Both high and low dispersal? Apparently contradictory genetic patterns in the Antarctic littorinid gastropod *Laevilacunaria antarctica*

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How the near-shore marine benthic communities survived Quaternary glaciations in Antarctica is a major question for Southern Ocean biogeographers. Several hypotheses that consider life-history traits, such as bathymetric ranges and developmental modes, have been proposed. Near-shore species with high dispersive potential are expected to show star-like genealogies, with broadly distributed haplotypes surrounded by closely related variants at low frequencies, a consequence of rapid population post-glacial expansion mediated by larvae. By contrast, shallow-water taxa with low dispersal potential are expected to evidence marked genetic structure or even consist of separate cryptic species, due to their survival in small refugial populations subject to repeated bottlenecks. In this study we performed phylogeographic analyses on the high-latitude littorinid *Laevilacunaria antarctica* across its distribution from maritime Antarctica to South Georgia. This species is a highly abundant near-shore gastropod, commonly found on macroalgae, with low autonomous vagility and exhibits benthic protected development with no free-living dispersive stages. Such characteristics make this species a suitable model to test the above hypotheses. Contrary to low dispersal potential predictions, *L. antarctica* comprises a single unstructured unit across its distribution in maritime Antarctica, exhibiting a typical high-dispersal star-like, short-branched genealogy centered on a dominant broadly distributed haplotype. The South Georgia population, however, consists of a separate genetic unit, strongly differentiated from those of the maritime Antarctic. We estimate that these two genetic groups separated ~1.2 My, long before the Last

Glacial Maximum and evolved independently in these areas. Our results thus provide partial support for the expected pattern for a near-shore marine benthic species with low innate dispersive potential. On a wider geographic scale (maritime Antarctica vs South Georgia), our results in *L. antarctica* provide support for the expected pattern for a near-shore marine benthic species with low innate dispersive potential. However, on a narrower scale (maritime Antarctica) life-history characters including the close association of the species with macroalgae, would allow dispersal and a species with low dispersal potential exhibits paradoxically an absence of genetic structure across hundreds of kilometers probably mediated by rafting.

Introduction

How the shallow benthic marine biota endured Quaternary glaciations, particularly the Last Glacial Maximum (LGM), is a major question in Antarctic biogeography (Barnes et al., 2006; Thatje et al., 2008; Allcock and Strugnell, 2012; Fraser et al., 2014; Riesgo et al., 2015; Chenuil et al., 2018; Crame, 2018; Lau et al., 2020; Baird et al., 2021). Ice advances across the Antarctic continental platform generated major landscape shifts and a drastic reduction of available habitats (Zachos et al., 2001; Pollard and DeConto, 2009; Fraser et al., 2012; González-Wevar et al., 2012; González-Wevar et al., 2013; McCay et al., 2016; Halanych and Mahon, 2018);. Accordingly, near-shore marine benthic communities would have been especially vulnerable as continental ice sheets extended over most of the narrow Antarctic shelf to about 500 m in depth (Ingólfson, 2004; Thatje et al., 2005; Barker et al., 2007; Dambach et al., 2012).

Quaternary glaciations severely impacted the abundance, demographic dynamics, structure, and spatial distribution of Antarctic (Convey et al., 2009; McGaughran et al., 2011; Fraser et al., 2012; González-Wevar et al., 2013; Riesgo et al., 2015; Chenuil et al., 2018; Guillemin et al., 2018; Halanych and Mahon, 2018; Maturana et al., 2020; Cakil et al., 2021; Levicoy et al., 2021) and sub-Antarctic (Waters, 2008; Fraser et al., 2009; Nikula et al., 2010; González-Wevar et al., 2011; González-Wevar et al., 2012; Cumming et al., 2014; Moon et al., 2017; González-Wevar et al., 2018; González-Wevar et al., 2021) near-shore marine organisms. Moreover, ice advances during glacial maxima would have enhanced speciation, particularly in species with nonpelagic developmental modes (direct developers), as populations became fragmented into small and isolated refugia across the Antarctic continental shelf (Brey et al., 1996; Pearse et al., 2009; Chenuil et al., 2018). Following this, interglacial periods generated the expansion of newly available habitats where populations and species would have expanded their distributions (Provan and Bennett, 2008;

Marko et al., 2010; González-Wevar et al., 2013). Under a basic expansion-contraction model of Pleistocene biogeography (Provan and Bennett, 2008), refugial populations are expected to harbor higher levels of intraspecific genetic diversity compared to those that were heavily impacted by ice and/or recently recolonized ones (Marko, 2004; Maggs et al., 2008; Marko et al., 2010). Moreover, in some cases glaciations have been associated with the radiation and speciation of different marine near-shore invertebrates (Thornhill et al., 2008; Wilson et al., 2009; Allcock et al., 2011; Baird et al., 2011; Baird et al., 2021), a process also known as 'the Antarctic diversity pump' (Clarke and Crame, 1989; Clarke and Crame, 1992; Chenuil et al., 2018).

In a review of genetic signatures induced by Quaternary events in Antarctic taxa, Allcock and Strugnell (2012) proposed that two life-history traits, bathymetric ranges and developmental modes, played a key role. Shallow species with dispersive potential would exhibit star-like haplotype networks, with broadly distributed common haplotypes and closely related variants occurring at low frequencies. Such a pattern is associated with rapid population expansion, probably mediated by larvae dispersal, and has been recorded in the sea urchin *Sterechinus neumayeri* (Diaz et al., 2011), the nemertean *Parborlasia corrugatus* (Thornhill et al., 2008), the Antarctic limpet (González-Wevar et al., 2013) and the shrimp *Chorismus antarcticus* (Raupach et al., 2010). By contrast, the genetic signature predicted for shallow species with low dispersal potential includes disjunct haplotypes networks comprising multiple smaller genetic units, indicative of small populations isolated in glacial refugia that underwent bottlenecks. Due to the absence of free dispersive stages, recolonization is slow, allowing time for genetic drift and possible adaptive differentiation before secondary contact of isolated populations. Examples of taxa displaying this pattern include the amphipods *Eusirus* (Baird et al., 2011), the pycnogonid *Colossendeis megalonyx* (Krabbe et al., 2010), cephalopods (Allcock et al., 2011), and the nudibranch *Doris kerguelensis* (Wilson et al., 2009).

The high-latitude littorinid species *Laevilacunaria antarctica* (Martens van and Pfeffer, 1886) is one of the most abundant inter- and subtidal gastropods on macro-algae and rocky shores across ice-free areas of the Antarctic Peninsula and sub-Antarctic island of South Georgia (Cantera and Arnaud, 1984; Iken, 1999; Amsler et al., 2015; Martin et al., 2016; Amsler et al., 2019; Amsler et al., 2022; Schmider-Martínez et al., 2023). As with other Laevilitorininae genera such as *Laevilitorina* (Simpson and Harrington, 1985), *L. antarctica* exhibits low autonomous vagility, benthic protected development with the absence of free-living dispersive stages, a mode or reproduction where crawling juveniles emerge directly from egg masses. Generally, high-latitude littorinids are considered to be poor dispersers, with most species having narrow distributions restricted to particular areas of the Southern Ocean (González-Wevar et al., 2022; Rosenfeld et al., 2023). Based on its broad distribution and abundance across the maritime Antarctica, its narrow bathymetric range, and its reproductive mode, *L. antarctica* represents a suitable model to test the hypotheses proposed for Antarctic Quaternary biogeography of shallow marine benthic organisms.

In this study, we performed population-based molecular analyses of *Laevilacunaria antarctica* across its known distribution. We included more than 320 specimens collected from across the Antarctic Peninsula (AP) and the nearby South Shetland Islands (SSI), as well as from sub-Antarctic South Georgia (SG). Comparative mitochondrial (COI) and nuclear (28S rRNA) genetic analyses allowed us to unravel the legacy of Quaternary glaciations in the patterns of genetic diversity and structure of this species. Considering the lack of larval dispersive stages in the species and its narrow bathymetric range, we predicted that *L. antarctica* would display several disjunct haplotype networks separated into smaller genetic units, as recorded in other Antarctic species with similar life history traits. Through this research we aimed to understand how key elements of the near-shore Antarctic marine benthic communities endured Quaternary climate shifts associated with glacial and interglacial periods.

Materials and methods

Sample collection, DNA extraction and amplifications

Specimens of *L. antarctica* were collected by hand and by scuba divers from intertidal and subtidal rocky-shore ecosystems between 2015 and 2021 during the Chilean Scientific Antarctic Expeditions (ECAs) across the species distribution in the maritime Antarctica (AP and SSI) and SG (Figure 1; Table 1). The identification of specimens was done following the original descriptions and revisions (Martens van and Pfeffer, 1886; Smith, 1879; Preston, 1916; Powell, 1951) as well as recent literature (Arnaud and Bandel, 1979; Cantera and Arnaud, 1984; Engl, 2012). Individuals were fixed *in situ* using 95% ethanol and transported for further molecular analyses. All the specimens were photographed and measured for future comparative morphological studies. Preparation of DNA from the whole animal used the standard salting-out methodology (Aljanabi and Martinez, 1997) and the QIAGEN DNEasy Blood & Tissue kit (QIAGEN Inc.). We amplified a partial fragment of the mitochondrial cytochrome c subunit I gene (COI) using universal primers LCO1490 and HCO2198 (Folmer et al., 1994). Similarly, we used the universal primers 900F and 1600R (Littlewood et al., 2000) to amplify a fragment of the nuclear 28S rRNA gene. PCR amplicons were purified and sequenced in both directions at Macrogen Inc (Seoul, South Korea). Forward and reverse sequences were assembled and edited for each marker independently using GENEIOUS (http://www.geneious.com). Alignments and base composition of nucleotide sequences analyses were done for each marker in MUSCLE (Edgar, 2004) in MEGA 11 (Tamura et al., 2021), respectively. Wright's
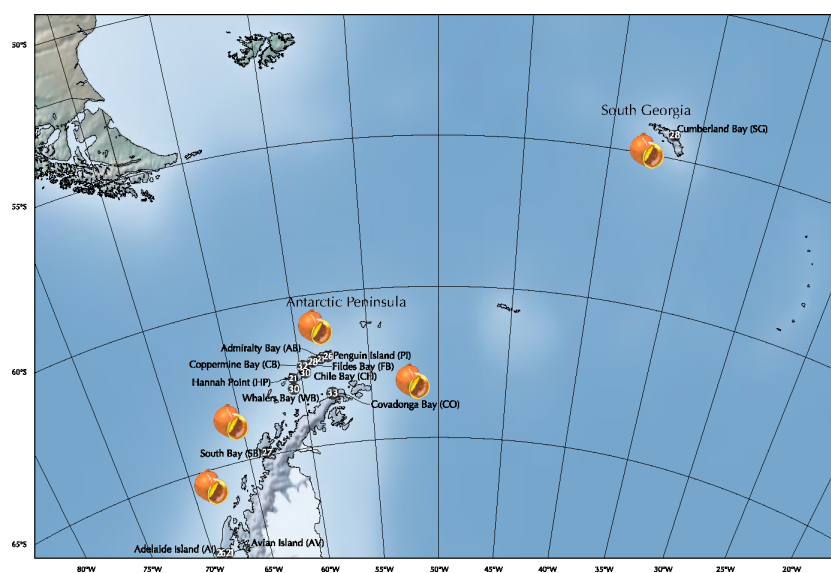

FIGURE 1
Sampling localities of *Laevilacunaria antarctica* populations across its distribution in maritime Antarctica (SSI/AP) and South Georgia (SG).

TABLE 1 Sampled localities of *Laevilacunaria antarctica* across its distribution in maritime Antarctica (MP) and South Georgia.

Locality	Latitude	Longitude	Area	Main Area
Penguin Island (PI)	62°06′00′′S	57°55′41′′W	King George Island	South Shetland Islands
Admiralty Bay (AB)	62°09′41′′S	58°28′10′′W	King George Island	South Shetland Islands
Fildes Bay (FB)	62°12′57″S	58°57′35″W	King George Island	South Shetland Islands
Coppermine Bay (CB)	62°22′17″S	59°42′53″W	Robert Island	South Shetland Islands
Hannah Point (HP)	62°39′19″S	60°36′48″W	Livingston Island	South Shetland Islands
Whalers Bay (WB)	62°59′57′′S	60°40′28′′W	Deception Island	South Shetland Islands
Chile Bay (CH)	62°28′43″S	59°39′48″W	Greenwich Island	South Shetland Islands
Covadonga Bay (CO)	63°19′15″S	57°53′59″W	Covadonga Bay	Antarctic Peninsula
South Bay (SB)	64°52′55″S	63°35′03″W	Doumer Island	Antarctic Peninsula
Adelaide Island (AI)	67°46′23′′S	68°55′53′′W	Marguerite Bay	Antarctic Peninsula
Avian Island (AV)	67°46′15′′S	68°53′10′′W	Marguerite Bay	Antarctic Peninsula
Cumberland Bay (SG)	54°14′12′′S	36°28′32′′W	South Georgia	Scotia Arc

mitochondrial codon usage was computed using the Effective Number of Codons (ENC) in DnaSP v5 (Librado and Rozas, 2009). The data presented in the study are deposited in the GenBank repository, accession number COI (ORT701885 - ORT701931) and 28S rRNA (ORT711532 - ORT711533).

Genetic diversity and population structure in *L. antarctica*

We performed a DNA saturation analysis following Xia and Xie (2001) to evaluate how transitions accumulate in relation to nucleotide divergence in the complete *L. antarctica* COI data set. We then estimated levels of population mtDNA polymorphism through standard diversity indices including number of haplotypes (k), haplotype diversity (H), number of segregating sites (S) and the number of private haplotypes (p. hap) per locality. Moreover, we also determined the average number of pairwise differences (Π) and the nucleotide diversity (π) across the species distribution using DnaSP.

Patterns of population differentiation were estimated following Pons and Petit (1996), using haplotype frequencies (G_{ST}) and mean pairwise differences (N_{ST}) in Arlequin v.3 (Excoffier et al., 2005). The statistical significance of genetic pairwise differences was calculated using permutation tests (20,000 iterations) and the adjustment for multiple testing was done through False Discovery Rate (FDR) correction (Narum, 2006). Moreover, we estimated levels of genetic differentiation using the nearest-neighbor statistic (S_{nn}), which measures how often nearest-neighbor (in sequence space) sequences are from the same locality in geographic space (Hudson, 2000). The statistical significance of S_{nn} was determined using a permutation test (20,000 iterations).

We used two different clustering methods to determine the spatial genetic structure of *L. antarctica*. First, we determined the number and the composition of panmictic groups and the spatial boundaries using a Bayesian model computed in GENELAND v.2.0.0 (Guillot et al., 2005) in the R environment (Ihaka and Gentleman, 1996). This analysis implements a Markov Chain Monte Carlo (MCMC) procedure to estimate the best clustering of samples considering genetic and geographic information. Analyses were run using 50 x 10⁶ MCMC iterations sampled every 1,000 steps. Assembled scores were graphed against generations in Tracer v.1.5. (Rambaut et al., 2018) to identify stationarity and the number of generations to be discarded as burn-in. A maximum number of clusters (K = 13) were run to estimate the model parameters and posterior probabilities of group membership. Second, we determined the spatial genetic structure in *L. antarctica* by estimating the number and composition of groups that were most differentiated based on sequence data set using Spatial Analysis of Molecular Variance (SAMOVA) (Dupanloup et al., 2002). This analysis partitions the genetic variance into i) within populations, ii) among populations within groups and iii) among groups.

Demographic analyses in *L. antarctica*

Haplotype genealogical relationships in *L. antarctica* populations were reconstructed using median-joining and maximum-parsimony networks in Network 10 (Forster et al., 2001) and Hapview (Salzburger et al., 2011), respectively. Moreover, we performed neutrality statistical tests (Tajima's D and Fu's FS) using DnaSP for the whole COI data set, for each recognized group, and for each locality to estimate whether sequences deviate from mutation-drift equilibrium. Population demographic histories were estimated comparing the distribution of pairwise differences between haplotypes (mismatch distribution) for each recognized group to the expected distribution under the sudden expansion growth model of Rogers and Harpending (1992). Finally, for comparative purposes, we also constructed genealogical

relationships in *L. antarctica* using sequences of the nuclear marker 28S rRNA.

We reconstructed past population dynamics through time in the recognized *L. antarctica*'s genetic clusters using a Bayesian skyline plot method implemented in BEAST v.1.10.4 (Drummond and Rambaut, 2007; Drummond et al., 2012). Three evolutionary models (strict clock, uncorrelated lognormal and uncorrelated relaxed clock) were tested and compared statistically using a Bayes factor test (Suchard et al., 2001) in Tracer and the strict clock model was the best fit for each of the COI data-set clusters. Following this, we performed three independent Bayesian MCMC runs for 250×10^6 generations (sampled every 1000 iterations), using the GTR + I + Γ model, previously estimated in MrModeltest v.2.3 (Nylander, 2004). Molecular evolutionary studies have yielded a wide range of rate estimates for different genes and taxa. Several studies based on population-level and intraspecific data have generated remarkably high estimates of mutation rate, which contrast with substitution rates inferred in phylogenetic studies. Consequently, BSP analyses were done using a tenfold evolutionary rate estimated for littorinids (Reid et al., 2012). Such a rate correction was assumed considering the time-dependence of molecular rate proposed by Ho et al (2005; 2007; 2011). The first 10% of the parameter values were discarded as a burn-in and the convergence of runs was confirmed with Tracer, ensuring a minimum of 1000 effective sampling for each statistic (ESSs > 1000).

Results

Genetic diversity in *L. antarctica*

The whole COI data set in *L. antarctica* included 323 specimens (SG = 28; SSI = 188; AP = 107) and comprised 687 nucleotide positions coding for 229 amino acids. No insertion/deletions or stop codons were detected. Moreover, mitochondrial (mtDNA) and nuclear (nucDNA) sequences were not saturated and no evidence of mtDNA codon bias was found (ENC = 46.75). Four amino-acid substitutions (positions 11, 44, 144, and 171) were recorded in *L. antarctica* using the invertebrate mitochondrial table. Low levels of genetic diversity characterized populations of *L. antarctica*, with 48 polymorphic characters (6.9%) and 29 of them (60.4%) were parsimony informative. Sequences were A – T rich (67.2%). The haplotype diversity (*H*) varied between 0.123 (Robert Island, SSI) and 0.675 (Cumberland Bay, SG) (Table 2). The number of polymorphic sites (*S*) varied between 1 (Admiralty Bay, SSI) and 10 (Cumberland Bay, SG). Similarly, the number of haplotypes/private haplotypes varied among the analyzed localities between 2/0 (Admiralty Bay, SSI) and 11/11 (Cumberland Bay, SG) (Table 2). Finally, the average number of nucleotide differences (*Π*) and the mean nucleotide diversity (*π*) were very low in most localities across the maritime Antarctica (SSI and AP), whereas the diversity of these indices was higher in SG (Table 2). The mean level of mtDNA genetic divergence between SSI/AP and SG populations of *L.*

TABLE 2 Diversity indices, private alleles, and neutrality tests in *Laevilacunaria antarctica* populations across its distribution in maritime Antarctica and South Georgia.

Locality	*n*	*k*	*H*	*S*	*Π*	*π*	p. hap.	Tajima´s D	Fu´s FS
Penguin Island (SSI)	26	4	0.222	4	0.308	0.0015	1	-1.88*	-2.45
Admiralty Bay (SSI)	21	2	0.095	1	0.095	0.0001	0	-1.16	-0.919
Fildes Bay (SSI)	28	6	0.437	6	0.556	0.0008	2	-1.85*	-3.66*
Hannah Point (SSI)	21	4	0.414	3	0.448	0.0006	1	-1.21	-1.77
Coppermine Bay (SSI)	32	3	0.123	3	0.188	0.0002	1	-1.72	-1.70
Whalers Bay (SSI)	30	7	0.464	6	0.524	0.0007	5	-1.86*	-5.35**
Chile Bay (SSI)	30	7	0.366	7	0.467	0.0006	6	-2.17**	-5.88**
SSI	188	23	0.311	23	0.380	0.0005	n/a	-2.48**	-40.27***
Covadonga Bay (AP)	33	2	0.061	2	0.121	0.0001	1	-1.50	-0.48
South Bay (AP)	27	5	0.396	5	0.507	0.0007	4	-1.70	-2.57
Adelaide Island (AP)	26	9	0.578	7	0.745	0.0010	5	-1.81*	-7.45***
Avian Island (AP)	21	4	0.271	3	0.286	0.0004	2	-1.72	-2.80*
AP	107	17	0.324	14	0.408	0.0005	n/a	-2.29**	-25.57***
MA	295	36	0.405	34	0.405	0.0005	n/a	-2.56***	-75.32***
Cumberland Bay (SG)	28	11	0.675	10	1.167	0.0017	11	-1.91*	-7.86***
Total	**323**	**47**	**0.436**	**48**	**2.296**	**0.0033**	n/a	**-2.00***	**-42.19***

Where AP = Antarctic Peninsula; SSI = South Shetland Islands; MA = Maritime Antarctica (SSI + AP); SG = South Georgia.
n = number of analyzed individuals; *k* = number of haplotypes; *S* = polymorphic sites; *H* = haplotype diversity; *Π* = average number of pairwise differences; *π* = nucleotide diversity. *p<0.05, **p<0.01, *** p<0.001; n/a not applicable.

antarctica was 1,74%. Levels of nucDNA diversity were much lower in the species, SSI and AP specimens (n = 123) shared the same allele which was differentiated by two mutation steps from the sequences recorded at South Georgia.

Genetic structure in *L. antarctica*

Mean general values of differentiation estimated were low, especially considering average G_{ST} (0.112) and N_{ST} (0.170). In fact, general levels of differentiation among maritime Antarctic populations were extremely low, considering average G_{ST} (0.043) and N_{ST} (0.034). Pairwise population comparisons did not recognize significant structure among maritime Antarctica populations (Table 3). Nevertheless, some populations showed significant differences after FDR corrections. By contrast, G_{ST} and N_{ST} comparisons recorded significant differences between SG and the rest of the analyzed populations from the maritime Antarctica (Table 3).

The nearest neighbor statistic in *L. antarctica* (S_{nn} = 0.199) showed low but significant levels of phylogeographic signal (p < 0.0001). Nevertheless, when this analysis was carried out considering the main pattern of genetic differentiation recorded in the species (maritime Antarctic vs South Georgia), S_{nn} became extremely high (S_{nn} = 1.00) and significant, showing the high degree of phylogeographic signal found between maritime Antarctica and SG. The pattern of genetic structure was supported by the model based on the Bayesian clustering algorithm, which detected two main clusters (K = 2). Again, the first cluster included all localities from the maritime Antarctica (Figure 2A), while the second one comprised only South Georgia (Figure 2B). Values of cluster membership were very high (P = 1.000) for all the individuals and the mean probability value (P = 0.5) corresponds to the boundary between these clusters and runs across the Scotia Ridge

located between South Georgia and the Antarctic Peninsula. Similarly, SAMOVA analyses detected two maximally differentiated genetic groups – maritime Antarctica and South Georgia – accounting for 59.35% of the total variance, in comparison with only 0.83% due to within-group variation among localities (Table 4). Considering the level of mtDNA genetic divergence (1.74%) and the strong phylogeographic signal recorded between SSI/AP and SG we performed divergence time estimations analysis under a phylogenetic framework. For this a relaxed molecular-clock using an uncorrelated-lognormal (ucld) model of molecular evolutionary rate heterogeneity and the GTR + I + G model of substitution was implemented in BEAST v.1.7.5 (Drummond & Rambaut, 2007; Drummond et al., 2012). Four chains were run for 200 x 10^6 generations and trees were sampled every 10,000 generations. For divergence time estimations we include in the analyses several calibration points within the evolution of Littorinidae estimated for the most common ancestors (TRMCA) of the genera *Echinolittorina*, *Littorina*, *Littoraria*, *Austrolittorina*, *Tectarius* and *Afrolittorina*) following Reid et al. (2012), and with special emphasis on higher latitude groups (González-Wevar et al., 2022). According to our divergence-time analyses the separation between SSI/AP and SG genetic clusters occurred around 1.1 Ma (2.0 – 0.55 Ma).

Demographic reconstructions

The parsimony mtDNA network of *L. antarctica* included 47 haplotypes and clearly discriminated two main groups: maritime Antarctica and South Georgia, separated by nine substitutional steps (Figure 3A). Both maritime Antarctica and South Georgia clusters exhibited typical starlike topologies and short genealogies. In maritime Antarctica, the central haplotype (H01) was the most frequent (82%), broadly distributed and surrounded by 35

TABLE 3 Pairwise G_{ST} (below the diagonal) and N_{ST} (above the diagonal) values calculated among the analyzed populations of *Laevilacunaria antarctica*. 20,000 iterations.

Locality	PI	AB	FB	HP	CB	WB	CH	CO	SB	AI	AV	SG
Penguin Island (PI)	-	0.000	0.017	0.038	0.001	0.009	0.000	0.003	0.013	0.017	0.000	**0.938**
Admiralty Bay (AB)	0.000	-	0.014	0.050	0.000	0.004	0.000	0.000	0.010	0.019	0.000	**0.941**
Fildes Bay (FB)	0.021	0.057	-	0.000	0.017	0.022	0.006	0.027	0.024	0.030	0.014	**0.930**
Hannah Point (HP)	0.028	0.071	0.000	-	0.044	0.035	0.029	0.065	0.039	0.039	0.037	**0.930**
Coppermine Bay (CB)	0.000	0.000	0.058	0.076	-	0.013	0.000	0.000	0.019	0.031	0.000	**0.947**
Whalers Bay (WB)	0.021	0.057	0.000	0.003	0.062	-	0.009	0.016	0.019	0.027	0.000	**0.932**
Chile Bay (CH)	0.000	0.022	0.000	0.002	0.024	0.000	-	0.001	0.011	0.017	0.000	**0.934**
Covadonga Bay (CO)	0.013	0.000	**0.097**	**0.120**	0.000	**0.096**	0.053	-	0.015	0.036	0.000	**0.950**
South Bay (SB)	0.009	0.043	0.000	0.005	0.046	0.000	0.000	0.051	-	0.028	0.002	**0.931**
Adelaide Bay (AI)	0.047	0.045	0.004	0.009	0.064	0.000	0.008	**0.144**	0.088	-	0.018	**0.922**
Avian Island (AV)	0.000	0.001	0.005	0.011	0.002	0.000	0.000	0.031	0.000	0.023	-	**0.935**
Cumberland Bay (SG)	**0.546**	**0.588**	**0.444**	**0.446**	**0.613**	**0.432**	**0.482**	**0.649**	**0.463**	**0.372**	**0.510**	-

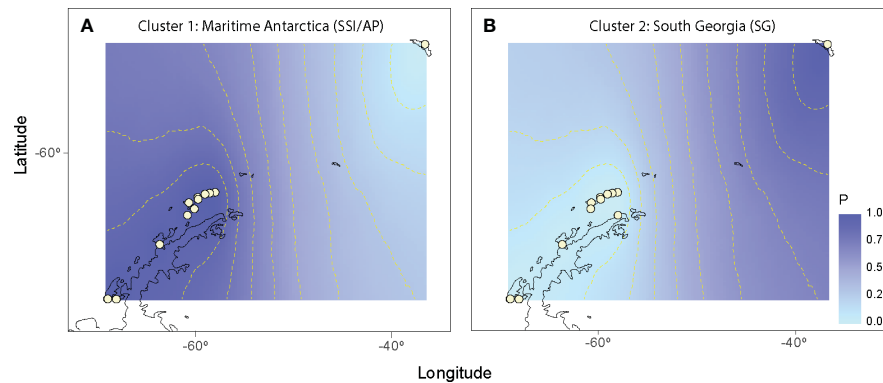Statistical significant FDR differences are marked in bold.

FIGURE 2
Spatial output from Geneland using *Laevilacunaria antarctica* populations. White circles indicate the relative positions of the sampling localities across maritime Antarctica and South Georgia. Darker and lighter shadings are proportional to posterior probabilities of membership to particular genetic cluster (**A** = maritime Antarctica and **B** = South Georgia). Posterior probabilities were plotted against the shapefile of the Antarctic coastline available in GEOdas (NOAA) and filtered using GEOdas Coastline Extractor v.1.1.3.1 (https://www.ngdc.noaa.gov/mgg/geodas/geodas.html).

haplotypes of low frequency (fewer than four individuals). A second dominant haplotype (H02) was found in 57% of the SG individuals and was surrounded by ten closely derived ones (Figure 3A). As expected, for star-like genealogies, global Tajima's D and Fu's neutrality tests were negative and significant for each recognized cluster and for the whole COI data set (Table 2). The distribution of pairwise differences depicted for the maritime Antarctica showed a typical L-shaped distribution. In contrast, the distribution of pairwise differences for South Georgia was bimodal. As stated above, nucDNA parsimony network showed that the maritime Antarctica populations specimens exhibited the same allele (HI) while SG specimens carried another one (HII) separated by two mutational steps (Figure 3B).

Bayesian skyline-plot analyses identified similar trajectories in terms of the time since the most recent common ancestors (trmca) and populations expansions between maritime Antarctica and South Georgia (Figure 4). Population expansion of the maritime

TABLE 4 Spatial Analysis of Molecular Variance (SAMOVA) depicting the percentage of variation explained among groups (maritime Antarctica and South Georgia), among populations within groups and within populations.
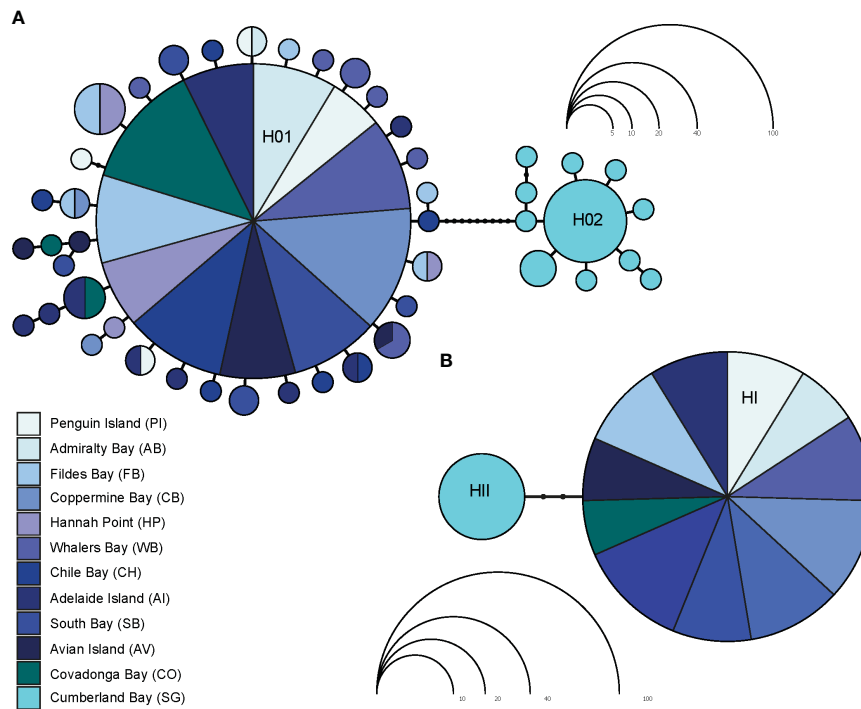
Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	13.232	0.25343 Va	59.35
Among populations within groups	10	2.654	0.00357 Vb	0.83
Within populations	311	52.867	0.16999 Vc	39.81
Total	322	68.752		

F_{SC} = differentiation within populations among groups; F_{CT} = Differentiation among groups (*** p<0.001).
Fixation Indices.
F_{SC}: 0.02054***.
F_{CT}: 0.59354***.

Antarctica populations occurred approximately 5,000 years ago while the expansion at South Georgia occurred around 9,000 years ago. The tmrca of SSI/AP occurred around 12,000 years ago while the tmrca for SG occurred 17,000 years ago (Figure 4).

Discussion

Our results show that *Laevilacunaria antarctica* has a remarkably low level of genetic diversity across much of its known range, with the presence of a single dominant COI haplotype in our sampled populations from Penguin Island (62° 06'), the farthest north locality in the South Shetland Islands, to the southernmost point in Avian Island (67°46') in the Antarctic Peninsula. There is marginally more diversity further afield, in South Georgia, although again one haplotype, albeit a very different one, dominates. The only population structure is between South Georgia and the remaining populations, with no shared haplotypes and at least nine substitutional steps between haplotypes from these two parts of the species' range. Effectively, the haplotype map is one of two star-like genealogies linked by a nine-step branch. Nevertheless, such results should be taken with caution, considering that we only included one locality from South Georgia. In this context, future samplings in South Georgia are required to increase our sampling effort across this island and corroborate the diversity pattern here recorded.

Our findings provide only limited support for the predictions of Allcock and Strugnell (2012), and indeed some evidence to the contrary. As *Laevilacunaria* lacks any pelagic dispersal stage, yet the low levels of diversity and the absence of genetic structure showed by star-like networks in the maritime Antarctica across hundreds of kilometers are more congruent with the pattern of genetic diversity these authors envisaged in highly dispersive species. Nevertheless, our analyses detected a marked phylogeographic signal between sub-Antarctic South Georgia and maritime Antarctic localities, fitting more closely with a poor dispersal capacity at large geographical scale. The absence of shared haplotypes, together

FIGURE 3
Parsimony mtDNA **(A)** and nucDNA **(B)** networks in *Laevilacunaria antarctica* across its distribution in maritime Antarctica and South Georgia. Each haplotype/allele is represented by a colored circle indicating the locality. The size the haplotypes/alleles is proportional to their frequencies.

with the number of fixed mutations (n = 9) between these areas are strong evidence of distinct genetic and evolutionary units in *L. antarctica*. Indeed, divergence-time estimations of these Significant Evolutionary Units (ESUs) suggest that they were separated ~ 1.1 million years ago (Ma), long before the LGM and, ever since, have evolved independently in these areas of the Southern Ocean.

How do we explain these apparently contradictory results? The absence of population structure in the maritime Antarctica matches previous molecular studies of near-shore marine benthic invertebrates such as the Antarctic limpet *Nacella concinna* (Hoffman et al., 2010; González-Wevar et al., 2013), the trochoid gastropod *Margarella antarctica* (González-Wevar et al., 2021), and the sea urchin *Sterechinus neumayeri* (Díaz et al., 2011; Díaz et al., 2018). *Nacella* and *Sterechinus* species possess a pelagic larval stage and such patterns are explained by their respective higher dispersal potentials. By contrast, *Margarella* and *Laevilacunaria* species exhibit benthic protected development, but they live closely associated with macroalgae on which they graze and reproduce. Accordingly, it is possible that *Margarella* and *Laevilacunaria* could maintain connectivity through rafting of adults and/or egg-masses attached to macroalgae. Alternatively, the low levels of genetic diversity and the absence of genetic structure in *L. antarctica* across the maritime Antarctica is probably associated to strong founder effects from bottlenecked refugial population. This same argument would apply to the South Georgian populations of *L. antarctica*. The lower frequency of buoyant macroalgae in the maritime Antarctic represented by *Crystosphaera jacquinotti*, however, presumably limits the extent of this rafting, precluding

dispersal of *L. antarctica* (and, indeed, also occur in the trochoid *M. antarctica*) to the more distant areas such as South Georgia. Events of long-distance colonization by rafting have been documented among non-buoyant macroalgae (Fraser et al., 2013; McCay et al., 2016). Evidence of macroalgae fronds enclosed in drift ice have been reported at sea (Guillemin M-L pers. comm.) and these fronds have been recovered far away from the coast (Guillemin et al., 2018). In fact, across the same study are, several non-buoyant red algae also exhibit very low levels of genetic diversity and a complete absence of genetic structure (Guillemin et al., 2018). During Quaternary glaciations, the study area, SSI/AP and SG, have been located south of the Antarctic Polar Front (APF). Hence, the positition of the APF can be excluded as a potential barrier to gene flow between these areas. Drifter-based data have demonstrated that the Antarctic Circumpolar Current flows from the maritime Antarctica to South Georgia with a velocity that would allow to drift between these two areas around two months (Matschiner et al., 2009).

The lack of genetic variation in the maritime Antarctica, with one dominant haplotype in the centre of a star-like genealogy, fits well with the idea that *L. antarctica* survived a bottleneck induced by the LGM in one or (possibly) more shelf refugia. The slightly less star-like pattern, and the smoother historical demographic curve in South Georgia implies, perhaps, a less drastic reduction in population size and/or an earlier population expansion.

We note that the levels of nucleotide diversity recorded in *L. antarctica* are significantly lower than those found in temperate (Doellman et al., 2011; Silva et al., 2013; Sotelo et al., 2020; Blakeslee
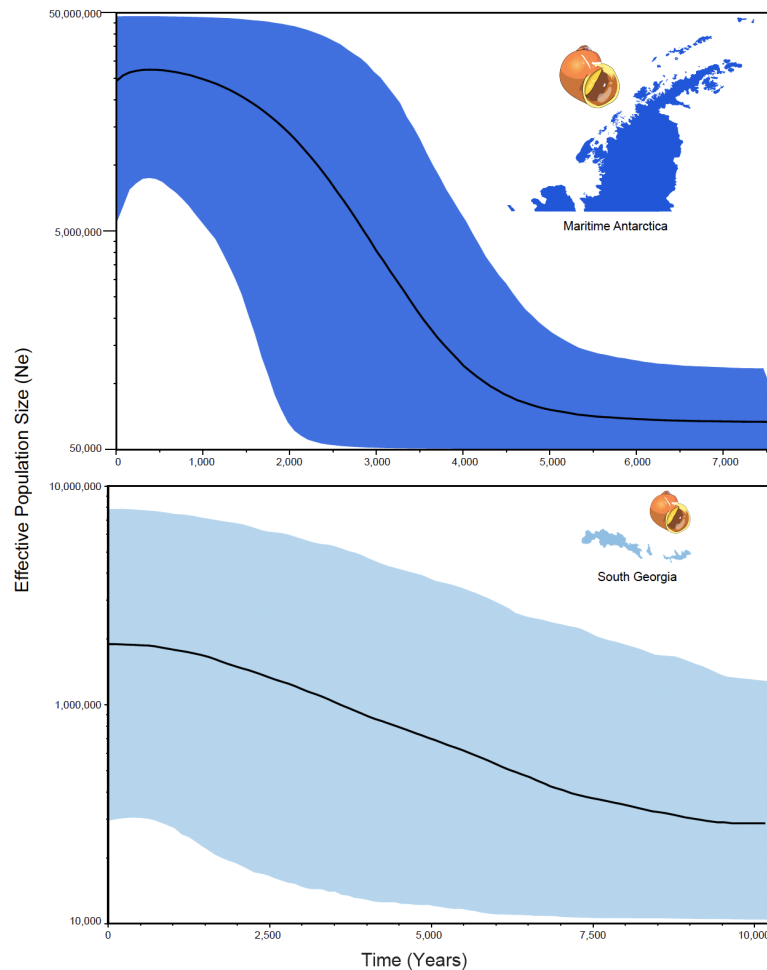
FIGURE 4
Historical demographic trajectories of the effective population sizes (N_e) estimated under a Bayesian skyline-plot approach based on COI sequences of *L. antarctica*. The y-axis represents the effective population size (N_e) while the x-axis shows the time since the present. The median estimates are shown for each genetic cluster recorded in the species (maritime Antarctica and South Georgia).

et al., 2021) and sub-Antarctic littorinids (González-Wevar et al., 2022). In fact, levels of genetic diversity in the species are much lower than those found in most other groups of Antarctic marine molluscs (Wilson et al., 2009; González-Wevar et al., 2013; Fassio et al., 2019; Levicoy et al., 2021) and fishes (Parker et al., 2002; Damerau et al., 2012; Damerau et al., 2014; Hüne et al., 2015). Nevertheless, several species of Antarctic near-shore marine invertebrates are characterized by low genetic diversity including the nemertean *Parborlasia* (Thornhill et al., 2008), the crustacean *Chorismus antarcticus* (Raupach et al., 2010), the echinoid *Sterechinus neumayeri* (Díaz et al., 2011) and the trochoid *Margarella antarctica* (González-Wevar et al., 2021). Drastic reduction of habitats has presumably affected populations of these Antarctic species and left characteristic signatures of drastic bottlenecks and/or founder effects (Aronson et al., 2007; Clarke and Crame, 2010; Allcock and Strugnell, 2012; González-Wevar et al., 2013; Riesgo et al., 2015; Guillemin et al., 2018).

Genetic structure

Our study adds to the diversity of biogeographic patterns seen in different taxa across maritime Antarctica and South Georgia in different taxa. This variation ranges from the absence of genetic differentiation, as in nototheniod fishes like *Gobionotothen gibberifrons* (Matschiner et al., 2009) and *Notothenia rossi* (Young et al., 2015), through marked phylogeographic structure, as in the limpet *Nacella concinna* (Hoffman et al., 2011; González-Wevar et al., 2013) and the notothenioid fish *Champsocephalus gunnari* (Young et al., 2015), to the presence of clearly divergent species-level clades, as in the gastropod *Margarella* (González-Wevar et al., 2021), the nudibranch *Doris* (Wilson et al., 2009), the crinoids of the genus *Promachocrinus* (Wilson et al., 2007), and the notothenioid genus *Lepidonotothen* (Dornburg et al., 2016).

The mtDNA (COI) levels of genetic divergence recorded between maritime Antarctic and South Georgian populations

(1.74%) is lower than those recorded between clearly recognized littorinid species (Williams et al., 2003; Williams and Reid, 2004; Reid et al., 2012) and particularly in higher latitude groups (González-Wevar et al., 2022; Rosenfeld et al., 2023). Under this molecular-based criteria, we would not treat the SSI/AP and SG populations as different species. Appropriate names would be available, however: the type locality of *L. antarctica* is South Georgia, whereas Deception Island, one of the South Shetland Islands, is the type locality for *L. bransfieldensis*, considered a junior synonym by Simone (2018). Nevertheless, our results in *Laevilacunaria* could probably be interpreted as an incipient speciation process, as has been hypothesized for other groups of marine organisms including *Nacella* (González-Wevar et al., 2011; González-Wevar et al., 2017), octocorals (Dueñas et al., 2016), *Doris* (Wilson et al., 2009), *Pareledone* (Allcock et al., 2011), nothothenioid fishes (Near et al., 2012; Dornburg et al., 2016) and even penguins (Vianna et al., 2017; Frugone et al., 2019). To further understand such evolutionary and biogeographic hypotheses in *Laevilacunaria*, future studies through morphological (radular and geometric morphometrics) analyses are needed. Addressing this important question in *Laevilacunaria* will provide new insights concerning biogeographical and diversity patterns in this widespread maritime Antarctica gastropod group. Moreover, integrative systematic studies including phylogenetic, genomic and morphological analyses of the whole genus are also required to evaluate the potential role of vicariance and long-distance dispersal in the biogeography of *Laevilacunaria*. The inclusion of the poorly known Kerguelen species *L. pumilio* and the partially sympatric Antarctic species *L. bennetti* will allow us to determine the origin and diversification of *Laevilacunaria* in different provinces of the Southern Ocean. Finally, through geometric morphometric it will be possible to evaluate the relevance of cryptic speciation in the evolution of the group across the Southern Ocean, a region of the planet that has been relatively neglected in spite of its global relevance.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: GenBank under the following Accession Numbers: COI (ORT701885 - ORT701931) and 28S rRNA (ORT711532 - ORT711533).

Ethics statement

The animal study was approved by Comité de Ética, Facultad de Ciencias, Universidad Austral de Chile. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration,

Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. YP: Formal analysis, Investigation, Methodology, Software, Writing – original draft. NS: Formal analysis, Methodology, Software, Writing – review & editing. SR: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. CM: Writing – original draft, Writing – review & editing. VJ: Formal analysis, Methodology, Writing – original draft. AS: Formal analysis, Methodology, Writing – original draft. KG: Writing – original draft. HS: Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. EP: Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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