over time. Population size is given on a logarithmic scale. Outer envelope (grey curves) represents the 95% highest posterior density intervals (95% HPDIs)

nyas) existed during the last glacial (Thatje et al. 2008). Glaciations had consequences such as population bottlenecks followed by rapid population expansions, as described for the Antarctic population of the limpet *Nacella concinna* (González-Wevar et al. 2011), benthic shrimps *Chorismus antarcticus*, and *Nematocarcinus lanceopes* (Raupach et al. 2010), among others. The pattern of genetic diversity that we identified in *L. antarctica* also deviates from that expected for stable populations. A 'star-like' genealogy, the neutrality test results and demography parameters strongly support the existence of a recent demographic expansion. The results of mismatch analysis suggest that *L. antarctica* experienced expansion at 260 000 yr BP (CI = 192 000–303 000 yr BP), which corresponds to the mid Pleistocene. Bayesian skyline plot analysis also indicates a *L. antarctica* population growth event during the Pleistocene, but does not indicate the time of the beginning of expansion. These estimations by far predated the Last Glacial Maximum (LGM). However, the impact of extending ice sheets on pelagic habitat is smaller than on benthic, and the onset of demographic expansion predating LGM has been described for other pelagic organisms (Janko et al. 2007). For example, the krill *Euphausia superba* have experienced population expansion dated to 205 000–304 000 yr BP (Zane et al. 1998). Conversely, Ho et al. (2005) showed that rates of microevolutionary change (measured between successive generations) could be 10-fold higher than rates of macroevolutionary change (inferred from the fossil records). Thus, when we applied a simple 10-fold correction of mutation rate, the expansion time is estimated at 26 000 yr BP (CI = 19 200–30 300 yr BP), which corresponds well to the last glacial-interglacial transition period, after the LGM (~21 000 yr, Huybrechts 2002). The above estimations do not give a clear answer to the question of the exact expansion time and, therefore, the molecular clock estimates should be regarded with caution. However, they all clearly indicate significant influence of glaciations on the population size during the Pleistocene. The recent demographic expansion is also reflected in the absence of genetic structure in *L. antarctica* populations, which is also presently maintained by strong circumpolar currents in the Southern Ocean. The homogenous population of Antarctic species has been demonstrated by several studies. For example, an analysis of mitochondrial DNA and nuclear DNA intron markers revealed no regional differentiation in the Antarctic toothfish *Dissostichus mawsoni* (Smith & Gaffney 2005). Evidence for a single well-mixed lineage in the Antarctic was also found for the nemertean worm *Parborlasia corrugatus* (Thornhill et al. 2008) and 2 benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes* (Raupach et al. 2010). All these species are able to disperse widely, like *Limacina antarctica*, due to presence of pelagic larval stages. Although there was a clear influence of glaciation on demography and genetic geographical structure in *L. antarctica*, the level of nucleotide and haplotype diversity in *L. antarctica* (H = 0.967, π = 0.0061) is higher than those found in other molecular studies of Antarctic invertebrates with pelagic larvae, e.g. nemertea *Parborlasia corrugatus* (H = 0.762, π = 0.00098, Thornhill et al. 2008), *Nacella concinna* (H = 0.630, π = 0.00128, González-Wevar et al. 2011), *Chorismus antarcticus* (H = 0.639, π = 0.00209, Raupach et al. 2010). Nucleotide and haplotype diversity of planktonic *L. antarctica* is more comparable to deep-sea shrimp *Nematocarcinus lanceope* (H = 0.9025, π = 0.00568, Raupach et al. 2010) or pelagic silverfish *Pleuragramma antarcticum* (H = 0.941, π = 0.009069, Zane et al. 2006). It can be argued that the destruc-

tion of benthic habitat by glacial cycles has a less prominent impact on genetic diversity of deep-sea species and pelagic species (Allcock & Strugnell 2012), and for *L. antarctica*, this is probably the case.

Notably, the level of genetic diversity in the mitochondrial COI gene was higher in *L. antarctica* than the Arctic species *L. helicina*; diversity indices were almost 2-fold higher in *L. antarctica*. It is likely that, in the case of *L. antarctica,* larger populations were maintained due to more efficient refuges available during glaciation events. In the case of Arctic *L. helicina,* displacement to small refugial areas by polar ice sheets have led to a more drastic reduction of population size and thus caused significant loss of genetic diversity, as was also shown for several other Arctic species (e.g. Bernatchez & Wilson 1998, Marko 2004, Maggs et al. 2008, Grant et al. 2011). Together, the 'star-like' genealogy and the neutrality test results strongly support the existence of a recent demographic expansion of *L. helicina*. Bayesian skyline plot analysis also indicates that *L. helicina* underwent a population growth event during the Pleistocene and suggests larger reductions in the effective population size of *L. helicina* compared to *L. antarctica*. The results of mismatch analysis indicate that *L. helicina* probably experienced expansion at 131 000 yr BP (CI = 44 000–225 000 yr BP). The average time of expansion estimated for *L. helicina* corresponds to the Eemian interglacial in the Arctic. However, after a correction for short-term mutation rate was applied, time estimation at 13 100 yr BP (CI = 4400–22 500 yr BP) corresponds to the initial deglaciation recognized along the Spitsebergen margin (~14 100 yr, Lloyd et al. 1996). A more recent expansion of *L. helicina* than *L. antarctica,* thus longer time for accumulation of new mutations, could be an additional factor responsible for much higher level of genetic diversity in *L. antarctica*.

On the other hand, we cannot rule out the possibility that more expansive sampling might reveal phylogeographic breaks. Additional samples from other regions (e.g. Ross Sea, Greenland Sea) would be useful to confirm lack of genetic structure of *Limacina* mollusks. It should be also noted that phylogeographic study based on the sole use of mitochondrial DNA reveal only a small part of the evolutionary history. Nuclear markers with a greater resolution on the population level should be employed in further analysis.

In summary, our data provide a first insight into the population genetic structure of 2 highly abundant and important polar species of pelagic mollusks. We observed the signature of population bottlenecks dating to the late Pleistocene. Overall, the effect of climate changes appears less prominent for genetic diversity of the Antarctic than Arctic species. Further work combining geological, paleontological and genetic data on Antarctic and Arctic organisms with different ecological characteristics and dispersal abilities is required to more fully understand these pole-oriented contrasts. In addition, the observed difference in genetic diversity between *L. antarctica* and *L. helicina* may be of interest for researchers examining the influence of ocean acidification on marine organisms and ecosystems, since these species may respond differently to global climatic changes.

Data archive. Sequence data have been submitted to the DNA Data Bank of Japan as accession numbers AB859484-AB859610.

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Appendix. Additional information for pairwise Φ_{ST} comparisons for *Limacina antarctica* and *Limacina helicina* at each collection station

Table A1. Pairwise Φ_{ST} values for *Limacina antarctica*. Significances were tested through 10 100 permutations; bold indicates $p < 0.05$

Collection station	1	2	3	4	5	6	7
1	0.00000						
2	0.04117	0.00000					
3	0.01273	0.00000	0.00000				
4	0.05081	0.01190	0.04762	0.00000			
5	0.00895	0.00392	0.00749	0.06183	0.00000		
6	0.00107	0.00451	0.02528	**0.11934**	0.03978	0.00000	
7	0.03166	0.03424	0.03424	0.00116	0.01821	0.02827	0.00000

Table A2. Pairwise Φ_{ST} values for *Limacina helicina*. Significances were tested through 10 100 permutations; no values were significant at $p < 0.05$

Collection station	Hornsund	Isjorden 1	Isfjorden 2	Isfjorden 3
Hornsund	0.00000			
Isfjorden 1	−0.03163	0.00000		
Isfjorden 2	−0.03250	−0.02724	0.00000	
Isfjorden 3	0.03915	0.01987	0.02620	0.00000

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