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Biodiversity of Antarctic and Subantarctic ecosystems

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Biodiversity of Antarctic and Subantarctic ecosystems

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Editorial: Biodiversity of Antarctic and Subantarctic ecosystems

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Editorial on the Research Topic
Biodiversity of Antarctic and Subantarctic ecosystems

The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) adopted a work program (2019–2030) that included, under its first objective, the interlinkages between biodiversity and climate change (IPBES, 2019). Because most ecosystems worldwide face multiple anthropogenic pressures, however, it is difficult to disentangle the specific effects of climate change from those of others such as habitat degradation, pollution or overexploitation. Against this backdrop, the Antarctic and sub-Antarctic (ASA) regions —including some of the most pristine environments remaining on Earth— offer unparalleled opportunities to understand, evaluate and predict the impacts of climate change on biodiversity in the general absence of other confounding anthropogenic drivers. These regions serve as natural laboratories for two main reasons: first, ASA biodiversity has already endured repeated and drastic climatic oscillations over timescales ranging from decades to tens of millions of years, providing a unique archive of responses to past change; second, some ASA areas are now experiencing some of the fastest rates of warming on the planet. The South Shetland Islands, for example, have undergone profound transformations over the past four decades, including the emergence of new ice-free areas, streams and freshwater bodies (Lee et al., 2017; Petsch et al., 2022; Tóth et al., 2025). Studying these transitions provides essential insights into how climate change reshapes ecosystems when other anthropogenic drivers remain minimal.

Biodiversity under change

Evidence from terrestrial, freshwater and marine systems across the ASA highlights both the rapid pace and the complexity of ecological transformations. For instance, predictive models forecast shifts in the distribution of snow algae, predicting expansions at higher elevations but a loss of habitats on low-lying islands under warming scenarios (Gray et al.). Microbiome studies on Antarctic fairy shrimps show how host-associated microbial diversity contributes to adaptation and eco-evolutionary dynamics (Schwob et al.). In terrestrial ecosystems, nematodes (Jackson et al.) and insects (Maturana et al.) reveal how glacial history and dispersal capacity are imprinted within strong population structures. Freshwater communities, including microarthropods, respond not only to temperature but also to habitat type and vegetation structure (Bokhorst et al.). Research in the Southern Ocean reveals how dispersal, connectivity, and human activities collectively shape the resilience of benthic communities. Genetic analyses of *Siphonaria lateralis*, indicate that rafting provides only occasional dispersal opportunities, insufficient to prevent significant population differentiation but enough to prevent speciation, across Patagonia, the Falkland Islands/Islas Malvinas, South Georgia and Îles Kerguelen (Millán-Medina et al.). Complementary surveys in the Crozet archipelago uncover unexpectedly high shallow-water marine biodiversity, including new records and likely endemic species, while also revealing connections with other sub-Antarctic regions (Jossart et al.). Studies on the brittle star, *Amphiura belgicae*, reveal it to be a species complex with regionally isolated lineages, challenging the idea of a homogeneous Antarctic benthic fauna. Comparative phylogeography emerges as a valuable tool to identify shared historical processes shaping assemblages and to inform conservation efforts beyond simple species inventories (Sands et al.). Additionally, applied assessments, such as evaluating the impact of wharf construction at Rothera Research Station on Adelaide Island, demonstrate that, with effective mitigation, human activities can have minimal long-term effects on Antarctic macroepifaunal communities (Robinson et al.). Finally, phylogeographic studies of the gastropod *Laevilacunaria antarctica* reveal contrasting patterns of both high and low dispersal, suggesting that rafting may enable widespread connectivity despite the lack of pelagic larvae (González-Wevar et al.). Collectively, these studies depict a mosaic of unique and dynamic communities across Antarctica and the Southern Ocean, where evolutionary history, dispersal mechanisms, and human activities intersect to influence vulnerability and resilience in the face of accelerating climate change.

Conservation and governance challenges

Translating scientific advances into effective conservation policy for ASA biodiversity remains an urgent challenge (Coetzee et al., 2017; Lee et al., 2022). While many sub-Antarctic islands and their surrounding waters receive various forms of protection under the national systems of their sovereign authorities, the Southern Ocean and all land and ice south of the sixty-degree latitude parallel form the area of governance of the international Antarctic Treaty System (ATS), which was negotiated in 1959 and has been in force since 1961. The ATS provides the mechanism and means to designate and manage areas of protection (Antarctic Specially Protected Areas and Antarctic Specially Managed Areas; APSAs and ASMAs) within the regions under its jurisdiction. This process is currently achieved through the Protocol on Environmental Protection to the Antarctic Treaty, which has been in force since 1998. Despite its achievements, however, the ATS relies on consensus-based decision-making, which has hindered progress on critical issues such as climate-change recognition, ASPA and ASMA establishment, and the designation of Marine Protected Areas through the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (Coetzee et al., 2017). Recent years have highlighted the risk of stalemate in this governance system, raising concerns about its capacity to adapt under mounting geopolitical pressures. In terrestrial and freshwater environments, additional linked policy challenges include management of non-native species, biosecurity and the conservation of rapidly emerging habitats, such as new lakes and streams revealed by glacier retreat. Effective safeguarding of ASA biodiversity requires integration of evolutionary history, ecological processes and the unique vulnerabilities of both marine and terrestrial systems.

Contribution from this Research Topic

This Research Topic addresses this challenge and emphasizes the importance of ASA biodiversity, as well as the integration of its evolutionary history across terrestrial and marine systems. The twelve contributions, including notable work from early-career researchers, cover a broad spectrum from microorganisms to top predators, illustrating how environmental factors and climate change influence ecological and evolutionary processes. Studies show how past climate history has shaped terrestrial species, while microbial and freshwater research highlight the role of diversity in adaptation. Marine-focused contributions reveal both limited dispersal patterns and unexpected endemism in benthic invertebrates, challenging assumptions of widespread connectivity across sub-Antarctic islands and the Southern Ocean. Comparative phylogeography proves to be a powerful tool for identifying shared historical processes across groups, providing a stronger foundation for conservation planning. Overall, these studies depict dynamic and diverse communities where historical legacies, dispersal mechanisms and human activities intersect, affecting vulnerability and resilience.

Looking forward

Taken together, the contributions to the Research Topic highlight the intricate interactions between historical, biological, and environmental factors that shape current ASA biodiversity. They portray a landscape of change, where climate-driven ecological reorganization manifests in shifts in microbiome diversity, species distribution and community restructuring. These findings emphasize the pressing need for long-term monitoring, adaptive conservation strategies and governance frameworks that can bridge the gap between science and policy. By integrating insights across ecosystems and taxa, this article collection provides a vital scientific foundation for confronting the challenges that climate change poses to some of the planet's most fragile and unique ecosystems.

Author contributions

CM: Supervision, Writing – original draft, Conceptualization, Writing – review & editing, Validation, Data curation. HS: Methodology, Supervision, Validation, Writing – review & editing. JO: Validation, Data curation, Supervision, Writing – review & editing. PC: Supervision, Writing – review & editing, Investigation, Validation, Data curation. TC: Supervision, Writing – review & editing, Validation, Data curation.

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References

Coetzee, B. W. T., Convey, P., and Chown, S. L. (2017). Expanding the protected area network in Antarctica is urgent and readily achievable. *Conserv. Lett.* 10, 670–680. doi: 10.1111/conl.12342

IPBES (2019). *Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services*. Bonn, Germany: IPBES secretariat.

Lee, J. R., Terauds, A., Carwardine, J., Shaw, J. D., Fuller, R. A., Possingham, H. P., et al. (2022). Threat management priorities for conserving Antarctic biodiversity. *PLoS Biol.* 20, e3001921. doi: 10.1371/journal.pbio.3001921

Petsch, C., Da Rosa, K. K., De Oliveira, M. A. G., Velho, L. F., Silva, S. L. C., Sotille, M. E., et al. (2022). An inventory of glacial lakes in the South Shetland Islands (Antarctica): temporal variation and environmental patterns. *Ann. Braz. Acad. Sci.* 94, e20210683. doi: 10.1590/0001-3765202220210683

Tóth, A. B., Terauds, A., Chown, S. L., Hughes, K. A., Convey, P., Hodgson, D. A., et al. (2025). A dataset of Antarctic ecosystems in ice-free lands: classification, descriptions, and maps. *Sci. Data* 12, 133. doi: 10.1038/s41597-025-04424-y

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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.

KEYWORDS

maritime Antarctica, sub-Antarctic, South Georgia, *Laevilacunaria antarctica*, quaternary glaciations, postglacial recolonization, rafting, dispersal potential

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 dispersion, and has been recorded in the sea urchin *Sterechinus neumayeri* (Díaz 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

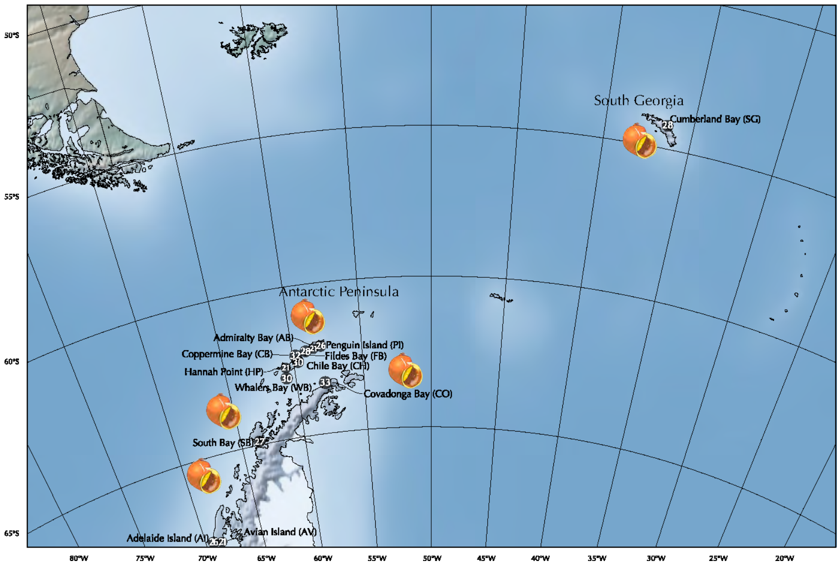

FIGURE 1
Sampling localities of *Laevilacunaria antarctica* populations across its distribution in maritime Antarctica (SSI/AP) and South Georgia (SG).

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 10^6 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 x 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**		**-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.

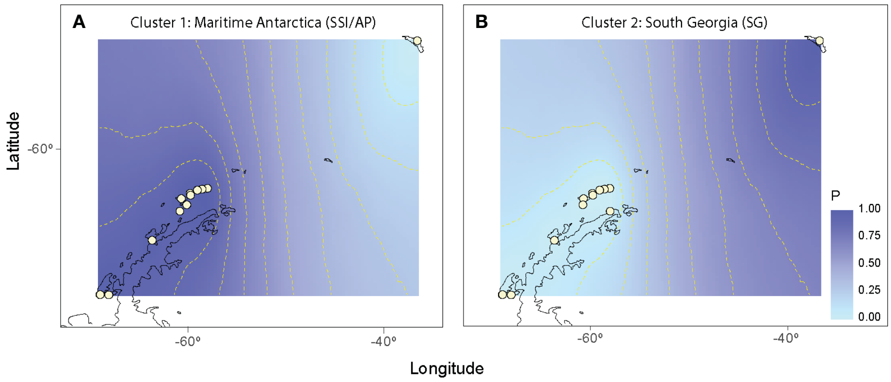

FIGURE 2
Spatial output from Geneland using *Laevilacunaria antarctica* populations. White circles indicate the relative positions of the sampling localities across maritime Antarctica and South Georgia. Darker and lighter shadings are proportional to posterior probabilities of membership to particular 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 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).

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***.

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

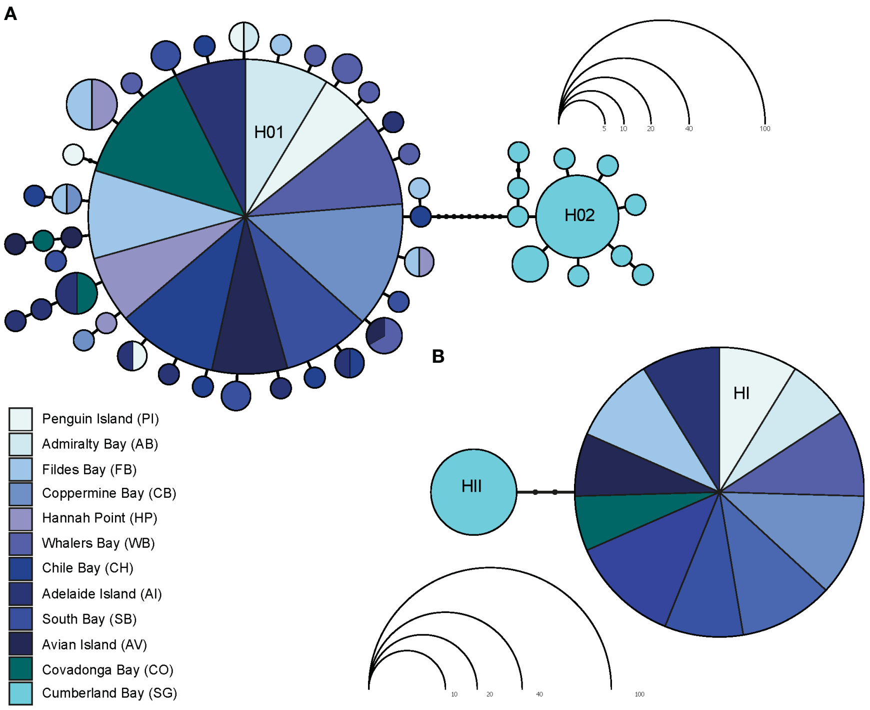

FIGURE 3
Parsimony mtDNA **(A)** and nucDNA **(B)** networks in *Laevilacunaria antarctica* across its distribution in maritime Antarctica and South Georgia. Each haplotype/allele is represented by a colored circle indicating the locality. The size the haplotypes/alleles is proportional to their frequencies.

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

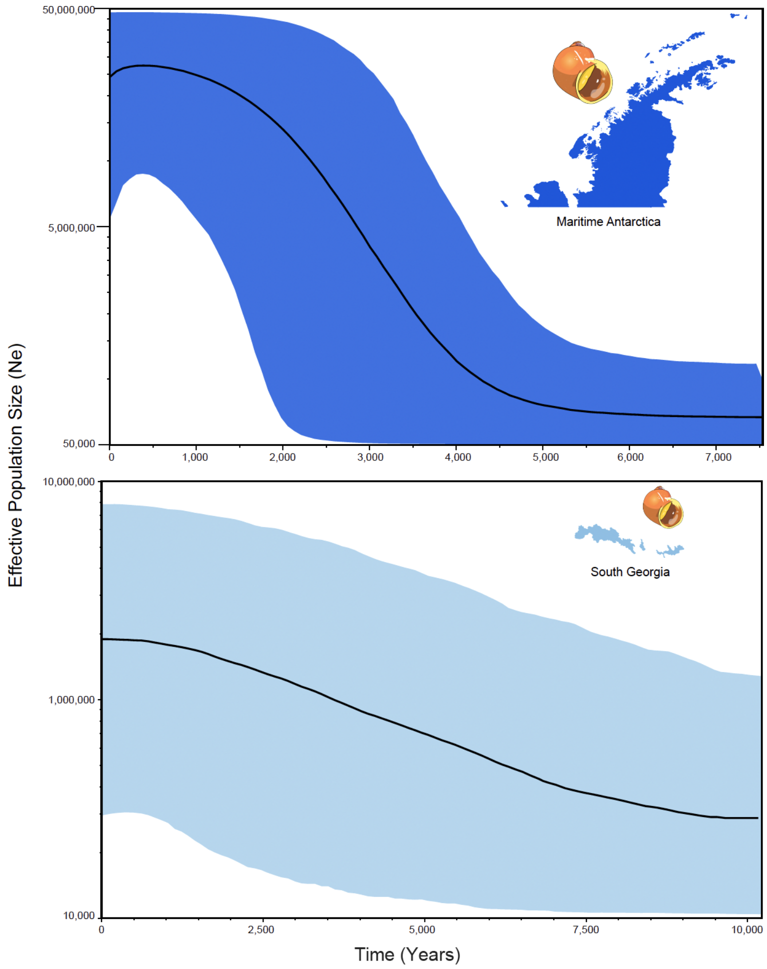

FIGURE 4
Historical demographic trajectories of the effective population sizes (N_e) estimated under a Bayesian skyline-plot approach based on COI sequences of *L. antarctica*. The y-axis represents the effective population size (N_e) while the x-axis shows the time since the present. The median estimates 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 notothenioid 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

References

Aljanabi, S. M., and Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res.* 25, 4692–4693. doi: 10.1093/nar/25.22.4692

Allcock, A. L., Barratt, I., Eléaume, M., Linse, K., Norman, M. D., Smith, P. J., 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 Res. Pt II* 58, 242–249. doi: 10.1016/j.dsr2.2010.05.016

Allcock, A. L., and Strugnell, J. M. (2012). Southern Ocean diversity: new paradigms from molecular ecology. *Trends Ecol. Evol.* 27, 520–528. doi: 10.1016/j.tree.2012.05.009

Amsler, C. D., Amsler, M. O., Curtis, M. D., McClintock, J. B., and Baker, B. J. (2019). Impacts of gastropods on epiphytic microalgae on the brown macroalga *Himantothallus grandifolius*. *Ant Sci.* 31, 89–97. doi: 10.1017/S095410219000014

Amsler, C. D., Miller, L. R., Edwards, R. A., Amsler, M. O., Engl, W., McClintock, J. B., et al. (2022). Gastropod assemblages associated with *Himantothallus grandifolius*, *Sarcopeltis Antarctica* and other subtidal macroalgae. *Ant Sci.* 34, 246–255. doi: 10.1017/S0954102022000153

Amsler, M. O., Huang, Y. M., Engl, W., McClintock, J. B., and Amsler, C. D. (2015). Abundance and diversity of gastropods associated with dominant subtidal macroalgae from western Antarctic Peninsula. *Polar Biol.* 38, 1171–1181. doi: 10.1007/s00300-015-1681-4

Arnaud, P. M., and Bandel, K. (1979). Comments on six species of marine Antarctic Littorinacea (Mollusca, Gastropoda). *Tethys* 8, 213–230.

Aronson, R. B., Thatje, S., Clarke, A., Peck, L. S., Blake, D. B., Wilga, C. D., et al. (2007). Climate change and invasibility of the Antarctic benthos. *Ann. Rev. Ecol. Evol. Syst.* 38, 129–154. doi: 10.1146/annurev.ecolsys.38.091206.095525

Baird, H. P., Miller, K. J., and Stark, J. S. (2011). Evidence of hidden biodiversity, ongoing speciation and diverse patterns of genetic structure in giant Antarctic amphipods. *Mol. Ecol.* 20, 3439–3454. doi: 10.1111/j.1365-294X.2011.05173.x

Baird, H. P., Shin, S., Oberprieler, R. G., Hullé, M., Vernon, P., Moon, K. L., et al. (2021). Fifty million years of beetle evolution along the Antarctic Polar Front. *Proc. Natl. Acad. Sci. U.S.A.* 118, e2017383118. doi: 10.1073/pnas.2017384118

Barker, P. F., Diekmann, B., and Escutia, C. (2007). Onset of cenozoic antarctic glaciation. *Deep-Sea Res. Pt II* 54, 2293–2307. doi: 10.1016/j.dsr2.2007.07.027

Barnes, D. K. A., Hodgson, D. A., Convey, P., Allen, C. S., and Clarke, A. (2006). Incursion and excursion of Antarctic biota: past, present and future. *Global Ecol. Biogeogr.* 15, 121–142. doi: 10.1111/j.1466-822x.2006.00216.x

Blakeslee, A. M. H., Whitman Miller, A., Ruiz, G. M., Johannesson, K., André, C., and Panova, M. (2021). Population structure and phylogeography of two North Atlantic *Littorina* species with contrasting larval development. *Mar. Biol.* 168, 117. doi: 10.1007/s00227-021-03918-8

Brey, T., Dahm, C., Gorny, M., Klages, M., Stiller, M., and Arntz, W. E. (1996). Do Antarctic benthic invertebrates show an extended level of eurybathy? *Ant Sci.* 8, 3–6.

Cakil, Z. V., Garlasché, G., Iakovenko, N., Di Cesare, A., Eckert, E. M., Guidetti, R., et al. (2021). Comparative phylogeography reveals consistently shallow genetic diversity in a mitochondrial marker in Antarctic bdelloid rotifers. *J. Biogeogr.* 48, 1797–1809. doi: 10.1111/jbi.14116

Cantera, J. R., and Arnaud, P. M. (1984). Les gastéropodes prosobranches des Iles Kerguelen et Crozet (Sud de L'Océan Indien) comparaison écologique et particularités biologiques. *CNFRA [Comité Natl. Francais Recherches Antarctiques]* 56, 1–170.

Chenuil, A., Saucède, T., Hemery, L. G., Eléaume, M., Féral, J.-P., Améziane, N., et al. (2018). Understanding processes at the origin of species flocks with focus on the marine Antarctic fauna. *Biol. Rev.* 93, 481–504. doi: 10.1111/brv.12354

Clarke, A., and Crame, J. A. (1989). The origin of the Southern Ocean marine fauna. *Geol. Soc London* 47, 253–268. doi: 10.1144/GSL.SP.1989.047.01.19

Clarke, A., and Crame, J. (1992). The Southern-Ocean benthic fauna and climate change – a historical-perspective. *Philos. T R. Soc B* 338, 299–309. doi: 10.1098/rstb.1992.0150

Clarke, A., and Crame, J. (2010). Evolutionary dynamics at high latitudes: speciation and extinction in polar marine faunas. *Phil Trans. R. Soc B* 365, 3655–3666. doi: 10.1098/rstb.2010.0270

Convey, P., Stevens, M. I., Hodgson, D. A., Smellie, J. L., Hillenbrand, C.-D., Barnes, D. K. A., et al. (2009). Exploring biological constraints on the glacial history of Antarctica. *Quat. Sci. Rev.* 28, 3035–3048. doi: 10.1016/j.quascirev.2009.08.015

Crame, J. (2018). Key stages in the evolution of the Antarctic marine fauna. *J. Biogeogr.* 45, 986–994. doi: 10.1111/jbi.13208

Cumming, R. A., Nikula, R., Spencer, H. G., and Waters, J. M. (2014). Transoceanic genetic similarities of kelp-associated sea slug populations: long-distance dispersal via rafting? *J. Biogeogr.* 41, 2357–2370. doi: 10.1111/jbi.12376

Dambach, J., Thatje, S., Rödder, D., Basher, Z., and Raupach, M. J. (2012). Effects of Late-Cenozoic glaciation on habitat availability in Antarctic benthic shrimps (Crustace: Decapoda: Caridae). *PloS One* 7, e46283. doi: 10.1371/journal.pone.0036283

Damerau, M., Marschiner, M., Salzburger, W., and Hanel, R. (2012). Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica. *Polar Biol.* 35, 1073–1086. doi: 10.1007/s00300-012-1155-x

Damerau, M., Salzburger, W., and Hanel, R. (2014). Population genetic structure of Lepidonotothen larseni revisited: cyb and microsatellites suggest limited connectivity in the Southern Ocean. *Mar. Ecol. Prog. Ser.* 517, 251–263. doi: 10.3354/meps11061

Díaz, A., Féral, J.-P., David, B., Saucède, T., and Poulin, E. (2011). Evolutionary pathways among shallow and deep-sea echinoids of the genus *Sterechinus* in the Southern Ocean. *Deep Sea Res. Pt II* 58, 205–211. doi: 10.1016/j.dsr2.2010.10.012

Díaz, A., Gérard, K., González-Wevar, C., Maturana, C., Féral, J.-P., David, B., et al. (2018). Genetic structure and demographic inference of the regular sea urchin *Sterechinus neumayeri* (Meissne 1900) in the Southern Ocean: The role of the last glaciation. *PloS One* 13, e0197611. doi: 10.1371/journal.pone.0197611

Doellman, M. M., Trussel, G. C., Grahame, J. W., and Vollmer, S. V. (2011). Phylogeographic analysis reveals a deep lineage split within North Atlantic *Littorina saxatilis*. *Proc. R. Soc B* 278, 3175–3183. doi: 10.1098/rspb.2011.0346

Dornburg, A., Eytan, R. I., Federman, S., Pennington, J. N., Stewart, A. L., Jones, C. D., et al. (2016). Molecular data support the existence of two species of the Antarctic fish genus *Cryodraco* (Channicthyidae). *Polar Biol.* 39, 1369–1379. doi: 10.1007/s00300-015-1859-9

Drummond, A. J., and Rambaut, A. (2007). BEAST: bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214. doi: 10.1186/1471-2148-7-214

Drummond, A. J., Suchard, M. A., Xie, D., and Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973. doi: 10.1093/molbev/mss075

Dueñas, L. F., Tracey, D. M., Crawford, A. J., Wilke, T., Alderslade, P., and Sánchez, J. A. (2016). The Antarctic Circumpolar Current as a diversification trigger for deep-sea octocorals. *BMC Evol. Biol.* 16, 2. doi: 10.1186/s12862-015-0574-z

Dupanloup, I., Schneider, S., and Excoffier, L. (2002). A simulated annealing approach to define the genetic structure of populations. *Mol. Ecol.* 11, 2571–2581. doi: 10.1046/j.1365-294x.2002.01650.x

Edgar, R. C. (2004). MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinf.* 5, 113. doi: 10.1186/1471-2105-5-113

Engl, W. (2012). *Shells of Antarctica* Hackenheim, Conchbooks Publishing, 402pp.

Excoffier, L., Laval, G., and Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol. Bioinform. Online* 1, 37–50. doi: 10.1177/117693430500100003

Fassio, G., Modica, M. V., Alvaro, M. C., Buge, B., Salvi, D., Oliverio, M., et al. (2019). An Antarctic flock under the Thorson's rule: Diversity and larval development of Antarctic Velutinidae (Mollusca: Gastropoda). *Mol. Phylogenet. Evol.* 132, 1–13. doi: 10.1016/j.ympev.2018.11.017

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biotech* 3, 294–299.

Forster, P., Torroni, A., Renfrew, C., and Röhl, A. (2001). Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. *Mol. Biol. Evol.* 18, 1864–1881. doi: 10.1093/oxfordjournals.molbev.a003728

Fraser, C. I., Nikula, R., Ruzzante, D. E., and Waters, J. M. (2012). Poleward bound: biological impacts of Southern Hemisphere glaciation. *Trends Ecol. Evol.* 27, 462–471. doi: 10.1016/j.tree.2012.04.011

Fraser, C. I., Nikula, R., Spencer, H. G., and Waters, J. M. (2009). Kelp genes reveal effects of subantarctic sea during the Last Glacial Maximum. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3249–3253. doi: 10.1073/pnas.0810635106

Fraser, C. I., Zuccarello, G. C., Spencer, H. G., Salvatore, L. C., Garcia, G. R., Waters, J. M., et al (2013). Genetic affinities between trans-oceanic populations of non-buoyant macroalgae in the high latitudes of the southern hemisphere. *PLoS ONE* 8, e69138. doi:10.1371/journal.pone.0069138

Fraser, C. I., Terauds, A., Smellie, J., Convey, P., and Chown, S. L. (2014). Geothermal activity helps life survive glacial cycles. *Proc. Natl. Acad. Sci. U.S.A.* 111, 5634–5639. doi: 10.1073/pnas.1321437111

Frugone, M. J., López, M. E., Segovia, N. I., Cole, T. L., Lowther, A., Pistorius, P., et al. (2019). More than the eye can see: Genomic insights into the drivers of genetic differentiation in Royal/Macaroni penguins across the Southern Ocean. *Mol. Phylogenet. Evol.* 139, 106563. doi: 10.1016/j.ympev.2019.106563

González-Wevar, C. A., Hüne, M., Cañete, J. I., Mansilla, A., Nakano, T., and Poulin, E. (2012). Towards a model of postglacial biogeography in shallow marine species along the Patagonian province: lessons from the limpet *Nacella magellanica* (Gmelin 1791). *BMC Evol. Biol.* 12, 139. doi: 10.1186/1471-2148-12-139

González-Wevar, C. A., Nakano, T., Cañete, J. I., and Poulin, E. (2011). Concerted genetic, morphological and ecological diversification in *Nacella* limpets in the Magellanic Province. *Mol. Ecol.* 20, 1936–1951. doi: 10.1111/j.1365-294X.2011.05065.x

González-Wevar, C. A., Saucède, T., Morley, T., Chown, S. L., and Poulin, E. (2013). 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. *Mol. Ecol.* 22, 5221–5236. doi: 10.1111/mec.12465

González-Wevar, C. A., Hüne, M., Segovia, N. I., Nakano, T., Spencer, H. G., Chown, S. L., et al (2017). Following the Antarctic Circumpolar Current: patterns and processes in the biogeography of the limpet Nacella(Mollusca: Patellogastropoda) across the Southern Ocean. *J. Biogeogr.* 44, 861–874. doi: 10.1111/jbi.12908

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Maturana, C. S., Jeldres, V., Pinochet, R., et al. (2022). Seven snail species hidden in one: Biogeographic diversity in an apparently widespread periwinkle in the Southern Ocean. *J. Biogeogr.* 49, 1521–1534. doi: 10.1111/jbi.14453

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Noll, D., Maturana, C. S., Hüne, M., et al. (2021). Contrasting biogeographical patterns in *Margarella* (Gastropoda: Calliostomatidae: Margarellinae) across the Antarctic Polar Front. *Mol. Phylogenet. Evol.* 156, 107039. doi: 10.1016/j.ympev.2020.107039

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Ojeda, J., Hüne, M., Naretto, J., et al. (2018). Unexpected absence of island endemics: Long-distance dispersal in higher latitude sub-Antarctic *Siphonaria* (Gastropoda: Euthyneura) species. *J. Biogeogr.* 45, 874–884. doi: 10.1111/jbi.13174

Guillemin, M.-L., Dubrasquet, H., Reyes, J., and Valero, M. (2018). Comparative phylogeography of six red algae along the Antarctic Peninsula: extreme genetic depletion linked to historical bottlenecks and recent expansion. *Polar Biol.* 41, 827–837. doi: 10.1007/s00300-017-2244-7

Guillot, G., Mortier, F., and Estoup, A. (2005). GENELAND: a computer package for landscape genetics. *Mol. Ecol. Notes* 5, 712–715. doi: 10.1111/j.1471-8286.2005.01031.x

Halanych, K. M., and Mahon, A. R. (2018). Challenging the dogma concerning biogeographic patterns of Antarctica and the Southern Ocean. *Annu. Rev. Ecol. Evol. Syst.* 49, 355–378. doi: 10.1146/annurev-ecolsys-121415-032139

Ho, S. Y. W., Lanfear, R., Bromham, L., Phillips, M. J., Soubrier, J., Rodrigos, A. G., et al. (2011). Time-dependent rates of molecular evolution. *Mol. Ecol.* 20, 3087–3101. doi: 10.1111/j.1365-294X.2011.05178.x

Ho, S. Y. W., Phillips, M. J., Cooper, A., and Drummond, A. J. (2005). Time dependency of molecular rate estimated and systematic overestimation of recent divergence times. *Mol. Biol. Evol.* 22, 1561–1568. doi: 10.1093/molbev/msi145

Ho, S. Y. W., Shapiro, B., Phyllips, M. J., Cooper, A., and Drummond, A. J. (2007). Evidence for time dependency of molecular rate estimates. *Syst. Biol.* 56, 517–522. doi: 10.1080/10635150701435401

Hoffman, J. I., Peck, L. S., Hillyard, G., Zieritz, A., and Clark, M. S. (2010). No evidence for genetic differentiation between Antarctic limpet *Nacella concinna* morphotypes. *Mar. Biol.* 157, 765–778. doi: 10.1007/s00227-009-1360-5

Hoffman, J. I., Peck, L. S., Linse, K., and Clarke, A. (2011). Strong population genetic structure in a broadcast-spawning Antarctic marine invertebrate. *J. Hered* 102, 55–66. doi: 10.1093/jhered/esq094

Hudson, R. R. (2000). A new statistic for detecting genetic differentiation. *Genetics* 155, 2011–2014. doi: 10.1093/genetics/155.4.2011

Hüne, M., González-Wevar, C., Poulin, E., Mansilla, A., Fernández, D. A., and Barrera-Oro, E. (2015). Low levels of genetic divergence between *Harpagifer* fish species (Perciformes: Notothenioidei) suggests a Quaternary colonization of Patagonia from the Antarctic Peninsula. *Polar Biol.* 38, 607–617. doi: 10.1007/s00300-014-1623-6

Ihaka, R., and Gentleman, R. (1996). R: A language for data analysis and graphics. *J. Comp. Graph Stat.* 5, 299–314. doi: 10.2307/1390807

Iken, K. (1999). Feeding ecology of the Antarctic herbivorous gastropod *Laevilacunaria Antarctica* Martens. *J. Exp. Mar. Biol. Ecol.* 236, 133–148. doi: 10.1016/S0022-0981(98)00199-3

Ingólfson, Ó. (2004). "Quaternary glacial and climate history of Antarctica," in *Quaternary glaciations – extent and chronology, part III: South America, Asia, Australia and Antarctica*. Eds. J. Ehlers and P. L. Gibbard (Development in Quaternary Science. Elsevier ScienceDirect), 3–43.

Krabbe, K., Leese, F., Mayer, C., Tollrian, R., and Held, C. (2010). Cryptic mitochondrial lineages in the widespread pycnogonid *Colossendeis megalonyx* Hoek 1881 from Antarctic and Subantarctic waters. *Polar Biol.* 33, 281–292. doi: 10.1007/s00300-009-0703-5

Lau, S. C. Y., Wilson, N. G., Silva, C. N. S., and Strugnell, J. M. (2020). Detecting glacial refugia in the Southern Ocean. *Ecography* 43, 1–18. doi: 10.1111/ecog.04951

Levicoy, D., Flores, K., Rosenfeld, S., and Cárdenas, L. (2021). Phylogeography and genetic diversity of the microbivalve *Kidderia subquadrata*, reveals new data from West Antarctic Peninsula. *Sci. Rep.* 11, 5705. doi: 10.1038/s41598-021-85042-7

Librado, P., and Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452. doi: 10.1093/bioinformatics/btp187

Littlewood, D. T. J., Curini-Galletti, M., and Herniou, E. A. (2000). The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Mol. Phylogenet. Evol.* 16, 449–466. doi: 10.1006/mpev.2000.0802

Maggs, C. A., Castilho, R., Foltz, D., Henzler, C., Jolly, M. T., Kelly, J., et al. (2008). Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. *Ecology* 89, S108–S122. doi: 10.1890/08-0257.1

Marko, P. B. (2004). 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropod with identical dispersal potential. *Mol. Ecol.* 13, 597–611. doi: 10.1046/j.1365-294X.2004.02096.x

Marko, P. B., Hoffman, J. M., Emme, S. A., McGovern, T. M., Keever, C. C., and Cox, N. (2010). The 'Expansion–Contraction' model of Pleistocene biogeography: rocky shores suffer a sea change? *Mol. Ecol.* 19, 146–167. doi: 10.1111/j.1365-294X.2009.04417.x

Martens van, E., and Pfeffer, G. (1886). Die Mollusken von Süd–Georgien nach der Ausbeute der Deutschen Station 1882–83. *Jahr Hamb Wiss. Anst* 3, 65–135.

Martin, A., Miloslavich, P., Díaz, Y., Ortega, I., Klein, E., Troncoso, J., et al. (2016). Intertidal benthic communities associated with the macroalgae *Iridaea cordata* and *Adenocystis utricularis* in King George Island, Antarctica. *Polar Biol.* 39, 207–220. doi: 10.1007/s00300-015-1773-1

Matschiner, M., Hanel, R., and Salzburger, W. (2009). Gene flow by larval dispersal in the Antarctic notothenioid fish *Gobionotothen gibberifrons*. *Mol. Ecol.* 18, 2574–2587. doi: 10.1111/j.1365-294X.2009.04220.x

Maturana, C. S., Segovia, N. I., González-Wevar, C. A., Díaz, A., Rosenfeld, S., Poulin, E., et al. (2020). Evidence of strong small-scale population structure in the Antarctic freshwater copepod *Boeckella poppei* in lakes on Signy Island, South Orkney Islands. *Limnol Oceanogr* 65, 2024–2040. doi: 10.1002/lno.11435

McCay, R. M., Barrett, P. J., Levy, R. S., Naish, T. R., Golledge, N. R., and Pyne, A. (2016). Antarctic Cenozoic climate history from sedimentary records: ANDRILL and beyond. *Phil Trans. R. Soc A* 374, 20140301. doi: 10.1098/rsta.2014.0301

McGaughran, A., Stevens, M. I., Hogg, I. D., and Carapelli, A. (2011). Extreme glacial legacies: A synthesis of the Antarctic springtail phylogeographic record. *Insects* 2, 62–82. doi: 10.3390/insects2020062

Moon, K. L., Chown, S. L., and Fraser, C. I. (2017). Reconsidering connectivity in the sub-Antarctic. *Biol. Rev.* 92, 2164–2181. doi: 10.1111/brv.12327

Narum, S. R. (2006). Beyond Bonferroni: Less conservative analyses for conservation genetics. *Cons Genet.* 7, 783–787. doi: 10.1007/s10592-005-9056-y

Near, T. J., Dornburg, A., Kuhn, K. L., Eastman, J. T., Pennington, J. N., Patarnello, T., et al. (2012). Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3434–3439. doi: 10.1073/pnas.1115169109

Nikula, R., Fraser, C. I., Spencer, H. G., and Waters, J. M. (2010). Circumpolar dispersal by rafting in two subantarctic kelp-dwelling crustaceans. *Mar. Ecol-Prog Ser.* 405, 221–230. doi: 10.3354/meps08523

Nylander, J. A. A. (2004). *MrModeltest Version 2. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University, Uppsala.*

Parker, R. W., Paige, K. N., and DeVries, A. L. (2002). Genetic variation among populations of the Antarctic toothfish: evolutionary insights and implications for conservation. *Polar Biol.* 25, 256–261. doi: 10.1007/s00300-001-0333-z

Pearse, J. S., Mooi, R., Lockhart, S. J., and 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. I. Krupnik, M. A. Lang and S. E. Miller Smithsonian Institution Scholarly Press, Washington, DC USA.

Pollard, D., and DeConto, R. M. (2009). Modeling West Antarctic ice sheet growth and collapse through the past five million years. *Nature* 458, 329–332. doi: 10.1038/nature07809

Pons, O., and Petit, R. J. (1996). Measuring and testing genetic differentiation with ordered Versus unordered alleles. *Genetics* 144, 1237–1245. doi: 10.1093/genetics/144.3.1237

Powell, A. W. B. (1951). Antarctic and subantarctic mollusca: pelecypoda and gastropoda. *Discovery Rep.* 26, 47–196. doi: 10.5962/bhl.part.16335

Preston, H. B. (1916). Descriptions of eight new species of marine Mollusca from the South Shetland Islands. *Ann. Mag Nat. Hist* 18, 269–272. doi: 10.1080/00222931608693847

Provan, J., and Bennett, K. D. (2008). Phylogeographic insights into cryptic glacial refugia. *Trends Ecol. Evol.* 23, 564–571. doi: 10.1016/j.tree.2008.06.010

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., and Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer. *Syst. Biol.* 67, 901–904. doi: 10.1093/sysbio/syy032

Raupach, M. J., Thatje, S., Dambach, J., Rehm, P., Misof, B., and Leese, F. (2010). Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes*. *Mar. Biol.* 157, 1783–1797. doi: 10.1007/s00227-010-1451-3

Reid, D. G., Dyal, P., and Williams, S. T. (2012). A global molecular phylogeny of 147 periwinkle species (Gastropoda, Littorininae). *Zool. Scripta* 41, 125–136. doi: 10.1111/j.1463-6409

Riesgo, A., Taboada, S., and Avila, C. (2015). Evolutionary patterns in Antarctic marine invertebrates: An update on molecular studies. *Mar. Genomics* 23, 1–13. doi: 10.1016/j.margen.2015.07.005

Rogers, A. R., and Harpending, H. C. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol.* 9, 552–569. doi: 10.1093/oxfordjournals.molbev.a04727

Rosenfeld, S., Segovia, N. I., Maturana, C. S., Aldea, C., Saucède, T., Brickle, P., et al. (2023). A revision of the higher latitude periwinkle species *Laevilitorina caliginosa* sensu lato. *Zool. J. Linn. Soc.*. (in press). doi: 10.1093/zoolinnean/zlad171

Salzburger, W., Ewing, G. B., and Von Haessler, A. (2011). The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Mol. Ecol.* 20, 1952–1963. doi: 10.1111/j.1365-294X.2011.05066.x

Schmider-Martínez, A., Maturana, C. S., Poveda, Y., López-Farran, Z., Saucède, T., Poulin, E., et al. (2023). Laevilacunaria (Mollusca: Gastropoda) in the Southern Ocean: a comprehensive occurrence dataset. *Biodivers. Data J.* 11, e111982.

Silva, S. E., Silva, I. C., Madeira, C., Sallema, R., Paulo, O. S., and Paula, J. (2013). Genetic and morphological variation in two littorinid gastropods: evidence for recent population expansions along the East African coast. *Biol. J. Linn. Soc* 108, 494–508. doi: 10.1111/j.1095-8312.2012.02041.x

Simone, L. R. (2018). Convergence with naticids: phenotypic phylogenetic study on some Antarctic littorinoideans, with description of the zerotulid new genus *Pseudonatica*, and its presence in Brazil (Mollusca, Caenogastropoda). *J. Mar. Biol. Ass UK* 98, 1365–1381. doi: 10.1017/S002531541700025X

Simpson, R. D., and Harrington, S. A. (1985). Egg masses of three gastropods, *Kerguelenella lateralis* (Siphonariidae), *Laevilitorina caliginosa* and *Macquariella hamiltoni* (Littorinidae), from Macquarie Island (sub-Antarctic). *J. Malacol Soc Aust.* 7, 17–28. doi: 10.1080/00852988.1985.10673973

Smith, E. A. (1879). Mollusca. An account of the petrological, botanical and zoological collection made in Kerguelen´s Island and Rodriguez during the Transit of "Venus" Expedition in the years 1874–75. *Philos. Trans. R. Soc Lond. B* 168, 167–192.

Sotelo, G., Duvetorp, M., Costa, D., Panova, M., Johannesson, K., and Faria, R. (2020). Phylogeographic history of flat periwinkles, *Littorina fabalis* and *L. obtusata*. *BMC Evol. Biol.* 20, 23. doi: 10.1186/s12862-019-1561-6

Suchard, M. A., Weiss, R. E., and Sinsheimer, J. S. (2001). Bayesian selection of continuous-time Markov Chain evolutionary models. *Mol. Biol. Evol.* 18, 1001–1013. doi: 10.1093/oxfordjournals.molbev.a003872

Tamura, K., Stecher, G., and Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 38, 3022–3027. doi: 10.1093/molbev/msab120

Thatje, S., Hillenbrand, C.-D., and Larter, R. (2005). On the origin of Antarctic marine benthic community structure. *Trends Ecol. Evol.* 20, 534–540. doi: 10.1016/j.tree.2005.07.010

Thatje, S., Hillenbrand, C.-D., Mackensen, A., and Larter, R. (2008). Life hung by a thread: endurance of Antarctic fauna in glacial periods. *Ecology* 89, 682–689. doi: 10.1890/07-0498.1

Thornhill, D. J., Mahon, A. R., Norenburg, J. L., and Halanych, K. (2008). Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nemertea: Lineidae). *Mol. Ecol.* 17, 5104–5117. doi: 10.1111/j.1365-294X.2008.03970.x

Vianna, J. A., Noll, D., Dantas, G. P. M., Petry, M. V., Barbosa, A., González-Acuña, D., et al. (2017). Marked phylogeographic structure of Gentoo penguin reveals an ongoing diversification process along the Southern Ocean. *Mol. Phylogenet. Evol.* 107, 486–498. doi: 10.1016/j.ympev.2016.12.003

Waters, J. M. (2008). Driven by the West Wind Drift? A synthesis of southern temperate marine biogeography, with new directions for dispersalism. *J. Biogeogr.* 35, 417–427. doi: 10.1111/j.1365-2699.2007.01724.x

Williams, S. T., and Reid, D. G. (2004). Speciation and diversity on tropical rocky shores: a global phylogeny of snail of the genus *Echinolittorina*. *Evolution* 58, 2227–2251. doi: 10.1111/j.0014-3820.2004.tb01600.x

Williams, S. T., Reid, D. G., and Littlewood, D. T. J. (2003). A molecular phylogeny of the Littorininae (Gastropoda: Littorinidae): unequal evolutionary rates, morphological parallelism, and biogeography of the Southern Ocean. *Mol. Phylogenet. Evol.* 28, 60–86. doi: 10.1016/S1055-7903(03)00038-1

Wilson, N. G., Hunter, R. L., Lockhart, S. J., and Halanych, K. M. (2007). Multiple lineage and absence of panmixia in the "circumpolar" crinoid *Promachocrinus kerguelensis* from the Atlantic sector of Antarctica. *Mar. Biol.* 152, 895–904. doi: 10.1007/s00227-007-0742-9

Wilson, N. G., Schrödl, M., and Halanych, K. M. (2009). Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). *Mol. Ecol.* 18, 965–984. doi: 10.1111/j.1365-294X.2008.04071.x

Xia, X., and Xie, Z. (2001). DAMBE: Software package for data analysis in Molecular Biology and Evolution. *J. Hered.* 92, 371–373. doi: 10.1093/jhered/92.4.371

Young, E. F., Belchier, M., Hauser, L., Horsburg, G. J., Meredith, M. P., Murphy, E. J., et al. (2015). Oceanography and life history predict contrasting genetic population structure in two Antarctic fish species. *Evol. Appl.* 8, 486–509. doi: 10.1111/eva.12259

Zachos, J., Pagani, M., Sloan, L., Thomas, E., and Billups, K. (2001). Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292, 686–693. doi: 10.1126/science.1059412

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Measuring the impact of wharf construction on the Antarctic benthos

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Shallow water Antarctic marine macroepifaunal assemblages live in one of the most naturally disturbed marine environments due to the impact of icebergs scouring the seafloor. They are, however, amongst the least anthropogenically impacted assemblages and are afforded protection under the Antarctic Treaty system. When the British Antarctic Survey's Rothera Research Station wharf needed extending to accommodate the newly constructed UK polar research vessel, the RRS *Sir David Attenborough*, a Comprehensive Environmental Evaluation (CEE) was conducted to assess the impact. The macroepifaunal likely to be impacted by the construction was surveyed through ROV videos of five transects, centered on the middle of the construction zone, from 10–100 m deep. A pre-construction survey was completed in March 2017, as part of the CEE impact assessment, and a post-build survey in 2022 (delayed from 2021, and reduced in scope, due to the COVID-19 pandemic). Sedimentation rates were also measured before and during construction and were high during the second summer when the wharf pilings were being back filled with crushed rock. The measured differences between pre- and post-construction assemblages were minor and were not reflected in the overall number of taxa (operational taxonomic units – OTU), or diversity, but there were subtle shifts in species composition. The largest differences in the macroepifauna were a reduction in the number of the common urchin, *Sterechinus neumayeri*, and seastar, *Odontaster validus*, and were within expected variability. The small changes detected in the macroepifauna indicate it was minimally impacted and/or recovered in the subsequent two years, therefore during wharf construction the accompanying mitigation measures were robust.

KEYWORDS

disturbance, Antarctic, Antarctic Treaty, environmental impact, construction

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1 Introduction

The Antarctic and sub-Antarctic regions are often viewed as isolated from direct anthropogenic disturbance due to their remoteness (Rogers et al., 2020); however, Southern Ocean ecosystems have experienced major human impacts over many hundreds of years (see Bonner and Laws, 1964, Forcada and Staniland, 2009 and Rogers et al., 2020). There is a strong case, however, that Antarctic shallows (continental shelf >500 m) are among the least historically anthropogenically impacted ecosystems (Zwerschke et al., 2021), and therefore require protection and measures to prevent additional impact. Anthropogenic disturbance is regulated through the Antarctic Treaty system, and specifically the Protocol on Environmental Protection to the Antarctic Treaty (entered into force 1998). Signatories to the Protocol agreed to comply with the Annex I to the Protocol that requires a Comprehensive Environmental Evaluation (CEE) to be undertaken and notified to all Parties before the commencement of any project that is likely to have an impact considered to be greater than minor or transitory (see https://www.ats.aq/e/protocol.html).

The shallow Antarctic benthos is one of the most naturally disturbed environments on Earth, due to frequent iceberg scour disturbance (Smale, 2008). Iceberg scour is caused when the keel of an iceberg (or any ice of sufficient size) impacts the seafloor (Convey et al., 2014) causing mass mortality in the zoobenthos (Barnes, 2017). The frequency of iceberg scour is typically higher in the shallows and lower at depth, but with far greater impact per scour, due to the scarcity of larger icebergs (Barnes and Conlan, 2007). Iceberg scour, therefore, presents a particular challenge to disentangling any anthropogenic impact from the natural stochastic background of high intensity natural disturbance.

This study reports the findings of a CEE for the extension of the Biscoe Wharf at the British Antarctic Survey Rothera Research Station (67° 34’ S, 68° 08’ W) to accommodate the UK’s new Polar research vessel, the RSS *Sir David Attenborough* (Fothergill, 2018). This new vessel is longer and has a deeper draft than the vessels it replaced (Rogan-Finnemore et al., 2021) and so the wharf had to be extended to accommodate the ship (Figure 1).

The sea floor adjacent to the Biscoe Wharf (~10 m depth) is subject to high natural disturbance from frequent and consistent iceberg scour (e.g., see Smale et al., 2006). Further from the wharf, the sea floor drops steeply away into deep water, at an approximate 45° angle. The Biscoe wharf experiences periods of seasonal sea ice, long period light and darkness during austral summer and winter respectively and stable temperatures year round, as has been reported from approximately 30 years of year round measurements (review available here: Venables et al., 2023) Due to the bathymetry of the site, it was also important to measure any impact on macroepifauna composition at depths below the site of wharf construction as rockfall and increased sedimentation into the water column could have impacted the deeper macroepifaunal assemblage. At these greater depths, where the sea floor is exposed to lower rates of iceberg scour disturbance, the macroepifaunal assemblage consists of a slower growing, less

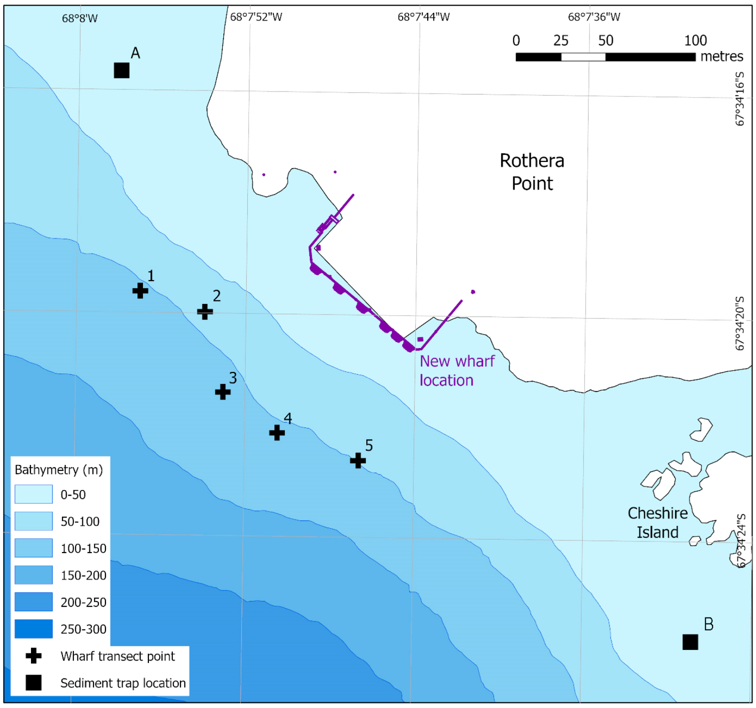

FIGURE 1
The location of the Biscoe Wharf extension at Rothera Research Station, showing the original and new (in purple) wharf footprint. Crosses 1–5 indicate the deepest point from which the five wharf transects started. The transects ran perpendicular to the wharf (NE), finishing at 10 m depth. A and B indicate locations of the two sediment traps. Coastline data from the SCAR Antarctic Digital Database, 2022. Bathymetry from Retallick et al. (2021).

disturbance-resilient, species (Robinson et al., 2021). Deeper water assemblages may, therefore, not be able to recover as rapidly as the more disturbance-tolerant species found in the shallows (Robinson et al., 2020, 2022).

Across the study area, previous studies (see Robinson et al., 2021) have found that between 10–30 m depth, the macroepifauna are dominated by mobile grazers, coincident with highest abundances of encrusting and macroalgae species. Between 40 and 60 m depth there is a combination of sessile suspension feeders including ascidians and anthozoans, as well as mobile grazers and scavengers such as ophiuroids and asteroids. At depths between 70–100 m sessile suspension feeders dominate in particular bryozoans and porifera. The macroepifaunal assemblages however show no distinct depth zonation, with multiple species having wide depth ranges that can encompass 10–100 m depth and high spatial heterogeneity (Smale et al., 2008), with the general trends described above often overlapping and combining. In this study, we aimed to measure the impact of the extension of the Biscoe Wharf construction on the surrounding Antarctic benthos (Figure 2). To describe any changes to macroepifauna composition pre- and post-construction video transects were recorded adjacent to, and alongside, the wharf construction sites. Due to the rocky substratum and depth of the site meiofauna and infauna assemblages were not sampled. Sediment traps were also deployed to monitor any changes in sedimentation rates associated with construction. Any changes detected were considered within the context of the naturally high levels of iceberg disturbance and natural macroepifauna variability seen within this environment (Robinson et al., 2021).

2 Materials and methods

2.1 Macroepifauna video transects

A pre-construction video survey was completed in March 2017 to provide baseline assemblage data for the CEE. A post build survey was completed in March 2022 to assess the impact of the construction on the benthic assemblage. This survey was delayed by one year, from 2021, and reduced to 50% of the original scope, due to constraints resulting from the COVID-19 pandemic (Hughes and Convey, 2020). The survey was conducted using a Deeptrekkor DTG2 ROV (Remotely Operated Vehicle) which recorded video transects from 0–100 m depth along five transects centered on the wharf construction zone and covering the area that was expected to be impacted by the construction (Figure 1). Video was recorded on the DTG2's internal 1/3 Sony CCD colour 700HD camera, lit by the inbuilt 1350 lumen lights with an additional pair of SOLA600 lights providing a total of 2550 lumen.

From the videos, abundance was scored in the range 0–10 by summing the presence or absence of each OTU (Operational Taxonomic Unit)in every one meter increment, within each 10 m depth interval. For example, OTUs present at every meter within the 10–20 m depth interval would be scored 10, whereas OTUs present at only three depths would be scored 3. This provided a rapid and repeatable way of measuring abundance, while preventing hyper-abundant OTUs, or species that occur in clusters, from dominating the analysis.

For each sample all mega and macrofauna were identified to the lowest possible taxonomic resolution; however, due to no specimens

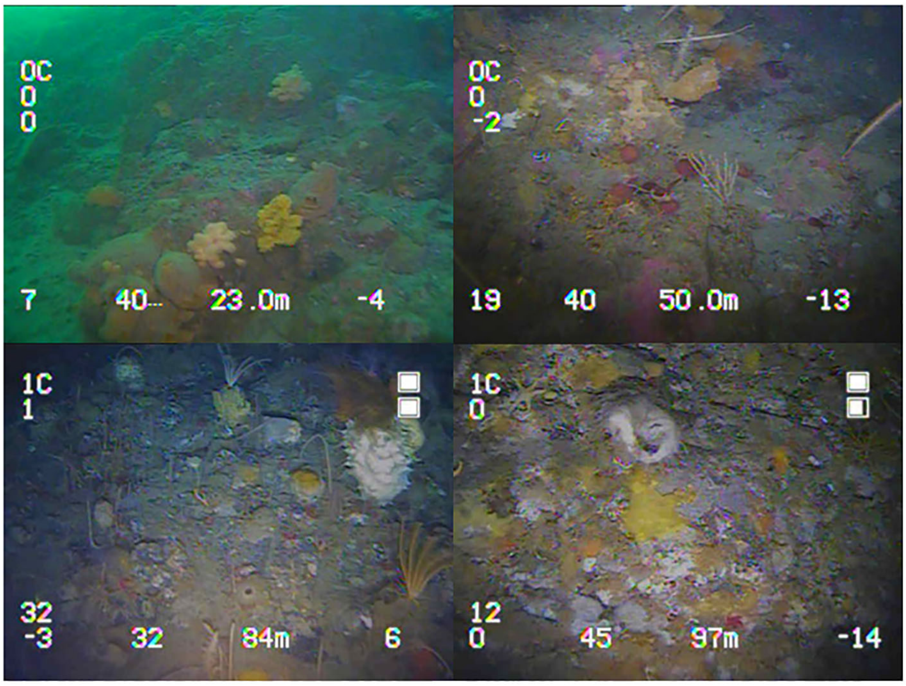

FIGURE 2
Frames from the video of the benthic macroepifaunal assemblages captured during ROV wharf surveys, from top right moving clockwise 23 m, 50 m, 97 m and 84 m depth.

being retrieved for identification and the constraints of video resolution, a reduced and modified OTU guide was adopted (see Supplementary Table S1 – OTU guide). The benthic biota of the study area has been well-characterised, many collected and an extensive catalogue of high-quality benthic images has been created, and so OTUs in video images could be identified and grouped together, based on their morphology. However, it was not always possible to identify OTUs to species level, as some species have a similar morphology, and would require collection or closer examination to differentiate.

2.2 Sedimentation rates

Sediment traps were deployed and collected by SCUBA divers to measure if the construction activity added sediment to the water column (Supplementary Figure S1). Traps were deployed two locations either side of the construction zone in July 2017 (locations A: 67°S 34.261, 068°W 07.967 and location B: 67°S 34.430, 068°W 07.514) (Figure 1). Sediment traps were placed at 30 m depth, to reduce the chances of them being hit by icebergs and were designed to have a small profile within the water column without a surface buoy that would be at risk of entanglement by seasonal sea ice and icebergs. By reducing the sediment trap profile in the water and allowing a degree of flexibility in the mooring itself, only a direct hit by icebergs on the anchor point (a concrete weight) would destroy the sediment trap, thereby reducing the risk of sample loss. Despite these precautions, the western trap (location A) was destroyed by an iceberg after redeployment in October 2018 so only pre-construction sedimentation data were available from this trap. The south-eastern sediment trap (location B) remained in place until it was removed in May 2021. Once collected the sediment dry mass (after 24 h at 60°C) and ash free dry mass (after 24 h at 480°C) were measured.

2.3 Macroepifauna biodiversity

Pre- and post- construction diversity was determined by comparing OTU richness, the number of morphotypes identified within each sample and the Shannon-Wiener diversity index (Pielou, 1966). A Friedman's non-parametric two-way ANOVA was implemented due to the non-parametric and non-balanced constraints of the post-construction samples for both OTU richness and Shannon-Wiener diversity index across depth. Both OTU richness data and Shannon-Wiener diversity index data, were averaged across depth to accommodate the requirements of the Friedman's test, which was performed using *R* v4.3.2.

2.4 Multivariate analysis

Multivariate analysis was used to describe the macroepifauna composition change between pre- and post-construction surveys between 10–100 m depth using Primer 7 with the PERMANOVA+ software package (Clarke and Gorley, 2015). An nMDS plot was constructed to describe the variance between all samples after 999 permutations using a Bray-Curtis similarity matrix. nMDS stress, a measure of how representative nMDS is of the underlying high dimension data, was reported at 0.12, which is considered a fair presentation of the underlying data. Hierarchical cluster analysis was overlayed on top of the nMDS to demonstrate groups sharing 45% similarity. Two-way ANOSIM tests were implemented to test macroepifauna composition change for pre- and post-construction and across depth, with SIMPER (SIMilarity PERcentage) tests investigating which taxa were responsible for any dissimilarity (Supplementary Table S2).

3 Results

3.1 Macroepifauna biodiversity and composition

Both pre- and post-construction diversity metrics, i.e., OTU richness and Shannon-Wiener diversity index, describe a general unimodal trend across depth peaking ~70 m depth (Figure 3). Species OTU richness ($\chi^2 = 12.0$, df = 8, P-values = 0.15; Friedman test) and Shannon-Wiener diversity index values ($\chi^2 = 13.07$, df = 8, P-values = 0.11; Friedman test) were not significantly different during the pre- and post-construction. Pre- and post-construction values had a similar peak diversity of 42 and 40 OTU richness, respectively. Nine unique OTUs and 12 unique OTUs were found in the pre- and post-construction surveys, respectively. All occurred with low abundance, i.e., < 10 counts, except for ALG005 and ALG006 which are both species of macroalgae.

Across the study area the macroepifauna was characterised by mobile grazers and scavengers between 10–30 m depth, in particular *Nacella concinna* (GAS001), *Sterechinus neumayeri* (ECH001) and *Odontaster validus* (AST001) (Figure 4). Between 40 and 60 m depth a mixture of groups including suspension feeders including *Primnoella* sp. (ANT001) and *Cnemidocarpa verrucosa* (ASC009), as well as grazers and scavengers such as *S. neumayeri* (ECH001). Calcareous pink encrusting algae (ALG002), was dominate at all depth between 10–50m depth. At 60 m to 100 m depth, sessile suspension feeders were found in higher abundances including *Perinsiana littoralis* (POL002), yellow encrusting porifera (POR001), and encrusting and foliose bryozoans (BRY001–BYR003), these bryozoan OTUs do contain multiple different species … As observed in previous studies (see Smale et al., 2008) there was no clear depth zonation, within the Antarctic benthos with assemblages overlapping at various depths, within the above description of the overall trend. Individual species also could demonstrate wide depth ranges, for example *Nacella concinna* (GAS001) a species of grazing limpet which dominated at 10 m depth could be found, in low abundances, in the deepest samples (100 m depth).

3.2 Multivariate analysis

Across all depths there was a high degree of overlap between samples, indicating similar macroepifauna composition amongst those samples, with the greatest degree of overlap between pre- and post-construction observed at depths greater than 50 m (Figure 5). While the differences in assemblages between depths ($F_{8,78} = 9.1$, $P < 0.01$; Table 1) and transects ($F_{5,78} = 4.4$, $P < 0.01$) remained the same after construction (i.e., there were no interaction terms), there were small but

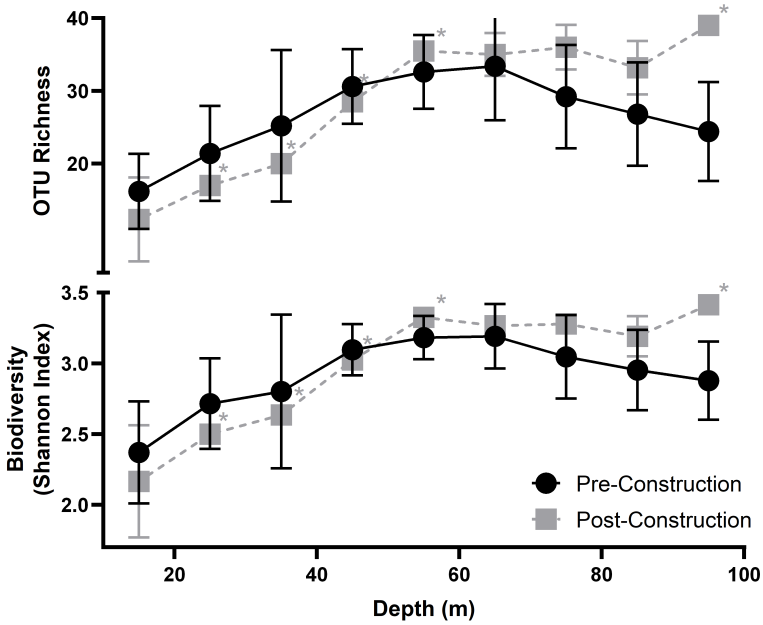

FIGURE 3
Average OTU richness (top) and Average Shannon-Wiener diversity index (bottom) measured across each 10 m depth interval; points are placed at the mid-point of each transect. Pre-construction data are shown in black and post-construction in grey, with the errors bars showing standard deviation. Asterisks indicated averages with <3 transects and no error bars.

significant differences between the assemblages measured pre- and post-construction ($F_{1,78} = 5.3$, $P = 0.03$). The biggest difference between pre- and post-construction assemblages was attributed to a reduction in both *Sterchinus neumayeri* and *Odontaster validus* (Figure 4) after the construction, with SIMPER attributing 5.4 and 4.2%, respectively, of the difference to these OTUs (Supplementary Table S2).

3.3 Sedimentation rates

Sediment settlement remained below 5 mg day between July 2017 and May 2020, for both settlement traps and remained at this level throughout the first construction period between December 2018 and March 2019 (Supplementary Figure S2). A large pulse in sedimentation was recorded in the south-eastern sediment trap (location B), during the final measurement period February 2020 to May 2021; however, as the western sediment trap (at location A) was lost to iceberg scour, this observation is the only result available (Figure 6). The pulse in sedimentation coincided with the backfilling of the wharf pilings with crushed rocks during the second construction period October 2019 to March 2020.

4 Discussion

There were small differences in the assemblage on the steep rocky slope in front of the Biscoe Wharf between the pre- and post-construction survey. The largest difference was a small (9%) shift in

FIGURE 4
Specimen images of *Sterechinus neumayeri* (ECH001) (left) and *Odontaster validus* (AST001) (right), from survey are in front of Biscoe Wharf at Rothera Research station.

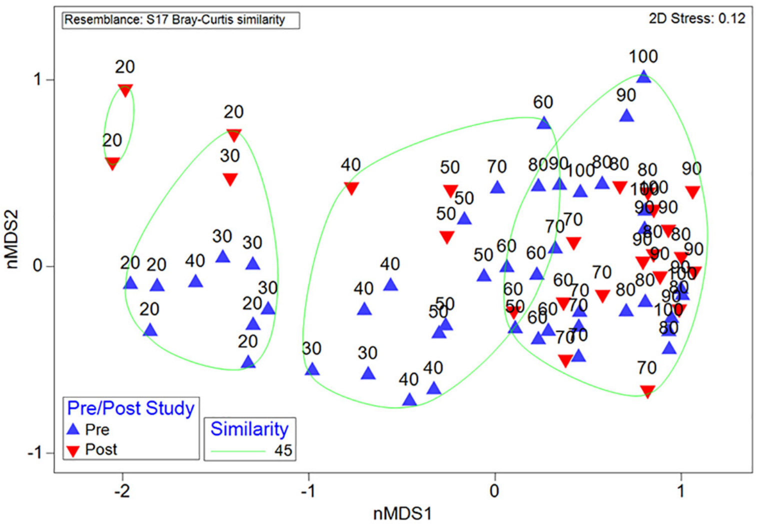

FIGURE 5
Macroepifauna structure from pre- and post-construction surveys, displayed with non-metric MultiDimensional Scaling (nMDS) with similarity bands based on hierarchical cluster analysis. Samples consist of 10 m depth intervals; samples are labelled with the greatest depth in the range (i.e., '20' represents the transect with the 10–20 m depth range).

community composition driven by a reduction in the presence of the sea urchin, *Sterechinus neumayeri*, and the seastar, *Odontaster validus*, in the shallows (10–20 m depth). These species are mobile and are known to migrate into recent iceberg scours to scavenge on the carcases and organic material generated by the impact (Robinson et al., 2020). The reduction in these two species is contrary to what we would expect from a site recently disturbed from construction, however in the two years since the impact, the high recovery capability (resilience) of the macroepifauna in the shallows (Robinson et al., 2020; Zwerschke et al., 2021) would have likely led to return to pre-construction state from a local disturbance event, such as the wharf construction. The natural and frequent impacts of iceberg scours within this area (Smale et al., 2006, 2008) could account for the variation in *S. neumayeri* and *O. validus*. With similar variations in abundance detected within the same area from previous studies (Robinson et al., 2021).

TABLE 1 PERMANOVA table of results after 999 unique permutations.

Factor	df	SS	MS	Pseudo F	P (perm)
Construction	1	941.3	941.3	5.3	0.03*
Transect	5	3882	776.6	4.4	<0.001**
Depth	8	12865	1608	9.1	<0.001**
Construction*Transect	4	1930	482.6	2.7	0.05
Construction*Depth	8	1311	163.9	0.93	0.51
Transect*Depth	40	9117	227.9	1.3	0.26
Res	12	2115	176.3		
Total	78	43654			

Significant differences are highlighted with asterisks.

Macroalgae species, such as OTUs ALG005 and ALG006, were only found in the post-construction survey, but are particularly variable in presence and abundance in the polar environment, as they rely on downwelling light levels that can be restricted or even blocked, by sea ice (Vernet et al., 2008). Many Antarctic species also have episodic recruitment events (e.g., sponges; Dayton et al. (2016)) and the shift in macroalgal presence is likely to represent one such recruitment event (Barnes and Souster, 2011) and with additional removal due frequent iceberg scours increasing natural variability.

A large pulse of sediment was recorded during the final measurement period, which started during the second half of the construction in 2020. This coincided with the backfilling of the wharf pilings with crushed rocks. The sediment traps gave an average measure of sedimentation over their whole period of underwater deployment but it is likely that the increased sedimentation came in a pulse from the rock as it was dumped into the water behind the pilings. No shifts in the overall pattern of macroepifaunal assemblages with depth was observed either between pre and post construction surveys or from previous data collected from this site, including amongst the sessile filter feeding assemblages. The sessile filter feeding assemblage includes a diverse range of taxa such as *Perinsiana littoralis* (POL002), yellow encrusting porifera (POR001), and encrusting and foliose bryozoans (BRY001–BYR003), which were found at high abundance at depth (70–100 m depth). These macroepifaunal communities would be expected to have the greatest vulnerability to increased sedimentation, detected during construction (Riegl and Branch, 1995; Tompkins-MacDonald and Leys, 2008; Bannister et al., 2012). However, with no knowledge of the local current regime, the impact for remote down-current filter feeding assemblages is unknown. While a single point measurement does not support speculation on the wider impacts of this sedimentation

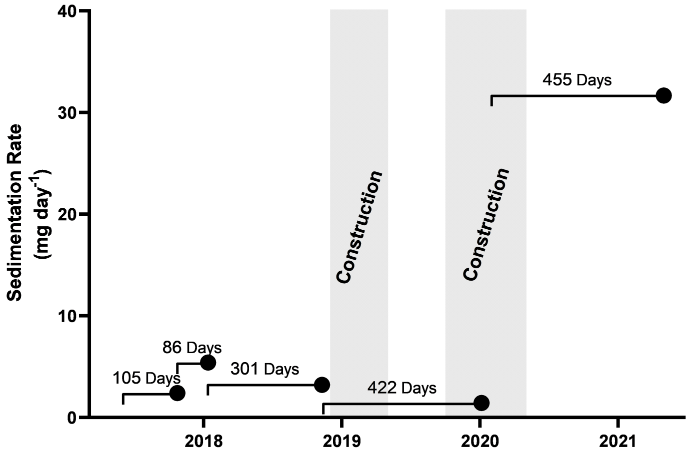

FIGURE 6
Sedimentation rate recorded by sediment trap B. Each point indicates the retrieval date of the sediment trap and the line represents the deployment time and date. Sedimentation rates were averaged across the entire deployment. The two periods of construction activity between Dec-2018 to May-2019 and Oct-2019 to May-2020 are represented in grey.

event, it does indicate that those undertaking extended construction activities should consider putting measures in place to mitigate the impact of such marine sedimentation events.

The mitigating measures undertaken during construction of the wharf included the reducing the footprint of the new wharf, while still accommodating the larger vessel and choosing a design to lower the amount of seabed milling and preparation that was required (drilling or changing seabed topography) (Fothergill, 2018). These mitigation measures were designed to reduce the direct loss of benthic habitat, reduce rock/boulder displacement (rock falls), and decrease sediment input into the marine environment.

Due to the delay of the post-construction survey, this report cannot comment on the initial impact of the wharf construction, only that any impact was transient and had become indistinguishable from natural state two years after construction had finished. Below 30 m depth, there was also no significant shift in macroepifauna composition, this is despite macroepifaunal groups with lesser recovery capability, such as *Stylocordyla chupachups* (POR016), *yellow encrusting porifera* (POR001) *and Phorbas areolatus* (POR026), dominating at greater depths (i.e., 70–100 m; Robinson, 2021). Any impact on deeper zoobenthos, e.g., due to rock falls or sediment smothering, would have been particularly evident, even two years after construction. Elsewhere, similar impacts from naturally occurring iceberg scours have been detectable a decade after the initial impact (Gutt et al., 1996). Supporting the conclusion that the mitigating efforts were successful and the environment outside the footprint remains unchanged.

The Antarctic and sub-Antarctic benthos remain some of the least human impacted macroepifauna assemblages on Earth (Rogers et al., 2020). It is therefore important to consider how to mitigate and measure any anthropogenic impact within the Southern Ocean. The monitoring project, although constrained as a consequence of the COVID-19 pandemic, demonstrates the low to negligible impact that the Biscoe Wharf extension has had on the local marine environment, which indicates that the various mitigation measures enacted were successful (Fothergill, 2018). When considering best practices and procedures for future monitoring of construction on seafloor biodiversity within Antarctica, we suggest the implementation of comprehensive monitoring regime, including monitoring of impacts during the construction phase and monitoring of the wider area. However, this study demonstrates the practical need for long term science to understand variability within the marine environment and how the understanding of the underlying mechanisms controlling biodiversity can be applied to quantifying the impact of anthropogenic activities.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

BR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. KH: Conceptualization, Funding acquisition, Project administration, Resources, Writing – review & editing. DS: Funding acquisition,

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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.

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References

Bannister, R. J., Battershill, C. N., and De Nys, R. (2012). Suspended sediment grain size and mineralogy across the continental shelf of the great barrier reef: Impacts on the physiology of a coral reef sponge. *Continental Shelf Res.* 32, 86–95.

Barnes, D. K. A. (2017). Iceberg killing fields limit huge potential for benthic blue carbon in Antarctic shallows. *Glob Chang Biol.* 23, 2649–2659. doi: 10.1111/gcb.13523

Barnes, D. K., and Conlan, K. E. (2007). Disturbance, colonization and development of Antarctic benthic communities. *Philos. Trans. R Soc. Lond B Biol. Sci.* 362, 11–38. doi: 10.1098/rstb.2006.1951

Barnes, D. K. A., and Souster, T. (2011). Reduced survival of Antarctic benthos linked to climate-induced iceberg scouring. *Nat. Climate Change* 1, 365–368. doi: 10.1038/nclimate1232

Bonner, W. N., and Laws, R. M. (1964). Seals and sealing. In: Priestly, R., Adu, R. J., du, G., and Robin, Q. (editors). *Antarctic research*. London: Butterworth, 163–190.

Convey, P., Chown, S. L., Clarke, A., Barnes, D. K., Bokhorst, S., CummingS, V., et al. (2014). The spatial structure of Antarctic biodiversity. *Ecol. Monogr.* 84, 203–244. doi: 10.1890/12-2216.1

Clarke, K. R., and Gorley, R. N. (2015). "Getting started with PRIMER v7," in *PRIMER-e: plymouth*, (Plymouth Marine Laboratory) 20, 1.

Dayton, P., Jarrell, S., Kim, S., Thrush, S., Hammerstrom, K., Slattery, M., et al. (2016). Surprising episodic recruitment and growth of Antarctic sponges: implications for ecological resilience. *J. Exp. Mar. Biol. Ecol.* 482, 38–55. doi: 10.1016/j.jembe.2016.05.001

Forcada, J., and Staniland, I. J. (2009). "Antarctic fur seal Arctocephalus gazella," in *Encyclopedia of marine mammals 2nd ed.*, eds. W. F. Perrin, B. Würsig and J. G.M. Thewissen (London: Academic Press), 36–42.

Fothergill, C. (2018). *Final comprehensive environmental evaluation for rother wharf reconstruction & Coastal stabilisation*. Available online at: https://www.bas.ac.uk/about/Antarctica/environmental-protection/environmental-policy-and-management/environmental-impact-assessments-eias-in-Antarctica/eias-for-proposed-activities-in-Antarctica-prepared-by-the-uk/ (Accessed 08/12/2023).

Gutt, J., Starmans, A., and Dieckmann, G. (1996). Impact of iceberg scouring on polar benthic habitats. *Mar. Ecol. Prog. Ser.* 137, 311–316. doi: 10.3354/meps137311

Hughes, K. A., and Convey, P. (2020). Implications of the COVID-19 pandemic for Antarctica. *Antarctic Sci.* 32, 426–439. doi: 10.1017/S095410202000053X

Pielou, E. (1966). Shannon's formula as a measurement of species diversity: it's use and disuse. *Am. Nat.* 100, 463–465. doi: 10.1086/282439

Retallick, K., Van Landeghem, K., Fremand, A., Howard, F., Sands, C., Roman-Gonzalez, A., et al. (2021). *Seafloor bathymetry of Sheldon Cove, Börgen Bay and Marian Cove, merged and gridded from EM122 multibeam echosounder data collected for the project NE/P003087/1 (2017–2020) (Version 1.0)* (NERC EDS UK Polar Data Centre). doi: 10.5285/1B4AB7BC-4272-4B16-A642-565E40544B0A

Riegl, B., and Branch, G. M. (1995). Effects of sediment on the energy budgets of four scleractinian (Bourne 1900) and five alcyonacean (Lamouroux 1816) corals. *J. Exp. Mar. Biol. Ecol.* 186, 259–275.

Robinson, B. J. (2021). Disturbance ecology of the shallow Antarctic Benthos. (Doctoral dissertation). University of Southampton.

Robinson, B. J., Barnes, D., Grange, L., and Morley, S. (2021). Intermediate ice scour disturbance is key to maintaining a peak in biodiversity within the shallows of the Western Antarctic Penisula. *Sci. Rep.* 11, 16712.

Robinson, B. J., Barnes, D. K., Grange, L. J., and Morley, S. A. (2022). The extremes of disturbance reduce functional redundancy: Functional trait assessment of the shallow Antarctic benthos. *Front. Mar. Sci.* 8, 2149. doi: 10.3389/fmars.2021.797112

Robinson, B. J., Barnes, D. K., and Morley, S. A. (2020). Disturbance, dispersal and marine assemblage structure: A case study from the nearshore Southern Ocean. *Mar. Environ. Res.*, 105025. doi: 10.1016/j.marenvres.2020.105025

Rogan-Finnemore, M., Ojeda, M., Acosta, J. M. P., Bretel, P., Browne, N., Flått, S., et al. (2021). Icebreaking polar class research vessels: New Antarctic fleet capabilities. *Polar Rec.* 57, e46. doi: 10.1017/S003224742100067X

Rogers, A., Frinault, B., Barnes, D., Bindoff, N., Downie, R., Ducklow, H., et al. (2020). Antarctic futures: An assessment of climate-driven changes in ecosystem structure, function, and service provisioning in the Southern Ocean. *Annu. Rev. Mar. Sci.* 12, 87–120. doi: 10.1146/annurev-marine-010419-011028

Smale, D. A. (2008). Continuous benthic community change along a depth gradient in Antarctic shallows: evidence of patchiness but not zonation. *Polar Biol.* 31, 189–198. doi: 10.1007/s00300-007-0346-3

Smale, D. A., Barnes, D. K. A., and Fraser, K. P. P. (2006). The influence of depth, site exposure and season on the intensity of iceberg scouring in nearshore Antarctic waters. *Polar Biol.* 30, 769–779. doi: 10.1007/s00300-006-0236-0

Smale, D. A., Brown, K. M., Barnes, D. K., Fraser, K. P., and Clarke, A. (2008). Ice scour disturbance in Antarctic waters. *Science* 321, 371. doi: 10.1126/science.1158647

Tompkins-MacDonald, G. J., and Leys, S. P. (2008). Glass sponges arrest pumping in response to sediment: implications for the physiology of the hexactinellid conduction system. *Mar. Biol.* 154, 973–984.

Venables, H., Meredith, M. P., Hendry, K. R., Hoopen, P. T., Peat, H., Chapman, A., et al. (2023). Sustained year-round oceanographic measurement from Rother Research Station, Antarctica 1997–2017. *Sci. Data* 10, 265. doi: 10.1038/s41597-023-02172-5

Vernet, M., Martinson, D., Iannuzzi, R., Stammerjohn, S., Kozlowski, W., Sines, K., et al. (2008). Primary production within the sea-ice zone west of the Antarctic Peninsula: I—Sea ice, summer mixed layer, and irradiance. *Deep Sea Res. Part II: Topical Stud. Oceanography* 55, 2068–2085. doi: 10.1016/j.dsr2.2008.05.021

Zwerschke, N., Morley, S. A., Peck, L. S., and Barnes, D. K. (2021). Can Antarctica's shallow zoobenthos 'bounce back'from iceberg scouring impacts driven by climate change? *Global Change Biol.* 27, 3157–3165. doi: 10.1111/gcb.15617

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Ancient diversification in extreme environments: exploring the historical biogeography of the Antarctic winged midge *Parochlus steinenii* (Diptera: Chironomidae)

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The terrestrial fauna of Antarctica consists of a limited number of species, notably insects, small crustaceans and other micro-invertebrates. Over long periods of evolutionary isolation, these organisms have developed varying degrees of tolerance to multifaceted environmental stresses. Recent molecular biogeographical research highlights the enduring persistence of much of Antarctica's current terrestrial fauna, with estimates spanning from hundreds of thousands to millions of years. *Parochlus steinenii*, commonly known as the Antarctic winged midge, stands out as one of the only two insect species native to Antarctica. Distributed across three biogeographic regions, southern South America and the Falkland/Malvinas Islands, sub-Antarctic South Georgia and the Maritime Antarctic South Shetland Islands, this midge raises questions about the temporal isolation of its populations and their divergence. Employing mitochondrial and nuclear genetic markers, we conducted phylogeographic and demographic analyses on 151 individuals of *P. steinenii* obtained across the three main biogeographic regions including the Magellanic sub-Antarctic Ecoregion (MSE) of southern South America, the sub-Antarctic Island of South Georgia (SG) and the South Shetland Islands (SSI) within the Maritime Antarctic (MA). Our data support the diversification of *P. steinenii* during the mid-Pleistocene around 1.46 Mya. This period included a branching event between a clade containing only specimens from the MSE and a clade containing individuals from a broader range of locations including the SSI and SG. Based on intraspecific phylogeographic and demographic inferences, we detected strong evolutionary divergence between the three main biogeographic regions. We also detected a signal of population growth during the deglaciation process in SSI and SG, contrary to the pattern seen in the MSE. The different demographic and phylogeographic histories between the sampled biogeographic regions could result from the MA and SG experiencing a strong genetic bottleneck due to a reduction in population size during the Last Glacial Maximum, while the MSE maintained a significant effective population size. The high level of divergence detected between individuals from the MSE and the remaining biogeographic regions supports the hypothesis of a speciation process taking place in *P. steinenii*.

KEYWORDS

Antarctic–Magellan connection, Diptera, Cape Horn, insect, past climate oscillations, phylogeography, speciation

Introduction

Today's Antarctic terrestrial fauna is largely restricted to ice-free areas of the continent, which contribute only ~0.3% of the total continental area, increasing to ~3% in the milder Antarctic Peninsula region. The fauna of the Maritime Antarctic, including the Antarctic Peninsula and Scotia Arc archipelagos, as well as the sub-Antarctic region, exhibits a low diversity characterized by isolated populations. It primarily consists of insects and small crustaceans representing the arthropods, along with tardigrades, rotifers, nematodes, and protozoans (Chown and Convey, 2016; Convey and Biersma, 2024). Over the course of millions of years, this distinct group of organisms has developed and diversified in response to a wide range of environmental stresses. Recent phylogeographic studies strongly suggests that many species within Antarctica's terrestrial biota have persisted for long periods, with estimated persistence ranging from hundreds of thousands to multi-million year timescales (Convey et al., 2008, 2020; Verleyen et al., 2021; Maturana et al., 2022). Conversely, a smaller number of studies have reported evidence supporting more recent mid- to post-Pleistocene colonization from lower latitudes (van de Wouw et al., 2008; Biersma et al., 2020). Contemporary terrestrial biodiversity in Antarctica therefore consists of a mixture of species that have survived the repeated glacial maxima in local refugia and then recolonized subsequently deglaciated areas, or have arrived more recently through inter-and post-glacial dispersal from lower latitude areas that remained ice-free, or are present through a combination of both mechanisms (Maturana et al., 2022).

The terrestrial fauna of the Antarctic region is closely related to South America, with intimate geographical, biological, geological and glaciological histories (Mercer, 1976; Clapperton and Sugden, 1988; Clapperton, 1990; Rodbell et al., 2009) as evidenced by the presence of shared non-biting midges (Diptera: Chironomidae), and crustaceans (Copepoda and Anostraca). Even though much still remains to be resolved in clarifying the relationships of sub-Antarctic and Antarctic taxa to their sister-groups in South America, the available evidence allows the development of hypotheses and predictions relating to the temporal scale of species divergence and subsequent colonization of the Antarctic region, such as the long-term presence of Antarctic biota and persistence of populations in ice-free areas (Convey et al., 2008), or the dispersal patterns in *Belgica antarctica* Jacobs, 1900, the endemic wingless Antarctic midge (Allegrucci et al., 2012).

Although there has been significant scientific effort on certain taxonomic groups, understanding in this area remains limited. This lack of knowledge makes it challenging to comprehensively analyze evolutionary patterns in the region. One such poorly studied taxon is *Parochlus steinenii* (Gercke, 1889) (Diptera: Chironomidae), commonly known as the Antarctic winged midge and one of only two insect species native to the Antarctic continent, along with *B. antartica*. *Parochlus steinenii* occurs naturally in southern South America, the Falkland/Malvinas Islands, sub-Antarctic South Georgia, and the South Shetland Islands in Maritime Antarctica (Gañán et al., 2021). Brundin (1966) redescribed the species from adults and pupae collected in Tierra del Fuego, along with the first description of *Parochlus steinenii brevipennis* Brundin, 1966, a sub-species found in the Andes of Central Chile, south of the Argentinian city of Bariloche (34°S, 893 m.a.s.l.) (Brundin, 1966). Various aspects of the species' biology have been studied, including occurrences, morphology, phenology and physiology (Brundin, 1966; Edwards and Usher, 1985; Rauschert, 1985; Shimada et al., 1991; Convey et al., 1996; Gañán et al., 2021; Pertierra et al., 2021; Contador et al., 2023) but its genetic diversity, phylogeographic structure and historical demographic processes have yet to be assessed, other than in the very preliminary data presented by Allegrucci et al. (2006).

Despite being capable of flight, *P. steinenii* may face challenges in dispersing across larger distances due to unfavorable abiotic conditions such as strong winds and cold temperatures. Its different life stages are found in both terrestrial and aquatic environments (Hahn and Reinhardt, 2006; Contador et al., 2023). The larvae and pupae are aquatic, inhabiting deeper permanent lakes where they can avoid entrapment in ice during winter, while the short-lived adults are terrestrial and are found in high abundance and density (approximately 600-800 individuals/m^2) during the Antarctic summer at the edge of lakes and streams, where copulation and oviposition occur (Contador et al., 2023). Some freshwater ecosystems in these regions may be adversely affected by predicted future climatic changes, particularly if these lead to local drying or loss of water sources, which could impact the persistence of *P. steinenii* in its current distribution. In this context, the Antarctic winged midge may act as an effective sentinel of climate change across past, present and future scenarios in Antarctic and sub-Antarctic terrestrial and freshwater ecosystems.

The wide distribution of *P. steinenii*, and its apparently highly conserved morphology, raise questions about temporal isolation and potential speciation among regional populations. We hypothesize that molecular phylogeographic and demographic analyses using two genetic markers may reveal cryptic divergence and the timescales of persistence of Maritime and sub-Antarctic populations. Our results are important for understanding the long-term evolution of Antarctic terrestrial species and contribute to the debate surrounding evolutionary patterns in the sub- and Maritime Antarctic biota.

Materials and methods

Sampling

Specimens of *P. steinenii* were collected from freshwater bodies within three recognized biogeographic regions (Figure 1, for more detail see Supplementary Table S1): 1) Magellanic sub-Antarctic Ecoregion (MSE), in particular in Navarino Island, within the Cape Horn Biosphere Reserve; 2) the sub–Antarctic island of South Georgia (SG), including individuals from Bird Island (BI) and the South Georgian mainland (TP, STR and HB); and 3) the Maritime Antarctic (MA) specifically King George Island (KGI), Deception Island (DCI) and Livingston Island (LVI) in the South Shetland Islands (SSI). Adult flies were sampled during the austral summer from 2017 to 2023 from their natural habitats around lakes with the use of an entomological aspirator. Additionally, larvae and adults were also manually collected on Navarino Island from aquatic mosses in a lake near the summit of Bandera Hill, located close to the north coast of the island. All individuals were preserved in 95% ethanol and their geolocation was later included in GBIF (see Gañan et al., 2020). Additional searches were conducted in the

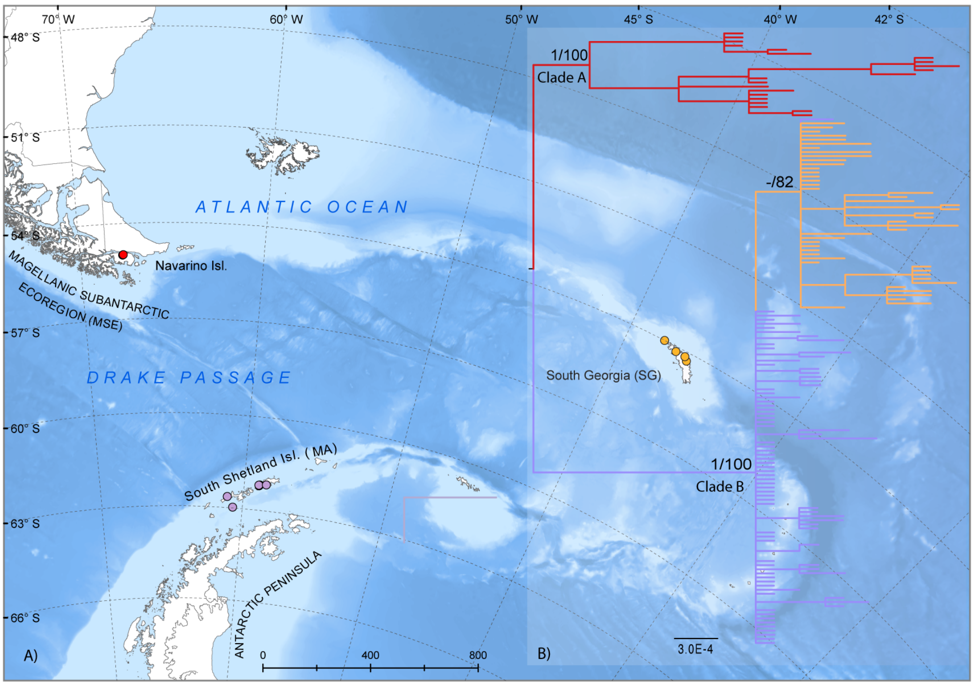

FIGURE 1
Historical biogeography reconstruction based on 151 *cox*1 sequences of *Parochlus steinenii* across the sampling areas. **(A)** Map of the sampling region in the Magellanic Subantarctic regions (MSE, red); sub-Antarctic Island of South Georgia (SG, orange), and Maritime Antarctic (MA, violet); **(B)** Bayesian Inference reconstruction using *P. steinenii* individuals. The values for node support are indicated for posterior probability/bootstrap from BI and ML analyses, respectively.

Falkland/Malvinas Islands and South Orkney Islands, but no *P. steinenii* were observed. Taxonomic identity of the specimens was confirmed following Edwards and Usher (1985).

Data preparation and sequence editing

DNA was extracted using two main protocols, QIAGEN DNeasy Blood & Tissue, following (Maturana et al., 2022), and the QiAMP Extraction Kit, following the manufacturer's protocols. All individuals were fully submerged in proteinase K+ATL buffer solution for 4 h at 56°C or overnight at 40°C; adults were not crushed to retain them as complete as possible for morphological examination, while larvae were partially or fully crushed before extraction.

Two DNA loci were amplified, two segments of the mitochondrial cytochrome c oxidase subunit I (*cox*1), and one segment of the nuclear DNA 28S rRNA gene. For the two segments of *cox*1, we used the universal primers LCO1490 and HCO2198 (Folmer et al., 1994) and UEA5 (AGTTTTAGCAGGAGCAATTA CTAT) and UEA10 (TCCAATGCACTAATCTGCCATATTA) (Lunt et al., 1996), while for the 28S rRNA we used rD1.2a (CCCSSGTAATTTAAGCATATTA) and rD5a (GGYGTTGGT TGCTTAAGACAG) (Whiting, 2002). Detailed descriptions of the protocol and techniques used in the Polymerase Chain Reaction are given in Supplementary Material (S2). The products obtained were commercially sequenced by LGC Genomics (Germany) and Macrogen (South Korea).

Phylogenetic reconstruction

For the phylogenetic reconstructions we incorporated the sequences of *P. steinenii* into a comprehensive dataset depicting the main lineages of the genus *Parochlus* Enderlein1912 (Supplementary Table S3). Homologous DNA sequences were independently aligned for each genetic locus using MAFFT v7.505 (Katoh and Standley, 2013) using the web server (Katoh et al., 2019). We conducted traditional phylogenetic estimations using both Maximum Likelihood (ML) and Bayesian Inference (BI) algorithms. The appropriate sequence evolution model for each genetic locus was determined using bModelTest v1.2.1 (Bouckaert and Drummond, 2017) and ModelFinder (Kalyaanamoorthy et al., 2017). The GTR+Γ model was used for *cox*1 and the HKY+I+Γ model for 28S rRNA. We prepared a phylogenetic tree for each locus to check congruence between both datasets (Supplementary Figure S4). Subsequently, the sequences were concatenated, and we performed posterior analyses on a partitioned dataset, considering each genetic locus. ML reconstruction was carried out using RAxML v8.2.12 (Stamatakis, 2014) and the node support was assessed through a bootstrap analysis with 1000 pseudo-replicates. The tree was rooted with *Zavrelimyia* sp. following Cranston et al. (2010). Bayesian reconstruction was performed in MrBayes3 v2.6.7 (Ronquist et al., 2012). The BI analysis was run three times for 100 million generations each time, with tree sampling every 10,000 generations. The consensus tree considered a burn-in period of 25%. For the intraspecific phylogenetic tree using the complete *cox*1 dataset, we make a midpoint rooted Bayesian tree reconstruction based on GTR+CAT model for ML and GTR+Γ for BI, respectively. Convergence was assessed by checking that split frequencies had an average standard deviation below 0.01 and all parameters had effective sample sizes (ESS) > 200 using Tracer v.1.6 (Rambaut et al., 2014).

Genetic diversity and genealogical reconstruction

Levels of genetic polymorphism were determined for each locus using standard diversity indices including number of haplotypes/alleles (*K*), number of segregation sites (*S*), haplotype/allelic diversity (*H*), average number of pairwise differences (Π) and nucleotide diversity (π) for each of the sampling areas, including the entire MA (KGI, DCI and LVI), sub-Antarctic (TP, BI), and the MSE using DnaSP v6.12.3 (Rozas et al., 2017). We estimated the number of private alleles for each sampling area and region as a proxy to assess the potential existence of refugia (Maggs et al., 2008). Neutrality tests (Tajima's *D* and Fu's F_S) were performed to assess deviation from the neutral model. Significant negative values for these tests provide evidence of excess of rare polymorphism in a population, indicating either recent demographic expansion or positive selection. Genealogical relationships were estimated for mitochondrial sequences by constructing a median-joining haplotype network (Bandelt et al., 1999) using PopArt v1.7 (Leigh et al., 2015).

Genetic and phylogeographic structure

We estimated levels of genetic differentiation among the recognized groups (MSE, SG and SSI), among populations within those groups, and within populations, through mean pairwise differences (Φ_{ST}, using Kimura-2P genetic distances) and haplotype frequencies (F_{ST}) in ARLEQUIN v3.5.2.2 (Excoffier and Heckel, 2006). To test statistical significance of differentiation, we performed a permutation test (20,000 iterations). The *p*-value for pairwise Φ_{ST} and F_{ST} between populations was corrected using the false discovery rate correction (FDR; Benjamini et al., 2005). We used two different clustering methods to determine the spatial genetic structure and the spatial boundaries among them in *P. steinenii.* First, we used the GENELAND v4.0.7 package (Guillot et al., 2005) in the software R v3.6.1 (R Development Core Team, 2019). This Bayesian approach uses the Markov Chain Monte Carlo (MCMC) procedure to estimate the optimal clustering of samples based on geographic information. The analysis considers that spatially structured clusters are more probable than randomly distributed clusters in space. The most probable number of populations (K) was identified through ten independent MCMC analyses, each consisting of 10 million iterations with a thinning interval of 1000 iterations using the correlated frequency model. The range was limited between K = 1 and K = 5, with a burn-in of 25%. Second, we conducted an analysis of molecular variance

(AMOVA) in Arlequin v3.5.2.2 to estimate the proportion of genetic variation explained by partitioning sampling sites into the different demographic units detected. This analysis characterizes spatial genetic structure by partitioning the variance within populations, among populations within groups and among groups.

Inference of demographic history of *P. steinenii*

We examined historical demography of the different previously identified genetic clusters (GENELAND and AMOVA), based on the *cox*1 marker. First, we compared observed mismatch distributions (Rogers and Harpending, 1992) implemented in DnaSP. We calculated Harpending's Raggedness index (rH) in Arlequin v3.5.2.2 to test the unimodality of observed data. Second, we estimated population growth trends over time, through Bayesian Skyline Plots (BSP, Drummond and Bouckaert, 2014) implemented in BEAST v2.5 (Bouckaert et al., 2019). The running conditions considered 100 million iterations with parameter sampling every 10,000 steps, discarding the initial 25% of steps in the analysis. We used a population substitution rate of tenfold the evolutionary rate (7.5% per million year, Martin et al., 2002a, b) to estimate the most recent common ancestor (TMRCA) of each genetic group, as this more accurately reflects the rate at which new haplotypes appear (Ho et al., 2011, 2015). This better accounts for the time-dependence of molecular evolution at population level, brings divergence estimates closer to the present and avoids over-estimation of recent divergences when analysing intraspecific lineages.

Divergence time estimations

To estimate divergence times, we utilized BEAST v2.5 with a reduced dataset comprising mitochondrial haplotypes retrieved using DnaSP. We performed a phylogenetic reconstruction combining a phylogenetic mutation rate with a population substitution rate (Ho et al., 2008). We estimated divergence times between the two main clades (Clade A and Clade B) using a phylogenetic rate, as it relates to the substitution rate at which mutation are fixed among clades. This was conducted using an optimized relaxed clock (ORC) model (Douglas et al., 2021) with a substitution rate of 0.75% per million years, a rate proposed for Chironomidae (Martin et al., 2002a, b) and previously used for *Parochlus* (Allegrucci et al., 2012). For the mutation rate, we used a population rate obtained to estimate the TMRCA of each clade, as it considers the rate at which new haplotypes appear in the clade. Three independent runs of 100 million generations each were conducted, with samples collected every 10,000 iterations. Convergence of results was assessed using Tracer v1.7.2 (Rambaut et al., 2018), and the results were summarized in a single ultrametric tree using TreeAnnotator v2.5.1 (Drummond et al., 2012). The final tree was constructed using the results of both approaches with the Chronos function in ape v5.7-1package (Paradis, 2013) in R, using a sequential secondary age calibration with the time of divergence of the main clades and TMRCA of each lineage (see another example in Pérez-Alvarez et al., 2021).

Results

Occurrence of *Parochlus steinenii* and molecular sequence data

Within the framework of this study, we sampled from 16 freshwater sites within the species' reported distribution across Maritime and sub-Antarctic regions and southern South America (Figure 1A, for more details see Supplementary Material S1, and Gañán et al., 2021). Two sites were in the MSE (IN = 9, NI = 12 sequences), four in sub-Antarctic South Georgia (BI = 5, TP = 16, STR = 14, HB = 11), and 10 in the South Shetland Islands (KGI = 40, DCI = 18, LVI = 26). The results of Xia's test showed low substitution saturation (Iss = 0.2; Iss.c = 0.8; $p < 0.05$) under the assumption of both symmetrical and asymmetrical tree topologies for all OTU subsets. Similarly, PhyloMad results indicated a low risk of substitution saturation for cox1, considering codon positions 1–2 and position 3 independently. Regarding the simple linear regression plots of genetic distances (uncorrected vs. model corrected) identified deviations for cox1 codon position 3 only (R2 = 0.784). Plots for codons 1 and 2 combined indicated strong linear correlations between uncorrected and model-corrected genetic distances (R2 = 1). When codon 3 was combined with codons 1 and 2, the coefficient of determination remained high (R2 = 0.995), supporting the inclusion of codon 3 for estimating evolutionary relationships. For interspecific phylogenetic tree we used a concatenated data considering one fragment of 699 bp length of cox1 (under the Folmer universal primers) and 28S rRNA, resulting in 1347 bp. This concatenated dataset included 264 and 213 segregating sites for cox1, and 28S rRNA, respectively. For the intraspecific analyses, a total of 151 sequences for *cox*1 and 26 sequences for 28S rRNA were obtained. However, there was no variation in the 28S rRNA sequences within *P. steinenii*, so no further population analyses were carried out using this locus. We included a final alignment of two *cox*1 fragments – considering two mitochondrial fragments obtained from Folmer's universal and UEA set of primers – of a total length of 1470 bp, with 80 variable sites (5.4%), and did not include insertions/deletions or stop codons.

Phylogenetic reconstruction

The haplotype tree topology of *P. steinenii* identified the divergence of two main clades, (A) and (B), with high posterior probability (PP) and bootstrap support (BS) (Figure 1B). Clade B showed a well-supported SG clade, separated from remaining but unresolved SSI specimens (Figure 1B). The concatenated 28S rRNA and cox1 phylogenetic reconstruction recovered the relationships within the subfamily Podonominae and the monophyly of *P. steinenii* (Figure 2). At the same time, individuals from the Maritime Antarctic (MA) populations were more closely related to those from sub-Antarctic South Georgia (SG) than to those from the

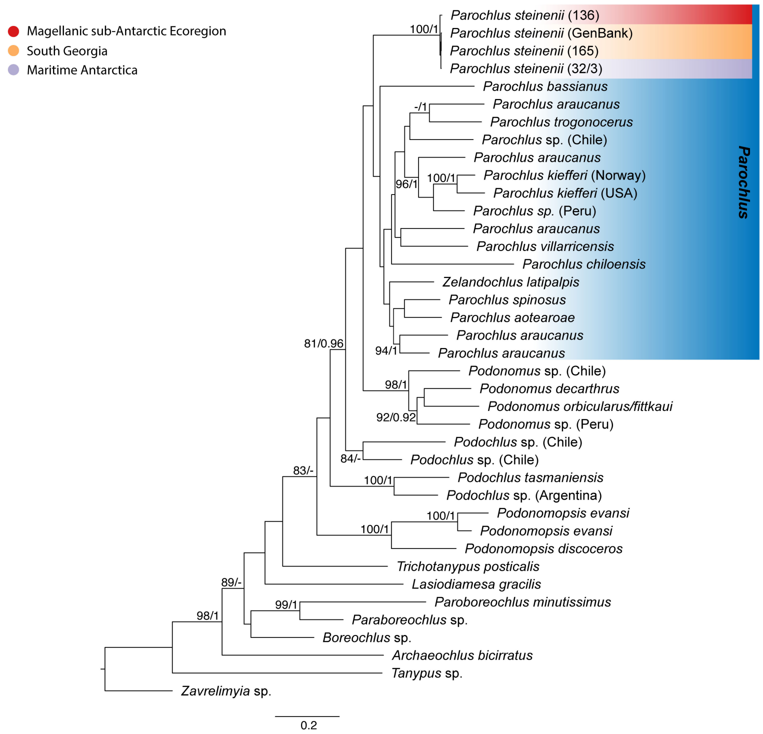

FIGURE 2
Phylogenetic reconstruction of Podonominae based on concatenated data. Maximum Likelihood reconstruction, including members of the subfamily Podonominae with emphasis on *Parochlus* spp. Information in brackets represent the sequence code used for the analysis (within *P. steinenii*), and sampling site for each sequence. Values for the nodes support are indicated for posterior probability/bootstrap, respectively.

MSE (Allegrucci et al., 2006). Within the *Parochlus* clade, MA, MSE and SG groups formed a monophyletic group together with previously published sequences of *P. steinenii* from SG (Cranston et al., 2010). This clade was recovered in an early branching event relative to the other available *Parochlus* species sequences from northern latitudes of South America (Peru, Chile, Argentina) and New Zealand (Figure 2).

Global diversity

Global mitochondrial haplotype diversity (Hd) was 0.922, with no substantial variation among the three biogeographic regions analyzed (Table 1). The MSE population exhibited the highest level of diversity, in terms of nucleotide diversity (Π = 7.44) and fewer haplotypes (K = 11), despite having a smaller sample size (n = 21). The sub-Antarctic SG and the Maritime Antarctic SSI populations showed similar genetic diversity patterns in terms of nucleotide diversity (Π = 3.04 and 1.2, respectively) and numbers of haplotypes (K = 27 and 28, respectively).

The median-joining haplotype network showed three main groups, without any shared haplotypes (Figure 3). The MSE was the most distinct haplogroup, separated by 10 mutational steps from MA and SG. It also displayed a more expanded genealogy, with several mutational steps between each haplotype, contrasting

TABLE 1 Diversity indices and neutrality tests for *Parochlus steinenii* sampled across three biogeographic regions.

Biogeographic regions	Locations	*n*	S	Π	Hd	K	π	p.a	Tajima's D	Fu and Fs
Magellan Subantarctic Ecoregion	Navarino Island	21	22	7.438	0.919	11	0.005	11	0.819	−0,122
sub-Antarctic Islands	South Georgia	46	35	3.043	0.94	28	0.002	28	−2,1073*	−25,500**
Antarctica	South Shetland Islands	84	26	1.295	0.768	27	0.001	27	−2,297**	−31,910**
	Total	151	80	6.513	0.922	66	0.004		−1,728	−50,874*

n, number of sequences; S, segregation sites; Π, mean number of pairwise differences; Hd, haplotype diversity; K, number of haplotypes; π, nucleotide diversity; p.a., private alleles. *p < 0.05; **p < 0.02.

with those in MA and SG, which displayed a star-like topology and short genealogy.

Genetic and phylogeographic structure

The Bayesian analysis of the number of genetic groups and the spatial clustering algorithm obtained with GENELAND detected three genetic clusters (K = 3, PP = 0.65, Figure 3), in agreement with the three biogeographic regions (i.e., MSE, SG and MA). The posterior probabilities associated with the definition of populations was on average 0.7, corresponding to the boundary between these clusters across the Polar Front (PF), which is located northern of SSI and SG (Figure 4). Similarly, AMOVA of comparison of pairwise differences (Supplementary Table S5), also confirmed the greatest differentiation between MSE and the combination of SG and SSI (70.41%, F_{CT} = 0.704, $p < 0.01$), while differentiation within groups only explained 2.72% (F_{SC} = 0.092, $p < 0.01$) of the total genetic variance. Pairwise comparison based on two statistics related to genetic (F_{ST}) and phylogeographic (Φ_{ST}) differentiation, showed significant structure across the three main biogeographic areas (Supplementary Table S6). In particular, the levels of genetic differentiation within SSI and SG were very low (SSI: F_{ST} = 0.145, Φ_{ST} = 0.052; SG: F_{ST} = 0.106, Φ_{ST} = 0.199, for more details see Supplementary Table S7).

Past demographics events

As expected for star-like genealogy, both neutrality tests Tajima's D and Fu's Fs were highly negative and significant (Table 1) for MA and SG, but positive and not significant for the MSE. The distribution of pairwise differences analyzed through Mismatch Analyses (MMA), varied between the different demographic units of *P. steinenii*. The MA and SG showed a typical unimodal curve and smooth distribution (Figure 5), representing a classical population expansion. In case of the SG populations, the curve moved to the right, indicating more mutation accumulation over time. As expected, the Harpending's raggedness index was not significant in SG (rH = 0.027, $p > 0.05$) or MA (rH = 0.079, $p > 0.05$). Likewise, the analysis revealed a non-significant SSD value in SG (SSD = 0.001, $p > 0.05$) and SSI (SSD = 0.003, $p > 0.05$), supporting a spatial expansion for both populations In contrast, the MSE population exhibited a multimodal distribution (Figure 5) and its Harpending's raggedness index was significant (rH = 0.099, $p < 0.05$). Likewise, the analysis revealed a significant SSD value (SSD = 0.060, $p < 0.05$), refuting the demographic model of spatial expansion for the South American population and, rather, supporting population equilibrium. Based on Bayesian Inference phylogenetic reconstruction, haplotype network and the genetic differentiation index, we identified the three haplogroups aligning with the three specific geographic areas as separated and independent demographic units. Bayesian Skyline Plot analyses were conducted using the 10× corrected substitution rate (Figure 5), providing historical population dynamic patterns and dates for TMRCA for the three demographic and evolutionary units (MA, SG and MSE). MSE had the oldest TMRCA of *ca.* 112,000 YBP (95% highest posterior density 95%HPD = 47.8 – 200 ka), followed by SG (*ca.* 50,000 YBP; 95%HPD: 19.6 – 92.1 ka), and finally MA (*ca.* 30,000 YBP; 95%HPD: 10.2 – 65.9 ka). The date of the onset of population expansion in SSI was estimated at *ca.* 10,000 YBP, while SG expanded earlier at *ca.* 20,000 YBP. For the MSE population, we did not detect a population expansion signal. Values of TMRCA were within the same range values obtained from the $\tau = 2\mu t$ values from the Mismatch Analyses (MSE: τ = 4.005, 36,035 YBP; SG: τ = 3.043, 13,500 YBP; MA: τ = 1.295, 11,655 YBP) (Figure 5).

Divergence time estimations

The haplotype phylogenetic reconstruction based on *cox*1 shows a clear assortment of haplotypes according to their geographic origin (MSE, SG and MA), as previously observed in the concatenated phylogenetic reconstruction (Figure 1), and the haplotype network (Figure 2). Using sequential calibration points, the divergence times estimated among the main clades of *Parochlus*

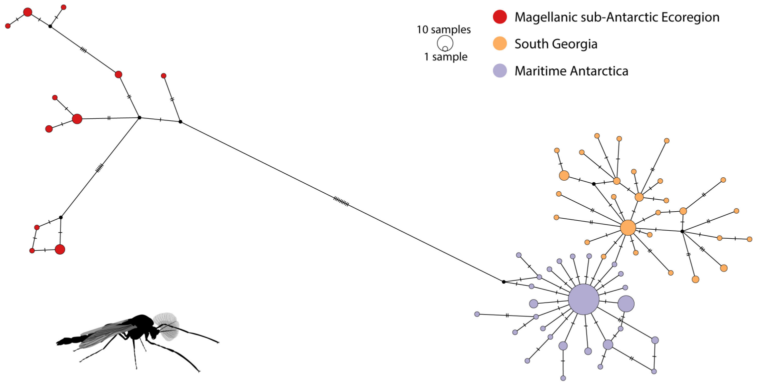

FIGURE 3
Haplotype network for *Parochlus steinenii* based on 151 mtDNA *cox*1 sequences spanning the species' distribution. Neighbor-joining network illustrating the distribution of haplotypes across lakes in the Magellanic Subantarctic Ecoregion, South Georgia and the South Shetland Islands. Circles sizes are proportional to haplotype frequency.

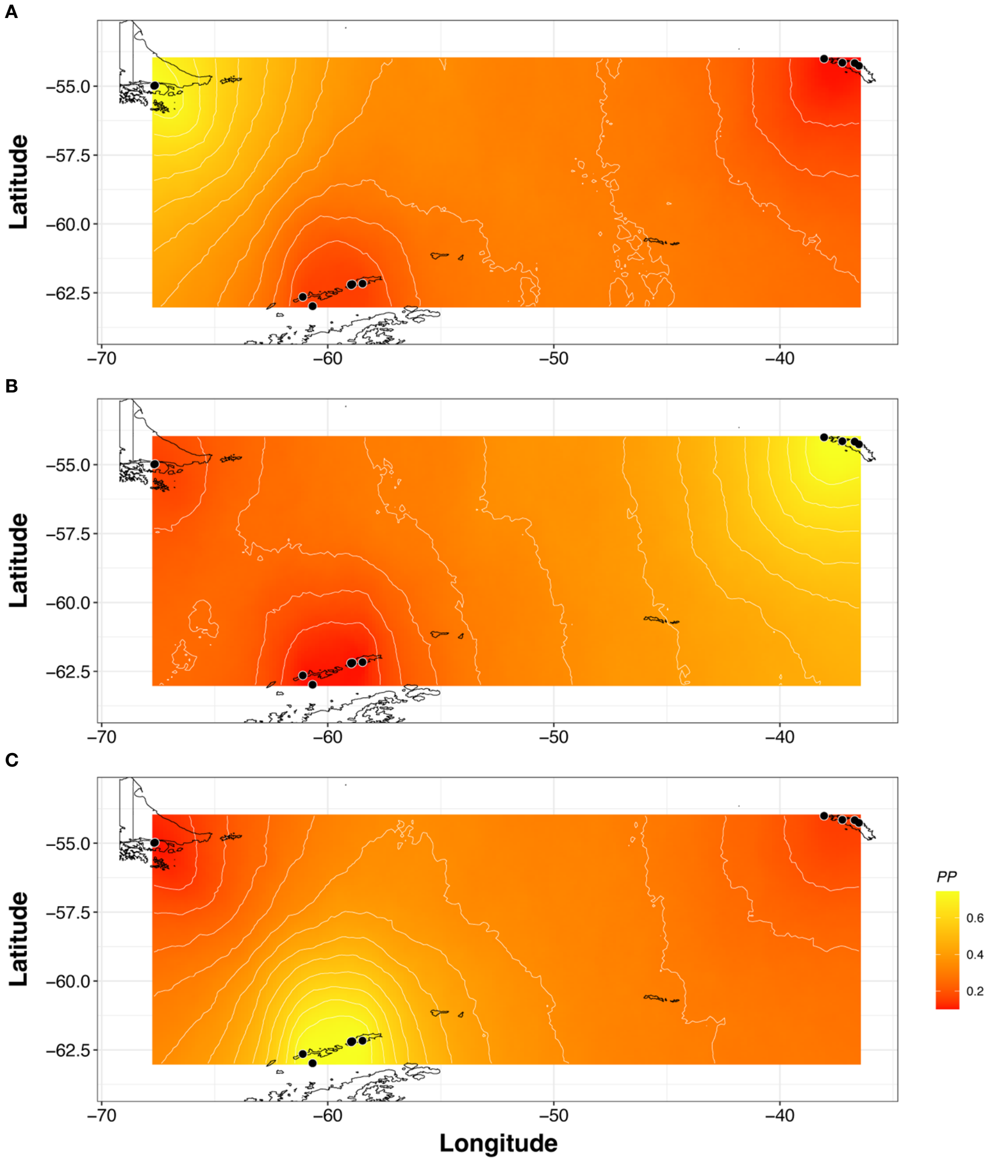

FIGURE 4
Spatial genetic structure of *Parochlus steinenii* from the spatial model in Geneland across the three biogeographic regions sampled. Higher posterior probabilities of population membership are indicated in yellow for each sampling site **(A)** MSE, **(B)** SG, **(C)** MA. Black circles indicate the relative position of the sampling localities. Posterior probabilities of membership were plotted with the shapefiles of Scotia Arc coastline available in the repository in the Antarctic digital database from the British Antarctic survey (BAS). https://add.data.bas.ac.uk.

suggest the MSE lineage as the first clade-group to diverge (Clade A), around 1.46 Mya (95%HPD): 0.87 – 2.10 Mya), followed by the divergence between MA and SG lineages (Clade B) around 0.84 Mya (95%HPD: 0.50 – 1.25 Mya). The age for each population node was obtained from the TMRCA in BSP analyses (Figure 6).

Additionally, we detected a second branching event within Clade B, differentiating specimens from SG and MA. Based on intraspecific phylogeographic, demographic inference and genetic structure, this finding suggests three distinct evolutionary and demographic units, matching the three sampled biogeographic regions.

Discussion

Our molecular data are consistent with previous phylogenetic analyses of *Parochlus* (Allegrucci et al., 2006; Cranston et al., 2010), in suggesting a sub-Antarctic and Antarctic clade that is distinct from all remaining *Parochlus* species. We identified two distinct lineages currently distributed in Clade (A) MSE, and Clade (B) MA and SG.

Long-term persistence of *Parochlus steinenii* in multiple refugia

Given the existence of two clades whose origin is during the Pliocene (1.46 Mya), the presence of private alleles in each population, the deeply diverged haplogroups/clades and distinct reciprocally monophyletic cluster, suggest the presence of separated

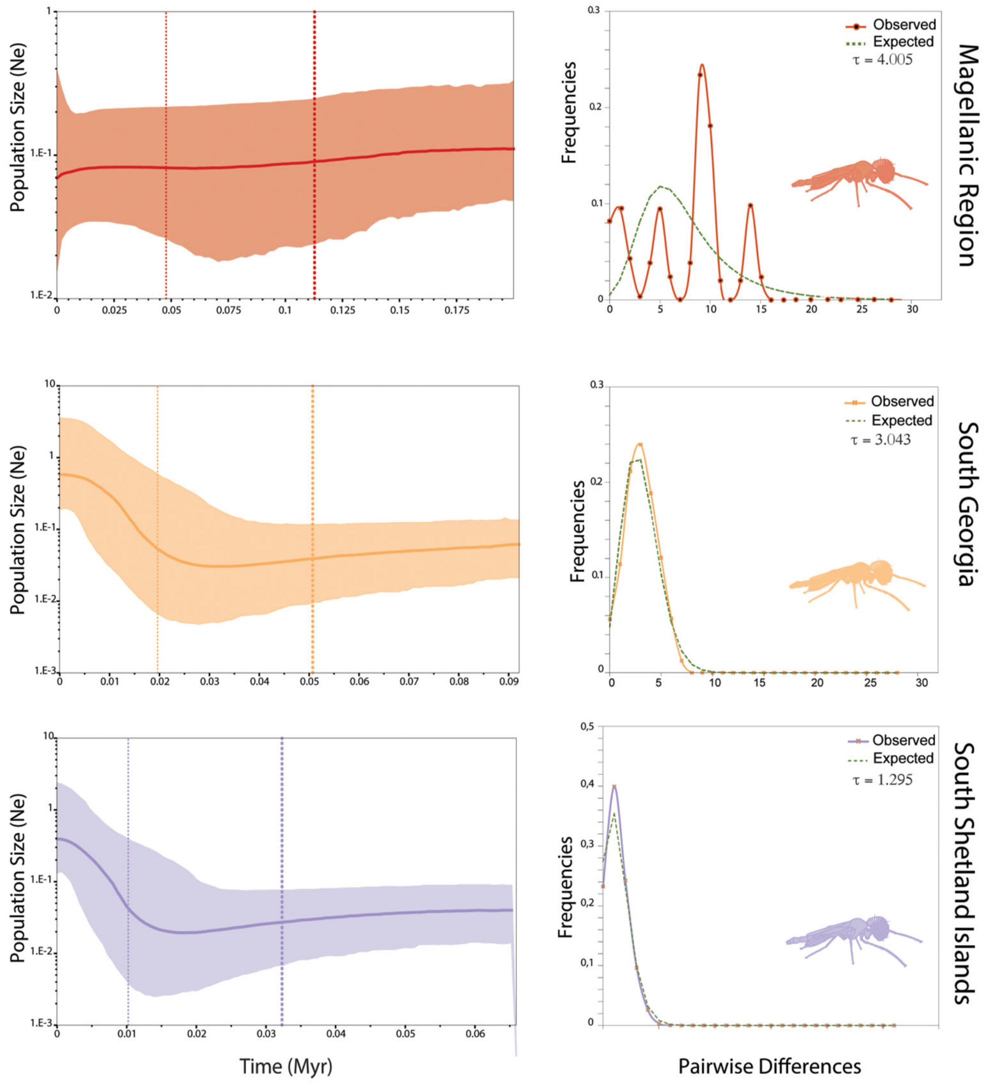

FIGURE 5
Historical demographic trajectories of *Parochlus steinenii* within its distribution in the Magellanic Subantarctic Ecoregion (red), South Georgia (yellow) and South Shetland Islands (violet). Left panels: Past demographic changes constructed using Bayesian Skyline Plot approach based on cox1 haplotypes. The y-axis is the product of the effective population sizes (Ne) and generation length in a log scale. The x-axis is the time before present (Myr). The median estimate (solid black line) and 95% highest probability density (HPD) limits (colored area) are shown. The thick dashed line represents the time of the most recent common ancestor (TMRCA). Right panels: Distribution of pairwise differences of cox1 for each demographic unit. Values of Tau are shown.

and multiple refugia within the present distribution of the species. The diversification of *P. steineni* agrees with the start of the intensification of the glacial cycles during the Pliocene–Pleistocene (Zachos et al., 2001). These results support the long-term persistence of *P. steinenii* in sub-Antarctic and Maritime Antarctic regions, adding further supporting evidence to the recurring pattern of long-term persistence across a range of Antarctic terrestrial and freshwater invertebrates (see Convey et al., 2020 and citation therein; Verleyen et al., 2021; Maturana et al., 2022; Short et al., 2022). Though phylogeographic approaches can identify one of the outcomes of isolation from ancestral panmixia due to refugia (allopatry among populations), we must also consider factors that maintain regional distinction during interglacial periods, such as physical, environmental or selective mechanisms that may limit gene flow among populations (Maggs et al., 2008).

Contrasting historical demographic processes

The representatives of Clade A were collected on Navarino Island in the Cape Horn Biosphere Reserve (CHBR), south of Tierra del Fuego. The lake sampled is located below Bandera Hill at 700 m a.s.l, and represents the only population sampled for genetic analyses within the MSE. The species has also been recorded at Horn Island, slightly further south in the Cape Horn archipelago, from a lake at similar altitude (see Gañan et al., 2020). The MSE population is characterized by high genetic diversity, an extended network topology and older TMRCA, with no signal of past demographic growth. Such genetic and demographic patterns suggest that this population maintained a significant effective

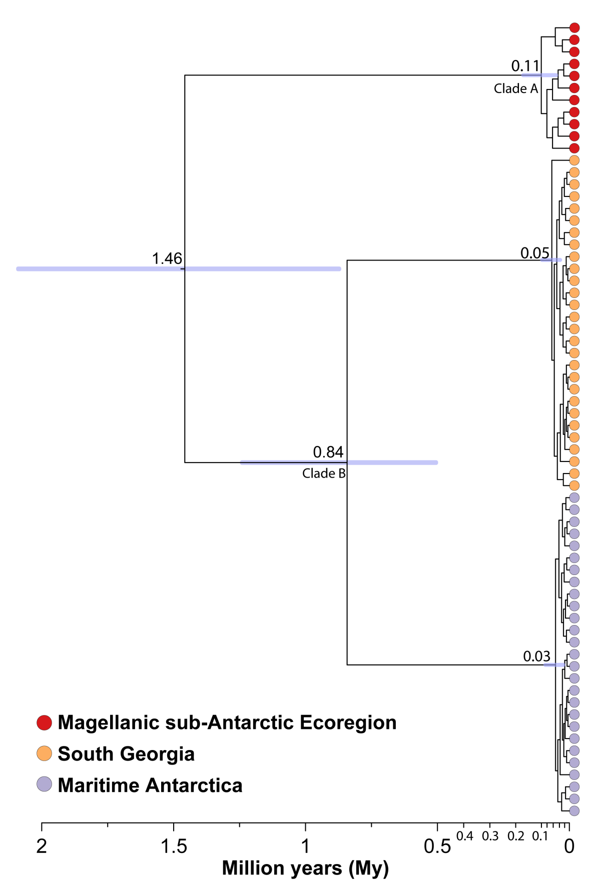

FIGURE 6
Phylogenetic reconstruction based on haplotypes cox1 data showing divergence times of *Parochlus steinenii* across its distribution in sub- and maritime Antarctica. Nodes ages are the median values from both Bayesian Molecular Clock analyses and TMRCA of each clade estimated with Bayesian Skyline Plot. In each clade of interest (MSE, SG and MA), nodes bar indicated the 95% HPD. The colored tip represents the colors of the sampling region.

population size during the intensification of glacial cycles throughout the Pleistocene, and has not experienced an interglacial population expansion unlike SG and MA. Additionally, the MSE population had a positive neutrality test, meaning a deficit of low frequency haplotypes and high divergence between them. Therefore, we proposed that *P. steinenii* is a polar/cold-adapted species with effective interglacial refugia at high altitude in MSE (see cryptic southern refugia section, Stewart et al., 2010), as described for other terrestrial examples in the Northern Hemisphere (Hirao et al., 2015). This plausible scenario of *P. steinenii* as a cold-adapted species allows us also to explain 1) the disjunct distribution and high percentage of divergence from representatives from SG and MA (which represent polar refugia at higher southern latitudes); 2) the persistence in high altitude ecosystems such as small rivers and marginal meltwater channels, similar to the habitats in which they are found today in their current MSE distribution range (Contador et al., 2023); and 3) the abundance and frequency of occurrences within South Shetland Islands, in particular Kitiesh Lake in King George Island where the population density is extremely high (T. Contador personal observations).

Conversely, the contemporary distribution of clade B included a broader allocation (SG, MA). In both populations, we found low levels of nucleotide diversity, unimodal curves in their Mismatch Analyses, and significant demographic expansion. Such a demographic pattern may reflect past contraction-expansion processes in response to climate oscillations, as has been frequently cited in phylogeographic studies of high-latitudes affected by the LGM (Provan and Bennett, 2008; Marko et al., 2010; Maturana et al., 2020). Considering that both SG and SSI are located south of the oceanic Antarctic Polar Front (APF), they would experience greater impact from ice-sheet advances during the LGM (Fraser et al., 2012; Hodgson et al., 2014). In consequence, these populations may have experienced strong bottlenecks due to drastic reductions in their population sizes. Further and ongoing research at small geographic scale, the use of more molecular markers (e.g. mitochondrial genomes), or more contemporary and polymorphic markers (e.g. SNPs), will be required to generate a possible scenarios with greater resolution for *P. steinenii.*

The archipelagoes of the Scotia Arc form a geological connection between the southern part of South America and the Antarctic Peninsula. The region is also one of Earth's major ocean gateways and is critical for understanding the behavior of the main oceanographic currents and their influence on Southern Hemisphere biodiversity (Livermore et al., 2004; Scher and Martin, 2006). It is particularly complex in terms of its geology, glacial processes and the spatial distribution of biodiversity (Terauds et al., 2012). Studies based on available distribution records of vegetation (primarily bryophyte and lichen species) and certain groups of terrestrial invertebrates erected 16 distinct Antarctic Conservation Biogeographic Regions (ACBR, Terauds et al., 2012) in the region of Antarctic Treaty governance, three of which cover the western Antarctic Peninsula, South Shetland Islands and South Orkney Islands. This terrestrial biogeographic classification does not extend to the maritime Antarctic South Sandwich Islands, sub-Antarctic South Georgia, or the Magellan sub-Antarctic Ecoregion, contrary to the complex marine biogeographic regions, which have identified patterns and linkages across the Scotia Arc region (De Broyer and Koubbi, 2014; Koubbi et al., 2014; González-Wevar et al., 2022). Understanding the extent to which these biogeographical regions apply to any particular taxon, and how distributions may change given the appearance of new ice-free areas (Lee et al., 2022), are key to informing how biosecurity protocols should be implemented to minimize risks of anthropogenic transfer of native biota between different parts of this region (van Vuuren et al., 2018; Cukier et al., 2023) and in particular in freshwater ecosystems, which have not previously been included in such studies.

Regional differentiation as a common pattern in inland invertebrates

Based on our *P. steinenii* mtDNA sequences obtained from 16 locations across Maritime Antarctica (SSI) and different sub-Antarctic regions (SG, MSE), we detected strong genetic and phylogeographic differentiation combined with ancient population divergence. Our data support the deep divergence between Clade A and B, which may provide evidence of a speciation processes (genealogical lineages). This hypothesis of species differentiation within the overall distribution was also noted and discussed by Allegrucci et al. (2006). However, speciation processes must be considered carefully, since the nDNA did not detect any differentiation between both clades. The lack of any differentiation in the nuclear 28S rRNA gene versus the mtDNA is likely to reflect lower mutation rates in the nuclear gene and should be addressed in future studies by the inclusion of more nuclear genes.

Finally, our phylogeographic and genetic divergence estimates for *P. steinenii* are comparable to those reported in studies of other sympatric Maritime Antarctic invertebrates (McGaughran et al., 2010; van Vuuren et al., 2018; McGaughran et al., 2019), and the endemic Antarctic midge *B. antarctica* (Allegrucci et al., 2012; Edgington et al., 2023). Additionally, these divergence time estimates are consistent with those recently reported for the freshwater copepod, *Boeckella poppei*, based on *cox*1 sequences obtained across the same three biogeographic regions (Maturana et al., 2022).

Conclusions

The populations of *P. steinenii* examined in this study exhibited strong genetic and phylogeographic structure across the Maritime Antarctic, South Georgia and the Magellanic sub-Antarctic Ecoregion. This structure is also reflected in the phylogenetic reconstruction carried out, in which we found two main clades separating the MSE and SG/MA, with a subsequent branching between the latter. Based on the contrasting phylogeographic and demographic patterns between southern South America and Antarctica, we propose that *P. steinenii* is a cold-adapted polar species with interglacial refugia in the high mountains of southern Patagonia. The strong genetic and phylogeographic structure, as well as the timing of divergence, are in agreement with patterns reported in other sympatric terrestrial invertebrates. Further analyses focusing on multiple nuclear loci are required to confirm the possible speciation processes and document contemporary fine scale patterns within these three regions to fully understand the connectivity and dispersal mechanisms behind the different biogeographic scenarios proposed for *P. steinenii.* The movement of individuals between local populations is a crucial factor in the persistence and dynamics of entire meta-populations, especially in changing, fragmented and extreme environments (Lakovic et al., 2015). Understanding past and present connectivity between populations of the Antarctic winged midge can provide valuable insights into how resilient or responsive the species may be in the face of ongoing climate change.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: NCBI GenBank, accession PP824188–PP824338.

Ethics statement

The animal study was approved by Instituto Antartico Chileno Instituto de Biodiversidad Antártica y Subantártica. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CSM: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing, Investigation, Software, Visualization. TCM: Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing. FS: Data curation, Writing – review & editing. MAV: Methodology, Software, Visualization, Writing – review & editing. PV: Data curation, Writing – review & editing. MG: Visualization, Writing – review & editing. CG-W: Funding acquisition, Validation, Writing – review & editing. EP: Investigation, Resources, Validation, Writing – review & editing. CS: Validation, Writing – review & editing. PC: Funding acquisition, Investigation, Supervision, Validation, Writing – original draft, 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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2024.1393376/full#supplementary-material

References

Allegrucci, G., Carchini, G., Convey, P., and Sbordoni, V. (2012). Evolutionary geographic relationships among orthocladine chironomid midges from marine Antarctic and sub-Antarctic islands. *Biol. J. Linn. Soc* 106, 258–274. doi: 10.1111/bij.2012.106.issue-2

Allegrucci, G., Carchini, G., Todisco, V., Convey, P., and Sbordoni, V. (2006). A molecular phylogeny of antarctic chironomidae and its implications for biogeographical history. *Polar Biol.* 29, 320–326. doi: 10.1007/s00300-005-0056-7

Bandelt, H. J., Forster, P., and Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16, 37–48. doi: 10.1093/oxfordjournals.molbev.a026036

Benjamini, Y., Krieger, A. M., and Yekutieli, D. (2005). Adaptive linear step-up procedures that control the false discovery rate. *Biometrika* 93, 491–507. doi: 10.1093/biomet/93.3.491

Biersma, E. M., Torres-Diaz, C., Molina-Montenegro, M. A., Newsham, K. K., Vidal, M. A., Collado, G. A., et al. (2020). Multiple late-Pleistocene colonisation events of the Antarctic pearlwort *Colobanthus quitensis* (Caryophyllaceae) reveal the recent arrival of native Antarctic vascular flora. *J. Biogeogr.* 47, 1663–1673. doi: 10.1111/jbi.13843

Bouckaert, R. R., and Drummond, A. (2017). Bmodeltest: bayesian phylogenetic site model averaging and model comparison. *BMC Evol. Biol.* 17, 42. doi: 10.1186/s12862-017-0890-6

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchene, S., Fourment, M., Gavryushkina, A., et al. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PloS Comput. Biol.* 15, e1006650. doi: 10.1371/journal.pcbi.1006650

Brundin, L. (1966). Transantarctic relationship and their significance, as evidenced by chironomid midges. With a monograph of the subfamilies Podonominae and Aphroteniinae and the austral Heptagyiae. *K. Svenska Vetensk Akad. Handl.* 11, 1–472.

Chown, S. L., and Convey, P. (2016). Antarctic entomology. *Annu. Rev. Entomol.* 61, 119–137. doi: 10.1146/annurev-ento-010715-023537

Clapperton, C. M. (1990). Quaternary glaciation in the Southern Ocean and Antarctic Peninsula area. *Quater. Sci. Rev.* 9, 229.252. doi: 10.1016/0277-3791(90)90020-B

Clapperton, C. M., and Sugden, D. E. (1988). Holocene glacier fluctuations in South America and Antarctica. *Quater. Sci. Rev.* 7, 185–198. doi: 10.1016/0277-3791(88)90005-4

Contador, T., Ganan, M., Rendoll-Carcamo, J., Maturana, C. S., Benitez, H. A., Kennedy, J., et al. (2023). A polar insect's tale: Observations on the life cycle of Parochlus steinenii, the only winged midge native to Antarctica. *Ecology* 104, e3964. doi: 10.1002/ecy.3964

Convey, P., and Biersma, E. M. (2024). "Antarctic Ecosystems," in *Encyclopedia of Biodiversity*, *3rd ed.* Ed. S. M. Scheiner (Elsevier, Oxford), 133–148.

Convey, P., Biersma, E. M., Casanova-Katny, A., and Maturana, C. S. (2020). "Refuges of Antarctic Diversity," in *Past Antarctica*. Ed. M. Oliva (Elsevier (Barcelona, Barcelona).

Convey, P., Gibson, J. A., Hillenbrand, C. D., Hodgson, D. A., Pugh, P. J., Smellie, J. L., et al. (2008). Antarctic terrestrial life – challenging the history of the frozen continent? *Biol. Rev. Cambridge Philos. Soc.* 83, 103–117. doi: 10.1111/j.1469-185X.2008.00034.x

Convey, P., Greenslade, P., Richard, K. J., and Block, W. (1996). The terrestrial arthropod fauna of the Byers Peninsula, Livingston Island, South Shetland Islands - Collembola. *Polar Biol.* 16, 257–259. doi: 10.1007/s003000050052

Cranston, P. S., Hardy, N. B., Morse, G. E., Puslednik, L., and McCluen, S. R. (2010). When molecules and morphology concur: the 'Gondwanan' midges (Diptera: Chironomidae). *Syst. Entomol.* 35, 636–648. doi: 10.1111/j.1365-3113.2010.00531.x

Cukier, S., Fudala, K., and Bialik, R. J. (2023). Are Antarctic aquatic invertebrates hitchhiking on your footwear? *J. Nat. Conserv.* 72, 126354. doi: 10.1016/j.jnc.2023.126354

De Broyer, C., and Koubbi, P. (2014). "Chapter 1.1: The Biogegraphy of the Southern Ocean," in *Biogeographic Atlas of the Southern Ocean*. Eds. C. De Broyer, P. Koubbi, H. J. Griffiths and B. U. Raymond (Scientific Committee on Antarctic Research, Cambridge), 2–5.

Douglas, J., Zhang, R., and Bouckaert, R. (2021). Adaptive dating and fast proposals: Revisiting the phylogenetic relaxed clock model. *PloS Comput. Biol.* 17, e1008322. doi: 10.1371/journal.pcbi.1008322

Drummond, A. J., and Bouckaert, R. R. (2014). *Bayesian evolutionary analysis with BEAST 2* (Cambridge: C.U. Press).

Drummond, A. J., Suchard, M. A., Xie, D., and and Rambaut, A. (2012). Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973. doi: 10.1093/molbev/mss075

Edgington, H., Pavinato, V. A. C., Spacht, D., Gantz, J. D., Convey, P., Lee, R. E., et al. (2023). Genetic history, structure and gene flow among populations of Belgica Antarctica, the only free-living insect in the western Antarctic Peninsula. *Polar Sci.* 36. doi: 10.1016/j.polar.2023.100945

Edwards, M., and Usher, M. B. (1985). The winged Antarctic midge Parochlus steinenii (Gerke) (Diptera: Chironomidae) in the South Shetland Islands. *Biol. J. Linn. Soc* 26, 83–93. doi: 10.1111/j.1095-8312.1985.tb01553.x

Excoffier, L., and Heckel, G. (2006). Computer programs for population genetics data analysis: a survival guide. *Nat. Rev. Genet.* 7, 745–758. doi: 10.1038/nrg1904

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. C. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.

Fraser, C. I., Ruzzante, D. E., and Waters, J. M. (2012). Poleward bound: biological impacts of Southern Hemisphere glaciation. *Trends Ecol. Evol.* 27, 462–471. doi: 10.1016/j.tree.2012.04.011

Gañán, M., Contador, T., Rendoll, J., Simoes, F., Carolina, P., Graham, G., et al. (2021). Records of Parochlus steinenii in the Maritime Antarctic and sub-Antarctic regions. *Zookeys* 1011, 63–71. doi: 10.3897/zookeys.1011.56833

Gañan, M., Contador, T., Rendoll, J., Simoes, F., Pérez, C., Graham, G., et al. (2020). *Records of Parochlus steinenii in the Maritime Antarctic and sub-Antarctic regions*. doi: 10.15468/2cfwd7

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Maturana, C. S., Jeldres, V., Pinochet, R., et al. (2022). Seven snail species hidden in one: Biogeographic diversity in an apparently widespread periwinkle in the Southern Ocean. *J. Biogeogr.* 49, 1521–1534. doi: 10.1111/jbi.14453

Guillot, G., Mortier, F., and Estoup, A. (2005). Geneland: a computer package for landscape genetics. *Mol. Ecol. Notes* 5, 712–715. doi: 10.1111/j.1471-8286.2005.01031.x

Hahn, S., and Reinhardt, K. (2006). Habitat preference and reproductive traits in the Antarctic midge Parochlus steinenii (Diptera: Chironomidae). *Antarct. Sci.* 18, 175–181. doi: 10.1017/S0954102006000204

Hirao, A. S., Watanabe, Y. Y., Liu, Q.-J., Li, X., Masuzawa, T., Ohara, M., et al. (2015). Low genetic diversity and high genetic divergence in southern rear edge populations of *Dryas octopetala* in the high mountains of far East Asia. *Japan. Soc. Plant System.* 66, 11–22.

Ho, S. Y., Duchene, S., Molak, M., and Shapiro, B. (2015). Time-dependent estimates of molecular evolutionary rates: evidence and causes. *Mol. Ecol.* 24, 6007–6012. doi: 10.1111/mec.13450

Ho, S. Y., Lanfear, R., Bromham, L., Phillips, M. J., Soubrier, J., Rodrigo, A. G., et al. (2011). Time-dependent rates of molecular evolution. *Mol. Ecol.* 20, 3087–3101. doi: 10.1111/j.1365-294X.2011.05178.x

Ho, S. Y., Saarma, U., Barnett, R., Haile, J., and Shapiro, B. (2008). The effect of inappropriate calibration: three case studies in molecular ecology. *PloS One* 3, e1615. doi: 10.1371/journal.pone.0001615

Hodgson, D. A., Graham, A. G. C., Roberts, S. J., Bentley, M. J., Cofaigh, C.Ó., Verleyen, E., et al. (2014). Terrestrial and submarine evidence for the extent and timing of the Last Glacial Maximum and the onset of deglaciation on the maritime-Antarctic and sub-Antarctic islands. *Quater. Sci. Rev.* 100, 137–158. doi: 10.1016/j.quascirev.2013.12.001

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., and Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589. doi: 10.1038/nmeth.4285

Katoh, K., Rozewicki, J., and Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinform.* 20, 1160–1166. doi: 10.1093/bib/bbx108

Katoh, K., and Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780. doi: 10.1093/molbev/mst010

Koubbi, P., De Broyer, C., Griffiths, H. J., Raymond, B., d'Udekem d'Acoz, C., Van de Putte, A. P., et al. (2014). "Chapter 12: Conclusions: Present and future of Southern Ocean Biogeography," in *Biogeographic atlas of the Southern Ocean*. Eds. P. Koubbi, C. De Broyer, H. J. Griffiths, B. Raymond, C. d'Udekem d'Acoz, A. P. Van de Putte, B. D.B. Danis, S. G.J. Grant, C. Held, G. Hosie, F. Huettmann, A. Post, Y. Ropert- Coudert, M. Stoddart and K. M. Swadling (Scientific Committee on Antarctic Research, Cambridge).

Lakovic, M., Poethke, H. J., and Hovestadt, T. (2015). Dispersal timing: emigration of insects living in patchy environments. *PloS One* 10, e0128672. doi: 10.1371/journal.pone.0128672

Lee, J. R., Waterman, M. J., Shaw, J. D., Bergstrom, D. M., Lynch, H. J., Wall, D. H., et al. (2022). Islands in the ice: Potential impacts of habitat transformation on Antarctic biodiversity. *Glob Chang Biol.* 28, 5865–5880. doi: 10.1111/gcb.16331

Leigh, J. W., Bryant, D., and Nakagawa, S. (2015). popart: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6, 1110–1116. doi: 10.1111/2041-210X.12410

Livermore, R., Eagles, G., Morris, P., and Maldonado, A. (2004). Shackleton Fracture Zone: No barrier to early circumpolar ocean circulation. *Geology* 32, 797. doi: 10.1130/G20537.1

Lunt, D. H., Zhang, D. X., Szymura, J. M., and Hewitt, G. M. (1996). The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Mol. Biol.* 5, 153–165. doi: 10.1111/j.1365-2583.1996.tb00049.x

Maggs, C. A., Castilho, R., Foltz, D., Henzler, C., Jolly, M. T., Kelly, J., et al. (2008). Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. *Ecology* 89, S108–S122. doi: 10.1890/08-0257.1

Marko, P. B., Hoffman, J. I., Emme, S. A., Mcgovern, T. M., Keever, C. C., and Cox, L. N. (2010). The 'Expansion–Contraction' model of Pleistocene biogeography: rocky shores suffer a sea change? *Mol. Ecol.* 19, 146–169. doi: 10.1111/mec.2009.19.issue-1

Martin, J., Guryev, V., and Blinov, A. (2002a). Population variability in Chironomus (Camptochironomus) species (Diptera, Nematocera) with a Holarctic distribution: evidence of mitochondrial gene flow. *Insect Mol. Biol.* 11, 387–397. doi: 10.1046/j.1365-2583.2002.00348.x

Martin, J., Guryev, V., Blinov, A., and Edward, D. H. D. (2002b). A molecular assessment of the extent of variation and dispersal between Australian populations of the genus *Archaeochlus* Brundin (Diptera: Chironomidae). *Invertebr. Syst.* 16, 599–603. doi: 10.1071/IT01040

Maturana, C. S., Biersma, E. M., Díaz, A., González-Wevar, C., Contador, T., Convey, P., et al. (2022). Survivors and colonizers: Contrasting biogeographic histories reconciled in the Antarctic freshwater copepod Boeckella poppei. *Front. Ecol. Evol.* 10. doi: 10.3389/fevo.2022.1012852

Maturana, C. S., Segovia, N. I., González-Wevar, C. A., Díaz, A., Rosenfeld, S., Poulin, E., et al. (2020). Evidence of strong small-scale population structure in the Antarctic freshwater copepod *Boeckella poppei* in lakes on Signy Island, South Orkney Islands. *Limnol. Oceanogr.* 65, 2024–2040. doi: 10.1002/lno.11435

McGaughran, A., Terauds, A., Convey, P., and Fraser, C. I. (2019). Genome-wide SNP data reveal improved evidence for Antarctic glacial refugia and dispersal of terrestrial invertebrates. *Mol. Ecol.* 28 (22), 4941–4957. doi: 10.1111/mec.15269

McGaughran, A., Torricelli, G., Carapelli, A., Frati, F., Stevens, M. I., Convey, P., et al. (2010). Contrasting phylogeographical patterns for springtails reflect different evolutionary histories between the Antarctic Peninsula and continental Antarctica. *J. Biogeogr.* 37, 103–119. doi: 10.1111/j.1365-2699.2009.02178.x

Mercer, J. H. (1976). Glacial history of southermost South America. *Quat. Res.* 6, 125–166. doi: 10.1016/0033-5894(76)90047-8

Paradis, E. (2013). Molecular dating of phylogenies by likelihood methods: a comparison of models and a new information criterion. *Mol. Phylogenet. Evol.* 67, 436–444. doi: 10.1016/j.ympev.2013.02.008

Pérez-Alvarez, M., Kraft, S., Segovia, N. I., Olavarría, C., Nigenda-Morales, S., Urbán R, J., et al. (2021). Contrasting phylogeographic patterns among northern and southern hemisphere fin whale populations with new data from the southern Pacific. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.630233

Pertierra, L. R., Escribano-Álvarez, P., and Olalla-Tárraga, M. Á. (2021). Cold tolerance is similar but heat tolerance is higher in the alien insect Trichocera maculipennis than in the native Parochlus steinenii in Antarctica. *Polar Biol.* 44, 1203–1208. doi: 10.1007/s00300-021-02865-w

Provan, J., and Bennett, K. D. (2008). Phylogeographic insights into cryptic glacial refugia. *Trends Ecol. Evol.* 23, 564–571. doi: 10.1016/j.tree.2008.06.010

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., and Suchard, M. A. (2018). Posterior summarization in bayesian phylogenetics using tracer 1.7. *Syst. Biol.* 67, 901–904. doi: 10.1093/sysbio/syy032

Rambaut, A., Suchard, M. A., Xie, D., and Drummond, A. J. (2014). *Tracer v1.6.* Available at: http://beast.bio.ed.ac.uk/Tracer.

Rauschert, M. (1985). Beobachtungen an der Chironomide Parochlus steineni auf der Insel King George (Südshetlandinseln, Antarktis)(Diptera, Chironomidae). *Dtsch. Entomol. Z.* 32, 183–188. doi: 10.1002/mmnd.v32:1/3

R Development Core Team. (2019). *R: A Language and Environment for Statistical Computing* (Vienna, Austria: R Foundation for Statistical Computing).

Rodbell, D. T., Smith, J. A., and Mark, B. G. (2009). Glaciation in the andes during the lateglacial and holocene. *Quater. Sci. Rev.* 28, 2165–2212. doi: 10.1016/j.quascirev.2009.03.012

Rogers, A. R., and Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol.* 9, 552–569. doi: 10.1093/oxfordjournals.molbev.a040727

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Hohna, S., et al. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542. doi: 10.1093/sysbio/sys029

Rozas, J., Ferrer-Mata, A., Sanchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., et al. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* 34, 3299–3302. doi: 10.1093/molbev/msx248

Scher, H. D., and Martin, E. E. (2006). Timing and Climatic consequences of the opening of Drake Passage. *Science* 312, 428–430. doi: 10.1126/science.1120044

Shimada, K., Ohyama, Y., and Pan, C. (1991). Cold-hardiness of the Antarctic winged midge Parochlus steinenii during the active season at King George Island. *Polar Biol.* 11, 311–314. doi: 10.1007/BF00239023

Short, K. A., Sands, C. J., McInnes, S. J., Pisani, D., Stevens, M. I., and Convey, P. (2022). An ancient, Antarctic-specific species complex: large divergences between multiple Antarctic lineages of the tardigrade genus *Mesobiotus*. *Mol. Phylogenet. Evol.* 170, 107429. doi: 10.1016/j.ympev.2022.107429

Stamatakis, A. (2014). version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313. doi: 10.1093/bioinformatics/btu033

Stewart, J. R., Lister, A. M., Barnes, I., and Dalen, L. (2010). Refugia revisited: individualistic responses of species in space and time. *Proc. Biol. Sci.* 277, 661–671. doi: 10.1098/rspb.2009.1272

Terauds, A., Chown, S. L., Morgan, F., Peat, H. J., Watts, D. J., Keys, H., et al. (2012). Conservation biogeography of the Antarctic. *Divers. Distrib.* 18, 726–741. doi: 10.1111/j.1472-4642.2012.00925.x

van de Wouw, M., van Dijk, P., and Huiskes, A. H. L. (2008). Regional genetic diversity patterns in Antarctic hairgrass (*Deschampsia Antarctica* Desv.). *J. Biogeogr.* 35, 365–376. doi: 10.1111/j.1365-2699.2007.01784.x

van Vuuren, B. J., Lee, J. E., Convey, P., and Chown, S. L. (2018). Conservation implications of spatial genetic structure in two species of oribatid mites from the Antarctic Peninsula and the Scotia Arc. *Antarct. Sci.* 30, 105–114. doi: 10.1017/S0954102017000529

Verleyen, E., Van de Vijver, B., Tytgat, B., Pinseel, E., Hodgson, D. A., Kopalová, K., et al. (2021). Diatoms define a novel freshwater biogeography of the Antarctic. *Ecography* 44, 548–560. doi: 10.1111/ecog.05374

Whiting, M. F. (2002). Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zool. Scr.* 31, 93–104. doi: 10.1046/j.0300-3256.2001.00095.x

Zachos, J., Pagani, M., Sloan, L., Thomas, E., and Billups, K. (2001). Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292, 686–693. doi: 10.1126/science.1059412

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Comparative phylogeography, a tool to increase assessment efficiency of polar assemblage resilience and vulnerability

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The Southern Ocean benthos is remarkably rich and diverse, and managed under a complexity of treaties and conventions, further complicated by geopolitical boundaries. Traditionally, conservation management is largely informed by species lists augmented, when data are available, by known vulnerability of the taxa. Species presence absence database resources are valuable tools with proven and positive management outcomes, however, in a vast, difficult to access and thus understudied region such as the Southern Ocean, there are large gaps in knowledge regarding the ecology, ecophysiology, life history and even species identity. Conservation biogeography identifies regions of conservation concern, rather than a species-by-species approach, but also relies on the availability of high-quality presence data from species lists and thus both approaches are undermined when species lists are inaccurate or species in general are poorly described. In addition, the data provide a snapshot of the current species diversity and have inadequate power to identify the processes underlying the patterns uncovered. Identifying historical processes common to shaping diversity (species or genetic) can be generalized across assemblages and regions, providing a more robust basis for conservation policy and decisions. In this study, largely based on consideration of Southern Ocean ophiuroids, we discuss the challenges inherent in using species lists, the power and limitations of genetic analyses, and revisit previous suggestions of building a spatial model of diversity that includes underlying evolutionary relationships transcending the simple species diversity approach, and that is applicable to assemblages, rather than just to individual taxa.

KEYWORDS

conservation biology, historical demographics, IUCN Red List, comparative phylogeography, Ophiuroidea, Southern Ocean

1 Introduction

The Southern Ocean region and its rich natural resources are governed and managed by various international treaties and conventions further complicated by geopolitical boundaries. These agreements generally adopt a precautionary approach to management, conservation and exploitation, with areas south of 60 degrees of latitude falling under the Antarctic Treaty governance. However, areas under individual national governments are managed according to their specific interests and specific policy pressures. Where population sizes of species in assemblages are abundant, widespread and well connected, poor management or strong exploitation from small regional territories is unlikely to negatively impact the wider assemblage elements. However, small, regionally localized and isolated species or populations are vulnerable to pressures at small regional scales, whether they be anthropogenic or stochastic. Given that many of the nation states with territories or interests in the Southern Ocean are signatories of the Convention on Biological Diversity that explicitly defines biodiversity as including within and between species variation (Article 2), it is essential that some understanding of the biological systems is generated in order to best approach conservation management if required.

The benthic faunal assemblage of the Southern Ocean is traditionally viewed as “Antarctic”, with some influence from the South American shelf fauna around the sub-Antarctic islands (Hedgpeth, 1969; Griffiths et al., 2009). Under this paradigm, there is little need for concern regarding the small, regionally managed locations in the Southern Ocean as any regional assemblages in decline at this scale can be continually seeded from the vast and well-connected populations surrounding them. However, the Southern Ocean benthos is very difficult to study, and as a result poorly understood in regard to any aspect of its ecology, ecophysiology, even species identity. The past 20 years have seen a change in the way biodiversity is assessed and a wealth of genetic data is challenging the widespread assumption of a general “Antarctic” fauna (Linse et al., 2007; Leese and Held, 2008; Havermans et al., 2011; Dietz et al., 2015; Dömel et al., 2017; Guzzi et al., 2022). Rather than well-connected assemblages with large population sizes, genetic data – largely mitochondrial sequences – imply that biogeographically structured populations may be the norm, potentially with many cryptic or currently unrecognized species, or at least isolated populations following independent evolutionary trajectories.

The resources available for assessing diversity patterns used in first pass conservation assessment across the vast and relatively unknown Southern Ocean largely consist of species lists such as the Global Biodiversity Information Facility (GBIF, 2024) and the International Union for Conservation of Nature’s (IUCN) Red List (IUCN Red List, 2024), the latter augmented by information regarding each species’ vulnerability status (Hilton-Taylor and Brackett, 2000; Rodrigues et al., 2006; Vié et al., 2009). These are demonstrably valuable resources (Heberling et al., 2021) and freely available online. Their full value relies heavily on the extent of data being collected (Huettmann, 2020) and its quality (Abe, 2005; Zizka et al., 2020; Rocha-Ortega et al., 2021). Unreliable taxonomic identification is certainly a significant issue, but a more pressing and perhaps damaging challenge is given by formal taxonomic descriptions that are inadequate for subsequent confident identification or that inadvertently underestimate species diversity. These taxonomic limitations will overestimate species distributions, population sizes and connectivity, the three key elements that inform assessment of a species’ vulnerability status. Genetic techniques can ameliorate these limitations, but associating genetic diversity with species diversity is difficult and controversial (Blaxter, 2004; DeSalle et al., 2005; Ebach and Holdrege, 2005; Cook et al., 2010). Complicating this further is the ongoing debate regarding what a species is and its relevance in biology (Aldhebiani, 2018; Stanton et al., 2019). With species lists requiring accurate species descriptions, many available species descriptions are currently inadequate in relation to the distribution of genetic diversity, and genetic diversity in turn not reflected in species descriptions required for species lists, a problem exists that is set to confound efforts to assess diversity patterns essential for raising conservation awareness.

Here we share a perspective for an alternative strategy to reliance on presence/absence species lists, based on a proven technique that uses information relating to the processes that underly geographic patterns observed in genetic sequence data (Taberlet and Bouvet, 1994; Moritz and Faith, 1998; Emerson et al., 2001). Shared historical processes can be inferred from genetic data of selected target species across a landscape. Such processes are likely to have been experienced by all species in any given assemblage. Recognition of this provides this provides a model that can help inform managers of the geographic extent of diversity, the size of local populations and the connectivity between populations, allowing inference of the potential for resilience in any given species or population. The benefit of such an approach is that it circumvents the need for accurate “traditional” species identifications. At the same time, it helps define potential species boundaries, highlights patterns of variation along the species continuum, and provides both geographic, ecological and evolutionary information regarding population (and by extension, assemblage) resilience.

As conservation management and policy are currently tightly bound to formally described species, traditional taxonomy is time intensive and relies on a rapidly dwindling skill pool (Wägele et al., 2011; Saucède et al., 2020), the number of putative new species “discovered” in molecular studies vastly exceeds the rate at which new species descriptions are possible. This creates a profound challenge that undermines the key importance of the IUCN Red List and the multitude of conventions it informs. In essence the bulk of the diversity on Earth is likely to be a large underestimate, and while the focus of conservation efforts is directed on a species-by-species basis, this is at a cost to regional assemblages holding unappreciated, unique diversity.

2 The species challenge: do species matter?

The term “species” is used frequently in biology and by non-biologists referring to biological units. Very few would argue with this and yet an acceptable definition, or concept, of a "species" remains elusive leading to a long running debate that has been described as "…sometimes boring and seemingly fruitless, but is not wholly futile" (Simpson, 1951). The debate continues with the concept of a species recognized as fundamental to biology, but controversial amongst biologists (Wheeler and Meier, 2000). There are currently at least 28 competing species concepts (Wilkins, 2018), each providing arguments justifying the specific groupings as recognized by the responsible authors. The challenge, with so many views on what a species may be, and the variety of concepts created, is that they are often incompatible in their outcomes, with individuals or populations being included in or excluded from a given species depending on which definition is adopted. Underlying this debate is the fact that the process of speciation is a continuum over time, that the time taken to "speciate" differs among species, the species or population under examination may be anywhere along the speciation continuum and the species concept criterion applied may also vary along that continuum. For example, where ecological or sexual selection is strong, such as with cichlid fish (Barluenga and Meyer, 2004), speciation as determined by discrete morphology or ecology may be readily apparent, fitting the ecological and morphological species concept, and yet the same groupings may be impossible to detect using genetic sequence data, thereby not satisfying the phylogenetic, Hennigian or cladistic species concept.

To enable meaningful comparison of species across space and time, species concepts matter (Cracraft, 2000), especially within a management context. The term "species" is critical for conservation biology, as it has been almost universally adopted as the base level of diversity. Of particular note in this context is the IUCN Red List, widely adopted by international conventions and treaties such as the Convention on International Trade in Endangered Species (CITES), Convention on Migratory Species (CMS), Convention on Biological Diversity (CBD), Ramsar Convention and Intergovernmental Science-policy Platform on Biodiversity and Ecosystem Services (IPBES). The Red List influences resource allocation (e.g. Global Environment Facility) and informs conservation planning (e.g. Key Biodiversity Areas; IUCN Red List, 2022). The central criterion required before initiating a Red List assessment is that the unit being assessed is "at or below the species level" (IUCN Red List Categories and Criteria V3.1 second edition, Preamble, paragraph 1), confirming that the base level of diversity is the species, with the recognition that further subdivision is possible.

On this basis, "species" do matter, specifically formally described species. This leads to the question: to what extent does the accurate parameterization of a species affect conservation biology, particularly in the light of confusing concepts and definitions? Answering this question is long and complex and depends on perspective and context. In the context of biodiversity management across a large realm managed by multiple states, treaties and conventions, such as the Southern Ocean, we suggest that any consequences of overestimating diversity based on "species" assemblages would be negligible, whereas underestimating diversity could be catastrophic.

3 Does DNA hold the solution?

The advent of DNA sequencing and phylogenetic reconstruction has resulted in an understanding of the systematic structure of the tree of life (Li et al., 2021) far exceeding the expectations of the evolutionary biologists of previous generations (Dobzhansky, 1935). In some groups, the use of multiple genetic markers and sophisticated analyses has resulted in very high resolution of relationships. Here, we focus as an exemplar on the work being carried out on class Ophiuroidea, known as brittle stars (O'Hara et al., 2017), where phylogenetic relationships have been reconciled with previously unappreciated informative morphological characters (Thuy and Stöhr, 2016) to formally restructure the taxonomy of the class (O'Hara et al., 2018). However, taking genetic inference at face value, without independent supporting evidence, can lead to the proposal of false relationships and incorrect interpretations.

The start of the twenty-first century saw a movement towards DNA based taxonomy, species delimitation and identification (Tautz et al., 2002; Wiens and Penkrot, 2002; Tautz et al., 2003; Blaxter, 2004; Hebert and Gregory, 2005). With the investment of substantial funding, initiatives such as the International Barcode of Life (International Barcode of Life – Illuminate Biodiversity, 2024) have resulted in many thousands of studies contributing data to resources such as the Barcode of Life Database (Ratnasingham and Hebert, 2007), which currently displays a total of 255,000 animal species with sequence data, relating to 1,046,000 "BINS" or putative DNA barcode species from 15,910,000 DNA barcodes (http://www.boldsystems.org accessed 28th February 2024). Whether this is an appropriate strategy for determining species or not is debatable, but the publicly available resource of a DNA sequence alongside a georeferenced specimen that has been unambiguously identified is precious, widely comparable and growing. There has been considerable resistance to the concept of DNA barcoding and its use in taxonomy (Mallet and Willmott, 2003; DeSalle et al., 2005; Ebach and Holdrege, 2005; Will et al., 2005). A synopsis of this debate is that DNA barcoding as a step in taxonomic discovery is useful but with exceptions. Incomplete lineage sorting and introgression can confuse genetic signals. Furthermore, population theory suggests that large, stable populations will hold large amounts of diversity over long periods of time (Pfaffelhuber et al., 2011; Nordborg, 2019), which could be confused with deeply divergent clades on a phylogeny. For a large number of well-characterized species, mitochondrial DNA sequences do indeed accurately define individual species groups (Hogg and Hebert, 2004; Rock et al., 2008; Ward et al., 2008; Jossart et al., 2021), but results and interpretations should be viewed with caution (Havermans et al., 2010, 2011).

4 Repeated patterns in genes and geography

Given the difficulties surrounding defining a species, the challenges of describing species and general uncertainty in assigning species using molecular techniques, a change in perspective is required. Patterns shared across taxa indicate shared historical events, in which case they probably represent the structure of assemblages (Moritz and Faith, 1998). The subdiscipline that specifically addresses the spatial distribution of genetic diversity is known as phylogeography (Avise et al., 1987; Avise, 2000). When analyses of co-distributed species are involved it is termed comparative phylogeography (Bermingham and Moritz, 1998). Linking gene trees, or evolutionary relationships, to geography provides historical information relevant to the formation of discrete spatial distributions (Taberlet et al., 1998; Hewitt, 2004). The information generated from such analyses transcends that of species diversity or richness (Taberlet et al., 2012) as it spans the divergence continuum from individuals to species and beyond.

Benthic species diversity is high in the Southern Ocean (Arntz et al., 1994; Brey et al., 1994; Clarke and Johnston, 2003) with representatives of the class Ophiuroidea (Figure 1) often the most abundant in terms of numbers and biomass (Martín-Ledo and López-González, 2013; Sands et al., 2013). Several studies have identified cryptic diversity in Southern Ocean ophiuroids (Hunter and Halanych, 2008; Martín-Ledo et al., 2013; Sands et al., 2015; Galaska et al., 2017a, b; Jossart et al., 2019; Sands et al., 2021). For example, the sub-Antarctic species *Ophiuroglypha lymani* Ljungman, 1871 forms several island-specific clades, including one in which its sister species, the Antarctic shelf endemic *Ophiuroglypha carinifera* Koehler, 1901 is subsumed (Sands et al., 2015). Similarly, *Astrotoma agassizii* Lyman, 1875 has several discrete clades across the Southern Ocean, one which includes its sister species *Astrotoma drachii* Guille, 1979 (Jossart et al., 2019).

There is strong evidence that both *Astrotoma agassizii* and *Ophiuroglypha lymani* represent species complexes. In these two examples, this results in increased overall diversity while individual population sizes have decreased. Both species groups appear to have congruent phylogeographic structure – in that it appears that clades are specific to island shelf regions and the Antarctic shelf region (Sands et al., 2015; Jossart et al., 2019). Most interesting perhaps is the sister-species/outgroup problem as, in both cases, the sister-species appears to be an element of the species complex rather than an outgroup, and this pattern has been found again with respect to *Amphiura belgicae* Koehler, 1900 and *A. eugeniae* Ljungman, 1867, its likely sister-species. (Sands et al. This Volume). This again suggests shared demographic histories and provides a starting point for comparative analyses.

5 A generalized model of genetic variation across the Southern Ocean

The concept that regions of conservation value can be identified based on co-distribution of discrete genetic variation between species is not new (Faith, 1992, 1993). Over the past 30 years the field has matured (Garrick et al., 2015; Edwards et al., 2022; McGaughran et al., 2022), with the cost of laboratory work much reduced and the sophistication and sensitivity of analyses greatly increased. Based on some of the above citations we can generalize that the island shelves around the Southern Ocean, particularly South Georgia, appear to contain discrete genetic types distinct from those around the Antarctic (Linse et al., 2007; Sands et al., 2015; Jossart et al., 2019). This is the basis of a model and more taxa should be added to strengthen it. The effects of the shared historical process may not be recorded in the genetic regions of all members of an assemblage, as life history difference in particular having a strong influence on retained polymorphisms (Avise, 2009; da Silva Ribeiro et al., 2020). For example, decapods with high dispersal capacity show a Southern Ocean wide panmictic distribution (Raupach et al., 2010; Dambach et al., 2016). Most *individuals* across the Southern Ocean are from species with some developmental stage with high dispersal capacity, but most "*species*" tend to have low dispersal in general (Poulin et al., 2002). For this reason choosing an appropriate taxon that is likely to have retained ancestral signal in its DNA is important for adequately building and testing a model such as we are suggesting.

Choosing taxa likely to hold historical information is one consideration. Others are the sample size and geographic spread of the study collection (Ruiz-García et al., 2021). Sufficient sample size within each region is required to compare the diversity of genetic types within a region as well as between regions. For example, in Sands et al. (2015) the included samples of *Ophiuroglypha lymani* from the South Sandwich Islands and Bouvetøya were distinctly divergent from other regions sampled. However, the sample sizes of these two regions were three and two respectively. If sample sizes for all regions were equally small there would be little confidence in their distinctness, and it is the larger sample sizes of the target regions in this study that indicate they are likely to be discrete populations. Broad geographic collections are more likely to hold more power for accurate inference. The studies comparing *Astrotoma agassizii* from Patagonian shelf and Antarctic shelf (Hunter and Halanych, 2008; Galaska et al., 2017a) identified divergences between populations, but the true extent of the diversity within *A. agassizii* was not appreciated until samples were collected and analyzed from across the Southern Ocean (Jossart et al., 2019).

Using gene trees for demographic inference using a single marker is prone uncertainty and risks over interpretation (Knowles and Maddison, 2002). The ability to use multiple genetic markers as a method of within-group replication is a powerful option for refining demographic inference for a single species (Brito and Edwards, 2009; Garrick et al., 2015; Edwards et al., 2022). However, the costs and complexity associated with the huge amount of data collected for these types of studies are substantial. For the purpose of this particular exercise – descriptive model of spatial diversity of the Southern Ocean – we suggest that it is by replicating species groups rather than loci that power and rigor can be obtained. Southern Ocean collection expeditions are infrequent, usually geographically limited, and costly in time, man power and environmental cost. Collections are not selective, so it is likely that they include a substantial number of different taxa from which to build relatively cost effective comparative studies. Inference from single marker gene trees

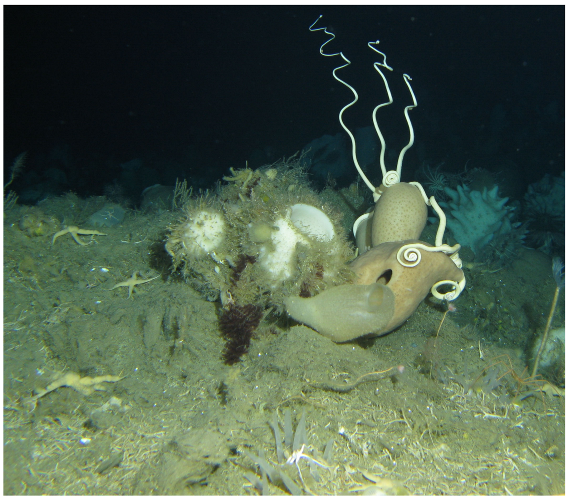

FIGURE 1
Image of the sea floor from the Eastern Weddell Sea at a depth of ~300m. The large brittle stars attached to hexactinellid sponges are *Astrotoma agassizii*, the brittle stars covered in *lophon* sponge symbiont are *Ophioplinthus gelida*, and the white brittle star in the foreground is *Ophiostiera antarctica*. The photograph was taken using an ROV by Tomas Lundäv and used with permission.

should be treated with caution, but they are still a powerful tool in their own right (Avise, 2009) and once a comparative model can be generated, more definitive, if expensive, methods can and should be used to test the models rigor.

6 Conclusions

The diversity of the Southern Ocean benthic fauna, described as remarkably rich (Clarke and Johnston, 2003), is underestimated. The reliance of species lists and databases obscures the true extent of diversity, fails to capture the uniqueness of shelf assemblages and does nothing to promote awareness of small, isolated populations likely in need of conservation management. Furthermore, it ignores the genetic variation within species that is within the definition of Biological Diversity according to the Convention on Biological Diversity Article 2. A model developed on a series of phylogeographic studies of benthic taxa sampled across the Southern Ocean would quickly identify patterns and hint at shared processes underlying genetic diversity, providing a robust tool that highlights regions conservation priorities.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

CS: Conceptualization, Writing – original draft, Writing – review & editing. WG-C: Conceptualization, Supervision, Writing – review & editing. SS: Supervision, Writing – review & editing. BN: Supervision, Writing – review & editing. PC: 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.

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Publisher's note

References

Abe, W. (2005). *Insuetifurca austronipponica*, a new tardigrade (Eutardigrada : Macrobiotidae) from Kagoshima, Southern Japan. *Zool. Sci.* 22, 1295–1299. doi: 10.2108/zsj.22.1295

Aldhebiani, A. Y. (2018). Species concept and speciation. *Saudi J. Biol. Sci.* 25, 437–440. doi: 10.1016/j.sjbs.2017.04.013

Arntz, W. E., Brey, T., and Gallardo, V. (1994). Antarctic zoobenthos. *Oceanogr. Mar. Biol.* 32, 241–304.

Avise, J. C. (2000). *Phylogeography: The History and Formation of Species*. doi: 10.2307/j.ctv1nzfgj7

Avise, J. C. (2009). Phylogeography: retrospect and prospect. *J. Biogeogr.* 36, 3–15. doi: 10.1111/j.1365-2699.2008.02032.x

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., et al. (1987). Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Ann. Rev. Ecol. Syst.* 18, 489–522. doi: 10.1146/annurev.es.18.110187.002421

Barluenga, M., and Meyer, A. (2004). The Midas cichlid species complex: incipient sympatric speciation in Nicaraguan cichlid fishes? *Mol. Ecol.* 13, 2061–2076. doi: 10.1111/j.1365-294X.2004.02211.x

Bermingham, E., and Moritz, C. (1998). Comparative phylogeography - concepts and applications. *Mol. Ecol.* 7, 367–369. doi: 10.1046/j.1365-294x.1998.00424.x

Blaxter, M. L. (2004). The promise of a DNA taxonomy. *Philos. Trans. R. Soc Lond. B. Biol. Sci.* 359, 669–679. doi: 10.1098/rstb.2003.1447

Brey, T., Klages, M., Dahm, C., Gorny, M., Gutt, J., Hain, S., et al. (1994). Antarctic benthic diversity. *Nature* 368, 297–297. doi: 10.1038/368297a0

Brito, P. H., and Edwards, S. V. (2009). Multilocus phylogeography and phylogenetics using sequence-based markers. *Genetica* 135, 439–455. doi: 10.1007/s10709-008-9293-3

Clarke, A., and Johnston, N. M. (2003). Antarctic marine benthic diversity. *Oceanogr. Mar. Biol. Annu. Rev.* 41, 47–114.

Cook, L. G., Edwards, R. D., Crisp, M. D., and Hardy, N. B. (2010). Need morphology always be required for new species descriptions? *Invertebr. Syst.* 24, 322–326.

Cracraft, J. (2000). Species concepts in theoretical and applied biology: a systematic debate with consequences.

Dambach, J., Raupach, M. J., Leese, F., Schwarzer, J., and Engler, J. O. (2016). Ocean currents determine functional connectivity in an Antarctic deep-sea shrimp. *Mar. Ecol.* 37, 1336–1344. doi: 10.1111/maec.12343

da Silva Ribeiro, T., Batalha-Filho, H., Silveira, L. F., Miyaki, C. Y., and Maldonado-Coelho, M. (2020). Life history and ecology might explain incongruent population structure in two co-distributed montane bird species of the Atlantic Forest. *Mol. Phylogenet. Evol.* 153, 106925. doi: 10.1016/j.ympev.2020.106925

DeSalle, R., Egan, M. G., and Siddall, M. (2005). The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philos. Trans. R. Soc B Biol. Sci.* 360, 1905–1916. doi: 10.1098/rstb.2005.1722

Dietz, L., Arango, C. P., Dömel, J. S., Halanych, K. M., Harder, A. M., Held, C., et al. (2015). Regional differentiation and extensive hybridization between mitochondrial clades of the Southern Ocean giant sea spider *Colossendeis megalonyx*. *Open Sci.* 2, 140424. doi: 10.1098/rsos.140424

Dobzhansky, T. (1935). A critique of the species concept in biology. *Philos. Sci.* 2, 344–355. doi: 10.1086/286379

Dömel, J. S., Melzer, R. R., Harder, A. M., Mahon, A. R., and Leese, F. (2017). Nuclear and Mitochondrial Gene Data Support Recent Radiation within the Sea Spider Species Complex *Pallenopsis patagonica*. *Front. Ecol. Evol.* 4. doi: 10.3389/fevo.2016.00139

Ebach, M. C., and Holdrege, C. (2005). DNA barcoding is no substitute for taxonomy. *Nature* 434, 697–697. doi: 10.1038/434697b

Edwards, S. V., Robin, V. V., Ferrand, N., and Moritz, C. (2022). The evolution of comparative phylogeography: putting the geography (and more) into comparative population genomics. *Genome Biol. Evol.* 14, evab176. doi: 10.1093/gbe/evab176

Emerson, B. C., Paradis, E., and Thébaud, C. (2001). Revealing the demographic histories of species using DNA sequences. *Trends Ecol. Evol.* 16, 707–716. doi: 10.1016/S0169-5347(01)02305-9

Faith, D. (1993). Biodiversity and systematics: the use and misuse of divergence information in assessing taxonomic diversity. *Pac Cons Biol.* 1, 53–57.

Faith, D. P. (1992). Conservation evaluation and phylogenetic diversity. *Biol. Conserv.* 61, 1–10. doi: 10.1016/0006-3207(92)91201-3

Galaska, M. P., Sands, C. J., Santos, S. R., Mahon, A. R., and Halanych, K. M. (2017a). Crossing the divide: admixture across the antarctic polar front revealed by the brittle star astrotoma agassizii. *Biol. Bull.* doi: 10.1086/693460

Galaska, M. P., Sands, C. J., Santos, S. R., Mahon, A. R., and Halanych, K. M. (2017b). Geographic structure in the Southern Ocean circumpolar brittle star *Ophionotus victoriae* (Ophiuridae) revealed from mtDNA and single-nucleotide polymorphism data. *Ecol. Evol.* 7, 475–485. doi: 10.1002/ece3.2617

Garrick, R. C., Bonatelli, I. A. S., Hyseni, C., Morales, A., Pelletier, T. A., Perez, M. F., et al. (2015). The evolution of phylogeographic data sets. *Mol. Ecol.* 24, 1164–1171. doi: 10.1111/mec.13108

GBIF (2024). Available online at: https://www.gbif.org/ (Accessed February 29, 2024).

Griffiths, H. J., Barnes, D. K. A., and Linse, K. (2009). Towards a generalized biogeography of the Southern Ocean benthos. *J. Biogeogr.* 36, 162–177. doi: 10.1111/j.1365-2699.2008.01979.x

Guille, A. (1979). Astrotoma drachi, nouvelle espéce bathyale d'Ophiuride, Gorgonocephalidae des îles Philippines. *Vie Milieu*, 437–442.

Guzzi, A., Alvaro, M. C., Danis, B., Moreau, C., and Schiaparelli, S. (2022). Not all that glitters is gold: barcoding effort reveals taxonomic incongruences in iconic ross sea stars. *Diversity* 14, 457. doi: 10.3390/d14060457

Havermans, C., Nagy, Z., Sonet, G., De Broyer, C., and Martin, P. (2010). Incongruence between molecular phylogeny and morphological classification in amphipod crustaceans: A case study of Antarctic lysianassoids. *Mol. Phylogenet. Evol.* 55, 202–209. doi: 10.1016/j.ympev.2009.10.025

Havermans, C., Nagy, Z. T., Sonet, G., De Broyer, C., and Martin, P. (2011). DNA barcoding reveals new insights into the diversity of Antarctic species of *Orchomene* sensu lato (Crustacea: Amphipoda: Lysianassoidea). *Deep Sea Res. Part II Top. Stud. Oceanogr.* 58, 230–241. doi: 10.1016/j.dsr2.2010.09.028

Heberling, J. M., Miller, J. T., Noesgaard, D., Weingart, S. B., and Schigel, D. (2021). Data integration enables global biodiversity synthesis. *Proc. Natl. Acad. Sci.* 118, e2018093118. doi: 10.1073/pnas.2018093118

Hebert, P. D. N., and Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. *Syst. Biol.* 54, 852–859. doi: 10.1080/10635150500354886

Hedgpeth, J. W. (1969). *Introduction to Antarctic zoogeography. Distribution of selected groups of marine invertebrates in waters south of 35° S.* (New York: American Geographical Society), 1–29.

Hewitt, G. M. (2004). The structure of biodiversity – insights from molecular phylogeography. *Front. Zool.* 1, 4. doi: 10.1186/1742-9994-1-4

Hilton-Taylor, C., and Brackett, D. (2000). *2000 IUCN Red List of threatened species*.

Hogg, I. D., and Hebert, P. D. N. (2004). Biological identification of springtails (Hexapoda : Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *Can. J. Zool.-Rev. Can. Zool.* 82, 749–754. doi: 10.1139/z04-041

Huettmann, F. (2020). "The Forgotten Data: A Rather Short but Deep Story of Museums and Libraries in HKH and Similar Information Sources in Support of the Global Biodiversity Information System (GBIF.org) and Model-Predictions for Improved Conservation Management," in *Hindu Kush-Himalaya Watersheds Downhill: Landscape Ecology and Conservation Perspectives*. Eds. G. R. Regmi and F. Huettmann (Springer International Publishing, Cham), 497–520. doi: 10.1007/978-3-030-36275-1_25

Hunter, R. L., and Halanych, K. M. (2008). Evaluating Connectivity in the Brooding Brittle Star *Astrotoma agassizii* across the Drake Passage in the Southern Ocean. *J. Hered.* 99, 137–148. doi: 10.1093/jhered/esm119

International Barcode of Life – Illuminate Biodiversity (2024). Available online at: https://ibol.org/ (Accessed March 7, 2024).

IUCN Red List (2022). *The IUCN Red List of threatened species*. Available online at: https://www.iucnredlist.org/en (Accessed August 29, 2022).

IUCN Red List (2024). *The IUCN Red List of threatened species*. Available online at: https://www.iucnredlist.org/en (Accessed March 4, 2024).

Jossart, Q., Kochzius, M., Danis, B., Saucède, T., and Moreau, C. V. E. (2021). Diversity of the Pterasteridae (Asteroidea) in the Southern Ocean: a molecular and morphological approach. *Zool. J. Linn. Soc* 192, 105–116. doi: 10.1093/zoolinnean/zlaa097

Jossart, Q., Sands, C. J., and Sewell, M. A. (2019). Dwarf brooder versus giant broadcaster: combining genetic and reproductive data to unravel cryptic diversity in an Antarctic brittle star. *Heredity* 1. doi: 10.1038/s41437-019-0228-9

Knowles, L. L., and Maddison, W. P. (2002). Statistical phylogeography. *Mol. Ecol.* 11, 2623–2635. doi: 10.1046/j.1365-294X.2002.01637.x

Koehler, R. (1900). Note préliminaire sur les Echinides et les Ophiures de l'Expédition antarctique belge … *impr*, Hayez.

Koehler, R. (1901). Expédition Antarctique Belge. Résultats du vogage de S. Y. "Belgica" en 1897-98-99. *Echinides Ophiures. Zoologie*, 1–42.

Leese, F., and Held, C. (2008). Identification and characterization of microsatellites from the Antarctic isopod *Ceratoserolis trilobitoides*: nuclear evidence for cryptic species. *Conserv. Genet.* 9, 1369–1372. doi: 10.1007/s10592-007-9491-z

Li, Y., Shen, X.-X., Evans, B., Dunn, C. W., and Rokas, A. (2021). Rooting the animal tree of life. *Mol. Biol. Evol.* 38, 4322–4333. doi: 10.1093/molbev/msab170

Linse, K., Cope, T., Lörz, A.-N., and Sands, C. J. (2007). Is the Scotia Sea a centre of Antarctic marine diversification? Some evidence of cryptic speciation in the circum-Antarctic bivalve *Lissarca notorcadensis* (Arcoidea: Philobryidae). *Polar Biol.* 30, 1059–1068. doi: 10.1007/s00300-007-0265-3

Ljungman, A. V. (1867). *Ophiuroidea viventia huc usque cognita, enumerat* (PA Norstedt & Filii).

Ljungman, A. V. (1871). Om tvänne nya arter Ophiurider. Öfversigt Af Kongl Vetensk.-Akad. *Förh. Stockh.* 27, 471–475.

Lyman, T. (1875). *Ophiuridae and Astrophytidae: including those dredged by the late Dr. William Stimpson* (University Press).

Mallet, J., and Willmott, K. (2003). Taxonomy: renaissance or tower of babel? *Trends Ecol. Evol.* 18, 57–59. doi: 10.1016/S0169-5347(02)00061-7

Martín-Ledo, R., and López-González, P. J. (2013). Brittle stars from Southern ocean (Echinodermata: ophiuroidea). *Polar Biol.*, 1–16. doi: 10.1007/s00300-013-1411-8

Martín-Ledo, R., Sands, C. J., and López-González, P. J. (2013). A new brooding species of brittle star (Echinodermata: Ophiuroidea) from Antarctic waters. *Polar Biol.* 36, 115–126. doi: 10.1007/s00300-012-1242-z

McGaughran, A., Liggins, L., Marske, K. A., Dawson, M. N., Schiebelhut, L. M., Lavery, S. D., et al. (2022). Comparative phylogeography in the genomic age: Opportunities and challenges. *J. Biogeogr.* 49, 2130–2144. doi: 10.1111/jbi.14481

Moritz, C., and Faith, D. P. (1998). Comparative phylogeography and the identification of genetically divergent areas for conservation. *Mol. Ecol.* 7, 419–429. doi: 10.1046/j.1365-294x.1998.00317.x

Nordborg, M. (2019). “Coalescent theory,” in *Handb. Stat. Genomics Two Vol. Set*, 145–130.

O’Hara, T. D., Hugall, A. F., Thuy, B., Stöhr, S., and Martynov, A. V. (2017). Restructuring higher taxonomy using broad-scale phylogenomics: The living Ophiuroidea. *Mol. Phylogenet. Evol.* 107, 415–430. doi: 10.1016/j.ympev.2016.12.006

O’Hara, T. D., Stöhr, S., Hugall, A. F., Thuy, B., and Martynov, A. (2018). Morphological diagnoses of higher taxa in Ophiuroidea (Echinodermata) in support of a new classification. *Eur. J. Taxon*. doi: 10.5852/ejt.2018.416

Pfaffelhuber, P., Wakolbinger, A., and Weisshaupt, H. (2011). The tree length of an evolving coalescent. *Probab. Theory Relat. Fields* 151, 529–557. doi: 10.1007/s00440-010-0307-6

Poulin, E., Palma, A. T., and Féral, J.-P. (2002). Evolutionary versus ecological success in Antarctic benthic invertebrates. *Trends Ecol. Evol.* 17, 218–222. doi: 10.1016/S0169-5347(02)02493-X

Ratnasingham, S., and Hebert, P. D. N. (2007). BOLD: the barcode of life data system (http://www.barcodinglife.org). *Mol. Ecol. Notes* 7, 355–364. doi: 10.1111/j.1471-8286.2007.01678.x

Raupach, M., Thatje, S., Dambach, J., Rehm, P., Misof, B., and Leese, F. (2010). Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes*. *Mar. Biol.* 157, 1783–1797. doi: 10.1007/s00227-010-1451-3

Rocha-Ortega, M., Rodriguez, P., and Córdoba-Aguilar, A. (2021). Geographical, temporal and taxonomic biases in insect GBIF data on biodiversity and extinction. *Ecol. Entomol.* 46, 718–728. doi: 10.1111/een.13027

Rock, J., Costa, F. O., Walker, D. I., North, A. W., Hutchinson, W. F., and Carvalho, G. R. (2008). DNA barcodes of fish of the scotia sea, Antarctica indicate priority groups for taxonomic and systematics focus. *Antarct. Sci.* 20, 253–262. doi: 10.1017/S0954102008001120

Rodrigues, A. S., Pilgrim, J. D., Lamoreux, J. F., Hoffmann, M., and Brooks, T. M. (2006). The value of the IUCN Red List for conservation. *Trends Ecol. Evol.* 21, 71–76. doi: 10.1016/j.tree.2005.10.010

Ruiz-García, M., Jaramillo, M. F., Sánchez-Castillo, S., Castillo, M. I., Pinto, C. M., and Shostell, J. M. (2021). “Effects of Sample Size in the Determination of the True Number of Haplogroups or ESUs Within a Species with Phylogeographic and Conservation Purposes: The Case of Cebus albifrons in Ecuador, and the Kinkajous and Coatis Throughout Latin America,” in *Molecular Ecology and Conservation Genetics of Neotropical Mammals*. Eds. M. Nardelli and J. I. Túnez (Springer International Publishing, Cham), 101–148. doi: 10.1007/978-3-030-65606-5_6

Sands, C. J., Griffiths, H. J., Downey, R. V., Barnes, D. K. A., Linse, K., and Martín-Ledo, R. (2013). Observations of the ophiuroids from the West Antarctic sector of the Southern Ocean. *Antarct. Sci.* 25, 3–10. doi: 10.1017/S0954102012000612

Sands, C. J., O’Hara, T., Barnes, D. K. A., and Martín-Ledo, R. (2015). Against the flow: evidence of multiple recent invasions of warmer continental shelf waters by a Southern Ocean brittle star. *Evol. Popul. Genet.* 3. doi: 10.3389/fevo.2015.00063

Sands, C. J., O’Hara, T. D., and Martín-Ledo, R. (2021). Pragmatic assignment of species groups based on primary species hypotheses: the case of a dominant component of the Southern ocean benthic fauna. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.723328

Saucède, T., Eléaume, M., Jossart, Q., Moreau, C., Downey, R., Bax, N., et al. (2020). Taxonomy 2.0: computer-aided identification tools to assist Antarctic biologists in the field and in the laboratory. *Antarct. Sci.*, 1–13. doi: 10.1017/S0954102020000462

Simpson, G. G. (1951). The species concept. *Evolution* 5, 285–298. doi: 10.2307/2405675

Stanton, D. W. G., Frandsen, P., Waples, R. K., Heller, R., Russo, I.-R. M., Orozco-terWengel, P. A., et al. (2019). More grist for the mill? Species delimitation in the genomic era and its implications for conservation. *Conserv. Genet.* 20, 101–113. doi: 10.1007/s10592-019-01149-5

Taberlet, P., and Bouvet, J. (1994). Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear *ursus arctos* in Europe. *Proc. R. Soc B Biol. Sci.* 255, 195–200.

Taberlet, P., Fumagalli, L., Wustsaucy, A. G., and Cosson, J. F. (1998). Comparative phylogeography and postglacial colonization routes in Europe. *Mol. Ecol.* 7, 453–464. doi: 10.1046/j.1365-294x.1998.00289.x

Taberlet, P., Zimmermann, N. E., Englisch, T., Tribsch, A., Holderegger, R., Alvarez, N., et al. (2012). Genetic diversity in widespread species is not congruent with species richness in alpine plant communities. *Ecol. Lett.* 15, 1439–1448. doi: 10.1111/ele.12004

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H., and Vogler, A. P. (2002). DNA points the way ahead in taxonomy. *Nature* 418, 479. doi: 10.1038/418479a

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H., and Vogler, A. P. (2003). A plea for DNA taxonomy. *Trends Ecol. Evol.* 18, 70–74. doi: 10.1016/S0169-5347(02)00041-1

Thuy, B., and Stöhr, S. (2016). A new morphological phylogeny of the ophiuroidea (Echinodermata) accords with molecular evidence and renders microfossils accessible for cladistics. *PloS One* 11, e0156140. doi: 10.1371/journal.pone.0156140

Vié, J.-C., Hilton-Taylor, C., Pollock, C., Ragle, J., Smart, J., Stuart, S. N., et al. (2009). “The IUCN Red List: a key conservation tool,” in *Wildl. Chang. World–An Anal. 2008 IUCN Red List Threat. Species*, vol. 1.

Wägele, H., Klussmann-Kolb, A., Kuhlmann, M., Haszprunar, G., Lindberg, D., Koch, A., et al. (2011). The taxonomist - an endangered race. A practical proposal for its survival. *Front. Zool.* 8, 25. doi: 10.1186/1742-9994-8-25

Ward, R. D., Holms, B. H., and O’Hara, T. D. (2008). DNA barcoding discriminates echinoderm species. *Mol. Ecol. Resour.* 8, 1202–1211. doi: 10.1111/j.1755-0998.2008.02332.x

Wheeler, Q., and Meier, R. (2000). *Species Concepts and Phylogenetic Theory: A Debate* (Columbia University Press).

Wiens, J. J., and Penkrot, T. A. (2002). Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (Sceloporus). *Syst. Biol.* 51, 69–91. doi: 10.1080/106351502753475880

Wilkins, J. S. (2018). *Species: The Evolution of the Idea. 2nd ed.* (CRC Press). doi: 10.1201/b22202

Will, K. W., Mishler, B. D., and Wheeler, Q. D. (2005). The perils of DNA barcoding and the need for integrative taxonomy. *Syst. Biol.* 54, 844–851. doi: 10.1080/10635150500354878

Zizka, A., Carvalho, F. A., Calvente, A., Baez-Lizarazo, M. R., Cabral, A., Coelho, J. F. R., et al. (2020). No one-size-fits-all solution to clean GBIF. *PeerJ* 8, e9916. doi: 10.7717/peerj.9916

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The diverse and widespread Southern Ocean ophiuroid *Amphiura belgicae* should be considered a species complex

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Accurate knowledge of geographic ranges of species is essential for effective conservation management. Species with large distributions and good connectivity are presumed to be resilient to adverse localized/regional conditions, whereas those with small ranges and, thus, smaller population sizes are more likely to be vulnerable. The rich benthic assemblages across the Southern Ocean are generally considered "Antarctic" with some input from South America contributing to diversity on the sub-Antarctic island shelves. However, molecular work over the past two decades is challenging the paradigm of a general Antarctic benthic fauna, with evidence mounting for assemblages being regionally unique in terms of genetic diversity, regardless of formal taxonomic species composition. The widely distributed brittle star, *Amphiura belgicae*, is one element of the Southern Ocean benthic assemblage that has a complex historical taxonomic background hinting that it may in reality be a complex of species, each with small geographic range and little connectivity. Our study identified deep genetic divisions between geographically isolated populations, particularly between those on the Patagonian shelf and elsewhere. Indeed, populations on the Patagonian shelf were more closely related to the sympatric sister species *A. eugeniae* than to any other Southern Ocean population. We compare our data with a study of *Ophiuroglypha lymani* from similar collections, highlighting that both show highly regionalized populations, particularly on Island shelves, and both share the curious phenomenon of a presumed outgroup species being an element of the ingroup. We suggest that the isolated populations of both these species are following distinct evolutionary and ecological trajectories and that both should be treated as species complexes.

KEYWORDS

diversity, taxonomy, conservation biology, speciation, benthos, Ophiuroidea, Southern Ocean

1 Introduction

We are in an age of rapid biodiversity decline due primarily to anthropogenic impacts on habitat quality and climate (Dirzo et al., 2022). At the same time, scientific discoveries are highlighting that mature, diverse assemblages are providing ecosystem services that are essential to maintaining human quality of life and in some cases acting as "nature-based solutions", such as carbon capture and sequestration, to human induced global problems (Sands et al., 2023). Identifying and managing mature assemblages requires sound taxonomic knowledge of the assemblage elements, as well as understanding of the geographical distributions of these elements and the connectivity between assemblages. In general, marine conservation management relies on species presence/absence information held in databases, and assesses distributions as indicated by records of presence. Identifications in databases are rarely confirmed by expert taxonomic examination, rather relying on field-based observations, thereby providing a major source of error. This is not offered as a criticism of field identifications, rather a frank acknowledgement of the difficulties of taxonomic identifications in general, particularly when characters are both subtle and variable, and original descriptions are difficult to interpret and reconcile with the specimen on the table. A case in point was highlighted as early as Mortensen (1936), regarding the ophiuroid (brittle star) species *Amphiura belgicae* Koehler, 1900, and is the focus of this study.

The genus *Amphiura* Forbes, 1843 is exceptionally species rich (471 species registered in WoRMS as of 3 April 2024) and has a global distribution. It is represented in the Southern Ocean with a total of 2114 records of 24 species (GBIF search greater than 50°S, 3 April 2024), and although not comparatively as species rich, the individual brittle stars can be locally abundant. The most abundant and widespread of these species is *Amphiura belgicae*, which is recorded as far north as the coast around Cape of Good Hope, South Africa, and as far south as the Ross and Weddell Seas, Antarctica. It was first described from material collected by the Belgica expedition, 1897–1899, from sampling locations in the Bellingshausen Sea. In describing *Amphiura belgicae*, Koehler used phrases (translated from French) such as "the disc is flattened, sometimes depressed in the center, most often rounded, but sometimes pentagonal and even somewhat excavated in the interbrachial spaces", indicating this species can vary in general shape. He concluded by stating "*Amphiura belgicae* is easily distinguished from all known species of genus *Amphiura*".

In 1908 Koehler examined material sampled from stations around the South Orkney Islands as part of the Scottish National Antarctic Expedition 1902–1904, from which he described *Amphiura mortenseni* Koehler, 1908b. He reported that it was "… easily characterized by the shape of its mouth shields provided with a prominent and well accentuated distal lobe … ventral surface covered with plates and two tentacular scales". *Amphiura belgicae* mouth shields are also described as "sometimes offering a small lobe…", the ventral surface is covered in small, interlocking, plates, and "The tentacular scales are two in number" (Koehler, 1900).

Koehler also examined material from the Swedish Antarctic Expedition 1901–1903, in which he noted the presence of both *A. belgicae* and *A. mortenseni* from South Georgia stations, as well as describing *Amphiura alternans* Koehler, 1923, also rounded or sub-pentagonal, sometimes convex disc, the oral shields also with a distal lobe, ventral surface covered with plates and variable tentacle scale presence. In his description he noted similarities and differences between *A. alternans* and several other *Amphiura* species, but not specifically *A. belgicae* or *A. mortenseni*.

Material from the German South-polar Expedition 1901–1903 was examined by Hertz (1927). She considered *A. mortenseni* a subspecies of *Amphiura eugeniae* Ljungman, 1867, and further described a second subspecies of *A. eugeniae* as *Amphiura eugeniae gracilis* Hertz, 1927.

The ophiuroids from the British National Antarctic (Discovery) Expedition 1901–1904 were examined by Mortensen (1936). When considering *A. belgicae*, he also examined and compared type material of *A. eugeniae gracilis*, *A. mortenseni* and *A. alternans* and concluded that the main characters overlapped substantially and with sufficient variation that these taxa should be synonymized to the single species *A. belgicae*. Finally, a further species, *Amphilepis gymnopora* Hertz, 1927 from the Kerguelen plateau has more recently been synonymized with *A. belgicae* by Clark (1970).

Given the difficulties of communication in the early 1900's compared with today's "information age", it is understandable that the same species may have been described on more than one occasion by different taxonomists. However, a further complication is provided when the same taxonomist describes two or three distinct species from the same collection that are then eventually synonymized.

Cryptic or unrecognized diversity appears to be common in Antarctic ophiuroids (Hunter and Halanych, 2008; Martín-Ledo et al., 2013; Sands et al., 2015, 2021; Jossart et al., 2019). In this case it is possible that there may be subtle morphological differences seen by the taxonomists of the last century that were then inadequately expressed in their formal descriptions. We therefore set out to test the null hypothesis of Mortensen (1936), that *A. belgicae* and *A. eugeniae* are two distinct species, with the expectation of genetic diversity divided into two distinct, well supported clades. The alternative hypothesis is that these species are taxonomically/morphologically complex, with cryptic variation and genetic divergence present, as hinted at by the multiple species' descriptions of Koehler and Hertz.

2 Methods

Specimens were collected during several research expeditions across the western sector of the Southern Ocean covering the Patagonian Shelf, Scotia Sea, Weddell Sea, Bellingshausen Sea and Amundsen Sea. Figure 1 illustrates the sampling range and details of specimens can be found at dx.doi.org/10.5883/DS-260324. Collections were made using Agassiz Trawls with a 1 cm inner mesh. Specimens were identified to general morphotype (e.g. *Amphiura*-like) before being placed in cold 100% ethanol and stored at −20°C for transport to the UK.

Specimens were diagnosed following relevant descriptions (e.g. Koehler, 1923; Hertz, 1927, 1922, 1908a, 1908b, 1900; Ljungman,

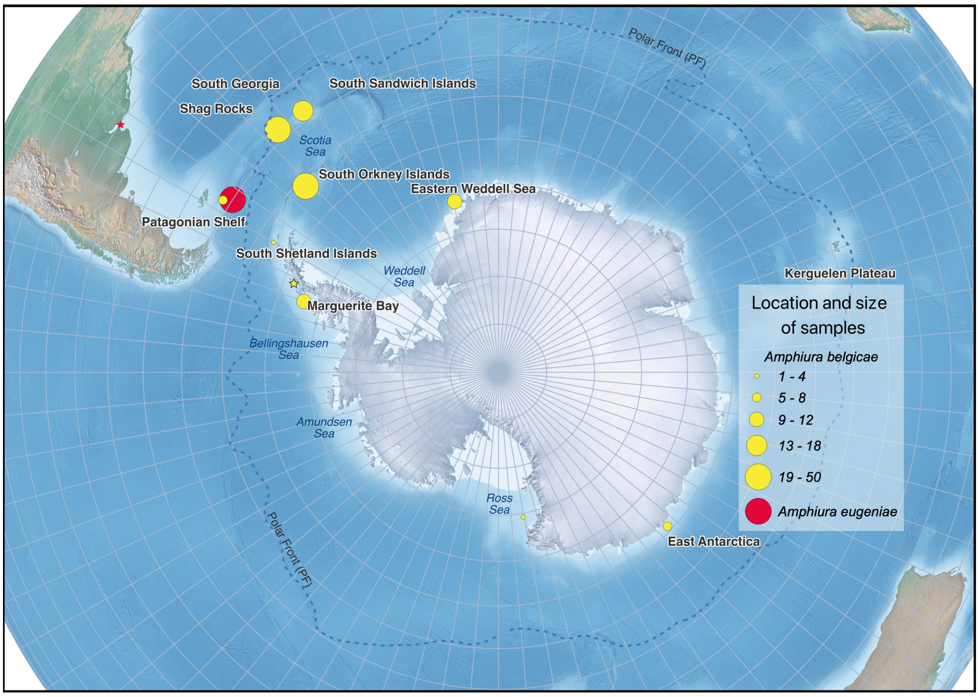

FIGURE 1
Map of Antarctica and the Southern Ocean showing where collections were made of *Amphiura belgicae* (yellow dots) and *Amphiura eugeniae* (red dot). The size of each dot is proportional to the sample size from that location. Stars indicate the type locality of each species.

1867; Mortensen, 1936, 1924) using a Leica M65 microscope. *Amphiura* specimens were photographed and ~3 mm^3 of tissue removed from an arm tip for molecular analyses. DNA extraction and bidirectional sequencing of mitochondrial cytochrome c oxidase subunit 1 (CO1) were carried out at the Canadian Centre for DNA Barcoding, University of Guelph.

Sequences were base-called using Phred in CodonCode Aligner V5.0.1, trimmed and assembled into individual contigs that were checked by eye before multiple alignment using the MUSCLE algorithm (Edgar, 2004). The alignment was translated using mitochondrial echinoderm code to verify an open reading frame. Summary statistics were generated in DNAsp v5 (Rozas et al., 2003; Librado and Rozas, 2009) to explore signals of diversity, selection and population dynamics. Within and among group distances were calculated in MEGA v10.2.4 (Kumar et al., 2018) using percentage differences (uncorrected P) and maximum composite likelihood.

Relationships between sequences were initially explored by building a statistical parsimony network (Templeton et al., 1992) as implemented in PopArt (Leigh and Bryant, 2015).

Phylogenetic analyses using identified *Amphiura* species were then conducted to assess diversity and the inferred systematic position of *Amphiura belgicae* in relation to other species. Outgroups (*Ophiacantha wolfarntzi* Martín-Ledo et al., 2013, *Ophiacantha pentactis* Mortensen, 1936, *Ophiacantha vivipara* Ljungman 1871, *Amphilepis* sp., *Ophiuroglypha carinifera* Koehler, 1901, *Ophiuroglypha lymani* Ljungman 1871, *Ophiocamax gigas* Koehler, 1900, *Ophiactis asperula* Philippi 1858) were included to root the phylogeny providing polarity and some systematic order. Phylogenetic reconstruction was conducted using both Bayesian (Mr Bayes v3.2.1) and Maximum Likelihood (RAxML v8) analyses (Ronquist et al., 2012; Stamatakis, 2014) using a GTR+G model of site evolution in each of three codon partitions. The resulting phylogenies were edited in Figtree v1.4.4 (Rambaut, 2012) and further edited in Graphic v3.1 (Picta inc.) to manually collapse poorly supported branches (bootstrap support < 50, posterior probabilities < 0.8). A reduced dataset of three to four representative sequences from each clade was used to estimate clade divergence times. The analysis was conducted in BEAST2 v2.7.5 under a GTR+G model, a Yule speciation prior and an optimized relaxed clock set to 2.48×10^{-8} per lineage per million years (Naughton et al., 2014; Sands et al., 2015). To enable direct comparison with a previous study (Sands et al., 2015), a dataset of *Ophiuroglypha lymani* was reanalyzed using the same clock rate and including extra individuals sampled from Tristan da Cunha seamounts.

Demographic exploration was conducted using the Bayesian Skyline Plot (Drummond et al., 2005) function in BEAST2 v2.7.5 (Bouckaert et al., 2014) for each spatial-genetic group. Working files were set up with an HKY+G model, strict clock with rate 2.48×10^{-8} (Naughton et al., 2014), and a coalescent Bayesian skyline prior. Runs included 10^8 generations sampling every 1000 states. Results were first checked for effective sample size, convergence and mixing in Tracer v1.7.1, with the same package used to generate the skyline plots.

3 Results

A total of 139 specimens of *Amphiura belgicae* and 26 specimens of *A. eugeniae* were examined in this study. *A. eugeniae* differs very subtly from *A. belgicae*, primarily the presence of a small papilla on the outside of the outer mouth papilla, the attitude of the tentacle pore scales in which the pair at each scale are characteristically perpendicular to each other as opposed to a more acute angle typically seen with *A. belgicae* and shorter arm spines for *A. eugeniae.* These characters were subtle and there was considerable variation particularly in the shape and number of tentacle scales observed in *A. belgicae*. The type locality for *A. eugeniae* is the bay north of Buenos Aires, in the far northern part of the Patagonian shelf. All our samples were collected on the southernmost region of the Patagonian shelf, the Burdwood Bank. Direct comparison of our samples to the holotype stored at the Swedish Natural History Museum satisfies us that they have been identified correctly. The neat genetic clustering of *A. belgicae* (n=19) sampled from Burdwood Bank from *A. eugeniae* (see below) supports our taxonomic observations.

The *Amphiura* sequences analyzed here were not strongly grouped in respect to the *Ophiactis asperula* outgroup (BS=38 Figure 2). While some GenBank sequences contributed to our analyses, several sequences attributed to *Amphiura* on GenBank were excluded as they did not cluster with the included sequences. This reinforces our position on the need for careful taxonomic determination prior to database submission, but it limited the taxonomic depth of the analysis. Within the *Amphiura* sequences, *A. belgicae* together with *A. eugeniae* form a well-supported clade (BS=87) separate from all other *Amphiura* sampled.

From the other localities sampled *A. belgicae* was collected around South Georgia (n=14) and Shag Rocks (n=49), the South Orkney Islands (n=30), along the shelf and offshore island regions of the Antarctic Peninsula (n=14), and locations on the East Antarctic shelf including the Ross Sea, and West Antarctic shelf (n=17). Although there was some subtle variation in characters across this sampling range, morphological variation had no regional patterns and corresponds with Mortensen (1936) observations of a broadly variable species.

Summary molecular statistics are presented in Table 1. Haplotype diversity was high ($H_D > 0.9$) in most locations apart from the Antarctic Peninsula where it was quite low ($H_D<0.5$). Nucleotide diversity (π) was very high ($\pi > 0.02$) for the total dataset and the *A. belgicae* dataset. High nucleotide diversity in relation to haplotype diversity in mtCO1 has been shown to be a good predictor of taxonomic complexity (Goodall-Copestake et al., 2012) and this ratio alone provides strong evidence supporting that *A. belgicae* variation does not conform to the expectation of a panmictic species. South Orkney Island and Antarctic Peninsula groupings also had higher than expected nucleotide diversity ($\pi > 0.01$), along with significantly negative values for Tajima's D statistic, indicating rare variates are more common than expected and suggesting purifying selection or, perhaps, recent population expansion (Tajima, 1989). Fu's S statistic also identifies population size changes (Fu, 1996, 1997) and the highly significant value for Shag Rocks indicates recent population expansion. However, Ramos-Onsins and Roza's R^2 statistic (Ramos-Onsins and Rozas, 2002), designed to identify population expansion, was not significant for any of these groupings.

The depth of divergence between locations was highlighted by examining pairwise distances, particularly between the Patagonian shelf population and other populations. The population of *A. belgicae* from Burdwood Bank on the Patagonian shelf was more similar to the sympatric *A. eugeniae* population (7.39% uncorrected P, 8.7% corrected) than to any other *A. belgicae* population, where distances ranged from 10.29% uncorrected P or 13.22% corrected between Patagonia and Antarctic Peninsula, and 12.07 uncorrected P or 16.33 corrected between Patagonia and

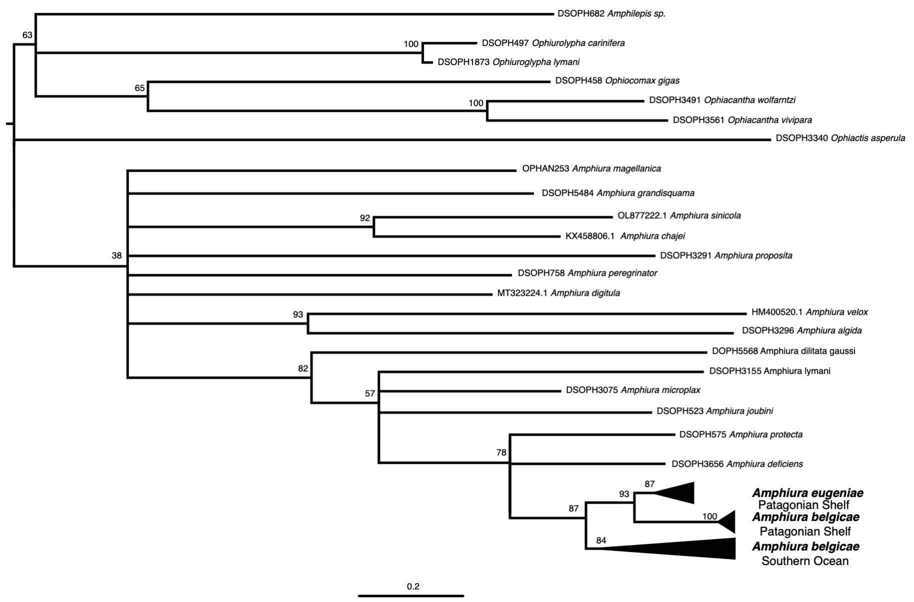

FIGURE 2

Maximum likelihood phylogeny produced with cytochrome c oxidase subunit 1 sequences of *Amphiura* species in relation to *Amphiura belgicae* and outgroups. Node support values were generated by bootstrap pseudoreplicates. Nodes with less than 50% bootstrap support were collapsed, apart from the *Amphiura* clade node that was left for context only as it has no support in this analysis.

TABLE 1 Summary statistics of molecular diversity in geographic regions of *Amphiura belgicae* and *A. eugeniae* based on cytochrome c oxidase subunit 1 sequences.

Population	n	Prob	No. Haplo	H_D	S	π	D_T	F_S	R_2	Max K
Total Dataset	159	0.9875	83	0.978	102	0.0539	0.7626	−23.2**	0.1079	53
Total *Amphiura eugeniae*	26	0.9259	16	0.948	41	0.0101	−1.615*	−4.097	0.0958	29
Total *Amphiura belgicae*	139	0.9857	69	0.972	93	0.0407	−0.0165	−22.321**	0.0886	53
Patagonian Shelf	19	0.9000	7	0.917	16	0.0098	0.459	−0.653	0.1788	12
Shag Rocks	49	0.9600	29	0.948	41	0.0091	−1.3824	−15.868***	0.0612	19
South Georgia	14	0.8667	8	0.923	23	0.0084	−1.2969	−0.423	0.1334	19
South Orkney Islands	30	0.9355	20	0.913	70	0.01242	−2.1511**	−5.92*	0.087	53
Antarctic Peninsula	14	0.8667	4	0.495	29	0.0115	−1.8932**	4.298	0.1721	26
Antarctic Shelf	17	0.8889	9	0.831	13	0.0052	−0.5722	−2.006	0.1146	9

*0.05–0.01, **0.01–0.001, ***<0.001.
n, Number of individuals; Prob, Probability of having captured the deepest coalescent event; No. Haplo, Number of haplotypes;
HD, Haplotype diversity; S, number of segregating sites; π, Nucleotide diversity; DT, Tajima's D; Fs, Fu's Fs Statistic;
R_2, RamosOnsins & Rozas' R2 statistic; Max K, Maximum number of nucleotide differences between any two sequences within the population.

Shag Rocks (Table 2). The most similar populations were Shag Rocks and South Georgia (2.48% uncorrected P, 2.6% corrected P), which are also the populations separated by the smallest distance (approximately 30 km of deep water separating the two shelf regions). Within-population variation ranged from 0.052% within Antarctic Shelf and 1.23% within South Orkney individuals.

The divergence between groups is further highlighted in the network analysis (Figure 3) where, in general, region-specific haplotypes are clearly grouped together.

Amphiura belgicae genetic diversity was strongly partitioned into geographic groupings with strongly supported clades for each of the regions sampled (Figure 4). Individuals from the Patagonian Shelf clustered into a clade along with *Amphiura eugeniae*. Again, the two singleton sequences, one from the South Orkney Island shelf, and one from the South Shetland Islands (closely associated with the Antarctic Peninsula shelf) did not cluster with any of the clades in either likelihood or Bayesian analyses. Two other individuals, one from the South Orkney Islands, the other from the South Shetland Islands, were sequence identical. A third South Shetland Island individual shared identical sequence with some individuals from Marguerite Bay, further south along the Antarctic Peninsula shelf. These relationships are also clearly visualized in Figure 3 and help explain the very high haplotype diversity: nucleotide diversity ratios. Details of the phylogeny at an individual sequence level are given in Supplementary Figure 1.

Assuming the accuracy of the clock rate used, diversification within *Amphiura belgicae* began in the Pliocene, with the current geographic lineages forming in the mid- to late-Pleistocene (Figure 5). Although error associated with these analyses is large, this is similar to those divergence times estimated for the diversification of *Ophiuroglypha lymani*. Estimates of mean divergence times and associated error are provided in Supplementary Table 1.

Population size changes as estimated by Bayesian skyline plots were associated with wide error bars, but most suggest stable or increasing population sizes over the last 300,000 years, apart from the Antarctic Peninsula which shows a slow population decline that has subsequently increased over the past 100,000 years (Figure 6). The island shelf regions of Shag Rocks, South Georgia and South Orkney Islands all show rapid population growth over the past

TABLE 2 Pairwise distance between geographic clades of *Amphiura belgicae* and *A. eugeniae*.

	Antarctic Peninsula	South Orkney	South Georgia	Shag Rocks	Antarctic Shelf	Patagonian Shelf	*A. eugeniae*
Antarctic Peninsula	**0.0122**	0.0510	0.0498	0.0607	0.0464	0.1322	0.1031
South Orkney	0.0463	**0.0123**	0.0441	0.0590	0.0260	0.1531	0.1084
South Georgia	0.0454	0.0405	**0.01**	0.0260	0.0378	0.1597	0.1220
Shag Rocks	0.0545	0.0530	0.0248	**0.0093**	0.0510	0.1633	0.1265
Antarctic Shelf	0.0426	0.0244	0.0351	0.0465	**0.0052**	0.1558	0.1127
Patagonian Shelf	0.1029	0.1147	0.1191	0.1207	0.1162	**0.0098**	0.0870
A. eugeniae Patagonian Shelf	0.0842	0.0872	0.0961	0.0988	0.0902	0.0739	**0.0105**

Below the diagonal is uncorrected P distance, above is model corrected distance. The diagonal values in bold are within clade (uncorrected) distances.

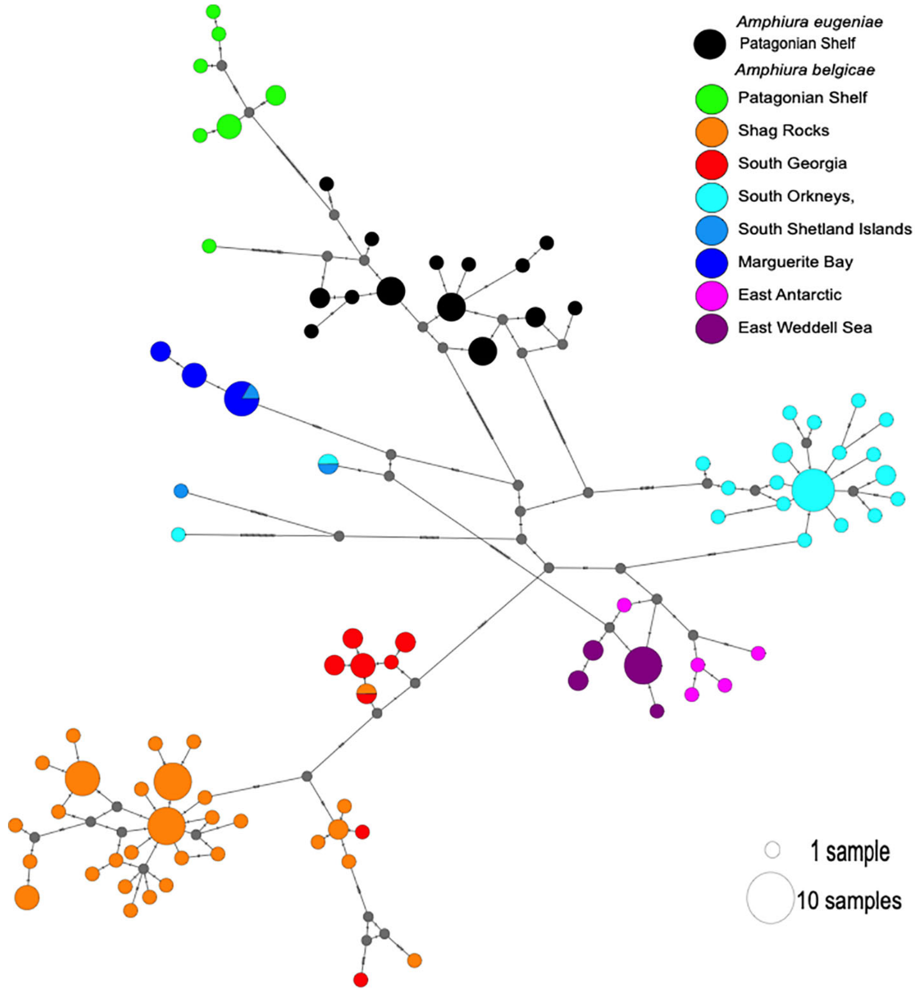

FIGURE 3
Haplotype network as produced by TCS procedure in PopArt. Circle colour indicates sampling location. Circle size is proportional to frequency of the haplotype it represents. Each hash through a connecting line represents a base change between haplotypes, which are unsampled haplotypes, possibly ancestral states. Small grey circles are nodes in the network also representing unsampled haplotypes.

50,000 years. Patagonian shelf, Antarctic shelf and *A. eugeniae* exhibit a slower population increase over the 300,000 years.

4 Discussion

Species level divergences that are difficult to distinguish using morphology alone appear common across the assemblages of the Southern Ocean (Hemery et al., 2012; Dietz et al., 2015; Dömel et al., 2017; Hauquier et al., 2017; González-Wevar et al., 2019). Generally, studies of Southern Ocean diversification have identified geographically structured genetic populations (Raupach and Wägele, 2006; Linse et al., 2007; Leese and Held, 2008), although there are notable exceptions where some species appear to have true circumpolar distributions (Wilson et al., 2009; McLaughlin et al., 2023). Patterns of geographically explicit probable species level diversity have also been demonstrated in previous studies of Southern Ocean brittle stars. The large snake star *Astrotoma agassizi* Lyman, 1875 was shown to have distinct genetic groups across the Drake Passage, with a clade found among a sample of individuals from the Antarctic Peninsula being distinct from two clades recovered from individuals sampled from the Patagonian shelf (Hunter and Halanych, 2008). Shortly after this study, evidence was found that suggested two different life history strategies were present in *A. agassizi*, with the Patagonian Shelf population described as brooding their young (Bernasconi, 1965), and yet lecithotrophic ophiopleutus larvae were recovered from the Ross Sea with Antarctic clade *A. agassizi* mitochondrial DNA (Heimeier et al., 2010). A further study extended the sample collection region around the Antarctic shelf and used single nucleotide polymorphisms (SNPs) to test the relatedness between the Antarctic clade and the two Patagonian clades (Galaska et al., 2017), confirming that the regions indeed hosted distinct populations, albeit with some hybrids present. The two Patagonian clades also appeared to represent two distinct populations. These findings prompted a further study with much broader geographic sampling across the Southern Ocean, including island shelf regions and samples of the sister species *Astrotoma drachii* Guille, 1979 from New Zealand waters along with other outgroups

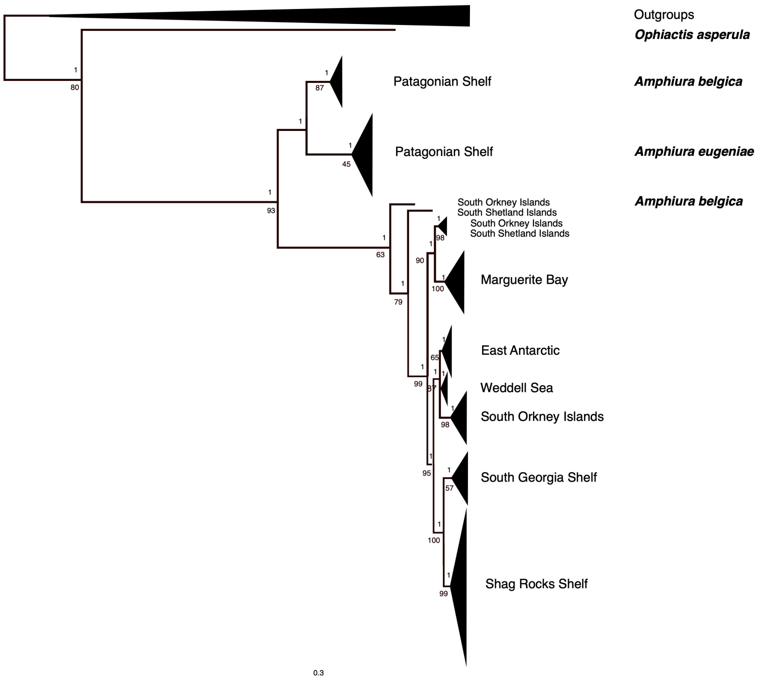

FIGURE 4
Maximum likelihood phylogeny of cytochrome c oxidase subunit 1 sequences of *Amphiura belgicae*, *A. eugeniae* and outgroups. Geographically supported clades were collapsed with the size of the resulting triangle proportional to the number of sequences included, and the depth of the triangle corresponds to the depth of diversity within the clade. Node support values on the top of the line are Bayesian posterior probabilities, on the bottom of the line are bootstraps. The unexpanded tree with all tip sequence identifications is available in Supplementary Figure 1.

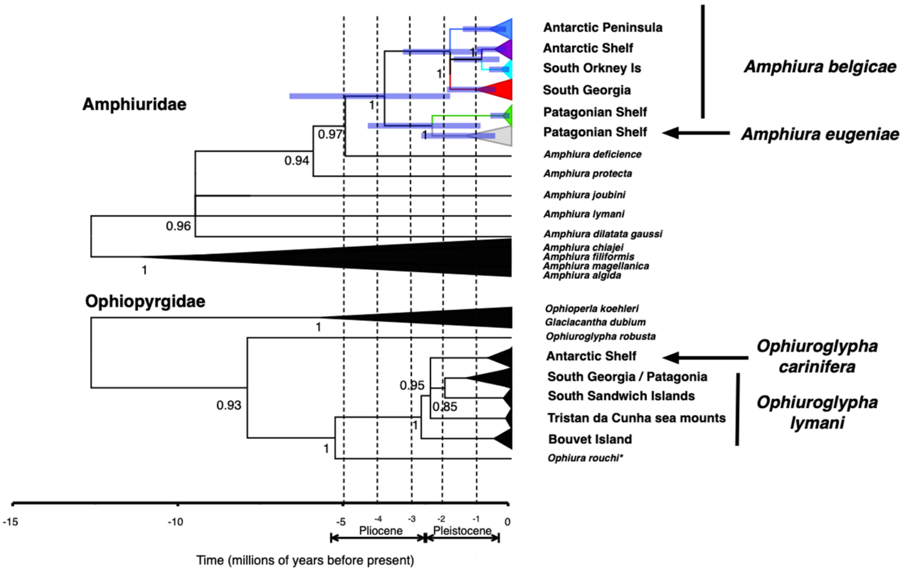

FIGURE 5
Bayesian phylogenies of *Amphiura belgicae* complex with Amphiuridae outgroups directly compared with *Ophiuroglypha lymani* complex and other Ophiopyrgidae outgroups. Both analyses used the same clock rate. The purple node bars represent the error at the assessed nodes. More detail for error associated with *Ophiuroglypha lymani* nodes can be found in Figure 7 of Sands et al. (2015). **Ophiura rouchi* does not fit the characters of genus *Ophiura*, rather it exhibits the form of *Ophiuroglypha* and should be redescribed as *Ophiuroglypha rouchi*. This is likely to be the case for all Southern Ocean "*Ophiura*" species.

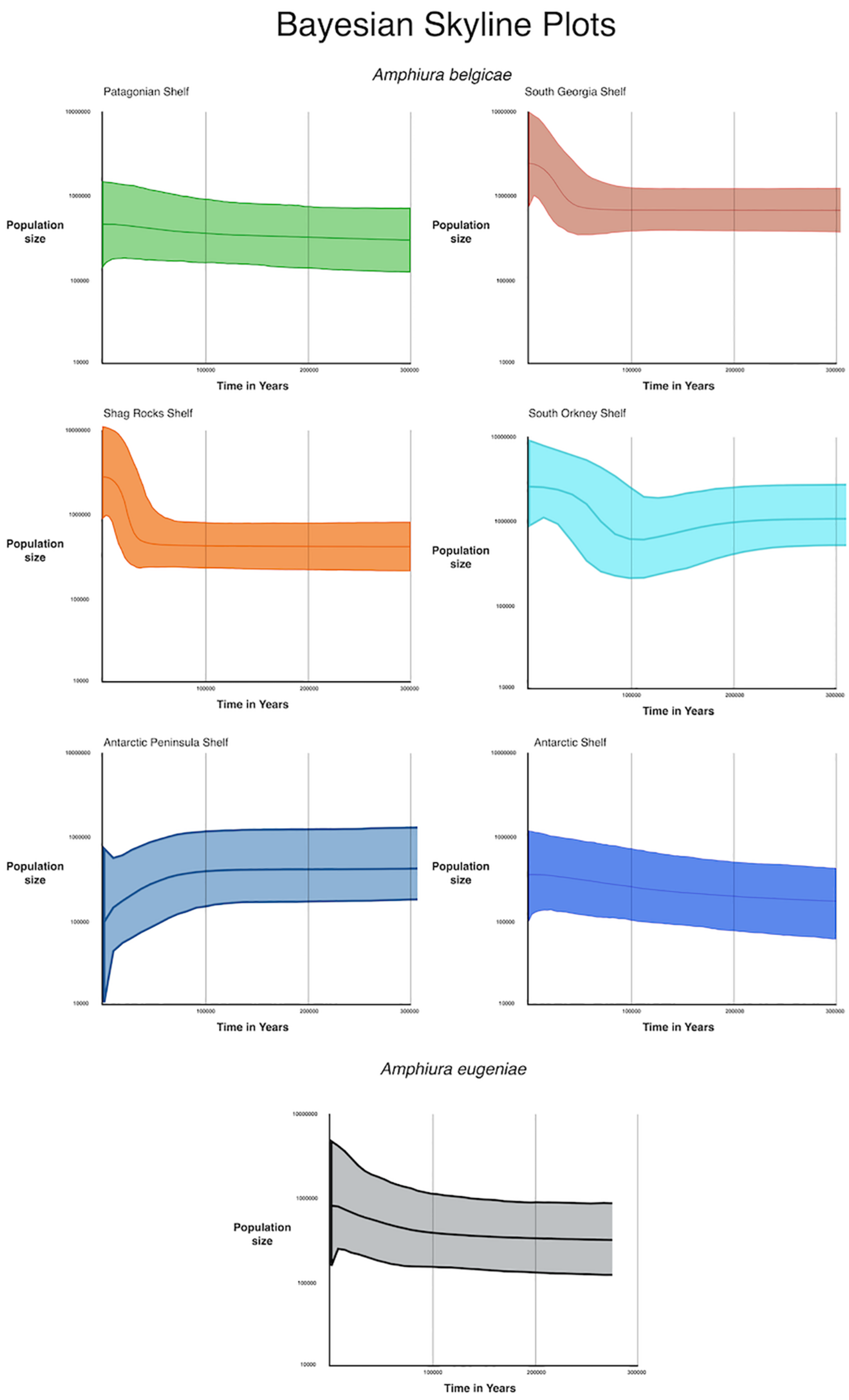

FIGURE 6
Bayesian skyline plots showing the estimated change in population size, with error, over time for the geographically descrete clades of *Amphiura belgicae* and *A. eugeniae*. The present is at the Y axis (X=0). The Y axis is population size in a log scale.

(Jossart et al., 2019). Results obtained from the latter study suggested that *A. agassizi* was far more diverse than previously suspected, with up to seven divergent clades. Some of these clades were specific to regions and, in one case where clade distributions overlapped, size dimorphism between clades was observed suggesting character displacement, an ecological phenomenon that occurs between sympatric sister species (Brown and Wilson, 1956). The final piece of evidence suggesting that the diversity observed represents species level distinctions was that the sister species, *A. drachii* mentioned above, grouped as a clade amongst all the others and was not distinct from *A. agassizi*.

The divergences observed in this study between geographically discrete clades of *Amphiura belgicae* could be considered sufficiently deep to represent species groupings, particularly that of *A. belgicae* sampled from the Patagonian shelf. These individuals were genetically closer to samples of *A. eugeniae* than they were to any of the other clades of *A. belgicae*. Indeed, *A. eugeniae*, as with *Astrotoma drachii*, is paraphyletic in relation to *A. belgicae* and should be considered as an element of the *A. belgicae* complex. This is a pattern that has also been recorded in the species complex of *Ophiuroglypha lymani* (Sands et al., 2015). In this example the sister species, *Ophiuroglypha carinifera*, is found only around the Antarctic continental shelf and is morphologically distinct from *O. lymani*, which is located on island shelves across the Southern Ocean and Patagonia. *O. lymani* was inferred to be a species complex as the depth of divergence among some geographically explicit clades exceeded that between *O. lymani* and *O. carinifera*.

Given the depth of divergence and the sister species being included as an ingroup, we suggest that *A. belgicae* is a species complex of which *A. eugeniae* is one element. If this is the case, and particularly if each clade were confirmed as a unique species, it presents a challenge to estimates of Southern Ocean biodiversity and richness. At present it is generally accepted that *A. belgicae*, *O. lymani* and *A. agassizii* are three species found across the Southern Ocean, implicitly meaning that they each have large population sizes that are well connected. Such species would be considered resilient and of little conservation concern. Our data suggest that the *A. belgicae* sampled in this study show regionally isolated populations on the sampled islands, which are distinctly different from the Patagonian shelf population, and even the Antarctic Peninsula shelf population is distinct from that of the shelf around the continent proper. From a conservation perspective this interpretation shifts the emphasis from a single resilient species to what would be multiple more vulnerable species. Furthermore, if this is the case for *A. belgicae*, *O. lymani* and *A. agassizii*, how many more members of the Southern Ocean benthic assemblage follow this pattern? Perhaps one viable approach to address this question is to consider whether there is a shared mechanism behind the pattern that can be extrapolated more widely.

Whether each clade is treated as a separate species, or only the Patagonian clade is accepted to be a distinct species, may have little effect on conservation management, at least until each species element is thoroughly morphologically investigated and formally described, which is inevitably a long and uncertain process. Methods of species delimitation may be applied but these are not always accurate, particularly in mitochondrial DNA studies (Sands et al., 2021) and have little influence in a conservation management context. However, if it can be shown that entire assemblages share patterns of unappreciated diversity, particularly if a common mechanism or process can be generally applied, then a robust model of biogeographic diversity can be presented as a management tool (Garrick et al., 2006).

To begin to explore this process, we considered some simple historical demographic characteristics of *A. belgicae* with *O. lymani*. Molecular clock analyses in general are assumption heavy and interpretation should be made with caution. This is particularly the case using a single genetic marker, but by assuming and applying a shared clock rate across studies the results can broadly be compared. Both *A. belgicae* and *O. lymani* began to diverge in the Pliocene, with clade level variation originating at the beginning of the Pleistocene (Figure 5). Although uncertainty prevents any claims of shared timings, there is sufficient evidence to further pursue this line of reasoning in more robust multi-marker studies. Poulin et al. (2014) surveyed studies of divergences between Southern Ocean invertebrates occurring either side of the Drake Passage and found divergences ranging from 6.7 to 10.9% which was equated to divergence times between 1 and 14.5 million years. Overall, it was inferred that the early Pleistocene was likely to be the onset of diversification, at least between Antarctic Peninsula and Patagonian Shelf, for most groups. This date could be seen as a minimum given the clock-rates underlying it were all faster than that used in this and other recent ophiuroid studies (Naughton et al., 2014; Sands et al., 2015; O'Hara et al., 2017; Weber et al., 2019).

Analyses of population size change show that populations of *A. belgicae* have been growing rapidly over the past 50,000 to 100,000 years on the island shelves of Shag Rocks, South Georgia and the South Orkney Islands. Stable or slowly growing populations are indicated on the Patagonian shelf for both *A. belgicae* and A. *eugeniae*, and also for the Antarctic shelf population of *A. belgicae*. Conversely, although, again, error bars are wide, the Antarctic Peninsula population shows a decrease in population size over the past 100,000 years. Due to small sample sizes only Patagonian Shelf and South Georgia shelf populations of *O. lymani* were analyzed, along with *O. carinifera* (Sands et al., 2015). All three of these populations showed signals of growth over the past 100,000 years of similar magnitudes to those seen with the rapidly growing *A. begicae* populations. Again, uncertainty and a strictly pattern-based observation do not give definitive answers but may be considered a pilot study providing a sound basis for further research.

Amphiura as a genus is polyphyletic in relation to the related genera *Amphioplus* and *Amphipholis* (O'Hara et al., 2017, 2018), and we suggest that it should be considered for revision. However, *Amphiura* is a difficult genus in general to work with, and, even in this putative species complex, the traditional characters of mouth shields, mouth papillae and tentacle papillae vary in shape, size and attitude sufficiently within each geographic region that creating a reproducible diagnosis for each genetic/geographic group will prove difficult. The species and subspecies raised by Koehler and Hertz may well be among the genetic clades identified here, but the vague language used in the descriptions, along with the character variation observed, failed to convince Mortensen (1936), and are equally unhelpful here despite our new results. Although our data appear to reject the null hypothesis of Mortensen's single *A. belgicae* species, more rigorous studies using multiple nuclear markers to better clarify modern day gene flow and species tree relationships would provide a clearer and more robust assessment of the true depth of divergence of the geographic clades.

There is no doubt that the Southern Ocean benthic assemblage is old and rich, performing many important and probably also many unrecognized ecosystem services. Our results add to evidence that there is not one single Southern Ocean benthic assemblage, but rather a mosaic of assemblages, each one following an internally shared evolutionary and ecological trajectory in isolation from other assemblages. Given the difficulties in morphologically distinguishing or describing species with varying diagnostic characters, we suggest using simple phylogeographic techniques to identify regions where there is shared cryptic diversity and singling out these as biogeographic regions in need of conservation management.

Data availability statement

The data presented in the study are deposited in BOLD and accessible via the DOI:dx.doi.org/10.5883/DS-260324.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because it was not required for the specimens used in this study.

Author contributions

CS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. WG-C: Supervision, Validation, Writing – review & editing. SS: Supervision, Validation, Writing – review & editing. BN: Supervision, Validation, Writing – review & editing. PC: Supervision, Validation, Writing – review & editing. TO: Data curation, Resources, Validation, Writing – review & editing. RM-L: Conceptualization, Validation, 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.

The author P. Convey declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2024.1416418/full#supplementary-material

References

Bernasconi, I. (1965). *Astrotoma agassizii* Lyman, especie vivípara del Atlántico sur (Ophiuroidea, Gorgonocephalidae). *Contrib. Científica* 015, 1Á5.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., et al. (2014). BEAST 2: A software platform for bayesian evolutionary analysis. *PloS Comput. Biol.* 10, e1003537. doi: 10.1371/journal.pcbi.1003537

Brown, W. L.Jr., and Wilson, E. O. (1956). Character displacement. *Syst. Biol.* 5, 49–64. doi: 10.2307/2411924

Clark, A. M. (1970). Notes on the family Amphiuridae (Ophiuroidea). *Bull. Br. Museum (Natural History) Zoology.* 19, 1–81. doi: 10.5962/bhl.part.24085

Dietz, L., Arango, C. P., Dömel, J. S., Halanych, K. M., Harder, A. M., Held, C., et al. (2015). Regional differentiation and extensive hybridization between mitochondrial clades of the Southern Ocean giant sea spider *Colossendeis megalonyx*. *Open Sci.* 2, 140424. doi: 10.1098/rsos.140424

Dirzo, R., Ceballos, G., and Ehrlich, P. R. (2022). Circling the drain: the extinction crisis and the future of humanity. *Philos. Trans. R. Soc B Biol. Sci.* 377, 20210378. doi: 10.1098/rstb.2021.0378

Dömel, J. S., Melzer, R. R., Harder, A. M., Mahon, A. R., and Leese, F. (2017). Nuclear and mitochondrial gene data support recent radiation within the sea spider species complex *Pallenopsis patagonica*. *Front. Ecol. Evol.* 4. doi: 10.3389/fevo.2016.00139

Drummond, A. J., Rambaut, A., Shapiro, B., and Pybus, O. G. (2005). Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol. Biol. Evol.* 22, 1185–1192. doi: 10.1093/molbev/msi103

Edgar, R. C. (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinf.* 5, 113. doi: 10.1186/1471-2105-5-113

Fu, Y. (1996). New statistical tests of neutrality for DNA samples from a population. *Genetics* 143, 557–570. doi: 10.1093/genetics/143.1.557

Fu, Y.-X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147, 915–925. doi: 10.1093/genetics/147.2.915

Galaska, M. P., Sands, C. J., Santos, S. R., Mahon, A. R., and Halanych, K. M. (2017). Crossing the divide: admixture across the antarctic polar front revealed by the brittle star *Astrotoma agassizii*. *Biol. Bull.* 198–211. doi: 10.1086/693460

Garrick, R. C., Sands, C. J., and Sunnucks, P. (2006). The use and application of phylogeography for invertebrate conservation research and planning, in *Insect biodiversity and dead wood: Proceedings of a symposium for the 22nd International Congress of Entomology* (Asheville, NC, USA), 15–22.

González-Wevar, C. A., Gérard, K., Rosenfeld, S., Saucède, T., Naretto, J., Díaz, A., et al. (2019). Cryptic speciation in Southern Ocean *Aequiyoldia eightsii* (Jay 1839): Mio-Pliocene trans-Drake Passage separation and diversification. *Prog. Oceanogr.* 174, 44–54. doi: 10.1016/j.pocean.2018.09.004

Goodall-Copestake, W. P., Tarling, G. A., and Murphy, E. J. (2012). On the comparison of population-level estimates of haplotype and nucleotide diversity: a case study using the gene cox1 in animals. *Heredity.* 109 (1), 50–56. doi: 10.1038/hdy.2012.12

Hauquier, F., Leliaert, F., Rigaux, A., Derycke, S., and Vanreusel, A. (2017). Distinct genetic differentiation and species diversification within two marine nematodes with different habitat preference in Antarctic sediments. *BMC Evol. Biol.* 17, 120. doi: 10.1186/s12862-017-0968-1

Heimeier, D., Lavery, S., and Sewell, M. A. (2010). Molecular species identification of *Astrotoma agassizii* from planktonic embryos: further evidence for a cryptic species complex. *J. Hered.* 101, 775–779. doi: 10.1093/jhered/esq074

Hemery, L. G., Eléaume, M., Roussel, V., Améziane, N., Gallut, C., Steinke, D., et al. (2012). Comprehensive sampling reveals circumpolarity and sympatry in seven mitochondrial lineages of the Southern Ocean crinoid species *Promachocrinus kerguelensis* (Echinodermata). *Mol. Ecol.* 21, 2502–2518. doi: 10.1111/j.1365-294X.2012.05512.x

Hertz, M. (1927). Die ophiuroiden der deutschen südpolar-expedition 1901-1903. *Dtsch. Südpolar-Exped.* 19, 1–56.

Hunter, R. L., and Halanych, K. M. (2008). Evaluating connectivity in the brooding brittle star *Astrotoma agassizii* across the Drake Passage in the Southern Ocean. *J. Hered.* 99, 137–148. doi: 10.1093/jhered/esm119

Jossart, Q., Sands, C. J., and Sewell, M. A. (2019). Dwarf brooder versus giant broadcaster: combining genetic and reproductive data to unravel cryptic diversity in an Antarctic brittle star. *Heredity* 123, 622–633. doi: 10.1038/s41437-019-0228-9

Koehler, R. (1900). *Note préliminaire sur les Echinides et les Ophiures de l'Expédition antarctique belge*. impr: Hayez.

Koehler, R. (1901). Expédition Antarctique Belge. Résultats du voyage de S. Y. "Belgica" en 1897-98-99. Echinides et Ophiures. *Zoologie*, 1–42.

Koehler, R. (1908a). Astéries, Ophiures et Échinides de l'Expédition Antarctique Nationale Écossaise. *Trans. R. Soc Edinb.* 46, 529–649.

Koehler, R. (1908b). Asteries, Ophiures et Echinides recueillis dans les mers australes par la "Scotia. *(1902-1904. Zool. Anz.* 32, 140–147.

Koehler, R. (1922). *Echinodermata ophiuroidea* (Sydney: Printed by John Spence). Available at: https://www.biodiversitylibrary.org/item/44167. doi: 10.5962/bhl.title.11722

Koehler, R. (1923). Astéries et ophiures. *Furth. Zool. Results Swed. Antarct. Exped.* 1, 1–145.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549. doi: 10.1093/molbev/msy096

Leese, F., and Held, C. (2008). Identification and characterization of microsatellites from the Antarctic isopod *Ceratoserolis trilobitoides*: nuclear evidence for cryptic species. *Conserv. Genet.* 9, 1369–1372. doi: 10.1007/s10592-007-9491-z

Leigh, J. W., and Bryant, D. (2015). popart: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6, 1110–1116. doi: 10.1111/2041-210X.12410

Librado, P., and Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452. doi: 10.1093/bioinformatics/btp187

Linse, K., Cope, T., Lörz, A.-N., and Sands, C. J. (2007). Is the Scotia Sea a centre of Antarctic marine diversification? Some evidence of cryptic speciation in the circum-Antarctic bivalve *Lissarca notorcadensis* (Arcoidea: Philobryidae). *Polar Biol.* 30, 1059–1068. doi: 10.1007/s00300-007-0265-3

Ljungman, A. V. (1871). Om tvänne nya arter. *Ophiurider Öfversigt af Kungliga Vetenskapsakademiens Förhandlingar*. 1870, 5, 471–476.

Ljungman, A. V. (1867). *Ophiuroidea viventia huc usque cognita, enumerat* (Öfversigt af Kgl. Vetenskaps-Akademiens Förhandlingar 1866) 23, 303–336.

Lyman, T. (1875). *Zoological results of the Hassler expedition: ophiuridae and astrophytidae*. (Vol. 2). (Cambridge USA: University Press).

Martín-Ledo, R., Sands, C. J., and López-González, P. J. (2013). A new brooding species of brittle star (Echinodermata: Ophiuroidea) from Antarctic waters. *Polar Biol.* 36, 115–126. doi: 10.1007/s00300-012-1242-z

McLaughlin, E. L., Wilson, N. G., Rouse, G. W., McLaughlin, E. L., Wilson, N. G., and Rouse, G. W. (2023). Resolving the taxonomy of the Antarctic feather star species complex *Promachocrinus 'kerguelensis'* (Echinodermata: Crinoidea). *Invertebr. Syst.* 37, 498–527. doi: 10.1071/IS22057

Mortensen, T. (1924). *Echinoderms of New Zealand and the auckland-campbell islands: II. Ophiuroidea* (Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening) 77, 91–177.

Mortensen, T. (1936). Echinoidea and Ophiuroidea, in *Discovery reports* (Cambridge, UK: Cambridge), 199–348.

Naughton, K. M., O'Hara, T. D., Appleton, B., and Cisternas, P. A. (2014). Antitropical distributions and species delimitation in a group of ophiocomid brittle stars (Echinodermata: Ophiuroidea: Ophiocomidae). *Mol. Phylogenet. Evol.* 78, 232–244. doi: 10.1016/j.ympev.2014.05.020

O'Hara, T. D., Hugall, A. F., Thuy, B., Stöhr, S., and Martynov, A. V. (2017). Restructuring higher taxonomy using broad-scale phylogenomics: The living Ophiuroidea. *Mol. Phylogenet. Evol.* 107, 415–430. doi: 10.1016/j.ympev.2016.12.006

O'Hara, T. D., Stöhr, S., Hugall, A. F., Thuy, B., and Martynov, A. (2018). Morphological diagnoses of higher taxa in Ophiuroidea (Echinodermata) in support of a new classification. *Eur. J. Taxon.* 1–35. doi: 10.5852/ejt.2018.416

Poulin, E., González-Wevar, C., Díaz, A., Gérard, K., Hüne, M., and Part, B. (2014). Divergence between Antarctic and South American marine invertebrates: What molecular biology tells us about Scotia Arc geodynamics and the intensification of the Antarctic Circumpolar Current. *Glob. Planet. Change* 123, 392–399. doi: 10.1016/j.gloplacha.2014.07.017

Rambaut, A. (2009). *FigTree. Tree figure drawing tool.* http://tree.bio.ed.ac.uk/software/figtree.

Ramos-Onsins, S. E., and Rozas, J. (2002). Statistical properties of new neutrality tests against population growth. *Mol. Biol. Evol.* 19, 2092–2100. doi: 10.1093/oxfordjournals.molbev.a004034

Raupach, M. J., and Wägele, J. W. (2006). Distinguishing cryptic species in Antarctic Asellota (Crustacea: Isopoda)-a preliminary study of mitochondrial DNA in *Acanthaspidia drygalskii*. *Antarct. Sci.* 18, 191–198. doi: 10.1017/S0954102006000228

Ronquist, F., Teslenko, M., van der, M. P., Ayres, D. L., Darling, A., Höhna, S., et al. (2012). MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542. doi: 10.1093/sysbio/sys029

Rozas, J., Sanchez-DelBarrio, J. C., Messeguer, X., and Rozas, R. (2003). *DnaSP, DNA polymorphism analyses by the coalescent and other methods.* (Oxford, UK: Oxford Univ Press). doi: 10.1093/bioinformatics/btg359

Sands, C. J., O'Hara, T., Barnes, D. K. A., and Martín-Ledo, R. (2015). Against the flow: evidence of multiple recent invasions of warmer continental shelf waters by a Southern Ocean brittle star. *Evol. Popul. Genet.* 3. doi: 10.3389/fevo.2015.00063

Sands, C. J., O'Hara, T. D., and Martín-Ledo, R. (2021). Pragmatic assignment of species groups based on primary species hypotheses: the case of a dominant component of the Southern Ocean benthic fauna. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.723328

Sands, C. J., Zwerschke, N., Bax, N., Barnes, D. K. A., Moreau, C., Downey, R., et al. (2023). PERSPECTIVE • The growing potential of antarctic blue carbon. *Oceanography* 36. doi: 10.5670/oceanog.2023.s1.5

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313. doi: 10.1093/bioinformatics/btu033

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123, 585–595. doi: 10.1093/genetics/123.3.585

Templeton, A. R., Crandall, K. A., and Sing, C. F. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonucleaase mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132, 619–633. doi: 10.1093/genetics/132.2.619

Weber, A. A.-T., Stöhr, S., and Chenuil, A. (2019). Species delimitation in the presence of strong incomplete lineage sorting and hybridization: Lessons from *Ophioderma* (Ophiuroidea: Echinodermata). *Mol. Phylogenet. Evol.* 131, 138–148. doi: 10.1016/j.ympev.2018.11.014

Wilson, N. G., Schrodl, M., and Halanych, K. M. (2009). Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). *Mol. Ecol.* 18, 965–984. doi: 10.1111/j.1365-294X.2008.04071.x

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A first glimpse into the biogeographic affinities of the shallow benthic communities from the sub-Antarctic Crozet archipelago

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Sub-Antarctic islands are expected to show a high degree of endemicity due to their remoteness. However, biogeographic affinities in the sub-Antarctic remain poorly understood, especially in the marine realm. Sub-Antarctic islands being at the crossroads between Antarctic and cold temperate regions, biodiversity characterization and biogeographic analyses are a priority for monitoring and rapidly assessing variations associated with environmental changes. One underexplored sub-Antarctic area is Crozet, a protected archipelago located halfway between Antarctica and South Africa. In this study, we investigated the shallow-water Crozet macrofaunal diversity, distribution patterns and biogeographic affinities based on the examination of fieldwork specimens via a thorough morphological identification and a genetic characterisation. The resulting dataset provides an important baseline for further studies and conservation strategies, compiling the first genetic and taxonomic database for the Crozet archipelago. In total, 100 morphotypes were found, belonging to nine different phyla, among which arthropods (32), molluscs (18) and echinoderms (17) were the richest. Forty-seven morphotypes were identified to the species level, among which 20 were reported in Crozet for the first time. This confirms that Crozet is a poorly known region, even compared to other sub-Antarctic areas. A large proportion of species (62%) had circum Southern Ocean or circum sub-Antarctic distributions. These species were mostly shared with Kerguelen (72%), the Magellan Province (64%), and Prince Edward Islands (64%), confirming the patterns found in macroalgae and specific macrofaunal groups. However, this large-distribution statement needs to be counterbalanced by the detection (genetic data) of more restricted distributions than expected in four study cases (the tanaid *Apseudes spectabilis*, the nudibranch *Doris kerguelenensis*, the polychaete *Neanthes kerguelensis* and the chiton *Hemiarthrum setulosum*). Considering that most morphotypes had no genetic data available from other regions, the proportion of morphotypes with restricted distribution is likely to increase alongside future investigations. In addition, we also found a few cases of unrecognized diversity that might lead to the descriptions of new species, some likely to be endemic to Crozet (e.g., within the polychaete genus *Harmothoe* and the bryozoan genus *Antarctothoa*). Altogether, this stresses the need to maintain conservation efforts in Crozet and pursue integrative investigations in order to highlight and protect its unusual diversity.

KEYWORDS

biodiversity, Southern Ocean, taxonomy, barcoding, marine

Introduction

In the Southern Ocean, the sub-Antarctic region is located between the subtropical and the Antarctic polar marine fronts, comprising a series of remote archipelagos and small islands (De Broyer et al., 2014). Due to the geographic remoteness of sub-Antarctic islands, a high degree of endemicity can be expected in both terrestrial and marine taxa (Frenot et al., 2001). Several studies from the last decades have highlighted contrasting patterns of distribution in various taxonomic groups, from single island endemicity to species exhibiting broad distributions (e.g., Poulin et al., 2014; Figuerola et al., 2017, Figuerola et al., 2018; Féral et al., 2021; Vantomme et al., 2023). Overall, biodiversity and biogeographic affinities of the different sub-Antarctic areas remain poorly understood, with a contrast of knowledge existing among sub-Antarctic islands (Griffiths and Waller, 2016; Féral et al., 2021). It is thus particularly important to better characterize their biodiversity and biogeographical affinities to improve our understanding of marine life dynamics, especially considering their key location at the crossroads between high Antarctic and cold temperate areas (Hemmings and Stephens, 2010; De Broyer et al., 2014). It is also central to conservation considerations, given that high endemicity can mean increased sensitivity to global change and risk of biodiversity loss (Griffiths, 2010; Park et al., 2014; Jossart et al., 2019; Féral et al., 2021). In particular, an improved biogeographic baseline would thus detect distribution shifts that are expected in response to current and future environmental changes (Saucède et al., 2017; Guillaumot et al., 2018). In response to these conservation concerns, some sub-Antarctic islands have already been given the status of Marine Protected Areas (Crozet MPA, Heard Island and McDonald Islands MPA, Kerguelen MPA, Prince Edward Islands MPA; Brooks et al., 2020) or have received a significant extension of their marine parks (e.g., Macquarie Island Marine Park in 2023, parksaustralia.gov.au).

Crozet's benthic marine biodiversity is notably underexplored. This five-island archipelago is located in the Indian Ocean sector of the Southern Ocean (45°48'S–46°26'S; 50°14'E–52°15'E) and is about 2,300 km away from both Antarctica and South Africa. Crozet originated from three main cycles of volcanism, among which the first one initiated nine million years ago (Chevallier and Nougier, 1981). The archipelago is strongly influenced by the Antarctic Circumpolar Current (ACC), which is expected to increase its connectivity with other sub-Antarctic areas (Pollard et al., 2007; De Broyer et al., 2014). Previous terrestrial studies have been undertaken on Crozet terrestrial biodiversity (e.g., Frenot et al., 2001; Convey, 2007; Hullé and Vernon, 2021), however, marine benthic ecosystems have received far less attention (Canteras and Arnaud, 1985; Sicinski and Gillet, 2002; Griffiths and Waller, 2016; Lelièvre et al., 2023). The waters around Crozet are part of the National Nature Reserve of the French Southern Territories MPA and recognized as a UNESCO World Heritage site (whc.unesco.org/en/list/1603). Marine coastal areas have also additionally received an IUCN "enhanced protection" status (Féral et al., 2021). In this context of conservation initiatives, the French polar institute project #1044 Proteker was implemented to monitor nearshore marine benthic communities of the French Southern Territories (Kerguelen, Saint-Paul & Amsterdam, and Crozet). The main goal of this ongoing project is to establish a baseline for monitoring biodiversity dynamics and assess the impact of climate change on coastal marine ecosystems (Féral et al., 2016). Since its initiation, samples collected from Crozet during the Proteker campaigns have been invaluable for the investigation of biogeographic patterns in selected taxa (e.g., González-Wevar et al., 2021; Rosenfeld et al., 2023). At the community level, using underwater video-imagery, Lelièvre et al. (2023, 2024) investigated the taxonomic and functional diversities of shallow benthic communities associated with hard substrates. The authors highlighted high taxonomic diversity and low functional richness, evenness, and redundancy. This suggests a potential vulnerability to current and future environmental changes, which, again, stresses the need for further investigations based on additional biological samples (Mason et al., 2005; Lelièvre et al., 2023).

In our study, we investigated the diversity and biogeography of macrofauna (>1 mm) at two sites (*Baie du Marin* and *Crique du Sphinx*) around Possession island (*"Ile de la Possession"*, 46°25'S; 51° 45'E), the largest island of the Crozet archipelago. Based on the examination of specimens collected in a recent fieldwork campaign, we combined a thorough morphological identification by taxonomic experts with a genetic characterisation based on the COI barcode region. The joint use of morphological and genetic data has been demonstrated to be effective in species identification of various taxonomic groups (e.g., Gostel and Kress, 2022; Jossart et al., 2023). In addition, comparing recorded biogeographic patterns (relying on morphology-based taxonomy) with genetic data was also important in the revision and clarification of species distributions (Christiansen et al., 2018; Jossart et al., 2019; Vantomme et al., 2023). In Lelièvre et al. (2023), Crozet faunal communities exhibited a high-level diversity, notably in echinoderms. Regarding its geographic situation and oceanic features (strong influence by the ACC), we expect benthic marine communities of Crozet to be composed of both endemic and circum sub-Antarctic species, with high-level faunal affinities to both Kerguelen and Prince Edward Islands. In fact, while remoteness can explain endemicity after rare colonisation and settlement events, a certain homogenisation can also be expected due to the role of the ACC in larval or even adult (kelp rafting) transport (Moore et al., 2018; Fraser et al., 2022). By compiling the first genetic and taxonomic database including specimen images and occurrence records for Crozet, this study's newly generated dataset provides an important identification tool and baseline for further studies and conservation purposes.

Materials and methods

Fieldwork

Specimen sampling occurred during November 2021 as part of the Proteker 9 campaign onboard the R/V *Marion Dufresne II*. Two geographically close sites (< 2 km), *Baie du Marin* and *Crique du Sphinx*, were investigated on the east coast of Possession Island (*"Ile de la Possession"*, Crozet archipelago). This investigation was part of an impact study on benthic communities after the recent installation of the International Monitoring System (IMS) hydroacoustic station HA04 in the area (Lelièvre et al., 2023). Biological samples were opportunistically collected by hand during six dives at depths ranging from 4 to 20 m. Specimens were then preserved in 96% ethanol for subsequent analyses.

Morphological identification

Each specimen was examined under a stereomicroscope (Leica LK300 LED), assigned to a morphotype (or putative species) and a preliminary identification was obtained using available taxonomic resources (e.g., Féral et al., 2019; Lelièvre et al., 2023). Each morphotype was also photographed using the same stereomicroscope and an Olympus OMD-EM1 camera with a 60 mm macro lens. Preliminary identifications of morphotypes were then confirmed or refined by taxonomic experts (Supplementary Material 1). Macro photographs were sent to taxonomists as well as voucher specimens when required in the identification process.

Genetic characterization

The barcode region of the cytochrome c oxidase subunit I (COI; 658 base pairs) was obtained for each morphotype (one to twenty individuals sequenced per morphotype). DNA extractions were undertaken on a small piece of tissue, following the salting-out protocol of Sunnucks and Hales (1996). For COI amplification, we used a PCR mix of 12.5 µL Accustart Toughmix (QuantaBio), 10 µL ultrapure water, 0.5 µL each primer (10 µM) and 1.5 µL DNA extract. PCR conditions consisted of 40 cycles for the following temperature steps: 30 s at 94°C (denaturation), 30 s at 45–49°C (annealing, see details below) and 45 s at 72°C (elongation). These cycles were preceded by 3 min at 94°C and followed by 3 min at 72°C. Amplifications occurred using either universal or taxon-specific primers. Bryozoans, chordates, cnidarians, nemerteans and sponges were amplified using the universal primer pair "F-LCO1490 + R-HCO2198" at an annealing temperature of 45°C (Folmer et al., 1994). Amphipods and pycnogonids were amplified with the same primers at an annealing temperature of 49°C. Isopods and tanaids were amplified using the primer pair "F-CrustCOIF + R-HCO2198" at an annealing temperature of 47°C (Teske et al., 2006). Echinoderms were amplified using the primer pair "F-LCOech1aF1+ R-HCO2198" at an annealing temperature of 45°C (Folmer et al., 1994; Layton et al., 2016). Annelids were amplified using either the primer pair "F-LCO1490+ R-HCO2198" and the primer pair "F-polyHCO + R-polyLCO" at an annealing temperature of 47°C (Carr et al., 2011). Molluscs were amplified using either the primer pair "F-LCO1490 + R-HCO2198" (annealing temperature: 45°C) or the primer pair "F-COI-mol & R-COI-mol" (annealing temperature: 49°C; Owada et al., 2013). The sequences of each of the aforementioned primer were F-LCO1490: GGTCAACAAATCATAAAGATATTGG; R-HCO2198: TAAACTTCAGGGTGACCAAAAAATCA; F-CrustCOIF: TCAACAAATCAYAAAGAYATTGG; F-LCOech1aF1: TTTTTTCTACTAAACACAAGGATATTGG; F-polyHCO: TAMACTTCWGGGTGACCAAARAATCA; R-polyLCO: GAYTATWTTCAACAAATCATAAAGATATTGG; F-COI-mol: TCWACAAATCAYAAAGATATTGG; R-COI-mol: ACYTCMGGRTGMCCAAAAAATCA. The purification step and Sanger sequencing were carried out at the sequencing centre of

Azenta/Genewiz Germany. Sequences were edited and checked for stop codons in Geneious Prime 2023.2.1 (Kearse et al., 2012). Barcodes were then aligned (Muscle algorithm), compared among them within classes/orders (e.g., amphipods) and to the ones available on GenBank and Barcode of Life (BOLD) databases (Ratnasingham and Hebert, 2007). Combining morphological and genetic data, a final identification was then determined at the most accurate taxonomic level possible. In addition to the COI sequences aforementioned, additional genetic barcodes from non-Crozet areas were obtained from either scientific publications or public BOLD records. Combining all the barcodes for each taxon, alignments were then realized in Geneious Prime 2023.2.1. Templeton Crandall Singh (TCS) haplotype networks were then constructed in PopART 1.7 (Leigh and Bryant, 2015) and mean pairwise genetic distances (uncorrected p-distances) were calculated between clades in the software MEGA X (Kumar et al., 2018). We then used a genetic distance threshold of 2% (among clades of the haplotype network) to consider that an initial morphotype represents more than one putative species. This threshold is based on the most common interspecific genetic distances observed in the groups of interest [crustaceans: Vieira et al., 2016, molluscs: Layton et al., 2014, polychaetes: Carr et al., 2011, sea stars: Ward et al., 2008 and Moreau et al., 2021)]. Species delimitation methods, such as ASAP and ABGD, were not used considering their low suitability for datasets with limited number of sequences (Puillandre et al., 2012, Puillandre et al., 2021) A public Barcode of Life (BOLD) project (HAOIV: Shallow benthic communities of Crozet archipelago) was created on the platform boldsystems.org. Macro pictures were uploaded for each specimen as well as COI barcodes, when successfully obtained (Table 1).

Biogeographic and phylogeographic analyses

The biogeographic distribution of taxa identified up to the species level was analysed based on the following bioregionalization of the Southern Ocean: Magellan Province, South Georgia/South Sandwich Islands, Bouvet (= Atlantic Sector of the Southern Ocean); Prince Edward Islands (Prince Edward and Marion islands), Crozet, Kerguelen, Heard Island (= Indian Ocean Sector of the Southern Ocean); Macquarie/sub-Antarctic New Zealand region (= Pacific sector of the Southern Ocean); West Antarctica; East Antarctica). Species occurrence data were either based on: 1) occurrences mentioned in scientific publications; 2) GBIF occurrences (Global Biodiversity Information Facility; https://www.gbif.org); 3) additional unpublished occurrences obtained from direct contacts with taxonomic experts. If a contradiction arose, occurrences obtained from direct contacts with taxonomists or dedicated publications had precedence over GBIF records. COI barcodes were also used to refine the species distribution of four species (e.g., the expected presence of *Hemiarthrum setulosum* P. P. Carpenter, 1876 in Antarctica was not considered as it appeared that Antarctic specimens were highly divergent from the ones of Crozet, see results). Four different distribution patterns were then considered: 1) restricted sub-Antarctic distribution (i.e., recorded in a single sector of the sub-Antarctic); 2) broad sub-Antarctic distribution (i.e., recorded in two sectors of the sub-Antarctic); 3) circum sub-Antarctic (i.e., recorded in three sector of the sub-Antarctic); 4) circum Southern Ocean (recorded both in the Antarctic and in either two or three sub-Antarctic sectors). Based on identified species occurrence data, a similarity network was produced using Gephi 0.10.1 (Bastian et al., 2009). In the network, nodes (circles; n = 56) represent both geographic regions and species that are connected by edges (lines; n = 241). The graph type was undirected, and no edge merging strategy was used. The layout algorithm “ForceAtlas2” (Jacomy et al., 2014) was then used to spatialize the network. The following input settings were used: tolerance: 0.05, approximate repulsion: checked, approximation: 1.2, scaling: 50, stronger gravity: checked, gravity: 0.01. Considering the low number of occurrences at Bouvet, this bioregion was not taken into account in the similarity network analysis.

Results

Overall taxonomic diversity

In total, 1050 specimens representing 100 morphotypes were collected in the study area (Table 1), and 173 individual genetic barcodes were obtained from 67 distinct morphotypes, indicating a barcoding success rate of 67%. The 100 morphotypes belonged to nine different phyla, among which Arthropoda (32 morphotypes) was the richest, followed by Mollusca (18), Echinodermata (17), Annelida (8), Chordata (8), Bryozoa (6), Cnidaria (5), Porifera (4) and Nemertea (2) (Figure 1). In Arthropoda, the diversity was split between amphipods (19 morphotypes), isopods (8 morphotypes), pycnogonids (3 morphotypes) and tanaids (2 morphotypes) (Figure 1). The phylum Mollusca was composed of gastropods (13 morphotypes), bivalves (4 morphotypes) and chitons (1 morphotype) (Figure 1). Within the Echinodermata, asteroids were the most speciose class (9 morphotypes), followed by holothuroids (6 morphotypes), echinoids (1 morphotype), and ophiuroids (1 morphotype) (Figure 1). From the 100 different morphotypes, 47 were identified to the species level (Table 1) among which 20 species were reported at Crozet for the first time (Table 1): three holothuroid species (*Cladodactyla crocea* var. *croceoides* (Vaney, 1908), *Echinopsolus splendidus* (Gutt, 1990), *Scoliorhapis massini* O'Loughlin & VandenSpiegel, 2010); two polychaete species (*Neoleprea streptochaeta* (Ehlers, 1897), *Platynereis australis* (Schmarda, 1861)); two bryozoan species (*Antarctothoa* cf. *bougainvillei* (d'Orbigny, 1842), *Beania magellanica* (Busk, 1852)); two gastropod species (*Doris kerguelenensis* (Bergh, 1884); *Fusinella jucunda* (Thiele, 1912)); one chiton species (*Hemiarthrum setulosum*); ten amphipod species (*Acontiostoma marionis* Stebbing, 1888, *Atyloella* cf. *magellanica* (Stebbing, 1888), *Haplocheira barbimana* (Thomson, 1879), *Iphimediella paracuticoxa* Andres, 1988, *Jassa* cf. *alonsoae* Conlan, 1990, *Jassa* cf. *hartmannae* Conlan, 1990, *Jassa* cf. *justi* Conlan, 1990, *Oradarea* cf. *unidentata* Thurston, 1974, *Pagetina monodi* (Nicholls, 1938), *Podocerus capillimanus* Nicholls, 1938); and one isopod species (*Cryosignum lunatum* (Hale, 1937)).

TABLE 1 List of the 100 taxa found in the studied area, ranked by alphabetical order of phyla (then by class or order).

Taxa	Code	BOLD	Distribution
Annelida – Clitellata			
Piscicolidae sp. Johnston, 1865	ANE_Q	HAOIV064-24 *	–
Annelida – Polychaeta			
Eulalia sp. Savigny, 1822	ANE_R	HAOIV065-24 *	–
Harmothoe spp. Kinberg, 1856	ANE_B-X-Y-Z	HAOIV029-24 *	–
Neanthes kerguelensis (McIntosh, 1885)	1 - ANE_D	HAOIV033-24 *	Circum SUB
Neoleprea streptochaeta (Ehlers, 1897) #	2 - ANE_A	HAOIV028-24 *	Circum SO
Platynereis australis (Schmarda, 1861) #	3 - ANE_E	HAOIV046-24 *	Circum SUB
Thelepus spectabilis Ehlers, 1897	4 - ANE_O	HAOIV174-24	Circum SO
Serpulidae sp. Rafinesque, 1815	UND_A	HAOIV175-24	–
Arthropoda – Amphipoda			
Acontiostoma marionis Stebbing, 1888 #	5 - AMP_V	HAOIV176-24	Circum SO
Atyloella cf. *magellanica* (Stebbing, 1888) #	6 - AMP_27	HAOIV002-24 *	Circum SO
Corophioidea sp. Leach, 1814	AMP_Q	HAOIV024-24 *	–
Haplocheira barbimana (Thomson, 1879) #	7 - AMP_G	HAOIV011-24 *	Circum SO
Haplocheira sp. Haswell, 1879	AMP_K	HAOIV023-24 *	–
Iphimediella paracuticoxa Andres, 1988 #	8 - AMP_21	HAOIV177-24	HANT+Rest.SUB
Ischyrocerus sp. Krøyer, 1838	AMP_H	HAOIV178-24	–
Jassa cf. *alonsoae* Conlan, 1990 #	9 - AMP_ZZ	HAOIV179-24	Circum SUB
Jassa cf. *hartmannae* Conlan, 1990 #	10 - AMP_A	HAOIV004-24 *	Broad SUB
Jassa cf. *justi* Conlan, 1990 #	11 - AMP_E	HAOIV180-24	Circum SUB
Oedicerotidae sp. Lilljeborg, 1865	AMP_P	HAOIV181-24	–
Oradarea cf. *unidentata* Thurston, 1974 #	12 - AMP_B	HAOIV007-24 *	Circum SO
Pagetina monodi (Nicholls, 1938) #	13 - AMP_12	HAOIV182-24	Broad SUB
Paramoera fissicauda (Dana, 1852)	14 - AMP_X	HAOIV025-24 *	Circum SO
Parawaldeckia kidderi (S.I. Smith, 1876)	15 - AMP_18	HAOIV001-24 *	Circum SO
Podocerus capillimanus Nicholls, 1938 #	16 - AMP_I	HAOIV012-24 *	Circum SO
Proboloides sp. Della Valle, 1893	AMP_ZF	HAOIV183-24	–
Prostebbingia sp. Schellenberg, 1926	AMP_J	HAOIV013-24 *	–
Tryphosella sp. Bonnier, 1893	AMP_ZC	HAOIV027-24 *	–
Arthropoda – Isopoda			
Cassidinopsis emarginata (Guérin-Méneville, 1843)	17 - ISO_C	HAOIV142-24 *	Circum SUB
Cryosignum lunatum (Hale, 1937) #	18 - ISO_8	HAOIV137-24 *	Circum SO
Iathrippa sp. Bovallius, 1886	ISO_G	HAOIV147-24 *	–
Limnoriidea sp. Brandt & Poore in Poore, 2002	ISO_I	HAOIV149-24 *	–
Septemserolis septemcarinata (Miers, 1875)	19 - ISO_J	HAOIV185-24	Circum SO
Sphaeromatidae sp. 1 Latreille, 1825	ISO_A	HAOIV184-24	–
Sphaeromatidae sp. 2 Latreille, 1825	ISO_D	HAOIV145-24 *	–
Spinoserolis latifrons (White, 1847)	20 - ISO_B	HAOIV139-24 *	Circum SO

(Continued)

TABLE 1 Continued

Taxa	Code	BOLD	Distribution
Arthropoda – Pycnogonida			
Endeis viridis Pushkin, 1976	21 - PYC_B	HAOIV166-24 *	Rest. SUB
Nymphon cf. *brevicaudatum* Miers, 1875	22 - PYC_A	HAOIV165-24 *	Circum SO
Tanystylum neorhetum Marcus, 1940	23 - PYC_D	HAOIV186-24	Circum SO
Arthropoda – Tanaidacea			
Apseudes spectabilis Studer, 1884	24 - TAN_2	HAOIV167-24 *	Circum SUB
Pancoloides litoralis (Vanhöffen, 1914)	25 - TAN_A	HAOIV168-24 *	Circum SO
Bryozoa – Gymnolaemata			
Antarctothoa cf. *bougainvillei* (d'Orbigny, 1842) #	26 - BRY_F	HAOIV187-24	Circum SO
Antarctothoa sp. Moyano, 1987	BRY_B	HAOIV188-24	–
Beania magellanica (Busk, 1852) #	27 - BRY_E	HAOIV189-24	Broad SUB
Chaperiopsis sp. Uttley, 1949	BRY_D	HAOIV190-24	–
Fenestrulina sp. 1 Jullien, 1888	BRY_1	HAOIV104-24*	–
Fenestrulina sp. 2 Jullien, 1888	BRY_C	HAOIV191-24	–
Chordata – Actinopterygii			
Gobionotothen marionensis (Günther, 1880)	28 - VER_B	HAOIV173-24 *	Broad SUB
Harpagifer sp. Richardson, 1844	VER_A	HAOIV192-24	–
Chordata – Ascidiacea			
Ascidiacea sp. 1 Blainville, 1824	TUN_B	HAOIV193-24	–
Ascidiacea sp. 2 Blainville, 1824	TUN_D	HAOIV194-24	–
Polyclinidae sp. 1 Milne Edwards, 1841	TUN_A	HAOIV195-24	–
Polyclinidae sp. 2 Milne Edwards, 1841	TUN_C	HAOIV196-24	–
Polyclinidae sp. 3 Milne Edwards, 1841	TUN_E	HAOIV171-24 *	–
Sycozoa sp. Lesson, 1832	TUN_F	HAOIV197-24	–
Cnidaria			
Actiniaria sp. 1 Hertwig, 1882	CNI_B	HAOIV198-24	–
Actiniaria sp. 2 Hertwig, 1882	CNI_E	HAOIV115-24 *	–
Alcyoniidae sp. Lamouroux, 1812	CNI_C	HAOIV112-24 *	–
Staurocladia sp. Hartlaub, 1917	CNI_A	HAOIV111-24 *	–
Tubulariidae sp. Goldfuss, 1818	CNI_D	HAOIV113-24 *	–
Echinodermata – Asteroidea			
Anasterias rupicola (Verrill, 1876)	29 - AST_K	HAOIV098-24 *	Broad SUB
Asteriidae sp. Gray, 1840	AST_H	HAOIV090-24 *	–
Diplasterias meridionalis (Perrier, 1875)	30 - AST_G	HAOIV088-24 *	Broad SUB
Glabraster antarctica (E. A. Smith, 1876)	31 - AST_C	HAOIV199-24	Circum SO
Henricia obesa (Sladen, 1889)	32 - AST_D	HAOIV084-24 *	Circum SO
Henricia cf. *spinulifera* (E. A. Smith, 1876)	33 - AST_1	HAOIV078-24 *	Rest. SUB
Leptychaster kerguelenensis E. A. Smith, 1876	34 - AST_J	HAOIV095-24 *	Broad SUB
Pteraster affinis Smith, 1876	35 - AST_F	HAOIV087-24 *	Circum SO

(Continued)

TABLE 1 Continued

Taxa	Code	BOLD	Distribution
Echinodermata – Asteroidea			
Smilasterias triremis (Sladen, 1889)	36 - AST_A	HAOIV081-24 *	Circum SO
Echinodermata – Echinoidea			
Pseudechinus sp. Mortensen, 1903	ECH_A	HAOIV117-24 *	–
Echinodermata – Holothuroidea			
Cladodactyla crocea var. *croceoides* (Vaney, 1908) #	37 - HOL_A	HAOIV129-24 *	Broad SUB
Cucumariidae sp. Ludwig, 1894	HOL_C	HAOIV134-24 *	–
Echinopsolus splendidus (Gutt, 1990)	38 - HOL_B	HAOIV130-24 *	HANT+Rest.SUB
Pentactella laevigata Verrill, 1876	39 - HOL_E	HAOIV135-24 *	Rest. SUB
Pentactella sp. Verrill, 1876	HOL_F	HAOIV136-24 *	–
Scoliorhapis massini O'Loughlin & VandenSpiegel, 2010 #	40 - UND_23	HAOIV172-24 *	Broad SUB
Echinodermata – Ophiuroidea			
Ophiosabine vivipara (Ljungman, 1871)	41 - OPH_A	HAOIV158-24 *	Broad SUB
Mollusca – Bivalvia			
Bivalvia sp. Linnaeus, 1758	BIV_D	HAOIV200-24	–
Gaimardia sp. A. Gould, 1852	BIV_B	HAOIV201-24	–
Imparidentia sp. Bieler, P. M. Mikkelsen & Giribet, 2014	BIV_A	HAOIV202-24	–
Lissarca sp. E. A. Smith, 1877	BIV_E	HAOIV103-24 *	–
Mollusca – Gastropoda			
Aeolidiidae sp. Gray, 1827	NUD_A	HAOIV153-24 *	–
Chlanidota sp. E. von Martens, 1878	GAS_C	HAOIV120-24 *	–
Doris kerguelenensis (Bergh, 1884) #	42 - NUD_B	HAOIV154-24 *	Broad SUB
Eatoniella sp. Dall, 1876	GAS_I	HAOIV124-24 *	–
Falsimohnia sp. A. W. B. Powell, 1951	GAS_L	HAOIV128-24 *	–
Fusinella jucunda (Thiele, 1912) #	43 - GAS_12	HAOIV118-24 *	Rest. SUB
Laevilacunaria pumilio (E. A. Smith, 1877)	44 - GAS_G	HAOIV122-24 *	Rest. SUB
Margarella sp. Thiele, 1893	GAS_D	HAOIV203-24	–
Marseniopsis sp. Bergh, 1886	GAS_J	HAOIV126-24 *	–
Nacella delesserti (R. A. Philippi, 1849)	45 - GAS_A	HAOIV204-24	Rest. SUB
Nudibranchia sp. Cuvier, 1817	NUD_C	HAOIV155-24 *	–
Pellilitorina setosa (E. A. Smith, 1875)	46 - GAS_H	HAOIV123-24 *	Circum SO
Xanthodaphne sp. A. W. B. Powell, 1942	GAS_M	HAOIV205-24	–
Mollusca – Polyplacophora			
Hemiarthrum setulosum P. P. Carpenter, 1876 #	47 - CHI_A	HAOIV108-24 *	Circum SUB
Nemertea – Hoplonemertea			
Antarctonemertes sp. Friedrich, 1955	UND_C	HAOIV206-24	–
Monostilifera sp. Brinkmann, 1917	NEM_U	HAOIV150-24 *	–

(Continued)

TABLE 1 Continued

Taxa	Code	BOLD	Distribution
Porifera			
Hemigellius sp. Burton, 1932	POR_C	HAOIV162-24 *	–
Chalinidae sp. Gray, 1867	POR_A	HAOIV207-24	–
Myxillidae sp. Dendy, 1922	POR_B	HAOIV208-24	–
Rossella sp. Carter, 1872	POR_D	HAOIV209-24	–

after the taxon name indicates a species reported for the first time in Crozet by the current study. BOLD: Public accession number of one specimen from each species, * indicates that a genetic barcode is available. Distribution: Circum SO (circum Southern Ocean, presence in both Antarctica and the sub-Antarctic), Circum SUB (circum sub-Antarctic, presence in three sub-Antarctic sectors), Broad SUB (broad sub-Antarctic, presence in two sub-Antarctic sectors), Rest. SUB (restricted sub-Antarctic, presence in a single sub-Antarctic sector), HANT + Rest. SUB (presence in the high Antarctic and in a single sub-Antarctic sector). Numbers prior to code of taxa identified to the species levels correspond to the unique numbers reported in Figure 4.

Biogeographic and phylogeographic patterns

Out of the 47 taxa identified to the species level, 35 were successfully barcoded in our study. Previous reference databases with COI sequence data were available for only 14 species (Miya et al., 2016; Moreau et al., 2021; Sands et al., 2021). The four following phylogeographic patterns were observed (Figure 2): (1) a circum Southern Ocean distribution such as in the sea stars *Pteraster affinis* Smith, 1876 and *Smilasterias triremis* (Sladen, 1889), and the pycnogonid *Nymphon brevicaudatum* Miers, 1875; (2) a circum sub-Antarctic distribution such as in the amphipod *Parawaldeckia kidderi* (S.I. Smith, 1876) and the brittle star *Ophiosabine vivipara* (Ljungman, 1871); (3) a broad sub-Antarctic distribution such as for the fish species *Gobionotothen marionensis* (Günther, 1880), and the sea stars *Anasterias rupicola* (Verrill, 1876) and *Diplasterias meridionalis* (Perrier, 1875); and (4) a restricted sub-Antarctic distribution such as in *Henricia* cf. *spinulifera* (E. A. Smith, 1876).

Based on COI data, some unrecognized species diversity was found in six taxa (genetic distance among clades > 2%, cf. Materials and Methods; Figure 3; Supplementary Material 2). In the chiton *Hemiarthrum setulosum* P. P. Carpenter, 1876, specimens showed an important genetic distance from sequences known from the Antarctic Peninsula (11.19%). Analysed specimens of the nudibranch *Doris kerguelenensis* are found within a clade with

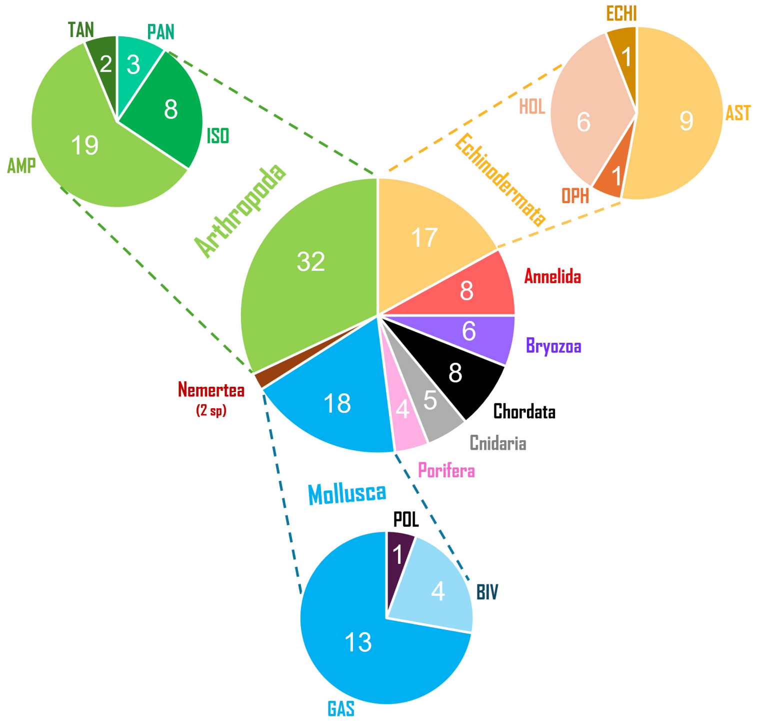
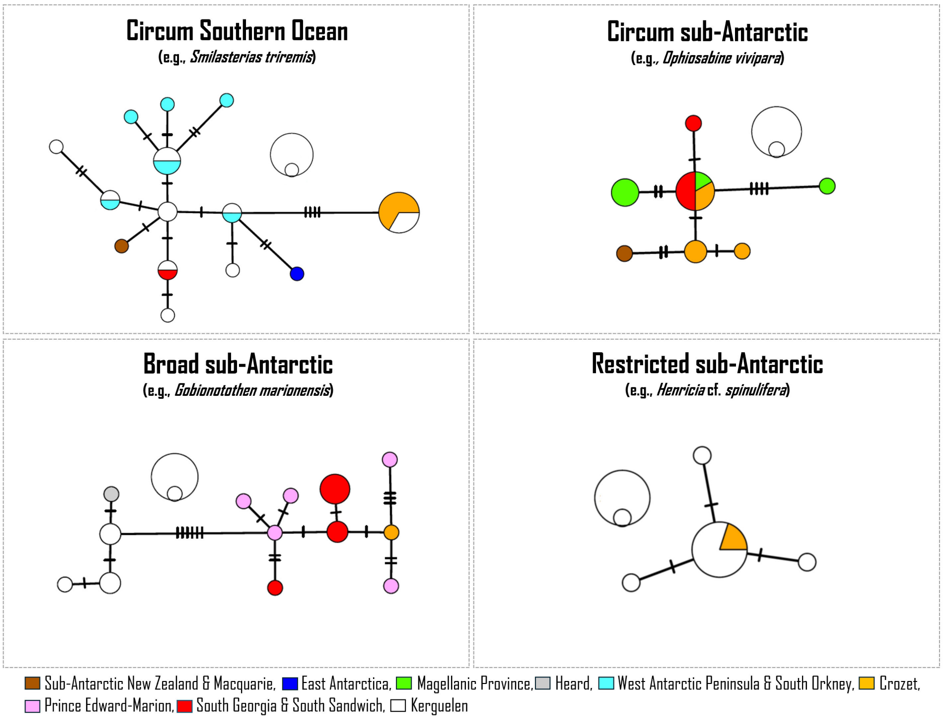


FIGURE 1
Overall taxonomic diversity for the 100 morphotypes found in the current Crozet investigation. Centre chart depicts phyla, top-left chart depicts arthropod orders (AMP, Amphipoda; ISO, Isopoda; TAN, Tanaidacea; PAN, Pantopoda), top-right chart depicts echinoderm classes (AST, Asteroidea; ECHI, Echinoidea; HOL, Holothuroidea; OPH, Ophiuroidea) and bottom chart depicts mollusc classes (BIV, Bivalvia; GAS, Gastropoda; POL, Polyplacophora). Numbers in each slice indicate the number of morphotypes for the specific group.



Circum Southern Ocean
(e.g., *Smilasterias triremis*)

Circum sub-Antarctic
(e.g., *Ophiosabine vivipara*)

Broad sub-Antarctic
(e.g., *Gobionotothen marionensis*)

Restricted sub-Antarctic
(e.g., *Henricia* cf. *spinulifera*)

FIGURE 2
Haplotype networks illustrating the most common biogeographic and phylogeographic patterns. Sizes of imbricated circles close to each network denote the number of specimens exhibiting the haplotype (outer circle: 10 specimens, inner circle: 1 specimen). All the Crozet sequences are from the current study. Non-Crozet sequences are from Miya et al., 2016 (*Gobionotothen marionensis*), Moreau et al., 2021 (*Henricia* cf. *spinulifera*, *Smilasterias triremis*) and Sands et al., 2021 (*Ophiosabine vivipara*).

specimens from South Georgia (genetic distance of 2.82% with the closest related clade; Figure 3). In the tanaid *Apseudes spectabilis* Studer, 1884, the single Crozet specimen showed a p-distance of 2.32% with a specimen from Kerguelen. For the polychaete *Neanthes kerguelensis* (McIntosh, 1885), Crozet specimens clustered with specimens from South Georgia and Kerguelen and are well-differentiated from two distinct Antarctic groups (3.93% et 4.82%, respectively; Figure 3). In addition, potential species complexes were found in the polychaete *Harmothoe* Kinberg, 1856 and the pycnogonid *Nymphon brevicaudatum* (Supplementary Material 2). Finally, while the level of genetic divergence was lower than the defined threshold (1.65%), the Crozet specimens of the sea star *Leptychaster kerguelenensis* interestingly clustered into a clade sharing no haplotype with other sub-Antarctic and Antarctic specimens.

After updating distribution information for four species based on available genetic barcodes (cf. results above), we found that nearly half of the 47 morphotypes identified to the species level were reported to have a circum Southern Ocean distribution (47%), 15% to have a circum sub-Antarctic distribution, 21% to have a broad sub-Antarctic distribution, and 13% to have a distribution restricted to one sector of the Southern Ocean (Table 1; Supplementary Material 3). Two species (the holothurian *Echinopsolus splendidus* (Gutt, 1990) and the amphipod *Iphimediella paracuticoxa* Andres, 1988) also showed a peculiar distribution across the polar front, being present in the high Antarctic and a single sub-Antarctic sector (4%). As highlighted in the similarity network (Figure 4), 34 of these 47 taxa were also found to occur in Kerguelen, 30 in the Magellan Province, 30 in Prince Edward (Marion) Islands, 25 in Macquarie/sub-Antarctic New Zealand region, 22 in South Georgia/South Sandwich Islands, 23 in West Antarctica, 16 in Heard Island, and 13 in East Antarctica. The faunal similarity with Kerguelen was notably marked in most echinoderms (10/13), molluscs (4/6), arthropods (14/21) and in all annelids (4/4) (Supplementary Material 3). This faunal similarity pattern was also observed when comparing with Prince Edwards Islands and the Magellan province (Figure 4; Supplementary Material 3). The similarity of Crozet with Macquarie and sub-Antarctic New Zealand was associated to different shared taxa: it was very high in amphipods (11/12) and in arthropods in general (16/21), but far less marked in echinoderms (5/13) and molluscs (1/6) (Supplementary Material 3).

Discussion

High levels of overlooked diversity

The current study has highlighted the presence of one hundred macrofaunal species in the shallow waters of Crozet. Among these 100 species, we reported 20 species for the first time in Crozet. In addition, 17 taxa identified to the family level (2), genus level (5) or species level (10) were already inventoried by Lelièvre et al. (2023), based on imagery transects of the same area. This included two annelids: Serpulidae Rafinesque, 1815 and *Thelepus spectabilis* Ehlers, 1897;

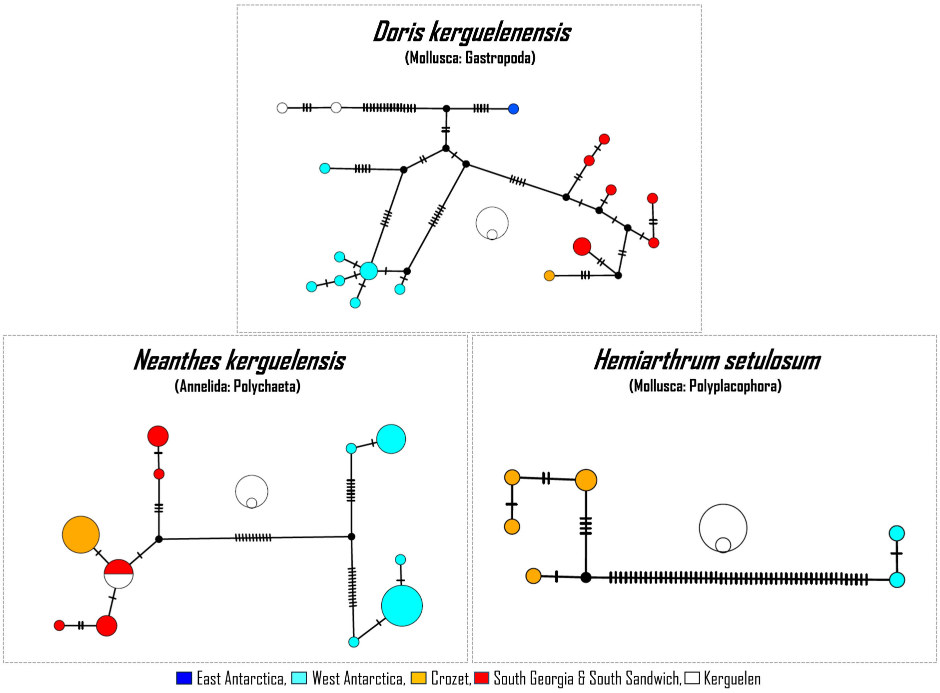


FIGURE 3
Haplotype networks illustrating three cases of unrecognized species diversity. Sizes of imbricated circles close to each network denote the number of specimens exhibiting the haplotype (outer circle: 10 specimens, inner circle: 1 specimen). All the Crozet sequences are from the current study. Non-Crozet sequences are from Maroni et al., 2022 (*Doris kerguelenensis*), Leiva et al. (2022) (*Neanthes kerguelensis*), Irisarri et al. (2014) and Jossart et al. (2023) (*Hemiarthrum setulosum*).

the isopod *Spinoserolis latifrons* (White, 1847); six sea stars: *Anasterias rupicola*, *Diplasterias meridionalis*, *Glabraster antarctica* (E. A. Smith, 1876), *Henricia* sp. Gray, 1840, *Leptychaster kerguelenensis* E. A. Smith, 1876 and *Smilasterias triremis*; the sea urchin *Pseudechinus* sp. Mortensen, 1903, two holothuroids: *Echinopsolus splendidus* (Gutt, 1990) and *Pentactella* sp. Verrill, 1876; the brittle star *Ophiosabine vivipara*; the bivalve *Gaimardia* sp. Gould, 1852; the nudibranch Aeolidiidae sp. Gray, 1827; the gastropods *Margarella* sp. Thiele, 1893 and *Nacella delesserti*; and the sponge *Hemigellius* sp. Burton, 1932. The 100 taxa belonged to nine different phyla, with 75% belonging to either arthropods (32 species), molluscs (18), echinoderms (17) or annelids (8; Figure 1). At lower taxonomic levels, amphipods and gastropods were particularly represented (31% of the species; Figure 1). The high-level species richness found in echinoderms is similar to the value previously reported by Lelièvre et al. (2023) (17 *versus* 14 species). However, our results contrast with the former study regarding the diversity of amphipods (19 *versus* 0) and gastropods (13 *versus* 3 species). Such contrast among sampling and imagery, are related to the higher detection of smaller specimens in physical inventory, which is not surprising and already highlighted in other studies (e.g., Hanafi-Portier et al., 2021).

Interestingly, several taxa found during our investigation might be species that are new to science. First, some specimens could not be assigned to known species by taxonomic experts based on morphology. This was notably the case in amphipods (*Prostebbingia* sp. Schellenberg, 1926), bryozoans (*Antarctothoa* sp. Moyano, 1987, *Fenestrulina* sp. Jullien, 1888) and echinoids (*Pseudechinus* sp). Second, the genetic analysis revealed an unexpected diversity in some taxa. This included species that exhibited more restricted distribution than initially expected (see details below) as well as taxa for which we found more putative species than formerly inventoried in Crozet. For instance, three species of the polychaete genus *Harmothoe* were previously reported around Crozet (*Harmothoe crosetensis* (McIntosh, 1885), *Harmothoe magellanica* (McIntosh, 1885), and *Harmothoe spinosa* Kinberg, 1856). However, our genetic analyses indicated that none of the studied specimens belong to either *Harmothoe crosetensis* or *Harmothoe magellanica* (based on specimens from Cowart et al., 2022) alongside a pattern of species complex (Supplementary Material 2). The exact number of species within this complex remains to be determined, but it is likely that more species than initially expected occur in Crozet. Altogether, our results have significant taxonomic implications for our knowledge of the diversity of various taxa, and definitively call for additional investigations, which are out of the scope of the current study.

Biogeography of Crozet

Among the 47 taxa identified to species level, we found that nearly half of the species (47%) were reported to have a circum Southern Ocean distribution. The rest had a circum sub-Antarctic

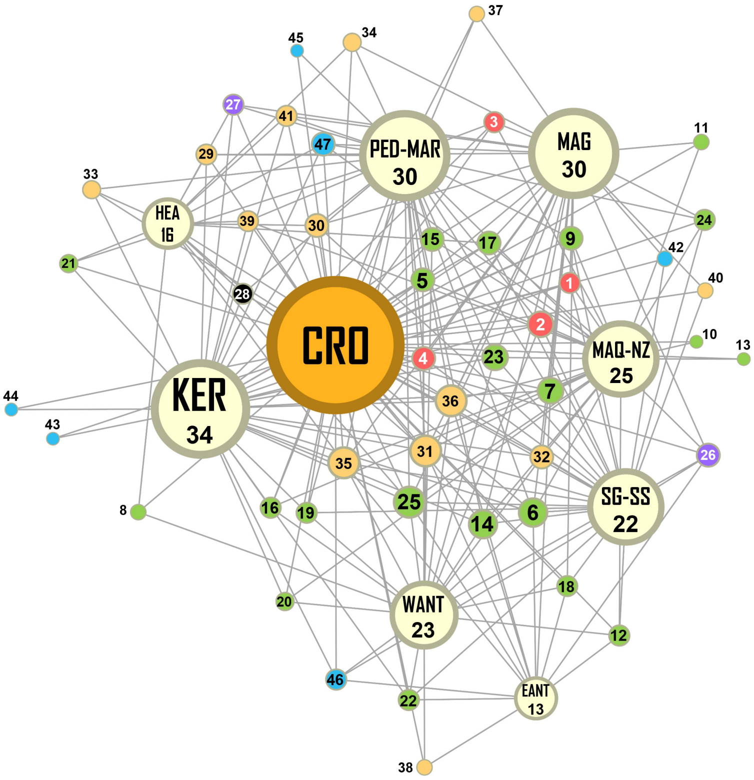


FIGURE 4
Similarity network produced with Gephi 0.10.1. Bioregions (except Crozet in orange) are represented by pale-yellow circles (CRO, Crozet; EANT, East Antarctica; HEA, Heard Island; KER, Kerguelen; MAG, Magellan Province; MAQ-NZ, Macquarie/sub-Antarctic New Zealand region; PED-MAR, Prince Edward (Marion) Islands; SG-SG, South Georgia/South Sandwich Islands; WANT, West Antarctica). The size of each circle is proportional to the total number of species shared by this bioregion with Crozet (indicated under the bioregion acronym). The smaller coloured circles indicate the 47 taxa identified up to the species level: their colours denoting their phyla (black: Chordata; blue: Mollusca; green: Arthropoda; purple: Bryozoa; red: Annelida; yellow: Echinodermata), their sizes denoting the number of bioregions where the species is found; their unique numbers indicating the species referenced in Table 1 (e.g., "45" refers to the gastropod *Nacella delesserti* (R. A. Philippi, 1849) that occurs in two bioregions).

(15%), broad sub-Antarctic (21%), restricted sub-Antarctic (13%) distributions or a peculiar distribution across the polar front (4%, see results). These 47 species were mostly found in three other bioregions: Kerguelen (72%), the Magellan Province (64%), and Prince Edward Islands (64%), and notably associated with high-level sharing of echinoderms (9 to 10 species shared). Such biogeographic affinity of Crozet with other sub-Antarctic areas has been highlighted from historical and recent studies (e.g., Hedgepth, 1969; Griffiths et al., 2009). More specifically, the high affinity with Kerguelen was recently highlighted for marine macroalgae (Féral et al., 2021) while not found for deeper water polychaetes (Sicinski and Gillet, 2002). The high-level similarity with Prince Edward Islands was already found in sea stars at both species and genus levels (Moreau et al., 2017). Interestingly, some species are also reported a few latitudinal degrees above the subtropical front, and therefore outside the Southern Ocean (Supplementary Material 3). For example, several species occur in Tristan da Cunha archipelago (37°S; e.g., the pycnogonid *Tanystylum neorhetum* Marcus, 1940; the amphipod *Parawaldeckia kidderi*) or around Australia and New Zealand (e.g., the polychaete *Platynereis australis*, the amphipod *Jassa* cf. *justi*) (Conlan, 1990; Hutchings and Reid, 1991). Some other species are also expected to occur in both hemispheres such as the sea star *Pteraster affinis* (confirmed by genetic data) or the tanaid *Apseudes spectabilis* (Larsen and Shimomura, 2006; Jossart et al., 2021). Overall, the investigated species therefore include a high proportion of widely distributed species, for which the known biogeographic distribution was confirmed by our genetic results for eight species in the current study (the sea stars *A. rupicola*, *D. meridionalis*, *P. affinis* and *S. triremis*, the pycnogonid *N. brevicaudatum*, the amphipod *P. kidderi*, the brittle star *O. vivipara*, and the fish *G. marionensis*). In contrast, it is very likely that more species with restricted distribution will be found as additional specimens and genetic data are obtained. This situation is illustrated in the present study by four study cases. The tanaid *Apseudes spectabilis*, the nudibranch *Doris kerguelenensis*, the polychaete *Neanthes kerguelensis*, and the chiton *Hemiarthrum setulosum* showed restricted distributions. The tanaid *A. spectabilis*, while expected to have a circum sub-Antarctic distribution (Schmidt and Brandt, 2001), also showed a significant genetic divergence (2.5%) between specimens from Crozet and those from Kerguelen. The nudibranch *D.*

kerguelenensis was shown to be composed of 60 putative species exhibiting contrasting distribution ranges (Maroni et al., 2022; Maroni and Wilson, 2022). The Crozet specimen belonged to a clade that might be distributed in two sub-Antarctic sectors (Atlantic and Indian oceans; Figure 3). The Crozet specimen of polychaete *N. kerguelensis* belongs to a sub-Antarctic clade and our results are in line with Leiva et al. (2022), highlighting an important divergence from an Antarctic clade (Figure 3). Finally, the chiton *H. setulosum*, the single species of the genus *Hemiarthrum*, was expected to have a circum Southern Ocean distribution (type locality: Kerguelen). Present genetic results indicate a high genetic distance between the specimens from Crozet and those from Antarctica (11.19%; Irisarri et al., 2014; Jossart et al., 2023; Figure 3). From now, it would be relevant to further explore whether this species is largely distributed in the sub-Antarctic, notably in the Macquarie-New Zealand region where another *Hemiarthrum* species was previously recognized (*H. hamiltonorum* Iredale & Hull, 1932; Sirenko, 2006).

Conclusions and perspectives

Our study shows the importance of integrative inventories to fill the gap of diversity knowledge existing in remote ecosystems that are facing important environmental changes. We found one hundred taxa, amongst which 20 were reported for the first time in the Crozet archipelago. This emphasizes the fact that shallow coastal waters around Crozet are an under-investigated area, even compared to most other sub-Antarctic areas. Regarding biogeographic patterns, a greater proportion of broadly distributed species was found but this statement needs to be counterbalanced by the detection (based on genetic data) of more restricted distributions than expected for several species. It is likely that future sampling will increase the proportion of species with restricted distribution. We also found a few cases of unrecognized diversity that might lead to the future descriptions of new species, some likely to have a restricted distribution or even be endemic to Crozet. Combined with the high diversity observed, this stresses the need to protect the biologically unusual Crozet archipelago. Additional sampling and barcoding efforts are necessary to better unravel marine faunal diversity and affinities with other sub-Antarctic islands and the overall Southern Ocean in the future. While this was not possible in the current study (due to the limited data available), bathymetry should notably be taken into account in such further investigations. In fact, for several taxonomic groups, species initially reported to occur over broad depth ranges has been showed to rather be distinct species with more restricted depth ranges (Barnes and Kuklinski, 2010; Neal et al., 2018; Moreau et al., 2019). Finally, another perspective includes the sharing of our data for conservation purposes. While the publicly available data will benefit to large scale projects related to conservation (genetic or occurrence data), they will also be directly shared with nature reserve managers of the French Southern Territories. In addition, the creation of a field guide of the most common species investigated is an ongoing work that would help in the monitoring of this area in the future.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The animal study was approved by TAAF-UB convention #2258 – French Polar Institute project #1044 Proteker – Project #2021-0882 “Nearshore Cable Inspection and Environmental Survey at IMS Hydroacoustic Station HA04 Crozet, France”. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

QJ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. YL: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. AK: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. BF: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. CM: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. DD: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. JM: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. MV: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. MM: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. RD: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. SR: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. SH: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. TS: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, 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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2024.1455329/full#supplementary-material

References

Barnes, D. K., and 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? *J. Biogeography* 37, 1648–1656. doi: 10.1111/j.1365-2699.2010.02320.x

Bastian, M., Heymann, S., and Jacomy, M. (2009). "Gephi: an open source software for exploring and manipulating networks," in *International AAAI Conference on Weblogs and Social Media*, Vol. 3. doi: 10.1609/icwsm.v3i1.13937

Brooks, C. M., Chown, S. L., Douglass, L. L., Raymond, B. P., Shaw, J. D., Sylvester, Z. T., et al. (2020). Progress towards a representative network of Southern Ocean protected areas. *PLoS One* 15, e0231361. doi: 10.1371/journal.pone.0231361

Canteras, J., and Arnaud, P. M. (1985). Les gastéropodes prosobranches des Îles Kerguelen et Crozet (Sud de l'Ocean Indien) comparaisons écologiques et particularités biologiques. *Comité Natl. Français Des. Recherches Antarctiques* 57, 1–169.

Carr, C. M., Hardy, S. M., Brown, T. M., Macdonald, T. A., and Hebert, P. D. (2011). A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS One* 6, e22232. doi: 10.1371/journal.pone.0022232

Chevallier, L., and Nougier, J. (1981). Première étude volcanostructurale de l'île de la Possession, iles Crozet (TAAF), Ocean Indien austral. C.R. *Acad. Sci. Paris* 292, 363–368.

Christiansen, H., Dettai, A., Heindler, F. M., Collins, M. A., Duhamel, G., Hautecoeur, M., et al. (2018). Diversity of mesopelagic fishes in the Southern Ocean-a phylogeographic perspective using DNA barcoding. *Front. Ecol. Evol.* 6. doi: 10.3389/fevo.2018.00120

Conlan, K. E. (1990). Revision of the crustacean amphipod genus *Jassa* Leach (Corophioidea: Ischyroceridae). *Can. J. Zoology* 68, 2031–2075. doi: 10.1139/z90-288

Convey, P. (2007). Influences on and origins of terrestrial biodiversity of the sub-Antarctic islands. *Papers Proc. R. Soc. Tasmania* 141, 83–93. doi: 10.26749/rstpp.141.1.83

Cowart, D. A., Schiaparelli, S., Alvaro, M. C., Cecchetto, M., Le Port, A. S., Jollivet, D., et al. (2022). Origin, diversity, and biogeography of Antarctic scale worms (Polychaeta: Polynoidae): a wide-scale barcoding approach. *Ecol. Evol.* 12, e9093. doi: 10.1002/ece3.9093

De Broyer, C., Koubbi, P., Griffiths, H. J., Raymond, B., Udekem d'Acoz, C., Van de Putte, A. P., et al. (2014). *Biogeographic Atlas of the Southern Ocean* (Cambridge: Scientific Committee on Antarctic Research). 498p.

Féral, J. P., Poulin, E., De Ridder, C., and Saucède, T. (2019). "A field guide to coastal echinoderms of the Kerguelen Islands," in *Proceedings of the 16th International Echinoderm Conference*, Nagoya, Japan, 2018 May 28-June 1, Vol. 15, Zoosymposia. doi: 10.11646/zoosymposia.15.1.6

Féral, J. P., Saucède, T., Poulin, E., Marschal, C., Marty, G., Roca, J. C., et al. (2016). PROTEKER: implementation of a submarine observatory at the Kerguelen islands (Southern Ocean). *Underwater Technol.* 34, 3–10. doi: 10.3723/ut.34.003

Féral, J. P., Verlaque, M., Rosenfeld, S., Poulin, E., Chenuil, A., and Saucède, T. (2021). The marine vegetation of the Kerguelen Islands: history of scientific campaigns, inventory of the flora and first analysis of its biogeographical affinities. *Cryptogamie Algologie* 42, 173–216. doi: 10.5252/cryptogamie-algologie2021v42a12

Figuerola, B., Barnes, D. K. A., Brickle, P., and Brewin, P. D. (2017). Bryozoan diversity around the Falkland and South Georgia Islands: Overcoming Antarctic barriers. *Mar. Environ. Res.* 126, 81–94. doi: 10.1016/j.marenvres.2017.02.005

Figuerola, B., Gordon, D. P., and Cristobo, J. (2018). New deep Cheilostomata (Bryozoa) species from the Southwestern Atlantic: shedding light in the dark. *Zootaxa* 4375, 211–249. doi: 10.11646/zootaxa.4375.2.3

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.

Fraser, C. I., Dutoit, L., Morrison, A. K., Pardo, L. M., Smith, S. D., Pearman, W. S., et al. (2022). Southern Hemisphere coasts are biologically connected by frequent, long-distance rafting events. *Curr. Biol.* 32, 3154–3160. doi: 10.1016/j.cub.2022.05.035

Frenot, Y., Gloaguen, J. C., Massé, L., and Lebouvier, M. (2001). Human activities, ecosystem disturbance and plant invasions in subantarctic Crozet, Kerguelen and Amsterdam Islands. *Biol. Conserv.* 101, 33–50. doi: 10.1016/S0006-3207(01)00052-0

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Noll, D., Maturana, C. S., Hüne, M., et al. (2021). Contrasting biogeographical patterns in *Margarella* (Gastropoda: Calliostomatidae: Margarellinae) across the Antarctic polar front. *Mol. Phylogenet. Evol.* 156, 107039. doi: 10.1016/j.ympev.2020.107039

Gostel, M. R., and Kress, W. J. (2022). The expanding role of DNA barcodes: Indispensable tools for ecology, evolution, and conservation. *Diversity* 14, 213. doi: 10.3390/d14030213

Griffiths, H. J. (2010). Antarctic marine biodiversity–what do we know about the distribution of life in the Southern Ocean? *PLoS One* 5, e11683. doi: 10.1371/journal.pone.0011683

Griffiths, H. J., Barnes, D. K., and Linse, K. (2009). Towards a generalized biogeography of the Southern Ocean benthos. *J. Biogeography* 36, 162–177. doi: 10.1111/j.1365-2699.2008.01979.x

Griffiths, H. J., and Waller, C. L. (2016). The first comprehensive description of the biodiversity and biogeography of Antarctic and Sub-Antarctic intertidal communities. *J. Biogeography* 43, 1143–1155. doi: 10.1111/jbi.12708

Guillaumot, C., Fabri-Ruiz, S., Martin, A., Eléaume, M., Danis, B., Féral, J. P., et al. (2018). Benthic species of the Kerguelen Plateau show contrasting distribution shifts in response to environmental changes. *Ecol. Evol.* 8, 6210–6225. doi: 10.1002/ece3.4091

Hanafi-Portier, M., Samadi, S., Corbari, L., Chan, T. Y., Chen, W. J., Chen, J. N., et al. (2021). When imagery and physical sampling work together: Toward an integrative methodology of deep-sea image-based megafauna identification. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.749078

Hedgepth, J. W. (1969). "Introduction to antarctic zoogeography," in *Distribution of selected groups of marine invertebrates in waters south of 35°S*. (New York, United States) 1–29.

Hemmings, A. D., and Stephens, T. (2010). The extended continental shelves of sub-Antarctic Islands: implications for Antarctic governance. *Polar Rec.* 46, 312–327. doi: 10.1017/S0032247409990532

Hullé, M., and Vernon, P. (2021). Terrestrial macro-arthropods of the sub-Antarctic islands of Possession (Crozet Archipelago) and Kerguelen: Inventory of native and non-native species. *Zoosystema* 43, 549–561. doi: 10.5252/zoosystema2021v43a22

Hutchings, P., and Reid, A. (1991). The nereididae (Polychaeta) from Australia-*leonnates*, *platynereis* and *solomononereis*. *Records Aust. Museum* 43, 47–62. doi: 10.3853/j.0067-1975.43.1991.40

Irisarri, I., Eernisse, D. J., and Zardoya, R. (2014). Molecular phylogeny of Acanthochitonina (Mollusca: Polyplacophora: Chitonida): three new mitochondrial genomes, rearranged gene orders and systematics. *J. Natural History* 48, 2825–2853. doi: 10.1080/00222933.2014.963721

Jacomy, M., Venturini, T., Heymann, S., and Bastian, M. (2014). ForceAtlas2, a continuous graph layout algorithm for handy network visualization designed for the Gephi software. *PLoS One* 9, e98679. doi: 10.1371/journal.pone.0098679

Jossart, Q., Bauman, D., Moreau, C. V., Saucède, T., Christiansen, H., Brasier, M. J., et al. (2023). A pioneer morphological and genetic study of the intertidal fauna of the Gerlache Strait (Antarctic Peninsula). *Environ. Monit. Assess.* 195, 514. doi: 10.1007/s10661-023-11066-3

Jossart, Q., Kochzius, M., Danis, B., Saucède, T., and Moreau, C. V. (2021). Diversity of the Pterasteridae (Asteroidea) in the Southern Ocean: a molecular and morphological approach. *Zoological J. Linn. Soc.* 192, 105–116. doi: 10.1093/zoolinnean/zlaa097

Jossart, Q., Sands, C. J., and Sewell, M. A. (2019). Dwarf brooder versus giant broadcaster: combining genetic and reproductive data to unravel cryptic diversity in an Antarctic brittle star. *Heredity* 123, 622–633. doi: 10.1038/s41437-019-0228-9

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., and Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649. doi: 10.1093/bioinformatics/bts199

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547. doi: 10.1093/molbev/msy096

Larsen, K., and Shimomura, M. (2006). Tanaidacea (crustacea: peracarida) from Japan. I. Apseudomorpha from the east China Sea, Seto inland Sea, and nansei islands. *Zootaxa* 1341, 29–48. doi: 10.11646/zootaxa.1341.1.2

Layton, K. K., Corstorphine, E. A., and Hebert, P. D. (2016). Exploring Canadian echinoderm diversity through DNA barcodes. *PLoS One* 11, e0166118. doi: 10.1371/journal.pone.0166118

Layton, K. K., Martel, A. L., and Hebert, P. D. (2014). Patterns of DNA barcode variation in Canadian marine molluscs. *PLoS One* 9, e95003. doi: 10.1371/journal.pone.0095003

Leigh, J. W., and Bryant, D. (2015). POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6, 1110–1116. doi: 10.1111/2041-210X.12410

Leiva, C., Riesgo, A., Combosch, D., Arias, M. B., Giribet, G., Downey, R., et al. (2022). Guiding marine protected area network design with comparative phylogeography and population genomics: An exemplary case from the Southern Ocean. *Diversity Distributions* 28, 1891–1907. doi: 10.1111/ddi.13590

Lelièvre, Y., Motreuil, S., Specq, L., Marschal, C., Dubois, P., Wauters, L., et al. (2024). MEDUSA: Marine benthic Ecological Data from Underwater imagery Surveys of sub-Antarctic Crozet environments. *Sci. Data* 11, 613. doi: 10.1038/s41597-024-03460-4

Lelièvre, Y., Specq, L., Lamy, T., Boyé, A., Downey, R. V., and Saucède, T. (2023). Taxonomic and functional diversity of subtidal benthic communities associated with hard substrates at Crozet archipelago (sub-Antarctic, Southern Ocean). *Front. Mar. Sci.* 10. doi: 10.3389/fmars.2023.1291038

Maroni, P. J., Baker, B. J., Moran, A. L., Woods, H. A., Avila, C., Johnstone, G. J., et al. (2022). One Antarctic slug to confuse them all: the underestimated diversity of *Doris kerguelenensis*. *Invertebrate Systematics* 36, 419–435. doi: 10.1071/IS21073

Maroni, P. J., and Wilson, N. G. (2022). Multiple *Doris "kerguelenensis"*(Nudibranchia) species span the Antarctic Polar Front. *Ecol. Evol.* 12, e9333. doi: 10.1002/ece3.9333

Mason, N. W., Mouillot, D., Lee, W. G., and Wilson, J. B. (2005). Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos* 111, 112–118. doi: 10.1111/j.0030-1299.2005.13886.x

Miya, T., Gon, O., Mwale, M., and Poulin, E. (2016). Molecular systematics and taxonomic status of three latitudinally widespread nototheniid (Perciformes: Notothenioidei) fishes from the Southern Ocean. *Zootaxa* 4061, 381–396. doi: 10.11646/zootaxa.4061.4.4

Moore, J. M., Carvajal, J. I., Rouse, G. W., and Wilson, N. G. (2018). The Antarctic Circumpolar Current isolates and connects: Structured circumpolarity in the sea star *Glabraster Antarctica*. *Ecol. Evol.* 8, 10621–10633. doi: 10.1002/ece3.4551

Moreau, C., Danis, B., Jossart, Q., Eléaume, M., Sands, C., Achaz, G., et al. (2019). Is reproductive strategy a key factor in understanding the evolutionary history of Southern Ocean Asteroidea (Echinodermata)? *Ecology and Evolution* 9, 8465-8478. doi: 10.1002/ece3.5280

Moreau, C., Jossart, Q., Danis, B., Eléaume, M., Christiansen, H., Guillaumot, C., et al. (2021). The high diversity of Southern Ocean sea stars (Asteroidea) reveals original evolutionary pathways. *Prog. oceanography* 190, 102472. doi: 10.1016/j.pocean.2020.102472

Moreau, C., Saucède, T., Jossart, Q., Agüera, A., Brayard, A., and Danis, B. (2017). Reproductive strategy as a piece of the biogeographic puzzle: a case study using Antarctic sea stars (Echinodermata, Asteroidea). *J. Biogeography* 44, 848–860. doi: 10.1111/jbi.12965

Neal, L., Linse, K., Brasier, M. J., Sherlock, E., and Glover, A. G. (2018). Comparative marine biodiversity and depth zonation in the Southern Ocean: evidence from a new large polychaete dataset from Scotia and Amundsen seas. *Mar. Biodiversity* 48, 581–601. doi: 10.1007/s12526-017-0735-y

Owada, M., Kasai, Y., Sato, S. I., and Hong, J. S. (2013). Shell morphology and genetic characteristics of *Corbula* (*Potamocorbula*) spp. (Bivalvia: Corbulidae) from the waters adjacent to Japan, Korea and San Francisco Bay. *Aquat. Biol.* 19, 297–305. doi: 10.3354/ab00539

Park, Y. H., Durand, I., Kestenare, E., Rougier, G., Zhou, M., d'Ovidio, F., et al. (2014). Polar Front around the Kerguelen Islands: An up-to-date determination and associated circulation of surface/subsurface waters. *J. Geophysical Research: Oceans* 119, 6575–6592. doi: 10.1002/2014JC010061

Pollard, R. T., Venables, H. J., Read, J. F., and Allen, J. T. (2007). Large-scale circulation around the Crozet Plateau controls an annual phytoplankton bloom in the Crozet Basin. *Deep Sea Res. Part II: Topical Stud. Oceanography* 54, 1915–1929. doi: 10.1016/j.dsr2.2007.06.012

Poulin, E., González-Wevar, C., Díaz, A., Gérard, K., and Hüne, M. (2014). Divergence between Antarctic and South American marine invertebrates: What molecular biology tells us about Scotia Arc geodynamics and the intensification of the Antarctic Circumpolar Current. *Global Planetary Change* 123, 392–399. doi: 10.1016/j.gloplacha.2014.07.017

Puillandre, N., Brouillet, S., and Achaz, G. (2021). ASAP: assemble species by automatic partitioning. *Mol. Ecol. Resour.* 21, 609–620. doi: 10.1111/1755-0998.13281

Puillandre, N., Lambert, A., Brouillet, S., and Achaz, G. J. M. E. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol. Ecol.* 21, 1864–1877. doi: 10.1111/j.1365-294X.2011.05239.x

Ratnasingham, S., and Hebert, P. D. (2007). BOLD: The Barcode of Life Data System (http:// www. barcodinglife.org). *Mol. Ecol. Notes* 7, 355–364. doi: 10.1111/j.1471-8286.2007.01678.x

Rosenfeld, S., Segovia, N. I., Maturana, C. S., Aldea, C., Saucède, T., Brickle, P., et al. (2023). A revision of the higher latitude periwinkle species *Laevilitorina caliginosa sensu lato*. *Zoological J. Linn. Soc.*, zlad171. doi: 10.1093/zoolinnean/zlad171

Sands, C. J., O'Hara, T. D., and Martín-Ledo, R. (2021). Pragmatic assignment of species groups based on primary species hypotheses: the case of a dominant component of the Southern Ocean benthic fauna. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.723328

Saucède, T., Guillaumot, C., Michel, L., Fabri-Ruiz, S., Bazin, A., Cabessut, M., et al. (2017). "Modelling species response to climate change in sub-Antarctic islands: echinoids as a case study for the Kerguelen Plateau," in *The Kerguelen Plateau: Marine Ecosystems and Fisheries*. (Paris, France) 95–116.

Schmidt, A., and Brandt, A. (2001). The tanaidacean fauna of the Beagle Channel (southern Chile) and its relationship to the fauna of the Antarctic continental shelf. *Antarctic Sci.* 13, 420–429. doi: 10.1017/S095410200100058X

Sicinski, J., and Gillet, P. (2002). Biogeographical affinities of polychaetes from Îles Crozet. *Antarctic Sci.* 14, 353–363. doi: 10.1017/S0954102002000172

Sirenko, B. (2006). Report on the present state of our knowledge with regard to the chitons (Mollusca: Polyplacophora) of the Magellan Strait and Falkland Islands (< Special Number> the 2nd International Chiton Symposium). *Venus (Journal Malacological Soc. Japan)* 65, 81–89. doi: 10.18941/venus.65.1-2_81

Sunnucks, P., and Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Mol. Biol. Evol.* 13, 510–524. doi: 10.1093/oxfordjournals.molbev.a025612

Teske, P. R., McQuaid, C. D., Froneman, P. W., and Barker, N. P. (2006). Impacts of marine biogeographic boundaries on phylogeographic patterns of three South African estuarine crustaceans. *Mar. Ecol. Prog. Ser.* 314, 283–293. doi: 10.3354/meps314283

Vantomme, L., Jossart, Q., Gérard, K., Danis, B., and Moreau, C. (2023). Preliminary assessment of sea star (Echinodermata, Asteroidea) diversity in the coastal magellanic Region (South Chile) and their geographical distribution. *Diversity* 15, 1129. doi: 10.3390/d15111129

Vieira, P. E., Gomes, N., Lobo, J., Borges, L., Queiroga, H., and Costa, F. (2016). A DNA barcode reference library for the superorder Peracarida (Crustacea) from the Southern European Atlantic coast. *Front. Mar. Sci.* 128. Conference Abstract: XIX Iberian Symposium on Marine Biology Studies.

Ward, R. D., Holmes, B. H., and O'Hara, T. D. (2008). DNA barcoding discriminates echinoderm species. *Mol. Ecol. Resour.* 8, 1202–1211. doi: 10.1111/j.1755-0998.2008.02332.x

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Does rafting promote contemporary gene flow? Global and regional patterns of population genetic diversity and structure on the false limpet *Siphonaria lateralis* in the Southern Ocean

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Rafting has been proposed as an effective mechanism for species without free-living pelagic larvae to achieve long-distance dispersal, theoretically preventing population differentiation over wide distributional ranges. Moreover, rafting has been advocated as a main dispersal mechanism for marine invertebrates with sub-Antarctic distributions, because of abundant buoyant kelps, driven by the Antarctic Circumpolar Current. Nonetheless, little attention has been given to the role of rafting to establish regular gene flow across the sub-Antarctic, and the geographic and temporal scales at which it occurs. Aiming to unravel these major questions about the extent of genetic connectivity across the Southern Ocean (SO), we studied the pulmonate limpet *Siphonaria lateralis*, a benthic species with encapsulated larvae, found on the rocky intertidal of sub-Antarctic islands and southern South America. Since *S. lateralis* is closely associated with *D. antarctica*, dispersal by rafting is plausible, as revealed by the absence of phylogeographic structure across the sub-Antarctic. We sampled 116 individuals from eight localities across the SO, and used 5,515 SNPs obtained through Genotyping-by-Sequencing, to determine contemporary genetic diversity, structure, and gene flow at two spatial scales; global, across the SO, and regional, within Kerguelen. Results identified substantial genetic structure, differentiating Patagonia, Falklands/Malvinas Islands, South Georgia and the Kerguelen archipelago, and low levels of contemporary gene flow. The most notable genetic differentiation was found between Patagonia/Falklands and South Georgia/Kerguelen. Structure was also significant between Patagonia and the

Falkland/Malvinas Islands. Conversely, South Georgia and Kerguelen exhibited closer genetic affinity, and indications of recent but limited gene flow. Moreover, historical gene flow estimates between the four populations were low. At regional scale, noteworthy genetic structure persisted, and gene flow was insufficient to prevent genetic differentiation within Kerguelen. Consequently, rafting's potential may be overestimated as a contemporary mechanism promoting gene flow across the SO, as these events may be sporadic, irregular, and unpredictable for marine invertebrates lacking a larval dispersal stage, since contemporary dispersal events don't seem to facilitate high gene flow at both scales. Accordingly, other oceanographic factors or processes may hinder the establishment of species associated with macroalgae, and as consequence, contemporary genetic connectivity in the sub-Antarctic.

KEYWORDS

long distance dispersal, sub-Antarctic, rafting, Antarctic Circumpolar Current, benthic protected development, Genotyping-by-Sequencing, gene flow

1 Introduction

Dispersal is a key trait for species from ecological and evolutionary perspectives, as movement plays a role in population dynamics, the extent of a species' geographic range, the degree of gene flow, and the potential for local adaptation (Slatkin, 1985; Palumbi, 1994; Cowen and Sponaugle, 2009; Manel et al., 2023). In marine ecosystems, fish and invertebrates have complex life cycles where at least one stage may be mobile and promotes a species' dispersal, either by active or passive movement, as dictated by life histories (Gaines et al., 2007). Because some of these earlier stages are often small sized, it's difficult to directly measure dispersal (Weersing and Toonen, 2009). However, genetic data has been used to study the exchange of individuals and their genes between populations, by estimating a direct consequence of effective dispersal: population connectivity (henceforth 'connectivity'), defined as the degree to which gene flow affects a species' evolutionary processes (Lowe and Allendorf, 2010).

For most benthic invertebrate species with complex life cycles, connectivity is usually associated with pelagic larval stages, which, aided by interactions with oceanic currents and processes, can travel and settle up to tens to thousands of kilometers away from their spawning populations (Cowen and Sponaugle, 2009; White et al., 2019). While numerous larval traits influence dispersal and connectivity (Blanco et al., 2019), pelagic larval duration (PLD) is key, as the longer larvae spend in the water column, the higher the chances to traverse longer distances, increasing dispersal potential, gene flow and limiting genetic structure (*i.e.* F_{ST}) (Waples, 1987; Haye et al., 2014; Pinsky et al., 2017; Álvarez-Noriega et al., 2020; Hernawan et al., 2021). On the contrary, restricted or null dispersal, low gene flow and marked genetic structure is to be expected from species with short PLD (*i.e.* lecithotrophic larvae) or without pelagic larval stages (*i.e.* brooders or direct developers). While this paradigm is widely accepted, there are plenty of exceptions to both these assumptions, as some species with high dispersal potential display unexpectedly high levels of genetic structure (Selkoe et al., 2016; Larsson et al., 2017; Gaeta et al., 2020), and on the other end of the spectrum, species with low or null dispersal potential report low genetic structure spanning great geographic distances (Selkoe et al., 2016; Fleming et al., 2018; Gélin et al., 2018; Bertola et al., 2020). These 'exceptions' further illustrate that dispersal in the marine ecosystem is complex and multidimensional and is subjected to intrinsic and extrinsic mechanisms and processes that influence its extent and consequences. As such, while larvae are undoubtedly important and PLD can guarantee a minimum dispersal distance, these traits may be a poor predictor of gene flow (D'Aloia et al., 2015; Hilário et al., 2015; Esser et al., 2023), because other factors such as ecology, physiology, oceanography or habitat selectivity limit dispersal and/or settlement rates (Waters et al., 2013; Sjöqvist et al., 2015; Pascual et al., 2017; Gaeta et al., 2020). Moreover, regardless of development mode, other life stages can play an important role in a species dispersal, such as adult migrations (Frisk et al., 2014), or other mechanisms or processes of natural or anthropogenic origin that may facilitate dispersal (Carlton et al., 2017).

One of such mechanisms is rafting, defined as marine dispersal mediated by buoyant elements of organic or inorganic origin, often described for organisms that live in close association with macroalgae (Highsmith, 1985; Thiel and Gutow, 2005; Winston, 2012). Numerous studies provide global evidence supporting the hypothesis that direct developers and brooders can disperse through rafting (Haye et al., 2012, Haye et al, 2014; Trickey et al., 2016; Bertola et al., 2020), arguing that this mechanism may be as relevant for dispersal and connectivity as having a pelagic larvae dispersal phase (Carlton et al., 2017; Simkanin et al., 2019; Pfaller et al., 2019). The Southern Ocean (SO) is a vast mass of open ocean encircling Antarctica and tens of relatively small islands, and influencing a portion of the Australian and South American continents (Koubbi et al., 2014; Chapman et al., 2020). Within this area, the past two decades of phylogenetic and phylogeographic research have pointed out that rafting may be the leading hypothesis to explain the current distribution and high levels of genetic connectivity that some sub-Antarctic benthic species display (Nikula et al., 2010; Cumming et al., 2014; Moon et al., 2017; González-Wevar et al., 2018; Waters et al., 2018b; Fraser et al., 2020a; Güller et al., 2020; Macaya et al., 2020), as buoyant macroalgae may help achieve long-distance dispersal (LDD), a highly relevant evolutionary and ecological process (Jokiel, 1990; Gillespie et al., 2012; Waters et al., 2018a; Macaya et al., 2020). This hypothesis is not only supported by genetic data, as even considering the extension of the SO, as many coastal and benthic taxa are shared within the sub-Antarctic biogeographical region (Griffiths et al., 2009; De Broyer et al, 2014; Koubbi et al., 2014). Since most of these land masses are of heterogeneous origins and ages (continental or volcanic, ranging from ~95Ma to ~500Ka) and are geographically isolated (Quilty, 2007; Hodgson et al., 2014), the most plausible mechanism by which these shared taxa could have colonized their coasts is via transoceanic LDD, largely associated to kelp rafts of *Durvillaea antarctica* and *Macrocystis pyrifera* (Waters et al., 2018a; Fraser et al., 2020a). These macroalgae not only dominate near-shore, hard-substrate ecosystems, but are frequently observed floating in open waters, and around 20 million of viable rafts have been estimated to be adriftin the Southern Ocean (Smith, 2002; Stevens et al., 2002). Moreover, phylogeographic studies illustrate common haplotypes between sub-Antarctic islands and southern South America (Patagonia and Falklands/Malvinas Islands), suggesting that *D. antarctica* (Fraser et al., 2009, Fraser et al, 2010; Bussolini and Waters, 2015) and *M. pyrifera* (Macaya and Zuccarello, 2010; Assis et al., 2023) have great ability to disperse across the Southern Ocean. Coupled with the fact that live, viable benthic invertebrates have been registered on beached rafts (Fraser et al., 2010; Layton et al., 2022), it is to be expected that species associated with rafting macroalgae are able to withstand floating adrift, settle into new populations, and achieve contemporary gene flow unrelated to their developmental mode, hinted by the scarce studies with SNPs (Single Nucleotide Polymorphisms) in the Southern Ocean (Leiva et al., 2019; Zbawicka et al., 2019; Lau et al., 2023).

However, knowledge of this mechanism is still lacking, and less attention has been given to the frequency of these events and its consequences on contemporary patterns of connectivity (Gillespie et al., 2012; Waters et al., 2018a). Are rafting events rare enough that there's only sporadic gene flow, to be able to sustain a species' integrity? Or are they frequent enough to establish a regular, contemporary gene flow amongst isolated, distant coasts? To address this issue, we studied the pulmonate false limpet *Siphonaria lateralis* (Gastropoda: Heterobranchia) as a model to re-evaluate connectivity and rafting in the Southern Ocean. Despite its benthic protected development with encapsulated larvae in egg masses (Zabala et al., 2020), this species is widely distributed across the rocky intertidal of Patagonia, Falkland/Malvinas Islands, South Georgia, Kerguelen and Macquarie (Dayrat et al., 2014; Güller et al., 2015; González-Wevar et al., 2018), a paradoxical distribution for a species with low dispersal potential. Nevertheless, as discussed above, such extended distribution is not considered rare for species that live in close association with *D. antarctica*, and rafting has been proposed as the main mechanism explaining such a broad distribution of *S. lateralis* (Morton and Miller, 1973; Simpson, 1976; González-Wevar et al., 2018). Moreover, phylogeographic studies with mtDNA and nucDNA markers evidenced shallow genetic differentiation across the sub-Antarctic region (>8,000 Km) suggesting that dispersal through rafting may preserve the genetic integrity of the species over large geographic distances (González-Wevar et al., 2018). However, recent studies aiming to explore connectivity with multilocus genetic markers such as SNPs (Single Nucleotide Polymorphisms) have demonstrated that, even in light of absent or low phylogeographic structure, marine species exhibit significant genetic subdivision and restricted gene flow across populations (Pazmiño et al., 2017; Teske et al., 2018; Green et al., 2022). Moreover, while *D. antarctica* across the Southern Ocean consists of a single broader, sub-Antarctic clade, this macroalgae is sub-structured and there is substantial differentiation between islands according to SNP data (Fraser et al., 2018), that nevertheless seem to be sporadically connected, as supported by distributional modeling and beached kelp rafts, which provide evidence of LDD events across the Southern Ocean (Fraser et al., 2018; Fraser et al, 2022). Considering this information, we expect that associated species such as *S. lateralis* will display moderate levels of genetic structure and low levels of recent gene flow, enough to maintain contemporary connectivity. Furthermore, our objective is twofold. Firstly, to evaluate whether the long-debated mechanism of rafting maintains substantial contemporary levels of connectivity throughout the distribution range of *S. lateralis*. Secondly, to ascertain whether this mechanism is effective at a regional scale, facilitating connectivity among disjoint yet geographically proximate coastal populations, particularly within the Kerguelen Islands. Clarifying these two aspects will help towards drawing robust conclusions about the extent and implications of rafting on the Southern Ocean, and its effects on evolutionary history, biogeographic patterns, and eventual conservation strategies.

2 Materials and methods

2.1 Sampling, sequencing and SNP filtering

Between the years 2013 and 2017, 190 adult *Siphonaria lateralis* individuals were sampled from eight locations, spanning most of the species' range (Figure 1). Specimens were stored in 96% ethanol for posterior genomic DNA extraction with Qiagen's DNeasy Blood & Tissue^{TM} Kit, following the manufacturer's instructions, and DNA concentration was assessed with Qubit^{TM} 3.0 fluorometer (Lifetechnologies). Samples were sent to the Biotechnology Center of the University of Wisconsin, USA. Sequencing and construction of genomic libraries was done by Genotyping by Sequencing (GBS) using

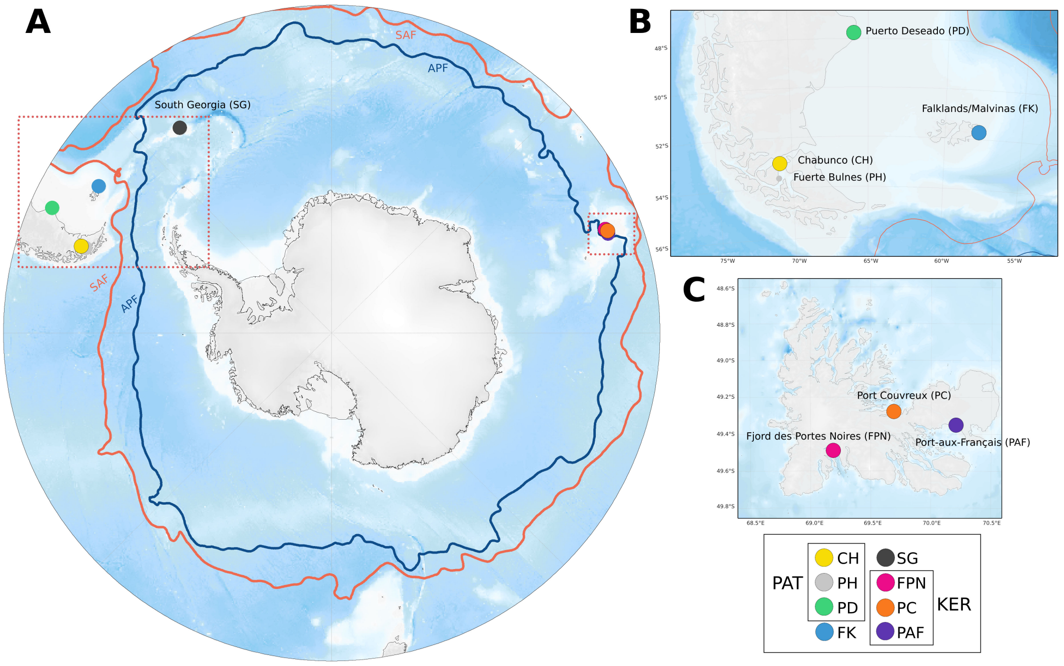

FIGURE 1
Map of the 8 sampled populations of *Siphonaria lateralis* **(A)**, with inset maps of South America **(B)** and Kerguelen Islands **(C)** sampling sites. Label box provides regional grouping information (PAT = Patagonia, KER = Kerguelen).

Illumina NovaSeq 6000 platform, following a Reduced Representation Sequencing (RRS) approach, which is a reliable and affordable method to study population structure of non-model species without a reference genome, and produce a large number of Single Nucleotide Polymorphisms (SNPs) (Rovelli et al., 2019; Kunvar et al., 2021). Under this approach, library preparation used two restriction enzymes to reduce the complexity of the genome (NsiI-MspI), and barcode adaptors for each individual. Single end reads were visualized in FastQC for quality checks. To generate our SNP dataset, our data was prepared and analyzed following the UNEAK pipeline (Universal Network-Enabled Analysis Kit) implemented in TASSEL v.5, which is useful for *de novo* locus identification for non-model organisms (Lu et al., 2013). With the UNEAK pipeline, the dataset was demultiplexed, barcodes were removed and reads were trimmed from ~100 bp to 64 bp. Identical tags were aligned with an error tolerance rate of 0.03, to minimize considering real tags as sequencing errors, and we kept only biallelic loci. Following this, we assembled two SNPs datasets according to our research objectives, one global, encompassing *S. lateralis* individuals sampled across different Southern Ocean sub-Antarctic provinces, and a regional dataset, considering three populations from Kerguelen Islands. We used a 80% mnC (minimum call rate), 5% MAF (minimum allele frequency) to filter the SNPs from each dataset (global and regional), and we allowed a maximum of 40% missing data per individual in TASSEL v.5. Then, we excluded those SNPs that failed the Hardy-Weinberg equilibrium (HWE) test implemented in Arlequin v3.5 (Excoffier and Lischer, 2010) at $p < 0.05$, and we used a False Discovery Rate (FDR) correction to avoid false positives (incorrectly considering as a SNP as out of HWE), in at least 60% of each of the dataset populations. Since the interest of the study was to focus on neutral evolutionary processes, we identified potential outlier SNPs with BAYESCAN 2.1 (Foll and Gaggiotti, 2008). For this, 500,000 MCMC iterations were run with a 10% burnin', a thinning interval of 10, and a prior odd of 1,000 (100 for the regional dataset), to minimize potential false positives, according to the large number of SNPs to test. Subsequently, we removed all SNPs after FDR q-value correction that had strong or very strong evidence to be under selection according to Jeffrey's criterion, to obtain a global and a regional neutral SNPs dataset for downstream analyses.

2.2 Genetic diversity

Analyses were performed for the global and regional datasets to evaluate the differences in genetic diversity at both spatial scales. For each population, expected heterozygosity (H_e), observed heterozygosity (H_o), average number of alleles per locus (allelic richness, A), the proportion of polymorphic loci (%Po), and the inbreeding coefficient (F_{IS}) were computed in GENETIX (Belkhir et al., 2004). Additionally, genetic diversity parameters were estimated for the four major geographic areas within the global dataset, and private alleles for each area, and within Kerguelen populations, were quantified in HP-RARE 1.0 (Kalinowski, 2005).Within Kerguelen populations, we estimated the effective population size (N_e) based on the linkage disequilibrium (LD) method using NeEstimator v2.1 (Do et al., 2014), and jackknife 95% confidence intervals (CIs) (Waples and Do, 2008).

2.3 Population structure

We used multiple approaches to assess population genetic structure at global and regional scale. First, pairwise population F_{ST} and their significance was estimated using a test of 10,000 permutations in Genodive v 3.0 (Meirmans, 2020). Corresponding F_{ST} p-values of multiple testing were adjusted through FDR (Narum, 2006). Secondly, we performed three individual-based population assignment tests to estimate genetic structure; 1) IBS analysis, which clusters together unrelated individuals based on similarities between genotypes at each locus, implemented in the R package *SNPRelate* (Zheng et al., 2012), 2) Principal Component Analysis (PCA), to determine the percentage of variation in allele frequencies explained by the major axes, implemented in *adegenet* (Jombart, 2008), and 3), STRUCTURE (Pritchard et al., 2000), a bayesian clustering method that assigns individuals to populations based on allele frequencies. Our aim with STRUCTURE was to discover the main genetic groups across the species' distribution across the Southern Ocean, and secondly, to determine substructure within each main group. Due to the large number of SNPs in our datasets, we used StrAuto, a Python script that reduces analysis time by allowing for parallel computing of STRUCTURE runs (Chhatre and Emerson, 2017). Number of estimated ancestral populations (K) varied from one to six. Ten replicate runs were performed for each estimated K under the admixture model, with 600,000 MCMC, a 10% burnin'. The most probable K value was inferred based on the ΔK method (Evanno et al., 2005), utilizing Structure Harvester online (Earl and vonHoldt, 2012). Post processing of STRUCTURE results and cluster graphs were drawn in CLUMPAK (Kopelman et al., 2015). Finally, we implemented an Analysis of Molecular Variance (AMOVA) in Arlequin (Excoffier and Lischer, 2010) to evaluate three grouping scenarios/hypothesis of *a priori* partitioning, to determine which grouping hypothesis maximized the difference between groups.

2.4 Gene flow

Two methods were used to estimate migration rates between the four major geographical areas, and at regional scale; 1) a multilocus approach based on the number of rare alleles, assuming that populations are under migration–drift equilibrium, to estimate the historical effective number of migrants (N_m) (Barton and Slatkin, 1986) in GENEPOP v.4.5 (Rousset, 2008), and 2) BayesAss V3, a bayesian approach that uses individual information to estimate recent migration events, asymmetric patterns of gene flow and directionality. At the same time, this analysis allows to identify first, second or third generation migrants between populations (Wilson and Rannala, 2003).A total of 100,000,000 MCMC iterations were run with a 25% burnin', sampling every 1,000 chains, to ensure chain independency, and convergence was confirmed in Tracer (Rambaut et al., 2018).

3 Results

3.1 Global and regional datasets

Raw data for each plate consisted of 260,135,231 and 263,506,046 reads, with 249,396,409 and 253,244,524 high-quality reads respectively. Using the UNEAK pipeline, 69,412 candidate SNP sites were detected across 140 individuals of *Siphonaria lateralis* from eight sampled populations. Out of the 140 individuals, only 116 had sufficient information to be included in the dataset after the bioinformatic filters, resulting in 6,277 SNPs. A total of 351 loci were removed due to being out of HWE in 60% or more of the populations after FDR correction, and another 411 loci were removed after Bayescan analysis identified these loci as outliers, with strong evidence of being under diversifying selection. The final global dataset was made up of 5,515 SNPs putatively neutral loci, for downstream diversity, structure, and gene flow analyses.

For the regional dataset, after implementing bioinformatic filters on 69,412 candidate SNPs from 53 individuals collected at Kerguelen Islands, 52 individuals showed sufficient data across a total of 1,646 SNPs. However, after HWE and FDR filters, we kept 1,298 SNPs, where a further 34 loci were removed for showing significant signals of being under selection. Accordingly, our putatively neutral working dataset consisted of 1,264 SNPs for diversity, structure and gene flow analyses on a smaller regional scale.

3.2 Population genetics across the Southern Ocean

3.2.1 Genetic diversity

All genetic diversity statistics were similar and low in each of the eight sampled populations of *S. lateralis*, except from Falklands/Malvinas Islands (for values and population codes hereinafter used, Table 1). Proportion of polymorphic loci ranged from 2.99% to 69.23%, where Falklands/Malvinas had the highest polymorphic loci, and contrarily, populations from South America (PH and PD) exhibited the lowest levels of genetic diversity. Observed heterozygosity (H_o) ranged from 0.0153 (FPN) to 0.2148 (FK), yet most values ranged from 0.0153 to 0.0288 (PAF), except from the FK population, which showed the highest H_o by more than an order of magnitude compared to all other populations. Expected heterozygosity (H_e) ranged from 0.0131 (PH) to 0.2406 (FK), and was slightly lower than H_o in half the populations (Table 1, values indicated in bold letters), and slightly higher than H_o in the other half. Allelic richness (A) ranged from 1.0299 (PH) to 1.6923 (FK), and was similar between all populations except for FK. Finally, F_{IS} ranged from -0.25854 (PH) to 0.39936 (SG), with negative values found only in continental sites (CH, PH, PD) and PAF.

TABLE 1 Genetic diversity of *Siphonaria lateralis* across the Southern Ocean.

Population	Coordinates	Code	N	%Po	H_o	H_e	A	F_{IS}
Chabunco	53°01’12.7S, 70°49’26.2W	CH	9	10.01	0.0217	0.0177	1.1001	-0.23809
Fuerte Bulnes	53°36’51.1S, 70°55’37.2W	PH	3	2.99*	0.0157	0.0131	1.0299	-0.25854
Puerto Deseado	47°45’06.9S, 65°51’53.1W	PD	4	5.33*	0.0215	0.0182	1.0533	-0.21810
Falklands/Malvinas	51°42’00.8S, 57°46’55.7W	FK	22	69.23	0.2148	0.2406	1.6923	0.10981
South Georgia	54°17’01.4S, 36°29’18.5W	SG	25	20.96	0.0197	0.0325	1.2096	0.39936
Fjord des Portes Noires	49°29’32.4S, 69°11’12.4E	FPN	16	10.61	0.0153	0.0162	1.1061	0.05779
Port Couvreux	49°16’49.6S, 69°41’31.1E	PC	17	11.66	0.0166	0.0180	1.1166	0.07914
Port-aux-Français	49°21’12.2”S, 70°13’0.1”E	PAF	20	21.12	0.0288	0.0262	1.2112	-0.10142

Table shows sampling populations, coordinates, codes (population abbreviation), sampling size (N), proportion of polymorphic loci (%Po), observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (A), and inbreeding coefficient (F_{IS}).

Grouping all populations by geographical area (Patagonia = PAT, Falklands/Malvinas = FK, South Georgia = SG, and Kerguelen = KER, Supplementary Table 1) illustrates similar diversity patterns, as FK remains the most diverse across all statistics. Polymorphism ranged from 14.84% (PAT) to 69.23% (FK), where at least 818 of the 5,515 loci were polymorphic. The H_e index ranged between 0.0193 (PAT) to 0.2350 (FK), and H_o ranged from 0.197 (SG) to 0.2148 (FK), where H_e was lower than H_o only in PAT. F_{IS} ranged from -0.02749 (PAT) to 0.39936 (SG), with PAT being the only negative value and SG more than doubled the value of other areas. The number of alleles per area ranged from 6334 (PAT) to 9333 (FK), with 1% (SG) to 36% (FK) of them being private. The number of private alleles from FK were three times higher than those recorded in other areas.

3.2.2 Population structure

All pairwise F_{ST} comparisons were significant amongst the eight sampled populations, with high and extremely high values across the studied distribution of *Siphonaria lateralis* (Table 2). The lowest F_{ST} value between sampled populations was found between CH-PH (F_{ST} = 0.236), the closest populations. In a general pattern, populations within the same geographical areas (within Patagonia and within Kerguelen islands) exhibited the lowest levels of differentiation amongst themselves (F_{ST} < 0.3). The highest F_{ST} value was found between FPN-PC (F_{ST} = 0.962), some of the most distant populations (8,000 Km). In fact, the highest comparisons (F_{ST} > 0.9) were found comparing Kerguelen populations (FPN, PC, PAF) with Patagonia populations (CH, PH, PD), and South Georgia with Patagonia populations. Genetic differentiation was slightly lower between South Georgia and Falkland/Malvinas (F_{ST} = 0.752), which are separated by approximately 1,500-2,200 Km of open ocean. South Georgia and Kerguelen populations displayed lower levels of genetic differentiation (F_{ST} = 0.579 - 0.604), despite being geographically more distant (>6,500 Km). Falklands/Malvinas Islands, on the other hand, was moderately-highly differentiated with all sampled populations (F_{ST} = 0.655 - 0.752).

TABLE 2 Pairwise F_{ST} values (below diagonal) for all Southern Ocean populations, and their statistical significance (above diagonal).

	CH	PH	PD	FK	SG	FPN	PC	PAF
CH	–	0.04	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PH	0.236	–	0.03*	<0.001	<0.001	0.001	0.001	0.001
PD	0.265	0.112	–	<0.001	<0.001	<0.001	<0.001	<0.001
FK	0.706	0.655	0.666	–	<0.001	<0.001	<0.001	<0.001
SG	0.933	0.927	0.928	0.752	–	<0.001	<0.001	<0.001
FPN	0.961	0.962	0.961	0.731	0.588	–	<0.001	<0.001
PC	0.958	0.959	0.958	0.739	0.604	0.286	–	<0.001
PAF	0.946	0.943	0.943	0.748	0.579	0.243	0.293	–

Cell shading delimit sampled geographical areas.

Individual-based analyses of global genetic structure suggest the existence of at least three, if not four genetic groups. Based on the number of shared alleles between individuals, the IBS analysis (Supplementary Figure 1) recognized four groups, two clearly defined and separate, and two relatively closer, similar to the PCA (Figure 2). The first two Principal Components explain most of the genetic variance (>70%): the first axis clearly differentiates FK from all other populations, and the second axis segregates CH, PH, PD (PAT) from a FPN, PC, PAF and SG (KER + SG) (Figure 2A). When considering a third axis, while it explains little of the genetic variance (3.41%), the PCA was able to distinguish South Georgia as a slightly differentiated group compared to Kerguelen Islands (Figure 2B). STRUCTURE's bayesian clustering and Evanno's ΔK indicate that two genetic clusters make up the first hierarchical level of genetic structure (Figure 3B, Supplementary Figure 2), highlighting the stark divergence between southern South America (CH, PH, PD and FK) and sub-Antarctic Islands (SG, FPN, PC and PAF). While further exploration into K = 3 distinguishes Falklands/Malvinas from the rest of South America (Figure 3C), matching results from the first two principal components of the PCA and the IBS, the second hierarchical level of genetic structure is accounted for when observing K = 4, which reveals significant substructure within the 2 main genetic clusters, southern South America and sub-Antarctic islands, providing a visual representation of what pairwise F_{ST} and PC3 suggest (Table 2, Figure 2B): South Georgia is a genetically distinct group, that, however, has a significant presence of a genetic component from

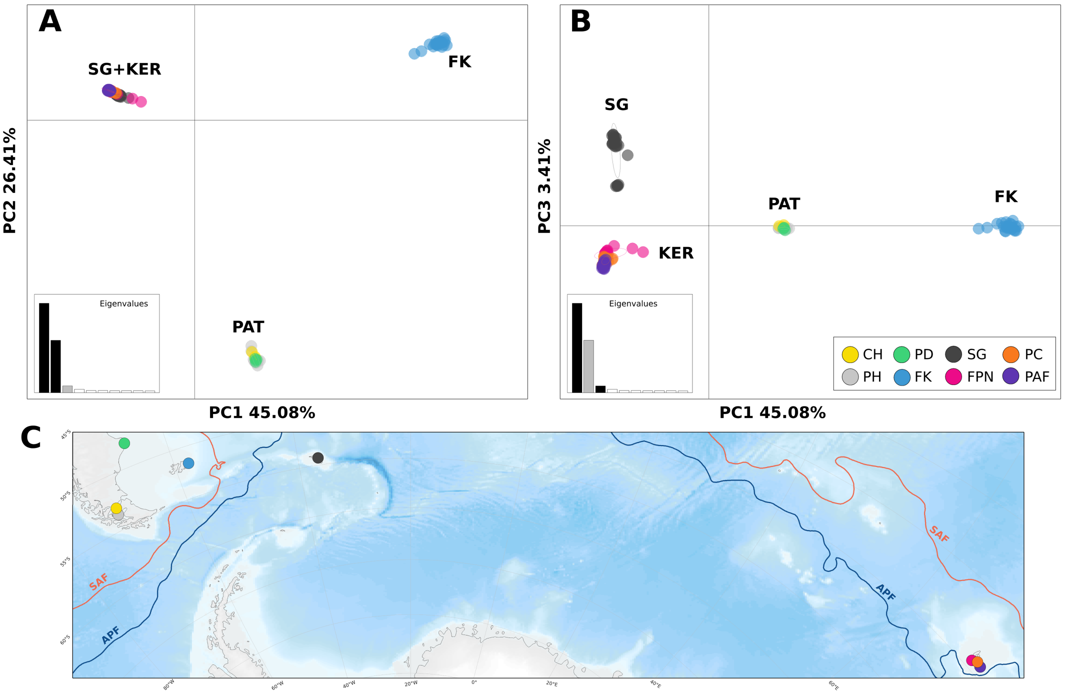

FIGURE 2
Global PCA scatterplot of the first 2 principal components **(A)**, and second and third principal components **(B)**, with percentage of explained variance by axis. Colors represent the sampling population of each individual, additional labels represent major geographical areas **(C)**, to reference the map (PAT = Patagonia (CH, PH, PD), SG = South Georgia, FK = Falklands/Malvinas, and KER = Kerguelen (FPN, PC, PAF)).

Kerguelen, which may correspond to third-generation migrants (Figure 3D).

Additionally, to further clarify the global genetic structure, we performed an AMOVA analysis considering the three alternative grouping hypotheses provided by the genetic structuring analyses as follows: H1 = two groups South America - Sub-Antarctic Islands (SA-IS), H2 = three groups Patagonia - Falklands/Malvinas - sub-Antarctic Islands (PAT-FK-IS), and H3 = four groups Patagonia - Falklands/Malvinas - South Georgia - Kerguelen (PAT-FK-SG-KER) (Supplementary Table 3). H1 was discarded, given that variation among groups (V_a = 57.56, F_{CT} = 0.5755) was comparatively lower than for the other two hypotheses. While F_{CT} was slightly higher for H2 than H3 (0.81269 *versus* 0.80529), we took into consideration the significant substructure within sub-Antarctic islands, illustrated by the percentage of variance among populations within groups in H2 (V_b = 4.69) and pairwise F_{ST} with

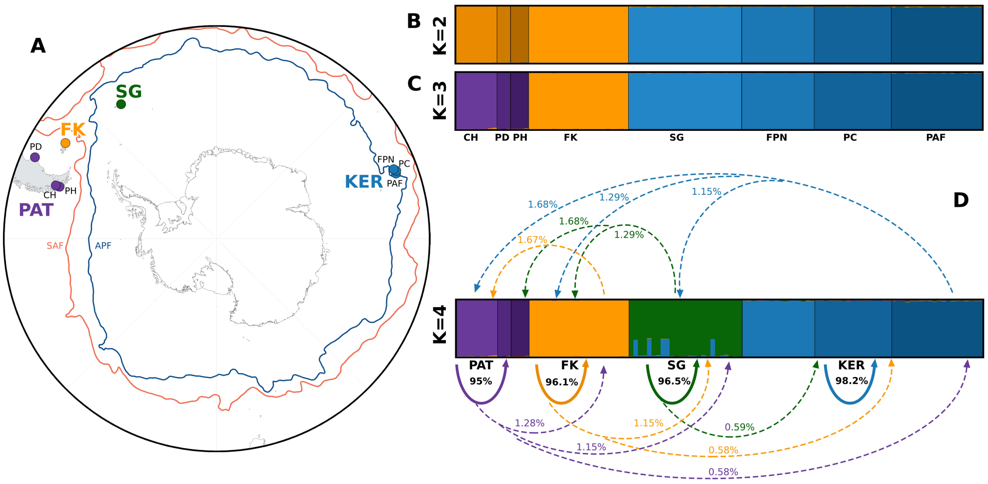

FIGURE 3
Southern Ocean map with the four major geographical areas **(A)**, and Bayesian clustering inferred with STRUCTURE for K = 2 **(B)**, K = 3 **(C)**, and K = 4 its respective recent migration rates inferred by BA3 for K=4 **(D)**. Colors represent geographical areas and are shaded to distinguish sampled populations. Solid arrows represent self-recruitment, dashed arrows represent migration towards other populations.

K = 4 (KER-PAT F_{ST} = 0.572, p-value <0.001, Supplementary Table 2). Therefore, we computed how much variation was truly just because of grouping choice, controlling for variation amongst populations within groups (V_a/V_a+V_b), which supported the four groups hypothesis over other grouping hypotheses (H3 = 98.33% versus H2 = 94.84%), considering South Georgia as a fourth group on its own.

3.2.3 Gene flow estimates

Two approaches were used to estimate gene flow between the four genetic groups, across >8,500 Km in the Southern Ocean (Figure 3A). Historical gene flow inferred by GENEPOP was low (N_m<1), and ranged from 0.0135 (between Patagonia and Kerguelen) to 0.305 (between South Georgia and Kerguelen) (Table 3). However, migration between South Georgia and Kerguelen Islands, albeit still low, was an order of magnitude higher than between the other genetic groups. On the other hand, recent migration rates between groups, as indicated by the BA3 results (Figure 3D), illustrate two scenarios; pairwise population migration rates range from 0.58% to 1.68%, whereas the highest rates consist on self-recruitment, ranging from 95% to 98.2%, meaning that >95% of individuals were genetically assigned to their population of origin. However, pairwise population migration rates were not symmetrical; inferred gene flow from Patagonia, Falklands/Malvinas and South Georgia towards Kerguelen is an order of magnitude lower than inferred gene flow from Kerguelen to the other three areas (*i.e.* KER towards PAT = 1.68%, PAT towards KER = 0.58%). Finally, BA3 did not identify migrants of first, second, or third generations, with all individuals being assigned as ~100% belonging to their population of origin, unlike the five individuals visually identified as possible third-generation migrants in STRUCTURE (Figure 3D).

3.3 Regional population genetics

3.3.1 Kerguelen genetic diversity

For the regional scale, which encompasses the Kerguelen Archipelago, genetic diversity was similar across populations, yet slightly higher in PAF across all statistics (Table 4). Proportion of polymorphic loci ranged from 75.16% to 91.07%, expected heterozygosity (H_e) ranged from 0.271 to 0.345, and observed heterozygosity (H_o) ranged from 0.280 to 0.449, where H_e was lower than H_o for FPN and PAF. Allelic richness (A) within populations ranged from 1.75518 to 1.9107. F_{IS} ranged from -0.278 to 0.055, negative only within PAF, suggesting a slight excess of heterozygotes and departure from HWE. Effective population size (N_e) was higher in PAF (224.4), and lowest in FPN (91.8). As for private alleles, FPN had the lowest value with 13 alleles, followed by PC with 64, and the highest value was observed in PAF with 155 alleles, representing between 1% and ~12% of the 1,264 loci in the regional dataset.

TABLE 3 Global pairwise estimates of the effective number of migrants (N_m) as determined with GENEPOP.

	PAT	FK	SG
PAT	–		
FK	0.0297	–	
SG	0.0209	0.0258	–
KER	0.0135	0.0162	0.305

3.3.2 Kerguelen structure and gene flow

The pairwise F_{ST} analyzes all resulted in significance ($p < 0.05$), with values ranging from 0.124 to 0.154 (Supplementary Table 4). The presence of three genetic groups corresponding to the sampled locations observed is suggested by IBS (Supplementary Figure 3), and the PCA, where the first two principal components (PC1 and PC2) explain >40% of the genetic variance results (Figure 4A). Likewise, STRUCTURE analyses determined an optimal K = 3, with three genetic groups clearly defined, each corresponding to the different sampled locations across Kerguelen, with only a slight signal of mixed genetic component of other groups, and without visual evidence of first, second or third generation migrants (Figure 4C).

Both historical and contemporary gene flow between these three groups is very low. According to GENEPOP, historical N_m between groups ranges from 0.06 to 0.077, the lowest genetic exchange being between PC and PAF (Supplementary Table 5). At contemporary scale, migration rates between the described populations ranged from 1.44% to 1.86%, were migration from FPN towards PAF (1.44%) was slightly lower than from PC to PAF (1.46%), providing evidence of low regional migration rates among the analyzed populations, which are mostly sustained by self-recruitment (>96%) (Figure 4B). Furthermore, BA3 did not provide any evidence for first, second, or third generation migrants.

4 Discussion

Through the analysis of genomic data (SNPs), we found that distant populations of *Siphonaria lateralis* distributed across the Southern Ocean are not sufficiently connected to prevent significant genetic differentiation. Buoyant kelp such as *Durvillaea antarctica* have probably played a crucial role in favoring transoceanic, long distance dispersal by rafting, and genetic homogeneity, of various coastal marine invertebrates, which largely match this macroalgae distribution (Fraser et al., 2010; Nikula et al., 2010; González-Wevar et al., 2018; Halanych and Mahon, 2018; Fraser et al., 2020a). This has allowed the colonization and re-colonization of sub-Antarctic coasts by different species (Leese et al., 2010; Nikula et al., 2010; Cumming et al., 2014; González-Wevar et al., 2018). Nonetheless, this dispersal mechanism would not necessarily prevent population differentiation. In light of our estimations of genetic structure and gene flow among populations at different geographical scales across the SO, based on more than 5,000 SNPs, we found that contemporary rafting does not promote sufficient ongoing gene flow in the pulmonate limpet *S. lateralis*, a species with benthic protected development, to homogenize distant populations. Furthermore, our results serve as evidence that connectivity is a process more complex than just accounting for dispersal potential, as Moon et al. (2017) argue, in which local dynamics, past and contemporary climate and physical barriers to dispersal, among other factors, may determine the extent of connectivity.

TABLE 4 Genetic diversity within Kerguelen Islands.

	N	%Po	H_o	H_e	A	F_{IS}	N_e (95% CI)	Private alleles	% of private alleles
FPN	15	75.16	0.296	0.271	1.7518	0.055	91.8 (12.9 - ∞)	13	1.03
PC	17	79.50	0.280	0.285	1.7950	0.055	130.2 (36.5 - ∞)	64	5.06
PAF	20	91.07	0.449	0.345	1.9107	-0.278	223.4 (65.0 - ∞)	155	12.26

Table shows population codes, sampling size (N), proportion of polymorphic loci (%Po), observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (A), inbreeding coefficient (F_{IS}), effective population size (N_e) with 95% confidence intervals (CI), number of private alleles and percentage of private alleles.

The eight sampled populations represent four major geographical areas; Patagonia, Falkland/Malvinas Islands, South Georgia, and Kerguelen. Genetic diversity was remarkably low across populations and geographical areas, in comparison to other genomic (SNPs) studies on gastropods across the world (*i.e.* H_o = 0.35-0.06) (Cortez et al., 2021; Maes et al., 2022; Morrissey et al., 2022; Quintero-Galvis et al., 2023). Moreover, values were low, regardless of whether populations were under-sampled or well-sampled. Falkland/Malvinas Islands was the only exception to this pattern, displaying genetic diversity values more than an order of magnitude higher than the rest of the populations, but falling in line with diversity from other gastropods. At global scale, patterns of low genetic diversity most likely reflect a founder takes all effect (Waters et al., 2013), where LDD rafting allow few founder alleles to colonize new populations, and genetic diversity remains low after demographic growth due to density dependent processes, with new alleles having little success in arriving and establishing in high-density populations. During Quaternary glaciations, the majority of the Pacific coast of southern South America was covered by the Patagonian Ice Sheet (Rabassa et al., 2005, Rabassa et al, 2011), extinguishing most of the benthic taxa (Clarke and Crame, 2010; Post et al., 2014), yet some taxa survived in pockets of glacial refugia (Montecinos et al., 2012; Trovant et al., 2015). On the contrary, the Atlantic coastline receded and exposed the continental shelf (Glasser and Jansson, 2008), causing local extinctions and/or range contractions, with refugium at lower latitudes for cold-temperate taxa. Moreover, SO islands had different glaciation scenarios; Falklands/Malvinas Islands had little to no ice during the Last Glacial Maximum (LGM), while South Georgia and Kerguelen Islands had terrestrial and marine glaciers (Fraser et al., 2009; Hodgson et al., 2014). While these scenarios offer different paths for recolonization events, phylogeographic patterns of *S. lateralis* support the hypothesis of two independent glacial refugia for the species (González-Wevar et al., 2018). One in Falklands/Malvinas, refugium illustrated by other benthic taxa (Leese et al., 2010; González-Wevar et al., 2012), which has thrived in isolation to this day, as illustrated by the rich genetic diversity found in our results (heterozygosity, polymorphism and number of private alleles). And a second refugium, in southern South America, with a reduced genetic diversity that recolonized both Patagonian coasts and distant populations across the SO such as South Georgia and Kerguelen Islands, which are less diverse in

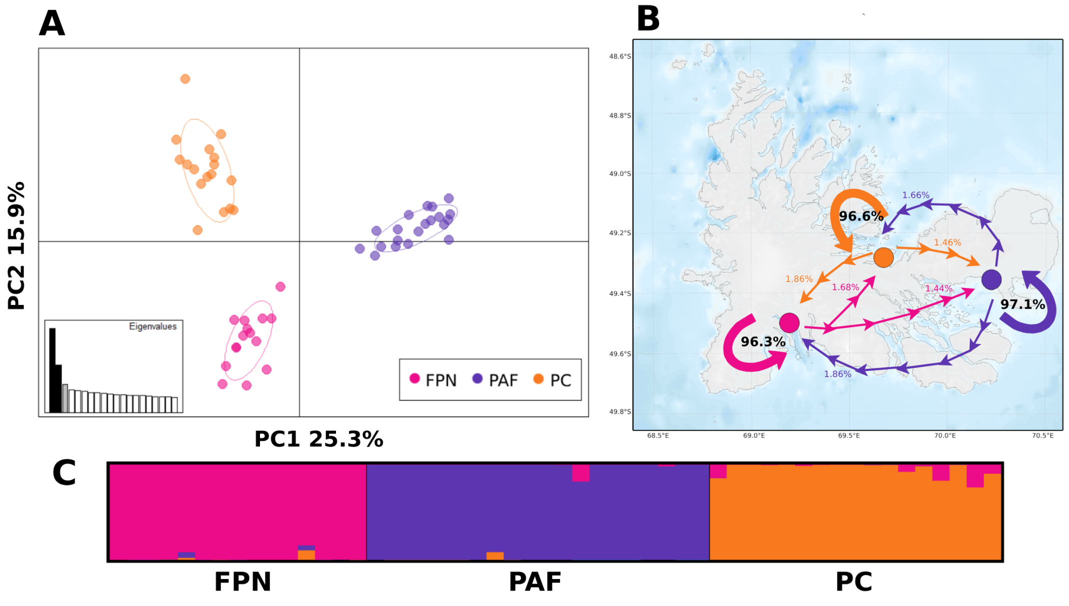

FIGURE 4
Genetic structure within Kerguelen Islands: PCA scatterplot showing the first 2 principal components and percentage of explained variance by axis **(A)**, BA3 recent migration rates between sampled populations **(B)**, and Bayesian clustering inferred with STRUCTURE **(C)**. Colors represent the sampling population of each individual. Solid arrows represent self-recruitment, dashed arrows represent migration towards other populations.

comparison to Falklands/Malvinas Islands, that have evolved separately with little to no allele surfing from other populations.

According to what we expected, we found significant genetic structure in *S. lateralis* across the SO. Results indicated that a substantial differentiation coincided with areas North and South of the Antarctic Convergence, an oceanographic feature that according to recent genomic studies, both represents a genetic barrier for gene flow on some species (Moore et al., 2018) or has no influence whatsoever (Galaska et al., 2017). However, we found strong evidence of four genetic clusters, matching the four major geographical areas sampled; Patagonia, Falklands/Malvinas Islands, South Georgia, and Kerguelen. The highest differentiation occurred between the two most distant populations, Patagonia and Kerguelen, however, Patagonia and South Georgia were also markedly differentiated, which is interesting to note, because it may hint at a deeper or older differentiation between these sub-Antarctic islands and the continent. On the other hand, the marked differentiation of Falklands/Malvinas Islands from the rest of the SO is most likely due to a historical differentiation (Quaternary glaciations) from the rest of southern South America, also evidenced in other studies that evaluate the phylogeographic structure of marine invertebrates without free-living larvae, which display significant phylogeographic structure between Falklands/Malvinas Islands and Patagonia (Leese et al., 2008; González-Wevar et al., 2018; González-Wevar et al., 2021). However, there are some exceptions, as some benthic brooders lack genetic structure between both populations (Cumming et al., 2014; Segovia et al., 2022), and few benthic broadcasters display significant differentiation (González-Wevar et al., 2021). Therefore, in addition to historical patterns, other factors must be sustaining and/or promoting this differentiation, as either environmental discontinuities arising from oceanographic characteristics, or species traits that could limit dispersal and act as gene flow barriers (Gillespie and Roderick, 2014; Moon et al., 2017), as seen in studies compending the notothenioid fish *Harpafiger*, which has reduced phylogeographic structure between Falklands/Malvinas Islands and Patagonia, yet its population discrimination analyses using SNPs clearly differentiate both populations (Segovia et al., 2022).

On the other hand, similar to what was found with traditional markers, multilocus markers exhibit significant, but lesser genetic structuring between South Georgia and Kerguelen. Our data provide scarce evidence of historical long-distance dispersal (LDD) on S. *lateralis* across sub-Antarctic islands approximately 7,000 Km apart, potentially via rafting, as previously hypothesized by González-Wevar et al. (2018). Despite noteworthy estimated historical migration rates between South Georgia and Kerguelen, in comparison to other pairwise estimates, these values are low (Nm < 1) and insufficient to prevent polymorphism loss and population differentiation (Wright, 1931; Vucetich and Waite, 2000). Moreover, self-recruitment dominates *S. lateralis* population dynamics, where 95% of individuals remain in their population of origin, and <2% migrate to other populations, insufficient gene flow to prevent genetic structuring across the SO. Interestingly, estimated contemporary migration rates, albeit marginal, evidence double the gene flow from Kerguelen to South Georgia, spanning ~16,000 Km, than from South Georgia towards Kerguelen, the shortest route, by ~10,000 Km less. This is plausible because the Antarctic Circumpolar Current flows eastward, connecting all SO land masses and remote islands by oceanic circulation and drifting macroalgae (Leese et al., 2010; Hunt et al., 2016), and simulated trajectories by Fraser et al. (2018) illustrate multiple pathways within ecologically viable timeframes for *D. antarctica* and the lifespan of associated hitchhiking invertebrates to reach Antarctica and sub-Antarctic islands (Fraser et al., 2010; López et al., 2017). However, as Moon et al. (2017) argue, and even in light of documented rafting events, these events may be insufficient to sustain levels of contemporary gene flow capable of preventing population structuring, as illustrated by *D. antarctica*, which displays significant genetic structure across the SO (Fraser et al., 2018; Fraser et al, 2022). Moreover, the current location and movement of the Antarctic Polar Front (APF) may limit dispersal between South Georgia and Kerguelen through different abiotic factors, such as temperature, which may affect survivorship or development and is a significant driver of genetic structure (Boulanger et al., 2022; Mendes et al., 2022), as rafting also depends on species traits and physiological tolerances (Simkanin et al., 2019). Therefore, while LDD through rafting remains a relevant dispersal mechanism at evolutionary timescales, our SNP data offer little support to the hypothesis that it promotes high levels of gene flow to sustain significant levels of contemporary connectivity, and population genetic structure may arise, as illustrated by our results.

Finally, at a regional scale within Kerguelen, genetic diversity was higher in PAF. Genetic structuring analyses within Kerguelen Islands reveal three similarly differentiated groups. Additionally, contemporary gene flow analyses failed to detect recent dispersal events by first, second or third generation migrants between the studied locations, with historical genetic flow being marginal at best. Few studies have evaluated contemporary genetic structure and gene flow within Kerguelen Islands, and yet both brooders and broadcasters exhibit marked genetic structure between North, South and East (Ledoux et al., 2012; Fraïsse et al., 2021). This is an even more interesting finding than the genetic structure of *S. lateralis across the sub-Antarctic region*, as rafting events across hundreds to thousands of kilometers have proven to be historically relevant, yet may be unpredictable and irregular at present day (Moon et al., 2017). But if macroalgae such as *D. antarctica* and *M. pyrifera* are as abundant as they are within Kerguelen Islands (Féral et al., 2021), surely, rafting at regional scale could be more frequent and/or less stochastic that at larger scales, as gene flow would offset genetic structuring. Studies within and around New Zealand provide evidence of sporadic rafting of direct developers across >500 Km (Fraser et al., 2020b; Donald et al., 2020). However, local upwelling processes, environmental discontinuities or habitat specificity are some of the potential drivers of genetic divergence regardless of developmental mode, leading to significant genetic fragmentation over short geographical distances (Johansson et al., 2008; Ayre et al., 2009; Gonzalez et al., 2016; Donald et al., 2020). Within Kerguelen, the intertidal zone has abundant and sub-divided kelp forests forming defined patches of habitat, all of which are differently exposed to the diverse influences of both open sea and land water (rivers and melting glaciers) (Koubbi et al., 2016). On the other hand, the Kerguelen coast is heterogeneous, as it has both jagged and linear coastlines, and exposed or sheltered bays, which in turn determine the spatial distribution of benthic species (Poulin and Féral, 1995; Améziane et al., 2011). Therefore, this oceanographic and environmental patchiness, even under the assumption that *S. lateralis* disperses by rafting within Kerguelen, could lead to the levels of genetic structure found in our study. On the other hand, currents around the Kerguelen Islands either transport waters coastward, eastward or northward of the Kerguelen Plateau (Park et al., 2008; Zhou et al., 2014), providing little means for kelp rafts to move within Kerguelen, which could explain the low levels of gene flow and high estimates of self-recruitment among sampled populations of *S. lateralis*. Furthermore, Fraïsse et al. (2021) tested for the role of habitat heterogeneity in explaining the North-South differentiation of *Mytilus* in Kerguelen, and found that the presence of *Macrocystis* kelps, substrate type and coastal slope had a low but significant role in genetic differentiation.

Therefore, considering the observed patterns at global and regional scale, multilocus markers such as SNPs provide further tools to comprehend the extent and reach of dispersal and connectivity paradigms in the SO. Rafting has been an important Quaternary dispersal mechanism for benthic fauna, which provided the means to colonize/recolonize the sub-Antarctic region, yet its present relevance in promoting high levels of gene flow is disputed by our data, which, albeit limited to a single species, demonstrates that rafting sustains low levels of gene flow, evidenced in significant contemporary genetic structure across SO populations. Nevertheless, further research is needed to clarify contemporary dispersal patterns across the SO, which need to consider studying SNPs or whole genome sequencing in other species with different dispersal potential as study models, as well as environmental/oceanographical characteristics as potential barriers to present-day dispersal and genetic connectivity in the SO, where seascape genomic studies could help clarify these patterns.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://figshare.com/, https://doi.org/10.6084/m9.figshare.25939687.v2.

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. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CM-M: Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. ML: Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. TS: Funding acquisition, Project administration, Resources, Writing – review & editing. EP: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. NS: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – review & editing. CG-W: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, 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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2024.1441397/full#supplementary-material

References

Álvarez-Noriega, M., Burgess, S. C., Byers, J. E., Pringle, J. M., Wares, J. P., and Marshall, D. J. (2020). Global biogeography of marine dispersal potential. *Nat. Ecol. Evol.* 4, 1196–1203. doi: 10.1038/s41559-020-1238-y

Améziane, N., Eléaume, M., Hemery, L., Monniot, F., Hemery, A., Hautecoeur, M., et al. (2011). Biodiversity of the benthos off Kerguelen Islands: overview and perspectives. *Cybium* 35 (SP), 157-167. doi: 10.26028/cybium/2011-35SP-016

Assis, J., Alberto, F., Macaya, E. C., Castilho Coelho, N., Faugeron, S., Pearson, G. A., et al. (2023). Past climate-driven range shifts structuring intraspecific biodiversity levels of the giant kelp (*Macrocystis pyrifera*) at global scales. *Sci. Rep.* 13, 12046. doi: 10.1038/s41598-023-38944-7

Ayre, D. J., Minchinton, T. E., and Perrin, C. (2009). Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? *Mol. Ecol.* 18, 1887–1903. doi: 10.1111/j.1365-294X.2009.04127.x

Barton, N. H., and Slatkin, M. (1986). A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* 56, 409–415. doi: 10.1038/hdy.1986.63

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., and Bonhomme, F. (2004). GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. *Laboratoire Génome Populations Interactions CNRS UMR*, 5000, *Université de Montpellier II, Montpellier (France).*

Bertola, L. D., Boehm, J. T., Putman, N. F., Xue, A. T., Robinson, J. D., Harris, S., et al. (2020). Asymmetrical gene flow in five co-distributed syngnathids explained by ocean currents and rafting propensity. *Proc. R. Soc. B: Biol. Sci.* 287, 20200657. doi: 10.1098/rspb.2020.0657

Blanco, M., Ospina-Álvarez, A., Navarrete, S. A., and Fernández, M. (2019). Influence of larval traits on dispersal and connectivity patterns of two exploited marine invertebrates in central Chile. *Mar. Ecol. Prog. Ser.* 612, 43–64. doi: 10.3354/meps12870

Boulanger, E., Benestan, L., Guerin, P.-E., Dalongeville, A., Mouillot, D., and Manel, S. (2022). Climate differently influences the genomic patterns of two sympatric marine fish species. *J. Anim. Ecol.* 91, 1180–1195. doi: 10.1111/1365-2656.13623

Bussolini, L. T., and Waters, J. M. (2015). Genetic analyses of rafted macroalgae reveal regional oceanographic connectivity patterns. *J. Biogeography* 42, 1319–1326. doi: 10.1111/jbi.12491

Carlton, J. T., Chapman, J. W., Geller, J. B., Miller, J. A., Carlton, D. A., McCuller, M. I., et al. (2017). Tsunami-driven rafting: transoceanic species dispersal and implications for marine biogeography. *Science* 357, 1402–1406. doi: 10.1126/science.aao1498

Chapman, C. C., Lea, M.-A., Meyer, A., Sallée, J.-B., and Hindell, M. (2020). Defining Southern Ocean fronts and their influence on biological and physical processes in a changing climate. *Nat. Climate Change* 10, 209–219. doi: 10.1038/s41558-020-0705-4

Chhatre, V. E., and Emerson, K. J. (2017). StrAuto: automation and parallelization of STRUCTURE analysis. *BMC Bioinf.* 18, 192. doi: 10.1186/s12859-017-1593-0

Clarke, A., and Crame, J. A. (2010). Evolutionary dynamics at high latitudes: speciation and extinction in polar marine faunas. *Philos. Trans. R. Soc. B: Biol. Sci.* 365, 3655–3666. doi: 10.1098/rstb.2010.0270

Cortez, T., Amaral, R. V., Sobral-Souza, T., and Andrade, S. C. S. (2021). Genome-wide assessment elucidates connectivity and the evolutionary history of the highly dispersive marine invertebrate *Littoraria flava* (Littorinidae: Gastropoda). *Biol. J. Linn. Soc.* 133, 999–1015. doi: 10.1093/biolinnean/blab055

Cowen, R. K., and Sponaugle, S. (2009). Larval dispersal and marine population connectivity. *Annu. Rev. Mar. Sci.* 1, 443–466. doi: 10.1146/annurev.marine.010908.163757

Cumming, R. A., Nikula, R., Spencer, H. G., and Waters, J. M. (2014). Transoceanic genetic similarities of kelp-associated sea slug populations: long-distance dispersal via rafting? *J. Biogeography* 41, 2357–2370. doi: 10.1111/jbi.12376

D'Aloia, C. C., Bogdanowicz, S. M., Francis, R. K., Majoris, J. E., Harrison, R. G., and Buston, P. M. (2015). Patterns, causes, and consequences of marine larval dispersal. *Proc. Natl. Acad. Sci.* 112, 13940–13945. doi: 10.1073/pnas.1513754112

Dayrat, B., Goulding, T. C., and White, T. R. (2014). Diversity of Indo-West Pacific *Siphonaria* (Mollusca: Gastropoda: Euthyneura). *Zootaxa* 3779, 246. doi: 10.11646/zootaxa.3779.2.7

De Broyer, C., Koubbi, P., Scientific Commitee on Antarctic Research (2014). "Chapter 1.1. The biogeography of the Southern Ocean," in *Biogeographic Atlas of the Southern Ocean*. Eds. C. De Broyer, P. Koubbi, H. J. Griffiths, B. Raymond, C. d'Udekem d'Acoz, A. P. Van de Putte and B. Danis (ublished by The Scientific Committee on Antarctic Research, Scott Polar reseach Institute, Cambridge), 1–9.

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., and Ovenden, J. R. (2014). NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Mol. Ecol. Resour.* 14, 209–214. doi: 10.1111/1755-0998.12157

Donald, K. M., McCulloch, G. A., Dutoit, L., and Spencer, H. G. (2020). Population structure of the New Zealand whelk, *Cominella glandiformis* (Gastropoda: Buccinidae), suggests sporadic dispersal of a direct developer. *Biol. J. Linn. Soc.* 130, 49–60. doi: 10.1093/biolinnean/blaa033

Earl, D. A., and vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the evanno method. *Conserv. Genet. Resour.* 4, 359–615. doi: 10.1007/s12686-011-9548-7

Esser, E. A., Pringle, J. M., and Byers, J. E. (2023). Neither larval duration nor dispersal distance predict spatial genetic diversity in planktonic dispersing species. *Mar. Ecol. Prog. Ser.* 721, 161–167. doi: 10.3354/meps14419

Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Mol. Ecol.* 14, 2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x

Excoffier, L., and Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under linux and windows. *Mol. Ecol. Resour.* 10, 564–567. doi: 10.1111/j.1755-0998.2010.02847.x

Féral, J.-P., Verlaque, M., Rosenfeld, S., Poulin, E., Chenuil, A., and Saucède, T. (2021). The marine vegetation of the Kerguelen Islands: history of scientific campaigns, inventory of the flora and first analysis of its biogeographical affinities. *Cryptogamie Algologie* 42, 173–216. doi: 10.5252/cryptogamie-algologie2021v42a12

Fleming, A. M., Dohner, M. M., Phillips, N. E., and Ritchie, P. A. (2018). Genetic Connectivity among Populations of Two Congeneric Direct-Developing Whelks Varies across Spatial Scales. *New Z. J. Mar. Freshw. Res.* 52, 100–117. doi: 10.1080/00288330.2017.1340899

Foll, M., and Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics* 180, 977–993. doi: 10.1534/genetics.108.092221

Fraïsse, C., Haguenauer, A., Gérard, K., Anh-Thu Weber, A., Bierne, N., and Chenuil, A. (2021). Fine-grained habitat-associated genetic connectivity in an admixed population of mussels in the small isolated Kerguelen Islands. *Peer Community J.* 1, e10. doi: 10.24072/pcjournal.18

Fraser, C. I., Dutoit, L., Morrison, A. K., Pardo, L. M., Smith, S. D. A., Pearman, W. S., et al. (2022). Southern hemisphere coasts are biologically connected by frequent, long-distance rafting events. *Curr. Biol.* 32, 3154–3160.e3. doi: 10.1016/j.cub.2022.05.035

Fraser, C. I., Morrison, A. K., A.M.c.M. Hogg, E. C., van Sebille, E., Ryan, P. G., Padovan, A., et al. (2018). Antarctica's ecological isolation will be broken by storm-driven dispersal and warming. *Nat. Climate Change* 8, 704–785. doi: 10.1038/s41558-018-0209-7

Fraser, C. I., Morrison, A., and Olmedo-Rojas, P. (2020a). "Biogeographic processes influencing Antarctic and sub-Antarctic seaweeds," in *Antarctic seaweeds: diversity, adaptation and ecosystem services*. Ed. I. Gómez and P. Huovinen (Springer International Publishing, Cham), 43–57. doi: 10.1007/978-3-030-39448-6_3

Fraser, C. I., Nikula, R., Spencer, H. G., and Waters, J. M. (2009). Kelp genes reveal effects of subAntarctic sea ice during the Last Glacial Maximum. *Proc. Natl. Acad. Sci. United States America* 106, 3249–3253. doi: 10.1073/pnas.0810635106

Fraser, C. I., Nikula, R., and Waters, J. M. (2010). Oceanic rafting by a coastal community. *Proc. R. Soc. B: Biol. Sci.* 278, 649–655. doi: 10.1098/rspb.2010.1117

Fraser, C. I., Velásquez, M., Nelson, W. A., Macaya, E. C., and Hay, C. H. (2020b). The biogeographic importance of buoyancy in macroalgae: A case study of the southern bull-Kelp genus *Durvillaea* (Phaeophyceae), including descriptions of two new species. *J. Phycology* 56, 23–365. doi: 10.1111/jpy.12939

Frisk, M. G., Jordaan, A., and Miller, T. J. (2014). Moving beyond the current paradigm in marine population connectivity: are adults the missing link. *Fish Fisheries* 15, 242–545. doi: 10.1111/faf.12014

Gaeta, J., Acevedo, I., López-Márquez, V., Freitas, R., Cruz, R., Maggioni, R., et al. (2020). Genetic differentiation among atlantic island populations of the brown spiny lobster *Panulirus echinatus* (Decapoda: Palinuridae). *Aquat. Conservation: Mar. Freshw. Ecosyst.* 30, 868–881. doi: 10.1002/aqc.3297

Gaines, S. D., Gaylord, B., Gerber, L. R., Hastings, A., and Kinlan, B. P. (2007). Connecting places: the ecological consequences of dispersal in the sea. *Oceanography* 20, 90–99. doi: 10.5670/oceanog

Galaska, M. P., Sands, C. J., Santos, S. R., Mahon, A. R., and Halanych, K. M. (2017). Crossing the divide: admixture across the Antartic Polar Front revealed by the brittle star *Astrotoma agassizii*. *Biol. Bull.* 232, 198–211. doi: 10.1086/693460

Gélin, P., Pirog, A., Fauvelot, C., and Magalon, H. (2018). High genetic differentiation and low connectivity in the coral *Pocillopora damicornis* type β at different spatial scales in the Southwestern Indian Ocean and Tropical Southwestern Pacifi. *Mar. Biol.* 165, 167. doi: 10.1007/s00227-018-3428-6

Gillespie, R. G., Baldwin, B. G., Waters, J. M., Fraser, C. I., Nikula, R., and Roderick, G. K. (2012). Long-distance dispersal: A framework for hypothesis testing. *Trends Ecol. Evol.* 27, 47–56. doi: 10.1016/j.tree.2011.08.009

Gillespie, R. G., and Roderick, G. K. (2014). Evolution: geology and climate drive diversification. *Nature* 509, 297–298. doi: 10.1038/509297a

Glasser, N., and Jansson, K. (2008). The glacial map of southern South America. *J. Maps* 4, 175–196. doi: 10.4113/jom.2008.1020

Gonzalez, E. B., Knutsen, H., and Jorde, P. E. (2016). Habitat discontinuities separate genetically divergent populations of a rocky shore marine fish. *PloS One* 11, e0163052. doi: 10.1371/journal.pone.0163052

González-Wevar, C. A., Hüne, M., Cañete, J. I., Mansilla, A., Nakano, T., and Poulin, E. (2012). Towards a model of postglacial biogeography in shallow marine species along the Patagonian Province: lessons from the limpet *Nacella magellanica* (Gmelin, 1791). *BMC Evolutionary Biol.* 12, 139. doi: 10.1186/1471-2148-12-139

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Noll, D., Maturana, C. S., Hüne, M., et al. (2021). Contrasting biogeographical patterns in *Margarella* (Gastropoda: Calliostomatidae: Margarellinae) across the Antarctic polar front. *Mol. Phylogenet. Evol.* 156, 107039. doi: 10.1016/j.ympev.2020.107039

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Ojeda, J., Hüne, M., Naretto, J., et al. (2018). Unexpected absence of island endemics: long-distance dispersal in higher latitude sub-Antarctic *Siphonaria* (Gastropoda: Euthyneura) species. *J. Biogeography* 45, 874–884. doi: 10.1111/jbi.13174

Green, M. E., Appleyard, S. A., White, W. T., Tracey, S. R., Heupel, M. R., and Ovenden, J. R. (2022). Updated connectivity assessment for the scalloped hammerhead (*Sphyrna lewini*) in Pacific and Indian oceans using a multi-marker genetic approach. *Fisheries Res.* 251, 106305. doi: 10.1016/j.fishres.2022.106305

Griffiths, H. J., Barnes, D. K.A., and Linse, K. (2009). Towards a generalized biogeography of the Southern Ocean benthos. *J. Biogeography* 36, 162–775. doi: 10.1111/j.1365-2699.2008.01979.x

Güller, M., Puccinelli, E., and Zelaya, D. G. (2020). The Antarctic Circumpolar Current as a dispersive agent in the Southern Ocean: evidence from bivalves. *Mar. Biol.* 167, 143. doi: 10.1007/s00227-020-03746-2

Güller, M., Zelaya, D. G., and Ituarte, C. (2015). How many *Siphonaria* species (Gastropoda: Euthyneura) live in southern South America. *J. Molluscan Stud.* 82 (1), eyv036. doi: 10.1093/mollus/eyv036

Halanych, K. M., and Mahon, A. R. (2018). Challenging dogma concerning biogeographic patterns of Antarctica and the *Southern Ocean. Annu. Rev. Ecology Evolution Systematics* 49, 355–378. doi: 10.1146/annurev-ecolsys-121415-032139

Haye, P. A., Segovia, N. I., Muñoz-Herrera, N. C., Gálvez, F. E., Martínez, A., Meynard, A., et al. (2014). Phylogeographic structure in benthic marine invertebrates of the Southeast Pacific coast of Chile with differing dispersal potential. *PloS One* 9, 88613. doi: 10.1371/journal.pone.0088613

Haye, P. A., Varela, A. I., and Thiel, M. (2012). Genetic signatures of rafting dispersal in algal-dwelling brooders *Limnoria* spp. (Isopoda) along the SE Pacific (Chile). *Mar. Ecol. Prog. Ser.* 455, 111–122. doi: 10.3354/meps09673

Hernawan, U. E., Lavery, P. S., Kendrick, G. A., van Dijk, K. J., Ulumuddin, Y. I., Triandiza, T., et al. (2021). Predictors of marine genetic structure in the Indo-Australian Archipelago. *Regional Stud. Mar. Sci.* 47, 101919. doi: 10.1016/j.rsma.2021.101919

Highsmith, R. (1985). Floating and algal rafting as potential dispersal mechanisms in brooding invertebrates. *Mar. Ecol. Prog. Ser.* 25, 169–179. doi: 10.3354/meps025169

Hilário, A., Metaxas, A., Gaudron, S. M., Howell, K. L., Mercier, A., Mestre, N. C., et al. (2015). Estimating dispersal distance in the deep sea: challenges and applications to marine reserves. *Front. Mar. Sci.* 2. doi: 10.3389/fmars.2015.00006

Hodgson, D. A., Graham, A. G. C., Roberts, S. J., Bentley, M. J., Cofaigh, C.Ó., Verleyen, E., et al. (2014). Terrestrial and submarine evidence for the extent and timing of the Last Glacial Maximum and the onset of deglaciation on the maritime-Antarctic and sub-Antarctic islands. *Quaternary Sci. Reviews Reconstruction Antarctic Ice Sheet Deglaciation (RAISED)* 100, 137–158. doi: 10.1016/j.quascirev.2013.12.001

Hunt, G. L., Drinkwater, K. F., Arrigo, K., Berge, J., Daly, K. L., Danielson, S., et al. (2016). Advection in polar and sub-polar environments: impacts on high latitude marine ecosystems. *Prog. Oceanography* 149, 40–81. doi: 10.1016/j.pocean.2016.10.004

Johansson, M. L., Banks, M. A., Glunt, K. D., Hassel-Finnegan, H. M., and Buonaccorsi, V. P. (2008). Influence of habitat discontinuity, geographical distance, and oceanography on fine-scale population genetic structure of copper rockfish (*Sebastes caurinus*). *Mol. Ecol.* 17, 3051–3061. doi: 10.1111/j.1365-294X.2008.03814.x

Jokiel, P. L. (1990). Long-distance dispersal by rafting: reemergence of an old hypothesis. *Endeavour* 14, 66–73. doi: 10.1016/0160-9327(90)90074-2

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics. *Bioinformatics* 24, 1403–1405. doi: 10.1093/bioinformatics/btn129

Kalinowski, S. T. (2005). HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. *Mol. Ecol. Notes* 5, 187–189. doi: 10.1111/j.1471-8286.2004.00845.x

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., and Mayrose, I. (2015). CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across K. *Mol. Ecol. Resour.* 15, 1179–1191. doi: 10.1111/1755-0998.12387

Koubbi, P., De Broyer, C., Griffiths, H. J., Raymond, B., d'Udekem d'Acoz, C., Van de Putte, A. P., et al. (2014). "Chapter 12. Conclusions: present and future of Southern Ocean biogeography," in *Biogeographic Atlas of the Southern Ocean*. Eds. C. De Broyer, P. Koubbi, H. J. Griffiths, B. Raymond, C. d'Udekem d'Acoz, A. P. Van de Putte, B. Danis, et al (Published by The Scientific Committee on Antarctic Research, Scott Polar reseach Institute, Cambridge), 1–9.

Koubbi, P., Guinet, C., Alloncle, N., Améziane, N., Azam, C. S., Baudena, A., et al. (2016). *Ecoregionalisation of the Kerguelen and Crozet islands oceanic zone. Part I: introduction and Kerguelen oceanic zone*. CCAMLR Report WG-EMM- 16/43. doi: 10.13140/RG.2.2.17278.18246

Kunvar, S., Czarnomska, S., Pertoldi, C., and Tokarska, M.a (2021). In search of species-Specific SNPs in a non-Model animal (European bison (*Bison bonasus*))- Comparison of de novo and reference-Based integrated pipeline of STACKS using genotyping-by-Sequencing (GBS) data. *Animals: Open Access J. MDPI* 11, 22265. doi: 10.3390/ani11082226

Larsson, J., Lind, E. E., Corell, H., Grahn, M., Smolarz, K., and Lönn, M. (2017). Regional genetic differentiation in the blue mussel from the Baltic sea area. *Estuarine Coast. Shelf Science Understanding Baltic Sea* 195, 98–109. doi: 10.1016/j.ecss.2016.06.016

Lau, S. C. Y., Strugnell, J. M., Sands, C. J., Silva, C. N. S., and Wilson, N. G. (2023). Genomic insights of evolutionary divergence and life history innovations in Antarctic brittle stars. *Mol. Ecol.* 32, 3382–3402. doi: 10.1111/mec.16951

Layton, C., Vermont, H., Beggs, H., Brassington, G. B., Burke, A. D., Hepburn, L., et al. (2022). Giant kelp rafts wash ashore 450 Km from the nearest populations and against the dominant ocean current. *Ecology* 103, e3795. doi: 10.1002/ecy.3795

Ledoux, J.-B., Tarnowska, K., Gérard, K., Lhuillier, E., Jacquemin, B., Weydmann, A., et al. (2012). Fine-scale spatial genetic structure in the brooding sea urchin *Abatus cordatus* suggests vulnerability of the Southern Ocean marine invertebrates facing global change. *Polar Biol.* 35, 611–623. doi: 10.1007/s00300-011-1106-y

Leese, F., Agrawal, S., and Held, C. (2010). Long-Distance Island Hopping without Dispersal Stages: Transportation across Major Zoogeographic Barriers in a Southern Ocean Isopod. *Naturwissenschaften* 97, 583–594. doi: 10.1007/s00114-010-0674-y

Leese, F., Kop, A., Wägele, J.-W., and Held, C. (2008). Cryptic speciation in a benthic isopod from Patagonian and Falkland Island waters and the impact of glaciations on its population structure. *Front. Zoology* 5, 19. doi: 10.1186/1742-9994-5-19

Leiva, C., Taboada, S., Kenny, N. J., Combosch, D., Giribet, G., Jombart, T., et al. (2019). Population substructure and signals of divergent adaptive selection despite admixture in the sponge *Dendrilla antarctica* from shallow waters surrounding the Antarctic Peninsula. *Mol. Ecol.* 28, 3151–3170. doi: 10.1111/mec.15135

López, B. A., Macaya, E. C., Tala, F., Tellier, F., and Thiel, M. (2017). The variable routes of rafting: stranding dynamics of floating bull kelp *Durvillaea antarctica* (Fucales, Phaeophyceae) on beaches in the SE Pacific. *J. Phycology* 53, 70–84. doi: 10.1111/jpy.12479

Lowe, W. H., and Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Mol. Ecol.* 19, 3038–3051. doi: 10.1111/j.1365-294X.2010.04688.x

Lu, F., Lipka, A. E., Glaubitz, J., Elshire, R., Cherney, J. H., Casler, M. D., et al. (2013). Switchgrass genomic diversity, ploidy, and evolution: novel insights from a network-based SNP discovery protocol. *PloS Genet.* 9, e10032155. doi: 10.1371/journal.pgen.1003215

Macaya, E. C., Tala, F., Hinojosa, I. A., and Rothäusler, E. (2020). "Detached seaweeds as important dispersal agents across the Southern Ocean," in *Antarctic seaweeds: diversity, adaptation and ecosystem services*. Ed. I. Gómez and P. Huovinen (New York: Springer International Publishing), 59–81. doi: 10.1007/978-3-030-39448-6_4

Macaya, E. C., and Zuccarello, G. C. (2010). Genetic structure of the giant kelp *Macrocystis pyrifera* along the Southeastern Pacific. *Mar. Ecol. Prog. Ser.* 420, 103–112. doi: 10.3354/meps08893

Maes, T., De Corte, Z., Vangestel, C., Virgilio, M., Smitz, N., Djuikwo-Teukeng, F. F., et al. (2022). Large-scale and small-scale population genetic structure of the medically important gastropod species *Bulinus truncatus* (Gastropoda, Heterobranchia). *Parasites Vectors* 15, 328. doi: 10.1186/s13071-022-05445-x

Manel, S., Boulanger, E., Benestan, L., Mouillot, D., and Dalongeville, A. (2023). Revisiting long-distance dispersal in a coastal marine fish. *Ecography* 2023, e06867. doi: 10.1111/ecog.06867

Meirmans, P. G. (2020). Genodive version 3.0: easy-to-use software for the analysis of genetic data of diploids and polyploids. *Mol. Ecol. Resour.* 20, 1126–1131. doi: 10.1111/1755-0998.13145

Mendes, C. B., Cortez, T., Santos, C. S. G., Sobral-Souza, T., Santos, A. D., Sasaki, D. K., et al. (2022). Seascape genetics in a polychaete worm: disentangling the roles of a biogeographic barrier and environmental factors. *J. Biogeography* 49, 2296–2308. doi: 10.1111/jbi.14504

Montecinos, A., Broitman, B. R., Faugeron, S., Haye, P. A., Tellier, F., and Guillemin., M. L. (2012). Species Replacement along a Linear Coastal Habitat: Phylogeography and Speciation in the Red Alga *Mazzaella laminarioides* along the South East Pacific. *BMC Evolutionary Biol.* 12, 975. doi: 10.1186/1471-2148-12-97

Moon, K. L., Chown, S. L., and Fraser, C. I. (2017). Reconsidering connectivity in the sub-Antarctic. *Biol. Rev.* 92, 2164–2181. doi: 10.1111/brv.12327

Moore, J. M., Carvajal, J. I., Rouse, G. W., and Wilson, N. G. (2018). The Antarctic Circumpolar Current isolates and connects: structured circumpolarity in the sea star *Glabraster antarctica*. *Ecol. Evol.* 8, 10621–10633. doi: 10.1002/ece3.4551

Morrissey, D., Goodall, J., Castilho, R., Cameron, T. C., and Taylor, M. L. (2022). Population genomics reveals a single semi-continuous population of a commercially exploited marine gastropod. *Fisheries Res.* 254, 106418. doi: 10.1016/j.fishres.2022.106418

Morton, J. E., and Miller, M. (1973). Collins, London: The New Zealand sea shore. 653.

Narum, S. R. (2006). Beyond bonferroni: less conservative analyses for conservation genetics. *Conserv. Genet.* 7, 783–787. doi: 10.1007/s10592-005-9056-y

Nikula, R., Fraser, C., Spencer, H., and Waters, J. (2010). Circumpolar dispersal by rafting in two subAntarctic kelp-dwelling crustaceans. *Mar. Ecol. Prog. Ser.* 405, 221–230. doi: 10.3354/meps08523

Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annu. Rev. Ecol. Systematics* 25, 547–572. doi: 10.1146/annurev.es.25.110194.002555

Park, Y.-H., Gasco, N., and Duhamel, G. (2008). Slope currents around the Kerguelen Islands from demersal longline fishing records. *Geophysical Res. Lett.* 35, L09604. doi: 10.1029/2008GL033660

Pascual, M., Rives, B., Schunter, C., and Macpherson, E. (2017). Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea. *PloS One* 12, 176419. doi: 10.1371/journal.pone.0176419

Pazmiño, D. A., Maes, G. E., Simpfendorfer, C. A., Salinas-de-León, P., and van Herwerden, L. (2017). Genome-wide SNPs reveal low effective population size within confined management units of the highly vagile galapagos shark (*Carcharhinus galapagensis*). *Conserv. Genet.* 18, 1151–1163. doi: 10.1007/s10592-017-0967-1

Pfaller, J. B., Payton, A. C., Bjorndal, K. A., Bolten, A. B., and McDaniel, S. F. (2019). Hitchhiking the high seas: global genomics of rafting crabs. *Ecol. Evol.* 9, 957–974. doi: 10.1002/ece3.4694

Pinsky, M. L., Saenz-Agudelo, P., Salles, O. C., Almany, G. R., Bode, M., Berumen, M. L., et al. (2017). Marine dispersal scales are congruent over evolutionary and ecological time. *Curr. Biol.* 27, 149–154. doi: 10.1016/j.cub.2016.10.053

Post, A. L., Meijers, A. J. S., Fraser, A. D., Meiners, K. M., Ayers, J., Bindoff, N. L., et al. (2014). "Chapter 4. Environmental setting," in *Biogeographic Atlas of the Southern Ocean*. Eds. C. De Broyer, P. Koubbi, H. J. Griffiths, B. Raymond, C. d'Udekem d'Acoz, A. P. Van de Putte, B. Danis, et al (Published by The Scientific Committee on Antarctic Research, Scott Polar research Institute, Cambridge), 46–64.

Poulin, E., and Féral, J.-P. (1995). Pattern of spatial distribution of a brood-protecting schizasterid echinoid, *Abatus cordatus*, endemic to the Kerguelen Islands. *Mar. Ecol. Prog. Ser.* 118, 179–186. doi: 10.3354/meps118179

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959. doi: 10.1093/genetics/155.2.945

Quilty, P. G. (2007). Origin and evolution of the sub-Antarctic islands: the foundation. *Papers and Proceedings of the Royal Society of Tasmania*, 14(1), 35–58. doi: 10.26749/rstpp.141.1.35

Quintero-Galvis, J. F., Ocampo-Zuleta, K., Castro, L. R., and Narváez-Barandica, J. C. (2023). Limited population genetic structure in the littoral gastropod *Nerita tessellata* (Neritimorpha, Neritidae) suggests high dispersal across the Caribbean Sea. *J. Exp. Mar. Biol. Ecol.* 568, 151942. doi: 10.1016/j.jembe.2023.151942

Rabassa, J., Coronato, A. M., and Martínez, O. (2011). Late Cenozoic glaciations in Patagonia and Tierra del Fuego: an updated review. *Biol. J. Linn. Soc.* 103, 316–335. doi: 10.1111/j.1095-8312.2011.01681.x

Rabassa, J., Coronato, A. M., and Salemme, M. (2005). Chronology of the Late Cenozoic Patagonian glaciations and their correlation with biostratigraphic units of the Pampean region (Argentina). *J. South Am. Earth Sciences Quaternary Paleontology biostratigraphy South. South Afr.* 20, 81–103. doi: 10.1016/j.jsames.2005.07.004

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., and Suchard, M. A. (2018). Posterior summarization in bayesian phylogenetics using tracer 1.7. *Systematic Biol.* 67, 901–904. doi: 10.1093/sysbio/syy032

Rousset, F. (2008). Genepop'007: A complete re-implementation of the genepop software for windows and linux. *Mol. Ecol. Resour.* 8, 103–106. doi: 10.1111/j.1471-8286.2007.01931.x

Rovelli, V., Ruiz-González, A., Vignoli, L., Macale, D., Buono, V., Davoli, F., et al. (2019). Genotyping-by-sequencing (GBS) of large amphibian genomes: A comparative study of two non-model species endemic to Italy. *Anim. Biol.* 69, 307–326. doi: 10.1163/15707563-00001094

Segovia, N. I., González-Wevar, C. A., Naretto, J., Rosenfeld, S., Brickle, P., Hüne, M., et al. (2022). The right tool for the right question: contrasting biogeographic patterns in the notothenioid fish *Harpagifer* spp. along the Magellan Province. *Proc. R. Soc. B: Biol. Sci.* 289, 20212738. doi: 10.1098/rspb.2021.2738

Selkoe, K. A., D'Aloia, C., Crandall, E., Iacchei, M., Liggins, L., Puritz, J. B., et al. (2016). A decade of seascape genetics: contributions to basic and applied marine connectivity. *Mar. Ecol. Prog. Ser.* 554, 1–19. doi: 10.3354/meps11792

Simkanin, C., Carlton, J. T., Steves, B., Fofonoff, P., Nelson, J. C., Clarke Murray, C., et al. (2019). Exploring potential establishment of marine rafting species after transoceanic long-distance dispersal. *Global Ecol. Biogeography* 28, 588–600. doi: 10.1111/geb.12878

Simpson, R. D. (1976). Physical and biotic factors limiting the distribution and abundance of littoral molluscs on Macquarje island (sub-Antarctic). *J. Exp. Mar. Biol. Ecol.* 21, 11–49. doi: 10.1016/0022-0981(76)90067-8

Sjöqvist, C., Godhe, A., Jonsson, P. R., Sundqvist, L., and Kremp, A. (2015). Local adaptation and oceanographic connectivity patterns explain genetic differentiation of a marine diatom across the North Sea-Baltic Sea salinity gradient. *Mol. Ecol.* 24, 2871–2885. doi: 10.1111/mec.13208

Slatkin, M. (1985). Gene flow in natural populations. Annual review of ecology and systematics. *Annu. Rev. Ecol. Evol. Syst.* 16, 393–430. doi: 10.1146/annurev.es.16.110185.002141

Smith, S. D. A. (2002). Kelp rafts in the Southern Ocean. *Global Ecol. Biogeography* 11, 67–69. doi: 10.1046/j.1466-822X.2001.00259.x

Stevens, C. L., Hurd, C. L., and Smith, M. J. (2002). Field measurement of the dynamics of the bull kelp *Durvillaea antarctica* (Chamisso) Heriot. *J. Exp. Mar. Biol. Ecol.* 2, 147–171. doi: 10.1016/S0022-0981(02)00007-2

Teske, P. R., Golla, T. R., Sandoval-Castillo, J., Emami-Khoyi, A., van der Lingen, C. D., Heyden, S.v. d., et al. (2018). Mitochondrial DNA is unsuitable to test for isolation by distance. *Sci. Rep.* 8, 84485. doi: 10.1038/s41598-018-25138-9

Thiel, M., and Gutow, L. (2005). The ecology of rafting in the marine environment. I. The floating subtrata. *Oceanography Mar. Biology: Annu. Rev.* 42, 181–264. doi: 10.1201/9780203507810.ch6

Trickey, J. S., Thiel, M., and Waters, J. M. (2016). Transoceanic dispersal and cryptic diversity in a cosmopolitan rafting nudibranch. *Invertebrate Systematics* 30, 290–301. doi: 10.1071/IS15052

Trovant, B., Orensanz, J. M., Ruzzante, D. E., Stotz, W., and Basso, N. G. (2015). Scorched mussels (Bivalvia: Mytilidae: Brachidontinae) from the temperate coasts of South America: phylogenetic relationships, trans-Pacific connections and the footprints of Quaternary glaciations. *Mol. Phylogenet. Evol.* 82, 60–74. doi: 10.1016/j.ympev.2014.10.002

Vucetich, J. A., and Waite, T. A. (2000). Is one migrant per generation sufficient for the genetic management of fluctuating populations? *Anim. Conserv.* 3, 261–266. doi: 10.1111/j.1469-1795.2000.tb00111.x

Waples, R. S. (1987). A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution* 41, 385–400. doi: 10.1111/j.1558-5646.1987.tb05805.x

Waples, R. S., and Do, C. (2008). Ldne: A program for estimating effective population size from data on linkage disequilibrium. *Mol. Ecol. Resour.* 8, 753–756. doi: 10.1111/j.1755-0998.2007.02061.x

Waters, J. M., Fraser, C. I., and Hewitt, G. M. (2013). Founder takes all: density-dependent processes structure biodiversity. *Trends Ecol. Evol.* 28, 78–85. doi: 10.1016/j.tree.2012.08.024

Waters, J. M., King, T. M., Fraser, C. I., and Craw, D. (2018a). An integrated ecological, genetic and geological assessment of long-distance dispersal by invertebrates on kelp rafts. *Front. Biogeography* 10, (3–4). doi: 10.21425/F5FBG40888

Waters, J. M., King, T. M., Fraser, C. I., and Garden, C. (2018b). Rafting dispersal in a brooding southern sea star (Asteroidea : *Anasterias*). *Invertebrate Systematics* 32, 253–258. doi: 10.1071/IS17037

Weersing, K., and Toonen, R. J. (2009). Population genetics, larval dispersal, and connectivity in marine systems. *Mar. Ecol. Prog. Ser.* 393, 1–12. doi: 10.3354/meps08287

White, J. W., Carr, M., Caselle, J., Washburn, L., Woodson, C. B., Palumbi, S., et al. (2019). Connectivity, dispersal, and recruitment: connecting benthic communities and the coastal ocean. *Oceanography* 32, 50–59. doi: 10.5670/oceanog.2019.310

Wilson, G. A., and Rannala, B. (2003). Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* 163, 1177–1915. doi: 10.1093/genetics/163.3.1177

Winston, J. E. (2012). Dispersal in marine organisms without a pelagic larval phase. *Integr. Comp. Biol.* 52, 447–457. doi: 10.1093/icb/ics040

Wright, S. (1931). Evolution in mendelian populations. *Genetics* 16, 97–159. doi: 10.1093/genetics/16.2.97

Zabala, S., Averbuj, A., Bigatti, G., and Penchaszadeh, P. E. (2020). Embryonic development of the false limpet *Siphonaria lateralis* from Atlantic Patagonia. *Invertebrate Biol.* 139, e12276. doi: 10.1111/ivb.12276

Zbawicka, M., Gardner, J. P. A., and Wenne, R. (2019). Cryptic diversity in smooth-shelled mussels on Southern Ocean islands: connectivity, hybridisation and a marine invasion. *Front. Zoology* 16, 32. doi: 10.1186/s12983-019-0332-y

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., and Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinf. (Oxford England)* 28, 3326–3328. doi: 10.1093/bioinformatics/bts606

Zhou, M., Zhu, Y., d'Ovidio, F., Park, Y.-H., Durand, I., Kestenare, E., et al. (2014). Surface currents and upwelling in Kerguelen Plateau regions. *Biogeosciences Discussions* 11, 6845–6876. doi: 10.5194/bgd-11-6845-2014

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Habitat type controls microarthropod community changes across a Magellanic sub-Antarctic elevation gradient

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Introduction: Elevation gradients are often used as a proxy for climate change as they allow comparisons of ecological responses over much larger temporal and spatial scales than is possible through experimental manipulations.

Methods: Here, we tested how microarthropod communities (Collembola and Acari) are affected by climatic differences between sea level and 600 m a.s.l. on Navarino Island, in the Magellanic sub-Antarctic ecoregion of southern Chile (mean annual temperatures of 5.6 vs 3.1°C, respectively). We quantified microarthropod abundance, richness and community trait characteristics in dominant moss (*Racomitrium lanuginosum* and *Polytrichum strictum*) and lichen (*Usnea trachycarpa, Pseudocyphellaria freycinetii* and *Stereocaulon alpinum*) vegetation growing at both elevations. These moss and lichen genera are characterized by large morphological differences and allow testing of how habitat characteristics affect microarthropod community response across elevation gradients.

Results: Collembola and Acari community composition differed between the low and high elevation sites. Total abundance levels of Acari were maintained in each habitat across elevation, whereas Collembola richness strongly declined (50%) at high elevation in the moss habitats. Acari community differences across elevation were driven by relative abundance changes whereas the Collembola community lost species at higher elevation. An anticipated decline of smaller eudaphic Collembola at high elevation was only observed in the moss *Racomitrium*, reflecting potentially lower temperature buffering capacity and shelter options compared to *Polytrichum*. Lichens mostly supported larger epigeic species irrespective of elevation. There were no consistent patterns linking microarthropod communities with habitat water holding capacity or water loss rates across the studied habitats and elevation.

Discussion: Habitat type and the genus of moss or lichen were associated with microarthropod community changes across elevation, including examples of declines, increases and no change. These findings highlight that community responses across gradients may not always relate to the generally hypothesized environmental variables (e.g. temperature variability) and that habitat characteristics should be taken into account when using elevation as a proxy for climate change.

KEYWORDS

Collembola, moss, lichen, microclimate, Acari (mites)

Introduction

Polar regions are experiencing some of the most rapid warming rates on Earth, resulting in potentially large changes in vegetation composition, soil communities and soil process rates (Elmendorf et al., 2012; Myers-Smith et al., 2015; IPCC, 2023). Understanding ecosystem response to warming is crucial for understanding future feedbacks to global climate change. Microarthropods, such as springtails (Collembola) and mites (Acari), are soil biotic drivers of soil nutrient and carbon cycling (Seastedt, 1984; Filser, 2002; Kampichler and Bruckner, 2009) and changes in their abundance and community composition affect decomposition rates (Wall et al., 2008; Handa et al., 2014). Predicting microarthropod response to changes in climate is complicated as various factors change simultaneously in the soil biotic and abiotic environment, all of which can potentially promote or restrict individual growth and reproduction. In addition, some microarthropod species may be more responsive to direct changes in microclimate conditions (Makkonen et al., 2011; Bokhorst et al., 2012), while others respond to indirect climate-driven changes in vegetation and associated soil characteristics (Krab et al., 2010; Bokhorst et al., 2017). Untangling this context specificity across species response requires complementary approaches, and will help to anticipate challenges in polar ecosystems resulting from climate change.

Experimental warming studies show large and variable responses by microarthropods (Hodkinson et al., 1998; Convey et al., 2002; Bokhorst et al., 2008; Kardol et al., 2011). This variability in part reflects the context dependency on where these studies were performed. In addition, methodological drawbacks exist with experimental approaches in terms of realism (such as altering the frequency of temperature extremes) or affect multiple microclimate conditions (e.g., moisture deficits, wind speed and shade) (Bokhorst et al., 2013a). This can complicate our ability to determine the nature of causality behind response variables. Using comparisons across elevation as a proxy for climate differences provides an alternative approach to quantify climate change effects and allow assessment of ecological responses over much larger temporal and spatial scales than is possible through experimental manipulations (Hodkinson, 2005; Sundqvist et al., 2013). Such elevation approaches have been used to characterize microarthropod community changes, but often generate contrasting response patterns in terms of abundance and diversity (Lamoncha and Crossley, 1998; Loranger et al., 2001; Sadaka and Ponge, 2003; Jing et al., 2005; Hasegawa et al., 2006; Illig et al., 2010; Nash et al., 2013) or community changes that were habitat dependent (Bokhorst et al., 2018). Recent studies have suggested that springtail communities show a higher proportion of larger species and individuals with increasing elevation (Bokhorst et al., 2018; Wu et al., 2023; Hishi et al., 2024), which probably reflects selection against smaller species with vulnerability to freezing intensity and variability around zero degrees (freeze-thaw) found at higher elevation (Leinaas, 1983; Sømme, 1989; Bokhorst et al., 2012; Bokhorst et al., 2013b; van Dooremalen et al., 2013). Mite communities typically show a decline in abundance with altitude across major taxonomic groups (Bokhorst et al., 2018). To further untangle patterns of microarthropod distribution patterns across environmental gradients, studies can take advantage of habitat types that change as little as possible with elevation, such as provided by mosses and lichens.

Mosses and lichens are key players in shaping boreal and polar terrestrial microarthropod communities by providing habitat, water, food and buffering against weather extremes (Cornelissen et al., 2007; Blok et al., 2011; Bokhorst et al., 2014; Roos et al., 2021). However, moss and lichen species typically differ in the microarthropod communities they support (Bokhorst et al., 2015; Trekels et al., 2017; Roos et al., 2022), resulting from the way each moss and lichen species creates its own microhabitat (Stoy et al., 2012; van Zuijlen et al., 2020). Moss and lichen water content affects the type of microarthropods supported and higher water holding capacity often results in greater arthropod abundance (Booth and Usher, 1984; Bokhorst et al., 2015). Cryptogam water holding capacity is unlikely to change markedly across environmental gradients (Roos et al., 2019), but water loss rates may be slower in exposed areas, such as a montane plateaus, to prolong photosynthetic capacity (Pintado et al., 1997) which, in turn, provides a longer duration of moist environmental conditions for associated microarthropods. By comparing how microarthropod communities change across elevation within the same moss and lichen habitat, we can identify if microarthropod responses are driven by the overall changes in temperature conditions at higher elevation (environmental selection) or by habitat conditions created by the moss and lichen habitats.

Here we tested how Collembola and Acari community composition responded to changes in climate conditions across elevation, while retaining the same habitat structure provided by moss and lichen species that grew at both high and low elevation sites. The study was carried out on Navarino Island in the Magellanic sub-Antarctic ecoregion of Southern Chile, whose high elevation fell-field plateaus face similar environmental conditions to the core sub-Antarctic islands and parts of the Antarctic Peninsula region (Rosenfeld et al., 2020; Francisco et al., 2021). We hypothesized that: a) microarthropod abundance will decline at higher elevation as a result of the colder environmental conditions, b) microarthropod communities will be more strongly affected by elevation in lichen than moss habitat as mosses provide stronger buffering against environmental extremes (Stoy et al., 2012), and c) communities will shift toward larger sized Collembola at higher elevation.

Methods

The study site (Figure 1) was located on Navarino Island in southern Patagonia (55° 07' S, 67° 66' W). The low elevation sampling site (54° 94' S, 67° 62' W) was situated close to sea level at the edge of *Nothofagus* forest (mixed stands of *N. pumilio* and *N. antarctica*) intermixed with peatland (*Sphagnum magellanicum*). The high elevation sampling site was situated above the tree line (600 m. asl. 54° 97' S, 67° 63' W) on Cerro Bandera and comprised a typical fellfield community consisting of a mosaic of lichens and mosses intermixed with dwarf shrubs (*Empetrum rubrum*), cushion plants (*Azorella* spp.) and grasses, similar to sub-Arctic mountainous regions and sub-Antarctic islands (Greene, 1964; Upson et al., 2017). Mean annual soil temperatures are 5.6°C and 3.1°C for the low and high elevation sites, respectively, and 0.5°C and -2.2°C during winter (Table 1). Snow frequently occurs during summer months at the high elevation site, but melts out within a few days, while at sea level precipitation mostly falls as rain (Rozzi and Jimenez, 2013).

During the austral summer (22-29th January 2020) we collected dominant moss and lichen species that occur at both low (sea level) and high (600 m a.s.l.) elevation. Twenty clumps each of *Racomitrium lanuginosum, Polytrichum strictum, Usnea trachycarpa, Pseudocyphellaria freycinetii* and *Stereocaulon alpinum* from the low (n = 10) and high (n = 10) elevation study site. Henceforth these species will be referred to by their genus name alone. *Polytrichum* (Polytrichaceae, bog haircap moss) is an erect growing, acrocarpic, moss species preferring moist habitats (peatlands), which is also found in the maritime Antarctic and the Arctic. Its erect growth form creates a compact moss mat which can develop to tens of cm depth. *Racomitrium* (Grimmiaceae, woolly fringe moss) is a common moss species of montane and polar tundra (also in the maritime Antarctic) growing as large mats of loose horizontal shoots, across dry exposed rock and in boulder scree. *Pseudocyphellaria* (Peltigeraceae, speckle belly lichens) is a foliose lichen growing on tree trunks and boulders where it forms extensive mats. Although Peltigeraceae are present in Antarctica, the genus *Pseudocyphellaria* is not reported from the Arctic or Antarctic. *Stereocaulon* (Stereocaulaceae, snow lichens) is a fruticose grey-white lichen, with densely branched thalli covered with grainy outer edges, which also occurs in the maritime Antarctic. *Usnea* (Parmelicaceae, beard lichen) is pale grey-green fruticose lichen that resembles a miniature shrub. Lichens of this genus have a worldwide distribution with several species also occurring in Antarctica.

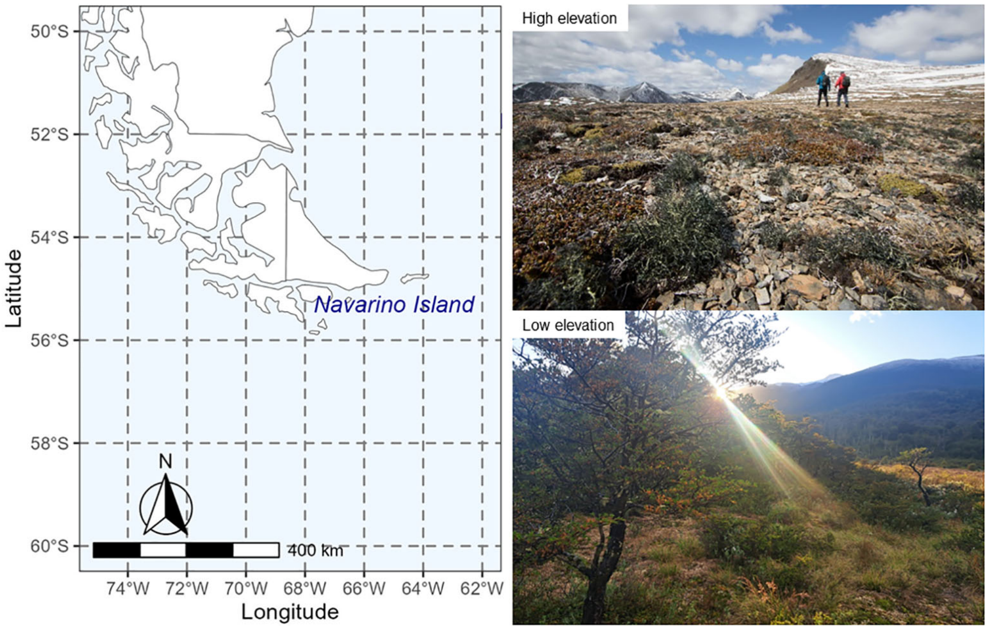

FIGURE 1
Map showing the location of Navarino Island in southern Patagonia (54° 97' S, 67° 63' W). The low elevation sampling site (lower right picture) was situated close to sea level at the edge of *Nothofagus* forest intermixed with peatland (*Sphagnum magellanicum*). The high elevation sampling site (upper right picture) was situated above the tree line (600 m. asl.) on Cerro Bandera and comprised a typical lichen fellfield community intermixed with dwarf shrubs and cushion plants.

TABLE 1 Microclimate temperature conditions and water traits among different moss and lichen habitats at low and high field sites.

	Temperature		Water	
	Low	High	Low	High
Site variables				
Mean T	5.6 (0.1)	3.1 (0.1)		
Minimum T	-3.2	-10.8		
Maximum T	44.0	47.6		
Degree days (sum)	1942 (42)	1409 (21)		
Freeze thaw cyles (No.)	80 (10.2)	227 (7.7)		
Habitat variables	Temperature range		WHC	
Polytrichum	15.4	17.5	432 (16)a	352 (16)
Racomitrium	24.2	22.2	511 (20)b	635 (15)*
Pseudocyphellaria	35.9	16.8	281 (18)c	298 (28)
Stereocaulon	33.8	23.6	356 (17)d	328 (20)
Usnea	–	23.4	144 (16)e	92 (6)
	Maximum temperature		Water loss (minutes)	
Polytrichum	21.8	21.3	1491 (119)a	1430 (76)
Racomitrium	30.4	25.2	861 (104)a	1518 (124)
Pseudocyphellaria	41.1	21.7	207 (18)b	244 (25)
Stereocaulon	39.5	27.5	363 (29)c	408 (45)
Usnea	–	25.9	152 (33)d	63 (12)*

Site variables are mean values (n = 5 and 3 for the high and low site, respectively) with SE between brackets. Habitat variables represent temperature conditions measured during summer (n = 1 at high and low site each). Water holding capacity (WHC) and time to 50% water loss for lichens and mosses are means of n = 10 samples with SE between brackets. WHC: habitat $F_{4,90}$ = 167.9, P < 0.001; elevation $F_{1,90}$ = 0.1, P < 0.720; H×E $F_{4,90}$ = 9.8, P < 0.001. Water loss: habitat $F_{4,90}$ = 135.1, P < 0.001; elevation $F_{1,90}$ = 0.1, P < 0.871; H×E $F_{4,90}$ = 5.2, P < 0.001. Significant (Tukey's HSD P < 0.05) differences in water variables between elevation are denoted by '*', while different letters indicate habitat differences.

Individual lichen thalli (c. 5 cm × 5 cm) were collected by hand, ensuring at least 50 cm distance between thalli. Mosses were collected using a 5 cm diameter, maximum depth of 5 cm, corer with a minimum of 1 m distance between cores. Mosses and lichens were obtained from patches dominated by the target species, to minimize potential edge effects. Each sample was immediately sealed in a sampling pot with lid and microarthropod extraction was started as soon as possible upon return to the field station (<5 h). Species replicate samples (n = 10) were always collected on the same day, but logistical practicalities prevented sampling all species across both study sites at the same day. However, given the short duration of the sampling campaign (7 d), we assume that any time differences between low and high site sampling dates (1-5 d) and species (3-6 d) did not affect microarthropod abundances.

A Tullgren heat extractor was used to collect Collembola, Acari and spiders (Araneae) from the vegetation samples. Extractions took place over a minimum of 48 h until the cryptogam habitat was dry, and the extracted animals were immediately preserved in ethanol (70%). Collembola species were identified to genus or species level following Heckman (2001) and grouped into life-form groups according to their known vertical distributions across soil-litter profiles: in soil, eudaphic; in litter, hemi-edaphic; on plants, epigeic; (Gisin, 1943). To determine whether Collembola species with specific traits would be differentially affected by elevation, community weighted trait means (CWM) were calculated (de Bello et al., 2011). For each trait the relative abundance of a Collembola species was multiplied by its trait value, and then summed over all species in the community. For Collembola we used the traits of life form eudaphic (trait value 1), hemidaphic (trait value 2) and epigeic (trait value 3), and CWM body length (mm). Trait information was obtained from the literature (Gisin, 1943; Fjellberg, 1998; Heckman, 2001). Acari were identified to major groups (Oribatida, Mesotigmata and Prostigmata-Astigmata) and, where possible, to family level. All spiders were counted, but not compared across functional trait groups as this type of information is lacking. All abundance data were expressed as individuals per gram oven dried substrate (70°C 48 h). Collembola species and Acari group frequency across sampled habitats are presented in Supplementary Table 1.

To determine if habitats differently buffer against environmental conditions, we measured hourly temperature and light conditions of each moss and lichen habitat at the high and low elevation site for 48 h in late January, using HOBO pendants (UA 002-64). Logger placement among mosses resulted in the logger being buried and not exposed to direct illumination, while among lichen thalli the loggers were partly exposed to direct illumination. Due to logger limitation there was no replication of microclimate logging at the respective elevation sites; the logger placed in *Usnea* at the low elevation site malfunctioned. For each habitat type (species) we calculated daily mean temperature, temperature range and the minimum and maximum temperature. Although the microclimate recording took place over only a short mid-summer period, it reflects the temperature and light conditions soil invertebrates are exposed to when living among these moss and lichen habitats. Year-round temperature and light data were obtained using HOBO pendants (UA 002-64) placed on the soil at the high (n = 5) and low (n = 3) elevation site.

Moss and lichen water holding capacity (WHC) was quantified by comparing the weight difference between fully hydrated and oven dried (70°C 48 h) tissue samples. Full hydration was achieved by wetting lichen thalli and moss plugs and placing them in a closed jar with moist tissue paper for 24 h. A thin layer (1-2 cm) of water was added to the moss plugs as these can take up substantially more water than lichens. To quantify water loss rates, fully hydrated samples were weighed at regular intervals (1.5-3 h) under standardized laboratory settings at room temperature for 7 h. Water loss rates followed a linear or exponential decline and the time to 50% water loss was calculated as a response variable (water retention time) for each individual moss and lichen sample.

Statistical analyses

A factorial ANOVA was used to test how microarthropod abundance (total, Collembola, Acari, spider, species and groups), taxonomic richness, community weighted trait values, water holding capacity (WHC) and water retention time were affected by habitat (n = 5 species) and elevation (low vs. high). We quantified how Collembola and Acari community composition (based on abundance) differed between elevation in each habitat using Bray-Curtis distance and compared habitat differences using the adonis-method from the R 'vegan package' (Oksanen, 2015). To identify effects of changing water conditions across elevation within each habitat we assessed the correlation (Pearson) of habitat WHC and water retention time with microarthropod abundance and richness. All data were log- or square root-transformed to improve homogeneity of variances. All analyses were performed using R (R-Core-Team, 2023). We used the online iNterpolation and EXTrapolation tool to check if sampling size was sufficient capture microarthropod diversity (Supplementary Figure S1) (Chao et al., 2014). This type of sampling curve plots the diversity estimates with respect to sample size. Although we tested for moss and lichen habitat differences in microarthropod composition measures, the results and discussion primarily focus on elevation comparisons between moss and lichen habitats.

Results

Microclimate differences

Mean annual soil temperature was 2.5°C lower at high elevation, and this was also reflected in lower moss (3.0 - 3.6°C) and lichen (5.4 - 5.7°C) summer temperatures (Table 1). The high elevation site was exposed to nearly three times higher frequency of freeze-thaw events annually, and experienced a larger temperature range due to colder winter and warmer summer temperatures (Table 1). The summer temperature range at the low elevation site was on average 7°C higher among the moss and lichen habitats compared to the high elevation site. Moss temperature range was about 10-20°C lower than that of lichens at low elevation, whereas this differences was reduced at high elevation (Table 1, Supplementary Figures 2, 3). Water holding capacity and water retention time were highest in the two moss habitats compared to the lichens, with the latter decreasing in the following order: *Stereocaulon* > *Pseudocyphellaria* > *Usnea*. *Racomitrium* had a 24% higher WHC at high elevation, and *Usnea* from high elevation lost water more than two times more rapidly than did specimens from low elevation. Other moss and lichen habitats did not differ in WHC or water retention time between high and low elevation (Table 1).

Microarthropod abundance and richness responses

Total microarthropod abundance was affected by elevation but not consistently across moss and lichen habitats, and a similar pattern was observed for Collembola and Acari abundance and taxonomic richness (Table 2). Collembola abundance was lower at high elevation in *Racomitrium* (64%) and *Usnea* (84%) compared to the low elevation site, as predicted, but no significant differences were observed among the other moss and lichen habitats. Acari abundance was 92% lower among *Usnea* at high elevation (Figures 2A, B). Collembola species richness was reduced by 50% at high elevation in *Polytrichum, Racomitrium* and *Usnea*, as predicted, while a doubling of Acari taxonomic richness was observed in *Polytrichum* at high elevation (Figures 2C, D). Spider abundance was low overall and spiders were absent from moss but not lichen at high elevation.

TABLE 2 ANOVA results (F and P-values) of micro-arthropod abundance, taxonomic richness and community weighted mean traits between different lichen and moss habitats across low and high elevation.

	Habitat		Elevation		Habitat × Elevation	
	F	*P*	*F*	*P*	*F*	*P*
Microarthropod abundance	4.5	0.002	11.6	<0.001	10.8	<0.001
Collembola abundance	22.0	<0.001	20.5	<0.001	3.4	0.012
Collembola R	38.4	<0.001	25.6	<0.001	2.5	0.047
Epigeic abundance	16.6	<0.001	14.7	<0.001	2.8	0.028
Hemidaphic abundance	12.4	<0.001	0.2	0.670	5.4	<0.001
Eudaphic abundance	10.6	<0.001	9.2	0.003	17.1	<0.001
CWM stratification	30.0	<0.001	0.3	0.618	8.2	<0.001
CWM length	34.0	<0.001	0.0	0.965	4.5	0.002
Epigeic (%)	24.7	<0.001	2.3	0.131	5.8	<0.001
Hemidaphic (%)	10.1	<0.001	5.9	0.018	1.1	0.384
Eudaphic (%)	16.4	<0.001	1.4	0.245	6.5	<0.001
Acari abundance	4.2	0.004	0.2	0.663	11.1	<0.001
Acari R	9.9	<0.001	0.3	0.564	4.6	0.002
Mesostigmata abundance	0.5	0.723	1.3	0.252	2.8	0.029
Oribatida abundance	6.9	<0.001	4.1	0.046	14.8	<0.001
Prostigmata abundance	7.1	<0.001	8.1	0.005	7.3	<0.001
Mesostigmata (%)	2.7	0.034	2.9	0.089	2.7	0.039
Oribatida (%)	4.6	0.002	0.2	0.640	9.1	<0.001
Prostigmata (%)	6.7	<0.001	3.3	0.070	8.8	<0.001

There was no consistent Collembola size response with elevation across habitats (Figures 3A, B). Epigeic Collembola abundance was decreased (84%) at high elevation in *Stereocaulon* (Supplementary Figure 4A). Eudaphic Collembola were absent at high elevation in *Racomitrium*, and hemidaphic Collembola abundance was reduced (62%) in this moss, resulting in a community with larger species (Figures 3A, B). No elevation

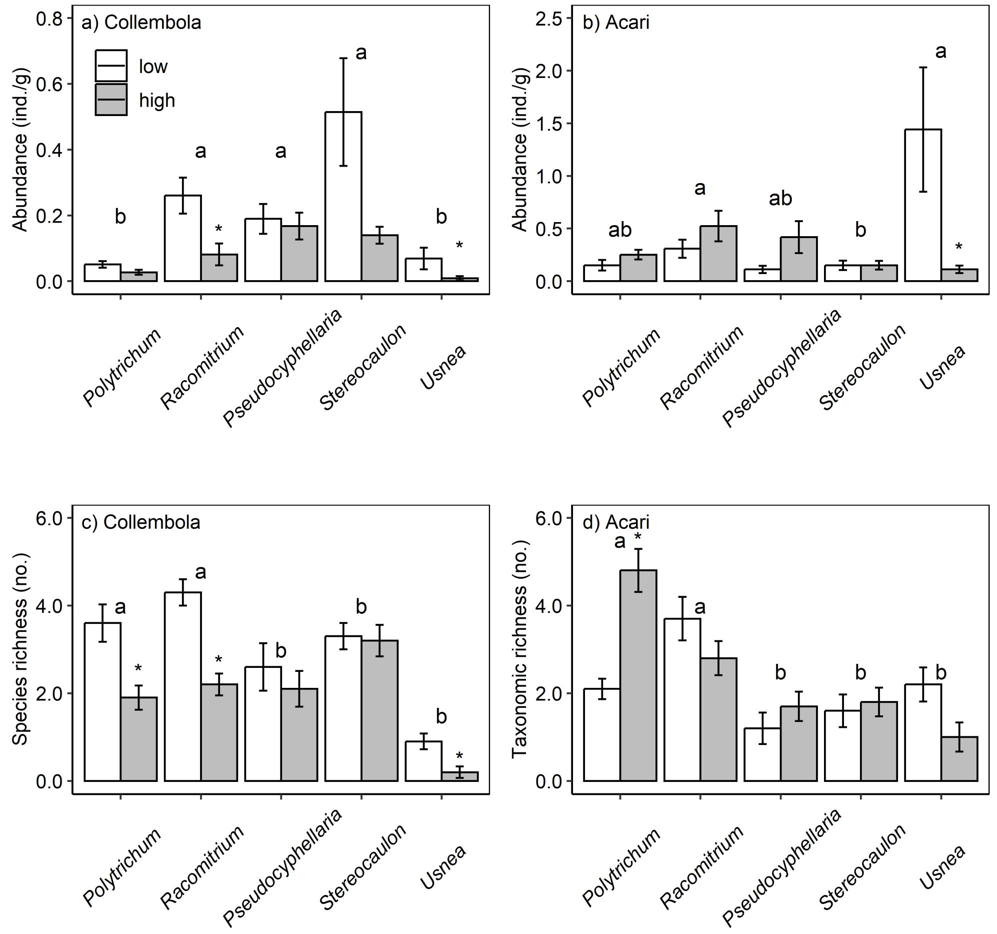

FIGURE 2
Micro-arthropod abundance **(A, B)** and taxonomic richness **(C, D)** across elevation and moss and lichen habitats. Mosses: *Polytrichum* and *Racomitrium*, lichens: *Pseudocyphellaria*, *Stereocaulon* and *Usnea*. Each bar is the mean of n = 10 replicates with SE as error bars. * denotes significant differences between elevation, while different letters indicate habitat differences.

effects were observed for any of the other moss or lichen habitats on these Collembola groups (Supplementary Figures 4B, C). Abundance of mesostigmatid mites was not consistently affected across elevation. Oribatid mite abundance was reduced (99%) at higher elevation in *Usnea*, while prostigmatid mite abundance increased ten-fold in *Racomitrium* at high elevation.

Community responses

Collembola community composition differed between the low and high elevation sites for four of the five sampled cryptogams, the exception being *Usnea* (Table 3, Figure 3). A similar pattern was observed for Acari community composition but no difference was found in *Pseudocyphellaria*. The proportion of epigeic Collembola increased with elevation in *Racomitrium*. Eudaphic Collembola species disappeared from *Racomitrium* at high elevation, but not from *Polytrichum*. The proportion of hemiedaphic Collembola was greater at high elevation (39% ± 5) compared to low sites (21% ± 5). The proportion of Oribatida declined at higher elevation in *Racomitrium* and a similar trend was found in *Usnea* (Figure 3D). The proportion of Prostigmata increased with elevation in both *Racomitrium* and *Usnea* but not in the other moss and lichen habitats. Mesostigmata were unaffected by elevation in any of the moss and lichen habitats. A small number of species were only observed at one elevation: the Collembola *Entomobrya lanuginosa, Setanodosa* sp. and *Sminthurinus patagonicus* were only observed at low elevation while a *Friesea* sp. was only observed at high elevation (Table 4). No such patterns were observed among the Acari families/groups (Table 5).

There were significant correlations between microarthropod (group) abundance and habitat WHC and water retention times. However, microarthropod abundance was never positively correlated, as anticipated. In addition, for the moss habitats these correlations resulted from a clear grouping of WHC and retention time between low and high elevation (Supplementary Figures 5–8). Total Acari abundance was negatively related to *Stereocaulon* WHC (R = -0.546, *P* = 0.027). Oribatida abundance was negatively related to *Stereocaulon* water retention time (R = -0.632, *P* = 0.007). Epigeic Collembola showed a negative correlation with *Polytrichum* water retention time (R = -0.459, *P* = 0.042).

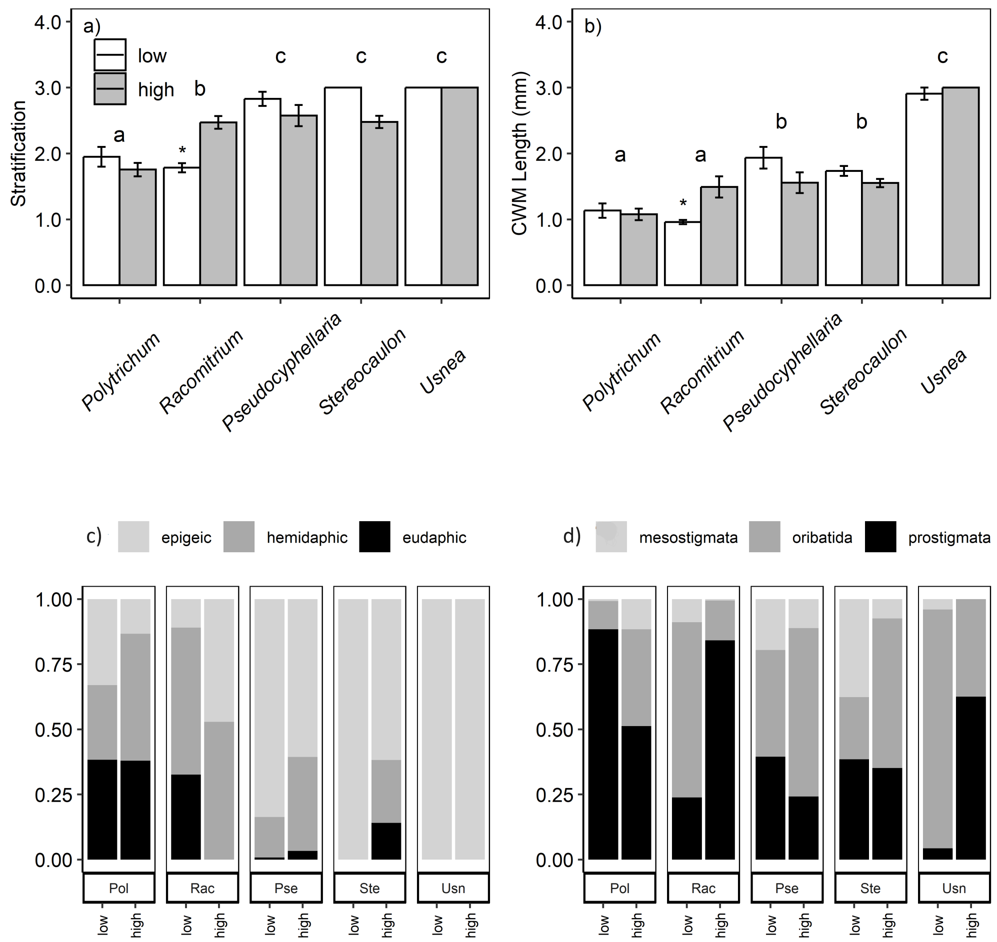

FIGURE 3
Micro-arthropod community composition in different habitats across elevation. Collembola community weighted mean vertical stratification **(A)**, and community weighted mean length **(B)**. The lower panels show the proportion of Collembola groups **(C)** and major Acari taxonomic groups **(D)**. mosses: *polytrichum* and *racomitrium*, lichens: *pseudocyphellaria*, *stereocaulon* and *usnea*. Bars show mean values of n = 10 replicates per habitat type with SE as error bars. Lowercase letters indicate significant community differences between mosses and lichens.

Discussion

Explaining and predicting microarthropod abundance and diversity patterns is one of the many challenges in ecology (Hodkinson, 2005). Our data showed that the colder and higher freezing frequency conditions of higher elevation did not consistently affect moss- and lichen-associated microarthropod communities, as hypothesized. Instead, abundance and diversity responses to elevation were dependent on habitat type, as also reported in a study of whole ecosystem change in sub-Arctic landscapes (Bokhorst et al., 2018). However, the community changes, or lack thereof, were also features of different habitats within the high elevation fell-field vegetation, indicating that small spatial scale heterogeneity affects community response within an ecosystem type. This was also apparent at the microarthropod species level, where species losses in one microhabitat at higher elevation were compensated by gains in a different microhabitat, thereby retaining the overall species pool, reflecting the relative roles of the spatial scale and heterogeneity of environmental factors on soil fauna community assembly (Widenfalk et al., 2016). Polar microarthropod community responses to climate change will, therefore, be habitat dependent.

TABLE 3 ANOVA output (*F* and *P*-values) community composition following Bray Curtis comparison of Collembola and Acari composition between low and high elevation for each moss and lichen habitat.

	Collembola		Acari	
	F	*P*	*F*	*P*
Polytrichum	2.1	0.080	7.6	0.001
Racomitrium	6.5	0.001	9.8	0.001
Pseudocyphellaria	3.3	0.003	1.1	0.438
Stereocaulon	10.3	0.001	4.8	0.002
Usnea	0.4	0.748	8.4	0.001

Elevation effect on microarthropod abundance

The high elevation study site was exposed to lower temperatures and a higher frequency of freezing and thawing, also during the summer

TABLE 4 Collembola species abundance (ind./g) across different moss and lichen habitat and elevation.

	Polytrichum		*Racomitrium*		*Pseudocyphellaria*		*Stereocaulon*		*Usnea*	
Species	low	high	Low	high	low	high	low	high	low	high
Cryptopygus cinctus	0.11 (0.04)	0.21 (0.08)	0.89 (0.30)	0.88 (0.42)	0.17 (0.17)	0.71 (0.23)	–	0.58 (0.19)	–	–
Entomobrya griseoolivata	–	–	–	0.02 (0.02)	0.20 (0.14)	–	–	–	0.99 (0.40)	0.14 (0.09)
Entomobrya lanuginosa	–	–	–	–	0.21 (0.07)	–	0.34 (0.14)	–	–	–
Friesea sp.	–	–	–	0.02 (0.01)	–	–	–	–	–	–
Hypogastrura purpurescens	–	–	0.31 (0.29)	–	1.14 (0.45)	–	5.33 (1.86)	0.15 (0.10)*	0.04 (0.04)	–
Isotoma punctata	0.28 (0.08)	0.13 (0.04)	0.62 (0.16)	–	0.03 (0.03)	–	–	0.07 (0.05)	–	–
Isotomidae sp. *(small)*	–	–	–	–	–	–	–	0.15 (0.09)	–	–
Isotomurus palustris	0.09 (0.04)	0.06 (0.02)	0.11 (0.04)	0.39 (0.18)	0.31 (0.17)	0.64 (0.29)	0.22 (0.11)	0.46 (0.14)	–	–
Isotomurus palustris ()?	0.01 (0.01)	–	–	–	0.06 (0.04)	0.16 (0.09)	0.65 (0.24)	0.11 (0.11)	–	–
Lepidocyrtus sp.	0.11 (0.05)	–	0.03 (0.02)	–	0.24 (0.12)	1.00 (0.35)	1.05 (0.45)	0.52 (0.10)	–	–
Protaphorura encarpta	0.01 (0.01)	–	0.38 (0.24)	–	–	0.08 (0.08)	–	0.03 (0.03)	–	–
Setanodosa sp.	0.04 (0.01)	–	1.40 (0.43)	–	0.17 (0.12)	–	–	–	–	–
Sminthurides hospes	0.11 (0.06)	–	0.03 (0.02)	0.05 (0.04)	–	0.20 (0.20)	0.16 (0.11)	–	–	–
Sminthurinus patagonicus	–	–	–	–	0.03 (0.03)	–	–	–	–	–
collembola total	0.77 (0.14) b	0.40 (0.07)	3.77 (0.95) a	1.36 (0.55) *	2.57 (0.64) a	2.79 (0.56)	7.74 (2.16) a	2.07 (0.27)	1.03 (0.40) b	0.14 (0.09) *
Number of species	3.6 (0.4) ab	1.9 (0.3)*	4.3 (0.3) ab	2.2 (0.3)*	2.6 (0.5) b	2.1 (0.4)	3.3 (0.3) a	3.2 (0.4)	0.9 (0.2) c	0.2 (0.1)*

Lichens and mosses sampled near sea level (low) and on a mountain plateau (high) on Navarino Island, southern Chile. Mosses: Polytrichum and Racomitrium, lichens: Pseudocyphellaria, Stereocaulon and Usnea. Values are mean of n = 10 samples with SE between brackets. '-' = not present. Moss and lichen habitat differences (Tukey's HSD P < 0.05) in total Collembola abundance and species richness are indicated by different letters, while '*' denote differences between low and high elevation.

growing season, which is generally expected to negatively affect the abundance of ectothermic organisms (Sinclair et al., 2003a). However, the studied moss and lichen habitats maintained similar Collembola and Acari population sizes across elevation. This contrasts with patterns observed across elevation in other montane regions, where Acari abundance has been reported to decline with elevation while Collembola abundance increased (Cutz-Pool et al., 2010; Bokhorst et al., 2018; Wu et al., 2023). Such differences may reflect habitat turnover changes across elevation (Cutz-Pool et al., 2010; Wu et al., 2023), substrate quantity and quality (Bokhorst et al., 2018), or differences in climate condition changes with elevation between studies. The current study sites have an annual mean temperature 2-3° C higher than those reported by Bokhorst et al. (2018) and larger microarthropod community differences may occur across greater elevation differences. However, the high frequency of freeze-thaw cycles (227 events at the high elevation site compared to 80 at the low site) are also detrimental for growth and survival (Bale et al., 2001; Sinclair et al., 2003b; Sinclair and Chown, 2005), and can be sufficient to induce equivalent changes in microarthropod abundance. The studied moss and lichen habitats appear, in most cases, to buffer micro-arthropod abundance patterns against temperature contrasts across elevation gradients, indicating that the population changes reported in other studies may in part result from larger habitat characteristic changes rather than changes in temperature conditions *per se*.

Micro-arthropod taxonomic richness was often reduced at higher elevation in the sampled habitats, indicating that fewer species were able to survive there, consistent with our prediction as well as reported in other studies of microarthropod communities across elevational gradients (Cutz-Pool et al., 2010; Illig et al., 2010; Bokhorst et al., 2018; Wu et al., 2023). However, total microarthropod diversity was sustained in the high-elevation landscape by the presence of the different moss and lichen species. These patterns may reflect physiological tolerance limits for species survival at this colder site with greater temperature fluctuations resulting in larger microarthropod population variability (Kuznetsova, 2006).

Responses in mosses and lichens

There was no support for our second hypothesis that microarthropod communities would change more among lichens

TABLE 5 Acari abundance (ind./g) across different moss and lichen habitats and elevation.

	Polytrichum		*Racomitrium*		*Pseudocyphellaria*		*Stereocaulon*		*Usnea*	
Group	low	high	low	high	low	high	low	high	low	high
Prostigmata-Astigmata	1.03 (0.27)	0.47 (0.11)	0.33 (0.10)	0.75 (0.32)	0.16 (0.11)	0.54 (0.24)	0.50 (0.22)	0.13 (0.09)	0.28 (0.13)	0.22 (0.12)
Oppiidae	–	0.06 (0.03)	0.04 (0.03)	0.30 (0.14)*	–	–	–	–	–	–
Oribatida spp.	0.14 (0.04)	0.36 (0.08)	1.31 (0.56)	0.11 (0.10)	0.32 (0.20)	0.54 (0.29)	–	0.45 (0.16)	10.34 (3.79)	0.09 (0.09)*
Oribatida (white)	–	–	–	–	–	–	–	–	0.55 (0.20)	–
Nothroidae	–	0.58 (0.30)	0.53 (0.22)	0.02 (0.02)	0.12 (0.12)	1.10 (0.99)	0.31 (0.23)	0.27 (0.18)	–	0.05 (0.05)
Phthiracaridae	–	–	0.09 (0.04)	0.03 (0.02)	–	0.96 (0.57)	0.12 (0.07)	–	0.04 (0.04)	0.24 (0.17)
Stereotydeus sp.	0.03 (0.02)	0.36 (0.06)	0.03 (0.02)	2.96 (0.72)*	0.20 (0.10)	0.03 (0.03)	–	0.28 (0.18)	0.08 (0.08)	0.31 (0.17)
Mesostigmata	0.01 (0.01)	0.18 (0.06)	0.13 (0.05)	0.02 (0.01)	0.09 (0.05)	0.18 (0.12)	0.27 (0.10)	0.07 (0.06)	0.24 (0.16)	–
Acari total	1.21 (0.29) ab	2.01 (0.45)	2.46 (0.85) a	4.19 (0.91)	0.89 (0.25) ab	3.36 (1.56)	1.21 (0.34) b	1.21 (0.27)	11.53 (3.86) a	0.90 (0.33) *
Taxonomic richness	2.10 (0.23) a	4.80 (0.49)	3.70 (0.50) a	2.80 (0.39)	1.20 (0.36) b	1.70 (0.33)	1.60 (0.37) b	1.80 (0.33)	2.20 (0.39) b	1.00 (0.33)
Spider abundance	0.04 (0.02)	–	0.10 (0.04)	–	0.31 (0.14)	0.12 (0.09)	0.05 (0.05)	0.02 (0.02)	–	0.04 (0.04)

Lichens and mosses sampled near sea level (low) and on a mountain plateau (high) on Navarino Island, southern Chile. Mosses: Polytrichum and Racomitrium, lichens: Pseudocyphellaria, Stereocaulon and Usnea. Values are mean of n = 10 samples with SE between brackets. '-' = not present. Moss and lichen habitat differences (Tukey's HSD, $P < 0.05$) in total Acari abundance and taxonomic richness are indicated by different letters, while '*' denote differences between low and high elevation.

than mosses, as the latter would provide stronger buffering against environmental variability (Stoy et al., 2012). Instead, almost all Collembola and Acari communities differed across elevation, irrespective of moss or lichen habitat. In addition, abundance differences were minimal between habitat types, despite clear differences in potential water status (Table 1), an important factor behind cryptogam-associated micro-arthropod communities (Booth and Usher, 1984; Bokhorst et al., 2015). Microarthropods most likely move across the vegetation mosaic at the high elevation site, from high population densities in favorable habitats to others, and so affect population and diversity patterns. Although, this study presents community data at a single point in time, there were clear habitat-specific microarthropod communities that likely derive from mobility and selection for a suitable habitat. Acari community differences primarily reflected changes in relative abundance instead of taxonomic group turnover between low and high elevation. These Acari community changes with elevation were inconsistent across moss and lichen habitats and the specific taxonomic groups involved, reflecting the context-dependency of Acari response to changes in environmental conditions (Nielsen et al., 2010; Wissuwa et al., 2012; Bokhorst et al., 2017). Collembola community differences between low and high elevation resulted from species losses, especially among the mosses, and turnover at higher elevation. The fruticose lichen *Usnea* supported only few species, and the Collembola species observed (*Entomobrya griseoolivata* and *Hypogastrura purpurescens*) at both elevations are generalist species commonly observed across many environments (Fjellberg, 1998, 2007). These results indicate that habitat type has a potentially strong role in shaping microarthropod community changes across environmental gradients and influences the conclusions that can be derived from such studies.

Community trait response

There was limited support for our third hypothesis, that larger-sized animals would dominate communities at higher elevation except in *Racomitrium*, where there was a clear increase in community weighted mean length at higher elevation which resulted from the loss of eudaphic Collembola. This community response has been observed in other elevation studies (Cutz-Pool et al., 2010; Bokhorst et al., 2018; Hishi et al., 2024), and most likely reflects a vulnerability of eudaphic species to the colder and more variable montane weather (van Dooremalen et al., 2013; Bokhorst et al., 2018). Interestingly this decline in eudaphic and smaller species was not observed in *Polytrichum*, possibly suggesting stronger temperature buffering capacity than *Racomitrium* (Table 1). The lichen habitats did not support eudaphic species to any great extent and, therefore, the associated microarthropod community is already adapted to 'harsher' conditions, with greater CWM-length and dominance of surface-dwelling species, irrespective of elevation. Overall, species identity and associated traits can provide insights into community shifts across elevation, but this cannot be easily disentangled from the habitat type under study. Future research should consider species physiological limits and the functional role (biotic and abiotic control) of microhabitats for microarthropods.

Conclusions

Many biotic and abiotic conditions change across elevation gradients (Hodkinson, 2005; Sundqvist et al., 2013), and associated changes in plant community structure and soil properties are underlying factors that shape animal communities, while at the same time making it hard to identify causal links with micro-arthropod patterns. By targeting the same habitat across elevation we attempted here to shed light on potential causal factors for micro-arthropod abundance and community changes across contrasting temperature conditions. Moss and lichen nutrient content was not taken into consideration but, given the isolation from industrial or agricultural activities and absence of bird colonies near the sampling sites that could provide major nutrient inputs (Bokhorst et al., 2019; van der Vegt and Bokhorst, 2024; Zmudczyńska-Skarbek et al., 2024), it is unlikely that this played a role in the observed microarthropod patterns across elevation. Overall, the responses seen appeared highly context-dependent, in that some lichen habitats maintained population levels even under colder conditions, while moss-associated communities appeared more responsive to changes associated with elevation. These differences appear to be driven by the habitat characteristics and the type of organisms associated with each habitat. While elevation gradients can provide useful insights into how ecosystem properties and the contained communities may change, the underlying causes for specific target groups may not always be obvious and care should be taken when extrapolating to other habitats and regions.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://npdc.nl/dataset/b20d02d3-63b4-5e8d-9b00-08bda0049337.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

SB: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. TC: Funding acquisition, Writing – review & editing. RM: Investigation, Resources, Writing – review & editing. PC: Conceptualization, Funding acquisition, Writing – review & editing. RA: Conceptualization, Funding acquisition, Investigation, 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.

The handling editor MM and reviewer CT declared a past co-authorship with the author PC.

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Publisher's note

Supplementary material

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References

Bale, J. S., Worland, M. R., and Block, W. (2001). Effects of summer frost exposures on the cold tolerance strategy of a sub-Antarctic beetle. *J. Insect Physiol.* 47, 1161–1167. doi: 10.1016/S0022-1910(01)00097-X

Blok, D., Heijmans, M., Schaepman-Strub, G., van Ruijven, J., Parmentier, F. J. W., Maximov, T. C., et al. (2011). The cooling capacity of mosses: Controls on water and energy fluxes in a Siberian tundra site. *Ecosystems* 14, 1055–1065. doi: 10.1007/s10021-011-9463-5

Bokhorst, S., Huiskes, A. H. L., Convey, P., Bodegom, P. M. v, and Aerts, R. (2008). Climate change effects on soil arthropod communities from the Falkland Islands and the Maritime Antarctic. *Soil Biol. Biochem.* 40, 1547–1556. doi: 10.1016/j.soilbio.2008.01.017

Bokhorst, S., Phoenix, G. K., Bjerke, J. W., Callaghan, T. V., Huyer-Brugman, F., Berg, M. P., et al. (2012). Extreme winter warming events more negatively impact small rather than large soil fauna: shift in community composition explained by traits not taxa. *Global Change Biol.* 18, 1152–1162. doi: 10.1111/j.1365-2486.2011.02565.x

Bokhorst, S., Huiskes, A. H. L., Aerts, R., Convey, P., Cooper, E. J., Dalen, L., et al. (2013a). Variable temperature effects of Open Top Chambers at polar and alpine sites explained by irradiance and snow depth. *Global Change Biol.* 19, 64–74. doi: 10.1111/gcb.2012.19.issue-1

Bokhorst, S., Metcalfe, D. B., and Wardle, D. A. (2013b). Reduction in snow depth negatively affects decomposers but impact on decomposition rates is substrate dependent. *Soil Biol. Biochem.* 62, 157–164. doi: 10.1016/j.soilbio.2013.03.016

Bokhorst, S., Wardle, D. A., Nilsson, M.-C., and Gunda, M. J. (2014). Impact of understory mosses and dwarf shrubs on soil micro-arthropods in a boreal forest chronosequence. *Plant Soil* 379, 121–133. doi: 10.1007/s11104-014-2055-3

Bokhorst, S., Asplund, J., Kardol, P., and Wardle, D. A. (2015). Lichen physiological traits and growth forms affect communities of associated invertebrates. *Ecology* 96, 2394–2407. doi: 10.1890/14-1030.1

Bokhorst, S., Kardol, P., Bellingham, P. J., Kooyman, R. M., Richardson, S. J., Schmidt, S., et al. (2017). Responses of communities of soil organisms and plants to soil aging at two contrasting long-term chronosequences. *Soil Biol. Biochem.* 106, 69–79. doi: 10.1016/j.soilbio.2016.12.014

Bokhorst, S., Wardle, D. A., Nilsson, M.-C., and Gundale, M. J. (2018). Contrasting responses of springtails and mites to elevation and vegetation type in the sub-Arctic. *Pedobiologia* 67, 57–64. doi: 10.1016/j.pedobi.2018.02.004

Bokhorst, S., Convey, P., and Aerts, R. (2019). Nitrogen inputs by marine vertebrates drive abundance and richness in Antarctic terrestrial ecosystems. *Curr. Biol.* 29, 1721–1727. doi: 10.1016/j.cub.2019.04.038

Booth, R. G., and Usher, M. B. (1984). Arthropod communities in a Maritime Antarctic Moss-Turf habitat: Effects of the Physical and Chemical Environment. *J. Anim. Ecol.* 53, 879–893. doi: 10.2307/4665

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., et al. (2014). Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecol. Monogr.* 84, 45–67. doi: 10.1890/13-0133.1

Convey, P., Pugh, P. J. A., Jackson, C., Murray, A. W., Ruhland, C. T., Xiong, F. S., et al. (2002). Response of Antarctic terrestrial microarthropods to long-term climate manipulations. *Ecology* 83, 3130–3140. doi: 10.1890/0012-9658(2002)083[3130:ROATMT]2.0.CO;2

Cornelissen, J. H. C., Lang, S. I., Soudzilovskaia, N. A., and During, H. J. (2007). Comparative cryptogam ecology: A review of bryophyte and lichen traits that drive biogeochemistry. *Ann. Bot.* 99, 987–1001. doi: 10.1093/aob/mcm030

Cutz-Pool, L. Q., Palacios-Vargas, J. G., Cano-Santana, Z., and Castano-Meneses, G. (2010). Diversity patterns of Collembola in an elevational gradient in the NW slope of Iztaccihuatl volvano, State of Mexico, Mexico. *Entomol. News* 121, 249–261. doi: 10.3157/021.121.0306

de Bello, F., Lavorel, S., Albert, C. H., Thuiller, W., Grigulis, K., Dolezal, J., et al. (2011). Quantifying the relevance of intraspecific trait variability for functional diversity. *Methods Ecol. Evol.* 2, 163–174. doi: 10.1111/j.2041-210X.2010.00071.x

Elmendorf, S. C., Henry, G. H. R., Hollister, R. D., Bjork, R. G., Bjorkman, A. D., Callaghan, T. V., et al. (2012). Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. *Ecol. Lett.* 15, 164–175. doi: 10.1111/j.1461-0248.2011.01716.x

Filser, J. (2002). The role of Collembola in carbon and nitrogen cycling in soil. *Pedobiologia* 46, 234–245. doi: 10.1078/0031-4056-00130

Fjellberg, A. (1998). *The Collembola of Fennoscandia and Denmark Part 1: Poduromorpha* (Brill: Leiden).

Fjellberg, A. (2007). *The Collembola of Fennoscandia and Denmark Part 2: Entomobryomorpha and Symphypleona* (Brill: Leiden).

Francisco,, Francisco, A., López, D., Grego, R. D., Buma, B., Carvajal, D., et al. (2021). *Climatic gradients and their high influences on terrestrial ecosystems of the Cape Horn Biosphere Reserve, Chile* (Anales del Instituto de la Patagonia), 49.

Gisin, H. (1943). [amp]]Ouml;kologie und lebensgmeinschaften der Collembolen im Schweizer exkursiongebiet Basels. *Rev. Suisse. Zool.* 50, 131–224.

Greene, S. W. (1964). "The vascular flora of South Georgia," in *British Antarctic Survey Scientific Reports*, 45.

Handa, I. T., Aerts, R., Berendse, F., Berg, M. P., Bruder, A., Butenschoen, O., et al. (2014). Consequences of biodiversity loss for litter decomposition across biomes. *Nature* 509, 218–221. doi: 10.1038/nature13247

Hasegawa, M., Ito, M. T., and Kitayama, K. (2006). Community structure of oribatid mites in relation to elevation and geology on the slope of Mount Kinabalu, Sabah, Malaysia. *Eur. J. Soil Biol.* 42, 191–196. doi: 10.1016/j.ejsobi.2006.07.006

Heckman, C. W. (2001). *Encyclopedia of South American aquatic insects: Collembola* (Dordrecht: Springer-Science+Business Media).

Hishi, T., Uemori, K., Tashiro, N., and Nakamura, T. (2024). Asymmetric environmental selection on intraspecific body size in Collembola communities along an elevational gradient in northern Japan. *Eur. J. Soil Biol.* 120, 103596. doi: 10.1016/j.ejsobi.2024.103596

Hodkinson, I. D. (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biol. Rev.* 80, 489–513. doi: 10.1017/S1464793105006767

Hodkinson, I. D., Webb, N. R., Bale, J. S., Block, W., Coulson, S. J., and Strathdee, A. T. (1998). Global change and Arctic ecosystems: Conclusions and predictions from experiments with terrestrial invertebrates on Spitsbergen. *Arctic. Alpine. Res.* 30, 306–313. doi: 10.2307/1551978

Illig, J., Norton, R. A., Scheu, S., and Maraun, M. (2010). Density and community structure of soil- and bark-dwelling microarthropods along an altitudinal gradient in a tropical montane rainforest. *Exp. Appl. Acarol.* 52, 49–62. doi: 10.1007/s10493-010-9348-x

IPCC (2023). *Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge: Cambridge University Press).

Jing, S., Solhøy, T., Huifuà, W., Vollan, I., and Rumei, X. (2005). Differences in Soil Arthropod Communities along a High Altitude Gradient at Shergyla Mountain, Tibet, China. *Arctic. Antarctic. Alpine. Res.* 37, 261–266. doi: 10.1657/1523-0430(2005)037[0261:DISACA]2.0.CO;2

Kampichler, C., and Bruckner, A. (2009). The role of microarthropods in terrestrial decomposition: a meta-analysis of 40 years of litterbag studies. *Biol. Rev.* 84, 375–389. doi: 10.1111/j.1469-185X.2009.00078.x

Kardol, P., Reynolds, W. N., Norby, R. J., and Classen, A. T. (2011). Climate change effects on soil microarthropod abundance and community structure. *Appl. Soil Ecol.* 47, 37–44. doi: 10.1016/j.apsoil.2010.11.001

Krab, E. J., Oorsprong, H., Berg, M. P., and Cornelissen, J. H. C. (2010). Turning northern peatlands upside down: disentangling microclimate and substrate quality effects on vertical distribution of Collembola. *Funct. Ecol.* 24, 1362–1369. doi: 10.1111/j.1365-2435.2010.01754.x

Kuznetsova, N. A. (2006). Long-term dynamics of Collembola in two contrasting ecosystems. *Pedobiologia* 50, 157–164. doi: 10.1016/j.pedobi.2005.12.004

Lamoncha, K. L., and Crossley, D. A. (1998). Oribatid mite diversity along an elevation gradient in a southeastern Appalachian forest. *Pedobiologia* 42, 43–55. doi: 10.1016/S0031-4056(24)00390-1

Leinaas, H. P. (1983). Winter strategy of surface dewlling Collembola. *Pedobiologia* 25, 235–240. doi: 10.1016/S0031-4056(23)05928-0

Loranger, G., Bandyopadhyaya, I., Razaka, B., and Ponge, J. F. (2001). Does soil acidity explain altitudinal sequences in collembolan communities? *Soil Biol. Biochem.* 33, 381–393. doi: 10.1016/S0038-0717(00)00153-X

Makkonen, M., Berg, M. P., Hal van, J. R., Callaghan, T. V., Press, M. C., and Aerts, R. (2011). Traits explain the responses of sub-arctic Collembola community to climate manipulation. *Soil Biol. Biochem.* 43, 377–384. doi: 10.1016/j.soilbio.2010.11.004

Myers-Smith, I. H., Elmendorf, S. C., Beck, P. S. A., Wilmking, M., Hallinger, M., Blok, D., et al. (2015). Climate sensitivity of shrub growth across the tundra biome. *Nat. Clim. Change* 5, 887–891. doi: 10.1038/nclimate2697

Nash, M. A., Griffin, P. C., and Hoffmann, A. A. (2013). Inconsistent responses of alpine arthropod communities to experimental warming and thermal gradients. *Climate Res.* 55, 227–237. doi: 10.3354/cr01136

Nielsen, U. N., Osler, G. H. R., Campbell, C. D., Burslem, D., and van der Wal , R. (2010). The influence of vegetation type, soil properties and precipitation on the composition of soil mite and microbial communities at the landscape scale. *J. Biogeogr.* 37, 1317–1328. doi: 10.1111/j.1365-2699.2010.02281.x

Oksanen, J. (2015). vegan: community ecology package. *R package version 2: 3.*

Pintado, A., Valladares, F., and Sancho, L. G. (1997). Exploring phenotypic plasticity in the lichen Ramalina capitata: Morphology, water relations and chlorophyll content in north- and south-facing populations. *Ann. Bot.* 80, 345–353. doi: 10.1006/anbo.1997.0453

R-Core-Team (2023). *R: A language and environment for statistical computing* (Vienna: R Foundation for Statistical Computing).

Roos, R. E., van Zuijlen, K., Birkemoe, T., Klanderud, K., Lang, S. I., Bokhorst, S., et al. (2019). Contrasting drivers of community-level trait variation for vascular plants, lichens and bryophytes across an elevational gradient. *Funct. Ecol.* 33, 2430–2446. doi: 10.1111/1365-2435.13454

Roos, R. E., Asplund, J., and van Zuijlen, K. (2021). Covered by a blanket of lichens: how mat-forming lichens affect microclimate and ecological processes. A commentary on: 'Lichens buffer tundra microclimate more than the expanding shrub Betula nana'. *Ann. Bot.* 128, i–iii.

Roos, R. E., Birkemoe, T., Bokhorst, S., Wardle, D. A., and Asplund, J. (2022). The importance of foundation species identity: A field experiment with lichens and their associated micro-arthropod communities. *Basic. Appl. Ecol.* 62, 45–54. doi: 10.1016/j.baae.2022.04.004

Rosenfeld, S., Convey, P., Contador, T., Rendoll, J., Poulin, E., Maturana, C., et al. (2020). "Magallanes, laboratorio del cambio global," in *Estrecho de Magallanes: Tres Descubrimientos, Museo de Chileno de Arte Precolombino*. Eds. R. Rozzi, F. Morello, F. M. and C. Aldunate (Colección Santander, Santiago Chile), 176–193.

Rozzi, R., and Jimenez, J. E. (2013). *Sub-Antarctic Magellanic Ornithology: The first decade of bird studies at Omora Ethnobotanical Park, Cape Horn Biosphere Reserve*. University of North Texas Press.

Sadaka, N., and Ponge, J. F. (2003). Soil animal communities in holm oak forests: influence of horizon, altitude and year. *Eur. J. Soil Biol.* 39, 197–207. doi: 10.1016/j.ejsobi.2003.06.001

Seastedt, T. R. (1984). The role of microarthropods in decomposition and mineralization processes. *Annu. Rev. Entomol.* 29, 25–46. doi: 10.1146/annurev.en.29.010184.000325

Sinclair, B. J., and Chown, S. L. (2005). Deleterious effects of repeated cold exposure in a freeze-tolerant sub-Antarctic caterpillar. *J. Exp. Biol.* 208, 869–879. doi: 10.1242/jeb.01455

Sinclair, B. J., Addo-Bediako, A., and Chown, S. L. (2003a). Climatic variability and the evolution of insect freeze tolerance. *Biol. Rev.* 78, 181–195. doi: 10.1017/S1464793102006024

Sinclair, B. J., Vernon, P., Klok, C. J., and Chown, S. L. (2003b). Insects at low temperatures: an ecological perspective. *Trends Ecol. Evol.* 18, 257–262. doi: 10.1016/S0169-5347(03)00014-4

Sømme, L. (1989). Adaptations of terrestrial arthropods to the alpine environment. *Biol. Rev.* 64, 367–407. doi: 10.1111/j.1469-185X.1989.tb00681.x

Stoy, P. C., Street, L. E., Johnson, A. V., Prieto-Blanco, A., and Ewing, S. A. (2012). Temperature, heat flux, and reflectance of common Subarctic mosses and lichens under field conditions: Might changes to community composition impact climate-relevant surface fluxes? *Arctic. Antarctic. Alpine. Res.* 44, 500–508. doi: 10.1657/1938-4246-44.4.500

Sundqvist, M. K., Sanders, N. J., and Wardle, D. A. (2013). Community and ecosystem responses to elevational gradients: processes, mechanisms, and insights for global change. *Annu. Rev. Ecol. Evol. Syst.* 44, 261–280. doi: 10.1146/annurev-ecolsys-110512-135750

Trekels, H., Driesen, M., and Vanschoenwinkel, B. (2017). How do patch quality and spatial context affect invertebrate communities in a natural moss microlandscape? *Acta Oecol.* 85, 126–135.

Upson, R., Myer, B., Floyd, K., Lee, J., and Clubbe, C. P. (2017). *Field guide to the introduced flora of South Georgia* (Kew: Royal Botanical Gardens, Kew).

van der Vegt, W., and Bokhorst, S. (2024). Bird traits and their nutrient impact on terrestrial invertebrate populations. *Polar. Biol.* 47, 821–832. doi: 10.1007/s00300-023-03161-5

van Dooremalen, C., Berg, M. P., and Ellers, J. (2013). Acclimation responses to temperature vary with vertical stratification: implications for vulnerability of soil-dwelling species to extreme temperature events. *Global Change Biol.* 19, 975–984. doi: 10.1111/gcb.2013.19.issue-3

van Zuijlen, K., Roos, R. E., Klanderud, K., Lang, S. I., and Asplund, J. (2020). Mat-forming lichens affect microclimate and litter decomposition by different mechanisms. *Fungal Ecol.* 44, 100905. doi: 10.1016/j.funeco.2019.100905

Wall, D. H., Bradford, M. A., John, M. G. S., Trofymow, J. A., Behan-Pelletier, V., Bignell, D. D. E., et al. (2008). Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. *Global Change Biol.* 14, 2661–2677. doi: 10.1111/j.1365-2486.2008.01672.x

Widenfalk, L. A., Malmström, A., Berg, M. P., and Bengtsson, J. (2016). Small-scale Collembola community composition in a pine forest soil - Overdispersion in functional traits indicates the importance of species interactions. *Soil Biol. Biochem.* 103, 52–62. doi: 10.1016/j.soilbio.2016.08.006

Wissuwa, J., Salamon, J. A., and Frank, T. (2012). Effects of habitat age and plant species on predatory mites (Acari, Mesostigmata) in grassy arable fallows in Eastern Austria. *Soil Biol. Biochem.* 50, 96–107. doi: 10.1016/j.soilbio.2012.02.025

Wu, Y., Xie, Z., Wan, Z., Ji, Q.-Q., Yang, J., Chen, T.-W., et al. (2023). Elevational changes in canopy Collembola community composition are primarily driven by species turnover on Changbai Mountain, northeastern China. *Biodivers Conserv.* 32, 4853–4872.

Zmudczyńska-Skarbek, K., Bokhorst, S., Convey, P., Gwiazdowicz, D. J., Skubała, P., Zawierucha, K., et al. (2023). The impact of marine vertebrates on polar terrestrial invertebrate communities. *Polar. Biol.* 47, 805–820. doi: 10.1007/s00300-023-03134-8

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Occurrence, residency, and habitat characterization of leopard seals in Chile

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Leopard seals have traditionally been considered Antarctic predators with a Southern Ocean distribution. Historically, sightings north of the Antarctic Polar Front were considered extralimital. However, recent studies suggest a significant presence of leopard seals in subantarctic regions. Here, we assess the spatial occurrence, residency status, and temporal trends of leopard seals in Chile using historical records, stranding reports, standardized monitoring data, photo-identification (photo ID) catalogs, and sightings from four research expeditions. We also characterize glaciers where sightings are concentrated, identifying glaciological and geomorphic attributes that prolong iceberg residency time, which is linked to high leopard seal concentrations. Based on these attributes, we evaluated other potential suitable glacial habitats in Patagonia. We obtained 438 sighting records of leopard seals from 1927 to 2023. Over the last 15 years, we documented a 4-18% annual increase in stranding events reported to national authorities. Most sightings (75%) were concentrated in two hotspots: National Park San Rafael Lagoon, located in Northern Patagonia, and Parry Fjord in Tierra del Fuego. Using photo ID catalogs, we identified 19 resident leopard seals, including 16 multi-year residents observed between 2010-2023 (10 in San Rafael, 6 in Tierra del Fuego) and 3 potential residents (observed multiple months in the same year in Tierra del Fuego). San Rafael monitoring data showed no inter-annual trend, but seasonal trends were observed. We also provide evidence of breeding in Chile, with records of at least 14 pups born and at least two females giving birth in multiple years. Our habitat characterization suggests that calving flux, fjord sinuosity, and fjord width variation are crucial for prolonging iceberg residency in hotspot areas. Based on these attributes, we identified 13 additional fjords in Patagonia as "very likely" suitable for leopard seals. Our study confirms that Patagonia is part of the species' breeding distribution, shifting the paradigm that leopard seals are merely visitors north of the Antarctic Polar Front. Given the limited number of suitable glaciers in Chile and the potential impacts of climate change, our assessment highlights glacial retreat as a major threat for the ecosystem of this pagophilic marine apex predator in South America.

KEYWORDS

***Hydrurga leptonyx*, pagophilic species, Antarctic seal, Southern Ocean pinnipeds, Patagonia biodiversity, species distribution, glaciers**

1 Introduction

Apex predators significantly affect ecosystem structure and function (Paine, 1980; Estes et al., 2011; Ripple et al., 2014). Changes in their abundance and distribution can trigger trophic cascades, affecting ecosystem-level processes such as prey population dynamics and nutrient cycling (Schmitz et al., 2010; Leo et al., 2019). Moreover, due to their high energetic demands and foraging efficiency, a small number of apex predators can rapidly modify the trophic composition of their habitat (Estes et al., 1998; Williams et al., 2004). Therefore, documenting the occurrence of apex predators, as well as monitoring changes in their populations, are crucial for understanding the trophic dynamics and functionality of ecosystems (Paine, 1980; Estes et al., 2011; Ripple et al., 2014).

Leopard seals (*Hydrurga leptonyx*, de Blainville, 1820) are one of the most conspicuous apex predators in the Southern Ocean (reviewed in Bester et al., 2017a). The species' effective population size is estimated at ~24,000 individuals (Bender et al., 2023). They are known to have a broad, generalist diet, including both ectothermic (e.g., krill, cephalopods, fish) and endothermic prey (e.g., seabirds, seals; reviewed in Krause et al., 2020). As Southern Ocean apex predators, leopard seals exert strong top-down control on other Antarctic mesopredators, including Antarctic fur seals (*Arctocephalus gazella*) and penguin species (Boveng et al., 1998; Forcada et al., 2009; Schwarz et al., 2013; Krause et al., 2022) and are responsible for more predation on endothermic prey than any other pinniped worldwide (Walker et al., 1998; Casaux et al., 2009). For instance, Krause et al. (2022) showed that a small number of leopard seals at Cape Shirreff in the South Shetland Islands has caused the local colony of Antarctic fur seal to collapse. Moreover, leopard seal diet can vary according to life history traits (e.g., sex, age, and mass; Krause et al., 2020; Sperou et al., 2023) and seasonality (Hall-Aspland et al., 2005; Krause et al., 2020) and they also exhibit a high degree of individual behavioral plasticity, employing strategies such as ambush tactics, kleptoparasitism, scavenging, and group prey processing (Krause et al., 2015; Krause and Rogers, 2019; Robbins et al., 2019). Together, their varied diet and behavioral plasticity likely enhances leopard seals' resilience to changes in prey availability and abundance.

Historically, leopard seals have been described as an Antarctic species predominately found in pack ice habitats around the Southern Ocean (Southwell et al., 2008; Meade et al., 2015; Bester et al., 2017a). In Antarctica, leopard seals are considered a pagophilic species that rely on sea ice for breeding, molting, and resting (Ray, 1970; Jessopp et al., 2004). However, leopard seals have long been observed in subpolar (and even ice-free) habitats across the South Atlantic, South Pacific, and Southern Indian Oceans, (reviewed in Bester et al., 2017b) and extensively observed in Patagonia (both Argentina and Chile; Rodríguez et al., 2003; Aguayo-Lobo et al., 2011; Cursach et al., 2011; Acevedo and Martinez, 2013; Acevedo et al., 2017). In Chile, some of the sightings have reached extreme northern latitudes such as the Juan Fernandez Archipelago and Easter Islands (Aguayo-Lobo et al., 2011; Hucke-Gaete et al., 2014; Stewart et al., 2020). Generally, sightings of leopard seals outside of the Southern Ocean have been considered as extralimital and were thought to be rare and/or isolated events.

Recently, research on leopard seals across the southern hemisphere has challenged the notion that leopard seals do not reside north of the Antarctic Polar Front (Hupman et al., 2020). Analyses combining historical records with contemporary sightings from subantarctic locations document the year-round presence of leopard seals in New Zealand, with more than 2,700 leopard seal sightings over the last eight centuries (1200-2018; Hupman et al., 2020). Furthermore, more recent data at both Marion Island—a subantarctic South African island—and South Australia suggest an increase in juvenile leopard seal sightings in the last two decades (Shaughnessy et al., 2023; de Bruyn and Ross, pers. comm.) compared to previous decades (Bester et al., 2006).

Similarly, in Chile, both historical and contemporary data demonstrate a centuries-long presence of leopard seals (Aguayo-Lobo et al., 2011; Torrejón et al., 2013; Stewart et al., 2020). For instance, anthropological evidence indicates that leopard seals have been occasional visitors to Easter Island (~3,500 km off the coast of Chile) since the arrival of Polynesians around 800 CE (Hucke-Gaete et al., 2014; Stewart et al., 2020). On the Chilean coast, a review by Aguayo-Lobo et al. (2011) provided compelling evidence of a nearly century-long periodic presence of leopard seals in Chile, documenting 118 sightings of leopard seals from 1927 to 2010. A later study documented a leopard seal in a Patagonian fjord of the Magallanes region in Chile making use of this location multiple times during five months, indicating potential residency (Acevedo and Martinez, 2013). Furthermore, observations of leopard seals since 2012 include sightings of adult-pup pairs and sexual behavior, indicating that mating and parturition also occur in Chilean waters (van der Linde et al., 2022; Kienle et al., 2024). Although these studies challenge the traditional view of leopard seals as episodic visitors in South America, there have been no systematic multi-year studies to examine occurrence and residency patterns of leopard seals in Chile, nor attempts to characterize their habitat outside Antarctica.

The main objective of this study was to determine the historical and current occurrence of leopard seals in Chile and assess residency patterns of individuals in areas with consistent sightings. We hypothesized that some leopard seals exhibit long-term site fidelity to ice-associated areas in Chile. To test this, we analyzed data from published historical sightings records, government reports, monitoring surveys, citizen science initiatives, and conducted boat-based expeditions to evaluate the spatial and temporal distribution of leopard seals from 1927 to 2023. In addition, we conducted a habitat characterization of leopard seals in Chile along with an assessment of potential habitats for the species across Patagonia. We hypothesized that at least half of the marine-terminating glaciers in Chile possess similar characteristics than those where most leopard seal sightings have been reported and may consist of additional suitable habitat. To test this, we characterized key glaciological and geomorphic attributes of marine-terminating glaciers where leopard seal sightings are most frequent and compared these with all other similar glaciers found throughout Chile. Our study thoroughly documents leopard seal occurrence, residency, and identify their potential habitats in Chile; we provide baseline information on the species distribution that would help to understand the impacts of climate change on this ice-dependent predator.

2 Methods

2.1 Historical sighting records

We conducted a literature review of peer-reviewed publications documenting the presence of leopard seals in Chile through Google Scholar and Web of Science using the following search terms: "leopard seals Chile", "*Hydrurga leptonyx* Chile", "leopard seals South America", "*Hydrurga leptonyx* South America", "leopard seals Patagonia", "leopard seals Tierra del Fuego", "leopard seals San Rafael", and "leopard seals Easter Island" from 2009 to 2023. We completed literature searches in both English and Spanish. We searched for additional peer-reviewed publications and grey literature by reviewing the cited references of each publication. Sightings reported before 2009 were previously reviewed and reported by Aguayo-Lobo et al. (2011). We extracted these sightings from Aguayo-Lobo et al. (2011) with the following consideration: Aguayo-Lobo et al. (2011) added up the recorded number of animals from all sightings to provide a "total number of leopard seals" in Chile. However, their methodology could not determine if the same animals were counted multiple times because seals were not tagged, nor were photo-ID methods used to avoid duplicate counts. Therefore, here we considered every seal from each sighting event as an independent sighting rather than reporting the total number of animals per sighting as did Aguayo-Lobo et al. (2011).

From each publication, we extracted all relevant biological data reported on the individual's life history (sex, age class, molting status), morphometrics (length), substrate (whether a seal was on land, ice, or in the water), location (name of the location, region, and coordinates transformed to decimal degrees), and date. We cross-referenced all data and identified, when possible, repeated individuals that were reported multiple times within the same publication or between publications. We also included any comment reported with each sighting. All sightings from our literature review and Aguayo-Lobo et al. (2011) are hereafter referred to as "Historical Records".

2.2 New sighting records

We compiled new sighting records—records that have not been previously reported in peer-reviewed literature—and referred to as "New Records". These new sighting records include strandings from government reports, monitoring data from San Rafael Lagoon National Park, photo-identification catalogs, and our expeditions.

2.2.1 Stranding data

Marine mammal stranding events in Chile are reported to the National Fisheries and Aquaculture Service of Chile (SERNAPESCA). These stranding events of dead or live animals are reported by citizens. We compiled all leopard seal sighting data publicly available online from SERNAPESCA (Sernapesca, 2023) from 2009 to 2023. SERNAPESCA stranding records up to 2009 were previously reviewed by Aguayo-Lobo et al. (2011).

2.2.2 San Rafael monitoring data

The National Park San Rafael Lagoon (henceforth "San Rafael") located in the Aysén Region (46°40'0" S, 73°58'0" W) is administered by the National Forestry Corporation (CONAF). It is one of the two areas of Chile where leopard seals are frequently observed. In 2013, CONAF began the first and only systematic leopard seal monitoring program for leopard seals in Chile. Each month, a boat with at least two trained observers surveyed the San Rafael lagoon exclusively searching for leopard seals. They documented the presence/absence of leopard seals throughout the entire lagoon. During boat surveys, observers recorded the following information: total number of leopard seals, geographic coordinates, and substrate (i.e., whether each seal was on ice, land, or in the water). When possible, age class (pup, juvenile, adult) and sex were visually assessed. Photographs were collected opportunistically for each seal whenever possible. Due to weather conditions, monitoring was not consistent over time and in some years, it was not conducted every month. The San Rafael monitoring program has near-monthly occurrence records for leopard seals from January 2013 to March 2020. Monitoring was paused after March 2020 due to the COVID-19 pandemic and restarted irregularly in 2021. For this study, we only included data from the period where standardized monitoring was performed (January 2013 to March 2020).

2.2.3 Leopard seal photo-identification catalogs

Leopard seals have unique spot patterns and distinguishable scars that facilitate individual identification from photographs, a method called photo-identification (Photo ID; Forcada and Robinson, 2006; Hupman et al., 2020; Visser et al., 2022; Grabham et al., 2023). We used the photographic data of two Photo ID regional catalogs for identifying individual leopard seals in Chile. The first catalog was developed as part of a citizen science initiative conducted by CONAF in collaboration with tour operators that visit San Rafael. The second catalog was an initiative of this study, conducted in the Magallanes y Antártica Chilena Region of Chile to identify leopard seals in this region. To create these regional catalogs, photographs of leopard seals were solicited from tour operators, local scientists/conservationists, and the general public.

The San Rafael catalog published by CONAF in 2021 (henceforth "SR catalog") was created for leopard seals exclusively observed in San Rafael. San Rafael is visited by hundreds of tourists year-round traveling with local tour operators. Thus, this catalog is the result of continuous sightings reported throughout the year and is composed of pictures collected from tour operators and tourists almost every month of the year.

The Tierra del Fuego catalog (henceforth "TF catalog") was created from leopard seals observed across the southwest portion of Patagonia (between latitudes 48°36´S and 56°30´S and longitudes 66°25´ and 75°40´W). Although most of the sightings are concentrated in the Coastal Marine Protected Area of Multiple Uses (Spanish acronym: AMCP-MU) "Seno Almirantazgo" (Almirantazgo Sound, 54°31'0" S, 69°15'0" W), some of the sightings are outside this geographical area (but still within western Tierra del Fuego) therefore, the entire area is henceforth referred to as "Tierra del Fuego".

We manually compared each leopard seal photograph to all others in our photographic database and used unique identifying spot patterns and scars to visually determine matches. A match was found when the same individual seal was documented in two or more images (or sets of images). Each image or set of images was visually inspected and manually manipulated if needed to maximize matching success; specifically, we cropped, and rotated each photo, and adjusted the exposure when necessary (Acevedo and Martinez, 2013; Cheeseman et al., 2017). Manual matching was validated by a second expert reviewer and if a disagreement occurred, a third expert reviewer decided. When available, we then extracted the location and dates from the metadata associated with each image and double checked the accuracy of the dates and locations with the photographer or project leaders of the initiatives. In pictures without location data, we estimated the coordinates using the location name provided by the photographer/project leaders. We used photographs to assign sex and age class when it was visually possible. Age class was simplified in three categories (pup, juvenile, adult) and estimated only when it was visually evident (pups: presence of lanugo or next to their mothers; juveniles: with no lanugo, unlikely to be accompanied by a mother, less than 2.0 meters, thin, and with minimum scars; and adults: over 2.5 meters with presence of several scars. Adapted from Laws, 1957; Forcada and Robinson, 2006; Hupman et al., 2020; van der Linde et al., 2022). We assigned each matched seal a unique identification number (Seal ID) and added them to each catalog. We added unmatched seals or leopard seals that did not have any matches to the database if there were high-quality, usable images that would allow for manually matching of the seal in the future as additional images are incorporated into each catalog.

We used the photographs of matched seals to identify resident individuals at each location. We classified a seal as 'Resident' if it was observed at least once per year across multiple years. We classified a seal as 'Potential Resident' if it was observed multiple months within a single year but not sighted in more than one year, and as 'Undetermined' if observers confirmed that the seal was not a match to any of the previously cataloged seals (thus, new to the catalog) and it had only been reported once. We assessed body condition (severe, poor, good, or excellent) of all cataloged individuals (i.e., Resident, Potential Resident and Undetermined) based on the last year with photographs available that included high resolution full-body profiles (following Hupman et al., 2020; see Supplementary Table 1).

In contrast to the systematic monitoring efforts in San Rafael (see Section 2.2.2 above), the remote and isolated characteristics of Tierra del Fuego make monitoring leopard seals extremely difficult. As a result, no standardized monitoring program exists. Furthermore, tour operators visit this location sporadically, mostly during the austral summer. Since 2010 limited expeditions have been conducted every few years by the Wildlife Conservation Society (WCS) to the Almirantazgo Sound, where observers record leopard seal sightings when possible. However, these expeditions, are not comparable to the regular visits of the monitoring program in San Rafael.

The contrasting differences in the accessibility and monitoring efforts between San Rafael and Tierra del Fuego led us to incorporate the catalog sightings data on our sighting database following different criteria. Due to limited records from Tierra del Fuego, we include all sightings reported by both major tour operators and past WCS expeditions. In contrast, because San Rafael has a monitoring program, first we included all the monitoring data in our database and then compared the monitoring photographs with the SR catalog to identify known individuals when possible. Next, we used additional San Rafael sightings from the SR catalog to provide data for months where: (1) CONAF did not perform any monitoring due to weather/logistic constraints; or (2) CONAF monitoring efforts found no leopard seals in a given month but photos from tour operators confirmed the presence of one or more leopard seals from other days of that month. We applied these criteria to San Rafael to avoid including repeated records of the same individual within a month. This criterion was applied only to include records in our overall database as we aimed to ascertain the degree of residency of some of the individuals within each year. However, the additional data extracted from the catalogs was not included in the analysis of seasonal trends of the San Rafael standardized monitoring data (section 3.3).

2.2.4 Expeditions

Here we report new leopard seal sightings data from four recent expeditions in Chile that were targeting leopard seals: two to Tierra del Fuego and two to San Rafael. Chronologically, the first expedition was led by WCS to the Almirantazgo Sound, Tierra del Fuego in November 2021 (henceforth “TF-21”). The next two expeditions were to San Rafael in December 2022 (henceforth “SR-22”) and November 2023 (henceforth “SR-23”). The fourth expedition was to Tierra del Fuego in December 2023 (henceforth “TF-23”). During each expedition, we navigated the area daily and recorded total counts of leopard seals, geographic coordinates, habitat characteristics (i.e., whether the seal was on ice, land, or in the water) and visually assess age class/sex as was done for the catalogs. We identified known individuals from the SR and TF catalogs and our national photographic database when possible. All daily records of leopard seals were added to a compiled table of sightings except when an individual was observed multiple times on the same day.

We compiled the historical and new sightings data and associated metadata in a table of sightings (Supplementary Table 2). To visually assess the distribution of leopard seal sightings across Chile, we used the GPS coordinates for each record or estimated coordinates from Google Earth based on the name of the location provided. We then created a heatmap of all leopard seal sightings in Chile using ArcGIS Pro.

2.3 National and local population trends in leopard seal counts

We evaluated population trends in leopard seals using two datasets. First, we analyzed the 15 years of stranding data to examine temporal trends in the number of leopard seals reported across Chile by evaluating the rate of change of stranding events. We excluded records where the same individual was reported multiple times both temporally and spatially (e.g. same animal observed twice in a week in adjacent beaches). Second, we used the San Rafael standardized monitoring data to identify seasonal and inter-annual leopard seal trends at this location. To accomplish this, we used a model-based approach to generate estimates associated with temporal parameter effects, with confidence intervals based on Monte Carlo (MC) simulations to enhance the robustness of model parameters.

2.3.1 Rate of change of stranding events

We analyzed the rate of change of stranding events reported over time from the stranding data using a generalized linear model with a Poisson distribution. To ensure robust parameter estimation, we employed MC simulation with 10,000 iterations. This allowed us to estimate the uncertainty associated with the expected rate of change of stranding events. Additionally, we applied bias correction and acceleration to the resulting 95% confidence intervals (CI 95%) from the simulated distribution of parameter values to enhance the accuracy of our inferences.

2.3.2 Seasonal and inter-annual population trends in San Rafael

We analyzed seasonal variations in leopard seal counts at San Rafael using generalized additive models (GAMs) to delineate linear and non-linear relationships of seal counts with time. These models incorporated year and month as explanatory variables and the count of observed animals as our response variable. The effect of year was estimated as a linear variable. However, to capture non-linear seasonal monthly trends on the leopard seal counts the effect of month was estimated using a smooth function (penalized regression splines; Wood, 2011). The estimation of smoothing parameters was achieved through restricted maximum likelihood method, using cyclic penalized cubic regression splines as the basis to model cyclic temporal dimensions (Wood, 2017).

To characterize seasonal trends in terms of linear seasonal variations, we computed first derivatives using finite differences method from the predicted count of animals by using grid approximation, accompanied by CI 95% (Makowski et al., 2020). Robustness confidence intervals were estimated via bootstrap resampling using 10,000 MC simulations.

We evaluated inter-annual trends in leopard seal counts in San Rafael using GAMs. We computed the linear and quadratic effect of year on leopard seal counts using non-orthogonal polynomial contrasts, while holding constant the intra-annual seasonal effect estimated using cyclic splines based on the number of months that the monitoring was performed, which vary per year. This approach facilitated the exploration of potential linear and quadratic trends associated with variations in observed animal counts across years. Due to irregular and interrupted monitoring in 2020, we excluded this year from the analysis.

For both stranding and monitoring datasets, all analyses were performed using R (version 4.3.2). GAMs and corresponding model estimates were conducted using the *mgcv* and *modelbased* packages (Wood, 2017; Makowski et al., 2020). Complementary R packages were used for data visualization purposes (Wickham, 2016; Lüdecke et al., 2021).

2.4 Glacial habitat characterization

Most leopard seal sightings in Chile involve animals hauling out on icebergs produced by glaciers whose ice fronts are in contact with water (sea or lake), suggesting the importance of marine-terminating glaciers with high iceberg production (Aguayo-Lobo et al., 2011). Patagonia is the region that spans the southernmost tip of South America between ~40° and ~56° S. This territory exhibits the largest number of glaciers on the continent, including the largest icefields of the southern hemisphere outside Antarctica; the so-called Northern and Southern Patagonian Icefields (Soteres et al., 2023). Consequently, we focused our glacial habitat characterization on the glacial basins that produce icebergs in the two areas where leopard seals are most frequently sighted in Patagonia: the San Rafael glacier at San Rafael, and the Parry Fjord glaciers in Tierra del Fuego. In both places, the occurrence and local residence time of icebergs appear to be a critical leopard seals haul out substrate. Our methodological approach involves assessing iceberg production and identifying potential geomorphic traps (i.e., specific landforms and morphometric parameters within the glacial basins and fjords that can prolong the local presence of icebergs; Figure 1).

Calving flux is the volume of ice detached from the front of water-terminating glaciers (i.e., marine-terminating and lake-terminating glaciers henceforth "marine-terminating glaciers") per unit of time and it is considered a key metric to estimate ice loss due iceberg formation (Meier and Post, 1987; Evans et al., 2022). We calculated calving flux for the San Rafael glacier and Parry Fjord glaciers following Meier and Post (1987); see the detailed methodology in the Supplementary Methods in Supplementary Material).

Calving flux account for iceberg production but iceberg residence timing is also partly affected by local geomorphology and morphometry of the fjords, particularly sinuosity (i.e. the degree of linearity of a fjord) and fjord width variations often linked to the presence of glacial landforms within the fjords that could promote iceberg stranding. Therefore, we assessed fjord sinuosity following Vérité et al. (2023); see Supplementary Methods in Supplementary Material for details) and fjord width variations by calculating a ratio between the maximum and minimum width of each glacier (Figure 1; minimum and maximum values used can be found in Supplementary Table 4 in Supplementary Material).

We then produced a simplified geomorphic map that broadly depicts the spatial arrangement of the most prominent landforms of glacial origin, such as moraine ridges and outwash plains, for the San Rafael glacier and the Parry Fjord glacial basins. We analyzed satellite imagery (Figure 1) provided by Google Earth (2016 CNES/SPOT, ~15 m spatial resolution), ESRI Imagery (2017, TerraColor, ~15 m spatial resolution and SPOT, ~2.5 m spatial resolution), and ALOS PALSAR digital elevation models (~12.5 m spatial resolution) to examined basic morphometric attributes and to map local geomorphic features of the San Rafael glacier at San Rafael and Parry Fjord glaciers at Tierra del Fuego using ArcGIS 10.4. We followed the identification criteria used in prior geomorphic maps from Patagonia by Leger et al. (2020); Soteres et al. (2020) and Soteres et al. (2022). Preliminary maps based on remote data were subsequently ground-truthed during expeditions.

2.5 Glacial suitability analysis for leopard seals in Patagonia

We used the glaciological (calving flux) and geomorphic attributes (ice-marginal landforms, fjord sinuosity, and fjord width variations) calculated for the San Rafael and Parry Fjord glaciers as reference values to evaluate the suitability of other glacial basins and fjords as potential habitats for leopard seals across Patagonia. First, we identified marine-terminating glaciers in the region according to the Chilean glacier inventory (DGA, 2022) and checked them visually in recent satellite imagery to assure their ice fronts are still resting on water. We focused our efforts on the major icefields and ice caps in Patagonia, specifically (1) the Northern Patagonian Icefield (NPI; ~46.5-47.5°S), (2) the Southern Patagonian Icefield (SPI; ~48.3-51.5°S), (3) Gran Campo Nevado (GCN; ~52.8°S), (4) Isla Santa Inés (ISI; ~53.8°S), and (5) the Cordillera Darwin Icefield (CDI; ~54.6°S). Second, we calculated the calving flux, fjord sinuosity, and fjord width variations of all marine-terminating glaciers identified following the described methods of the previous section (See also Supplementary Methods and Supplementary Table 4 in Supplementary Material). Third, we calculated the added weighted mean of the normalized values for calving flux, width, and sinuosity for all marine-terminating glaciers, and applied a proportional calculated weighted estimates (CWE) for each parameter (i.e. calving flux, fjord width, and fjord sinuosity) that was determined based on the estimated influence of each parameter over the iceberg retention time in San Rafael and the Parry Fjord Glaciers. Finally, fjords were classified as "very likely," "likely," and "less likely" to host leopard

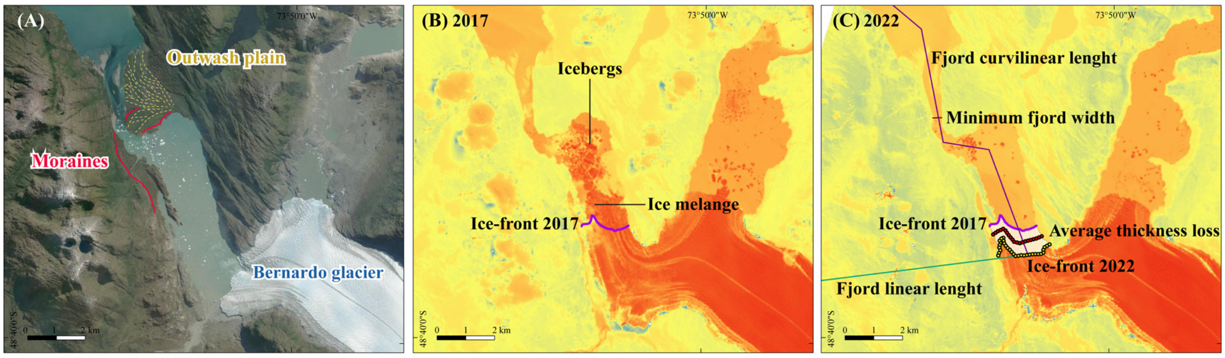

FIGURE 1
Schematic representation of variables used to calculate calving flux, fjord sinuosity, and fjord width in the study glaciers. **(A)** Satellite image illustrating geomorphic features (outwash plain and moraines) that affect the sinuosity and width of a fjord. Landsat 8 imagery in 2017 **(B)** and 2022 **(C)** with vectors used to calculate glacial and geomorphic attributes (list of Landsat 8 images used in ice flux calculation can be found in Supplementary Table 3).

seals, based on individual weighted mean values compared to the average weighted mean threshold determined from San Rafael Glacier and Parry Fjord.

3 Results

We documented 438 leopard seal sighting records in Chile from 1927 to 2023 (Supplementary Table 2). Two-thirds of the sightings were new records (66%, n=291), and one-third were historical records (34%, n=147). The new records included: (1) 15 years of SERNAPESCA stranding records (n=44), (2) San Rafael monitoring data (n=88), (3) Photo ID catalogs (n=108; SR catalog: 62 records, TF catalog: 46 records), and (4) expeditions data (n=51; TF-21: 4 records, TF-23: 15 records, SR-22: 12 records, SR-23: 20 records). From these sighting records, we were able to identify at least 41 leopard seal individuals that were observed two or more times.

Most leopard seal sightings (86%) were in Patagonia (between ~40° and ~56° S) where marine-terminating glaciers are found (Figure 2A). Two Patagonian locations accounted for 74% of sightings: San Rafael (15 historical and 183 new records) and Parry Fjord in Tierra del Fuego (63 historical and 63 new records). When substrate was reported for sightings in Patagonia (i.e. if the seal was observed on land, ice, or water), 84% of sightings were animals hauled out on floating ice.

Chile is among the few countries with recorded sightings of leopard seal pups outside Antarctica (van der Linde et al., 2022). The earliest pup record is from 2012 (Acevedo et al., 2017). Here, we documented 14 pups (Tierra del Fuego: 4, San Rafael: 10). Eight of these pup records were reviewed by van der Linde et al. (2022), and six are from our new records (four from the San Rafael monitoring program, two from our expeditions). In Chile, all pup records occurred in Patagonia. Four pups were initially observed alive (one was later found dead), seven were reported dead, and three had no recorded status.

Of the 438 records of leopard seals in Chile, there were 404 confirmed age class records; 75% of these leopard seals were adults and 25% were immature individuals (i.e., pups and juveniles). In Patagonia specifically, there were 352 confirmed age class records; 82% (n = 289) of these leopard seals were adults and 18% (n =63) were immature individuals. In contrast, among the 52 age class records north of Patagonia, only 23% (n =12) were adults, and 77% (n =40) were immature, including the northernmost sightings from Easter Island (Figure 2B; Stewart et al., 2020).

3.1 Temporal trends of stranding data

Leopard seal stranding numbers in Chile are low. There was a mean of two leopard seal strandings per year, with a range from 0-5

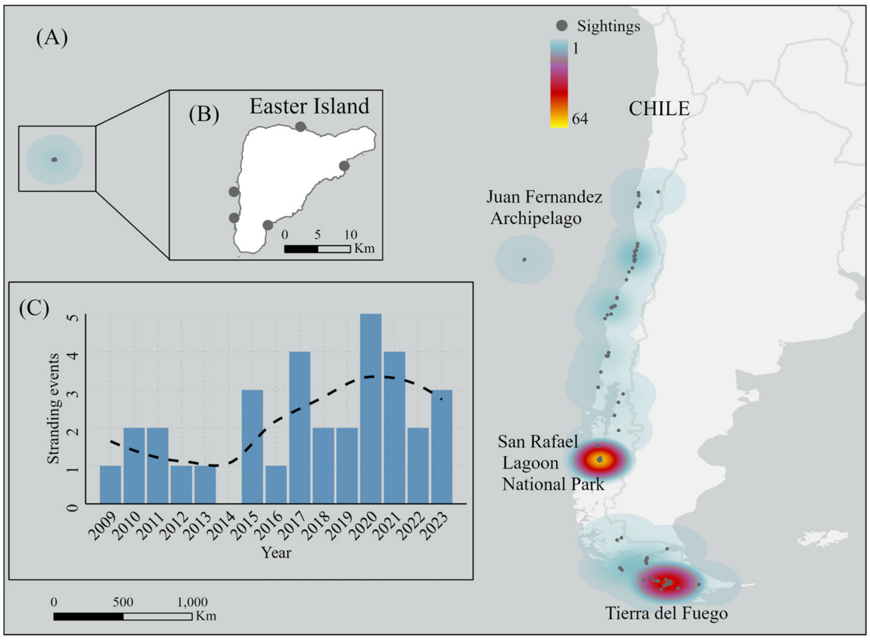

FIGURE 2
Historical and contemporary leopard seal sightings (gray circles) in Chile. **(A)** Heatmap of all 438 sightings, including those in Chilean oceanic insular territories (i.e., Juan Fernandez and Easter Island). **(B)** Map of Easter Island highlighting the northernmost sightings reported for Chile reviewed in Stewart et al. (2020) with some of the geolocations estimated from descriptions. **(C)** Annual leopard seal stranding events reported to the National Service of Fisheries and Aquaculture of Chile (SERNAPESCA) from 2009 to 2023. Stranding data are publicly available (Sernapesca, 2023). The dashed black line indicates the trend of the stranding data. Map Sources: Esri, DeLorme, HERE, MapmyIndia.

leopard seals reported annually between 2013-2019 (Figure 2C). Bias corrected and accelerated intervals from MC samples estimated from simulation-based Poisson regression suggested a 4-18% increase in leopard seal stranding events rate annually; with 99.9% of the density mass of MC outcomes indicating a probability of > 0 and supporting an increase in the number of stranding events of leopard seals over time (Figure 3).

3.2 Resident leopard seals

We reviewed ~2,500 photographs of leopard seals (catalogs and expedition photographs) from the two locations where most sightings were concentrated (i.e., San Rafael and Tierra del Fuego). 19 leopard seals had recurrent sightings over time (Table 1; Figure 4). After cross-referencing all images between San Rafael and Tierra del Fuego, we found no evidence of the same individuals visiting both locations. Both catalogs can be downloaded from the Supplementary Data in Supplementary Material.

3.2.1 San Rafael

We reviewed ~1,500 photographs of leopard seals from San Rafael taken from 2009 to 2023. We identified 10 leopard seal individuals with recurrent sightings over time (Table 1; Figure 4; Supplementary Data in Supplementary Material) and classified all 10 individuals as residents. The sex ratio was nearly 1:1, with four females and six males. In years when body condition was assessed, all seals were in excellent body condition (Supplementary Table 1). The longest resident leopard seals were two males (LSR05 and LSR06). LSR05 was observed five different years from 2009-2020, and LSR06 was observed 10 different years from 2012-2023. Females LSR02 and LSR01 each gave birth at least twice (LSR02 in October 2013 and October 2017; LSR01 in October 2021 and October 2023; Figure 4). The only year with multiple pup sightings was in 2019 when two pups were sighted simultaneously but mothers were not identified. Both sightings were reported on the same day (January 14th, 2019) by the same observers. Pup records (n=10) were all reported from mid-October to the end of November (Supplementary Table 2).

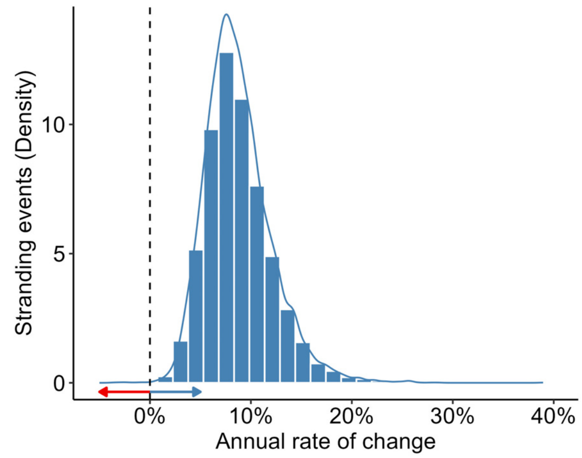

FIGURE 3
Probability distribution showing the annual rate of change in stranding events across Chile from Monte Carlo simulations of the Poisson model. The vertical dashed line indicates the null rate of change. The red arrow illustrates the probability of a decrease in stranding events (<0.1% of total Monte Carlo samples), while the blue arrow indicates the probability of an increase (>99.9% of total Monte Carlo samples).

3.2.2 Tierra del Fuego

We reviewed ~1,000 photographs from Tierra del Fuego between 2010 and 2023. We identified nine leopard seals with recurrent sightings over time (Table 1; Figure 4; Supplementary Data in Supplementary Material). We identified six residents, three potential residents, and five undetermined individuals. The longest resident recorded was a female (TF01) observed in eight different years over a 14-year period (2010-2023). We identified more females than males at this location (females: 10, males: 4). All seals were in excellent body condition in all years when body condition was assessed (Supplementary Table 2). Female TF01 gave birth in November 2017, and female TF13 gave birth in November 2021 (Figure 4). Pup records (n=4) were all reported between the beginning of October to beginning of December (Supplementary Table 1).

3.3 Standardized monitoring data of San Rafael

3.3.1 Seasonal trends

We analyzed monthly trends on the observed number of seals in San Rafael from 2013 to 2019. The highest number of leopard seals reported during one monitoring day was seven (January 2019). After accounting for between-year differences, we found a non-linear effect ($p = 0.003$), suggesting seasonal intra-annual variations in the number of leopard seals (Figure 5). Specifically, the non-linear seasonal pattern reveals two distinct intra-annual trends. First, there was a significant tendency of seal counts to decrease from March (Month = 3.2, linear slope β = -0.31, CI95% [-0.67, -0.00]) to May (Month = 5.3, β = -0.29, CI95%[-0.61, -0.01]), with counts likely remaining low throughout the austral winter (Figure 5). Second, we found a significant tendency of seal counts to increase in late August (Month = 8.5, linear slope β = 0.27, CI95% [0.00, 0.55]) to late October (Month = 9.8, β = 0.40, CI95%[0.00, 0.83]). These higher counts persisted through late austral spring (November-December; Figure 5).

3.3.2 Inter-annual trends

When analyzing the inter-annual trends from the San Rafael monitoring data, we found no significant negative (β = -0.17, CI95% [-0.98, 0.63], $p = 0.666$) or positive quadratic or linear trends (β = 0.28, CI95% [-0.56, 1.11], $p = 0.513$) even after accounting for seasonal variations (Figure 6).

TABLE 1 Summary of sighting records and biological information compiled from the two catalogs of leopard seals from Chile and our four expeditions.

Catalog Name	Seal ID	First Record	Last Record	Time Span Recorded	No. Years Recorded	Total Months Recorded	Latitude (S)	Longitude (W)	Location	Age	Sex	Residency Status	Original Source
SR	LSR01	2014	2023	10	8*	15	-46.67	-73.87	San Rafael Lagoon	Adult	F	Resident	CONAF 2021
SR	LSR02	2012	2020	9	8	11	-46.67	-73.87	San Rafael Lagoon	Adult	F	Resident	CONAF 2021
SR	LSR03	2013	2023	11	5	9	-46.67	-73.87	San Rafael Lagoon	Adult	M	Resident	CONAF 2021
SR	LSR04	2013	2023	11	8	15	-46.67	-73.87	San Rafael Lagoon	Adult	M	Resident	CONAF 2021
SR	LSR05	2009	2020	12	5	7	-46.67	-73.87	San Rafael Lagoon	Adult	M	Resident	CONAF 2021
SR	LSR06	2012	2023	12	10	17	-46.67	-73.87	San Rafael Lagoon	Adult	M	Resident	CONAF 2021
SR	LSR07	2012	2022	11	7	10	-46.67	-73.87	San Rafael Lagoon	Adult	F	Resident	CONAF 2021
SR	LSR08	2014	2023	10	7	11	-46.67	-73.87	San Rafael Lagoon	Adult	M	Resident	CONAF 2021
SR	LSR09	2019	2020	2	2	2	-46.67	-73.87	San Rafael Lagoon	Adult	M	Resident	CONAF 2021
SR	LSR10	2019	2023	5	4	5	-46.67	-73.87	San Rafael Lagoon	Adult	F	Resident	CONAF 2021
TF	TF01	2010	2023	14	8	11	-54.65	-69.45	Parry Fjord/Cuevas	Adult	F	Resident	This Study
TF	TF02	2010	2018	9	4	5	-54.65	-69.45	Parry Fjord/Cuevas	Adult	F	Resident	This Study
TF	TF03	2016	2016	1	1	1			Agostini Fjord	Adult	F	Undetermined	This Study
TF	TF04	2017	2017	1	1	2	-54.65	-69.45	Parry Fjord/Cuevas	Adult	F	Potential Resident	This Study
TF	TF05	2017	2017	1	1	2	-54.65	-69.45	Parry Fjord/Cuevas	Adult	M	Potential Resident	This Study
TF	TF06	2018	2021	4	3	3	-54.65	-69.45	Parry Fjord/Cuevas	Adult	F	Resident	This Study
TF	TF07	2018	2018	1	1	1	-54.65	-69.45	Parry Fjord/Cuevas	Adult	F	Undetermined	This Study
TF	TF08	2018	2018	1	1	2	-54.65	-69.45	Parry Fjord/Cuevas	Adult	F	Potential Resident	This Study
TF	TF09	2017	2017	1	1	1	-54.65	-69.45	Parry Fjord/Cuevas	Adult	F	Undetermined	This Study
TF	TF10	2018	2021	4	2	2	-54.65	-69.45	Parry Fjord/Cuevas	Adult	M	Resident	This Study
TF	TF11	2019	2019	1	1	1	-54.65	-69.45	Parry Fjord/Cuevas	Adult	M	Undetermined	This Study
TF	TF12	2017	2021	5	2	2	-54.65	-69.45	Parry Fjord/Cuevas	Adult	M	Resident	This Study
TF	TF13	2021	2021	1	1	1	-54.65	-69.45	Parry Fjord/Cuevas	Adult	F	Undetermined	This Study
TF	TF14	2015	2023	9	2	2	-54.65	-69.45	Parry Fjord/Cuevas	Adult	F	Resident	This Study

"Time Span Recorded" indicates the period between the first and last sighting of each individual seal. "No. Years Recorded" shows the total number of years within this period for which photographic records are available. "Total Months Recorded" details the total number of months with records within those years. Latitude and Longitude coordinates were estimated from the location names and are provided in decimal degrees. Age and Sex were visually assessed for each leopard seal. Residency Status is categorized as follows: "Resident" individuals observed one or more times per year across multiple years; "Potential Resident" those seals observed multiple months within a single year but not sighted in more than one year; and "Undetermined" animals with sufficiently clear photographs to confirm that they are not any other cataloged animals but have been reported only once. The "Original Source" column specifies the program responsible for each catalog's creation— the National Forestry Corporation (CONAF 2021) in San Rafael, and the initiative behind this manuscript (labeled as "This Study") for those leopard seals from Tierra del Fuego. Both catalogs are available in the Supplementary Data in Supplementary Material. *There is photographic record of LSR01 of seven years, however, we added an additional record from 2021 after verifying with co-authors of van der Linde et al. (2022) that the mother of a pup reported in that study was LSR01 (Fernandez-Ferrada pers. comm.).

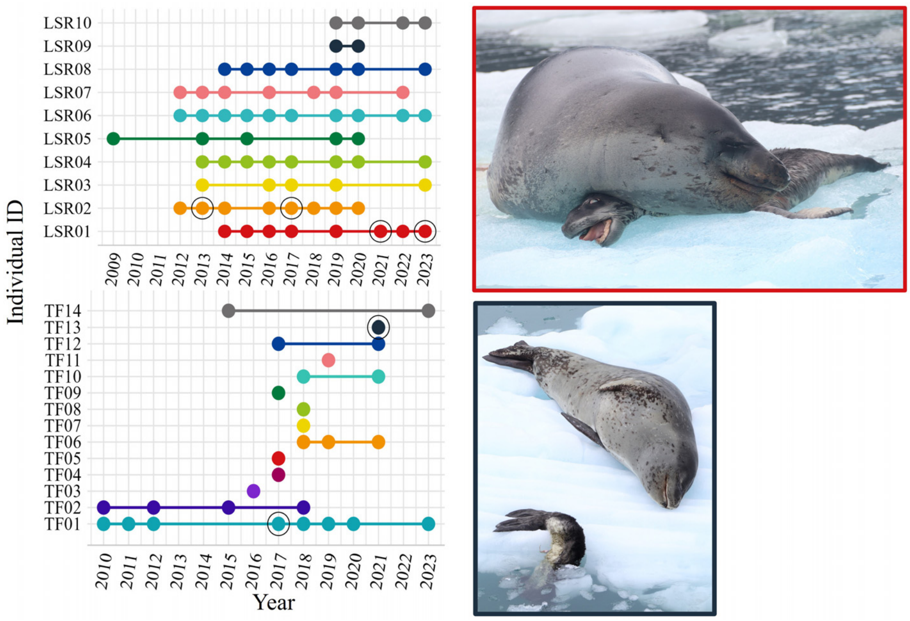

FIGURE 4
Annual presence of leopard seals in San Rafael (upper panel) from the photo-identification catalogs (until 2020) and expeditions (2021-2023). In the plots, each color represents a unique individual. Dots show years with photographic record of each individual. Colored lines connecting the dots represent the total time span that each seal has been observed. The 2021 sighting of LSR01 is the only data point that is not from the SR catalog or expeditions; the female was validated as the mother of a reported pup by van der Linde et al. (2022). Black unfilled circles show the year adult females were observed with pups. The right panels display photographs of the reported pups during our expeditions (both dead): the upper right picture is female LSR01 from San Rafael with her pup (SR-23), and the lower right picture is female TF13 from Tierra del Fuego with her pup (TF-21). Photo credits: Renato Borras-Chavez.

3.4 Glacial habitat characterization

The contrasting characteristics between the San Rafael glacier in the NPI and the glaciers in Parry Fjord in the CDI — particularly the Darwin and Cuevas glaciers — make these locations ideal for identifying characteristics that may define optimal glacial habitats for leopard seals (Figure 7A). Located at the northern (San Rafael) and southern (Parry Fjord glaciers) limits of Patagonia these glaciers experience different climate conditions, glaciological regimes, and topographical features (Sagredo and Lowell, 2012; Sagredo et al., 2014).

3.4.1 San Rafael glacier

The San Rafael glacier, flowing westwards from the NPI, is the northernmost marine-terminating glacier in Patagonia (Figure 7B). The local geomorphology features well-preserved arcuate moraine ridges known as "Témpanos" moraines, deposited following successive ice advances during the Holocene (Harrison et al., 2012). Extensive outwash plains extend westwards from these moraines, most likely connecting the Taitao peninsula with the mainland. This landform assembly creates a proglacial basin occupied by the fan-like-shaped San Rafael Lagoon, which receives inflow from the San Rafael glacier and drains into the main fjord through a narrow and shallow channel known as "Canal Témpanos". Recent geodetic measurements have estimated ice velocity in the glacier front ranging from ~7200 to 6900 m/yr, making it one of the fastest glaciers in the region and, in the middle latitudes of the Southern Hemisphere as well (Mouginot and Rignot, 2015; Millán et al., 2022). First-order estimations revealed that the San Rafael glacier calving flux reached ~2.7 Km^3/yr between 2017 and 2022 (Supplementary Table 4). Given the geographic setting of the San Rafael Lagoon, the distance between the glacier front and the Pacific Ocean is relatively short and linear, so the sinuosity is low (0.31; Supplementary Table 4). Furthermore, the drainage width is very narrow, so the change between the width of the proglacial basin and the main fjord is very high (0.86; Supplementary Table 4). In the San Rafael glacier, normalized values of glaciological and geomorphic attributes indicates that changes in fjord width (~52%) and calving flux (~34%) are the major contributing factors to iceberg residence time, while sinuosity (~14%) plays a secondary role.

3.4.2 Parry Fjord glaciers

The Parry Fjord glaciers are part of the CDI located in Tierra del Fuego, southernmost Patagonia. It is composed by two coalescing glacial valleys flooded by the ocean, the Parry fjord to the West and the Seno Cuevas to the East (Figure 7C). The Parry Fjord valley has at least six marine-terminating and one land-terminating glaciers. Satellite-derived ice motion measurements indicate that the marine-terminating glaciers move at velocities ranging from ~200 to 1750 m/yr, whereas the land-terminating glacier reaches velocities of ~30 m/yr (Millán et al., 2022). The Seno Cuevas is drained by only one marine-terminating glacier, the Cuevas glaciers, which reaches maximum velocities of ~1630 m/yr. However, there are several land-

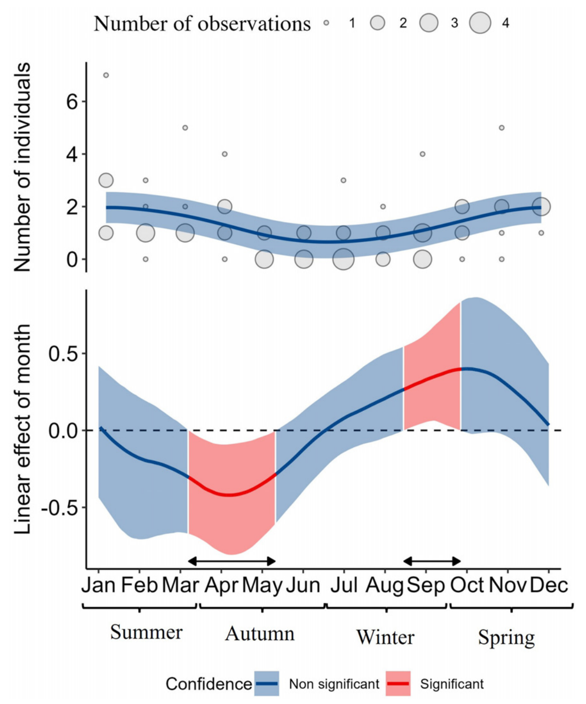

FIGURE 5
Seasonal variation and rate of change in leopard seal counts based on San Rafael standardized monitoring data. The upper panel displays the change in leopard seal counts across seasons. The lower panel illustrates the slope of the seasonal rate of change in the predicted number of leopard seals based on the first derivative of the fitted Generalized Additive Model (GAM) shown in the upper panel. In the lower panel, red areas indicate time segments where 95% of the Monte Carlo simulations for the first derivative of the GAM did not include the null effect, indicating a significant linear effect during these periods.

terminating glaciers that most likely transitioned from a marine to a terrestrial proglacial environment in the past millennia or centuries (Strelin et al., 2008; Menounos et al., 2020; Reynhout et al., 2021). At the mouth of the Seno Cuevas, we identified the remnants of a moraine ridge closing the fjord (Figure 7C). The Darwin glacier in Parry Fjord and the Cuevas glacier in Seno Cuevas—both locations with the highest record of leopard seals of the region—present the highest calving rate of their respective areas reaching ~0.15 and ~0.13 Km^3/yr between 2017 and 2022, respectively. Altogether glaciers of Parry Fjord (glaciers 45 through 51 of Supplementary Table 4) flowing towards the ocean exhibit a combined calving flux of ~0.48 Km^3/yr. Sinuosity of the Parry Fjord range from moderate to high (0.55 average; Supplementary Table 4). The average width ratio of local glaciers is very high (0.83) denoting that, geomorphic features such as moraine crest and subaequous bedrock knobs, are frequent in the area (Supplementary Table 4). In Parry Fjord, normalized values of fjord width (~57%) and fjord sinuosity (~35%) indicate that local geomorphology is the key factor for prolonged iceberg residence, while calving flux (~7%) exerts a secondary control on iceberg residence.

Given that leopard seal sighting records have historically been concentrated in San Rafael and Parry Fjord, we utilized the combined normalized values of the analyzed glaciological and geomorphic attributes of these two areas as references to assess the suitability of other fjords in Patagonia as leopard seal potential habitats. We calculated the weighted mean of these attributes for each location; San Rafael and Parry Fjord glaciers (Table 2). With these results, we hypothesized that calving flux, fjord sinuosity and fjord width ratio all contribute to increase iceberg residence time and, consequently, higher chances of being visited/inhabited by leopard seals.

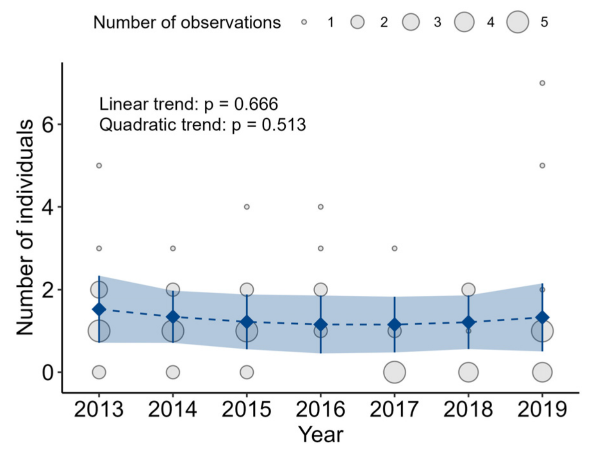

FIGURE 6
Inter-annual variations in the San Rafael monitoring data from 2013 to 2019. After accounting for intra-annual (seasonal) variation and based on non-orthogonal polynomial contrasts up to the second order, the fitted model shows no trends in the increase or decrease of individual numbers of leopard seals in San Rafael.

When considering the normalized values of glaciological and geomorphic attributes at both San Rafael and Parry Fjord together, the width ratio emerged as the primary factor contributing to prolonged iceberg residence at both locations, accounting for 50% of the observed variance. In contrast, calving flux played a more significant role in San Rafael (34%) compared to Parry Fjord (7%), while sinuosity was less influential in San Rafael (14%) but contributed considerably more in Parry Fjord (35%). Therefore, when evaluating other suitable fjords in Chile (discussed in section 3.5) we assigned a Calculated Proportional Weighted Estimate (CWE) to each calculated weighted mean of all fjords of 0.5 to width and 0.2 and 0.3 to calving flux and sinuosity respectively (Table 2).

3.5 Glacial suitability analysis for leopard seal in Patagonia

According to the Chilean Glacier Inventory (DGA, 2022), we identified 59 marine-terminating glaciers grouped within 27 fjords along the Pacific coast of Patagonia (Figure 8). In recent years, the surface area of these glaciers has receded at varying rates, ranging from 0.63 km^2/yr at Jorge Montt Glacier to nearly 0 km^2/yr at an unnamed minor glacier in the Parry Fjord (Supplementary Table 4). Exceptionally, the Europa Glacier has expanded by 0.20 km^2 between 2018 and 2021. Individually, Jorge Montt Glacier exhibits the highest calving flux in Patagonia, reaching 4.61 km^3/yr, followed by San

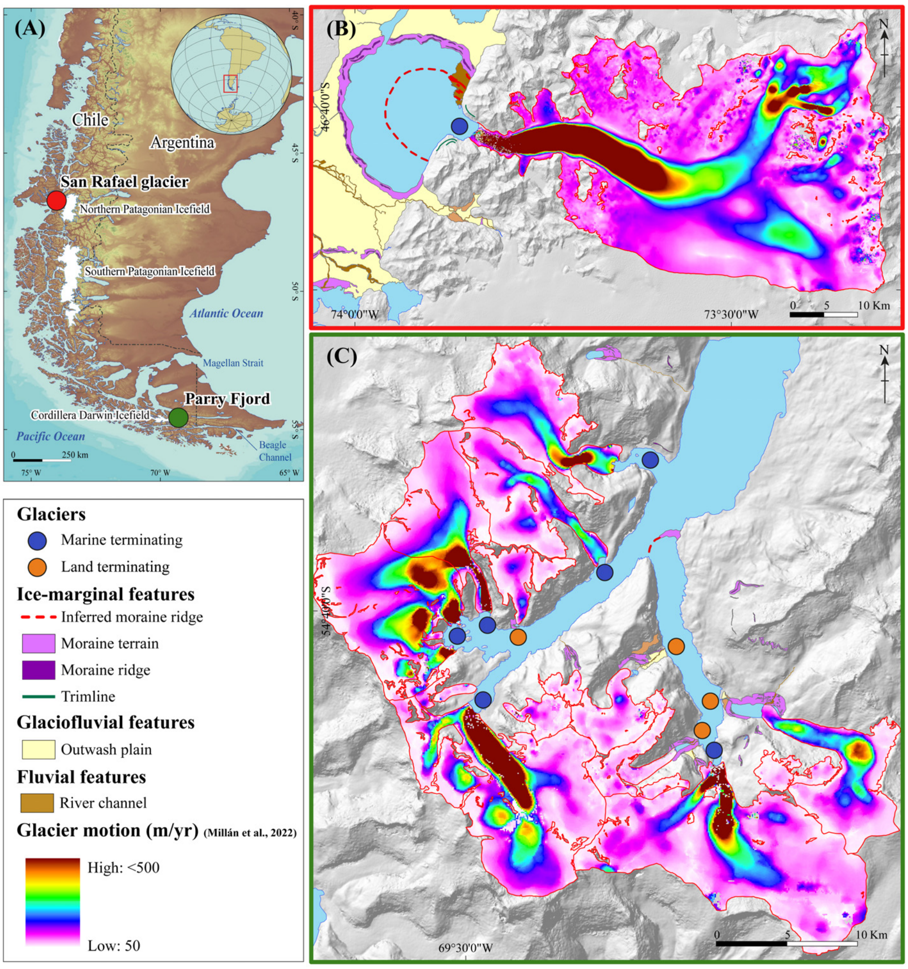

FIGURE 7
Characterization of the San Rafael and the Parry Fjord glaciers. **(A)** Locations of the San Rafael and the Parry Fjord glaciers in Patagonia. **(B, C)** are simplified geomorphic maps of the San Rafael and Parry Fjord glaciers, respectively. Glaciers flowing towards the main water bodies are outlined based on the Chilean Glacier Inventory (DGA, 2022). Surface glacier velocity is depicted in graded colors, with dark red indicating faster areas and white indicating slower areas of ice motion (Millán et al., 2022).

Rafael Glacier at 2.69 km^3/yr, Pinguino Glacier at 1.47 km^3/yr, and Europa Glacier at 1.35 km^3/yr (Supplementary Table 4). All these glaciers exceed the regional average calving flux of 0.29 km^3/yr.

Despite the geographical complexity of the Patagonian archipelago, regional fjords generally show low sinuosity, ranging from 0.16 to 0.99, with a mean of 0.34 (Supplementary Table 4). Many fjords exhibit geomorphic features, such as moraines and outwash plains, that create "geomorphic traps" enhancing iceberg residence time. The width ratios of these fjords vary from 0.23 to 0.96, with a mean of 0.68 (Supplementary Table 4). The normalized weighted mean of glaciological and geomorphic attributes in Patagonian fjords, aligned with values calculated for the San Rafael Glacier and those hosted in Parry Fjord, ranges from 0.62 to 0.11, averaging 0.39 (Table 2). Fjords were classified as "very likely," "likely," and "less likely" to host leopard seals if their weighted means were equal to or above 0.39, between 0.38 and 0.30, and below 0.29, respectively (Figure 8 and Table 2).

From 27 fjords encompassing all 59 marine-terminating glaciers, we found 13 fjords—in addition to San Rafael and Parry Fjord— with potential long-lasting iceberg residence and therefore classified as "very likely" suitable for leopard seals (Figure 8; see values in Table 2). In the SPI, Bernardo (fjord ID 3; 0.39) and the fjord hosting the HPS27, HPS28 and HPS29 (fjord ID 9; 0.57). To the south, in the GCN, the S/N glacier (fjord ID 14; 0.39) and the Snoring and Sarmiento de Gamboa glaciers in the ISI (fjord ID 15; 0.49 and fjord ID 16; 0.53, respectively). In the CDI at Tierra del Fuego, the Agostini Fjord (fjord ID 18; 0.48), Relander glacier and two glaciers without name (fjord ID 19; 0.52), Italia glacier (fjord ID 22 C. Beagle; 0.42), Pía Fjord (fjord ID 23; 0.43 and 24; 0.45), Torcido Fjord (fjord ID 25; 0.58), Garibaldi Fjord (fjord ID 26; 0.54), Grande glacier (27; 0.55). 4 fjords were classified as "likely" habitats for leopard seals: the Jorge Montt glacier (fjord ID 2; 0.34), Pingüino Fjord (fjord ID 7; 0.30), HPS30 Fjord (fjord ID 10; 0.38), and S/N Glacier in the South Patagonian Icefield (fjord ID 17; 0.36).

TABLE 2 Normalized glaciological and geomorphic attributes of marine-terminating glaciers in Patagonia (calving flux, fjord sinuosity, and width ratio).

Icefield Name	Fjord ID	Glacier ID	Glacier Name	Normalized Calving Flux	Normalized Fjord Sinuosity	Normalized Fjord Width Average	Weighted Mean
Northern Patagonian Icefield (2017-2022)	**1**	**1**	**San Rafael**	**0.58**	**0.24**	**0.87**	**0.62**
Southern Patagonian Icefield (2018-2021)	2	2	Jorge Montt	1	0.1	0.22	0.34
	3. Bernardo	3	Bernardo	0.17	0.19	0.61	0.39
	4. Témpano	4	Témpano	0.01	0.06	0.47	0.26
	5. Falcon	5	HPS12	0.06	0.31	0	0.11
		6	HPS13				
		7	HPS14				
		8	HPS15				
	6	9	HPS17	0.04	0.1	0.17	0.12
		10	HPS18				
	7	11	HPS19	0.4	0.06	0.41	0.3
		12	Pingüino				
	8	13	Europa*	0.29	0.07	0.31	0.23
	9	14	HPS27*	0.17	0.37	0.85	0.57
		15	HPS28				
		16	HPS29*				
	10	17	HPS30	0.29	0.24	0.51	0.38
		18	HPS31				
		19	S/N*				
		20	HPS32				
		21	Calvo*				
		22	HPS33				
		23	HPS34				
		24	HPS35				
	11	25	Asia	0.02	0.13	0.45	0.27
	12	26	Amalia*	0.16	0.08	0.12	0.12
Gran Campo Nevado (2015-2022)	13	27	S/N	0	0	0.46	0.23
	14	28	S/N*	0	0.65	0.4	0.39
Isla Santa Inés (2017-2022)	15	29	Snoring	0.01	1	0.37	0.49
	16	30	Sarmiento de Gamboa	0	0.35	0.85	0.53
Cordillera Darwin Icefield (2017-2022)	17	31	S/N	0.01	0.19	0.6	0.36
		32	S/N				
	18. Agostini	33	Andrés	0.08	0.15	0.83	0.48
		34	Bárbara				
		35	Navarro				

(Continued)

TABLE 2 Continued

Icefield Name	Fjord ID	Glacier ID	Glacier Name	Normalized Calving Flux	Normalized Fjord Sinuosity	Normalized Fjord Width Average	Weighted Mean
		36	S/N				
		37	S/N				
		38	Serrano				
		39	S/N				
		40	Rugidor				
	19. Brookes	41	Relander	0.08	0.52	0.69	0.52
		42	S/N				
		43	S/N				
	20. Marinelli	44	Marinelli*	0.05	0.36	0.05	0.15
	21. Parry	**45**	Luis de Saboya	**0.1**	**0.52**	**0.84**	**0.59**
		46	S/N				
		47	S/N				
		48	S/N				
		49	S/N				
		50	Darwin				
		51	Cuevas				
	22. C. Beagle	52	Italia	0	0.15	0.75	0.42
	23. Pía Este	53	Kalv	0.02	0.16	0.76	0.43
		54	S/N				
	24. Pía Oeste	55	Guilcher	0.03	0.21	0.76	0.45
	25. Torcido	56	Cattigara	0.06	0.23	1	0.58
	26. Garibaldi	57	Picos Azules	0.04	0.12	1	0.54
		58	Garibaldi				
	27. Ventisquero	59	Grande	0.13	0.13	0.98	0.55
CWE						**Max:**	0.62
Calving flux	0.2					**Min:**	0.11
Fjord Sinuosity	0.3					**Mean:**	0.39
Fjord Width	0.5					**SD:**	0.16

Glaciers highlighted in bold were used to define the Calculated Proportional Weighted Estimates (CWE) for calving flux, fjord sinuosity, and fjord width ratio. These estimates were then applied to calculate the weighted means for the remaining fjords. Some fjords consist of multiple glaciers, so the values represent the average per fjord. The table also provides descriptive statistics of these final weighted means. Glaciers marked with an asterisk (*) indicate expanding glaciers.

4 Discussion

Leopard seals have been observed across most regions of Chile: from Easter Island in the north/west to Tierra del Fuego in the south. Aguayo-Lobo et al. (2011) first compiled historical records of leopard seals to show that this species is a regular member of the marine fauna of southern Chile. Then, Acevedo and Martinez (2013) provided the first evidence of a potential multi-month resident leopard seal individual in Tierra del Fuego. Both studies hypothesized the existence of resident aggregations of leopard seals in Patagonia. Here, we provide evidence to support this hypothesis through multiyear observations of the same leopard seal individuals. Specifically, we confirmed two distinct resident aggregations of leopard seals in Chile: one in San Rafael and one in Parry Fjord. There was no evidence that the same individuals were at both sites. Our results also show that these resident aggregations in Patagonia have existed for decades at least.

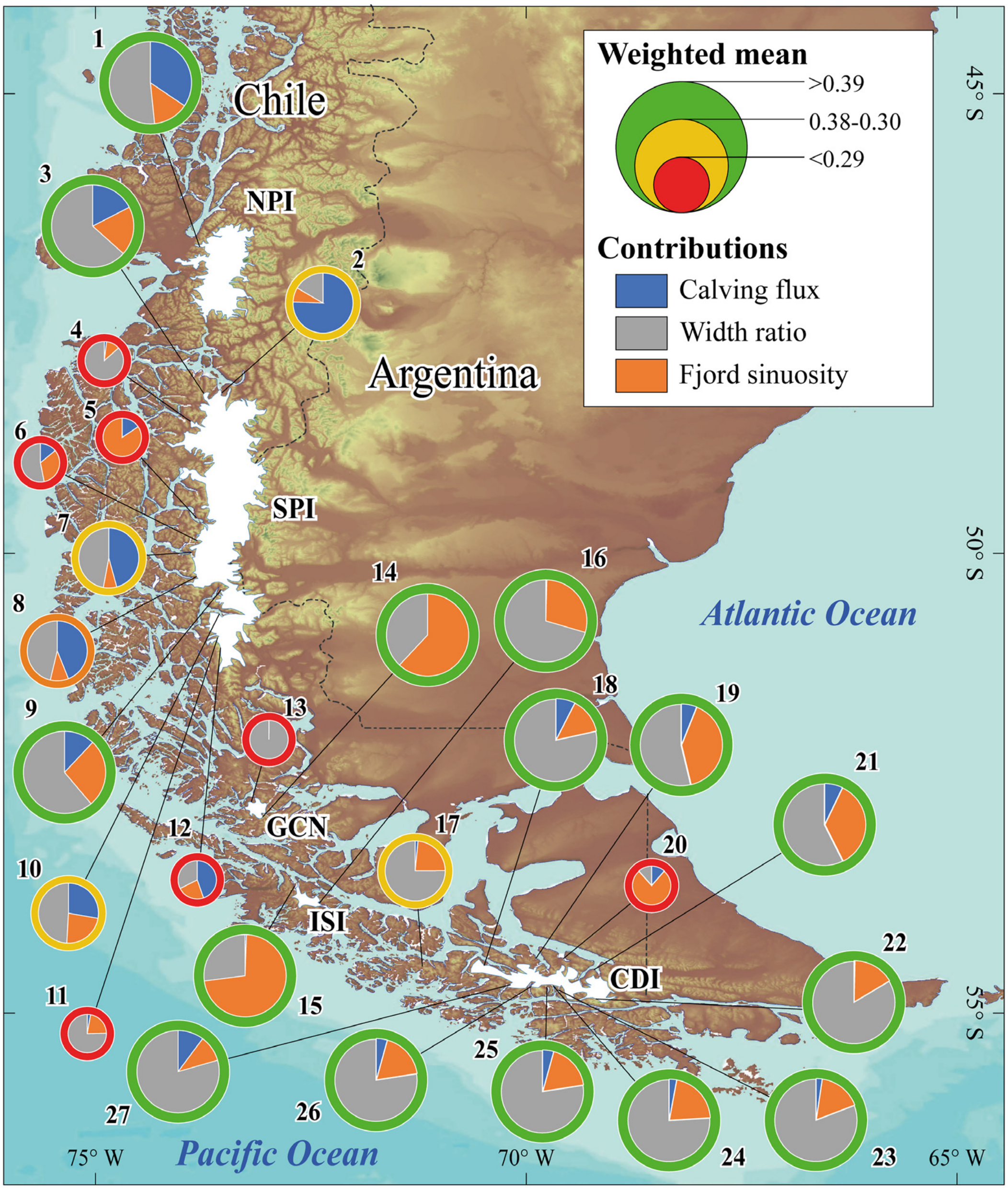

FIGURE 8
Fjords hosting marine-terminating glaciers of Patagonia. The figure displays pie charts corresponding to the fjord IDs listed in Table 2. The color and size of each pie chart indicate the likelihood of the site to host leopard seals; fjords were categorized as "very likely" when their weighted mean was equal or above 0.39, and "likely" or "less likely" between 0.38 and 0.30 and less than 0.29, respectively. Within the pie charts, different colors represent the contributions of calving flux (blue), fjord width ratio (grey), and fjord sinuosity (orange) to the potential of each glacier as habitat for leopard seals.

We documented an increase in pups and stranding events reported over the past 10-15 years in Chile (van der Linde et al., 2022; this study). Together, these pup records, as well as recent observations of leopard seal sexual behavior in San Rafael (Kienle et al., 2024), show that breeding is not limited to Antarctic pack ice as previously suggested (Bester et al., 2017a). Therefore, Patagonia should be considered as an integral part of the species breeding distribution range rather than continuing to report leopard seal sightings as occasional or extralimital.

4.1 Leopard seal distribution north from the Antarctic Polar Front

Leopard seal sightings have increased in Chile and other areas north of the Antarctic Polar Front. Our study reports 438 sightings in Chile, with 86% of sightings concentrated in Patagonia. When we correct and transform Aguayo-Lobo et al. (2011) "total animals reported" to individual sightings (118 records), we found a 271% increase in sightings from 2009-2023 compared to pre-2009

(Aguayo-Lobo et al., 2011). Similarly, Hupman et al. (2020) documented an increase in leopard seal sightings in New Zealand, from 19 records in 2000 to 781 records in 2018. Systematic monitoring at Marion Island also suggests an increase in leopard seals, from 19 immature animals reported over 25 years (1980-2005; Bester et al. (2006) to almost double over the last 15 years (2005-2020; De Bruyn and Ross, pers. comm.). Increasing numbers have also been reported in South Australia with a rate of 1.2 seals per year between 1979-2009 to 2.8 seals per year between 2017-2022 (Shaughnessy et al., 2023). The increase in leopard seal sightings north of the Polar Front may be driven by a shift in distribution patterns, with more seals potentially crossing the Antarctic Convergence and/or an increase in their abundance in subantarctic areas.

The origin of leopard seals north of the Polar Front has been widely discussed (reviewed in Bester, 2021; Stewart et al., 2020). For example, early reports for the Indian Ocean and Australasian sub-Antarctic waters suggested that leopard seals migrate north during the winter expansion of Antarctic pack ice (Rounsevell and Eberhard, 1980; Bester and Roux, 1986; Bester et al., 1995). Similarly, in Chile, researchers have suggested that leopard seals potentially migrate from the Western Antarctic Peninsula (WAP) as sea ice expands northward (Stewart et al., 2020). While leopard seals can travel long distances equivalent to those separating southern Chile and the WAP (~1,000 km; tagged leopard seals in the WAP are capable of travelling more than 1,500 km, Kienle et al., 2022; Staniland et al., 2018), most instrumented seals from Antarctica showed movement patterns restricted to a few hundred kilometers (Rogers et al., 2005; Kienle et al., 2022). In addition, consistent multi-year/multi-month observations of resident leopard seals in Patagonia (this study), along with increasing reports of newborns in subantarctic areas (van der Linde et al., 2022; this study), support the hypothesis that there are local resident aggregations of leopard seals in some subpolar locations (Aguayo-Lobo et al., 2011; Acevedo and Martinez, 2013).

Historically, leopard seals found north of the Polar Front were described as being in poor condition and usually as immature individuals (Siniff and Stone, 1985; Rounsevell and Pemberton, 1994; Bester et al., 2006). For instance, juvenile leopard seals have been reported in large numbers at Macquarie Island every 4-5 years, likely due to a northward winter dispersal in response to conditions in the pack ice to the south (Testa et al., 1991). Here, however, resident leopard seals in both San Rafael and Tierra del Fuego were in excellent body condition, and most seals from Patagonia (82%) were adults with only a few (18%) immature individuals. Similarly, in New Zealand, leopard seals were predominantly adults in good to excellent body condition (Hupman et al., 2020). The presence of adults in good body condition regularly observed north of the Polar Front indicates that Patagonia, and potentially New Zealand, provide secure habitats and abundant resources necessary for leopard seals to thrive.

Furthermore, Rounsevell and Pemberton (1994) hypothesized that the number of immature leopard seals increases with decreasing latitude. Like previous studies, we also identified changes in leopard seal age class across Chile. North of San Rafael in Chile, leopard seal sightings are mostly of immature individuals (82%). Sightings of these immature individuals that included mentions of their health status showed that 74% of these leopard seals were in suboptimal condition (out of 19 records, 14 were reported either poor, emaciated, injured, or dead). Similarly, in South Australia most leopard seal sightings are of juvenile animals reported in fair/thin body conditions (Shaughnessy et al., 2023). Further, the cyclical increase in leopard seals at Macquarie Island are mostly the result of immature individuals dispersing north, likely driven by poor environmental conditions (Testa et al., 1991). Our data therefore support the hypothesis that age class is correlated with latitude, but we found that, in Chile, this pattern starts much further north of the Polar Front than proposed in other locations (Rounsevell and Pemberton, 1994; Testa et al., 1991; van den Hoff et al., 2005; Bester et al., 2006).

4.2 Long term temporal trends in Chile

It is arguable whether leopard seal aggregations have increased or remained stable in San Rafael and Tierra del Fuego. In San Rafael, the first official historical record of leopard seals was of three individuals in 1979 (Torres et al., 1979; reviewed in Aguayo-Lobo et al., 2011). Afterwards, there was a 30-year absence of records (1979-2009) where no sightings were reported from San Rafael. Now, at least 10 unique leopard seals have been identified from >195 sighting records. Although this could suggest a potential local increase of leopard seals in San Rafael, the San Rafael monitoring data have shown a stable leopard seal aggregation, with no major increase in seal counts between 2013-2019 (Figure 6). This leads us to call for caution when interpreting sightings data. The lack of pre-2009 records in San Rafael may have been due to a reduced number of private tour companies operating before 2013, leading to no sightings reported during this time, rather than a true absence of leopard seals.

Similarly in Parry Fjord, only nine records of leopard seals existed before 2010 (Aguayo-Lobo et al., 2011) compared to >55 new sighting records after 2010. Moreover, at Parry Fjord, tour operators report that not observing leopard seals during their visits is rare (Moreno, pers. comm.). Like San Rafael, the recent increase in sightings in Parry Fjord may also reflect the rise of the tourism industry, leading to more people visiting the area. However, leopard seal aggregations have been reported there since 1971 (Markham, 1971; n=4) with numbers similar to our most recent expedition in the area (TF-23; n=6), suggesting that numbers may be at least stable at this location.

The overall stranding data did show a positive annual rate of change within the last two decades in Chile (Figure 3). This data indicates an overall increase in the number of leopard seals inhabiting Chile in the last 15 years. Stranding reports are more reliable than sighting records as they do not require photographic validation and are often verified by trained personnel. An increase in leopard seal numbers along the Chilean coast aligns with the expanding distribution and reappearance of other pinnipeds on the Pacific coast after centuries of exploitation. For example, southern elephant seals are recolonizing the eastern South Pacific where they were heavily depleted during the sealing era (Acevedo et al., 2016, 2019). As in our study, Cárcamo et al. (2019) showed an increase in elephant seal sighting records along the coast of Chile. Additionally, South American fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria flavescens*) populations are increasing in key breeding areas of South America, including Chile (Rodrigues et al., 2018; Milano et al., 2020; Sepúlveda et al., 2021).

4.3 San Rafael: a unique case study

The leopard seals at San Rafael may constitute an isolated population from other aggregations found in Chile. There are ten resident leopard seals at this location. Eight seals have been continuously observed over the last ten years, and only two new individuals (LSR09 and LSR10) were reported in recent years (2019-2020). In addition, our two most recent expeditions to San Rafael did not find new seals beyond those already reported. In contrast, we continuously obtain sighting reports of new individuals at Parry Fjord. For instance, our latest ten day expedition (TF-23) found four new individuals never reported before. We have also found consistency in the seasonality of individuals from San Rafael, stressing their resident nature. There is a seasonal trend, with numbers tending to decrease during the austral autumn (march–may) and increasing during the austral spring (August–October). The slight tendency of higher number of seals during the spring aligns with the hypothesized breeding season for the species in locations where residents are found (between September-November; van der Linde et al., 2022). Specifically, 8 of 10 pups of San Rafael were recorded between mid-October and early December with two resident females giving birth in San Rafael at least twice (LSR01 and LSR02). Moreover, evidence of mating behavior has been recently reported in San Rafael (Kienle et al., 2024). The temporal overlap among individuals in San Rafael, the lack of evidence of transient individuals, and the consistency in annual life history cyclic patterns such as reproduction, suggest that the seals living in San Rafael and nearby areas may be isolated from the rest of Patagonia.

During the Last Glacial Maximum (globally around 26-19 kya), the abundance of leopard seals in Antarctic increased likely driven by the abundance of ice (Bender et al., 2023). Similarly, the Patagonian ice sheet also expanded, covering much of southern Andes of Chile and Argentina; this was followed by a progressive fragmentation of the Patagonian ice sheet into today's multiple ice caps (Davies et al., 2020). The NPI separated from the SPI around 12.6-11.3 kya (Thorndycraft et al., 2019; Vásquez et al., 2022), isolating the NPI where the San Rafael Lagoon formed sometime after ~9.5 kya (Harrison et al., 2012). Furthermore, the San Rafael glacier constitutes the only marine-terminating glacier in the NPI. The nearest glacier, Jorge Montt Glacier, is not easily accessible from San Rafael. Leopard seals would need to swim ~630 km to reach the Jorge Montt Glacier. While leopard seals can travel long distances (Staniland et al., 2018), most tend to remain close to their origin (Rogers et al., 2005; Kienle et al., 2022). Altogether, this support the idea that leopard seals of the NPI may have been isolated during the separation of the NPI from the SPI with the San Rafael glacier being today's only glacier producing icebergs in the region.

The potentially isolated leopard seal population of the NPI was then severely decimated by the sealing industry during the 18th and 19th centuries, likely causing a local demographic reduction. The peak sealing period in the mid-19th century focused on southern sea lions also included southern elephant seals and leopard seals (Torrejón et al., 2013). Historical accounts indicate a significant reduction of all pinniped species by the end of the 19th century, including leopard seals. The navy captain E. Simpson mentioned "*(…) there was another seal larger than southern sea lions but smaller than elephant seals called leopards due to their spot patterns, and they have also disappeared from the region*" (Simpson, 1875). The progressive fragmentation of the Patagonian Ice Sheet after the Last Glacial Maximum, as well as the significant reduction of leopard seals during the sealing era, supports our hypothesis of a potential isolated leopard seal aggregation in San Rafael with little flux of new animals—at least— in the last decades. Genetic analyses are needed to assess inbreeding levels in San Rafael and compare the genetic make-up of these animals to other aggregations in Chile and elsewhere. These assessments will help clarify the history of this population and levels of past and contemporary gene flow among leopard seal aggregations.

4.4 Identifying potential habitats for leopard seals in Chile

Based on our characterization of the San Rafael and Parry Fjord glaciers, we find that fjord width ratio, which accounts for geomorphic traps (e.g., moraine ridges, outwash plains), is key to controlling residence of local icebergs (the most important substrate for leopard seals in Chile). Our simplified calving flux estimation for the San Rafael glacier (~2.7 km^3/yr) in the NPI and the Darwin and Cuevas glaciers (0.15 and 0.13 km^3/yr) in the CDI aligns with previous estimates of the same glaciers in the last decades (Koppes et al., 2011; Melkonian et al., 2013). More broadly, our regional calving flux estimation is consistent with previous estimates for Patagonian marine-terminating glaciers (Koppes et al., 2011; Melkonian et al., 2013; Schaefer et al., 2015). The minor differences observed between our values and those from the studies mentioned, may be explained by the challenges in outlining glacier ice fronts due to the lack of cloud-free satellite imagery and ice mélange (i.e., mixture of icebergs, sea-ice and snow) in the ice front. More important, glaciological parameters database assume uncertainties of ~30% in ice velocity estimations, and 25-35% for ice thicker than 100 m and >50% for thinner ice (Millán et al., 2022). Thus, we suggest that our calving flux assessments can be considered as a qualitative indicator.

Geomorphic attributes are crucial for iceberg retention at regional scale. Patagonian marine-terminating glaciers and fjords in the NPI and SPI show higher contributions from calving flux, while southern ice masses (GCN, ISI, and CDI) are more influenced by morphometric attributes and the existence of landforms closing fjords. However, winter sea ice formation in several fjords of CDI, including Marinelli and Parry Fjords (Salame et al., 2020), may compensate for limited calving flux.

Our habitat model aligns well with the leopard seal sighting records, thereby suggesting additional fjords and glaciers across Patagonia that should be explored in search of leopard seals. While most sighting records are concentrated in San Rafael and Parry Fjord, other locations are consistent with fjords predicted by our model as "very likely" suitable habitats. We identified 13 additional fjords in Patagonia (out of 27 fjords that included 59 glaciers in total) as "very likely" suitable habitats for leopard seal with many of them matching areas where leopard seals have also been reported. For instance, Agostini Fjord and Ballena Sound (fjords 18 and 15 in Figure 8) are both identified as very likely suitable habitats for leopard seals, and the historic records show that each location has had 11 leopard seal sighting records, including multiyear records (Aguayo-Lobo et al., 2011; Acevedo and Martinez, 2013, and this study). Similarly, Pia Sound (4 reported sightings near fjords 23 and 24), Helado Sound (2 sightings near Sarmiento Glacier, fjord 16), and the Beagle Channel (1 sighting near Italia Glacier, fjord 22) were all classified as "very likely" habitats for leopard seals.

Because of the challenges in identifying Patagonian glaciers, the 59 marine-terminating glaciers evaluated should be considered a minimum number of iceberg-prone glaciers existing in Patagonia. Overall, intense calving flux, accounting for iceberg production, and the existence of geomorphic landforms in the fjords—particularly moraines and outwash plains that create iceberg traps—are critical factors when suitable habitats for leopard seals are evaluated in Patagonia. Furthermore, other factors such as prey availability also play an important role defining suitable location for leopard seals, however, resources availability seems not to be a problem in Patagonia (Aguayo-Lobo et al., 2011; Friedlander et al., 2021; Castilla et al., 2023). The fact that 76% of sighting records were on icebergs, and the consistent match between our predicted suitable fjords and historical sighting locations, support the idea that iceberg availability and residency are crucial variables for evaluating suitable habitats for leopard seals in the Chilean fjords.

4.5 The role of leopard seals as apex predators

Apex predator populations are rapidly changing in response to widespread environmental changes (Roff et al., 2018). Their presence or absence can lead to significant restructuring of ecosystems (e.g., Wilmers and Post, 2006; Knopff et al., 2014; Jonsen et al., 2019). Introducing new predators often results in a redistribution and potential shift in local food web structures (Johnson et al., 2007; Letnic et al., 2009; Sherman et al., 2020), while the suppression of apex predators can trigger ecosystem restructuring through various ecophysiological pathways (e.g. Leo et al., 2019; Hammerschlag et al., 2022). This highlights the importance of monitoring the distribution of apex predators such as leopard seals.

We document the long-term presence of 10 resident leopard seals in San Rafael and 6-9 in Tierra del Fuego. While these numbers may seem low, small numbers of apex predators can significantly impact prey species abundance (Williams et al., 2004; Krause et al., 2022). For instance, fewer than 40 killer whales may be responsible of the decline of the Steller sea lion (*Eumetopias jubatus*) population in the Aleutian Islands and a pod of only five individuals could account for the regional decline of sea otters (Williams et al., 2004). Leopard seals are effective and efficient apex predators (Hiruki et al., 1999; Krause et al., 2015) and cause drastic reductions in their prey populations (Forcada et al., 2009). At Cape Shirreff in the WAP, leopard seals consumed on average 2.5 Antarctic fur seal pups per day during the 2004-2005 breeding season (Vera et al., 2004). The annual predation rate over Antarctic fur seal pups increased from 7.1% to 73.8% between 2002-2020, with a mean predation rate of nearly 70% annually since 2010. The average maximum number of leopard seals responsible for this high predation rate was only 20 individuals per year between 2011 and 2020 with this small average number contributing to the local population collapse of Antarctic fur seals at Cape Shirreff (Krause et al., 2022). This highlights the importance of monitoring occurrence of apex predators and distinguishing resident from transient individuals (movement patterns) to help predict changes in prey abundance and local food web dynamics (Andrews and Harvey, 2013).

We provide baseline data on the historical resilience of leopard seals in Patagonia. However, assessing the short- and long-term ecological role of leopard seals in these ecosystems is critical and requires information on their movement patterns, diet, and local behavioral flexibility. Our results confirmed that leopard seals are a permanent component of the food web in Patagonian ecosystems and enhanced our understanding of leopard seal ecology by providing evidence of their distribution and residency beyond Antarctica.

4.6 Conclusions

We present evidence that multiple resident leopard seal aggregations exist in Chile and confirm that their breeding distribution is not limited to Antarctic pack ice (Bester et al., 2017a). Specifically, we show long-term residence of leopard seals in two locations of Patagonia (San Rafael and Parry Fjord), evidenced by multiyear observations of adults. Additionally, we report an increase in stranding events and newborn sightings over the last 15 years, suggesting an overall growing population in Chile. Although the origin of these animals remains unknown, continued monitoring of leopard seals, particularly of newborn and juvenile animals, is essential, as including reproductive success in species distribution models can lead to more accurate predictions of population growth (Chuine and Beaubien, 2001).

Global warming is rapidly impacting the cryosphere (Schneider et al., 2020) especially in Patagonia, where marine-terminating glaciers are increasingly transitioning to land-terminating glaciers (Dussaillant et al., 2019). Consequently, this will cause a decrease in the number of potential habitats for leopard seals and likely impact their distribution. For instance, cyclical increases of juvenile leopard seal numbers in locations north of the Polar Front— likely due to changes in sea ice extent in the south—is an example of how the absence of sea ice may influence the dispersal of these animals (Testa et al., 1991). Similarly, climate change driven responses are already shaping the distribution of other pagophilic apex predators such as polar bears in the Arctic (Wiig et al., 2008; Sahanatien and Derocher, 2012). Future studies should conduct comprehensive habitat suitability analyses combining oceanographic variables, iceberg characteristics, local prey availability, and species movement patterns to assess the impact of climate change on ice-dependent apex predators' occurrence and distribution.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Subsecretary of Fisheries and Aquaculture of Chile (SUBPESCA). Permit PINV E-2022-394 and the CONAF Authorization N° XI-21-2022. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RB-C: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. RS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. GG: Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. FM: Conceptualization, Data curation, Methodology, Writing – review & editing. NF-F: Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – review & editing. MC-A: Formal analysis, Visualization, Writing – original draft, Writing – review & editing. FMA: Resources, Validation, Writing – review & editing. CD: Data curation, Resources, Validation, Writing – review & editing. CA: Data curation, Resources, Validation, Writing – review & editing. NB: Data curation, Investigation, Writing – review & editing. ES: Funding acquisition, Investigation, Resources, Writing – review & editing. CB: Funding acquisition, Investigation, Writing – review & editing. MG: Investigation, Resources, Writing – review & editing. AG: Investigation, Writing – review & editing. MD: Investigation, Writing – review & editing. SK: Funding acquisition, Investigation, Resources, 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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2024.1448098/full#supplementary-material

References

Acevedo, J., Aguayo-Lobo, A., Brito, J., Torres, D., Cáceres, B., Vila, A., et al. (2016). Review of the current distribution of southern elephant seals in the eastern south pacific. *New Z. J. Mar. Freshw. Res.* 50, 240–258. doi: 10.1080/00288330.2015.1132746

Acevedo, J., González, A., Garthe, S., González, I., Gómez, R., and Aguayo-Lobo, A. (2017). Births of leopard seals *Hydrurga leptonyx* in southern Chile. *Polar Biol.* 40, 713–717. doi: 10.1007/s00300-016-1968-0

Acevedo, J., and Martinez, F. (2013). Residence of the leopard seal in the Magellan strait: A potential sub-Antarctic population inhabiting the waters of southern Chile? *Polar Biol.* 36, 453–456. doi: 10.1007/s00300-012-1275-3

Acevedo, J., Vargas, R., Torres, D., and Aguayo-Lobo, A. (2019). Northerly births of the southern elephant seal (*Mirounga leonina*) in their former southeast pacific distribution. *Aquat. Mammals* 45, 293–298. doi: 10.1578/AM.45.3.2019.293

Aguayo-Lobo, A., Acevedo, J., Brito, J. L., Acuña, G. P., Bassoi, M., Secchi, E. R., et al. (2011). Presence of the leopard seal, *Hydrurga leptonyx* (de Blainville 1820), on the coast of Chile: An example of the Antarctica-South America connection in the marine environment. *Oecologia Australis* 15, 69–85. doi: 10.4257/oeco.2011.1501.07

Andrews, K. S., and Harvey, C. J. (2013). Ecosystem-level consequences of movement: Seasonal variation in the trophic impact of a top predator. *Mar. Ecol. Prog. Ser.* 473, 247–260. doi: 10.3354/meps10095

Bender, A. N., Krause, D. J., Goebel, M. E., Hoffman, J. I., Lewallen, E. A., and Bonin, C. A. (2023). Genetic diversity and demographic history of the leopard seal: A Southern Ocean top predator. *PloS One* 18, e0284640. doi: 10.1371/journal.pone.0284640

Bester, M. N. (2021). Status of pinnipeds on mid-Atlantic ridge islands, South Atlantic Ocean. *Polar Biol.* 44, 865–871. doi: 10.1007/s00300-021-02838-z

Bester, M. N., Bester, W. A., Wege, M., Schofield, R. A., and Glass, T. A. (2017b). Vagrant leopard seal at Tristan da Cunha Island, South Atlantic. *Polar Biol.* 40, 1903–1905. doi: 10.1007/s00300-017-2073-8

Bester, M. N., Bornemann, H., and McIntyre, T. (2017a). "Antarctic marine mammals and sea ice," in *Sea ice*. Ed. D. N. Thomas (The Atrium, Southern Gate, Chichester, West Sussex, UK: John Wiley & Sons, Ltd), 534–555.

Bester, M. N., Erickson, A. W., and Ferguson, J. W. H. (1995). Seasonal change in the distribution and density of seals in the pack ice off princess Martha coast, Antarctica. *Antarctic Sci.* 7, 357–364. doi: 10.1017/S0954102095000502

Bester, M., Hofmeyr, G., Kirkman, S., Chauke, L., De Bruyn, P., Ferreira, S., et al. (2006). The leopard seal at Marion Island, vagrant or seasonal transient?. *South Afr. J. Wildlife Research-24-month delayed Open Access* 36, 195–198. doi: abs/10.10520/EJC117238

Bester, M. N., and Roux, J. P. (1986). Summer presence of leopard seals *Hydrurga leptonyx* at the Courbet Peninsula, Iles Kerguelen. *South Afr. J. Antarctic Res.* 16, 29–32.

Boveng, P. L., Hiruki, L. M., Schwartz, M. K., and Bengtson, J. L. (1998). Population growth of Antarctic fur seals: Limitation by a top predator, the leopard seal? *Ecology* 79, 2863–2877. doi: 10.1890/0012-9658(1998)079[2863:PGOAFS]2.0.CO;2

Cárcamo, D., Pizarro, M., Orellana, M., Muñoz, L., Pavez, G., Sepúlveda, M., et al. (2019). Are southern elephant seals re-invading mid-latitude grounds? New sightings and first birth records off the Chilean coast. *Polar Biol.* 42, 433–440. doi: 10.1007/s00300-018-2433-z

Casaux, R., Baroni, A., Ramón, A., Carlini, A., Bertolin, M., and DiPrinzio, C. Y. (2009). Diet of the leopard seal *Hydrurga leptonyx* at the Danco coast, Antarctic peninsula. *Polar Biol.* 32, 307–310. doi: 10.1007/s00300-008-0567-0

Castilla, J. C., Armesto Zamudio, J. J., Martínez-Harms, M. J., and Tecklin, D. (2023). *Conservation in Chilean Patagonia: Assessing the state of knowledge, opportunities, and challenges*. (Cham, Switzerland: Springer Nature; Santiago, Chile: Ediciones UC) 504.

Cheeseman, T., Johnson, T., Southerland, K., and Muldavin, N. (2017). *Happywhale: Globalizing marine mammal photo identification via a citizen science web platform* (Santa Cruz, CA, USA: Happywhale). Rep. SC/67b/PH 2.

Chuine, I., and Beaubien, E. G. (2001). Phenology is a major determinant of tree species range. *Ecol. Lett.* 4, 500–510. doi: 10.1046/j.1461-0248.2001.00261.x

Cursach, J. A., Rau, J. R., Ojeda, J., Vilugrón, J., Tobar, C., Oyarzún, C., et al. (2011). Diversidad de aves y mamíferos marinos en bahía san Pedro, costa de Purranque, centro-sur de Chile. *Gayana (Concepción)* 75, 146–154. doi: 10.4067/S0717-65382011000200003

Davies, B. J., Darvill, C. M., Lovell, H., Bendle, J. M., Dowdeswell, J. A., Fabel, D., et al. (2020). The evolution of the Patagonian ice sheet from 35 ka to the present day (PATICE). *Earth-Science Rev.* 204, 103152. doi: 10.1016/j.earscirev.2020.103152

DGA (2022). "Inventario Público de Glaciares," in *Dirección General de Aguas*. Available at: https://dga.mop.gob.cl/Paginas/InventarioGlaciares.aspx.

Dussaillant, I., Berthier, E., Brun, F., Masiokas, M., Hugonnet, R., Favier, V., et al. (2019). Two decades of glacier mass loss along the Andes. *Nat. Geosci.* 12, 802–808. doi: 10.1038/s41561-019-0432-5

Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., et al. (2011). Trophic downgrading of planet earth. *Science* 333, 301–306. doi: 10.1126/science.1205106

Estes, J. A., Tinker, M. T., Williams, T. M., and Doak, D. F. (1998). Killer whale predation on sea otters linking oceanic and nearshore ecosystems. *science* 282, 473–476. doi: 10.1126/science.282.5388.473

Evans, E., Fraser, A. D., Cook, S., Coleman, R., and Joughin, I. (2022). An observation-based approach to calculating ice-shelf calving mass flux. *Remote Sens. Environ.* 272, 112918. doi: 10.1016/j.rse.2022.112918

Forcada, J., Malone, D., Royle, J. A., and Staniland, I. J. (2009). Modelling predation by transient leopard seals for an ecosystem-based management of Southern Ocean fisheries. *Ecol. Model.* 220, 1513–1521. doi: 10.1016/j.ecolmodel.2009.03.020

Forcada, J., and Robinson, S. L. (2006). Population abundance, structure and turnover estimates for leopard seals during winter dispersal combining tagging and photo-identification data. *Polar Biol.* 29, 1052–1062. doi: 10.1007/s00300-006-0149-y

Friedlander, A. M., Ballesteros, E., Goodell, W., Hüne, M., Muñoz, A., Salinas-de-León, P., et al. (2021). Marine communities of the newly created Kawésqar National Reserve, Chile: From glaciers to the Pacific Ocean. *PloS One* 16, e0249413. doi: 10.1371/journal.pone.0249413

Grabham, A. A., van der Linde, K., Visser, I. N., and Nelson, X. J. (2023). Initial insights into leopard seal moult in Aotearoa New Zealand. *New Z. J. Ecol.* 47, 3553. doi: 10.20417/nzjecol.47.3553

Hall-Aspland, S. A., Rogers, T. L., and Canfield, R. B. (2005). Stable carbon and nitrogen isotope analysis reveals seasonal variation in the diet of leopard seals. *Mar. Ecol. Prog. Ser.* 305, 249–259. doi: 10.3354/meps305249

Hammerschlag, N., Fallows, C., Meÿer, M., Seakamela, S. M., Orndorff, S., Kirkman, S., et al. (2022). Loss of an apex predator in the wild induces physiological and behavioural changes in prey. *Biol. Lett.* 18, 20210476. doi: 10.1098/rsbl.2021.0476

Harrison, S., Glasser, N. F., Duller, G. A., and Jansson, K. (2012). Early and mid-Holocene age for the Témpanos moraines, Laguna San Rafael, Patagonian Chile. *Quaternary Sci. Rev.* 31, 82–92. doi: 10.1016/j.quascirev.2011.10.015

Hiruki, L. M., Schwartz, M. K., and Boveng, P. L. (1999). Hunting and social behaviour of leopard seals (*Hydrurga leptonyx*) at seal island, south Shetland islands, Antarctica. *J. Zoology* 249, 97–109. doi: 10.1111/j.1469-7998.1999.tb01063.x

Hucke-Gaete, R., Aguayo-Lobo, A., Yancovic-Pakarati, S., and Flores, M. (2014). Marine mammals of Easter Island (Rapa Nui) and Salas y Gómez Island (Motu Motiro Hiva), Chile: A review and new records. *Latin Am. J. Aquat. Res.* 42, 743–751. doi: 10.3856/vol42-issue4-fulltext-5

Hupman, K., Visser, I. N., Fyfe, J., Cawthorn, M., Forbes, G., Grabham, A. A., et al. (2020). From vagrant to resident: Occurrence, residency and births of leopard seals (*Hydrurga leptonyx*) in New Zealand waters. *New Z. J. Mar. Freshw. Res.* 54, 1–23. doi: 10.1080/00288330.2019.1619598

Jessopp, M., Forcada, J., Reid, K., Trathan, P., and Murphy, E. (2004). Winter dispersal of leopard seals (*Hydrurga leptonyx*): Environmental factors influencing demographics and seasonal abundance. *J. Zoology* 263, 251–258. doi: 10.1017/S0952836904005102

Johnson, C. N., Isaac, J. L., and Fisher, D. O. (2007). Rarity of a top predator triggers continent-wide collapse of mammal prey: Dingoes and marsupials in Australia. *Proc. R. Soc. B: Biol. Sci.* 274, 341–346. doi: 10.1098/rspb.2006.3711

Jonsen, I., McMahon, C., Patterson, T., Auger-Méthé, M., Harcourt, R., Hindell, M., et al. (2019). Movement responses to environment: Fast inference of variation among southern elephant seals with a mixed effects model. *Ecology* 100, e02566. doi: 10.1002/ecy.2019.100.issue-1

Kienle, S. S., Bonin, C. A., Gómez, G., Goebel, M. E., Donke, M., Sperou, E. S., et al. (2024). First paired observations of sexual behavior and calls in wild leopard seals. *Polar Biol.* 47 (10), 1025–1037. doi: 10.1007/s00300-024-03275-4

Kienle, S. S., Goebel, M. E., LaBrecque, E., Borras-Chavez, R., Trumble, S. J., Kanatous, S. B., et al. (2022). Plasticity in the morphometrics and movements of an Antarctic apex predator, the leopard seal. *Front. Mar. Sci.* 9, 976019. doi: 10.3389/fmars.2022.976019

Knopff, A. A., Knopff, K. H., Boyce, M. S., and Clair, C. C. S. (2014). Flexible habitat selection by cougars in response to anthropogenic development. *Biol. Conserv.* 178, 136–145. doi: 10.1016/j.biocon.2014.07.017

Koppes, M., Conway, H., Rasmussen, L., and Chernos, M. (2011). Deriving mass balance and calving variations from reanalysis data and sparse observations, Glaciar San Rafael, northern Patagonia 1950–2005. *Cryosphere* 5, 791–808. doi: 10.5194/tc-5-791-2011

Krause, D. J., Bonin, C. A., Goebel, M. E., Reiss, C. S., and Watters, G. M. (2022). The rapid population collapse of a key marine predator in the northern Antarctic peninsula endangers genetic diversity and resilience to climate change. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.796488

Krause, D. J., Goebel, M. E., and Kurle, C. M. (2020). Leopard seal diets in a rapidly warming polar region vary by year, season, sex, and body size. *BMC Ecol.* 20, 1–15. doi: 10.1186/s12898-020-00300-y

Krause, D. J., Goebel, M. E., Marshall, G. J., and Abernathy, K. (2015). Novel foraging strategies observed in a growing leopard seal (*Hydrurga leptonyx*) population at Livingston Island, Antarctic peninsula. *Anim. Biotelemetry* 3, 1–14. doi: 10.1186/s40317-015-0059-2

Krause, D. J., and Rogers, T. L. (2019). Food caching by a marine apex predator, the leopard seal (*Hydrurga leptonyx*). *Can. J. zoology* 97, 573–578. doi: 10.1139/cjz-2018-0203

Laws, R. (1957). On the growth rate of the leopard seal *Hydrurga leptonyx* (de blainville 1820). *Saugetierkundliche Mitt.* 5, 49–55.

Leger, T. P., Hein, A. S., Bingham, R. G., Martini, M. A., Soteres, R. L., Sagredo, E. A., et al. (2020). The glacial geomorphology of the río Corcovado, Río Huemul and Lago Palena/General Vintter valleys, northeastern Patagonia (43 s, 71 w). *J. Maps* 16, 651–668. doi: 10.1080/17445647.2020.1794990

Leo, V., Reading, R. P., Gordon, C., and Letnic, M. (2019). Apex predator suppression is linked to restructuring of ecosystems via multiple ecological pathways. *Oikos* 128, 630–639. doi: 10.1111/oik.2019.v128.i5

Letnic, M., Koch, F., Gordon, C., Crowther, M. S., and Dickman, C. R. (2009). Keystone effects of an alien top-predator stem extinctions of native mammals. *Proc. R. Soc. B: Biol. Sci.* 276, 3249–3256. doi: 10.1098/rspb.2009.0574

Lüdecke, D., Patil, I., Ben-Shachar, M. S., Wiernik, B. M., Waggoner, P., and Makowski, D. (2021). see: An R package for visualizing statistical models. *J. Open Source Software* 6, 3393. doi: 10.21105/joss.03393

Makowski, D., Ben-Shachar, M. S., Patil, I., and Lüdecke, D. (2020). *Estimation of model-based predictions, contrasts and means* (CRAN). Available at: https://github.com/easystats/modelbased.

Markham, B. J. (1971). Observation on the elephant seal and the leopard seal seals on Brecknock Peninsula (Alberto M. de Agostini National Park). Tierra del Fuego. *Anales Instituto la Patagonia* 2, 160–163.

Meade, J., Ciaglia, M. B., Slip, D. J., Negrete, J., Márquez, M. E. I., Mennucci, J., et al. (2015). Spatial patterns in activity of leopard seals *Hydrurga leptonyx* in relation to sea ice. *Mar. Ecol. Prog. Ser.* 521, 265–275. doi: 10.3354/meps11120

Meier, M., and Post, A. (1987). Fast tidewater glaciers. *J. Geophysical Research: Solid Earth* 92, 9051–9058. doi: 10.1029/JB092iB09p09051

Melkonian, A., Willis, M., Pritchard, M., Rivera, A., Bown, F., and Bernstein, S. (2013). Satellite-derived volume loss rates and glacier speeds for the cordillera Darwin icefield, Chile. *Cryosphere* 7, 823–839. doi: 10.5194/tc-7-823-2013

Menounos, B., Maurer, L., Clague, J. J., and Osborn, G. (2020). Late Holocene fluctuations of Stoppani glacier, southernmost Patagonia. *Quaternary Res.* 95, 56–64. doi: 10.1017/qua.2019.87

Milano, V. N., Grandi, M. F., Schiavini, A. C., and Crespo, E. A. (2020). Sea lions (Otaria flavescens) from the end of the world: Insights of a recovery. *Polar Biol.* 43, 695–706. doi: 10.1007/s00300-020-02672-9

Millán, R., Mouginot, J., Rabatel, A., and Morlighem, M. (2022). Ice velocity and thickness of the world's glaciers. *Nat. Geosci.* 15, 124–129. doi: 10.1038/s41561-021-00885-z

Mouginot, J., and Rignot, E. (2015). Ice motion of the Patagonian icefields of South America: 1984–2014. *Geophysical Res. Lett.* 42, 1441–1449. doi: 10.1002/2014GL062661

Paine, R. T. (1980). Food webs: Linkage, interaction strength and community infrastructure. *J. Anim. Ecol.* 49, 667–685. doi: 10.2307/4220

Ray, C. (1970). Population ecology of Antarctic seals. *Antarctic Ecol.* 1, 398–414.

Reynhout, S. A., Kaplan, M. R., Sagredo, E. A., Aravena, J. C., Soteres, R. L., Schwartz, R., et al. (2021). Holocene glacier history of northeastern cordillera Darwin, southernmost South America (55° s). *Quaternary Res.* 105, 166–181. doi: 10.1017/qua.2021.45

Ripple, W. J., Estes, J. A., Beschta, R. L., Wilmers, C. C., Ritchie, E. G., Hebblewhite, M., et al. (2014). Status and ecological effects of the world's largest carnivores. *Science* 343, 1241484. doi: 10.1126/science.1241484

Robbins, J. R., Poncet, D., Evans, A. R., and Hocking, D. P. (2019). A rare observation of group prey processing in wild leopard seals (*Hydrurga leptonyx*). *Polar Biol.* 42, 1625–1630. doi: 10.1007/s00300-019-02542-z

Rodrigues, P., Seguel, M., Gutiérrez, J., Pavés, H., and Verdugo, C. (2018). Genetic connectivity of the South American fur seal (*Arctocephalus australis*) across Atlantic and pacific oceans revealed by mitochondrial genes. *Aquat. Conservation: Mar. Freshw. Ecosyst.* 28, 315–323. doi: 10.1002/aqc.2870

Rodríguez, D., Bastida, R., Morón, S., Heredia, S., and Loureiro, J. (2003). Occurrence of leopard seals in northern Argentina. *Latin Am. J. Aquat. Mammals* 2, 51–54. doi: 10.5597/lajam00031

Roff, G., Brown, C. J., Priest, M. A., and Mumby, P. J. (2018). Decline of coastal apex shark populations over the past half century. *Commun. Biol.* 1, 223. doi: 10.1038/s42003-018-0233-1

Rogers, T. L., Hogg, C. J., and Irvine, A. (2005). Spatial movement of adult leopard seals (*Hydrurga leptonyx*) in Prydz Bay, Eastern Antarctica. *Polar Biol.* 28, 456–463. doi: 10.1007/s00300-004-0703-4

Rounsevell, D., and Eberhard, I. (1980). Leopard seals, *Hydrurga leptonyx* (Pinnipedia), at Macquarie Island from 1949 to 1979. *Wildlife Res.* 7, 403–415. doi: 10.1071/WR9800403

Rounsevell, D., and Pemberton, D. (1994). The status and seasonal occurrence of leopard seals, *Hydrurga leptonyx*, in Tasmanian waters. *Aust. Mammalogy* 17, 97–102. doi: 10.1071/AM94010

Sagredo, E., and Lowell, T. (2012). Climatology of Andean glaciers: A framework to understand glacier response to climate change. *Global Planetary Change* 86, 101–109. doi: 10.1016/j.gloplacha.2012.02.010

Sagredo, E. A., Rupper, S., and Lowell, T. V. (2014). Sensitivities of the equilibrium line altitude to temperature and precipitation changes along the Andes. *Quaternary Res.* 81, 355–366. doi: 10.1016/j.yqres.2014.01.008

Sahanatien, V., and Derocher, A. E. (2012). Monitoring sea ice habitat fragmentation for polar bear conservation. *Anim. Conserv.* 15, 397–406. doi: 10.1111/j.1469-1795.2012.00529.x

Salame, C., Gonzalez, I., Gomez-Fell, R., Jaña, R., and Arigony-Neto, J. (2020). First record of the occurrence of sea ice in the cordillera Darwin fjords (54 s), Chile. *Ann. Glaciology* 61, 472–482. doi: 10.1017/aog.2021.3

Schaefer, M., Machguth, H., Falvey, M., Casassa, G., and Rignot, E. (2015). Quantifying mass balance processes on the southern Patagonia icefield. *Cryosphere* 9, 25–35. doi: 10.5194/tc-9-25-2015

Schmitz, O. J., Hawlena, D., and Trussell, G. C. (2010). Predator control of ecosystem nutrient dynamics. *Ecol. Lett.* 13, 1199–1209. doi: 10.1111/j.1461-0248.2010.01511.x

Schneider, C., Braun, M., Schaefer, M., Grießinger, J., and Casassa, G. (2020). Editorial: climate impacts on glaciers and biosphere in fuego-patagonia. *Front. Earth Sci.* 8. doi: 10.3389/feart.2020.00091

Schwarz, L. K., Goebel, M. E., Costa, D. P., and Kilpatrick, A. M. (2013). Top-down and bottom-up influences on demographic rates of Antarctic fur seals Arctocephalus gazella. *J. Anim. Ecol.* 82, 903–911. doi: 10.1111/jane.2013.82.issue-4

Sepúlveda, M., Carrasco, P., and Quiñones, R. (2021). Long-term abundance dynamics of the south American sea lion (*Otaria flavescens*) in the most important breeding colony in central Chile. *Austral Ecol.* 46, 1277–1286. doi: 10.1111/aec.13086

Sernapesca (2023). *Stranding events reports*. Available online at: https://www.sernapesca.cl/informacion-utilidad/registro-de-varamientos/. (Accessed [February 10th, 2023]).

Shaughnessy, P. D., Tomo, I., Gibbs, S. E., Kemper, C. M., and Stemmer, D. (2023). Records of leopard seals *Hydrurga leptonyx* ashore in south Australia 2017–2022. *Aust. Mammalogy* 46, AM22041. doi: 10.1071/AM22041

Sherman, C. S., Heupel, M. R., Moore, S. K., Chin, A., and Simpfendorfer, C. A. (2020). When sharks are away, rays will play: Effects of top predator removal in coral reef ecosystems. *Mar. Ecol. Prog. Ser.* 641, 145–157. doi: 10.3354/meps13307

Simpson, E. (1875). *Esploraciones hechas por la corbeta Chacabuco al mando del capitan de fragata D. Enrique M. Simpson en los archipiélagos de las Guaitecas, Chonos i Taitao* (Santiago, Chile: Anuario Hidrográfico de la Marina de Chile I), 3–147.

Siniff, D., and Stone, S. (1985). "The role of the leopard seal in the tropho-dynamics of the Antarctic marine ecosystem," in *Antarctic nutrient cycles and food webs* (Berlin, Heidelberg: Springer), 555–560.

Soteres, R. L., Peltier, C., Kaplan, M. R., and Sagredo, E. A. (2020). Glacial geomorphology of the strait of Magellan ice lobe, southernmost Patagonia, South America. *J. Maps* 16, 299–312. doi: 10.1080/17445647.2020.1736197

Soteres, R. L., Riquelme, F. M., Sagredo, E. A., and Kaplan, M. R. (2023). (Paleo) glacier studies in Patagonia over the past decades, (1976–2020): A bibliometric perspective based on the Web of Science. *J. South Am. Earth Sci.* 122, 104173. doi: 10.1016/j.jsames.2022.104173

Soteres, R. L., Sagredo, E. A., Moreno, P. I., Lowell, T. V., and Alloway, B. V. (2022). Glacial geomorphology of the central and southern Chilotan archipelago (42.2° s–43.5° s), northwestern Patagonia. *J. Maps* 18, 151–167. doi: 10.1080/17445647.2021.2008538

Southwell, C., Paxton, C. G., Borchers, D., Boveng, P., Rogers, T., and de la Mare, W. K. (2008). Uncommon or cryptic? Challenges in estimating leopard seal abundance by conventional but state-of-the-art methods. Deep Sea Research Part I. *Oceanographic Res. Papers* 55, 519–531. doi: 10.1016/j.dsr.2008.01.005

Sperou, E. S., Crocker, D. E., Borras-Chavez, R., Costa, D. P., Goebel, M. E., Kanatous, S. B., et al. (2023). Large and in charge: Cortisol levels vary with sex, diet, and body mass in an Antarctic predator, the leopard seal. *Front. Mar. Sci.* 10, 1179236. doi: 10.3389/fmars.2023.1179236

Staniland, I. J., Ratcliffe, N., Trathan, P. N., and Forcada, J. (2018). Long term movements and activity patterns of an Antarctic marine apex predator: The leopard seal. *PloS One* 13, e0197767. doi: 10.1371/journal.pone.0197767

Stewart, B. S., Grove, J. S., Kühlem, A., and Flores, M. (2020). Recent and historic occurrences of leopard seals (*Hydrurga leptonyx*) at easter island (Rapa Nui), Eastern Polynesia. *Polar Biol.* 43, 1899–1902. doi: 10.1007/s00300-020-02741-z

Strelin, J., Casassa, G., Rosqvist, G., and Holmlund, P. (2008). Holocene glaciations in the ema glacier valley, monte Sarmiento massif, Tierra del Fuego. *Palaeogeography Palaeoclimatology Palaeoecol.* 260, 299–314. doi: 10.1016/j.palaeo.2007.12.002

Testa, J. W., Oehlert, G., Ainley, D. G., Bengtson, J. L., Siniff, D. B., Laws, R. M., et al. (1991). Temporal variability in Antarctic marine ecosystems: periodic fluctuations in the phocid seals. *Can. J. Fisheries Aquat. Sci.* 48, 631–639. doi: 10.1139/f91-081

Thorndycraft, V. R., Bendle, J. M., Benito, G., Davies, B. J., Sancho, C., Palmer, A. P., et al. (2019). Glacial lake evolution and Atlantic-Pacific drainage reversals during deglaciation of the Patagonian ice sheet. *Quaternary Sci. Rev.* 203, 102–127. doi: 10.1016/j.quascirev.2018.10.036

Torrejón, F., Bizama, G., Araneda, A., Aguayo, M., Bertrand, S., and Urrutia, R. (2013). Descifrando la historia ambiental de los archipiélagos de Aysén, Chile: El influjo colonial y la explotación económica-mercantil republicana (siglos XVI-XIX). *Magallania (Punta Arenas)* 41, 29–52. doi: 10.4067/S0718-22442013000100002

Torres, D., Yañez, J., and Catán, P. (1979). Mamíferos Marinos de Chile. Antecedentes y Situación Actual. *Biología Pesquera (Chile)* 11, 49–81.

van den Hoff, J., Fraccaro, R., Mitchell, P., Field, I., Mcmahon, C., Burton, H., et al. (2005). Estimating body mass and condition of leopard seals by allometrics. *J. Wildlife Manage.* 69, 1015–1023. doi: 10.2193/0022-541X(2005)069[1015:EBMACO]2.0.CO;2

Visser, I. N., van der Linde, K., Richard, S. E., Cooper, T. E., Hardie, T. M., Bout, R., et al. (2022). Can a leopard seal, Hydrurga leptonyx de Blainville, 1820 (Mammalia Phocidae), change its spots? (Spoiler alert: at least one adult female can). *Biodiversity J.* 13, 685–693. doi: 10.31396/Biodiv.Jour.2022.13.3.685.693

van der Linde, K., Visser, I. N., Bout, R., Krause, D. J., Forcada, J., Siniff, D., et al. (2022). A review of leopard seal (*Hydrurga leptonyx*) births and pups using a standardized age-class classification system. *Polar Biol.* 45, 1193–1209. doi: 10.1007/s00300-022-03053-0

Vásquez, A., Flores-Aqueveque, V., Sagredo, E., Hevia, R., Villa-Martínez, R., Moreno, P. I., et al. (2022). Evolution of glacial lake C during the last glacial termination, central Chilean Patagonia (47°s). *Front. Earth Sci.* 10, 817775. doi: 10.3389/feart.2022.817775

Vera, C., Vargas, R., and Torres, D. (2004). El impacto de la foca leopardo en la población de cachorros de lobo fino antártico en cabo Shirreff, Antártica, temporada 2003-2004. *Boletín del Instituto Antártico Chileno* 23, 19–22.

Vérité, J., Ravier, É., Bourgeois, O., Bessin, P., and Pochat, S. (2023). New metrics reveal the evolutionary continuum behind the morphological diversity of subglacial bedforms. *Geomorphology* 427, 108627. doi: 10.1016/j.geomorph.2023.108627

Walker, T., Boyd, I. L., McCafferty, D., Huin, N., Taylor, R., and Reid, K. (1998). Seasonal occurrence and diet of leopard seals (*Hydrurga leptonyx*) at bird island, South Georgia. *Antarctic Sci.* 10, 75–81. doi: 10.1017/S0954102098000108

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis* (New York: Springer-Verlag). Available at: https://ggplot2.tidyverse.org.

Wiig, Ø., Aars, J., and Born, E. W. (2008). Effects of climate change on polar bears. *Sci. Prog.* 91, 151–173. doi: 10.3184/003685008x324506

Williams, T. M., Estes, J. A., Doak, D. F., and Springer, A. M. (2004). Killer appetites: Assessing the role of predators in ecological communities. *Ecology* 85, 3373–3384. doi: 10.1890/03-0696

Wilmers, C. C., and Post, E. (2006). Predicting the influence of wolf-provided carrion on scavenger community dynamics under climate change scenarios. *Global Change Biol.* 12, 403–409. doi: 10.1111/j.1365-2486.2005.01094.x

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J. R. Stat. Soc. (B)* 73, 3–36. doi: 10.1111/j.1467-9868.2010.00749.x

Wood, S. N. (2017). *Generalized additive models: An introduction with r. 2nd Ed* (Chapman: Hall/CRC).

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Which microbiome are we talking about? Contrasted diversity patterns and eco-evolutionary processes between gill and intestinal microbiomes of Antarctic fairy shrimps

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Metazoans comprise multiple physical niches ("microenvironments"), each colonized by unique microbiomes that contribute to their hosts' evolutionary dynamics, influencing their health, physiology, and adaptation to changing environments. Most wildlife microbiome studies focus on higher metazoans and multiple host microenvironments, while studies of lower species often concentrate on a single microenvironment, sometimes pooling whole bodies or specimens. This is particularly evident in small-sized animals, such as freshwater meiofaunal invertebrates, thus impeding a holistic understanding of microbiome assembly across host microenvironments and its relation with host population genetics. Leveraging the anostracan fairy shrimp *Branchinecta*, which has easily discernible organs and expected high levels of intraspecific genetic divergence, we aimed to investigate the microbiome assembly processes and test the phylosymbiosis signal in two microenvironments (gill and intestine) across four host populations of *Branchinecta gaini* within Maritime Antarctica, using 16S rRNA metabarcoding. Our results showed that the gill and intestine harbor strikingly different microbiomes resulting from the *B. gaini* ecological filtering of the surrounding environment microbial community. Both microenvironments exhibit their respective core microbiomes, yet the gill's core microbiome is narrower and constitutes a smaller proportion of the overall bacterial community compared to that of the intestine. Within each host population (*i.e.* each sampling site), the microbiome assembles through distinct eco-evolutionary processes in both microenvironments, mostly stochastically (ecological drift) in the gill and deterministically (variable selection) in the intestine. Across different *B. gaini* populations, variable selection dominates in driving compositional divergence of both microenvironment microbiomes, although to a lesser extent in the gill. Lastly, our study reveals robust correlation between host intraspecific genetic structure and intestine microbiome composition, providing evidence of phylosymbiosis in

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anostracans. Contrastingly, phylosymbiosis was less pronounced in the gill microbiome. We discuss the potential differences in ecological filtering between each host microenvironment that may underlie the difference in the strength of phylosymbiosis. Our study highlights the relevance of considering host microenvironment and intraspecific levels in testing the phylosymbiosis hypothesis to better understand the intricate eco-evolutionary relationships between hosts and their microbiomes.

KEYWORDS

invertebrate microbiome, gill, intestine, ecological filtering, ecological assembly processes, *Branchinecta*, West Antarctic Peninsula, freshwater ecosystems

1 Introduction

Most macroorganisms exist in close association with diverse microbial partners, colonizing various physical niches provided by their hosts, thereby conforming what is termed metaorganisms (McFall-Ngai et al., 2013; Ludington, 2024). The variability in microbiome composition and function across different body parts has been linked to various facets of the host ecology (Zilber-Rosenberg and Rosenberg, 2008; Alberdi et al., 2022; Ludington, 2024). An iconic example is the human body, where each organ constitutes a distinct ecological niche that hosts a unique microbiome carrying out specific functions essential for host health (Costello et al., 2009; Dekaboruah et al., 2020). However, most of the animal-associated microbiome studies remain confined to compositional description of single-body sites (Rosenberg and Zilber-Rosenberg, 2018; Woodhams et al., 2020; Wang et al., 2023). Considering various body sites within the same host is necessary to gain a holistic understanding of the eco-evolutionary processes driving microbiome assembly (Stegen et al., 2013; Zhou and Ning, 2017). One of these eco-evolutionary processes is the contribution of the host evolution to microbiome assembly. Indeed, the intimate and prolonged ecological interdependency of hosts and their microbiomes can result in continuous mutual adaptation, also known as co-evolution (Wilson and Duncan, 2015; Rosenberg and Zilber-Rosenberg, 2018; Hayward et al., 2021; Mazel et al., 2023). Co-evolution can leave a phylogenetic footprint of the host in the microbiome composition, meaning that closely related host species exhibit more similar microbiomes, which is known as phylosymbiosis (Bordenstein and Theis, 2015; Brooks et al., 2016; Groussin et al., 2020). Although the detection of phylosymbiosis signature does not explicitly underpin a common evolutionary history between hosts and their microbiomes (Moran and Sloan, 2015; Mazel et al., 2018; Mallott and Amato, 2021), it constitutes a relevant hypothesis to delve into the eco-evolutionary mechanisms that shape metaorganisms-associated microbiome (Lim and Bordenstein, 2020; Mazel et al., 2023; Mallott, 2024).

Phylosymbiosis has been extensively tested across a biased fraction of metazoan species (Woodhams et al., 2020; Mazel et al., 2023), primarily vertebrates, including mammals (Groussin et al., 2017; Moeller et al., 2017; Youngblut et al., 2019) and fishes (Doane et al., 2020; Pan et al., 2023; Sadeghi et al., 2023; Schwob et al., 2024a), and to a lesser extent in invertebrates, like insects (Qin et al., 2023), sponges and corals (Pollock et al., 2018; O'Brien et al., 2020; Pankey et al., 2022). Despite a growing body of research, the universality of phylosymbiosis still remains to be fully tackled (Mazel et al., 2018; Lim and Bordenstein, 2020). The frequency and magnitude of phylosymbiosis appear to be inconsistent among taxonomic groups (Lim and Bordenstein, 2020), being relatively common and strong across mammals (Mallott and Amato, 2021), while looser in other vertebrates, such as birds (Trevelline et al., 2020; Bodawatta et al., 2022), and even absent in most (though not all) of the terrestrial and aquatic invertebrates studied (Hammer et al., 2019; Huot et al., 2019; Eckert et al., 2021; Boscaro et al., 2022; Eckert et al., 2022). Consequently, general conclusions about the underlying mechanisms of phylosymbiosis may be skewed by the restricted range of investigated models (Hammer et al., 2019), emphasizing the need to consider understudied species (Leasi et al., 2023). Some authors suggested that the phylosymbiosis signal might be obscured by extended divergence time among studied species (Groussin et al., 2017; Kohl et al., 2018; Ross et al., 2018), as well as by taxonomically rich microbiomes (Mallott and Amato, 2021), which contain a relatively high proportion of transient microorganisms (Ross et al., 2018). Conversely, the phylosymbiosis signal seems to be more frequent in internal rather than external host body sites (Moran et al., 2019). Moreover, considering the intraspecific variability of hosts offers an essential, yet often overlooked, resolution level for generalizing conclusions about the consistency of phylosymbiosis across taxonomic resolution and understanding how intraspecific host genetic variation associates with microbiome variation (Mazel et al., 2018; Couch and Epps, 2022). To the best of our knowledge, no study combines intraspecific resolution and multiple body microenvironments to test the eco-evolutionary processes in general, and phylosymbiosis in particular.

Characterizing the microbiome assembly across multiple body sites can be challenging, particularly in small-sized animals. To date, the microbiome of meiofaunal freshwater invertebrates like copepod and cladoceran has only been investigated at the whole individual-scale. These invertebrates host a specific microbiome discernable from the surrounding water microbiome (Samad et al., 2020), confirming the host-driven ecological filtering (Mazel et al., 2018). In *Daphnia* (Anamopoda), specific bacteria were consistently associated with geographically and genetically separated host populations, suggesting a stable "core microbiome" shaped by host-microbiome interactions (Qi et al., 2009; Guivier et al., 2018). Additionally, the gut microbiome of *Daphnia magna* is shaped by host genotype and enhances host fitness by providing essential nutrients (Cooper and Cressler, 2020; Macke et al., 2020; Motiei et al., 2020). In the branchiopod genus *Artemia* (Anostraca), host species has been identified as a key determinant of microbiome composition. The *Artemia* microbiome influences host tolerance to low salinity, thereby potentially contributing to its evolutionary trajectory (Nougué et al., 2015; Lee et al., 2024). Despite the ecological significance of branchiopoda-associated microbiomes, their variability across body sites and their eco-evolutionary drivers are unclear (Eckert et al., 2021; Boscaro et al., 2022). In previous phylosymbiosis studies focusing on invertebrates, the relatively small size imposed to use the whole individuals (Eckert et al., 2021; Leasi et al., 2023), or even to pool several individuals for microbiome assembly (Callens et al., 2018; Eckert et al., 2022). Besides ignoring the microbiome variability across host body site and among individuals, these approaches may have masked the impact of host phylogeny on microbiome assembly (Mazel et al., 2018).

The anostracan fairy shrimp *Branchinecta gaini* Daday, (1910) is the largest (adult body size c. 16 mm) and most abundant crustacean species of Antarctic lacustrine habitats. *B. gaini* is widely distributed across Maritime Antarctica (*i.e.*, South Shetland Islands, the Antarctic Peninsula, and the islands of the Scotia Arc) and southern South America (Hawes, 2009). It plays a key role in the trophic chain of these freshwater ecosystems (Peck, 2004; Hawes, 2009). *B. gaini* is considered as a primarily non-selective detritivore and herbivorous filter feeder (Lukic et al., 2023), although occasional predation on copepods like *Boeckella poppei*, larval stages of *Parochlus steineni* and even conspecific organisms has been reported (Paggi, 1996). *B. gaini* is characterized by two main microenvironments: a prominent branchial lamina (referred to as gill) and a digestive tract (referred to as intestine) easily discernable through body transparency (Lynch, 1964), facilitating the dissection of these tissues despite the host's small size. The microbiome of *B. gaini* remains unexplored to date. Similarly, the intraspecific genetic variation of *B. gaini* in the Maritime Antarctica is uncharacterized, although other Branchinectidae species exhibit high intraspecific genetic divergences (Aguilar et al., 2017). Given the relative isolation of freshwater lentic ecosystems in Maritime Antarctica (*i.e.*, patchiness of suitable habitat), *B. gaini* is likely to exhibit geographic and genetic structuring at an intra-species level, as previously reported in other freshwater invertebrates [*e.g., B. poppei*, Maturana et al. (2020)]. Thus, the fairy shrimp *B. gaini* represents a suitable model for comprehensively investigating the microbiome assembly processes and testing the phylosymbiosis signal across different host microenvironments in relation with the intraspecific genetic structure of host populations.

This study aims to (i) evaluate the distinguishability of *B. gaini* microbiomes between gill and intestine, (ii) identify the core microbiomes of gill and intestine, and their keystone Operational Taxonomic Units (OTUs), across geographically-distant sites in Maritime Antarctica, (iii) compare the eco-evolutionary processes governing gill and intestine microbiome assembly at local and regional scales, and (iv) elucidate the relative effects of host phylogenetic diversity, geography and climate in shaping both *B. gaini* microbiomes.

2 Methods

2.1 Host and environmental samples recollection and preparation

Specimens of *B. gaini* were sampled from Maritime Antarctica lake water between 2018 and 2023, during the austral summer (January), using a hand-crafted net, with mesh size < 1mm (specimen body size of 15–20 mm) (Table 1). The sampling sites encompass four shallow (0.5–1 m-deep) freshwater lakes, located in Signy Island (PUM) in the South Orkney Islands, King George Island (KIT, WUK) in the South Shetland Islands, and Avian Island (ANT) in the West Antarctic Peninsula (Figure 1). Immediately following sampling, entire individuals of *B. gaini* were preserved in absolute ethanol with one 50-ml Falcon tube dedicated per sampling site, for subsequent morphological and molecular analyses. The ethanol-preserved *B. gaini* individuals (8 specimens per site) were dissected under binocular magnifier to separate the head, the gill and the entire intestine with its content. Due to logistical constraints, superficial sediment was collected at a single site (ANT) in sterile Ziploc® bags. Samples from all the microenvironments (*i.e.*, sediment, head, gill and intestine) were conserved into 2-ml sterile cryotubes at -20°C until further molecular analyses (Table 1).

2.2 Molecular procedures

Prior to molecular processing, host individuals were carefully rinsed with sterile water to remove the ethanol and minimize the risk of cross-contamination. For host analysis, the genomic DNA from *B. gaini* head samples was extracted using the DNAeasy Blood & Tissue Kit (Qiagen, CA, USA) protocol for small tissue samples with previous optimizations, including a preliminary incubation in proteinase K+ATL buffer solution overnight at 37°C (Maturana et al., 2020). One segment of the mitochondrial cytochrome c oxidase subunit I (cox1) was amplified using the universal primer LCO1490 and HCO2198 (Folmer et al., 1994). Detailed PCR protocol is given in Supplementary Material (Supplementary Material S1). The obtained PCR products obtained were sequenced in both directions at Macrogen Chile, using Sanger technology.

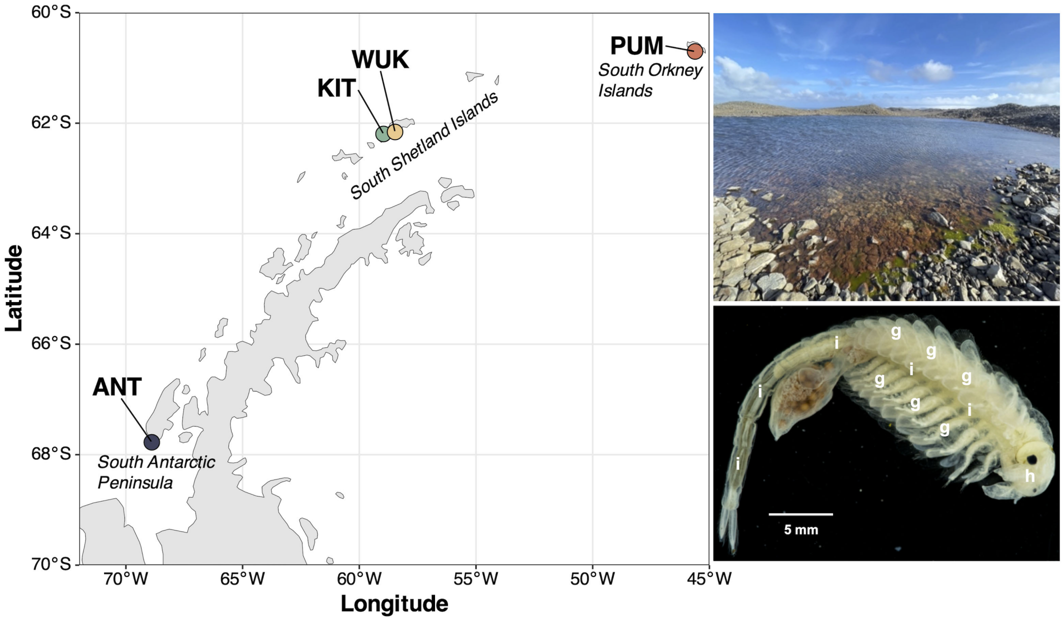

FIGURE 1
Localization of the sampling sites of *Branchinecta gaini* across Maritime Antarctica. From North to South, PUM: Lake 'Pumphouse' from South Orkney Islands, WUK and KIT: Lakes 'Wukja' and 'Kitiesh' from South Shetland Islands, and ANT: Lake 'Antena', from South Antarctic Peninsula (see Table 1 for details). The photos illustrate the characteristic habitat of *B. gaini* (above) and an individual of *B. gaini* (below). White letters "i", "g" and "h" indicate the intestine, the gill and the head, respectively. More detailed photos are provided in Supplementary Figure S1.

For microbiome analysis, the genomic DNA from gill and intestine samples was extracted using the DNAeasy PowerSoil Pro Kit (Qiagen, CA, USA) with a previous FastPrep-24® homogenization step (20 s at 4 m s^{-1}) (MP Biomedicals, USA) according to manufacturer's recommendations. Amplification, library preparation, and amplicon sequencing was carried out by Novogene (Beijing, China). Briefly, the hypervariable V3-V4 region of the 16S rRNA gene was amplified using the 341F-806R primers (Klindworth et al., 2013), and the Phusion® High-Fidelity PCR Master Mix (New England Biolabs, USA), following the PCR protocol detailed in Supplementary Material (Supplementary Material S2).

2.3 Bioinformatic treatment of sequencing data

Host cox1 partial sequences were manually quality controlled, assembled and edited using GENEIOUS 10.2.2 (Kearse et al., 2012).

TABLE 1 Sampling locations of *Branchinecta gaini* and overview of metabarcoding sequencing data.

Region	Locality	Site	Date	GPS coordinates	Microenv.	N	Nseq. (Relat. Abund.)
South Orkney Islands	Signy I.	Lake Pumphouse (PUM)	01-2018	60°42'05.00"S 45°36'51.31"W	Gill	4	681,376 (8.3%)
					Intestine	7	1,251,377 (15.2%)
South Shetland Islands	Fildes Peninsula, King George I.	Lake Wujka (WUK)	01-2019	62°09'32.25"S 58°28'01.77"W	Gill	6	722,924 (8.8%)
					Intestine	8	1,007,827 (12.3%)
South Shetland Islands	Fildes Peninsula, King George I.	Lake Kitiesh (KIT)	01-2019	62°11'41.14"S 58°57'40.71"W	Gill	6	1,023,860 (12.4%)
					Intestine	8	1,246,796 (15.2%)
South Antarctic Peninsula	Margarite Bay, Avian I.	Lake Antena (ANT)	01-2023	67°46'32.59"S 68°53'21.44"W	Water	1	147,072 (1.8%)
					Sediment	3	345,612 (4.2%)
					Gill	7	822,516 (10.0%)
					Intestine	8	975,076 (11.9%)

Microenv., microenvironment; N, number of samples successfully sequenced; Nseqs. (Relat. Abund.), total number of sequences per sample type, and relative abundance in the whole dataset, after sequence cleaning; I., island.

Alignments were obtained using MUSCLE (Edgar, 2004) with standard setting. The host phylogenetic tree was reconstructed from cox1 sequences with NGPhylogeny (Lemoine et al., 2019), using the HKY85+I model recommended by the implemented Smart Model Selection tool (SMS), and complemented by non-parametric SH-like branch supports. Subsequently, the pairwise phylogenetic distances between the pairs of host cox1 sequences within the host were calculated with the *cophenetic.phylo* function implemented in the APE R package (v5.7-1) (Paradis and Schliep, 2019).

Bacterial paired-end raw sequences were analyzed through the Mothur pipeline (v1.48.0) (Schloss et al., 2009), following the previously described procedure (Schwob et al., 2020). Chimeras were removed using Uchime implemented in Mothur (Edgar et al., 2011). Cleaned sequences were clustered into OTUs at 97%-identity, and low-abundance (< 0.0001%) OTUs were discarded according to (Bokulich et al., 2013). The final OTU table, representing a total of 688 OTUs, was rarefied at 80,000 sequences per sample and converted into a Bray-Curtis dissimilarity distance matrix using the *vegdist* function of the VEGAN R package (v2.6-2) (Oksanen et al., 2011). The representative sequences of the OTUs were used to calculate the phylogenetic tree with NGPhylogeny (Lemoine et al., 2019), using the GTR+G+I model recommended by the implemented Smart Model Selection tool (SMS), and complemented by non-parametric SH-like branch supports. The bacterial phylogenetic tree was rooted on the branch separating Cyanobacteria from the rest of the sequences (Coleman et al., 2021).

2.4 Microbiome diversity analysis

The observed richness, the Chao1, and the Shannon indices were estimated using the *plot_richness* function from the PHYLOSEQ R package (version 1.46) (McMurdie and Holmes, 2013), and Faith's phylogenetic diversity was estimated using the *estimate_pd* function from the BTOOLS R package (v0.0.1) (Battaglia, 2021). Mean values of alpha-diversity indices among microenvironments were compared using a non-parametric pairwise Wilcoxon test through the *geom_signif* function of the GGPUBR R package (Kassambara, 2020).

The beta-diversity pattern of *B. gaini* microbiomes across sites and microenvironments was visualized through a hierarchical clustering and a non-metric multidimensional scaling (NMDS) ordination, using the *hclust* function from the DENDEXTEND R package and the *plot_ordination* function in the PHYLOSEQ R package, respectively. Differences in community structure among sites and microenvironments were tested with PERMANOVA and pairwise PERMANOVA using the *adonis2* and *pairwise.adonis* functions (VEGAN and FUNFUNS R packages, respectively). The contribution of individual OTUs to the distinguishability of the microbiome among microenvironments was assessed using Linear Discriminant Analysis (LDA) Effect Size (LEfSe) analysis, performed via the *run_lefse* function in the MICROBIOMEMARKER R package (Cao et al., 2022).

The ecological niche breadth (B_N) was computed for each OTU within the gill and intestine microbiome using the Levins' method implemented in *levins.Bn* function of the MICRONICHE R package (Finn et al., 2020). To classify OTUs as generalists, specialists, or neutralists within gill and intestine microbiomes, their Levins' B_N values were compared to a null distribution, following the methodology described in Gao et al. (2023). The proportions of neutralists, generalists, and specialists were statistically compared between gill and intestine microenvironments using the DUNN.TEST R package (Dinno, 2017). The mean community-level niche breadth was calculated for each sample and the significance of the difference in means between the two microenvironments was assessed using the Wilcoxon test.

To characterize the core microbiome of *B. gaini* gill and intestine, we applied the 'common core' definition (Risely, 2020), which encompasses the OTUs present in a large proportion of hosts (*i.e.*, in > 75% of all replicates from each site), including rare OTUs (*i.e.* no abundance criteria) (Lahti et al., 2017). OTUs with an occurrence frequency of less than 75% were classified as part of the flexible microbiome. The OTU tables of gill and intestine core microbiomes were transformed into relative abundances (normalized by each total core abundance). Co-occurrence networks were computed for each core community using the SPIEC-EASI algorithm with default parameters in R (Kurtz et al., 2015). Nodes' features, including closeness centrality (*i.e.*, the proximity of a node to all other nodes in the network), betweenness centrality (*i.e.*, the extent to which a node lies on the shortest paths among other nodes in the network), and degree count, were used to assess their potential hub taxa status (Berry and Widder, 2014). The microbiome assembly processes within the gill and intestine of *B. gaini* were analyzed both at each individual site and across all sites, and then averaged by microenvironments, using an in-house R function of the framework from Stegen et al. (2015), and previously published in Delleuze et al. (2024).

For phylosymbiosis analysis, Mantel tests with 9,999 permutations were conducted for each microenvironment (gill, intestine) to independently test the correlation between the microbiome Bray-Curtis dissimilarity distance and the host phylogenetic distance matrix using the cophenetic function implemented in the APE R package. The Mantel correlations were visualized under scatterplots generated with the GGPLOT R package (Wickham, 2016). Tanglegrams were generated using the *tanglegram* function in the DENDEXTEND R package to illustrate the correspondence of samples' order between the dendrograms of gill and intestine microbiomes and the host tree. Partial Mantel tests were employed to determine the contribution of host phylogeny to beta-diversity in gill and intestine microbiomes, while controlling for geographic and climatic distances (Martiny et al., 2006). Geographic distances among sites were computed from the longitude and latitude coordinates using the *earth.dist* function of the FOSSIL R package (Vavrek, 2011). Climatic variables, including precipitation amount (pr), mean daily maximum air temperature (tasmax), mean daily minimum air temperature (tasmin), mean daily air temperature (tas), and surface downwelling shortwave flux (rsds) were obtained from the CHELSA database v2.1 (Karger et al., 2017), selecting January 2018 data as the most coincident with our sampling time. Standardization of climatic variables was performed using the *decostand* function (VEGAN). Next, automatic stepwise model selection for redundancy analysis was performed through the *ordiR2step* function (VEGAN) to select the climatic variables that explained most of the variance in the OTU composition of gill and intestine samples. To address collinearity among explanatory factors, principal component analysis (PCA) was conducted on the selected climatic variables (*i.e.*, pr, tasmax, and rsds), and the PCA axis scores were transformed into Euclidean distances using the *vegdist* function (VEGAN) that will be further used as the climatic distance matrix.

3 Results

3.1 Significant alpha-diversity variations across *B. gaini* microenvironments

Microbiome alpha diversity varied significantly across *B. gaini* microenvironments. Except for the WUK site, the observed and Chao1-estimated OTU richness was approximately twice higher in intestinal samples compared to gill samples (Figure 2; Supplementary Figure S2). Likewise, greater phylogenetic diversity was observed in intestine than in gill (Figure 2). Contrarily, the Shannon diversity values were more homogenous between gill and intestinal samples across sites (Supplementary Figure S2). At the ANT site, alpha diversity indices of the lake sediment (excluding the Shannon index) were comparable to those of the intestine and significantly higher than those of the gill samples (Supplementary Figure S3).

3.2 Unique microbiome features in gill vs. intestine of *B. gaini*

Within each site, clear dissimilarities in microbiome compositions were observed between gill and intestinal microenvironments (pairwise PERMANOVA, p-values < 0.005, Table 2; Figures 3A, B). For ANT and KIT, the microbial communities clustered primarily by microenvironments and secondly by site, indicating that microbiome compositions among samples from the same microenvironment (despite coming from different sites) were more similar than among samples from the same site (Figures 3A, B). Intestinal communities were on average more homogeneous than gill communities (mean pairwise dissimilarity in gill: 0.71 ± 0.006, vs. in intestine: 0.56 ± 0.006,

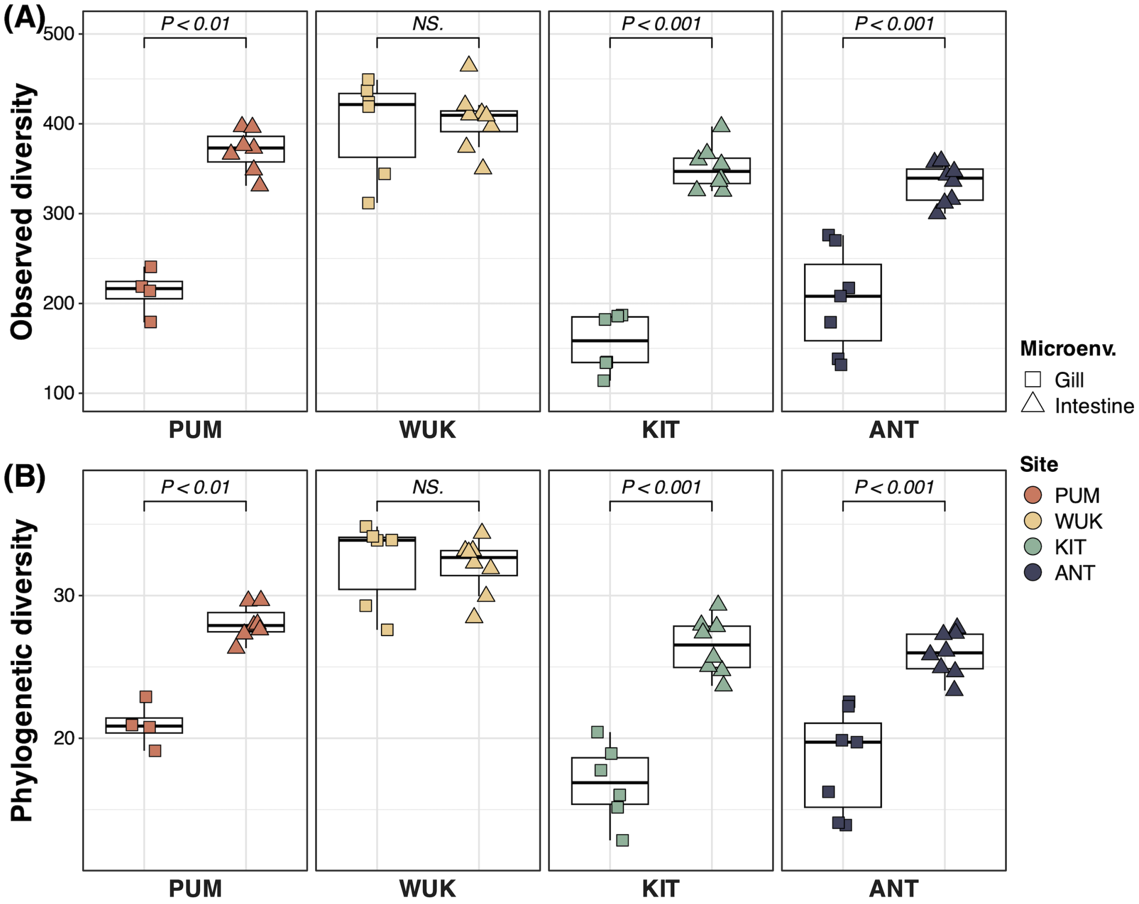

FIGURE 2
Bacterial alpha diversity in gill and intestine microbiomes of *Branchinecta gaini*. The shape and color correspond to the microenvironment and to the site, respectively. The significance of the differences was inferred through Wilcoxon tests, and p-values (*P*) are displayed. '*NS.*' indicates non-significant differences. **(A)** Observed diversity; **(B)** Chao Index.

Wilcoxon test, $p < 0.001$, Supplementary Figure S4). The effect of the sampling site in shaping *B. gaini* microbiome composition was twice as strong as that of the microenvironment ($R^2 = 0.39$, $p = 0.001$ and $R^2 = 0.19$, $p = 0.001$), and was about 25% stronger in intestine than in gill ($R^2 = 0.78$, $p = 0.001$ and $R^2 = 0.51$, $p = 0.001$, respectively, Table 2; Figure 3B). The effect of the host microenvironment on community composition differed depending on the levels of the site factor, as evidenced by the significant interaction in the PERMANOVA ($R^2 = 0.13$, $p = 0.001$, Table 2), and by the hierarchical clustering in which communities clustered either according to the microenvironment (*i.e.*, KIT and ANT) or by site (*i.e.*, PUM and WUK, Figure 3A). At the ANT site, sediment microbiome composition was more similar to intestine than gill samples (Figures 3A, B).

The partitioning of gill, intestinal, and sediment microbiomes was explained by the significant enrichment of discriminant bacterial taxa within each microenvironment, with a total of 375 discriminant OTUs identified through LEfSe analysis. The taxonomic affiliations of the top 20 OTUs with highest LDA scores within each microenvironment encompassed 12 bacterial classes (Figure 4). In the gill microbiome, discriminant OTUs were mainly from the *Bacteroidia* class, predominantly represented by *Flavobacterium*. In the intestine microbiome, most of discriminant OTUs were assigned to the *Clostridia* and *Alphaproteobacteria* classes, represented by *Clostridium* and *Tabrizicola*, respectively (Figure 4). The discriminant OTUs of sediment microbiomes predominantly belonged to the *Gammaproteobacteria* and *Bacteroidia* classes, represented by various taxa such as *Rhodoferax* and *Lentimicrobiaceae*, among others.

The Levins' niche breadth index (Bj) was roughly 30% higher in the intestine compared to the gill microenvironment (Wilcoxon test, $p < 0.001$, Figure 5, left panel). The proportion of specialist OTUs was strongly higher in the gill community (96%) than in the intestine community (52%). Generalist OTUs were nearly absent in both microenvironments (< 0.01%), and around half of the intestinal OTUs were neutralists (Figure 5, right panel).

3.3 Core microbiome detected but no hub taxa in gill and intestine of *B. gaini*

All core OTUs detected had a minimum prevalence of 87.5% across all samples and variable abundances, particularly within the intestine, as indicated by the abundance-occupancy curves (Figure 6A). Gill samples revealed a more flexible microbiome with only 12 core OTUs detected, whereas intestinal samples displayed 107 core OTUs (Figure 6B). In both microenvironments, the core OTUs accounted for substantial fractions of the entire bacterial communities, with cumulative relative abundances of 45.5% (gill) and 73.3% (intestine) of the community (Figure 5). The core composition was mostly distinct between both microenvironments. In line with the microenvironment-specific OTUs detected in the preceding section, the gill core microbiome was mostly dominated in abundance by OTUs belonging to genera like *Polaromonas* and *Flavobacterium*. Conversely, the intestine core microbiome was primarily dominated by OTUs belonging to *Intrasporangiaceae* and *Tabrizicola* (Figure 4; Figure 6C). The only bacterial genera (with relative abundance > 0.015) represented in both gill and intestine were *Clostridium sensu stricto 9* and *13* (Figure 6C), previously detected as significantly enriched solely within intestinal samples (Figure 4).

Co-occurrence networks computed for both gill and intestine core microbiomes did not reveal any hub taxa in either microenvironment. In the gill core microbiome, a single significant interaction was identified between *Acinetobacter* and *Microbacteriaceae* taxa, with betweenness centrality values below the threshold to be considered as hub taxa. Contrastingly, in the intestine microbiome, co-occurrence interactions among core

TABLE 2 Individual and pairwise PERMANOVA on Bray-Curtis dissimilarities assessing the effect of microenvironment and site on microbiome composition.

Dataset	Sample grouping	Df	Sums of square	F-statistics	R^2	*p*-value
ALL†	Microenv.	1	2.56	29.70	0.19	0.001
	Site	3	5.20	20.12	0.39	0.001
	Microenv. * Site	3	1.68	6.51	0.13	0.001
ANT	Microenv.	2	2.80	17.58	0.70	0.001
	↳ Sediment vs. Gill	–	–	13.51	0.63	0.010
	↳ Sediment vs. Intestine	–	–	22.85	0.72	0.010
	↳ Gill vs. Intestine	–	–	13.90	0.52	0.001
KIT	↳ Gill vs. Intestine	–	–	12.59	0.51	<0.001
WUK	↳ Gill vs. Intestine	–	–	10.49	0.47	<0.001
PUM	↳ Gill vs. Intestine	–	–	10.50	0.54	0.005
Gill	Site	3	3.97	32.26	0.51	0.001
Intestine	Site	3	2.92	6.47	0.78	0.001

Significance was inferred through 999 permutations. † Without sediment samples from ANT. Microenv., microenvironment.

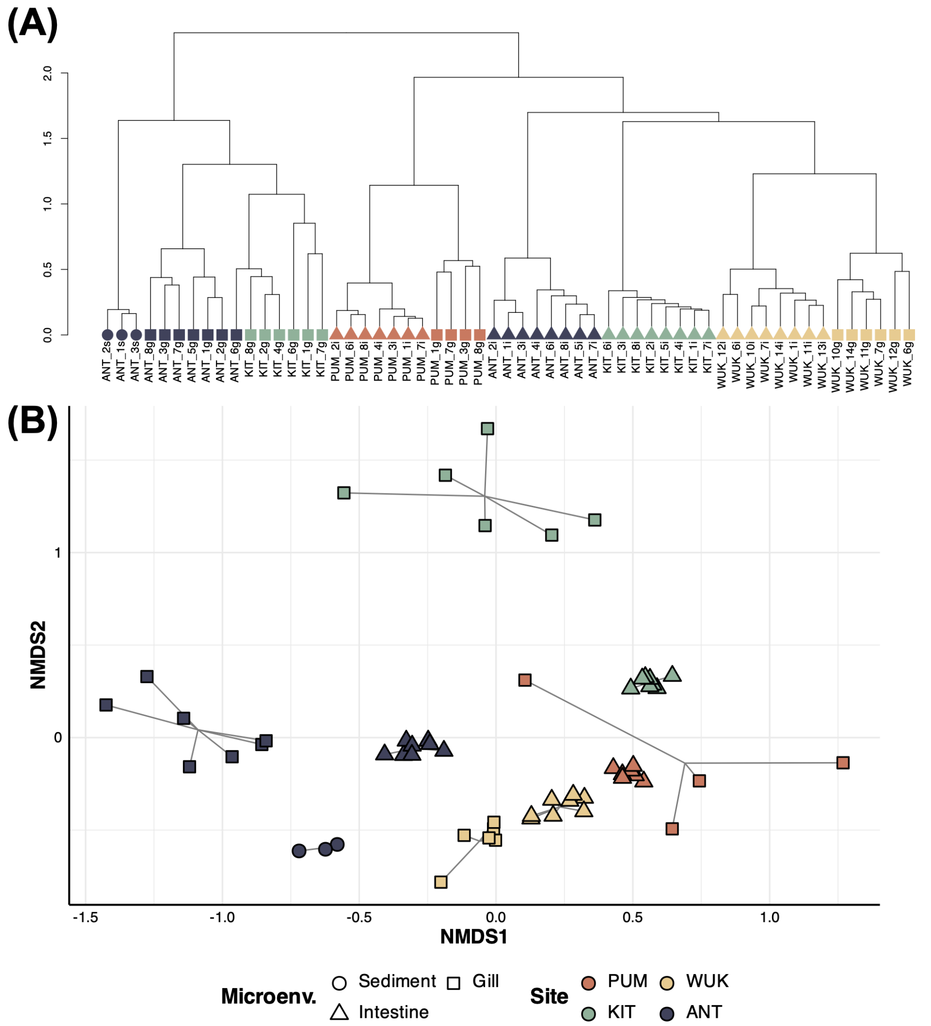

FIGURE 3
Bacterial beta diversity in *Branchinecta gaini*-associated and lake sediment microbiomes. Hierarchical clustering **(A)** and non-metric multidimensional scaling ordination **(B)** are based on Bray-Curtis dissimilarities among *B. gaini* microenvironments. Clustering is performed using the ward. D2 agglomeration method. The shapes and colors correspond to the microenvironment and to the site, respectively. In sample nomenclature, the first three letters, the number, and the last lowercase letter correspond to the site, the sample number, and the microenvironment, respectively.

OTUs were more abundant, with average betweenness and closeness centrality of 209.2 ± 20.0 and 0.002 ± $2.5e^{-5}$, respectively, and a degree count of 3.3 ± 0.2; however, none of the nodes in the core co-occurrence network reached the 97th percentile of closeness and betweenness centrality values, precluding them from being classified as hub OTUs (Supplementary Figure S5).

3.4 Divergence-driving processes dominate in *B. gaini* microbiome assembly

Ecological processes leading to microbiome divergence (*i.e.*, ecological drift, dispersal limitation, and variable selection) accounted for most (> 95%) of the community composition turnover in both microenvironments, regardless of the geographical scale (*i.e.*, intra- and inter-site) (Figures 7A, B). However, the respective contributions of these ecological processes were markedly different between the gill and intestine. Notably, at the intra-site scale, variable selection was the dominant process within the intestinal microbiome across *B. gaini* individuals, accounting for 60% of the community composition turnover, while it only explains about 6% of the turnover observed in the gill microbiome. In contrast, ecological drift was the most important process in the gill microbiome, driving more than 70% of the community composition turnover but only 36% in the intestine microenvironment. Additionally, the dispersal limitation contribution, absent in the intestine microbiome assembly processes, drove 18% of the intra-site turnover of the gill bacterial community (Figure 7A).

When examining the inter-site turnover, the contribution of variable selection increased to 95% and 58% in the gill and intestine microbiomes, respectively. The contribution of dispersal limitation

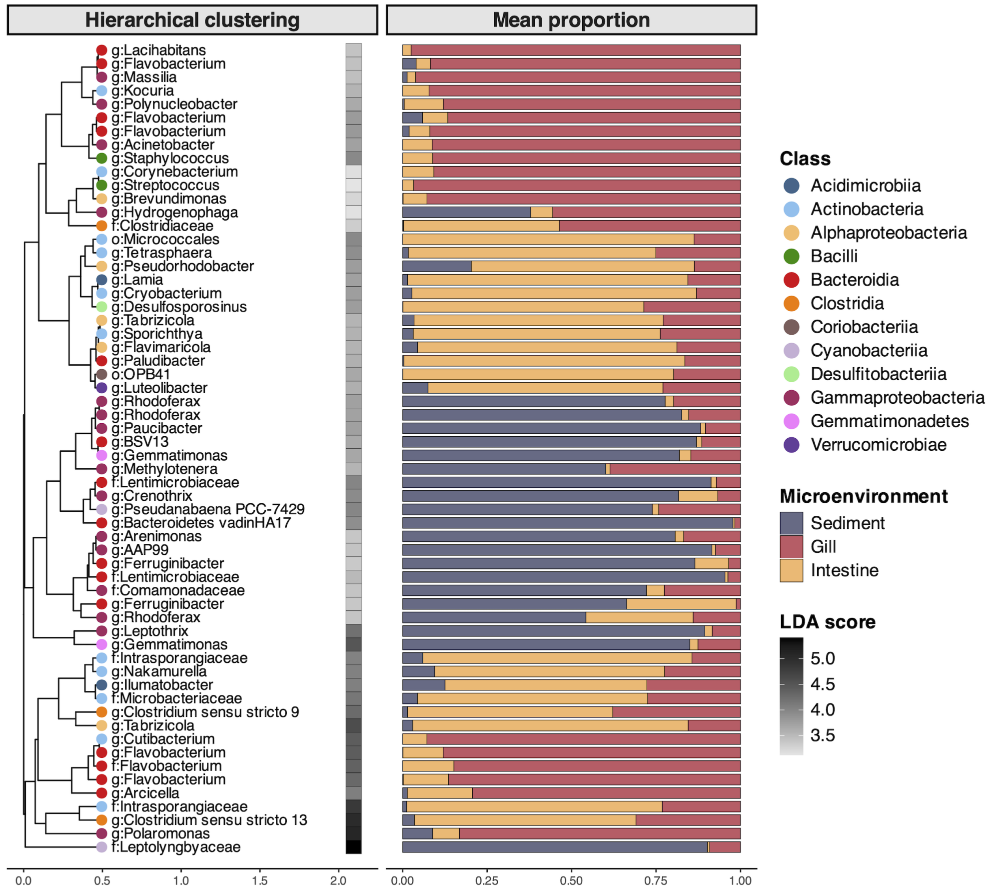

FIGURE 4
The twenty most enriched bacterial OTUs within each *B. gaini* and lake sediment microenvironments identified through Linear discriminant analysis (LDA) effect size (LEfSe) analysis. Taxonomic affiliations at the genus level (g): or at the family level (f): when genus was undetermined, and LDA score are presented for each OTU. The LDA score is represented by the color gradient in the heatmap. The barplot indicates the mean relative abundance of the indicator bacterial OTUs to the microbial community within each microenvironment (Microenv.). The hierarchical clustering is based on complete method from OTU relative abundance across microenvironments.

slightly increased, accounting for 21% and 5% in the gill and intestine microbiomes, respectively. Meanwhile, the contribution of ecological drift decreased to 16% and 0% in the gill and intestine microbiomes, respectively (Figure 7B).

3.5 Stronger host phylogenetic footprint in intestinal vs. gill microbiome of *B. gaini*

A substantial congruence was detected between microbiome composition dissimilarity and *B. gaini* phylogenetic distances, with a stronger correlation in the intestine ($R^2 = 0.64$, $p = 0.0001$) compared to the gill ($R^2 = 0.49$, $p < 0.0001$) (Figure 8A). Upon controlling for either climatic or geographic variability, the correlation strength slightly weakened for both microenvironments, but remained higher in the intestine than in the gill microbiome (Supplementary Table S3). In addition, while the *p*-values of partial Mantel tests remained largely significant in case of the intestine, they became less significant for the gill, and even marginally significant in the case of climatic distance-controlled correlation (Supplementary Table S3). Finally, the degree of alignment between the microbiome dendrogram and the host phylogenetic tree labels (*i.e.*, entanglement) was twice higher in the intestine compared to the gill (Figure 8B).

4 Discussion

We have limited understanding of how microbiome assembly vary across host microenvironments and among intraspecific populations of the same species. In this study, we surveyed the microbiome associated with two microenvironments of the fairy shrimp *B. gaini* across four populations distributed throughout Maritime Antarctica, and explored the eco-evolutionary mechanisms influencing the assembly of these microbiomes. Our results refine previous findings on meiofaunal host-microbiome relationships and verify several general hypotheses regarding the detection of phylosymbiosis (Woodhams et al., 2020).

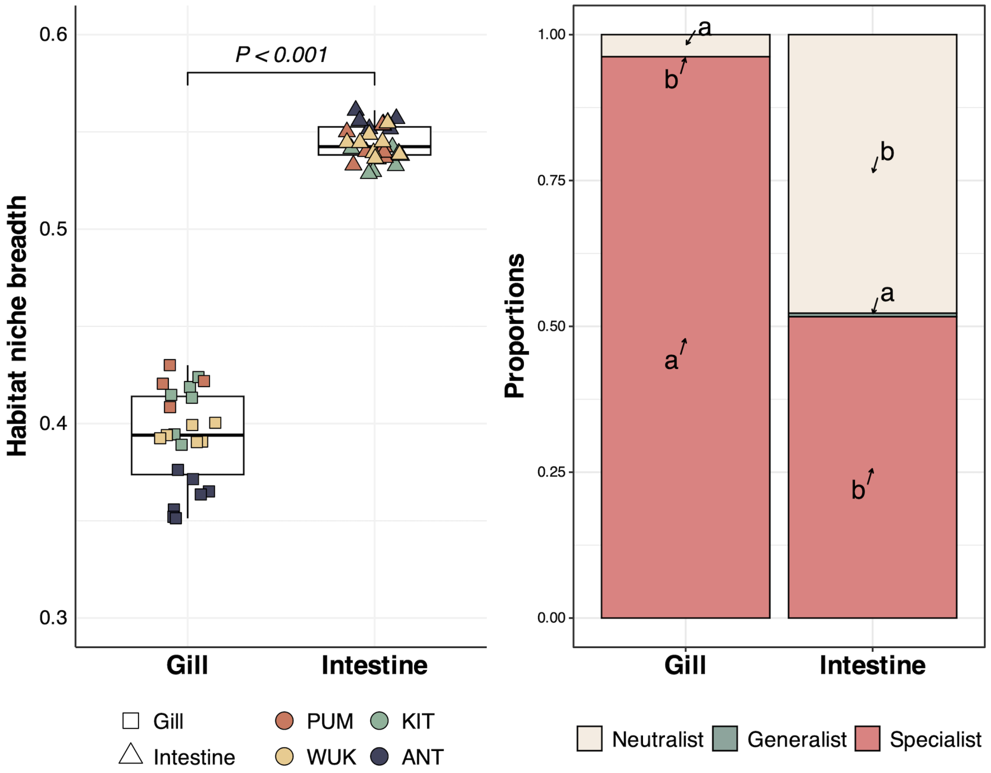

FIGURE 5
Habitat niche breadth (left) and proportions of habitat neutralists, generalists, and specialists (right) of the microbiome of *Branchinecta gaini* microenvironments. In the left panel, means of the Levins niche breadth index (Bj) were calculated for each OTU within a community (*i.e.*, sample). Differences between microenvironments were assessed for significance using the Wilcoxon test. In the right panel, arrows are used to indicate the different category within each microenvironment. Different lowercase letters between gill and intestine for the same category (*e.g.*, neutralists) indicate significant difference, as determined by the Dunn test. All comparisons were statistically different (p-values < 0.001).

4.1 *B. gaini* microbiome is shaped by host microenvironments

Assessing the distinguishability of bacterial community across host microenvironments and between the host and the surrounding environment is an essential first step to further unravel the microbiome significance in host ecology and evolution (Hammer et al., 2017; Schwob et al., 2024b). The composition of gill and intestine microbiomes of *B. gaini* significantly differed from each other. This finding demonstrates that fairy shrimps host body-site specific microbiomes, as previously observed in bigger crustaceans from Malacostraca class such as shrimps and crabs (Zhang et al., 2016; Cornejo-Granados et al., 2018). Our results underscore the importance of examining distinct organs individually, even in small and seemingly simple organisms like branchiopods. Moreover, the gill and intestine microbiomes were distinct from the lacustrine sediment environment. This first evidence of host ecological filtering in *B. gaini* suggests a selective enrichment of specific bacterial taxa able to colonize the gill and intestine tissues within *B. gaini* (Moran and Sloan, 2015; Mazel et al., 2018). A similar pattern has been previously reported through whole individual analysis in other small and microscopic freshwater organisms (Samad et al., 2020; Eckert et al., 2021; Boscaro et al., 2022).

4.2 Greater specificity and stochasticity in gill versus intestine microbiome assembly of *B. gaini*

Based on alpha-diversity results, host filtering seems to be stronger in the gill than in the intestine, limiting its colonization to a fewer number of, and more phylogenetically homogeneous, bacterial taxa compared to the intestine samples. This suggests that the gill may be a more selective habitat with a lower microbial carrying capacity compared to the intestine. Consistently, almost all the gill OTUs were identified as specialists according to the Levin's niche breadth framework. For instance, the most enriched and prevalent bacterial taxa in the gills is *Polaromonas*, a psychrophilic genus previously isolated from saline pond water, and various glacial environments, including lake microbial mat and glacier surfaces in Antarctica (Kapardar et al., 2010). Some authors propose that this genus can form dormant cells, facilitating its survival and dispersion over time and space (Darcy et al., 2011). Moreover, the *Polaromonas* genus is described as metabolically versatile due to high levels of horizontal gene transfer, allowing it to thrive during transient periods of higher temperatures and substrate availability (Yagi et al., 2009). This "opportunitroph" lifestyle (Polz et al., 2006) echoes the ecological strategy observed in *B. gaini*, which can survive extreme environmental fluctuations (Peck, 2004).

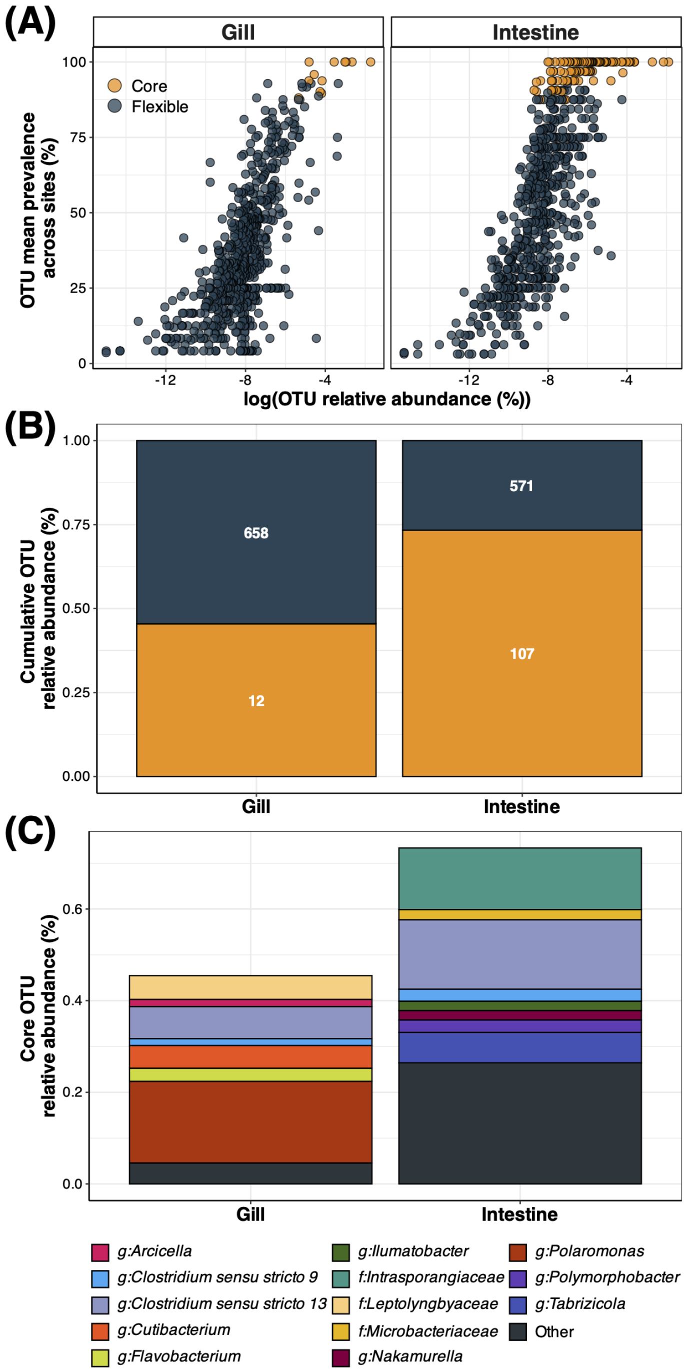

FIGURE 6
Bacterial core and flexible microbiomes of *B. gaini* gill and intestine. **(A)** represents the abundance-occupancy curves of all OTUs in *B gaini* gill and intestine. Colors indicate either the core (carrot orange) or flexible OTUs (charcoal blue). **(B)** represents the cumulative relative abundance of core (carrot orange) and flexible OTUs (charcoal blue) in the gill and intestine. The number inside each bar section indicates the number of OTUs. **(C)** displays the taxonomic composition at the genus level (or family level when genus information was not available) of the gill and intestine core microbiome OTUs. Core OTUs with relative abundance < 0.015 are represented under the label "Other".

B. gaini gills were also enriched in *Flavobacterium*, a genus associated with gill rot symptoms in various cold freshwater fish species (Farkas, 1985). The enrichment of these genera in the gills of freshwater invertebrates from the surrounding habitat is unprecedented and warrants further dedicated study to uncover their potential ecological roles.

The apparently stronger specificity observed in the gill versus the intestine aligns with the greater contribution of dispersal limitation processes in the gill, as previously shown in other organ-associated microbiomes of invertebrates and mammals (Mazel et al., 2024). This suggests that gill-associated OTUs possess phenotypic bacterial traits associated with reduced dispersal abilities (Mallott, 2024).

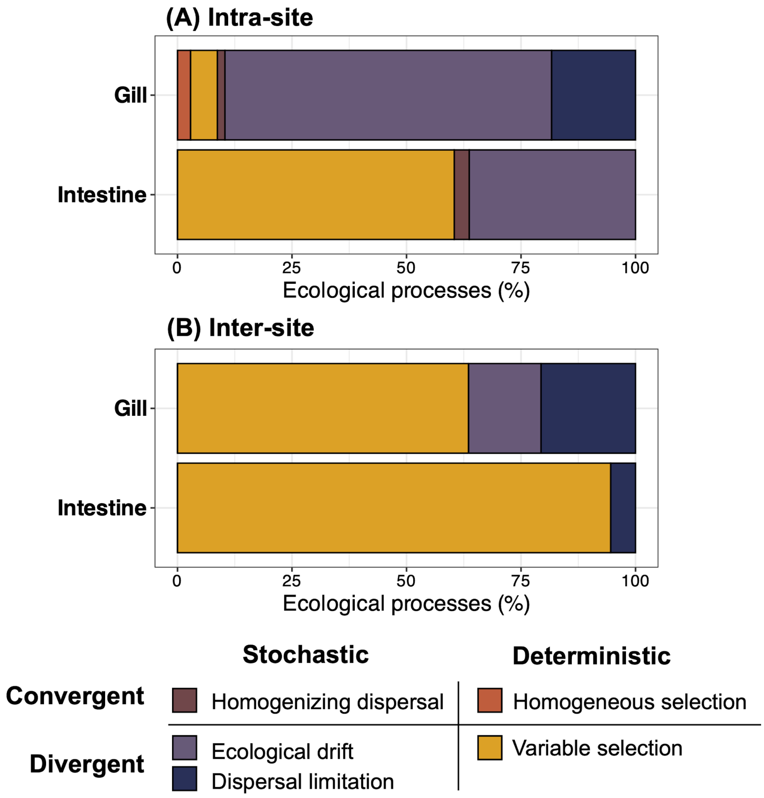

FIGURE 7
Estimation of the ecological processes governing the microbiome of *Branchinecta gaini* microenvironments within **(A)** and among **(B)** sites. The significance of the contribution changes in ecological processes was determined using a permutation test. The 'ecological processes' axis refers to the mean contribution of the community assembly processes.

Additionally, we identified a narrow core microbiome in the gill consisting of only 12 OTUs, yet accounting for almost 50% of all reads in the gill. This finding suggests that at least a fraction of the gill microbiome may be less transitory, reflecting some degree of host-symbiont fidelity over time and space (Risely, 2020).

However, the relatively higher variability in gill microbiome composition compared to the intestine, coupled with the greater proportion of flexible microbiome, challenges the notion of a highly specific gill microenvironment and prompts distinct scenarios to explain this discrepancy. First, in line with observations from other crustacean species, we propose that the microbial community colonizing *B. gaini* gill is periodically eliminated and gradually re-establishes after each host molting event (Corbari et al., 2008; Middlemiss et al., 2015; Zhang et al., 2021). Although the molting rate in *B. gaini* remains uncertain, estimates for the closely related species *B. giga* suggest approximately 18 molts per life cycle (Daborn, 1975). It is thus plausible that asynchronicity in molting and differences in the gill recolonization outcomes across individuals introduce variability in the microbiome composition.

Alternatively, the unique anatomical structure of the anastrocan gills, which lack a protective gill chamber unlike larger invertebrates, results in a more direct and continuous exposure to the environment (Paggi, 1996). Consequently, the gill microbiome may be more influenced by the lake water column than the intestine, leading to greater variability across sampling sites, potentially reflecting environmental differences among lakes. This aligns with the stronger increase of variable selection from intra-site to inter-sites in the gill compared to the more stable intestine microbiome. The high flexibility of microbiome in response to the external environment has been previously described in freshwater zooplankton, including rotifers, copepods, and cladocerans (Eckert et al., 2021). Although the lack of direct water sampling in this study limits our understanding of the water microbiome's influence on the gill microbiome, *Branchinecta* species primarily inhabit the surface of the nutrient-rich substrates at the bottom of Antarctic lakes, rather than the water column. These sediments can be considered as their primary habitat, as *Branchinecta* actively plow through superficial sediments using their thoracopods (Cáceres and Rogers, 2015). Additionally, the interstitial water present in the sediment samples provides an indirect representation of the water environment, compensating to some extent for the absence of explicit water samples in this study.

Finally, stochastic processes, primarily ecological drift, dominates in *B. gaini* gill microbiome assembly at a local scale (intra-site), suggesting that the microbiome is mostly acquired horizontally through pervasiveness uptake of environmental bacteria (Hammer et al., 2017, 2019; Rosenberg and Zilber-Rosenberg, 2021). Ecological assembly processes have not been specifically estimated in the past in crustacean models, but this finding intuitively contrasts with the typical pattern described in gill of other crustaceans, such as crabs, where the microbiome coating

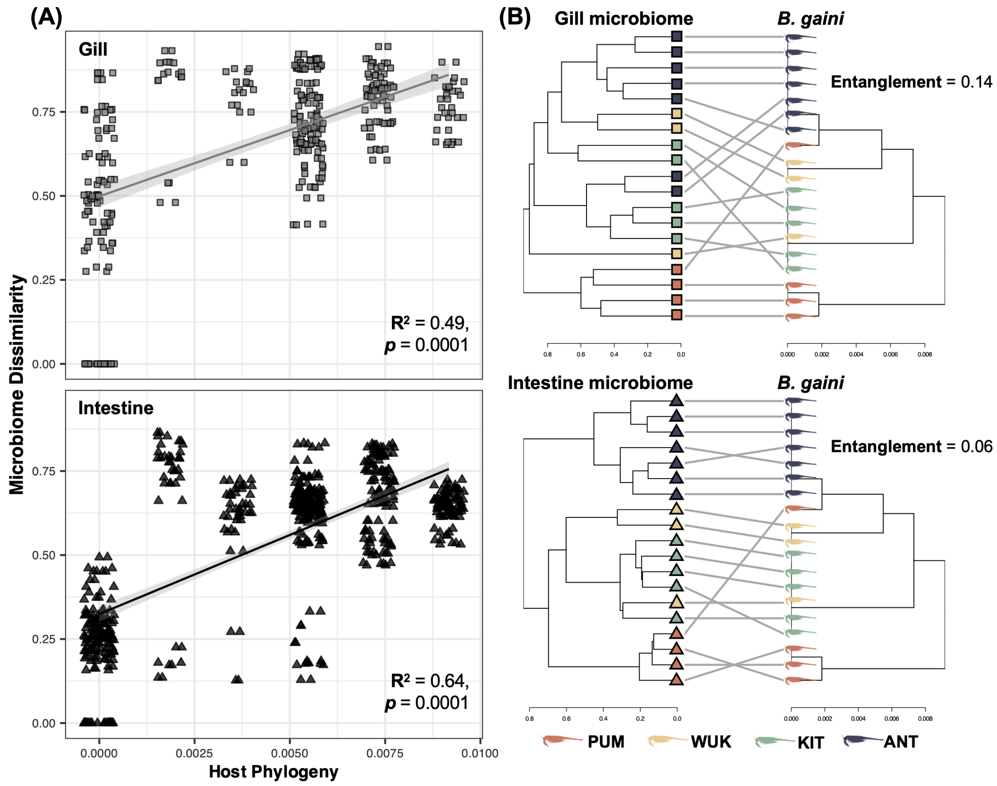

FIGURE 8
Congruence between *Branchinecta gaini* phylogeny and its microbiome composition. **(A)** Scatter plots of partial Mantel test results based on microbiome dissimilarity of *B. gaini* gill (gray square) and intestine (black triangle), using Bray-Curtis distances and host phylogeny. R^2 and *p*-values based on 10,000 permutations are provided. **(B)** Tanglegrams showing the association between the estimated *B. gaini* phylogeny and its gill (square) or intestine (triangle) microbiome composition. The scale and direction of the divergence are indicated by the scale bars, representing Bray-Curtis dissimilarity and pairwise phylogenetic distances for microbiome and host, respectively. The degree of alignment between the dendrograms is presented under a value of entanglement (0: fully aligned labels, 1: fully mismatched labels). Colors represent the sampling site as follows: Terra cotta; PUM, goldenrod yellow: WUK, asparagus green; KIT, dark slate blue; ANT.

the gills lamella is dominated by stable bacterial taxa across host populations (Bacci et al., 2023; Fusi et al., 2023). The importance of ecological drift during establishment of symbioses has been demonstrated to weaken/counteract deterministic selection through mechanisms such as priority or founder effect (Hagen and Hamrick, 1996; Kohl, 2020). Thus, the colonization outcome (*i.e.*, symbiont community composition) of a selective microenvironment may vary within and between host populations after the coarse ecological filter against taxa unable to colonize the tissue, even in the case of highly specific and vertically-transmitted symbionts (Vega and Gore, 2017; Lange et al., 2023; Chen et al., 2024). Such mechanisms may explain the detection of relatively variable community composition, the detection of stochastic assembly processes in the gill, in spite of the specificity of this microenvironment, which initially seemed contradictory.

4.3 Greater consistency and determinism in intestine versus gill microbiome assembly of *B. gaini*

We detected a predominant core microbiome across the four populations of *B. gaini* accounting for almost two thirds of the total community abundance, suggesting the existence of stable, permanent, and abundant microbial partners in the intestine of *B. gaini* (Astudillo-Garcia et al., 2017). Among the core microbial taxa, we reported the *Intrasporangiaceae* family, which has been characterized as highly abundant in the intestine of terrestrial earthworms and correlated with the digestive capacity of the host (Liu et al., 2023). This family has also been repeatedly detected in Antarctic ecosystems, mainly in soils and seawater (Giudice et al., 2007), but also associated with metazoan hosts such as tardigrades living on the surface of glaciers (Zawierucha et al., 2022). The *Clostridium sensu stricto 13* and *9* genera were also particularly prevalent and enriched in *B. gaini* intestines. Representatives of the *Clostridia* class, including *Clostridium*, are typically detected in the gut of freshwater invertebrates and fish (Zhao et al., 2018; Weingarten et al., 2019; Savard et al., 2023). *Clostridium* is a fermentative anaerobe known for its chitinolytic activity (Olsen et al., 1999). We suggest that the enrichment of these genera in the intestine of *B. gaini* might be attributed to their chitinolytic activity, enabling them to leverage the chitin-rich peritrophic matrix.

As in the gill microenvironment, the microbiome assembly processes within the intestine of *B. gaini* revealed a predominance of processes leading to a divergent microbiome. Yet, in contrast with the gill, these processes were mostly deterministic, dominated by variable selection and ecological drift at intra-site scale, and almost exclusively by variable selection at inter-sites scale. Our findings contrast with several studies of the macrofauna gut microbiomes, which have identified either deterministic processes leading to phylogenetically convergent gut microbiome (*i.e.*, homogenizing selection) (Xiong et al., 2017; Li et al., 2019), or mostly stochastic processes leading to divergent microbiome (*i.e.*, mainly ecological drift) (Yan et al., 2016; Ge et al., 2021; Schwob et al., 2021). Our results also diverge from the typical pattern observed in the micro-eukaryotic community of freshwater lakes on the King George Island (South Shetland Islands, Maritime Antarctica) (including the same sampling site as ours), which are dominated by homogenizing dispersal process (Zhang et al., 2022). In our case, the high contribution of variable selection, which causes the variability of intestinal microbiome, might be somewhat linked to *B. gaini* host-related factors (*e.g.*, ecology, diet, genetics, physiology) that selected, enriched or maintained bacteria adapted to the intestine microenvironment with site-specific differences (Stegen et al., 2013, 2015). We speculate that the trophic plasticity of *B. gaini*, coupled with potentially significant inter-sites variations in nutrient resources, may drive the gut microbial diversity toward various directions associated with different dietary intakes. This could lead to distinct enrichments within the intestine and consequently high intraspecific variations in microbiome compositions, similar to patterns observed in Tibetan herdsmen and birds (Li et al., 2018; Bodawatta et al., 2021). In line with this, the niche breadth in the intestine was wider than in the gill, with half of the OTUs being either neutralists or generalists, suggesting that the diet flexibility of *B. gaini* promotes a metabolically more flexible microbial community in the intestine (Jiao et al., 2020).

4.4 Intestinal microbiomes exhibit stronger phylosymbiosis than the gill microbiomes

Our study addressed three significant gaps in the current phylosymbiosis literature by (i) comparing the strength of phylosymbiosis in two host microenvironments, (ii) through host intraspecific resolution, and (iii) within an unexplored taxonomic group (Brachiopoda). To date, only a single phylosymbiosis study has been reported in this region (Schwob et al., 2024a). We detected robust correlations between within-species phylogenetic distances of *B. gaini* and its microbiomes' compositions. This result formally confirms the feasibility of detecting phylosymbiosis among genetically-structured intraspecies populations of freshwater invertebrates, thereby highlighting the importance of preserving the individual identity when considering different populations (Mazel et al., 2018; Lim and Bordenstein, 2020). Our results contrast with previous findings that mostly report the absence of phylosymbiosis in meiofaunal invertebrates, including rotifers, crustaceans, and flatworms, among others (Turgay et al., 2020; Eckert et al., 2021; Boscaro et al., 2022; Eckert et al., 2022; Rosa and Loreto, 2023). Several major methodological variances may explain the difference in phylosymbiosis detection with these previous works. First, ancient divergence time among different hosts tend to obscure the phylogenetic signal within their microbiomes, thus limiting the detection of phylosymbiosis (Brooks et al., 2016; Groussin et al., 2017). Consistently, Leasi et al. (2023) did not detect phylosymbiosis signal among the microbiomes of seven marine interstitial nemertean genera, but the correlation was positive when tested within the single genus *Ototyphlonemertes*. Secondly, we distinguished two different host microenvironments within *B. gaini*, while all the aforementioned studies used complete individuals for inspecting phylosymbiosis association. Mixing different microbiomes from whole individuals may lead to spurious conclusions about phylosymbiosis. Here, the strength of the phylosymbiosis pattern was markedly different between gill and intestine microbiomes. Specifically, the phylosymbiosis signal was weaker (and even marginally significant) in the *B. gaini* gill compared to intestine microenvironment when controlling for climatic and geographic distances. These findings suggest that, independently of climatic and geographic variables, host genetics shape more strongly the intestine microbiome than the gill microbiome, reflecting deterministic assemblages of bacteria. This is in accordance with the predominance of the variable selection process observed for the intestine microbiome assembly across sampling sites. This pattern is consistent with the general trend in phylosymbiosis studies showing that internal microbiomes (*e.g.*, gut) tend to harbor stronger and more frequent phylosymbiosis signals than external microbiomes (*e.g.*, skin), as reported in fishes (Minich et al., 2022), due to likely more direct influence of host physiology, immune system, and trophic diet in shaping the microbial communities (Moran and Sloan, 2015; Mazel et al., 2018; Woodhams et al., 2020).

We cannot discard a non-adaptive origin of the phylosymbiosis through host deterministic ecological filtering. Indeed, more phylogenetically closely related hosts might share more similar habitat, ecology and physiology, thus indirectly selecting similar microbial taxa from the surrounding environment (Moran and Sloan, 2015; Mazel et al., 2018). This hypothesis likely applies in the case of the intestine microenvironment, as the phylosymbiosis may result from diverging dietary intakes, related to diet preferences and/or the available resources (Hammer et al., 2020), which tend to be more homogenous within and between geographically closer sites, as observed in lake plankton (Soininen et al., 2011). This is in line with previous microscopic observations in another branchiopod genus (*Artemia*), suggesting that the intestinal microbial community is mostly transient and associated with the ingested material (Martin et al., 2020). The localization and density of the microbes in the intestine, as well as the functional host-symbiont complementarity remain to be fully resolved in *B. gaini*, and constitute promising avenues of research to further understand the gut microbial roles in freshwater crustaceans inhabiting lacustrine ecosystems in the rapidly changing region of Maritime Antarctica.

5 Conclusions

This study examines the microbiome assembly processes and phylosymbiosis signal in the Antarctic fairy shrimp *Branchinecta gaini*, relating to the host's intraspecific genetic structure. We found that the gill and intestine harbor distinct microbiomes, each with specific core OTUs that form site-dependent interaction networks, assembled through eco-evolutionary processes that result in divergent microbial communities. The host genetic structure correlates with the composition of both intestine and gill microbiomes of fairy shrimp, validating the phylosymbiosis hypothesis. The stronger phylosymbiosis signal in the intestine is linked to deterministic microbiome assembly processes associated with site-specific dietary variations that reflect the host's genetic structure. In contrast, the greater variability observed in the gill microbiome–due to stochastic assembly processes likely influenced by environmental exposure and/or random colonization–obscures this signal. These findings emphasize the importance of distinguishing between host microenvironments, even in small organisms, when studying the microbiome assembly.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA1090016 https://www.ncbi.nlm.nih.gov/, PP506203-PP506230.

Ethics statement

The animal study was approved by Comité de Bioética, Instituto de Ecología y Biodiversidad (IEB), Santiago, Chile. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

GS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration, Resources. LC: Writing – review & editing, Validation. PV: Methodology, Writing – review & editing. YT: Methodology, Writing – review & editing. FM: Visualization, Writing – review & editing, Methodology. TC: Writing – review & editing, Funding acquisition, Project administration, Resources. JO: Funding acquisition, Project administration, Resources, Writing – review & editing, Validation. CM: Project administration, Resources, Writing – review & editing, Validation, Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – original draft.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2024.1438057/full#supplementary-material

References

Aguilar, A., Maeda-Martínez, A. M., Murugan, G., Obregón-Barboza, H., Christopher Rogers, D., McClintock, K., et al. (2017). High intraspecific genetic divergence in the versatile fairy shrimp Branchinecta lindahli with a comment on cryptic species in the genus Branchinecta (Crustacea: Anostraca). *Hydrobiologia* 801, 59–69. doi: 10.1007/s10750-017-3283-3

Alberdi, A., Andersen, S. B., Limborg, M. T., Dunn, R. R., and Gilbert, M. T. P. (2022). Disentangling host-microbiota complexity through hologenomics. *Nat. Rev. Genet.* 23, 281–297. doi: 10.1038/s41576-021-00421-0

Astudillo-Garcia, C., Bell, J. J., Webster, N. S., Glasl, B., Jompa, J., Montoya, J. M., et al. (2017). Evaluating the core microbiota in complex communities: A systematic investigation. *Environ. Microbiol.* 19, 1450–1462. doi: 10.1111/1462-2920.13647

Bacci, G., Fratini, S., Meriggi, N., Cheng, C. L., Ng, K. H., Pindo, M., et al. (2023). Conserved organ-specific microbial assemblages in different populations of a terrestrial crab. *Front. Microbiol.* 14, 1113617. doi: 10.3389/fmicb.2023.1113617

Battaglia, T. (2021). *btools: A suite of R function for all types of microbial diversity analyses.* R package version 0.0.1. Available online at: https://scholar.google.com/

scholar?hl=fr&as_sdt=0%2C5&q=btools%3A+A+suite+of+R+function+for+all+types+of+microbial+diversity+analyses.&btnG=#d=gs_cit&t=1729863033392&u=%2Fscholar%3Fq%3Dinfo%3AQyGUFtacEcAJ%3Ascholar.google.com%2F%26output%3Dcite%26scirp%3D0%26hl%3Dfr

Berry, D., and Widder, S. (2014). Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Front. Microbiol.* 5. doi: 10.3389/fmicb.2014.00219

Bodawatta, K. H., Hird, S. M., Grond, K., Poulsen, M., and Jønsson, K. A. (2022). Avian gut microbiomes taking flight. *Trends Microbiol.* 30, 268–280. doi: 10.1016/j.tim.2021.07.003

Bodawatta, K. H., Koane, B., Maiah, G., Sam, K., Poulsen, M., and Jønsson, K. A. (2021). Species-specific but not phylosymbiotic gut microbiomes of New Guinean passerine birds are shaped by diet and flight-associated gut modifications. *Proc. R. Soc. B* 288, 20210446. doi: 10.1098/rspb.2021.0446

Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., et al. (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat. Methods* 10, 57–59. doi: 10.1038/nmeth.2276

Bordenstein, S. R., and Theis, K. R. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PloS Biol.* 13, e1002226. doi: 10.1371/journal.pbio.1002226

Boscaro, V., Holt, C. C., Van Steenkiste, N. W. L., Herranz, M., Irwin, N. A. T., Alvarez-Campos, P., et al. (2022). Microbiomes of microscopic marine invertebrates do not reveal signatures of phylosymbiosis. *Nat. Microbiol.* 7, 810–819. doi: 10.1038/s41564-022-01125-9

Brooks, A. W., Kohl, K. D., Brucker, R. M., van Opstal, E. J., and Bordenstein, S. R. (2016). Phylosymbiosis: relationships and functional effects of microbial communities across host evolutionary history. *PloS Biol.* 14, e2000225. doi: 10.1371/journal.pbio.2000225

Cáceres, C. E., and Rogers, D. C. (2015). Class branchiopoda. In *Thorp and Covich's Freshwater Invertebrates*. Academic Press, 687–708. Available online at: https://scholar.google.com/scholar?hl=fr&as_sdt=0%2C5&q=Cáceres%2C+C.E.%2C+and+Rogers%2C+D.C.+%282015%29.+%22Class+branchiopoda%2C%22+in+Thorp+and+Covich%27s+Freshwater+Invertebrates.+Elsevier%29%2C+687-708.&btnG=.

Callens, M., Watanabe, H., Kato, Y., Miura, J., and Decaestecker, E. (2018). Microbiota inoculum composition affects holobiont assembly and host growth in *Daphnia*. *Microbiome* 6, 1–12. doi: 10.1186/s40168-018-0444-1

Cao, Y., Dong, Q., Wang, D., Zhang, P., Liu, Y., and Niu, C. (2022). microbiomeMarker: an R/Bioconductor package for microbiome marker identification and visualization. *Bioinformatics* 2022, btac438. doi: 10.1093/bioinformatics/btac438

Chen, J. Z., Kwong, Z., Gerardo, N. M., and Vega, N. M. (2024). Ecological drift during colonization drives within-host and between-host heterogeneity in an animal-associated symbiont. *PloS Biol.* 22, e3002304. doi: 10.1371/journal.pbio.3002304

Coleman, G. A., Davín, A. A., Mahendrarajah, T. A., Szánthó, L. L., Spang, A., Hugenholtz, P., et al. (2021). A rooted phylogeny resolves early bacterial evolution. *Science* 372, eabe0511. doi: 10.1126/science.abe0511

Cooper, R. O., and Cressler, C. E. (2020). Characterization of key bacterial species in the Daphnia magna microbiota using shotgun metagenomics. *Sci. Rep.* 10, 652. doi: 10.1038/s41598-019-57367-x

Corbari, L., Zbinden, M., Cambon-Bonavita, M.-A., Gaill, F., and Compère, P. (2008). Bacterial symbionts and mineral deposits in the branchial chamber of the hydrothermal vent shrimp Rimicaris exoculata: relationship to moult cycle. *Aquat. Biol.* 1, 225–238. doi: 10.3354/ab00024

Cornejo-Granados, F., Gallardo-Becerra, L., Leonardo-Reza, M., Ochoa-Romo, J. P., and Ochoa-Leyva, A. (2018). A meta-analysis reveals the environmental and host factors shaping the structure and function of the shrimp microbiota. *PeerJ* 6, e5382. doi: 10.7717/peerj.5382

Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., and Knight, R. (2009). Bacterial community variation in human body habitats across space and time. *Science* 326, 1694–1697. doi: 10.1126/science.1177486

Couch, C. E., and Epps, C. W. (2022). Host, microbiome, and complex space: applying population and landscape genetic approaches to gut microbiome research in wild populations. *J. Heredity* 113, 221–234. doi: 10.1093/jhered/esab078

Daborn, G. R. (1975). Life history and energy relations of the giant fairy shrimp Branchinecta gigas Lynch 1937 (Crustacea: Anostraca). *Ecology* 56, 1025–1039. doi: 10.2307/1936144

Daday, J. (1910). Quelques Phyllopodes Anostracés nouveaux. *Annales des Sciences Naturelles* 12, 241–264. Available online at: https://scholar.google.com/scholar_lookup?&title=Quelques%20Phyllopodes%20Anostracés%20nouveaux&journal=Annales%20des%20Sciences%20Naturelles&volume=12&pages=241-264&publication_year=1910&author=Daday%2CJ

Darcy, J. L., Lynch, R. C., King, A. J., Robeson, M. S., and Schmidt, S. K. (2011). Global distribution of *Polaromonas* phylotypes-evidence for a highly successful dispersal capacity. *PloS One* 6, e23742. doi: 10.1371/journal.pone.0023742

Dekaboruah, E., Suryavanshi, M. V., Chettri, D., and Verma, A. K. (2020). Human microbiome: an academic update on human body site specific surveillance and its possible role. *Arch. Microbiol.* 202, 2147–2167. doi: 10.1007/s00203-020-01931-x

Delleuze, M., Schwob, G., Orlando, J., Gérard, K., Saucède, T., Brickle, P., et al. (2024). Habitat specificity modulates the bacterial biogeographic patterns in the Southern Ocean. *FEMS Microbiology Ecol*, fiae134. doi: 10.1093/femsec/fiae134

Dinno, A. (2017). dunn. test: Dunn's test of multiple comparisons using rank sums. *R Package version* 1, 1.

Doane, M. P., Morris, M. M., Papudeshi, B., Allen, L., Pande, D., Haggerty, J. M., et al. (2020). The skin microbiome of elasmobranchs follows phylosymbiosis, but in teleost fishes, the microbiomes converge. *Microbiome* 8, 93. doi: 10.1186/s40168-020-00840-x

Eckert, E. M., Anicic, N., and Fontaneto, D. (2021). Freshwater zooplankton microbiome composition is highly flexible and strongly influenced by the environment. *Mol. Ecol.* 30, 1545–1558. doi: 10.1111/mec.15815

Eckert, E. M., Cancellario, T., Bodelier, P. L. E., Declerck, S. A. J., Diwen, L., Samad, S., et al. (2022). A combination of host ecology and habitat but not evolutionary history explains differences in the microbiomes associated with rotifers. *Hydrobiologia* 850, 3813–3821. doi: 10.1007/s10750-022-04958-x

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797. doi: 10.1093/nar/gkh340

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., and Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194–2200. doi: 10.1093/bioinformatics/btr381

Farkas, J. (1985). Filamentous Flavobacterium sp. isolated from fish with gill diseases in cold water. *Aquaculture* 44, 1–10. doi: 10.1016/0044-8486(85)90037-7

Finn, D. R., Yu, J., Ilhan, Z. E., Fernandes, V. M. C., Penton, C. R., Krajmalnik-Brown, R., et al. (2020). MicroNiche: an R package for assessing microbial niche breadth and overlap from amplicon sequencing data. *FEMS Microbiol. Ecol.* 96. doi: 10.1093/femsec/fiaa131

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.

Fusi, M., Ngugi, D. K., Marasco, R., Booth, J. M., Cardinale, M., Sacchi, L., et al. (2023). Gill-associated bacteria are homogeneously selected in amphibious mangrove crabs to sustain host intertidal adaptation. *Microbiome* 11, 189. doi: 10.1186/s40168-023-01629-4

Gao, G., Li, G., Liu, M., Li, P., Liu, J., Ma, S., et al. (2023). Changes in soil stoichiometry, soil organic carbon mineralization and bacterial community assembly processes across soil profiles. *Sci. Total Environ.* 903, 166408. doi: 10.1016/j.scitotenv.2023.166408

Ge, Y., Jing, Z., Diao, Q., He, J.-Z., and Liu, Y.-J. (2021). Host species and geography differentiate honeybee gut bacterial communities by changing the relative contribution of community assembly processes. *MBio* 12, 00751–00721. doi: 10.1128/mBio.00751-21

Giudice, A. L., Bruni, V., and Michaud, L. (2007). Characterization of Antarctic psychrotrophic bacteria with antibacterial activities against terrestrial microorganisms. *J. basic Microbiol.* 47, 496–505. doi: 10.1002/jobm.200700227

Groussin, M., Mazel, F., and Alm, E. J. (2020). Co-evolution and co-speciation of host-gut bacteria systems. *Cell Host Microbe* 28, 12–22. doi: 10.1016/j.chom.2020.06.013

Groussin, M., Mazel, F., Sanders, J. G., Smillie, C. S., Lavergne, S., Thuiller, W., et al. (2017). Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. *Nat. Commun.* 8, 14319. doi: 10.1038/ncomms14319

Guivier, E., Martin, J. F., Pech, N., Ungaro, A., Chappaz, R., and Gilles, A. (2018). Microbiota diversity within and between the tissues of two wild interbreeding species. *Microbial Ecol.* 75, 799–810. doi: 10.1007/s00248-017-1077-9

Hagen, M., and Hamrick, J. (1996). Population level processes in Rhizobium leguminosarum bv. trifolii: the role of founder effects. *Mol. Ecol.* 5, 707–714. doi: 10.1111/j.1365-294X.1996.tb00367.x

Hammer, T. J., Dickerson, J. C., McMillan, W. O., and Fierer, N. (2020). Heliconius butterflies host characteristic and phylogenetically structured adult-stage microbiomes. *Appl. Environ. Microbiol.* 86, e02007–e02020. doi: 10.1128/AEM.02007-20

Hammer, T. J., Janzen, D. H., Hallwachs, W., Jaffe, S. P., and Fierer, N. (2017). Caterpillars lack a resident gut microbiome. *Proc. Natl. Acad. Sci.* 114, 9641–9646. doi: 10.1073/pnas.1707186114

Hammer, T. J., Sanders, J. G., and Fierer, N. (2019). Not all animals need a microbiome. *FEMS Microbiol. Lett.* 366. doi: 10.1093/femsle/fnz117

Hawes, T. (2009). Origins and dispersal of the Antarctic fairy shrimp. *Antarctic Sci.* 21, 477–482. doi: 10.1017/S095410200900203X

Hayward, A., Poulin, R., and Nakagawa, S. (2021). A broadscale analysis of host-symbiont cophylogeny reveals the drivers of phylogenetic congruence. *Ecol. Lett.* 24, 1681–1696. doi: 10.1111/ele.13757

Huot, C., Clerissi, C., Gourbal, B., Galinier, R., Duval, D., and Toulza, E. (2019). Schistosomiasis vector snails and their microbiota display a phylosymbiosis pattern. *Front. Microbiol.* 10. doi: 10.3389/fmicb.2019.03092

Jiao, S., Yang, Y., Xu, Y., Zhang, J., and Lu, Y. (2020). Balance between community assembly processes mediates species coexistence in agricultural soil microbiomes across eastern China. *ISME J.* 14, 202–216. doi: 10.1038/s41396-019-0522-9

Kapardar, R. K., Ranjan, R., Grover, A., Puri, M., and Sharma, R. (2010). Identification and characterization of genes conferring salt tolerance to *Escherichia* *coli* from pond water metagenome. *Bioresource Technol.* 101, 3917–3924. doi: 10.1016/j.biortech.2010.01.017

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., et al. (2017). Climatologies at high resolution for the earth's land surface areas. *Sci. Data* 4, 1–20. doi: 10.1038/sdata.2017.122

Kassambara, A. (2020). ggplot2" based publication ready plots [R package ggpubr version 0.4.0. *Compr. R Arch. Network.* 25, 2022.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., et al. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649. doi: 10.1093/bioinformatics/bts199

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., et al. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41, e1. doi: 10.1093/nar/gks808

Kohl, K. D. (2020). Ecological and evolutionary mechanisms underlying patterns of phylosymbiosis in host-associated microbial communities. *Philos. Trans. R. Soc. B* 375, 20190251. doi: 10.1098/rstb.2019.0251

Kohl, K. D., Dearing, M. D., and Bordenstein, S. R. (2018). Microbial communities exhibit host species distinguishability and phylosymbiosis along the length of the gastrointestinal tract. *Mol. Ecol.* 27, 1874–1883. doi: 10.1111/mec.14460

Kurtz, Z. D., Muller, C. L., Miraldi, E. R., Littman, D. R., Blaser, M. J., and Bonneau, R. A. (2015). Sparse and compositionally robust inference of microbial ecological networks. *PloS Comput. Biol.* 11, e1004226. doi: 10.1371/journal.pcbi.1004226

Lahti, L., Shetty, S., Blake, T., and Salojarvi, J. (2017). *Tools for microbiome analysis in R.* Version, Vol. 1. 28. Available online at: http://microbiome.github.iohttps://scholar.google.com/scholar?hl=fr&as_sdt=0%2C5&q=Lahti%2C+L.%2C+Shetty%2C+S.%2C+Blake%2C+T.%2C+and+Salojarvi%2C+J.+%282017%29.+Tools+for+microbiome+analysis+in+R.+Version+1%2C+28.&btnG=#d=gs_cit&t=1729863017070&u=%2Fscholar%3Fq%3Dinfo%3AOxH9VzHXxTAJ%3Ascholar.google.com%2F%26output%3Dcite%26scirp%3D0%26hl%3Dfr

Lange, C., Boyer, S., Bezemer, T. M., Lefort, M. C., Dhami, M. K., Biggs, E., et al. (2023). Impact of intraspecific variation in insect microbiomes on host phenotype and evolution. *ISME J.* 17, 1798–1807. doi: 10.1038/s41396-023-01500-2

Leasi, F., Eckert, E., Norenburg, J., Thomas, W. K., Sevigny, J., Hall, J., et al. (2023). Microbiota associated with meiofaunal nemerteans reveals evidence of phylosymbiosis. *Authorea Preprints*. doi: 10.22541/au.169339864.44365926/v1

Lee, E., Lee, K. W., Park, Y., Choi, A., Kwon, K. K., and Kang, H. M. (2024). Comparative microbiome analysis of artemia spp. and potential role of microbiota in cyst hatching. *Mar. Biotechnol.* 26, 50–59. doi: 10.1007/s10126-023-10276-7

Lemoine, F., Correia, D., Lefort, V., Doppelt-Azeroual, O., Mareuil, F., Cohen-Boulakia, S., et al. (2019). NGPhylogeny. fr: new generation phylogenetic services for non-specialists. *Nucleic Acids Res.* 47, W260–W265. doi: 10.1093/nar/gkz303

Li, H., Li, T., Li, X., Wang, G., Lin, Q., and Qu, J. (2018). Gut microbiota in Tibetan herdsmen reflects the degree of urbanization. *Front. Microbiol.* 9, 1745. doi: 10.3389/fmicb.2018.01745

Li, H., Zhou, R., Zhu, J., Huang, X., and Qu, J. (2019). Environmental filtering increases with elevation for the assembly of gut microbiota in wild pikas. *Microbial Biotechnol.* 12, 976–992. doi: 10.1111/1751-7915.13450

Lim, S. J., and Bordenstein, S. R. (2020). An introduction to phylosymbiosis. *Proc. R. Soc. B* 287, 20192900. doi: 10.1098/rspb.2019.2900

Liu, P., Zha, T., Xia, S.-Q., Sun, P., Wu, Y.-F., Zhao, H., et al. (2023). Repeated digestion by earthworms on tetracycline degrading and biological responses in tetracycline-contaminated sewage sludge. *Water Air Soil pollut.* 234. doi: 10.1007/s11270-023-06366-w

Ludington, W. B. (2024). The importance of host physical niches for the stability of gut microbiome composition. *Philos. Trans. R. Soc. B* 379, 20230066. doi: 10.1098/rstb.2023.0066

Lukic, D., Pormehr, N., Beladjal, L., Vad, C. F., Ptacnik, R., Van Stappen, G., et al. (2023). Life-history omnivory in the fairy shrimp *Branchinecta orientalis* (Branchiopoda: Anostraca). *Hydrobiologia* 850, 901–909. doi: 10.1007/s10750-022-05132-z

Lynch, J. E. (1964). Packard's and Pearse's species of *Branchinecta*: analysis of a nomenclatural involvement. *Am. Midland Nat.* 71 (2), 466–488. doi: 10.2307/2423306

Macke, E., Callens, M., Massol, F., Vanoverberghe, I., De Meester, L., and Decaestecker, E. (2020). Diet and genotype of an aquatic invertebrate affect the composition of free-living microbial communities. *Front. Microbiol.* 11, 503595. doi: 10.3389/fmicb.2020.00380

Mallott, E. K. (2024). Disentangling the mechanisms underlying phylosymbiosis in mammals. *Mol. Ecol.* 33, e17193. doi: 10.1111/mec.17193

Mallott, E. K., and Amato, K. R. (2021). Host specificity of the gut microbiome. *Nat. Rev. Microbiol.* 19, 639–653. doi: 10.1038/s41579-021-00562-3

Martin, G. G., Natha, Z., Henderson, N., Bang, S., Hendry, H., and Loera, Y. (2020). Absence of a microbiome in the midgut trunk of six representative Crustacea. *J. Crustacean Biol.* 40, 122–130. doi: 10.1093/jcbiol/ruz087

Martiny, J. B., Bohannan, B. J., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., et al. (2006). Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.* 4, 102–112. doi: 10.1038/nrmicro1341

Maturana, C. S., Segovia, N. I., González-Wevar, C. A., Díaz, A., Rosenfeld, S., Poulin, E., et al. (2020). Evidence of strong small-scale population structure in the Antarctic freshwater copepod *Boeckella poppei* in lakes on Signy Island, South Orkney Islands. *Limnology Oceanography* 65, 2024–2040. doi: 10.1002/lno.11435

Mazel, F., Davis, K. M., Loudon, A., Kwong, W. K., Groussin, M., and Parfrey, L. W. (2018). Is host filtering the main driver of phylosymbiosis across the tree of life? *Msystems* 3, e00097–e00018. doi: 10.1128/msystems.00097-18

Mazel, F., Guisan, A., and Parfrey, L. W. (2024). Transmission mode and dispersal traits correlate with host specificity in mammalian gut microbes. *Mol. Ecol.* 33, e16862. doi: 10.1111/mec.16862

Mazel, F., Knowles, S. C. L., Videvall, E., and Sweeny, A. R. (2023). Evolutionary patterns and processes in animal microbiomes. *J. evolutionary Biol.* 36, 1653–1658. doi: 10.1111/jeb.14248

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., et al. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl. Acad. Sci.* 110, 3229–3236. doi: 10.1073/pnas.1218525110

McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One* 8, e61217. doi: 10.1371/journal.pone.0061217

Middlemiss, K. L., Urbina, M. A., and Wilson, R. W. (2015). Microbial proliferation on gill structures of juvenile European lobster (Homarus gammarus) during a moult cycle. *Helgoland Mar. Res.* 69, 401–410. doi: 10.1007/s10152-015-0445-4

Minich, J. J., Härer, A., Vechinski, J., Frable, B. W., Skelton, Z. R., Kunselman, E., et al. (2022). Host biology, ecology and the environment influence microbial biomass and diversity in 101 marine fish species. *Nat. Commun.* 13, 6978. doi: 10.1038/s41467-022-34557-2

Moeller, A. H., Suzuki, T. A., Lin, D., Lacey, E. A., Wasser, S. K., and Nachman, M. W. (2017). Dispersal limitation promotes the diversification of the mammalian gut microbiota. *Proc. Natl. Acad. Sci.* 114, 13768–13773. doi: 10.1073/pnas.1700122114

Moran, N. A., Ochman, H., and Hammer, T. J. (2019). Evolutionary and ecological consequences of gut microbial communities. *Annu. Rev. Ecol. Systematics* 50, 451–475. doi: 10.1146/annurev-ecolsys-110617-062453

Moran, N. A., and Sloan, D. B. (2015). The hologenome concept: helpful or hollow? *PloS Biol.* 13, e1002311. doi: 10.1371/journal.pbio.1002311

Motiei, A., Brindefalk, B., Ogonowski, M., El-Shehawy, R., Pastuszek, P., Ek, K., et al. (2020). Disparate effects of antibiotic-induced microbiome change and enhanced fitness in *Daphnia magna*. *PloS One* 15, e0214833. doi: 10.1371/journal.pone.0214833

Nougué, O., Gallet, R., Chevin, L.-M., and Lenormand, T. (2015). Niche limits of symbiotic gut microbiota constrain the salinity tolerance of brine shrimp. *Am. Nat.* 186, 390–403. doi: 10.1086/682370

O'Brien, P. A., Tan, S., Yang, C., Frade, P. R., Andreakis, N., Smith, H. A., et al. (2020). Diverse coral reef invertebrates exhibit patterns of phylosymbiosis. *ISME J.* 14, 2211–2222. doi: 10.1038/s41396-020-0671-x

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., O'Hara, R., Simpson, G. L., et al. (2011). Vegan: Community ecology packag. *R Package version* 1, 17–18.

Olsen, M., Blix, A., Utsi, T., Sørmo, W., and Mathiesen, S. (1999). Chitinolytic bacteria in the minke whale forestomach. *Can. J. Microbiol.* 46, 85–94. doi: 10.1139/w99-112

Paggi, J. (1996). Feeding ecology of *Branchinecta gaini* (Crustacea: Anostraca) in ponds of south Shetland Islands, Antarctica. *Polar Biol.* 16, 13–18. doi: 10.1007/BF02388730

Pan, B., Han, X., Yu, K., Sun, H., Mu, R., and Lian, C. A. (2023). Geographical distance, host evolutionary history and diet drive gut microbiome diversity of fish across the Yellow River. *Mol. Ecol.* 32, 1183–1196. doi: 10.1111/mec.16812

Pankey, S. M., Plachetzki, D. C., Macartney, K. J., Gastaldi, M., Slattery, M., Gochfeld, D. J., et al. (2022). Cophylogeny and convergence shape holobiont evolution in sponge-microbe symbioses. *Nat. Ecol. Evol.* 6, 750–762. doi: 10.1038/s41559-022-01712-3

Paradis, E., and Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35, 526–528. doi: 10.1093/bioinformatics/bty633

Peck, L. S. (2004). Physiological flexibility: the key to success and survival for Antarctic fairy shrimps in highly fluctuating extreme environments. *Freshw. Biol.* 49, 1195–1205. doi: 10.1111/j.1365-2427.2004.01264.x

Pollock, F. J., McMinds, R., Smith, S., Bourne, D. G., Willis, B. L., Medina, M., et al. (2018). Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nat. Commun.* 9, 4921. doi: 10.1038/s41467-018-07275-x

Polz, M. F., Hunt, D. E., Preheim, S. P., and Weinreich, D. M. (2006). Patterns and mechanisms of genetic and phenotypic differentiation in marine microbes. *Philos. Trans. R. Soc. B: Biol. Sci.* 361, 2009–2021. doi: 10.1098/rstb.2006.1928

Qi, W., Nong, G., Preston, J. F., Ben-Ami, F., and Ebert, D. (2009). Comparative metagenomics of *Daphnia* symbionts. *BMC Genomics* 10, 1–21. doi: 10.1186/1471-2164-10-172

Qin, M., Jiang, L., Qiao, G., and Chen, J. (2023). Phylosymbiosis: the eco-evolutionary pattern of insect-symbiont interactions. *Int. J. Mol. Sci.* 24. doi: 10.3390/ijms242115836

Risely, A. (2020). Applying the core microbiome to understand host-microbe systems. *J. Anim. Ecol.* 89, 1549–1558. doi: 10.1111/1365-2656.13229

Rosa, M. T., and Loreto, E. L. (2023). Stenostomum leucops (Catenulida, Platyhelminthes) has a flexible microbiome in time and space. *Hydrobiologia* 850, 3675–3683. doi: 10.1007/s10750-022-04931-8

Rosenberg, E., and Zilber-Rosenberg, I. (2018). The hologenome concept of evolution after 10 years. *Microbiome* 6, 78. doi: 10.1186/s40168-018-0457-9

Rosenberg, E., and Zilber-Rosenberg, I. (2021). Reconstitution and transmission of gut microbiomes and their genes between generations. *Microorganisms* 10, 70. doi: 10.3390/microorganisms10010070

Ross, A. A., Müller, K. M., Weese, J. S., and Neufeld, J. D. (2018). Comprehensive skin microbiome analysis reveals the uniqueness of human skin and evidence for phylosymbiosis within the class Mammalia. *Proc. Natl. Acad. Sci.* 115, E5786–E5795. doi: 10.18653/v1/K18-2

Sadeghi, J., Chaganti, S. R., Johnson, T. B., and Heath, D. D. (2023). Host species and habitat shape fish-associated bacterial communities: phylosymbiosis between fish and their microbiome. *Microbiome* 11, 258. doi: 10.1186/s40168-023-01697-6

Samad, M. S., Lee, H. J., Cerbin, S., Meima-Franke, M., and Bodelier, P. L. E. (2020). Niche differentiation of host-associated pelagic microbes and their potential contribution to biogeochemical cycling in artificially warmed lakes. *Front. Microbiol.* 11. doi: 10.3389/fmicb.2020.00582

Savard, P., Fernandes, T., Dao, A., McMeans, B., and Lazar, C. S. (2023). Seasons influence the native gut microbiome of lake trout salvelinus namaycush. *Appl. Microbiol.* 3, 276–287. doi: 10.3390/applmicrobiol3010019

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541. doi: 10.1128/AEM.01541-09

Schwob, G., Cabrol, L., Poulin, E., and Orlando, J. (2020). Characterization of the gut microbiota of the Antarctic heart urchin (Spatangoida) *Abatus agassizii*. *Front. Microbiol.* 11. doi: 10.3389/fmicb.2020.00308

Schwob, G., Cabrol, L., Saucède, T., Gérard, K., Poulin, E., and Orlando, J. (2024a). Unveiling the co-phylogeny signal between plunderfish *Harpagifer* spp. and their gut microbiomes across the Southern Ocean. *Microbiol. Spectr.* 12 (4), e03830–e03823. doi: 10.1101/2023.04.18.537398

Schwob, G., Rosenfeld, S., González-Wevar, C., and Orlando, J. (2024b). Ecological filtering and phylogeographic structuring of *Psychrilyobacter* within two closely related limpet species from the Southern Ocean. *Ann. Microbiol.* 74 (1), 8. doi: 10.1186/s13213-024-01751-0

Schwob, G., Segovia, N. I., González-Wevar, C., Cabrol, L., Orlando, J., and Poulin, E. (2021). Exploring the microdiversity within marine bacterial taxa: toward an integrated biogeography in the southern ocean. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.703792

Soininen, J., Korhonen, J. J., Karhu, J., and Vetterli, A. (2011). Disentangling the spatial patterns in community composition of prokaryotic and eukaryotic lake plankton. *Limnology Oceanography* 56, 508–520. doi: 10.4319/lo.2011.56.2.0508

Stegen, J. C., Lin, X., Fredrickson, J. K., Chen, X., Kennedy, D. W., Murray, C. J., et al. (2013). Quantifying community assembly processes and identifying features that impose them. *ISME J.* 7, 2069–2079. doi: 10.1038/ismej.2013.93

Stegen, J. C., Lin, X., Fredrickson, J. K., and Konopka, A. E. (2015). Estimating and mapping ecological processes influencing microbial community assembly. *Front. Microbiol.* 6. doi: 10.3389/fmicb.2015.00370

Trevelline, B. K., Sosa, J., Hartup, B. K., and Kohl, K. D. (2020). A bird's-eye view of phylosymbiosis: weak signatures of phylosymbiosis among all 15 species of cranes. *Proc. R. Soc. B* 287, 20192988. doi: 10.1098/rspb.2019.2988

Turgay, E., Steinum, T. M., Eryalçın, K. M., Yardımcı, R. E., and Karataş, S. (2020). The influence of diet on the microbiota of live-feed rotifers (Brachionus plicatilis) used in commercial fish larviculture. *FEMS Microbiol. Lett.* 367, fnaa020. doi: 10.1093/femsle/fnaa020

Vavrek, M. J. (2011). Fossil: palaeoecological and palaeogeographical analysis tools. *Palaeontologia Electronica* 14, 16.

Vega, N. M., and Gore, J. (2017). Stochastic assembly produces heterogeneous communities in the Caenorhabditis elegans intestine. *PloS Biol.* 15, e2000633. doi: 10.1371/journal.pbio.2000633

Wang, J., Pan, Z., Yu, J., Zhang, Z., and Li, Y.-z. (2023). Global assembly of microbial communities. *Msystems* 8 (3), e01289–e01222. doi: 10.1128/msystems.01289-22

Weingarten, E. A., Atkinson, C. L., and Jackson, C. R. (2019). The gut microbiome of freshwater Unionidae mussels is determined by host species and is selectively retained from filtered seston. *PloS One* 14, e0224796. doi: 10.1371/journal.pone.0224796

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis* (Springer-Verlag New York: Springer).

Wilson, A. C., and Duncan, R. P. (2015). Signatures of host/symbiont genome coevolution in insect nutritional endosymbioses. *Proc. Natl. Acad. Sci.* 112, 10255–10261. doi: 10.1073/pnas.1423305112

Woodhams, D. C., Bletz, M. C., Becker, C. G., Bender, H. A., Buitrago-Rosas, D., Diebboll, H., et al. (2020). Host-associated microbiomes are predicted by immune system complexity and climate. *Genome Biol.* 21, 23. doi: 10.1186/s13059-019-1908-8

Xiong, J., Dai, W., Zhu, J., Liu, K., Dong, C., and Qiu, Q. (2017). The underlying ecological processes of gut microbiota among cohabitating retarded, overgrown and normal shrimp. *Microbial Ecol.* 73, 988–999. doi: 10.1007/s00248-016-0910-x

Yagi, J. M., Sims, D., Brettin, T., Bruce, D., and Madsen, E. L. (2009). The genome of *Polaromonas naphthalenivorans* strain CJ2, isolated from coal tar-contaminated sediment, reveals physiological and metabolic versatility and evolution through extensive horizontal gene transfer. *Environ. Microbiol.* 11, 2253–2270. doi: 10.1111/j.1462-2920.2009.01947.x

Yan, Q., Li, J., Yu, Y., Wang, J., He, Z., Van Nostrand, J. D., et al. (2016). Environmental filtering decreases with fish development for the assembly of gut microbiota. *Environ. Microbiol.* 18, 4739–4754. doi: 10.1111/1462-2920.13365

Youngblut, N. D., Reischer, G. H., Walters, W., Schuster, N., Walzer, C., Stalder, G., et al. (2019). Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. *Nat. Commun.* 10, 2200. doi: 10.1038/s41467-019-10191-3

Zawierucha, K., Trzebny, A., Buda, J., Bagshaw, E., Franzetti, A., Dabert, M., et al. (2022). Trophic and symbiotic links between obligate-glacier water bears (Tardigrada) and cryoconite microorganisms. *PloS One* 17, e0262039. doi: 10.1371/journal.pone.0262039

Zhang, C., Li, H., Zeng, Y., Ding, H., Wang, B., Li, Y., et al. (2022). Diversity and assembly processes of microbial eukaryotic communities in Fildes Peninsula Lakes (West Antarctica). *Biogeosciences* 19, 4639–4654. doi: 10.5194/bg-19-4639-2022

Zhang, M., Sun, Y., Chen, L., Cai, C., Qiao, F., Du, Z., et al. (2016). Symbiotic bacteria in gills and guts of Chinese mitten crab (*Eriocheir sinensis*) differ from the free-living bacteria in water. *PloS One* 11, e0148135. doi: 10.1371/journal.pone.0148135

Zhang, M., Zhang, X., Tran, N. T., Sun, Z., Zhang, X., Ye, H., et al. (2021). Molting alters the microbiome, immune response, and digestive enzyme activity in mud crab (Scylla paramamosain). *Msystems* 6 (5), 10–1128. doi: 10.1128/msystems.00917-00921

Zhao, Y., Duan, C., Zhang, X., Chen, H., Ren, H., Yin, Y., et al. (2018). Insights into the gut microbiota of freshwater shrimp and its associations with the surrounding microbiota and environmental factors. *Journal of Microbiology and Biotechnology* 28, 946–956. doi: 10.4014/jmb.1709.09070

Zhou, J., and Ning, D. (2017). Stochastic community assembly: does it matter in microbial ecology? *Microbiol. Mol. Biol. Rev.* 81 (4), 10–1128. doi: 10.1128/MMBR.00002-17

Zilber-Rosenberg, I., and Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.* 32, 723–735. doi: 10.1111/j.1574-6976.2008.00123.x

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Effect of climate history on the genetic structure of an Antarctic soil nematode

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Historical climate disturbances such as glacial cycling and fluctuating stream, lake, and sea levels strongly influence the distribution and evolutionary trajectories of Antarctic terrestrial species. Antarctic invertebrates, including the ubiquitous sentinel nematode species *Scottnema lindsayae*, are especially sensitive to climate disturbances. We tested hypotheses associated with the historical geographic and population genetic structure of this species as it occurs across the McMurdo Dry Valleys of Antarctica. To reconstruct the influence of climate disturbance and ecological conditions on this species, partial mitochondrial cytochrome c oxidase I gene sequences were analyzed from individual *S. lindsayae* collected from sites across the McMurdo Dry Valleys reflecting opposing gradients of climate disturbance during the Last Glacial Maximum. We found that populations were strongly demarcated by geomorphic barriers, with distinct haplotypes associated with valleys except among valleys that experienced glacial advance and retreat during the LGM. Our work shows that contemporary populations of these animals are strongly structured by prior climate history and reinforced by subsequent ecological conditions. Such findings can be useful for understanding the processes that shape the distribution and abundance of these ecologically important animals and interpreting long-term monitoring of demographic shifts in response to changing climate trends in the McMurdo Dry Valleys.

KEYWORDS

Antarctica, biogeography, climate disturbance, evolution, McMurdo Dry Valleys, population genetics, polar, sentinel species

1 Introduction

Large-scale climate disturbances have lasting effects on ecological conditions and evolutionary change of populations (e.g., Opdam and Wascher, 2004). Climate disturbances create opportunities for stochastic gene flow among populations within a species that are otherwise structured by geography (Moran and Ormond, 2015; Schierenbeck, 2017). The Last Glacial Maximum (LGM; 20-10 kya) caused large-scale extinctions, subsequent expansions, and range shifts apparent in the phylogeography of many species (Hewitt, 1996, 2001; Riddle, 1996; Shafer et al., 2010). Understanding these historical evolutionary responses is important when considering the resilience of ecological communities to climate change (Willis et al., 2010). Mitochondrial DNA has been used extensively to test hypotheses of evolutionary responses to climate disturbances (Avise et al., 1987; Avise, 2009; Hickerson et al., 2010). Utilizing this molecular marker, intraspecies responses to the LGM have been studied across ecosystems and throughout the animal kingdom (Lobon et al., 2016; Khanal et al., 2018; Collins et al., 2019; Guimarães et al., 2022). Climate disturbance events, past and present, are especially influential in the evolutionary patterns of non-vagile species (Malcolm et al., 2002; Normand et al., 2011), and exceptionally so for soil invertebrates faced with extinction by glacial advances during the LGM (McGaughran et al., 2011; Collins et al., 2019, 2020). The extent to which these disturbance events impact the genetic structure of soil invertebrates is less well known.

The extreme Antarctic environment is no exception to climate disturbances that have influenced contemporary biogeography and abiotic conditions (Convey et al., 2014). Though experiencing a relatively stable climate since the mid-Miocene (Sugden et al., 1993; Spector and Balco, 2021; Halberstadt et al., 2022), the LGM created lasting legacies of habitat quality (Campbell et al., 1998; Burkins et al., 2000) and dispersal patterns (Virginia and Wall, 1999; Convey et al., 2014). Work in both terrestrial (Baird et al., 2021) and marine ecosystems (Soler-Membrives et al., 2017) and more specifically on springtails (Stevens and Hogg, 2003; Nolan et al., 2006; Collins et al., 2019, 2020), mites (Marshall and Coetzee, 2000; Brunetti et al., 2021), and mosses (Biersma et al., 2018) have all corroborated the evolutionary impact of the LGM. Among taxonomic groups that have received less attention are Antarctic nematodes. The haplotype diversity of Antarctic soil nematodes has been demonstrated (Courtright et al., 2000; Adams et al., 2007; Velasco-Castrillón and Stevens, 2014). However, the impact of climate disturbance on endemic terrestrial Antarctic biota has yet to be examined using the phylogeographic patterns of broadly distributed nematode species.

The nematode *Scottnema lindsayae* (Timm, 1971) is found across the scant ice-free regions of Antarctica (Freckman and Virginia, 1998; Adams et al., 2014; Velasco-Castrillón and Stevens, 2014). The McMurdo Dry Valleys comprise the largest of these ice-free landscapes (MDVs; 78°S, 162°E; 4,500 km^2; Levy, 2013). Freezing temperatures (-18°C; Doran et al., 2002a), low soil moisture (1% water by volume; Campbell et al., 1998), high salinity (~1600 µS/cm; Nkem et al., 2006a), and a truncated growing season (~2 months per year) reinforce the extreme conditions for life to exist in the MDVs. Under such harsh conditions, terrestrial communities are composed of only a few taxa of soil invertebrates, fungi, and microbial flora (Adams et al., 2014; Dreesens et al., 2014; Feeser et al., 2018). In the 65% of soils suitable for colonization, the communities have minimal functional overlap and are sensitive to climate disturbances (Freckman and Virginia, 1998; Fountain et al., 2014, 2016; Andriuzzi et al., 2018; Franco et al., 2021). In this low diversity ecosystem, *S. lindsayae* is the most common and often the exclusive metazoan in high saline (EC < 700 mS cm^{-1}), low soil moisture (<5%), and high elevation habitats that are inhospitable to other metazoan taxa (Treonis et al., 1999; Nkem et al., 2006a; Poage et al., 2008; Adams et al., 2014; Zawierucha et al., 2019). As a result, populations of *S. lindsayae* are distributed across heterogenous landscapes, thus providing an ideal system to study the effects of contemporary and historical climate trends on population structure throughout the MDVs. *S. lindsayae* functions as an excellent sentinel of MDVs soil ecosystem structure and functioning for its sensitivity to climate disturbances (Freckman and Virginia, 1997; Doran et al., 2002b; Porazinska et al., 2002; Gooseff et al., 2017; Andriuzzi et al., 2018), indicator of soil habitability (Courtright et al., 2001; Poage et al., 2008), and its disproportionate role in carbon cycling (Barrett et al., 2008; Andriuzzi et al., 2018). As such, the evolutionary response of *S. lindsayae* to past climate disturbances is important for interpreting contemporary responses to ongoing environmental change and sensitivity to future climate disturbances.

The main objective of this study was to identify the phylogeographic response of the sentinel species *S. lindsayae* in the MDVs to historical climate disturbance. We hypothesized that physical and population processes associated with the LGM are responsible for contemporary patterns of nematode distribution and connectivity rather than present day proximal processes shaping phylogeographic patterns. To test the role of historical climate disturbance on the genetic structure of *S. lindsayae*, we focused on three potential drivers: 1) geomorphic barriers that restrict connectivity among populations, 2) legacies of glacial advance and paleolake inundation during the LGM that may have disrupted or homogenized population structure, and 3) contemporary ecological gradients that could shape intraspecific diversity.

2 Materials and methods

2.1 Study system

Scottnema lindsayae (Timm, 1971) is well studied in terms of abundance, distribution, and ecosystem functioning (Figure 1; Adams et al., 2006, 2007, 2014). This species is known to tolerate low soil moisture (<5%; Poage et al., 2008), high salt content (EC < 700 mS/cm; Nkem et al., 2006a; Poage et al., 2008), and high pH (Courtright et al., 2001). Population abundances and life stage diversity are clearly linked to suitable soil habitat (Courtright

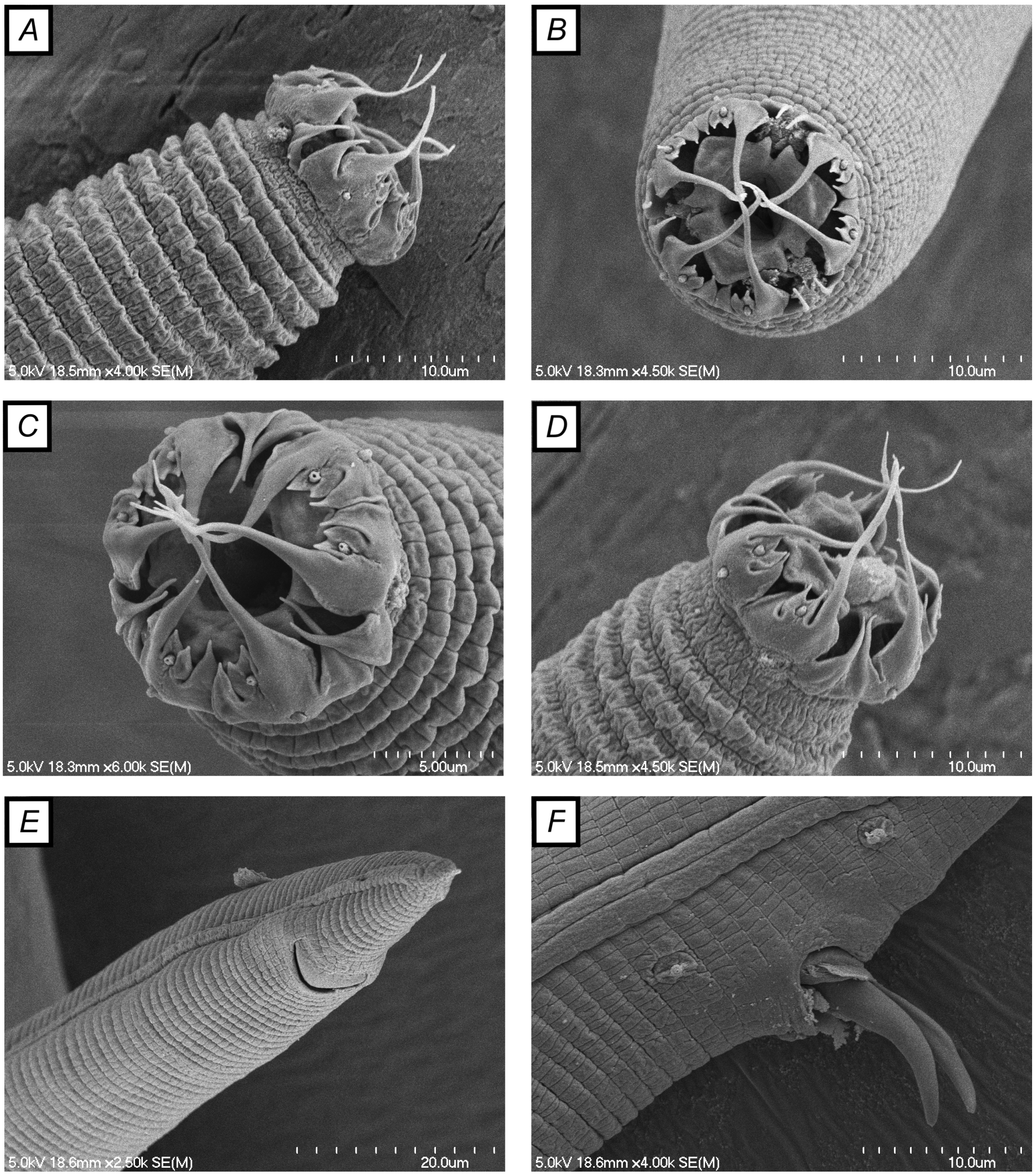

FIGURE 1
Scanning electron microscope images of *S. lindsayae* collected from Garwood Valley. Images **(A-D)**, adult anterior view. Image **(E)**, adult male posterior end. Image **(F)**, adult male with protruding spicula.

et al., 2001; Poage et al., 2008) and respond to contemporary environmental disturbances (Porazinska et al., 2002; Barrett et al., 2008; Andriuzzi et al., 2018).

2.2 Geographic sampling

To study the impacts of opposing disturbance histories during the LGM on nematode populations in the MDVs, we collected soil from 26 sites (Figure 2; Table 1) representing 8 valley systems. Of note, Taylor Valley is further divided into three well studied lake basins – Fryxell Basin, Hoare Basin, and Bonney Basin – that are distinct in their glacial histories. Of all the sites, 14 represent locations within valleys considered putative undisturbed refugia. These areas of refugia have likely existed as overlapping habitats with suitable abiotic conditions (Convey et al., 2020) throughout recurrent glaciations of the Pleistocene and even through the formation of the MDVs in the mid-Miocene (15-9 mya; Denton et al., 1984, 1993; Sugden et al., 1993; Schäfer et al., 1999). The remaining 12 sites represent locations in the MDVs that were disturbed during the LGM (10-21 kya; Table 1) either by local

FIGURE 2
Map sampling locations for *S. lindsayae* in the McMurdo Dry Valleys. Triangles indicate undisturbed sites (putative refugia), squares represent disturbed sites (impacted by glaciation or paleolake inundation during the LGM). Red diagonal hatching denotes the historical extent of the Ross Sea Drift incursion during the LGM Each valley or basin is represented by a different color. Where possible we use a consistent color scheme across figures, with darker variants to represent putative refugia when shape-based distinctions were not applicable. Soils collected adjacent to contemporary lakes are referred to as the lake basin they came from.

glacial expansion or paleolake inundation. We chose these sites based on published soil exposure ages (Table 1), proximity to recorded local glacier terminal moraines and paleolake shorelines, and presence of viable *S. lindsayae* communities.

2.3 Soil extraction

We collected 500 g of the top 10 cm of soil at each site using clean plastic scoops and sterile Whirlpak® bags during the Austral

TABLE 1 Sampling site descriptions and locations including the site name, valley or basin system, glacial history (disturbed or undisturbed) during the Last Glacial Maximum, *S. lindsayae* abundances as a proxy for soil habitability, number of individuals sanger sequenced from each site (sample size), latitude and longitude (decimal degrees), elevation (m), and estimated exposure ages based on the existing literature.

Site	Valley	Glacial History	Scottnema abundances (#/kg soil)	Sample Size	Lat	Long	Elevation (m)	Estimated Exposure ages	Citation
Levy Cirque	Beacon Valley	Undisturbed	358	16	-77.7972330	160.6154780	1522	2.3 mya	Schäfer et al., 2000
Beacon Valley, Lower		Undisturbed	3678	13	-77.8067660	160.7177160	1017		
Beacon Valley, Upper		Undisturbed	2320	8	-77.8805330	160.4578160	1386		
Battleship Promontory	Alatna Valley	Undisturbed	147	10	-76.9223500	161.0814330	1241	5 mya	Sugden and Denton, 2004; Margerison et al., 2005; Diaz et al., 2020
Alatna Valley		Undisturbed	1333	13	-76.8946500	161.1391500	981		Calkin, 1964
Hawkings Cirque	Wright Valley	Undisturbed	196	14	-77.5101920	160.5785970	1204		
Labyrinth		Undisturbed	351	7	-77.5500000	161.5166660	767	3 mya	Schäfer et al., 1999; Lewis et al., 2006
Dais		Undisturbed	132	7	-77.5500030	161.1847300	801	4 mya	Schäfer et al., 1999
Lake Brownworth		Disturbed	726	6	-77.4386330	162.7288030	241	26-5 kya	Hall et al., 2001
Mount Suess Peak	Mackay Glacier	Undisturbed	580	7	-77.0366280	161.7101190	970		Sugden and Denton, 2004; Jones et al., 2015
Mount Suess, Lower		Disturbed	3801	16	-77.0182700	161.7343100	517	25-5 kya	
Pegtop Mountain		Disturbed	3047	2	-77.0473530	161.4442960	823	25-5 kya	
Mount Falconer	Fryxell Basin, Taylor Valley	Undisturbed	446	11	-77.5731720	163.1585970	731		Stuiver et al., 1981; Denton et al., 1993; Burkins et al., 2000; Bockheim et al., 2008; Fountain et al., 2014
Gurkha Peak		Undisturbed	2308	9	-77.6509600	163.2515110	917		
Lake Fryxell		Disturbed	110	12	-77.6076940	163.2506830	9	21-12 kya	
Explorers cove		Disturbed	3150	5	-77.5665830	163.4934500	13	10 kya	Anderson et al., 2014; Fountain et al., 2014
Mount Rae	Hoare Basin, Taylor Valley	Disturbed	233	4	-77.6005850	162.8507150	1057		Bockheim et al., 2008
Lake Hoare		Disturbed	3007	11	-77.6349830	162.8888000	129	21-12 kya	Stuiver et al., 1981; Burkins et al., 2000; Hall et al., 2000; Bockheim et al., 2008
Campbell Terrace	Bonney Basin,	Undisturbed	1445	8	-77.6787330	162.5009830	957	120-70 kya	Burkins et al., 2000; Hall et al.,

(Continued)

TABLE 1 Continued

Site	Valley	Glacial History	Scottnema abundances (#/kg soil)	Sample Size	Lat	Long	Elevation (m)	Estimated Exposure ages	Citation
	Taylor Valley								2000; Bockheim et al., 2008
Lake Bonney		Disturbed	470	11	-77.7250000	162.3120000	77	21-12 kya	Stuiver et al., 1981; Burkins et al., 2000; Hall et al., 2000; Bockheim et al., 2008
Miers Valley, high	Miers Valley	Undisturbed	4831	10	-78.1166720	163.7507920	516		
Lake Miers		Disturbed	794	13	-78.1005970	163.8091190	167	26-5 kya	Clayton-Greene et al., 1988; Joy et al., 2017
Garwood Valley, high	Garwood Valley	Undisturbed	1647	7	-78.0379670	163.8748330	581		
Lake Garwood		Disturbed	528	12	-78.0264780	163.8512080	351	26-5 kya	Levy et al., 2013; Joy et al., 2017
Wall Valley	Victoria Valley	Undisturbed	237	10	-77.4955000	160.8651000	1617		
Lake Vida		Disturbed	90	7	-77.3822500	161.8178500	108	300-120 kya	Calkin, 1963; Bockheim and McLeod, 2013; McGowan et al., 2014

Fryxell Basin, Hoare Basin, and Bonney Basin comprise Taylor Valley.

summer between 1996 to 2022. A GPS point of each site was recorded and used for analyses. We transported soils within 48 hours to the Crary Science and Engineering Laboratory at McMurdo Station where they were stored at 4°C until soil invertebrate collection and geochemistry analyses could be performed within 48 hours. We performed a modified sugar centrifugation extraction (Freckman and Virginia, 1993) with a subsample of 100 g to calculate metazoan abundance. We counted soil invertebrates and identified to species, as living or dead, life stage (juvenile or adults), and sex under an inverted microscope (Olympus CKx41, Tokyo, Japan). We measured soil moisture, pH, and electrical conductivity following standard protocols of the MCM LTER project (https://mcm.lternet.edu/content/soil-elevational-transect-experiment). Subsequent subsamples of 100 g were extracted in the same way at Brigham Young University to collect individuals of *S. lindsayae* for molecular analyses. All soil samples from which *S. lindsayae* individuals were extracted for this study are archived in the Monte L. Bean Life Sciences Museum frozen soil repository at Brigham Young University. These samples remain available for subsequent morphological, molecular, or ecological analyses. While museum catalog numbers have not yet been assigned, each sample is uniquely identified by its site name and collection metadata. These identifiers, along with sample-specific information sufficient for retrieval, are provided in Supplementary Table 1.

2.4 PCR amplification and sanger sequencing

Following nematode DNA extraction methods of Powers and Harris (1993), we hand-picked individual *S. lindsayae* with an eyelash pick into 10 µl of sterile elution buffer on a cover slip. Using a sterilized 10 µl micropipette tip, we ruptured the nematode by gentle pressure and confirmed the lysate. To a 0.2 ml microcentrifuge tube we pipetted the lysate and 5.2 µl of sterile water used to clean the cover slip. We combined nematode lysate with 2.4 µl each of 20 µM diluted forward primer (COI-Scott-F1: 5'-GTTACAACTTTTTTGCTTATTCTCTCAC-3') and reverse primer (COI-Scott-R2: 5'-CTGTAAAATAAGCTCGACTRTCWG–3'). Some extractions were performed with an alternative reverse primer with higher fidelity but shorter sequence reads (COI-Scott-R4 5'-GCRTCRATACCTGTTACRTATATRTG-3'). This was done due to lack of successful amplification with the initial primer. We added 15 µl of JumpStart™ REDTaq® ReadyMix™ Reaction Mix (Sigma-Aldrich, DE) to the mixture, vortexed for 30 seconds (sec) to mix, and then placed the mixture into a DNA thermal cycler (Mastercycler X50s, Eppendorf, Germany) with the following cycling parameters: initial denaturation at 94°C for 5 min, 40 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, extension of 0.5°C/sec ramp to 72°C for 90 sec, followed by a final extension at 72°C for 5 min and held at 4°C.

Following PCR amplification, we screened the products on a 1% TAE agarose gel stained with ethidium bromide and visualized with a UV image capture (GelDoc Go, Bio-Rad Laboratories, USA). Successful DNA amplifications were cleaned with a magnetic bead cleaning protocol. We added Mag-Bind® TotalPure NGS (Omega Bio-tek, GA) in a 1.2× concentration to the remaining PCR product, vortexed for 10 min at 2,000 rpm, centrifuged for 5 sec, and followed the standard Omega Bio-tek protocol. We quantified DNA with a Quibit 4 Fluorometer (Invitrogen, USA). Finally, we diluted or concentrated products to 10 ng DNA for Sanger dideoxy sequencing at the Brigham Young University Sequencing Center (Provo, UT).

To ensure highly divergent populations based on COI data did not warrant further investigation as divergent species (Smythe et al., 2019; Ahmed et al., 2022), we sequenced the nuclear Large Ribosomal Subunit (LSU). With the exception of a 52°C annealing step, we used the same DNA template, extraction protocol, PCR cycling parameters, and sequencing chemistry used for the most genetically divergent COI populations: Beacon Valley upper, Lake Bonney, Campbell Terrace, Gurkha Peak, Lake Hoare, Levy Cirque, Mount Rae, Mount Suess peak, and Lake Vida. We used Nadler et al. (2006) primers: forward primer (F-LSU-391: 5'-AG CGGAGGAAAAGAAACTAA-3') and reverse primer (R-LSU-501: 5'-TCGGAAGGAACCAGCTACTA-3') to sequence 965 base pair (bp) of the LSU gene.

2.5 Genetic analysis

We verified sequences for the intended loci by comparing them using the NCBI reference database and confirmed the coding sequence did not contain early termination codons, numts (Bensasson et al., 2001), or indels. We aligned sequences in Geneious Prime® 2022.2.2 (https://www.geneious.com, New Zealand) using the default settings of MAFT Alignment v7.490. We subsequently trimmed consensus sequences and checked for ambiguities or missing nucleotides using Geneious Prime® 2022.2.2. Out of 838 individual nematodes, 249 yielded successful mtDNA amplificons of 284 base pairs in length which were subsequently aligned and mapped to the reference sequence for further analyses of populations' genetics. The number of sequences processed may not have captured all possible haplotypes (Supplementary Figure 1; Brown et al., 2012). Additionally, 30 LSU sequences, 922 base pairs in length were aligned and mapped to the reference genome. These were found to have no diversity and were not used for downstream analysis. We accessioned the partial COI and LSU sequences in the NCBI GenBank database (OP456699-OP456947 and OP692666-OP692695, respectively).

2.6 Population genetic analyses

All population genetic analyses were conducted using the aligned 284 bp COI mtDNA sequences. We calculated metrics on haplotype number (Hap), haplotype diversity (h), number of polymorphic sites (P), and nucleotide diversity (π) for each population using ARLEQUIN v3.5.2.2 2 (Excoffier and Lischer, 2010). To determine the qualitative population structure, we produced median-joining haplotype networks designating the sample site and the valley system using pegas package (Paradis, 2010) and haplotype (Aktas, 2020) following the code provided by Toparslan et al. (2020) using R Studio (R Core Team, 2002). We also constructed a dendrogram stratified by sampling site populations and valley system constructed in poppr 2.9.3 using Nei's D genetic distance measure (Nei, 1972) with 1,000 bootstrap replications. Although the data may have limited power to recover true phylogenetic relationships (Ballard and Rand, 2005), we reconstructed a maximum likelihood phylogenetic tree of all sequences to further represent the evolutionary relationships among sample sites using HKY+F+G4 as the evolutionary model determined the most appropriate for mitochondrial DNA sequence evolution by IQ-tree's model finder (Kalyaanamoorthy et al., 2017; Minh et al., 2020), 10,000 bootstrap resample iterations, and *Acrobeloides varius* COI as the outgroup (Accession number: KX889087.1). The resulting tree was visualized and manipulated using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

To quantitatively assess genetic structure, we calculated pairwise F_{ST} from pairwise nucleotide differences among sites and among valleys. We estimated the statistical significance with 1,000 permutations implemented in ARLEQUIN using the Kimura 2-parameter model (Kimura, 1980). We chose this model because it was most similar to the best fit model (see above). Pairwise uncorrected (*p* distance) and corrected (Kimura 2P with gamma 4 parameter rate; K2PG4) genetic distances were generated in Molecular Evolutionary Genetic Analysis (MEGA 11.0.13). Finally, we assessed the genetic structure among valleys and sampling sites by performing an analysis of molecular variance (AMOVA) with ARLEQUIN (Kimura 2P distance method) grouping by ten valleys (Table 1).

2.7 Demographic history

To evaluate the demographic history of *S. lindsayae* populations, we calculated Tajima's D and Fu's Fs in ARLEQUIN with 1,000 permutations. These statistics were used descriptively to explore departures from neutral expectations and to support patterns observed in Bayesian skyline plots, particularly regarding recent population expansions (significant negative values) or contractions (positive values). Because these tests were not used for formal hypothesis testing or multiple comparisons, and were interpreted in conjunction with other demographic metrics, we did not apply a multiple testing correction.

To further assess the effect of the LGM disturbance event on *S. lindsayae*, we used Bayesian skyline plots (BSPs) at each valley and for the combined valleys of Taylor, Miers, and Garwood given the lack of significant genetic differentiation and monophyly of the three valleys. BSPs (Drummond et al., 2005) were constructed in BEAST v2.6.7 (Bouckaert et al., 2019) using the following priors:

gamma site model with 4 categories, an HKY empirical evolutionary model as determined by IQ-tree, a strict clock with a molecular rate of 4.7 E -8 substitutions per site per year as determined by the evolutionary rate of *Caenorhabditis elegans* mitochondria DNA (Denver et al., 2000; Xu et al., 2012), and the calculated natural life span of *S. lindsayae* (Overhoff et al., 1993) and the number of days with an average temperature above freezing in the MDVs (Hudson et al., 2022) given that days below freezing pause the life cycle in a cryptobiotic state and must be accounted for in the *in situ* life span of *S. lindsayae*. All models were run with 20,000,000 permutations and reached an ESS score over 200. While mitochondrial DNA is not recommended as the best marker for assuming contemporary population size (Galtier et al., 2009), BSPs provide a rough estimate of effective population size change and are used here in a more relative manner to compare valleys.

2.8 Environmental parameters

To assess the statistical significance of environmental conditions on genetic variation, we analyzed the correlation of intrapopulation genetic differentiation (within population F_{ST}) and haplotype diversity within a population to elevation, soil moisture, electrical conductivity, distance to the coast, and glacial history with generalized linear models in the stats package in Program R (R Core Team, 2002). We then determined the best fitting model based on Akaike Information Criteria adjusted for small sample size (AIC_C; Akaike, 1973) using AICcmodavg package (Mazerolle, 2020) and MuMIn package (Bartoń, 2022) to average top models. Distance to the coast and elevation were correlated and not considered in the same models. We estimated distance to the coast with GPS and Google Earth Pro. Assumptions for residuals and normalcy were validated on the top model. Top models with a weight (W_i) above 0.05 were averaged and used to calculate the 85% confidence interval of the top parameters to determine the strength of the relationships between ecological conditions and genetic response.

3 Results

3.1 Genetic diversity

We identified high levels of genetic diversity among *S. lindsayae* across all valleys. Of the 249 sequences (~ 12 per site), 55 haplotypes were recovered with 39 (70%) being unique to a sampling site and 41 (75%) being unique to a valley. Haplotypes varied with 52 polymorphic sites (Supplementary Figure 2). The lowest haplotype diversity was found in isolated sites - Beacon Valley Lower, Battleship Promontory, Mount Suess lower, and Pegtop Mountain had one recovered haplotype each. Wright Valley's Dais population had the highest haplotype diversity. Garwood Lake had the highest nucleotide diversity. We found high levels of genetic distance (≤ 7.4%; Table 2; Supplementary Figure 4) for corrected

TABLE 2 Genetic differentiation (F_{ST}) matrix among valley or lake basin calculated in ARLEQUIN using Kimura 2P gamma 4 model.

	Beacon valley	Alatna valley	Wright valley	Mackay glacier	Fryxell basin	Miers valley	Garwood valley	Victoria valley	Hoare basin
Beacon Valley									
Alatna Valley	0.450						Top 15%		
Wright Valley	0.382	0.194					Bottom 15%		
Mackay Glacier	0.726	0.422	0.165						
Fryxell Basin	0.809	0.619	0.502	0.630					
Miers Valley	0.870	0.656	0.534	0.659	0.007				
Garwood Valley	0.638	0.370	0.287	0.470	0.095	0.154			
Victoria Valley	0.582	0.186	0.100	0.369	0.535	0.580	0.290		
Hoare Basin	0.904	0.669	0.540	0.667	0.023	0.040	0.182	0.595	
Bonney Basin	0.931	0.710	0.569	0.707	0.098	0.219	0.245	0.651	0.132

Locations reflect the pooled population from the respective valley or basin. Red-filled cells correspond to F_{ST} values in the bottom 15%. Blue-filled cells correspond to values in the top 15%. All comparisons are statistically significant except for those that are shaded in grey stripes.

TABLE 3 Molecular diversity metrics for each sampling site population including number of sequences (N), number of haplotypes with number of unique haplotypes (Hap()), number of polymorphic sites (P), haplotype diversity (h), nucleotide diversity (π), intra-population genetic differentiation (Kimura 2P F_{ST}).

Population	N	Hap	P	h ± SD	π ± SD	Within Pop F_{ST} ± SD
Levy Cirque	16	5 (2)	16	0.450 ± 0.151	0.0082 ± 0.0053	2.33 ± 1.34
Beacon Valley Upper	8	1 (0)	0	0	0.0005 ± 0.0009	0
Beacon Valley Lower	13	3 (1)	1	0.295 ± 0.156	0.0005 ± 0.0009	0.15 ± 0.23
Battleship Promontory	10	1 (0)	0	0	0	0.00
Alatna Valley	13	4 (2)	12	0.680 ± 0.112	0.0119 ± 0.0073	3.37 ± 1.85
Dais	7	6 (6)	16	0.952 ± 0.0955	0.0191 ± 0.0120	5.43 ± 2.98
Labyrinth	7	2 (0)	14	0.476 ± 0.171	0.0247 ± 0.0152	7.03 ± 3.76
Hawkings Cirque	14	3 (1)	2	0.648 ± 0.0813	0.0028 ± 0.0024	0.81 ± 0.62
Brownworth	6	3 (1)	18	0.600 ± 0.215	0.0338 ± 0.0209	9.59 ± 5.13
Mount Suess Top	7	2 (0)	11	0.571 ± 0.120	0.0233 ± 0.0143	6.61 ± 3.55
Mount Suess Bottom	16	1 (0)	0	0	0	0
Pegtop Mountain	2	1 (1)	0	0	0	0
Wall Valley	10	3 (1)	12	0.644 ± 0.101	0.0124 ± 0.0078	3.53 ± 1.96
Lake Vida	7	5 (1)	28	0.857 ± 0.137	0.0427 ± 0.0252	12.10 ± 6.25
High Miers	10	5 (1)	7	0.844 ± 0.080	0.0103 ± 0.0067	2.93 ± 1.68
Lake Miers	13	5 (2)	21	0.744 ± 0.087	0.0193 ± 0.0111	5.47 ± 2.82
High Garwood	7	3 (1)	5	0.667 ± 0.160	0.0061 ± 0.0052	2.03 ± 1.29
Garwood Lake	12	5 (1)	23	0.727 ± 0.113	0.0442 ± 0.0242	12.50 ± 6.09
Campbell Terrace	8	4 (1)	7	0.643 ± 0.184	0.0086 ± 0.0059	2.44 ± 1.47
Lake Bonney	11	5 (3)	2	0.764 ± 0.107	0.0022 ± 0.0021	0.62 ± 0.53
Mount Rae	4	3 (2)	2	0.833 ± 0.222	0.0035 ± 0.0035	1.00 ± 0.83
Lake Hoare	11	4 (2)	8	0.746 ± 0.098	0.0146 ± 0.0089	4.14 ± 2.23
Lake Fryxell	12	8 (4)	23	0.894 ± 0.078	0.0192 ± 0.0112	5.46 ± 2.83
Mount Falconer	11	6 (4)	20	0.836 ± 0.089	0.0224 ± 0.0130	6.35 ± 3.26
Gurkha Peak	9	3 (0)	5	0.556 ± 0.165	0.0068 ± 0.0048	1.92 ± 1.20
Explorers Cove	5	4 (2)	13	0.900 ± 0.161	0.0196 ± 0.0133	5.57 ± 3.22

SD indicates standard deviation from the mean. Sample sites with lake in the name are soils collected adjacent to contemporary lake shorelines.

K2PG4 genetic distance, differentiation ($F_{ST} \le 0.996$; Table 3), and haplotype diversity (h = 0.926; Table 3) across McMurdo Dry Valleys' nematodes.

From nine sampling sites with high genetic divergence based on COI sequences, 30 LSU sequences were analyzed. These sequences were 100% identical to the reference sequence from Boström et al. (2011), excepting two ambiguous base calls in two individuals. Accession number HM439773.1 (LSU sequence data not shown).

3.2 Phylogeography

Populations of *S. lindsayae* were highly structured in the MDVs based around valley systems and geographic proximity. The haplotype network revealed two high frequency haplotypes (haplotype 6 and 24; Figure 3). Haplotype 6 was shared among 13 sites (50% of the sampling sites) and was found in the Lake Brownworth basin and all of the Miers, Garwood and Taylor valley sample sites. Haplotype 24 was found only in Beacon sites, Hawkings Cirque, and Victoria Valley sites (see Table 1 for sites within each valley). In total, 39 haplotypes were restricted to a specific sampling site and the remaining 16 haplotypes were shared among more than one site. Haplotypes 21, 13, 9, 13, and 28 were shared only among the valleys disturbed during the LGM (Taylor, Miers, and Garwood) while haplotypes 30, 3, 54, 48, 4, and 25 were found in the more northerly and undisturbed valleys (Beacon, Alatna, Mackay Glacier, Victoria, and Wright). Only three haplotypes were shared across these two clusters, Haplotypes 23, 10, and 12.

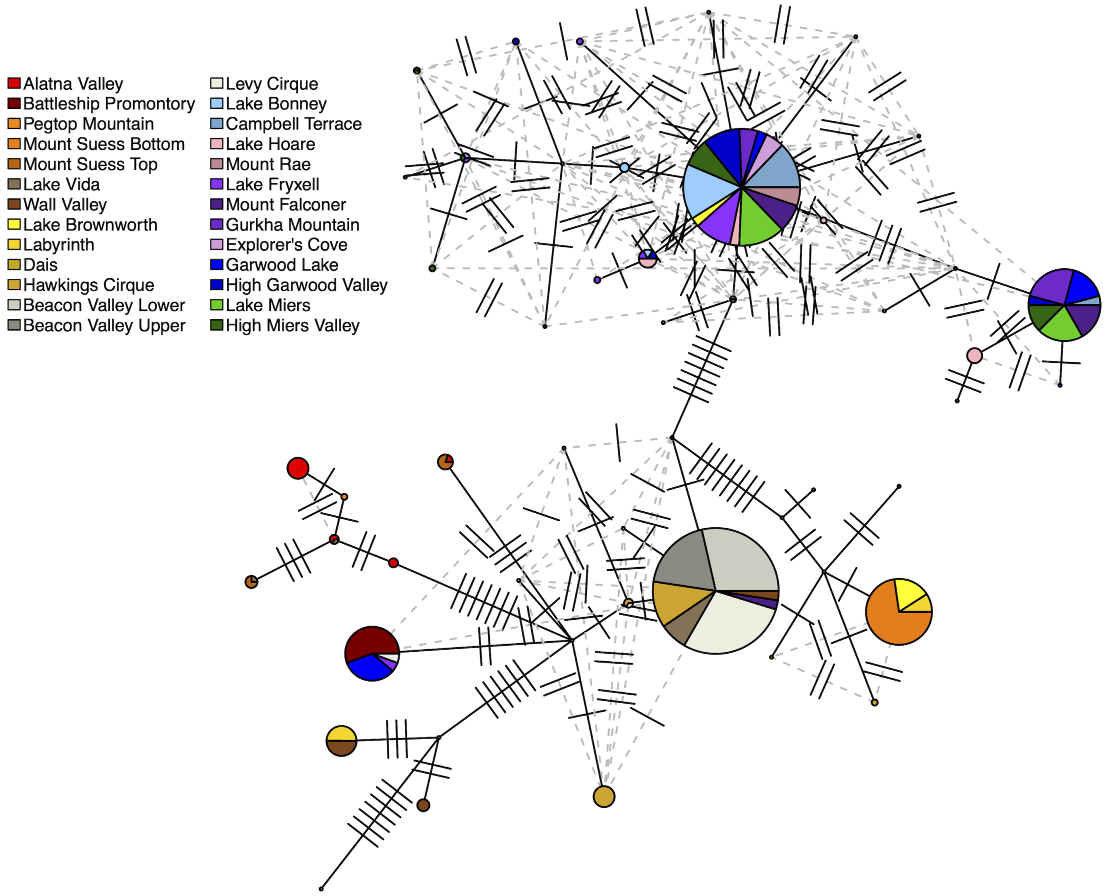

FIGURE 3
Haplotype network depicting haplotype associations and spread among sample site populations. Color corresponds to a sampling site; size of pie slice reflects the number of individuals with the designated haplotype; tick marks indicate a single mutation event distinguishing one haplotype from another. Warmer colors are associated with the northern valleys – Alatna, Mackay Glacier, Victoria, and Wright. The cooler colors designate Taylor, Miers, and Garwood valleys. White and grey designate haplotypes from the westerly isolated Beacon Valley.

The majority of sampled site populations had high genetic differentiation. Pairwise F_{ST} values ranged from 0.000 to 0.996 among sampling site populations (Figure 4, Supplementary Figure 5). F_{ST} values were highest among the sampling sites from undisturbed valleys (Mount Suess top and bottom, Battleship Promontory, Beacon Valley upper and lower, Levy Cirque, Hawkings Cirque, Pegtop Mountain, Alatna Valley, Wall Valley, Labyrinth, and Dais). Even sites within the same valley system contained statistically significant, high F_{ST} scores. Sample sites from Lake Vida, Taylor, Miers, and Garwood (Mount Rae, Miers mountainside, Garwood mountainside, Campbell Terrace, Lake Hoare, Gurkha Peak, Lake Bonney, Lake Fryxell, Lake Miers, Explorer's Cove, and Lake Garwood) had much lower F_{ST} scores, averaging 0.176. Lake Vida and Lake Garwood populations had lower pairwise genetic divergence across all sampled sites.

Among all valleys, pairwise F_{ST} ranged from 0.007 to 0.931 with all comparisons being significant except for Hoare Basin to Miers Valley, Hoare Basin to Fryxell Basin, and Miers Valley to Fryxell Basin (Table 2, Supplementary Figure 3). The greatest genetic differentiation was between Beacon and Bonney Basin (F_{ST} = 0.931; p = 0.000 E-5) and Beacon and Hoare Basin (F_{ST} = 0.904; p = 0.000 E-5). Pairwise genetic divergence from disturbed valleys – Taylor, Miers, Garwood, and Wright – contained F_{ST} values among the bottom 15%.

Based on the analysis of molecular variance, we found that *S. lindsayae* population structure was not part of a panmictic population as the majority of the variance did not arrive from within populations (Table 4). The majority of the variance was found among valleys (% variance = 41.56.3; σ = 2.72; p < 0.00). Variance among samples within populations accounted for the least variance (% variance = 28.34; σ = 1.85; p < 0.00. This provided evidence that populations were structured by valleys.

The dendrogram constructed with Nei's D and the ML tree additionally validated that populations were structured by valley system (Figure 5, Supplementary Figure 5). Both solutions revealed two broad clusters of populations. Clade B, composed of all sampled populations from Taylor, Miers, and Garwood valleys formed a well-supported monophyletic group (bootstrap support = 99.9%),

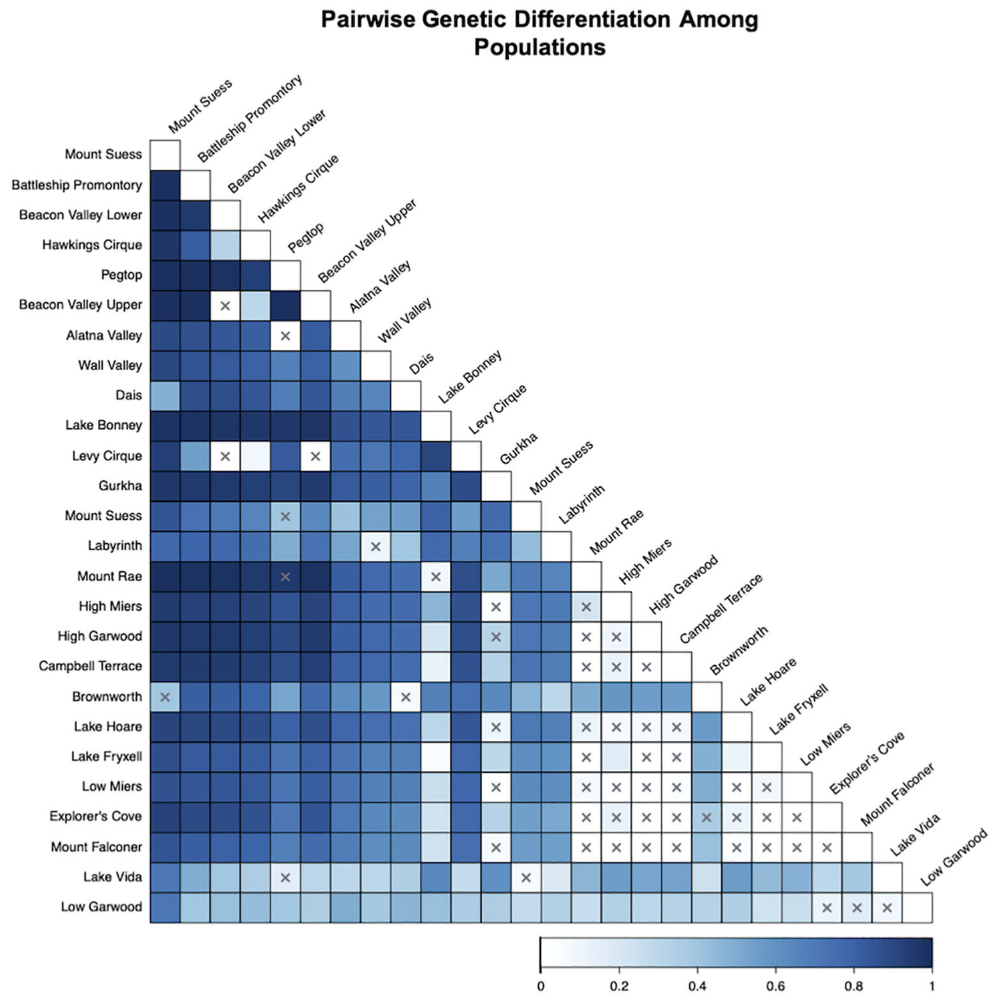

FIGURE 4
Genetic differentiation matrix calculated with Kimura 2P Gamma 4 test between sample site populations calculated in ARLEQUIN. Darker colors indicate larger F_{ST} values between two populations designated on the horizontal and vertical axes. "X" marks correspond to p-values < 0.05.

suggesting recent shared ancestry and genetic connectivity across these glacially disturbed valleys. In contrast, Clade A, which includes populations from Beacon, Alatna, Victoria, and Wright valleys, represents the best-supported grouping of undisturbed refugial sites. However, nodal support for the monophyly of Clade A is weak, and several internal nodes within the group are also poorly resolved. While this pattern is consistent with broad biogeographic structuring by glacial history, we interpret the monophyly of Clade A with caution due to limited statistical support.

3.3 Demographic history

Bayesian skyline plots (BSPs) indicated that all populations have experienced recent demographic shifts occurring in the time

TABLE 4 Analysis of molecular variance (ANOVA) indicating population structure is dominated by valley.

Source of variation	d.f.	Sum of squares	Variance components (Σ)	Percentage of variation	p value
Within Populations	223	412.88	1.85	28.34	p < 0.0001
Among sites within same valley	16	309.96	1.97	30.11	p < 0.0001
Among valleys	9	806.68	2.72	41.56	p < 0.0001
Total	248	1529.52	6.53		

Degrees of freedom (df) sum of squares indicate how much of the total variance can be attributed to each source.

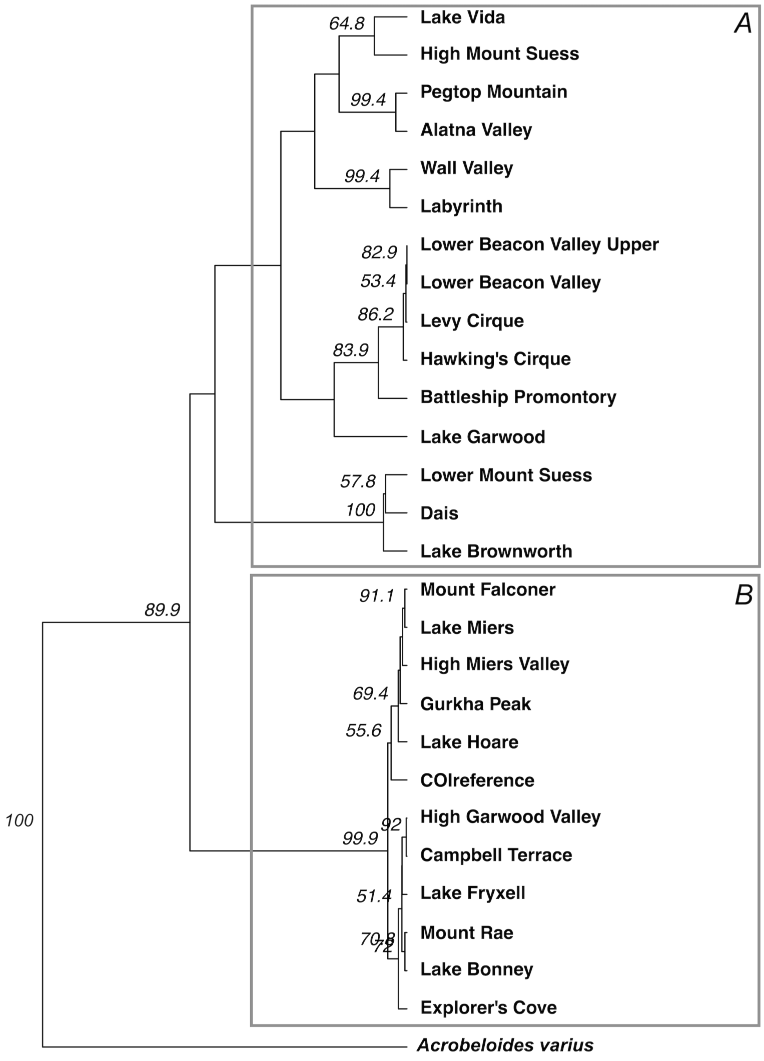

FIGURE 5
Dendrogram of pooled sequences using Nei's D from each sample site population (1,000 bootstrap replicates). The COI sequence of *Acrobeloides varius*, South Korea is used to root the tree. Populations in clade B include those from Taylor Valley sites: Mt Falconer, Gurkha Peak, Explorer's Cove, Lake Fryxell, Campbell Terrace, Lake Bonney, Mt Rae, Lake Hoar, Clade A is composed of sites from Alatna (Alatna Valley and Battleship Promontory), Victoria Valley (Lake Vida and Wall Valley), Mackay Glacier (Mt Suess and Pegtop Mountain), Wright Valley (Lake Brownworth, Dais, Labyrinth, Hawkings Cirque), and Beacon Valley (Lower and Upper Beacon, Levy Cirque).

since the LGM (Figure 6). Small sample sizes or loss of genetic information during population fluctuations may have contributed to the apparent stability of populations prior to the LGM, and may have caused an underestimation of population expansions in the BSPs (Grant, 2015). All undisturbed valleys (Alatna, Mackay Glacier, Victoria, and Wright) except Beacon Valley, shared a pattern of declining effective population size since the time of the LGM (20 kya to present). Conversely, Taylor Valley populations reported an increase in estimated population size following the LGM. Because Taylor, Miers, and Garwood form a monophyletic clade with limited genetic divergence, they were considered a population in BSP and likewise revealed a pattern of population increase around the time of the LGM. Beacon Valley also had a pattern of population increase since the LGM.

Patterns associated with the haplotype network likewise support the pattern of a recent population expansion in Taylor, Garwood, and Miers with one central haplotype and several satellite haplotypes only one or two mutations removed. Comparatively, the undisturbed northern and western valleys contain haplotypes associated with patterns of long-term isolation associated with several haplotypes with similar frequencies that were several mutations distant.

3.4 Environmental parameters

We ran generalized mixed linear models to test the influence of these environmental conditions (elevation, soil moisture, electrical conductivity, distance to the coast, and glacial history) on genetic diversity and report AIC_C model selection results with AIC_C, weight, delta, and independent variable correlation. We developed 19 models and advanced models with $W_i > 0.05$ for conditional model average

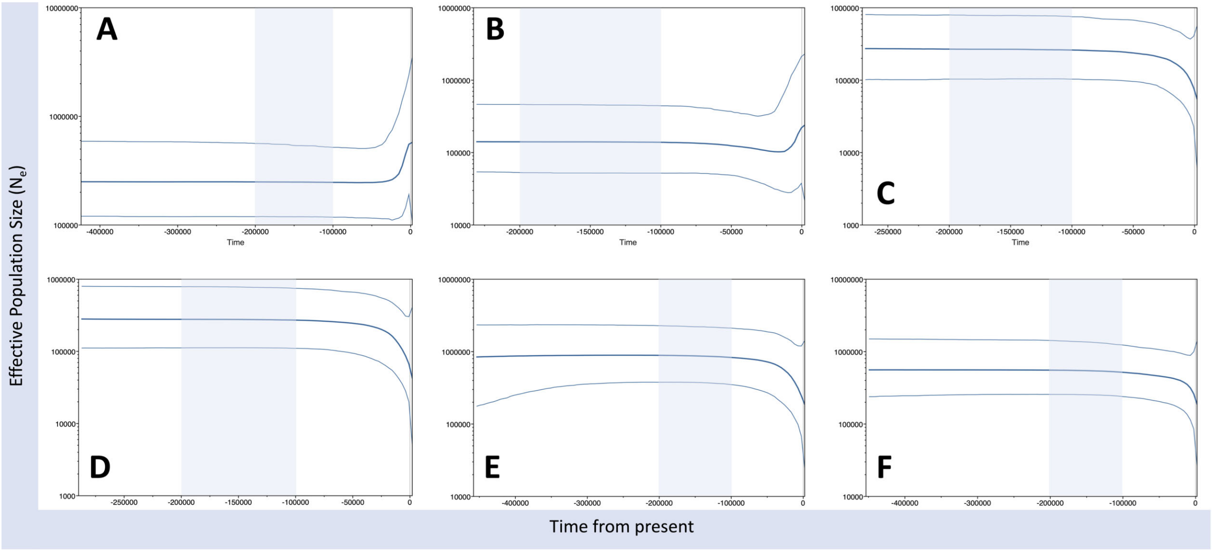

FIGURE 6
Bayesian skyline plots (BSPs) constructed for **(A)** Taylor, Miers, and Garwood (n = 71); **(B)** Beacon Valley (n = 37; **(C)** Alatna Valley (n = 23); **(D)** Mackay Glacier (n = 25); **(E)** Victoria (n = 17); and **(F)** Wright Valleys (n = 34). The x-axis represents time from the present with present day aligned on the right. The time of the LGM is shaded. The y-axis represents the effective population size. The center line is the median estimate. The upper and lower lines indicate highest posterior density intervals.

(Tables 5, 6; full list of models Supplementary Figure 6). Intra-population F_{ST} had a negative relationship with elevation across the top four models (ß = -0.006 ± 0.003, 85% CI = -0.010 to -0.002 W_i = 81%; Table 5). However, intra-population F_{ST} had a positive relationship with elevation where soils had higher water content (ß = 9.15e-4 ± 4.25 E -4, 85% CI = 2.81e-4 to 1.55e-3, W_i = 37%). Soil moisture had a negative relationship with intra-population F_{ST} and was a parameter in three of the top four models (ß = -0.590 ± 0.400, 85% CI = -1.18 to -0.003, W_i = 63%). Glacial history and electrical conductivity were also parameters in the top models but did not exhibit significant influence over intra-population genetic divergence. Ultimately elevation appears to explain the most variation in intra-population genetic divergence (R^2 = 0.240, p = 0.012).

Haplotype diversity was influenced by elevation and glacial history. The top model (W_i = 76%) included elevation, glacial history, and their interaction. While the model retained a positive relationship between elevation and haplotype diversity in undisturbed sites, the effect size was modest (ß = 7.82 X10^{-4}). This relationship is visually represented in Figure 7, though the trend line appears more pronounced than the spread of data points due to the compressed range of elevation values and limited number of undisturbed high-elevation sites. We interpret this effect as statistically significant but not strongly predictive on its own. The second-best model (W_i = 19%) had simplified parameters that included glacial history and elevation, but not the interaction (Table 6). From these top two models, haplotype diversity had a positive relationship with undisturbed glacial history but not strongly (ß = 0.165 ± 0.248, 85% CI = -0.200 to 0.531, W_i = 95%), a negative relationship with elevation (ß = -0.001 ± 3.43e-4, 85% CI = -1.61e-3 to -6.05e-4, W_i = 100%), and a positive relationship with elevation when the glacial history is undisturbed (ß = 7.82e-4 ± 3.54e-4, 85% CI = 2.89e-4 to 1.28e-3, W_i =76%). We included population abundance in a secondary model selection to determine if haplotype diversity or lineage divergence was confounded by abundance of individuals in a site. We found that population abundance was not a parameter in the top models for haplotype diversity. It was among the top two models for intra-population structure but was not significant.

4 Discussion

4.1 Phylogeographic structuring and taxonomic implications

We found that the MDV genetic structure of *S. lindsayae* is demarcated by valley system except where historical climate disturbance has created opportunity for migration and gene flow. Geomorphic barriers like glaciers and intervening mountain ranges have been effective barriers to inferred connectivity, causing accumulation of distinct genetic variations. Glacial recession following the LGM may have aided migration of soil nematodes. We also found that current ecological conditions were able to predict genetic diversity within populations. We found that *S. lindsayae* populations across the MDVs contained highly conserved rRNA sequences across valley systems, most likely indicating this is a single species despite high genetic divergences of the COI gene. Our results are consistent with observations by Courtright et al. (2000) who found a single species and several subpopulations in the MDVs and Velasco-Castrillón and Stevens

TABLE 5 Model selection for within population genetic divergence and ecological conditions.

Within population F_{ST}				
Model parameters	**df**	**AIC_C**	**Δ**	**W_i**
Elevation + Soil Moisture + Elevation × Soil Moisture	5	139.62	0.00	0.37
Elevation + Soil Moisture	4	141.49	1.87	0.15
Elevation + Glacial History	4	142.34	2.73	0.09
Elevation + EC	4	142.34	2.73	0.10

Model Parameter	Weight %	β	85% CI
Intercept	100	8.29 ± 2.08	5.24 to 11.3
Elevation	81	-5.65e-3 ± 2.69e-3	-0.010 to -0.002
Soil moisture	63	-0.593 ± 0.402	-1.18 to -0.003
Elevation × soil moisture	37	9.15e-4 ± 4.25e-4	2.81e-4 to 1.55e-3
Glacial history	32	0.376 ± 2.20	-2.90 to 3.66
Electrical Conductivity	22	-0.180 ± 1.09	-1.80 to 1.44

Ecological conditions included elevation (mamsl), soil moisture (%g/g), glacial history, electrical conductivity (EC; dS/m), and distance to the coast (km). We report (top) the models with $W_i > 0.05$ selected from 19 initial models showing each parameter per model (model parameter), degrees of freedom (df), Aikaike's Information criterion (AIC_C), difference in AIC_C value comparted to top model (Δ), and AIC_C model weight (W_i). Models with $W_i > 0.05$ were judged to contain informative parameters and were included in conditional model averaging (bottom). We report model weight, β estimates, and 85% confidence intervals for conditional averaged parameter estimates from the top models.

TABLE 6 Model selection for haplotype diversity and ecological conditions. Ecological conditions included elevation (mamsl), soil moisture (%g/g), glacial history, electrical conductivity (EC; dS/m), and distance to the coast (km).

Haplotype diversity				
Model parameters	**df**	**AIC_C**	**Δ**	**Weight**
Elevation + Glacial History + Elevation × Glacial History	5	2.63	0.00	0.76
Elevation + Glacial History	4	5.43	2.80	0.19

Model Parameter	Weight %	β	85% CI
Intercept	100	0.893 ± 0.108	0.732 to 1.05
Elevation	100	-1.11e-3 ± 3.43e-4	-0.002 to -6.05e-4
Glacial History	95	0.165 ± 0.248	-0.200 to 0.531
Elevation × Glacial History	76	7.82e-4 ± 3.31e-4	2.89e-4 to 0.001

We report (top) the models with $W_i > 0.05$ selected from 19 initial models showing each parameter per model (model parameter), degrees of freedom (df), Aikaike's Information criterion (AIC_C), difference in AIC_C value comparted to top model (Δ), and AIC_C model weight (W_i). Models with $W_i > 0.05$ were judged to contain informative parameters and were included in conditional model averaging (bottom). We report model weight, β estimates, and 85% confidence intervals for conditional averaged parameter estimates from the top models.

(2014) who identified limited genetic distance consistent with a single species across the continent. Our study represents a more geographically constrained and detailed synthesis of the population structure of *S. lindsayae* in the MDVs. We found high levels of genetic distance (*p* distance ≤ 7.4%), differentiation ($F_{ST} \leq 0.996$), and haplotype diversity ($h = 0.926$) that identify endemic subpopulations with long-term geographic isolation and population structure excepting large-scale climate disturbance events such as glacial advance and retreat. These results reveal the deterministic processes underlying metazoan population structure and specifically the strong influences of space, climate disturbance, and ecological conditions over genetic patterns.

Our phylogenetic analyses revealed a strongly supported clade (Clade B) composed of populations from valleys disturbed during the LGM, supporting a scenario of post-disturbance recolonization and recent gene flow. Populations from undisturbed valleys clustered into a second group (Clade A), though weak bootstrap support for this clade suggests that its monophyly remains tentative. Interestingly, the poor resolution of deeper nodes within Clade A may reflect a period of rapid diversification among populations in high-elevation valleys that remained isolated through the LGM. This pattern is consistent with long-term geographic isolation followed by early divergence and limited subsequent gene flow. Bayesian skyline plots for these valleys also support this

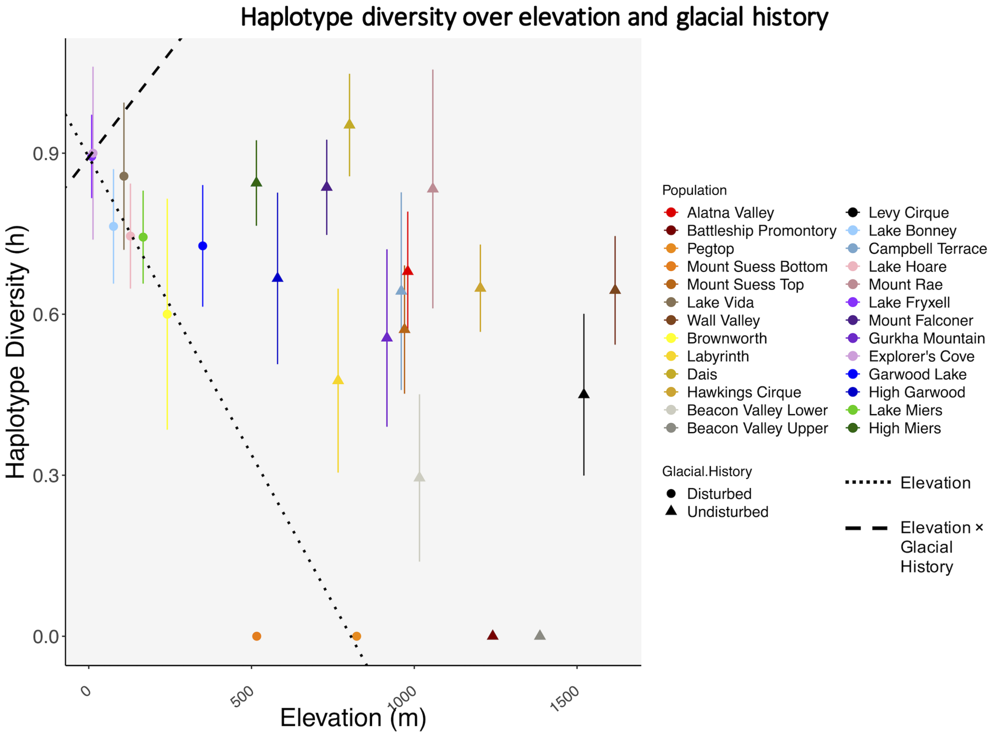

FIGURE 7
Distribution of haplotype diversity across sites by elevation and glacial history. Shapes indicate glacial history (triangles = undisturbed, circles = disturbed); colors represent sampling sites. The dashed line shows the fitted relationship between elevation and haplotype diversity for undisturbed sites, as estimated by the top mixed linear model. While the model indicates a positive relationship, the effect size is modest and visually subtle due to the limited range of high-elevation undisturbed sites. The dotted line shows the relationship for disturbed sites, which was not significant. Haplotype diversity was calculated from COI sequences for each site.

interpretation, showing demographic stability or gradual decline since the LGM—indicative of long-established, independently evolving populations rather than recent expansions. While this lends further support to the refugial history of these populations, the lack of deep phylogenetic resolution underscores the need for future work using multilocus or genomic data to more accurately reconstruct their divergence history and assess potential cryptic speciation.

Notably, the deep divergence among some COI haplotypes, particularly between the two major clades, approaches levels commonly associated with interspecific differences in other nematodes. These patterns raise the possibility that *S. lindsayae*, as currently described, may represent a complex of independently evolving lineages rather than a single cohesive species. While the conserved LSU sequences suggest genetic continuity, the COI divergence is consistent with emerging evidence in other Antarctic invertebrates where mitochondrial lineages reflect cryptic species. This warrants further investigation using multilocus nuclear markers and formal species delimitation analyses to determine whether the divergent lineages identified here correspond to reproductively isolated taxa.

While mitochondrial DNA offers valuable resolution for inferring population structure, we recognize that our reliance on a single maternally inherited locus imposes limitations. COI does not capture recombination and may be subject to lineage-specific selective sweeps, potentially biasing demographic or phylogenetic inferences. Moreover, mitochondrial markers alone cannot disentangle male-mediated gene flow or provide independent estimates of coalescent times. Though our findings are consistent with other Antarctic metazoan phylogeographic patterns, future studies using nuclear multilocus or genomic datasets (e.g., RADseq or target capture) will be necessary to validate these patterns and resolve fine-scale demographic histories.

4.2 Spatial and temporal patterns of genetic divergence

Using a portion of the mitochondrial COI gene, we found that valley origin, and to a lesser degree geographic proximity, predicted genetic similarity. Frequently, population genetic studies find a strong correlation of genetic distance to geomorphic barriers or geographic distance supporting regionally defined populations (Khanal et al., 2018; Van Der Valk et al., 2018). Populations structured heterogeneously by barriers and distance have been consistently observed throughout Antarctic marine and terrestrial ecosystems (Convey et al., 2014; Harder et al., 2016) including the MDVs (Stevens and Hogg, 2006; Dreesens et al., 2014; Bennett et al., 2016). Consistent with patterns of Antarctic phylogeography, *S. lindsayae* genetic variance was regionally defined and followed geomorphic barriers.

The strength of geomorphic barriers in shaping population structure is an important consideration in assessing the spatial structure of Antarctic biodiversity. Even on fine scales such as the distance from Wright Valley to Taylor Valley only 20 km apart, populations of *S. lindsayae* were distinct and have evolved significantly high levels of pairwise genetic differentiation and genetic divergence. The intervalley mountain ranges running east to west have the strongest isolating effect on metazoan populations in the MDVs. Results of our AMOVA analysis corroborate this conclusion where we found valley origin to be a strong predictor of genetic variance. While geologic features like mountain ranges and glaciers prevent gene flow, simple geographic distance, to a lesser degree, also increased genetic divergence. Populations close together, in the same valley system, for example, exhibited much lower pairwise genetic differentiation than populations from different valleys that were separated by geomorphic barriers. Wind and water movement are known mechanisms of gene flow among metazoans at the intravalley level in the MDVs and potentially explain the patterns found here (Nkem et al., 2006b; Gooseff et al., 2011; McGaughran et al., 2019; Schulte et al., 2022).

The high degree of intraspecies genetic variation between valleys imply high endemism of valley populations of MDV nematodes (Adams et al., 2007). It is reasonable to find intraspecies variation aligned with geomorphic barriers for non-vagile species like soil nematodes. Given the long-term environmental stability of many of these high elevation refugial valleys and the absence of mechanisms for active dispersal, these patterns are consistent with extended periods of geographic isolation. However, in the absence of formal divergence time estimates, we are not able to assign a specific temporal scale to the origin of this structure. Instead, we interpret the observed patterns as reflecting historical isolation maintained over long durations, disrupted primarily by large-scale events such as glacial advance and retreat during the LGM. Thus, the refugial valleys that remained undisturbed through the LGM contain locally endemic subpopulations that have been evolving independently throughout the Pleistocene. It is likely that other dispersal-limited metazoan taxa of the MDVs are similarly structured by the same valley and geomorphic barriers.

4.3 Climate disturbance legacies

We found that typical patterns of genetic structuring due to geomorphological features (e.g., divergence among valleys) were disrupted by large-scale climate disturbance events, specifically glacial expansion and paleolake inundation during the LGM. It is well established that historical climate is reflected in phylogeographic patterns, including population structures of organisms in Antarctica where the LGM caused population contractions to local refugia and subsequent recolonization (Rogers, 2007; Keppel et al., 2012; Convey et al., 2020). The impact of the LGM on Antarctic metazoan diversity and population structure has been well documented for mite and springtail species (Demetras et al., 2010; Collins et al., 2019, 2020; McGaughran et al., 2019). Our results corroborate the influence of glacial expansion on phylogeographic patterns in a sentinel Antarctic metazoan. Given the high degree of lineage diversity in the context of phylogenetic reconstruction, our results provide evidence that much of the MDVs have remained habitable through the LGM while those that were inundated by glacial advance and paleolakes – Taylor, Miers, and Garwood – experienced local extinctions and subsequent recolonization.

Our results suggest that the LGM disturbance event may have facilitated connectivity and homogenization of subpopulations as glaciers receded at the end of the LGM. These results are similar to those found in Taylor Valley springtails with lower genetic divergence along the shoreline of paleolake Washburn (Nolan et al., 2006). Similarly, there was less pairwise genetic divergence between populations of *S. lindsayae* from valleys that experienced some degree of disturbance during the LGM indicating a possible mechanism of intervalley gene flow following glacial recession. Additionally, an examination of the relationship among haplotypes suggested two distinct phylogenetic groups, which differs from the conclusions of Courtright et al. (2000) and Adams et al. (2007). The evolutionary pattern of these two clades suggests glaciers shape more than just the geology and geography of the MDVs, but also the distributional patterns of subpopulations. Demographic histories among *S. lindsayae* populations of the MDVs are likely concordant with the timings of glacial and paleolake recession and the progression of habitable soil after the LGM and should be explored in future studies. Bayesian skyline plots support this interpretation by revealing signals of post-LGM population expansion in disturbed valleys (e.g. Taylor, Miers, Garwood), while undisturbed refugial valleys generally show demographic stability or gradual decline. These patterns, drawn from the haplotype network and phylogenetic analysis, suggest that climate disturbance events during the LGM played a large role in shaping the phylogeographic patterns and facilitating intervalley migration, particularly along glacial margins and forefields. While active dispersal across valley systems is unlikely for *S. lindsayae*, historical glacial expansions and retreats, such as the incursion of the Ross Sea Drift, may have temporarily bridged otherwise isolated valleys. These events could have enabled passive migration via continuous ice cover, soil redistribution, or meltwater pathways across low passes. Such mechanisms offer a plausible explanation for the genetic connectivity observed among populations in disturbed valleys. While these results are consistent with historical isolation and post-disturbance recolonization, we note that formal divergence time estimates were not calculated, and future studies using time-calibrated, multilocus data would be necessary to refine the temporal dynamics of these patterns.

Our results indicate that endemic populations are vulnerable to large scale climate disturbances and provides a line of evidence for long-distance dispersal of Antarctic soil nematodes along the margins of receding glaciers and shorelines. The disturbed valley populations exhibit resilience in the face of climate disturbance and ability to recolonize glacial forefields, but forfeit unique genetic lineages as found in undisturbed valleys.

4.4 Environmental constraints on genetic structure

Environmental conditions are important but often overlooked factors in generating phylogeographic patterns (Knowles and Maddison, 2002; Alvarado-Serrano and Knowles, 2014). Results from our analysis of model selection indicated that some ecological drivers may influence intra-population genetic differentiation and haplotype diversity. The abiotic conditions of the MDVs are frequently cited as drivers of species distributions in the MDVs (Hogg et al., 2006; Caruso et al., 2019) and especially when determining the presence of *S. lindsayae* (Poage et al., 2008; Wlostowski et al., 2018; Franco et al., 2021). These studies often utilize elevation (Lee et al., 2019; Bottos et al., 2020), soil moisture (Poage et al., 2008; Van Horn et al., 2013), electrical conductivity (Nkem et al., 2006a), and more recently, glacial history (Magalhães et al., 2012; McGaughran et al., 2014; Chen et al., 2017; Jackson et al., 2022) to characterize deterministic factors of biogeography. To date, studies of how these ecological interactions shape the genetic diversity of MDV soil metazoans have been lacking but are likely significant (Schierenbeck, 2017; Caruso et al., 2019).

Using just a few, but widely tested environmental factors – elevation, soil moisture, distance to the coast, electrical conductivity, and glacial history – we found some factors that were relevant to genetic diversity in this system. The top models for intra-population genetic divergence indicated that elevation and soil moisture were negatively correlated. This trend was reversed when considering the interaction of elevation and soil moisture. Valley floor habitats are the sink to down valley aeolian-transported materials (Diaz et al., 2018). Thus, it follows that lower elevations accumulate intravalley lineages and have more genetic differentiation in any given population. These results indicate higher potential for migration at valley floors through physical processes. Soil moisture is necessary for habitable soil and would be required for wind mediated transport. The negative correlation was surprising but could indicate a more homogenized population where *S. lindsayae* could move further through the interstitial, fluid-filled spaces. Higher elevation soils are typically less conducive for life and sufficient soil moisture provides the first indication that *S. lindsayae* populations can establish at these higher elevations and support relict populations capable of developing higher degrees of within population genetic differentiation. This is consistent with higher elevations, where, mediated by soil moisture, they contain larger variability in community structure (Andriuzzi et al., 2018). Considering haplotype diversity, we found that elevation was negatively correlated until the disturbance history was considered. Sites at low elevations had higher haplotype diversity perhaps owing to the reasons described above. However, haplotype diversity also increased in high elevation populations that have remained undisturbed, as these sites have been afforded stable habitats and time to develop evolutionary mutations and thus higher haplotype diversity. Such trends provide further support for high-elevation habitats as putative refugia. Environmental and ecological conditions play a role in structuring the genetic diversity of these soil organisms and should be further considered as an important component of long-term monitoring.

Community composition and the distribution of organisms in the MDVs is clearly driven by the environmental and ecological factors responsible for habitat suitability. By extension, it is reasonable to expect that genetic diversity is influenced by these conditions as well. Given the simplified biotic communities, well documented heterogenous abiotic conditions of the MDVs, and nearly universal distribution of *S. lindsayae*, this system provides a unique opportunity to test the influence of basic abiotic properties like elevation, soil conditions, and geologic history on genetic diversity and structure.

4.5 Conclusions

Our primary aim in this study was to explore the evolutionary response of the sentinel species *S. lindsayae* to historical climate disturbance. We found that climate history had a lasting impact on its population and genetic structure, resulting in demographic shifts and the emergence of distinct, isolated, and endemic subpopulations. Understanding how such patterns arose provides foundational context for interpreting ongoing and future changes. We suggest that future long-term monitoring studies should incorporate a phylogeographic perspective, as this can help identify populations with unique evolutionary histories, inform strategies to preserve existing genetic structure, and predict how climate-driven disturbances may alter the functional and evolutionary trajectories of Antarctic terrestrial ecosystems.

Data availability statement

The full data package is published and available from the Environmental Data Initiative found here: https://portal.edirepository.org/nis/mapbrowse?packageid=knb-lter-mcm.269.1 (Jackson et al., 2023).

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

AJ: Conceptualization, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. SL: Writing – review & editing. DP: Writing – review & editing. DW: Resources, Writing – review & editing. TP: Methodology, Writing – review & editing. TH: Methodology, Writing – review & editing. BA: Conceptualization, Funding acquisition, Methodology, Writing – review & editing, Resources.

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This work is part of a thesis and can be accessed online in accordance with the author's university policy. This is the only form in which it has previously appeared. It can be accessed here: http://hdl.lib.byu.edu/1877/etd12622 (Jackson, 2022).

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.

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References

Adams, B. J., Bardgett, R. D., Ayres, E., Wall, D. H., Aislabie, J., Bamforth, S., et al. (2006). Diversity and distribution of Victoria Land biota. *Soil Biol. Biochem.* 38, 3003–3018. doi: 10.1016/j.soilbio.2006.04.030

Adams, B. J., Wall, D. H., Gozel, U., Dillman, A. R., Chaston, J. M., Hogg, I. D., et al. (2007). The southernmost worm, *Scottnema lindsayae* (Nematoda): Diversity, dispersal and ecological stability. *Polar Biol. Springer* 30, 809–815. doi: 10.1007/s00300-006-0241-3

Adams, B. J., Wall, D. H., Virginia, R. A., Broos, E., and Knox, M. A. (2014). Ecological biogeography of the terrestrial Nematodes of Victoria Land, Antarctica. *ZooKeys* 71, 29–71. doi: 10.3897/zookeys.419.7180

Ahmed, M., Roberts, N. G., Adediran, F., Smythe, A. B., Kocot, K. M., and Holovachov, O. (2022). "Phylogenomic analysis of the Phylum nematoda: conflicts and congruences with morphology, 18S rRNA, and mitogenomes," in *Frontiers in Ecology and Evolution*, vol. 9. . doi: 10.3389/fevo.2021.769565

Akaike, H. (1973). "Information theory and an extension of the maximum likelihood principle," in *International Symposium on Information Theory*. Eds. B. N. Petran and F. Csaki (Akademiai Kiado, Budapest), 267–281. doi: 10.1007/978-1-4612-0919-5_38

Aktas, C. (2020). haplotypes: manipulating DNA sequences and estimating unambiguous haplotype network with statistical parsimony. doi: 10.32614/CRAN.package.haplotypes

Alvarado-Serrano, D. F., and Knowles, L. L. (2014). Ecological niche models in phylogeographic studies: Applications, advances and precautions. *Mol. Ecol. Resour.* 14, 233–248. doi: 10.1111/1755-0998.12184

Anderson, J. B., Conway, H., Bart, P., Witus, A. E., Greenwood, S. L., Mckay, R., et al. (2014). "Ross Sea paleo-ice sheet drainage and deglacial history during and since the LGM," in *Quaternary Science Reviews*, vol. 100. (Elsevier Ltd), 31–54. doi: 10.1016/j.quascirev.2013.08.020

Andriuzzi, W. S., Adams, B. J., Barrett, J. E., Virginia, R. A., and Wall, D. H. (2018). Observed trends of soil fauna in the Antarctic Dry Valleys: early signs of shifts predicted under climate change. *Ecology* 99, 312–321. doi: 10.1002/ecy.2090

Avise, J. C. (2009). Phylogeography: retrospect and prospect. *J. Biogeography* 36, 3–15. doi: 10.1111/j.1365-2699.2008.02032.x

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., et al. (1987). Intraspecific phyloeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu. Rev. Ecol. Systematics* 18, 489–522. doi: 10.1146/annurev.es.18.110187.002421

Baird, H. P., Shin, S., Oberprieler, R. G., Hullé, M., Vernon, P., Moon, K. L., et al. (2021). Fifty million years of beetle evolution along the Antarctic Polar Front. *PNAS* 118(24), e2017384118. doi: 10.1073/pnas.2017384118

Ballard, J. W. O., and Rand, D. M. (2005). The population biology of mitochondrial DNA and its phylogenetic implications. *Annu. Rev. Ecology Evolution Systematics* 36, 621–642. doi: 10.1146/annurev.ecolsys.36.091704.175513

Barrett, J. E., Virginia, R. A., Wall, D. H., and Adams, B. J. (2008). Decline in a dominant invertebrate species contributes to altered carbon cycling in a low-diversity soil ecosystem. *Global Change Biol.* 14, 1734–1744. doi: 10.1111/j.1365-2486.2008.01611.x

Bartoń, K. (2022). *MuMIn: Multi-Model Inference*. R package version 1.48.11. Available online at: https://CRAN.R-project.org/package=MuMIn.

Bennett, K. R., Hogg, I. D., Adams, B. J., and Hebert, P. D. N. (2016). "High levels of intraspecific genetic divergences revealed for Antarctic springtails: evidence for small-scale isolation during Pleistocene glaciation," in *Biological Journal of the Linnean Society*, vol. 119. (Blackwell Publishing Ltd), 166–178. doi: 10.1111/bij.12796

Bensasson, D., Zhang, D. X., Hartl, D. X., and Hewitt, G. M. (2001). Mitochondrial pseudogenes: Evolution's misplaced witnesses. *Trends Ecol. Evol.* 16, 314–321. doi: 10.1016/S0169-5347(01)02151-6

Biersma, E. M., Jackson, J. A., Stech, M., Griffiths, H., Linse, K., and Convey, P. (2018). Molecular data suggest long-term in Situ Antarctic persistence within Antarctica's most speciose plant genus, *Schistidium*. *Front. Ecol. Evol.* 6. doi: 10.3389/fevo.2018.00077

Bockheim, J. G., and McLeod, M. (2013). "Glacial geomorphology of the Victoria valley system, ross sea region, Antarctica," in *Geomorphology*, vol. 193. (Elsevier B.V.), 14–24. doi: 10.1016/j.geomorph.2013.03.020

Bockheim, J. G., Prentice, M., and McLeod, M. (2008). Distribution of glacial deposits, soils, and permafrost in Taylor Valley, Antarctica. *Arctic Antarctic Alpine Res.* 40, 279–286. doi: 10.1657/1523-0430(06-057)[BOCKHEIM]2.0.CO;2

Boström, S., Holovachov, O., and Nadler, S. A. (2011). Description of *Scottnema lindsayae* Timm 1971 (Rhabditida: Cephalobidae) from Taylor Valley, Antarctica and its phylogenetic relationship. *Polar Biol.* 34, 1–12. doi: 10.1007/s00300-010-0850-8

Bottos, E. M., Laughlin, D. C., Herbold, C. W., Lee, C. K., McDonald, I. R., and Cary, S. C. (2020). Abiotic factors influence patterns of bacterial diversity and community composition in the Dry Valleys of Antarctica. *FEMS Microbiol. Ecology. Oxford Univ. Press* 96, 1–12. doi: 10.1093/FEMSEC/FIAA042

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., et al. (2019) BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* 15 (4), e1006650. doi: 10.1371/journal.pcbi.1006650

Brown, S., Collins, R., Boyer, S., Lefort, M., Malumbres-Olarte, J., Vink, C., et al. (2012). SPIDER: an R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. *Mol. Ecol. Resour.* 12, 562–565. doi: 10.1111/j.1755-0998.2011.03108.x

Brunetti, C., Siepel, H., Paolo, P. F., Nardi, F., Convey, P., Carapelli, A., et al. (2021). Two new species of the mite genus *Stereotydeus* Berlese 1901 (Prostigmata: Penthalodidae) from Victoria Land, and a key for identification of Antarctic and sub-Antarctic species. *Taxonomy* 1, 116–141. doi: 10.3390/taxonomy1020010

Burkins, M. B., Virginia, R. A., Chamberlain, C. P., and Wall, D. H. (2000). Origin and distribution of soil organic matter in Taylor Valley, Antarctica. *Ecology* 81, 2377–2391. doi: 10.1890/0012-9658(2000)081[2377:OADOSO]2.0.CO;2

Calkin, P. E. (1963). *Geomorphology and Glacial Geology of the Victoria Valley System, Southern Victoria Land, Antarctica.*

Calkin, P. E. (1964). Glacial geology of the mount gran area, southern Victoria land, Antarctica. *Bull. Geological Soc. America* 75, 1031–1036. doi: 10.1130/0016-7606(1964)75[1031:GGOTMG]2.0.CO;2

Campbell, I. B., Claridge, G. G. C., Campbell, D. I., and Balks, M. R. (1998). "Soil environment of the McMurdo dry valleys, Antarctica," in *Ecosystem Dynamics in a Polar Desert: the Mcmurdo Dry Valleys, Antarctica*, vol. 72 . Ed. J. C. Priscu (American Geophysical Union), 297–322. doi: 10.1029/AR072

Caruso, T., Hogg, I. D., Nielsen, U. N., Bottos, E. M., Lee, C. K., Hopkins, D. W., et al. (2019). "Nematodes in a polar desert reveal the relative role of biotic interactions in the coexistence of soil animals," in *Communications Biology*, vol. 2. (Springer US). doi: 10.1038/s42003-018-0260-y

Chen, J. H., Huang, C. L., Lai, Y. L., Chang, C. T., Liao, P. C., Hwang, S. Y., et al. (2017). "Postglacial range expansion and the role of ecological factors in driving adaptive evolution of Musa basjoo var. formosana," in *Scientific Reports*, vol. 7. (Springer US), 1–13. doi: 10.1038/s41598-017-05256-6

Clayton-Greene, J. M., Hendy, C. H., and Hogg, A. G. (1988). Chronology of a wisconsin age proglacial lake in the Miers Valley, Antarctica. *New Z. J. Geology Geophysics* 31, 353–361. doi: 10.1080/00288306.1988.10417781

Collins, G. E., Hogg, I. D., Convey, P., Barnes, A. D., and McDonald, I. R. (2019). Spatial and temporal scales matter when assessing the species and genetic diversity of springtails (Collembola) in Antarctica. *Front. Ecol. Evol.* 7. doi: 10.3389/fevo.2019.00076

Collins, G. E., Hogg, I. D., Convey, P., Sancho, L. G., Cowan, D. A., Lyons, W. B., et al. (2020). Genetic diversity of soil invertebrates corroborates timing estimates for past collapses of the West Antarctic Ice Sheet. *Proc. Natl. Acad. Sci.* 117, 202007925. doi: 10.1073/pnas.2007925117

Convey, P., Biersma, E. M., Casanova-Katny, A., and Maturana, C. S. (2014). The spatial structure of antarctic biodiversity. *Ecol. Monogr.* 84, 203–244. doi: 10.1890/12-2216.1

Convey, P., et al. (2020). "Refuges of Antarctic diversity," in *Past Antarctica*. Eds. M. Oliva and J. Ruiz-Fernández (Academic Press), 181–200. doi: 10.1016/b978-0-12-817925-3.00010-0

Courtright, E. M., Wall, D. H., Virginia, R. A., Frisse, L. M., Vida, J. T., and Thomas, W. K. (2000). Nuclear and mitochondrial DNA sequence diversity in the Antarctic nematode *Scottnema lindsayae*. *J. Nematol.* 32, 143–153.

Courtright, E., Wall, D. H., and Virginia, R. (2001). Determining habitat suitability for soil invertebrates in an extreme environment: the McMurdo Dry Valleys, Antarctica. *Antarctic Sci.* 13, 9–16. doi: 10.1017/S0954102001000037

Demetras, N. J., Hogg, I. D., Banks, J. C., and Adams, B. J. (2010). Latitudinal distribution and mitochondrial DNA (COI) variability of Stereotydeus spp. (Acari: Prostigmata) in Victoria Land and the central Transantarctic Mountains. *Antarctic Sci.* 22, 749–756. doi: 10.1017/S0954102010000659

Denton, G. H., Prentice, M. L., Kellogg, D. E., and Kellogg, T. B. (1984). Late Tertiary history of the Antarctic ice sheet: evidence from the Dry Valleys. *Geology* 12, 263–267. doi: 10.1130/0091-7613(1984)12<263:LTHOTA>2.0.CO;2

Denton, G. H., et al. (1993). East Antarctic ice sheet sensitivity to pliocene climatic change from a dry valleys perspective. *Geografiska Annaler: Ser. A Phys. Geogr.* 75 (4), 155–204. doi: 10.1080/04353676.1993.11880393

Denver, D. R., Morris, K., Lynch, M., Vassilieva, L. L., and Thomas, W. K. (2000). High direct estimate of the mutation rate in the mitochondrial genome of Caenorhabditis elegans. *Science* 289, 2342–2344. doi: 10.1126/science.289.5488.2342

Diaz, M. A., Adams, B. J., Welch, K. A., Welch, S. A., Opiyo, S. O., Khan, A. L., et al. (2018). Aeolian material transport and its role in landscape connectivity in the McMurdo dry valleys, Antarctica. *J. Geophysical Research: Earth Surface* 123, 3323–3337. doi: 10.1029/2017JF004589

Diaz, M. A., Welch, S. A., Sheets, J. M., Welch, K. A., Khan, A. L., Adams, B. J., et al. (2020). "Geochemistry of aeolian material from the McMurdo Dry Valleys, Antarctica: Insights into Southern Hemisphere dust sources," in *Earth and Planetary Science Letters*, vol. 547. (Elsevier B.V.), 116460. doi: 10.1016/j.epsl.2020.116460

Doran, P. T., McKay, C. P., Clow, G. D., Dana, G. L., Fountain, A. G., Nylen, T., et al. (2002a). Valley floor climate observations from the McMurdo dry valleys, Antarctica 1986-2000. *J. Geophysical Res. Atmospheres* 107, 1–12. doi: 10.1029/2001JD002045

Doran, P. T., Priscu, J. C., Lyons, B. W., Walsh, J. E., Fountain, A. G., McKnight, D. M., et al. (2002b). Antarctic climate cooling and terrestrial ecosystem response. *Nature* 415, 517–520. doi: 10.1038/nature710

Dreesens, L. L., Lee, C. K., and Cary, S. C. (2014). The distribution and identity of edaphic fungi in the McMurdo dry valleys. *Biology* 3, 466–483. doi: 10.3390/biology3030466

Drummond, A. J., Rambaut, A., Shapiro, B., and Pybus, O. G. (2005). Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol. Biol. Evol.* 22, 1185–1192. doi: 10.1093/molbev/msi103

Excoffier, L., and Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10, 564–567. doi: 10.1111/j.1755-0998.2010.02847.x

Feeser, K. L., Van Horn, D. J., Buelow, H. N., Colman, D. R., McHugh, T. A., Okie, J. G., et al. (2018). Local and regional scale heterogeneity drive bacterial community diversity and composition in a polar desert. *Front. Microbiol.* 9. doi: 10.3389/fmicb.2018.01928

Fountain, A. G., Levy, J. S., Gooseff, M. N., and Van Horn, D. (2014). "The McMurdo Dry Valleys: A landscape on the threshold of change," in *Geomorphology*, vol. 225. (Elsevier B.V.), 25–35. doi: 10.1016/j.geomorph.2014.03.044

Fountain, A. G., Saba, G., Adams, B. J., Doran, P., Fraser, W., Gooseff, M., et al. (2016). The impact of a large-scale climate event on Antarctic ecosystem processes. *BioScience* 66, 848–863. doi: 10.1093/biosci/biw110

Franco, A. L. C., Adams, B. J., Diaz, M. A., Lemoine, N. P., Dragone, N. B., Fierer, N., et al. (2021). Response of Antarctic soil fauna to climate-driven changes since the Last Glacial Maximum. *Global Change Biol.* 28, 644–653. doi: 10.1111/gcb.15940

Freckman, D. W., and Virginia, R. A. (1993). Extraction of nematodes from Dry Valley Antarctic soils. *Polar Biol.* 13, 483–487. doi: 10.1007/BF00233139

Freckman, D. W., and Virginia, R. A. (1997). Low-diversity antarctic soil nematode communities : distribution and response to disturbance. *Ecology* 78, 363–369. doi: 10.1890/0012-9658(1997)078[0363:LDASNC]2.0.CO;2

Freckman, D. W., and Virginia, R. A. (1998). "Soil biodiversity and community structure in the McMurdo dry valleys, Antarctica," in *Ecosystem Dynamics in a Polar Desert: the Mcmurdo Dry Valleys, Antarctica*, vol. 72 . Ed. J. C. Priscu (American Geophysical Union), 323–335. doi: 10.1029/AR072

Galtier, N., Nabholz, B., Glemin, S., and Hurst, G. D. D. (2009). Mitochondrial DNA as a marker of molecular diversity: A reappraisal. *Mol. Ecol.* 18, 4541–4550. doi: 10.1111/j.1365-294X.2009.04380.x

Gooseff, M. N., McKnight, D. M., Doran, P., Fountain, A. G., and Lyons, W. B. (2011). Hydrological connectivity of the landscape of the McMurdo Dry Valleys, Antarctica. *Geogr. Compass* 5, 666–681. doi: 10.1111/j.1749-8198.2011.00445.x

Gooseff, M. N., Barrett, J. E., Adams, B. J., Doran, P. T., Fountain, A. G., Lyons, W. B., et al. (2017). "Decadal ecosystem response to an anomalous melt season in a polar desert in Antarctica," in *Nature Ecology and Evolution*, vol. 1. (Springer US), 1334–1338. doi: 10.1038/s41559-017-0253-0

Grant, W. S. (2015). Problems and cautions with sequence mismatch analysis and Bayesian skyline plots to infer historical demography. *J. Heredity* 106, 333–346. doi: 10.1093/jhered/esv020

Guimarães, K. L. A., Lima, M. P., Santana, D. J., Belém de Souza, M. F., Barbosa, R. S., Rodrigues, L. R. R., et al. (2022). "DNA barcoding and phylogeography of the *Hoplias malabaricus* species complex," in *Scientific Reports*, vol. 12. (Nature Publishing Group UK), 1–15. doi: 10.1038/s41598-022-09121-z

Halberstadt, A. R. W., Kowalewski, D. E., and DeConto, R. M. (2022). Reconciling persistent sub-zero temperatures in the McMurdo Dry Valleys, Antarctica, with Neogene dynamic marine ice-sheet fluctuations. *Geology* XX, 7–11. doi: 10.1130/g49664.1

Hall, B. L., Denton, G. H., and Hendy, C. H. (2000). Evidence from Taylor Valley for a grounded ice sheet in the Ross Sea, Antarctica. *Geografiska Annaler Ser. A: Phys. Geogr.* 82, 275–303. doi: 10.1111/j.0435-3676.2000.00126.x

Hall, B. L., Denton, G. H., and Overturf, B. (2001). Glacial Lake Wright, a high-level Antarctic lake during the LGM and early holocene. *Antarctic Sci.* 13, 53–60. doi: 10.1017/S0954102001000086

Harder, A. M., Halanych, K. M., and Mahon, A. R. (2016). Diversity and distribution within the sea spider genus Pallenopsis (Chelicerata: Pycnogonida) in the Western Antarctic as revealed by mitochondrial DNA. *Polar Biol. Springer Berlin Heidelberg* 39, 677–688. doi: 10.1007/s00300-015-1823-8

Hewitt, G. M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* 58, 247–276. doi: 10.1006/bijl.1996.0035

Hewitt, G. M. (2001). Speciation, hybrid zones and phylogeography - Or seeing genes in space and time. *Mol. Ecol.* 10, 537–549. doi: 10.1046/j.1365-294X.2001.01202.x

Hickerson, M. J., Carstens, B. C., Cavender-Bares, J., Crandall, K. A., and Graham, C. H. (2010). "Phylogeography's past, present, and future: 10 years after Avis," in *Molecular Phylogenetics and Evolution*, vol. 54. (Elsevier Inc.), 291–301. doi: 10.1016/j.ympev.2009.09.016

Hogg, I. D., Cary, S. C., Convey, P., Newsham, K. K., O'Donnell, A. G., Adams, B. J., et al. (2006). Biotic interactions in Antarctic terrestrial ecosystems: Are they a factor? *Soil Biol. Biochem.* 38, 3035–3040. doi: 10.1016/j.soilbio.2006.04.026

Hudson, A. R., Peters, D. P. C., Blair, J. M., Childers, D. L., Doran, P. T., Geil, K., et al. (2002). Cross-site comparisons of dryland ecosystem response to climate change in the US long-term ecological research network. *Bioscience* 72 (9), 889–907. doi: 10.1093/biosci/biab134

Jackson, A. C. (2022). *Effect of Climate History on the Genetic Structure of an Antarctic Soil Nematode* (Theses and Dissertations), 9784. Available online at: https://scholarsarchive.byu.edu/etd/9784. (Accessed Deceber 14, 2022).

Jackson, A. C., Jorna, J., Chaston, J. M., and Adams, B. J. (2022). Glacial legacies : microbial communities of Antarctic Refugia. *Biology* 11(10), 1440. doi: 10.3390/biology11101440

Jackson, A. C., Wall, D. H., and Adams, B. J. (2023). Mitochondrial COXI sequences and associated metazoan abundances in soils collected from the McMurdo Dry Valleys, Antarctica from 1995 to 2022. *Environ. Data Initiative*. doi: 10.6073/pasta/45337fb617c40312ea55ea97133a4c5e

Jones, R., Mackintosh, A., Norton, K., Golledge, N. R., Fogwill, C. J., Kubik, P. W., et al. (2015). "Rapid Holocene thinning of an East Antarctic outlet glacier driven by marine ice sheet instability," in *Nature Communications*, vol. 6. (Nature Publishing Group). doi: 10.1038/ncomms9910

Joy, K., Fink, D., Storey, B., De Pascale, G. P., Quigley, M., and Fujioka, T. (2017). "Cosmogenic evidence for limited local LGM glacial expansion, Denton Hills, Antarctica," in *Quaternary Science Reviews*, vol. 178. (Elsevier Ltd), 89–101. doi: 10.1016/j.quascirev.2017.11.002

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K., Von Haeseler, A., and Jermiin, L. S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589. doi: 10.1038/nmeth.4285

Keppel, G., Van Niel, K. P., Wardell-Johnson, G. W., Yates, C. J., Byrne, M., Mucina, L., et al. (2012). "Refugia: identifying and understanding safe havens for biodiversity under climate change," in *Global Ecology and Biogeography*, vol. 21. (John Wiley & Sons, Ltd), 393–404. doi: 10.1111/j.1466-8238.2011.00686.x

Khanal, L., Chalise, M. K., Wan, T., and Jiang, X. (2018). Riverine barrier effects on population genetic structure of the Hanuman langur (Semnopithecus entellus) in the Nepal Himalaya. *BMC Evolutionary Biol. BMC Evolutionary Biol.* 18, 1–16. doi: 10.1186/s12862-018-1280-4

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16, 111–120. doi: 10.1007/BF01731581

Knowles, L. L., and Maddison, W. P. (2002). Statistical phylogeography. *Mol. Ecol.* 2623–2635.

Lee, C. K., Laughlin, D. C., Bottos, E. M., Caruso, T., Joy, K., Barrett, J. E., et al. (2019). Biotic interactions are an unexpected yet critical control on the complexity of an abiotically driven polar ecosystem. *Commun. Biol.* 2, 1–10. doi: 10.1038/s42003-018-0274-5

Levy, J. S., Fountain, A. G., O'Connor, J. E., Welch, K. A., and Lyons, W. B (2013). How big are the McMurdo Dry Valleys? Estimating ice-free area using Landsat image data. *Antarctic Sci.* 25, 119–120. doi: 10.1017/S0954102012000727

Levy, J. S., et al. (2013). Garwood Valley, Antarctica: A new record of last glacial maximum to holocene glaciofluvial processes in the Mcmurdo Dry Valleys. *Bull. Geological Soc. America* 125, 1484–1502. doi: 10.1130/B30783.1

Lewis, A. R., Marchant, D. R., Kowalewski, D. E., Baldwin, S. L., and Webb, L. E. (2006). The age and origin of the Labyrinth, western Dry Valleys, Antarctica: Evidence for extensive middle Miocene subglacial floods and freshwater discharge to the Southern Ocean. *Geology* 34, 513–516. doi: 10.1130/G22145.1

Lobon, I., Tucci, S., de Manuel, M., Ghirotto, S., Benazzo, A., Prado-Martinez, J., et al. (2016). Demographic history of the genus Pan inferred from whole mitochondrial genome reconstructions. *Genome Biol. Evol.* 8, 2020–2030. doi: 10.1093/gbe/evw124

Magalhães, C., Stevens, M. I., Cary, S. C., Ball, B. A., Storey, B. C.error, et al. (2012). At limits of life: multidisciplinary insights reveal environmental constraints on biotic diversity in continental Antarctica. *PloS One* 7(9), e44578. doi: 10.1371/journal.pone.0044578

Malcolm, J. R., Markham, A., Neilson, R. P., and Garaci, M. (2002). Estimated migration rates under scenarios of global climate change. *J. Biogeography* 29, 835–849. doi: 10.1046/j.1365-2699.2002.00702.x

Margerison, H. R., Phillips, W. M., Stuart, F. M., and Sugden, D. E. (2005). Cosmogenic 3He concentrations in ancient flood deposits from the Coombs Hills, northern Dry Valleys, East Antarctica: Interpreting exposure ages and erosion rates. *Earth Planetary Sci. Lett.* 230, 163–175. doi: 10.1016/j.epsl.2004.11.007

Marshall, D. J., and Coetzee, L. (2000). Historical biogeography and ecology of a Continental Antarctic mite genus, Maudheimia (Acari, Oribatida): evidence for a Gondwanan origin and Pliocene-Pleistocene speciation. *Zoolog. J. Linnean Soc.* 129 (1), 111–128. doi: 10.1111/j.1096-3642.2000.tb00011.x

Mazerolle, M. J. (2020).AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). Available online at: http://www.ran.r-project.org/package=AICcmodavg. (Accessed September 1, 2022).

McGaughran, A., Stevens, M. I., Hogg, I. D., and Carapelli, A. (2011). Extreme glacial legacies: A synthesis of the Antarctic springtail phylogeographic record. *Insects* 2, 62–82. doi: 10.3390/insects2020062

McGaughran, A., Terauds, A., Convey, P., and Fraser, C. I. (2019). Genome-wide SNP data reveal improved evidence for Antarctic glacial refugia and dispersal of terrestrial invertebrates. *Mol. Ecol.* 28, 4941–4957. doi: 10.1111/mec.15269

McGaughran, A., Morgan, K., and Sommer, R. J. (2014). Environmental variables explain genetic structure in a beetle-associated nematode. *PloS One* 9(1), e87317. doi: 10.1371/journal.pone.0087317

McGowan, H. A., Neil, D. T., and Speirs, J. C. (2014). A reinterpretation of geomorphological evidence for Glacial Lake Victoria, McMurdo Dry Valleys, Antarctica. *Geomorphology* 208, 200–206. doi: 10.1016/j.geomorph.2013.12.005

Minh, B. Q., et al. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* 37, 1530–1534. doi: 10.1093/molbev/msaa015

Moran, E. V., and Ormond, R. A. (2015). Simulating the interacting effects of intraspecific variation, disturbance, and competition on climate-driven range shifts in trees. *PloS One* 10, 1–21. doi: 10.1371/journal.pone.0142369

Nadler, S. A., De Ley, P., Mundo-Ocampo, M., Smythe, A. B., Stock, S. P., Bumbarger, D., et al. (2006). Phylogeny of Cephalobina (Nematoda): Molecular evidence for recurrent evolution of probolae and incongruence with traditional classifications. *Mol. Phylogenet. Evol.* 40, 696–711. doi: 10.1016/j.ympev.2006.04.005

Nei, M. (1972). Genetic distance between populations. *Am. Nat.* 106, 283–292. doi: 10.1086/282771

Nkem, J. N., Virginia, R. A., Barrett, J. E., Wall, D. H., and Li, G. (2006a). Salt tolerance and survival thresholds for two species of Antarctic soil nematodes. *Polar Biol.* 29, 643–651. doi: 10.1007/s00300-005-0101-6

Nkem, J. N., Wall, D. H., Virginia, R. A., Barrett, J. E., Broos, E. J., and Porazinska, D. L. (2006b). Wind dispersal of soil invertebrates in the McMurdo Dry Valleys, Antarctica. *Polar Biol.* 29, 346–352. doi: 10.1007/s00300-005-0061-x

Nolan, L., Hogg, I. D., Stevens, M. I., and Haase, M. (2006). Fine scale distribution of mtDNA haplotypes for the springtail Gomphiocephalus hodgsoni (Collembola) corresponds to an ancient shoreline in Taylor Valley, continental Antarctica. *Polar Biol.* 29, 813–819. doi: 10.1007/s00300-006-0119-4

Normand, S., Ricklefs, R. E., Skov, F., Bladt, J., Tackenberg, O., and Svenning, J. C. (2011). Postglacial migration supplements climate in determining plant species ranges in Europe. *Proc. R. Soc. B: Biol. Sci.* 278, 3644–3653. doi: 10.1098/rspb.2010.2769

Opdam, P., and Wascher, D. (2004). Climate change meets habitat fragmentation: Linking landscape and biogeographical scale levels in research and conservation. *Biol. Conserv.* 117, 285–297. doi: 10.1016/j.biocon.2003.12.008

Overhoff, A., Freckman, D. W., and Virginia, R. A. (1993). Life cycle of the microbivorous Antarctic Dry Valley nematode *Scottnema lindsayae* (Timm 1971). *Polar Biol.* 13, 151–156. doi: 10.1007/BF00238924

Paradis, E. (2010). pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics* 26, 419–420. doi: 10.1093/bioinformatics/btp696

Poage, M. A., Barrett, J. E., Virginia, R. A., and Wall, D. H. (2008). The influence of soil geochemistry on nematode distribution, Mcmurdo Dry Valleys, Antarctica. *Arctic Antarctic Alpine Res.* 40, 119–128. doi: 10.1657/1523-0430(06-051)[POAGE]2.0.CO;2

Porazinska, D. L., Wall, D. H., and Virginia, R. A. (2002). Population age structure of nematodes in the Antarctic dry valleys: perspectives on time, space, and habitat suitability. *Arctic Antarctic Alpine Res.* 34, 159–168. doi: 10.1080/15230430.2002.12003480

Powers, T. O., and Harris, T. S. (1993). A polymerase chain reaction method for identification of five major *Meloidogyne* species. *J. Nematol.* 25, 1–6.

R Core Team (2002). *R: A language and environment for statistical computing* (Vienna, Austria: R Foundation for Statistical Computing). Available online at: https://www.r-project.org/. (Accessed September 1, 2021).

Riddle, B. R. (1996). The molecular phylogeographic bridge between deep and shallow history in continental biotas. *Trends Ecol. Evol.* 11, 207–211. doi: 10.1016/0169-5347(96)10032-X

Rogers, A. D. (2007). Evolution and biodiversity of Antarctic organisms: A molecular perspective. *Philos. Trans. R. Soc. B: Biol. Sci.* 362, 2191–2214. doi: 10.1098/rstb.2006.1948

Schäfer, J. M., Ivy-Ochs, S., Wieler, R., Leya, I., Baur, H., Denton, G. H., et al. (1999). Cosmogenic noble studies in the oldest landscape on earth: Surface exposure ages of the Dry Valleys, Antarctica. *Earth Planetary Sci. Lett.* 167, 215–226. doi: 10.1016/S0012-821X(99)00029-1

Schäfer, J. M., Baur, H., Denton, G. H., Ivy-Ochs, S., Marchant, D. R., Schlüchter, C., et al. (2000). The oldest ice on Earth in Beacon Valley, Antarctica: New evidence from surface exposure dating. *Earth Planetary Sci. Lett.* 179, 91–99. doi: 10.1016/S0012-821X(00)00095-9

Schierenbeck, K. A. (2017). Population-level genetic variation and climate change in a biodiversity hotspot. *Ann. Bot.* 119, 215–228. doi: 10.1093/aob/mcw214

Schulte, N. O., Khan, A. L., Smith, E. W., Zoumplis, A., Kaul, D., Allen, A. E., et al. (2022). Blowin' in the wind: Dispersal, structure, and metacommunity dynamics of aeolian diatoms in the McMurdo Sound region, Antarctica. *J. Phycology* 58, 36–54. doi: 10.1111/jpy.13223

Shafer, A. B., Cullingham, C. I., Côté, S. D., and Coltman, D. W. (2010). Of glaciers and refugia: A decade of study sheds new light on the phylogeography of northwestern North America. *Mol. Ecol.* 19, 4589–4621. doi: 10.1111/j.1365-294X.2010.04828.x

Smythe, A. B., Holovachov, O., and Kocot, K. M. (2019). Improved phylogenomic sampling of free-living nematodes enhances resolution of higher-level nematode phylogeny. *BMC Evolutionary Biol. Cambridge Univ. Press* 19, 1–15. doi: 10.1186/s12862-019-1444-x

Soler-Membrives, A., Linse, K., Miller, K. J., and Arango, C. P. (2017). Genetic signature of last glacial maximum regional refugia in a circum-antarctic sea spider. *R. Soc. Open Sci.* 4, 1–16. doi: 10.1098/rsos.170615

Spector, P., and Balco, G. (2021). Exposure-age data from across Antarctica reveal mid-Miocene establishment of polar desert climate. *Geology* 49, 91–95. doi: 10.1130/g47783.1

Stevens, M. I., and Hogg, I. D. (2003). Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail Gomphiocephalus hodgsoni from Victoria Land, Antarctica. *Mol. Ecol.* 12, 2357–2369. doi: 10.1046/j.1365-294X.2003.01907.x

Stevens, M. I., and Hogg, I. D. (2006). Contrasting levels of mitochondrial DNA variability between mites (Penthalodidae) and springtails (Hypogastruridae) from the Trans-Antarctic Mountains suggest long-term effects of glaciation and life history on substitution rates, and speciation processes. *Soil Biol. Biochem.* 38, 3171–3180. doi: 10.1016/j.soilbio.2006.01.009

Stuiver, M., Yang, I. C., Denton, G. H., and Kellogg, T. B. (1981). "Oxygen isotope ratios of antarctic glacier permafrost and in glacie ice," in *Antarctic Research Series: Dry Valley Drilling Project*, 131–139.

Sugden, D., and Denton, G. (2004). Cenozoic landscape evolution of the Convoy Range to Mackay Glacier area, Transantartic Mountains: Onshore to offshore synthesis. *Bull. Geological Soc. America* 116, 840–857. doi: 10.1130/B25356.1

Sugden, D. E., Marchant, D. R., and Denton, G. H. (1993). The case for a stable east Antarctic ice sheet : the background. *Geografiska Annaler. Ser. A Phys. Geogr.* 75, 151–154. doi: 10.1080/04353676.1993.11880392

Timm, R. W. (1971). Antarctic soil and freshwater nematodes from the McMurdo Sound Region. *Proc. Helminth Soc. Washington* 38, 42–52.

Toparslan, E., Karabag, K., and Bilge, U. (2020). A workflow with R: Phylogenetic analyses and visualizations using mitochondrial cytochrome b gene sequences. *PloS One* 15, 1–17. doi: 10.1371/journal.pone.0243927

Treonis, A. M., Wall, D. H., and Virginia, R. A. (1999). Invertebrate biodiversity in Antarctic Dry Valley soils and sediments. *Ecosystems* 2, 482–492. doi: 10.1007/s100219900096

Van Der Valk, T., Sandoval-Castellanos, E., Caillaud, D., Ngobobo, U., Binyinyi, E., Nishuli, R., et al. (2018). Significant loss of mitochondrial diversity within the last century due to extinction of peripheral populations in eastern gorillas. *Sci. Rep.* 8, 1–11. doi: 10.1038/s41598-018-24497-7

Van Horn, D. J., Van Horn, M. L., Barrett, J. E., Gooseff, M. N., Altrichter, A. E., Geyer, K. M., et al. (2013). Factors controlling soil microbial biomass and bacterial diversity and community composition in a cold desert ecosystem: role of geographic scale. *PloS One* 8, e66103. doi: 10.1371/journal.pone.0066103

Velasco-Castrillón, A., and Stevens, M. I. (2014). Morphological and molecular diversity at a regional scale: A step closer to understanding Antarctic nematode biogeography. *Soil Biol. Biochem.* 70, 272–284. doi: 10.1016/j.soilbio.2013.12.016

Virginia, R. A., and Wall, D. H. (1999). How soils structure communities in the Antarctic Dry Valleys. *BioScience* 49, 973–983. doi: 10.1525/bisi.1999.49.12.973

Willis, K. J., Bailey, R. M., Bhagwat, S. A., and Birks, H. J. B. (2010). Biodiversity baselines, thresholds and resilience: Testing predictions and assumptions using palaeoecological data. *Trends Ecol. Evol.* 25, 583–591. doi: 10.1016/j.tree.2010.07.006

Wlostowski, A. N., Gooseff, M. N., and Adams, B. J. (2018). Soil moisture controls the thermal habitat of active layer soils in the McMurdo dry valleys, Antarctica. *J. Geophysical Research: Biogeosciences* 123, 46–59. doi: 10.1002/2017JG004018

Xu, S., Schaack, S., Seyfert, A., Choi, E., Lynch, M., Cristescu, M. E., et al. (2012). High mutation rates in the mitochondrial genomes of Daphnia pulex. *Mol. Biol. Evol.* 29, 763–769. doi: 10.1093/molbev/msr243

Zawierucha, K., Marshall, C. J., Wharton, D., and Janko, K. (2019). A nematode in the mist: *Scottnema lindsayae* is the only soil metazoan in remote antarctic deserts, at greater densities with altitude. *Polar Res.* 38, 1–12. doi: 10.33265/polar.v38.3494

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Modelling snow algal habitat suitability and ecology under extreme weather events on the Antarctic Peninsula

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Snow algae form extensive blooms within Antarctica's coastal snowpacks and are a crucial contributor to its scarce terrestrial ecosystems. There is limited knowledge about the factors that contribute to snow algal bloom occurrence, distribution, ecological niche thresholds, or the prevalence of suitable conditions for bloom formation. To address these knowledge gaps and gain a clearer understanding of the current and potential future distribution of blooms, a habitat suitability model, using a Bayesian additive regression tree approach, was established. The model incorporated remotely sensed observations of blooms, physical environmental predictor variables, and snow melt modelling based on different climate scenarios. This was used to describe the ecological niche of snow algae and predict its occurrence at a landscape scale across the Antarctic Peninsula. The findings revealed that most habitable snow was predicted north of latitude 66° S, with patch density, area, and habitable elevation decreasing poleward. Factors that strongly influenced bloom presence were days of snow melt and aspect, with blooms of red-colored algae being associated with longer seasons and north-facing slopes. The model outputs also suggested heterogeneous preferences for environmental conditions amongst red and green snow algae blooms, suggesting a diversity of ecological niches for bloom-forming algae. Long-term climate-change impacts were difficult to discern as extreme summer temperatures and melt during the timeframe of this study in 2021 exceeded the projected 2100 temperatures for parts of the Antarctic Peninsula. However, warmer conditions produced a greater area of potentially habitable snow at higher elevation and latitude. Conversely, small and low-lying islands were predicted to lose habitable snow under a warming scenario. Model and training imagery both indicated that algal blooms are forming on snow-covered icecaps in the South Shetland Islands, suggesting greater potential for glacier-based algal blooms in the future, should recent trends for extreme summer temperatures persist.

KEYWORDS

snow algae, remote sensing, species distribution model, extreme weather events, Antarctica, climate change

1 Introduction

The Antarctic Peninsula is a crucial indicator region for global climate change, having experienced exceptionally rapid warming in recent decades (González-Herrero et al., 2024; Clem et al., 2022). Increasing temperatures are changing the Peninsula's landscape, with significant consequences for its ecosystems, generating greater snow and ice melt, along with increased occurrence of extreme weather events (Siegert et al., 2023), increased precipitation and new ice-free areas becoming available (Lee et al., 2017; Robinson, 2022). Antarctic snow and ice habitats have been particularly affected by climate change as warming temperatures have driven accelerated melting, as well as changes to seasonal melt dynamics, snow structure, chemistry, and precipitation patterns (Colesie et al., 2023).

Terrestrial snow and ice habitats in Antarctica, particularly in coastal regions, can be host to a variety of microorganisms such as bacteria, fungi and algae, as well as meiofaunal and invertebrate species (Pugh and Convey, 2008; Davey et al., 2019; de Menezes et al., 2019; Chown and Convey 2016). The algal component of the snow ecosystem is useful as a sentinel system for climate change, as thawing conditions within the snow can cause blooms to form in high densities and over large areas (Figure 1). In this state, the snow appears green or red and can be detected and monitored using satellite imagery (Hashim et al., 2016; Huovinen et al., 2018; Gray et al., 2020, 2021). Predictive models have forecast increasing loss and retreat of ice over the next century, with up to a 300% increase (depending on warming scenario) in ice-free territories across the Peninsula region from 2014 to 2100 (Lee et al., 2017). At the same time the zero-degree isotherm, north of which snow melt occurs, is shifting southwards (González-Herrero et al., 2024), potentially opening up new areas for bloom formation. Warmer temperatures and loss of ice and summer snowpacks will change the timing of availability and distribution of snow habitats along the Peninsula and could result in the disappearance of these habitats in some areas. Terrestrial snow and ice algae play important ecological roles in their environment, ranging from contributing to primary production and wider biogeochemical cycles, to influencing the albedo and physical structure of the snow or ice surface (Hodson et al., 2008; Gray et al., 2020; Khan et al., 2021). The consequences of major changes or loss of such habitat could, therefore, be ecologically significant for the region.

Although snow algae occur in polar and alpine areas globally (Benning et al., 2014; Hoham and Remias, 2020), Antarctic snow algae display several unique features, including regular bloom occurrence in coastal, nutrient rich snowpacks, and a higher proportion of endemic species compared to elsewhere in the world (Vyverman et al., 2010). Their contribution to ecological function in Antarctic and sub-Antarctic areas is also highly significant given the extent of the cryospheric habitat, and the limited presence of other photosynthetic life in the region. Despite their significance in Antarctica, our understanding of the processes and factors governing snow algal bloom occurrence remain limited due to the paucity of and challenges in obtaining regular *in situ* or remote sensed datasets. Snow melt duration (Roussel et al., 2024), light (Lavoie et al., 2005) and nutrient availability (Lavoie et al., 2005; Gray et al., 2020; Phillips-Lander et al., 2020; Roussel et al., 2024) have all been identified as important elements for production and bloom formation, though we lack the explicit link between Antarctic blooms and environmental conditions to assess what role these factors play, or to understand how prevalent bloom forming conditions are in Antarctica.

Previous distribution maps of snow algal blooms using remote sensing methods (Walshaw et al., 2024; Gray et al., 2020; Hashim et al., 2016; Huovinen et al., 2018) show the presence of green snow algae (GSA) across large parts of the coastal western Antarctic Peninsula region. However, satellite detection has limitations, as freely available imagery is generally too spatially coarse to detect small blooms, and too spectrally coarse to detect red snow algae (RSA) over very large areas without confounding false positives. For example, strong signals arising from iron-rich mineral debris within snow, when using band ratios to detect RSA (Chen et al., 2023). Higher resolution satellites, such as the WorldView platforms, are valuable assets for studying Antarctic ecology (e.g., Gray et al., 2021; Jawak et al., 2019; Sun et al., 2021). Their higher spectral resolution (relative to Sentinel 2 and Landsat) enables the detection of RSA alongside GSA (Gray et al., 2021), but the images are costly, and snapshots of a specific point in time meaning that it is difficult to view detailed changes in the dynamic snow habitat. On a physical level, all optical remote sensing methods are hampered by frequent cloud cover over the Antarctic Peninsula, limiting the number of available snapshots.

Habitat suitability modelling can complement remote sensing studies, addressing limitations in temporal resolution, spatial coverage, and data availability. Such models can explore how different environmental predictor variables influence occurrence, predict ecological preferences and distributions over a landscape scale, and forecast responses to climatic variability. For RSA, for example, Onuma et al. (2022) developed a model to estimate occurrence and growth dynamics on a global scale. However, the model differed mechanistically from a habitat suitability model, and related to the ecology of red blooms common in Arctic and alpine areas rather than the unique coastal green and red blooms found in Antarctica. Chen et al. (2023) explored seasonal RSA bloom development over King George Island with respect to climatic conditions but included significant areas of mineral debris within snow in their analysis, due to difficulties isolating RSA in Sentinel 2 imagery. An alternative approach to exploring the relationship between environmental factors and snow algae occurrence in Antarctica is to use models such as Bayesian additive regression trees (BART). BART provides a flexible and robust framework for ecological analysis due to its ability to handle nonlinear relationships, incorporate prior knowledge, and quantify uncertainty (Carlson, 2020; Chipman et al., 2010). In addition, due to the workflow wrapper developed by Carlson (2020), BART models have been gaining traction as a tool for modelling species distributions, including those of trees (Ahmadi et al., 2023) and marine invasive species (Poursanidis et al., 2022).

Our aim in this study was to understand the key factors contributing to the distribution of snow habitats capable of supporting blooms of snow algae throughout the Antarctic Peninsula region. To achieve this, we combined high-resolution satellite observations of snow algal blooms into a BART-based habitat suitability model. This model was then used to explore how environmental variables influenced potential bloom formation as well as distribution shifts (losses and gains) associated with inter-annual variability caused by contemporary extreme weather events as well as long-term predicted trends in climate. To test the factors affecting distribution, variables were chosen to represent growth requirements such as liquid water availability (cumulative snow melt, days of melt), potential nutrient availability (distance to the coast, distance from an animal colony) as well as physical attributes (surface type, slope, aspect).

2 Methods

To construct our model, we derived presence and absence observations of GSA and RSA blooms using satellite imagery from six different locations (Robert Island, Nelson Island (for validation), Trinity Island, Melchior Island, Neumayer Channel, and Ryder Bay) across the Antarctic Peninsula (Figure 1A) and six summer growth seasons (2013, 2017, 2019, 2020, 2021 and 2023). These were used to train a BART-based species distribution model using seven predictor variables based upon abiotic and bioclimatic factors, as described below and in the workflow schematic (Figure 2). Posterior distributions, showing the modelled probability of occurrence, were then mapped across the Antarctic Peninsula using actual climate conditions for 2021 and projected climatic conditions for 2100.

2.1 Remote sensing presence/absence training data

WorldView imagery (Maxar Technologies) was used to detect snow algae for training the model. It has a high spatial resolution (1.84 m (WorldView 2); 1.24 m (WorldView 3)) and has previously been used to identify GSA and RSA blooms in Antarctica (Gray et al., 2021). Table 1 shows the dates and locations of the scenes used in this study. Each image was an 8-band multispectral scene from WorldView 2 or 3 and was corrected to surface reflectance using the Py6s-derived Atmospheric and Radiometric Correction of Satellite Imagery (ARCSI v4.0.0) (Bunting and Clewley, 2022) software. 6S parameters were set as in Gray et al. (2021).

Snow algae were detected within the images following the methodology of Gray et al. (2021). First, band thresholds were used to isolate snow and ice from ocean, rock, and vegetation. Algae were identified within snow- or ice-containing pixels utilizing the characteristic absorption of light by chlorophyll-*a* pigments within the algae, which occurs at around 680 nm, or Band 5 of the WorldView's multispectral sensor. Instead of using a spectral angle mapper (SAM) approach (Gray et al., 2021) to distinguish GSA from RSA, we adapted the red-green normalized difference index (RGND) approach of Engstrom et al. (2022) for the WorldView sensor i.e., $RGND = \frac{Band\ 5 - Band\ 3}{Band\ 5 + Band\ 3}$, to differentiate bloom color. This approach simplified classification and does not require *in situ* reference spectra. Clean and mineral-laden snow, for use as absence data in the model, were also identified within each image, and were defined as snow that did not contain a detectable chlorophyll signal. Remote sensed outputs underwent validation against *in situ* observations of 361 snow and ice algae blooms made between 30th January 2023 and 13th February 2023 on Robert Island, South Shetland Islands (locations shown in Figure 3A). A confusion matrix and Cohen's Kappa coefficient were used to assess classification accuracy against bloom location, recorded by handheld GPS at the bloom center.

Pixels for each class, GSA, RSA and snow with no visible algal presence, were randomly chosen from each image to use as training data within the model. For each image, about 1% of the classified pixels were used for training, with visual inspection removing obviously misclassified pixels. The final per-image pixel counts are shown in Table 1. In total, 1777 pixels containing GSA, 1273 pixels containing RSA, and 1226 containing clean snow were used to train the BART model.

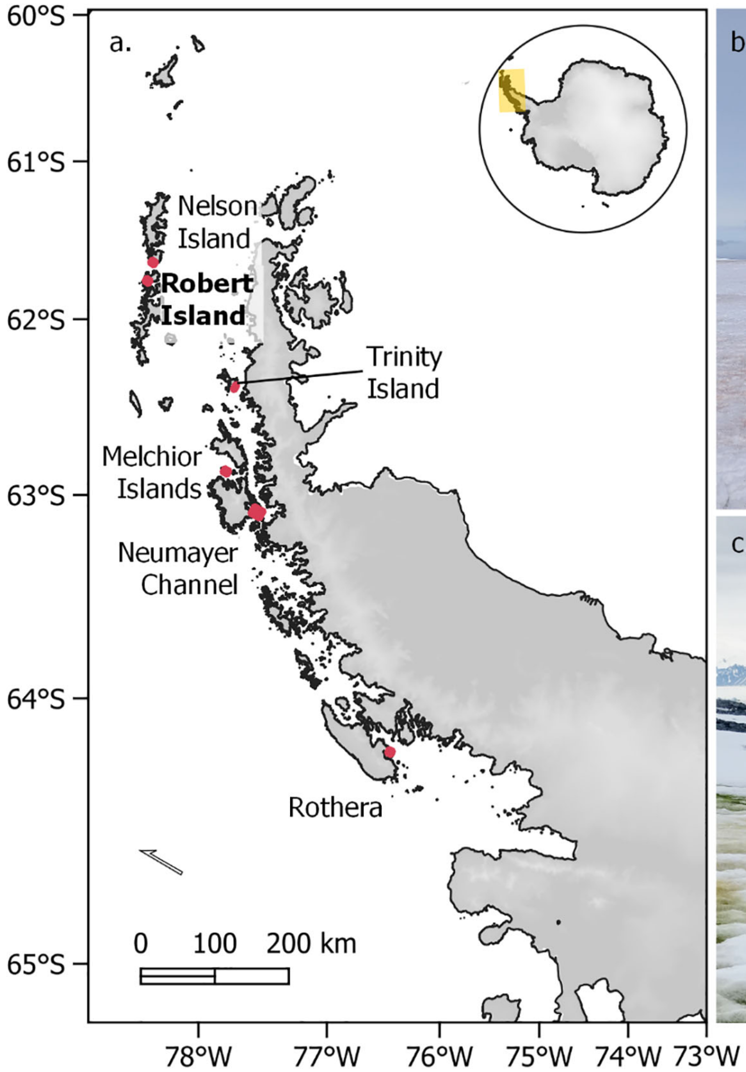

FIGURE 1

Study locations and bloom sites in the western Antarctic Peninsula region. **(A)** WorldView 2 and 3 image sites are shown in red, Robert Island ground validation site is highlighted in bold. Coordinates of these images are given in Table 1. **(B)** Example of a red snow algal bloom on Robert Island. **(C)** Example of a coastal green snow algal bloom at Anchorage Island, Ryder Bay. Photo credits **(B)** A. Gray, **(C)** M. Davey.

2.2 Assembly of predictor variables

Seven variables (summarized within Table 2) were selected to describe habitable snow conditions using the BART model. Principal component analysis (PCA) (RStudio (version 2022.02.3)) was used to ensure that predictor variables lacked collinearity across the training dataset (Supplementary Figure S1). The BART model was trained using 8 m resolution data at the locations shown in Figure 1, to more closely match the resolution of the remote sensed presence/absence data, and to maximize interpretation of microtopographic niches. Posterior predictive maps were produced at 100 m resolution for the Antarctic Peninsula, rather than 8 m resolution, due to the significant computational requirement. All predictors of snow algal blooms were processed as raster datasets and were derived as described below.

2.2.1 Physical-based variables

The 8 m Elevation Model of Antarctica (REMA) (Howat et al., 2019) and 100 m gapless REMA (Dong et al., 2022) were used to derive slope (degrees) and aspect (degrees azimuth) variables using the *gdaldem* tool (GDAL version 3.4.3). Distance to the coast (in meters) was calculated from the high-resolution coastline polygon (Gerrish et al., 2020) included in the *Quantarctica* dataset (Matsuoka et al., 2018). Distance to animal colonies (in meters) was calculated using combined data from the Mapping Application for Penguin Populations and Projected Dynamics (MAPPPD) database (Humphries et al., 2017), Important Bird Areas (Harris et al., 2015; Matsuoka et al., 2018), Emperor penguin colonies (Fretwell and Trathan, 2021; Matsuoka et al., 2018), petrel breeding sites (Schwaller et al., 2018) and Weddell seal population data

TABLE 1 The date, WorldView (WV) sensor, location and remote-sensed area estimates of red snow algae (RSA) and green snow algae (GSA) within the images used for training and evaluating the BART model.

Date	Sensor	Image location	GSA area (km^2)	RSA area (km^2)	Training pixels (n)		
					GSA	RSA	Absence
2013-03-13	WV2	Robert Island (62.4°S, 59.7°W)	0.09	0.07	0	114	358
2019-03-16	WV2	Robert Island (62.4°S, 59.7°W)	0.02	0.11	200	214	334
2023-02-06	WV2	Robert Island (62.4°S, 59.7°W)	0.03	0.95	207	213	324
2021-02-03	WV3	Nelson Island* (62.3°S, 59.2°W)	0.04	1.03	333	333	333
2020-03-07	WV2	Trinity Island (63.9°S, 60.8°W)	0.16	0.69	30	208	384
2020-02-09	WV3	Melchior Islands (64.3°S, 62.9°W)	0.03	0.16	61	105	378
2021-01-22	WV2	Neumayer Channel (64.8°S, 63.6°W)	0.01	0.02	184	206	634
2017-12-28	WV2	Ryder Bay (67.6°S, 68.2°W)	0.01	0.001	34	4	31
2020-02-16	WV3	Ryder Bay (67.6°S, 68.2°W)	0.22	0.06	61	180	40
		Totals			743†	1273†	2483†

Also shown are the numbers of pixels used from each image for training and validation. Rows in descending latitude order.

*Nelson Island data were for validation only.

†Not including validation data.

(LaRue et al., 2021). The surface type was a binary input, relating to whether a bloom occurred on permanent snow/ice or a seasonally snow-covered rocky outcrop or soil. For the 2021 model run, the rock outcrop map derived from Landsat 8 data was used to create an ice/rock vector layer (Burton-Johnson et al., 2016). For the 2100 run, modelled ice-free areas from Lee et al. (2017) under RCP 8.5 climate predictions (Lopez, 2016) were used.

2.2.2 Snow melt model variables – climate data and *GlacierSMBM* model

In trial model runs (data not shown), unprocessed air temperature or solar radiation variables were used, but were too spatially coarse to have predictive power in the complex mountainous terrain of the Antarctic Peninsula. We therefore include snow melt parameters in our model, using downsampled, high resolution climate data, as snow melt directly influences liquid water availability and habitat stability for snow algae. Simplified snow melt modelling was undertaken using the *GlacierSMBM* R package (Groos et al., 2017). This uses daily temperature (°C) and net radiation (W m^{-2}) data as factors within a degree day model to estimate the daily melt of snow and/or ice on a per-pixel basis. Note that we were unable to predict the melt-out and disappearance of snow patches, as initial snow depth and accumulation data were not available for the region at high enough resolution. Instead, we aimed to improve the predictive value of the climate variables within the BART model by enhancing their resolution and interpreting their effects upon physical processes within the snowpack.

Daily mean 2 m air temperature and net solar radiation data were generated from the fifth generation ECMWF atmospheric reanalysis of the global climate (ERA5) hourly dataset in 0.5 x 0.25 degrees resolution (Hersbach et al., 2020) for 2013, 2017, 2019, 2020, 2021 and 2023, to correspond to the WorldView image dates. For the 2100 analysis, 2 m air temperature data from the CMIP5 daily data on single levels (Lopez, 2016) were used (RCP 8.5 warming scenario; HadGEM2-CC UK Met Office model, 1.875 x 1.25 degrees resolution).

Over the area used for training, temperature data were downsampled from their native resolution using the SAGA Lapse Rate Based Temperature Downscaling tool (SAGA v8.5.1). The 8 m Elevation Model of Antarctica (REMA) (Howat et al., 2019) was used as an elevation layer for down-sampling in the training datasets, whereas the 100 m REMA dataset was used for the Peninsula-scale posterior analysis. Lapse rates were either set as 0.68 °C per 100 m (Martin and Peel, 1978) or, in the case of Robert Island and Nelson Island images (see Figure 1A for locations), set as 0.8 °C per 100 m, calculated using Hobo MX2202 loggers installed across Coppermine Peninsula on Robert Island, from 2 m elevation to 130 m elevation, during the 2023 austral summer season (see Figure 3A for locations).

Solar radiation was used over global radiation to simplify downsampling, as shortwave radiation is more affected by topographic shading effects (Robledano et al., 2022). Net solar radiation data were downsampled using a sky view factor, generated using the 8 m or 100 m elevation models in SAGA. The sky view factor is a measure of terrain shading and was used to improve the model's ability to simulate melt within topographic microclimates such as nival hollows and meltwater channels. Net solar radiation from 2021 was used in both 2021 and 2100 melt model runs, due to high uncertainty in future cloud cover and albedo conditions.

The Glacier SMBM melt model was run in RStudio on a daily interval at 8 m resolution from the 1st November (preceding the image date) up until the time of the WorldView image used for training. In the case of modelling melt across the Antarctic Peninsula for posterior analysis, this point was set to 21st January 2021 or 2100, which reflects austral mid-summer, and the model

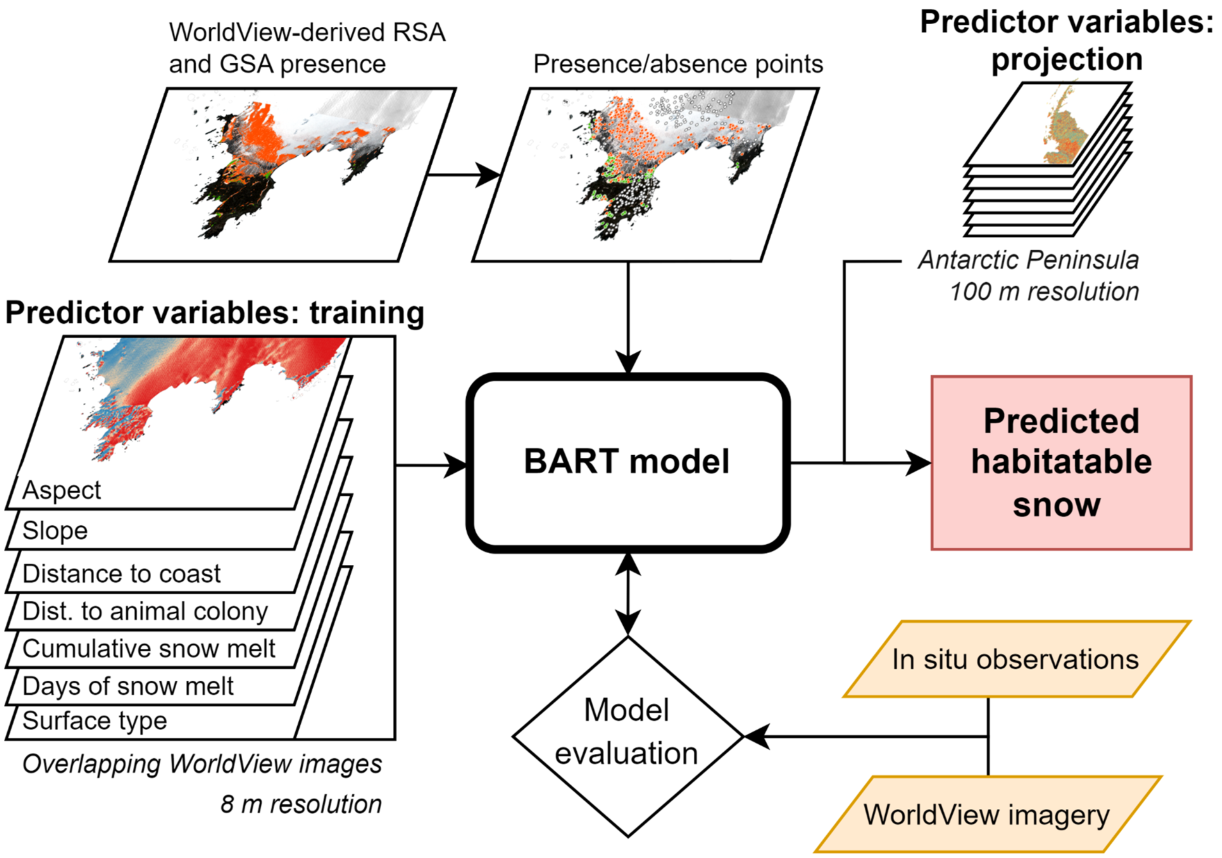

FIGURE 2
Workflow diagram of the construction of a BART model to produce current and future snow algae bloom probability distribution maps. Training variables overlap the spatial extent and temporal range of each WorldView image used to derive presence/absence data. Predictor variables, used to map habitable snow with the model, cover the Antarctic Peninsula. Model runs (inference) over the Antarctic Peninsula were performed using 2021 and 2100 data.

was run at 100 m resolution. The model outputs daily snow melt in meters, which was aggregated into a sum of melt for the "cumulative snow melt" predictor. The number of days where melt occurred in the snowpack on a per-pixel basis was aggregated into the "days of snow melt" predictor.

2.3 BART model processing

To build the training dataset, WorldView training pixels were converted into point data and used to sample each predictor variable raster layer. Cumulative snow melt and days of snow melt variables were specific to each image. For Robert Island and Ryder Bay imagery, this meant being mapped over the same location but using climate data corresponding to the date of each image. Sampled points were combined and exported as a table for input into our model. BART was run in RStudio using the *embarcadero* package (Carlson, 2020), a workflow wrapper for using BART for species distribution modelling. BART models were run using 200 trees and 1000 Markov Chain Monte Carlo (MCMC) iterations. Posterior model projections were mapped at 100 m resolution for the Antarctic Peninsula, using 2021 and 2100 temperature data as the basis of the snow melt variables in the raster stack used for prediction. The true skill statistics (TSS) and the area under the receiver operating characteristic curve (AUC) were used to assess model performance (Allouche et al., 2006). 95% credible intervals, i.e. the range over which a value has 95% probability were also produced using embarcadero and used to assess predictor and model performance. Binary maps of habitable snow were created using a threshold value based upon the maximum true skill statistic values (see Supplementary Figure S2). These values were 0.29 probability for GSA and 0.39 probability for RSA. Partial response curves for each predictor variable were constructed by running the model for a single variable whilst holding the other variables at their mean values.

We evaluated the model using three separate datasets. First, we ran model inference on a raster stack of training variables for Robert Island to produce a map of modelled habitable area that coincided with fieldwork conducted in 2023, then we compared this to the *in situ* sample locations for GSA and RSA taken between January and February 2023 (see Figures 3A, C, E). Secondly, we used remote-sensed locations of red and green blooms from within a WorldView image that wasn't used for training the model (Harmony Point, Nelson Island; see Table 1 and Figure 3 for details). Here, a

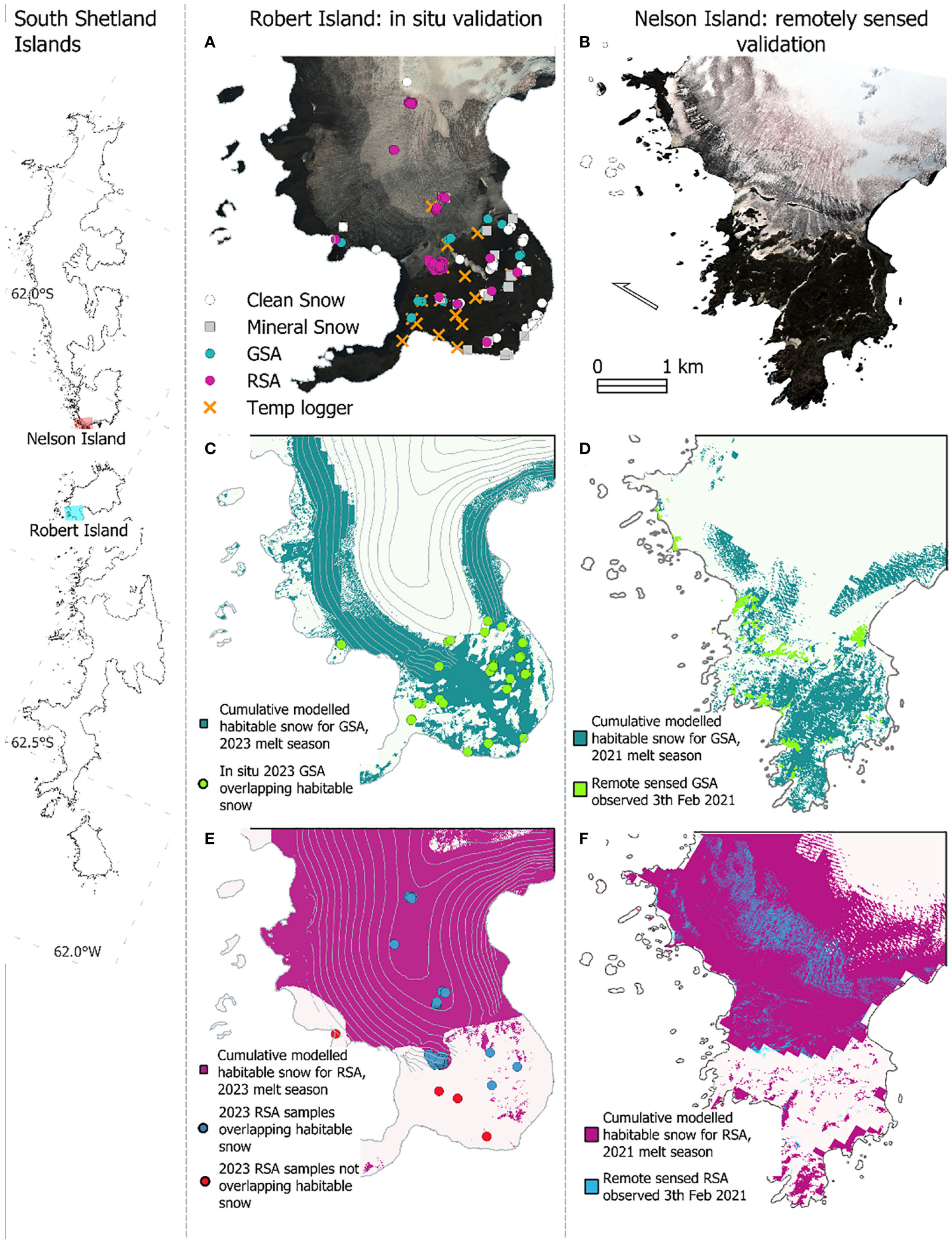

FIGURE 3
Model evaluation. **(A)** True color WorldView image of the Coppermine Peninsula region of Robert Island from 6th February 2023. Circles show the GPS sampling locations of in situ blooms, January and February 2023. **(B)** True color WorldView image of the Harmony Point region of Nelson Island from 3rd February 2021. **(C, E)** show cumulative modelled habitable snow for green snow algae (GSA) and red snow algae (RSA) on Robert Island. Points show the location of in situ observations of GSA **(C)** or RSA **(E)**, color coded based on agreement with the BART model. **(D, F)** show observed GSA and RSA on 3rd February 2021 as well as cumulative modelled habitable snow between 1st November 2020 and 3rd February 2021. Remote sensed blooms are shown by overlain GSA (bright green pixels) and RSA (blue pixels) to allow comparison of modelled and observed blooms. Inset shows the South Shetland Islands, with the extent of a, c, and e shown in blue and b, d and f shown in red.

TABLE 2 Model components, data source, processing summary, units, and rationale for inclusion within the modelling framework.

Variable	Data source	Processing	Units	Rationale for inclusion
WorldView imagery	Maxar Technologies. Imagery from 2013, 2017, 2019, 2020, 2021 and 2023.	Atmospheric correction, classification of GSA and RSA, extraction of training points	Binary presence/absence points	High resolution red and green algal bloom observations.
Aspect	8 m or 100 m Reference Elevation Model of Antarctica (REMA)	gdaldem	Degrees	Sunlight availability, controls on melt rate and melt onset
Slope	8 m or 100 m REMA	gdaldem	Degrees	Surface stability, liquid water availability, light availability.
Cumulative snow melt	ECMWF, ERA5 hourly dataset (2m temperature, solar radiation); CMIP5 daily data RCP 8.5 projection (2m air temperature) REMA	GlacierSMBM, daily melt rates generated using topographically downsampled temperature and solar radiation data	meters	Liquid water availability, surface stability
Days of snow melt	Glacier SMBM output	Derived from daily maps of melt.	days	Liquid water availability, surface stability
Surface type	Rock outcrop shapefile (Quantarctica). Training and 2021 projection. Modelled ice-free area under RCP 8.5 (Lee et al., 2017). 2100 projection.	Rasterization of vector layer	binary ice surface/rocky outcrop	Ice/snow patch preference
Distance to coast	High-resolution coastline polygon		meters	Nutrient input from marine sources
Distance to animal colony	MAPPPD database, Important Bird Areas, emperor penguin colonies, snow petrel breeding sites, Weddell seal population data		meters	Nutrient inputs from birds/seals

Where two resolutions are given, 8 m data was used for training, and 100 m data was used for prediction over the Antarctic Peninsula.

confusion matrix was applied using 1000 randomly chosen GSA, RSA, and clean snow pixels within the WorldView image and assessed against modelled habitable area using Cohen's Kappa coefficient. Finally, we assessed the map of 2021, Peninsula-scale GSA habitat suitability by testing cooccurrence of modelled habitable areas with remote sensed GSA locations, observed within 2021 Sentinel 2 imagery taken from Walshaw et al. (2024). There is no commensurate RSA dataset available for Peninsula-scale RSA validation.

3 Results

3.1 Remote-sensed training data

There was substantial agreement between remote-sensed bloom locations and *in situ* GPS measurements taken on Robert Island in 2023 (GSA: $\kappa = 0.78$; RSA: $\kappa = 0.73$). Combined, the maximum total area of GSA and RSA identified within the WorldView images (excluding overlapped regions) was 3.4 km^2. The overlapped regions in the training imagery, both on Robert Island and in Ryder Bay, had marked differences in bloom area between different years and at different stages of the growth season (see Table 1). Robert Island, for example, had substantially more RSA coverage on 6th February 2023 (0.95 km^2) compared to 16th March 2019 (0.11 km^2) and 13th March 2013 (0.07 km^2), despite the earlier timing of the image.

3.2 Model evaluation

The BART model performed well at predicting habitable snow for GSA and RSA across the Antarctic Peninsula. The true skill statistics (TSS) and the area under the receiver operating characteristic curve (AUC) values were high (i.e close to 1) and similar between GSA (TSS = 0.80; AUC = 0.96) and RSA (TSS = 0.84; AUC = 0.98) model runs, indicating good overall predictive accuracy with both sets of training data. Model performance is summarized in Supplementary Figure S2. Binary habitat suitability maps, where the mapped probability was above the model's maximum TSS value, had good agreement with validation datasets. Comparing to *in situ* bloom GPS positions on Robert Island in 2023, all observed green snow algae (n = 57) were within the predicted areas habitable for GSA (Figure 3C), whereas 91% of *in situ* RSA observations (n = 140) were within areas predicted habitable for RSA (Figure 3E). Similarly, there was good agreement between modelled habitat suitability and remote-sensed GSA or RSA observations, respectively, Kappa scores of 0.76 and 0.75 (n = 1000) were observed for the Nelson Island WorldView validation image (Figures 3B, D, F). For the spatially coarser 2021 Peninsula-scale habitat suitability maps, 76% of remote sensed GSA blooms (n = 784) (Walshaw et al., 2024) were within areas indicated as habitable by the BART model.

The modelled extent of habitable snow was larger than the extent of the remote-detected algal blooms (Figures 3D, F). This was expected, first as the model does not capture all the environmental parameters determining algal bloom formation, and second as the model output is aggregated for the 2021 summer season whereas the remote sensed blooms are a snapshot from 3rd February 2021. Crucially, the upper elevation range of modelled habitable snow was similar to that over which blooms were observed and so the model captured the environmental envelope where snow was melting, enabling blooms to form.

3.3 Predictor variable importance

Figure 4 shows the predictor variable partial response curves, visualizing their relative contribution to the model across the range of values used for each predictor in training. Higher 95% credible intervals for distance to the coast, distance to animal colonies, and slope variables towards the extreme ends of their ranges are a result of low sample density there. Otherwise, the 95% credible interval was low (< 0.2) across the range of values observed within the training dataset. Except for the distance to coast and distance to animal colony predictors, the ranges in Figure 4 reflect the range of values observed within the Peninsula-scale posterior analysis. However, the flattening out of the relationship between occurrence and these two variables, also shown in Figure 4, shows that the training data captured the full extent of variation. This indicates that the scope of the model is suitable for predicting probabilities across this larger spatial extent. Figure 4 also shows the relative importance of each variable, with days of snow melt and aspect identified as the most important predictors for both GSA and RSA blooms.

3.4 Antarctic Peninsula-scale mapping of habitable snow

Based on 2021 climate conditions, habitable snow for GSA (Figures 5B, C) and RSA (Figures 5F, G) was distributed across much of the coastal regions of the western and north-eastern Antarctic Peninsula, as well as the South Shetland Islands and South Orkney Islands. The 95% credible intervals for GSA and RSA are shown in Figures 5A, E, respectively, and were generally low (95% credible interval < 0.5) across most of the Peninsula (mean 95% CI: 0.51 GSA; 0.46 RSA), though uncertainty was greater (> 0.8) across the Larsen ice shelf on the east of the Peninsula, and the Wordie, George VI, Wilkins, Bach, and Stange ice shelves towards the south-west of the Peninsula. Due to high uncertainty, and a lack of *in situ* observations of algal blooms on ice shelves, they were masked out of the area calculations shown in Figure 5 and discussed herein. Total habitable area under 2021 conditions was 479 km^2 for GSA and 1194 km^2 for RSA.

The overall distribution trend across latitudes was similar in 2100 and 2021 for both GSA and RSA (Figures 5C, G), though a greater number of habitable areas were evident towards the south of the Peninsula in 2100. This was particularly true of RSA, which showed an increase in habitable area between 66°S and 70°S in 2100 versus 2021. Despite these trends, total habitable area for the

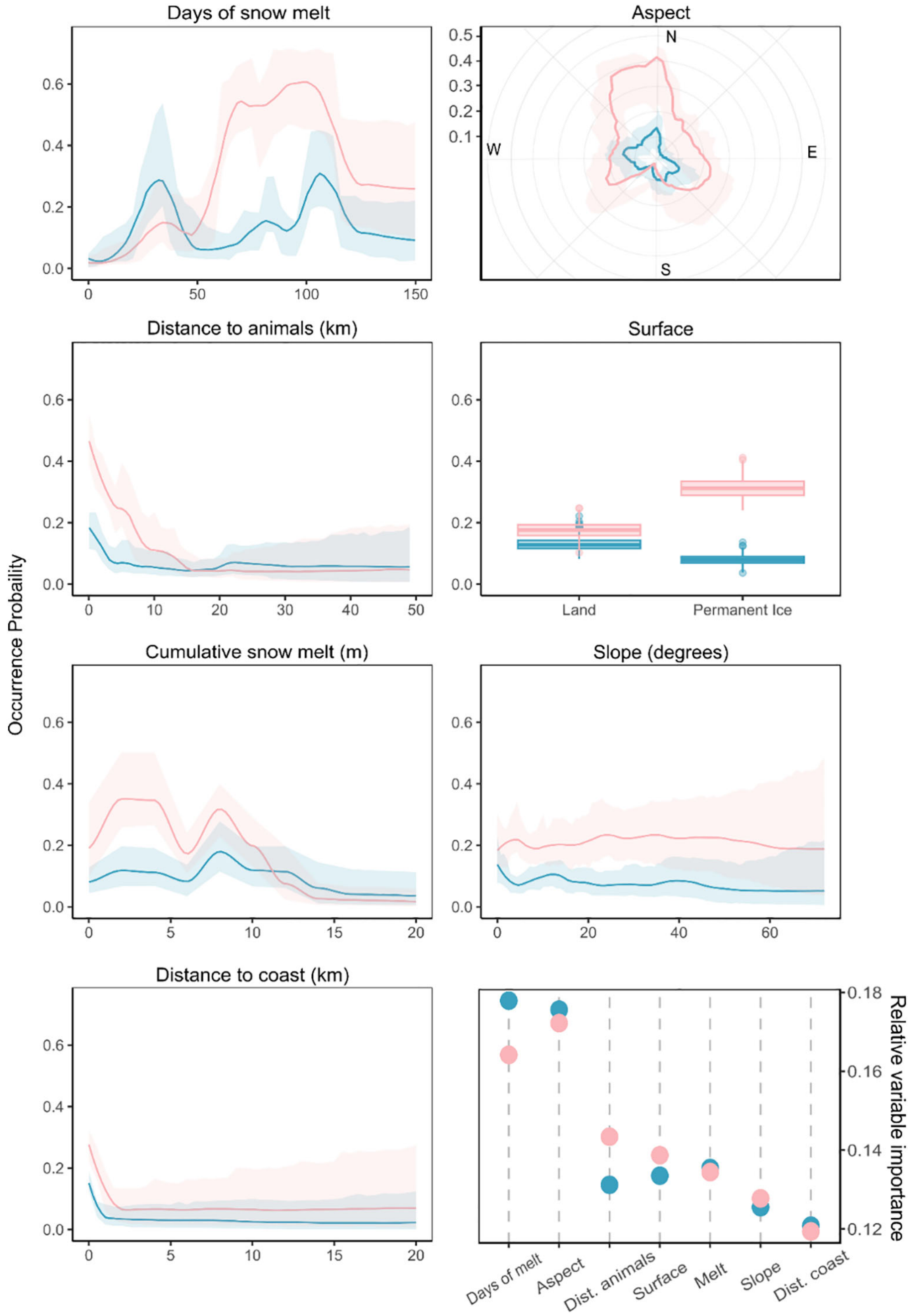

FIGURE 4
Partial response curves for the environmental variables used within the BART model. Individual predictor responses illustrate the shape of the relationship between the probability of occurrence and each environmental predictor for the green snow algae (GSA - light blue) and red snow algae (RSA - pink) model runs. Shaded area shows the 95% credible interval. Also shown, bottom right, is the relative predictive importance of the different environmental variables used within the BART model, shown for GSA and RSA.

Peninsula was smaller in the 2100 analysis than in 2021, with GSA predicted across 423 km² and RSA predicted across 924 km² in 2100. This is evident in Figures 5D, H, which show decreased habitable area concentrated in the north of the Peninsula. However, these findings should be viewed in the context of a strong, positive, temperature anomaly during the 2021 melt season, centered north-east of the Peninsula (Clem et al., 2022) which caused greater melt in some regions of the Peninsula in 2021 than predicted for 2100. The marker line in Figures 5D, H show the boundary between regions experiencing greater (east of the line) or lesser (west of the line) melt. This anomaly resulted in a mixed picture of change in habitable areas across the Peninsula, and masked Peninsula-wide trends.

The 2021 density distributions of GSA and RSA by latitude (Figures 6A, D, respectively) show that most habitable snow was found north of 66°S, with presence becoming patchier further south. However, the model predicted habitable snow for both GSA and RSA as far as 73°S, close to the 74°S extent of the analysis. Figures 6B, C (GSA) and 6e and 6f (RSA) also show a tendency for habitable areas to decrease in elevation and patch size at higher latitudes. The drop in habitable area density seen south of 66°S (Figure 6A) is accompanied by significantly lower lying ($t(6421) = 15.3$, $p < 0.01$) and smaller ($t(6421) = 1.8$, $p = 0.07$) patches of habitable snow, with the decreasing trend continuing from 66°S to 74°S. Most habitable area was at low elevation, with 54% occupying snow less than 50 m elevation. Smaller patches were picked out at higher elevations, particularly at around 64°S where there is a lot of higher elevation land adjacent to the ocean (e.g., Anvers Island, Brabant Island and mountainous terrain on the Danco Coast). The higher elevations shown in Figures 6B, E exceed field observations of bloom elevations in Antarctica, though not elsewhere (e.g., Onuma et al., 2022; Vimercati et al., 2019), but are predominantly (60%) one or two isolated pixels, indicative of model noise. RSA habitable areas (Figure 6F) were significantly higher (97 *versus* 48 m; $t(6421) = 4.6$, $p < 0.01$) and larger (0.038 *versus* 0.037 km^2 $t(6421) = 5.6$, $p < 0.01$) than GSA (Figure 6C).

To examine the influence of warming in context of the 2021 temperature anomaly, we focused our analysis on modelled habitable areas that were predicted to be warmer in 2100 than in 2021. Total area increased with warming for both GSA (270 km^2 versus 217 km^2) and RSA (711 km^2 versus 367 km^2). However, there was no significant increase in the area of individual habitable patches of snow (GSA: $t(8378) = 0.20$, p = 0.84; RSA: $t(9267) = 1.1$, p = 0.27), indicating that total area increased as a result of a greater number of regions along the Antarctic Peninsula becoming habitable for snow algae.

Blooms also increased in elevation within warmed areas of the Antarctic Peninsula in 2100. Average elevation was significantly higher, GSA rising from 43 m to 57 m ($t(8378) = -13.50$, $p < 0.01$) and RSA from 50 m to 70 m ($t(9267) = -7.91$, $p < 0.01$). Similar patch sizes between 2100 and 2021 indicate that this change resulted from a shift in range rather than an expansion of patch area. Snow at higher elevations became more habitable as more and longer melt occurred there, at the same time as blooms at lower elevations became less prevalent. In the case of islands that experienced warming, the model predicted GSA to decrease in area on 70% of them (n = 559) and disappear from 43%, and RSA to decrease in area on 40% of them and disappear from 20%. This loss was compounded on small and low-lying islands, with GSA and RSA decreasing 69% and 42%, respectively, for islands less than 5 km^2, and 87% and 64%, respectively, on islands lower than 50 m in elevation. This range shift is illustrated in Figure 7, which shows an area of islands south of Anvers Island (64.8°S to 65.1°S) and the change in distribution of GSA and RSA between 2021 and 2100.

4 Discussion

4.1 The ecological niche of Antarctic snow algae

We have included a wide range of physical variables in a habitat suitability model and compared those variables against a large dataset of snow algal bloom observations, collected over multiple years. Through this, we have been able to generate output response curves for predictor variables that offer insight into the habitat characteristics and ecological processes that govern the occurrence of seasonal snow algal blooms across the Antarctic Peninsula, the South Shetland Islands and South Orkney Islands. This work is the first landscape scale model to explore the mechanisms that influence the distribution of Antarctica's unique red and green snow algal ecosystems, as well as to map their potential future changes.

Days of snow melt was identified as an important predictor variable for both GSA and RSA (Figure 4). The influence of longer periods of snow melt, in particular for RSA, suggests the need for longer periods of melt and warm temperatures for blooms to develop. This has been noted previously, and likely relates to bloom requirements for time to grow and multiply, as well as time to undertake the processes of secondary carotenoid formation and encystment, which is potentially critical for year-on-year re-dispersal and seeding (Onuma et al., 2022; Soto et al., 2023). Melt reflected the intensity of warming, and therefore melt rate, within the snowpack. Modelling snow melt to derive total cumulative snow melt and days of snow melt predictors instead of using elevation, temperature, and solar radiation layers directly, provided the BART model with more directly relevant information with respect to conditions for algal growth. This was because the temperature within melting snow will not vary significantly from 0 °C, whereas the melt model allows quantification of the duration and intensity of melt. The response curves for cumulative snow melt and days of snow melt displayed an interesting interplay whereby habitable probability was reduced by higher amounts of melt, whereas a greater number of melt days was influential (Figure 4). This again suggests a scenario where lower daily melt over a longer period provides a more stable snow environment for blooms to develop and complete their life cycle (Soto et al., 2023).

Aspect was another important predictor for both GSA and RSA. The partial dependence plots (Figure 4) show that GSA had little preference for a specific aspect when other variables were set to their mean values, indicating interaction with other variables i.e. aspect may be interdependent with slope, or may depend on the time point within a melt season. RSA showed a preference for occurrence on north facing slopes. Secondary carotenoid pigments, which drive the coloration of RSA blooms, have been shown to confer additional UV-A and UV-B photo-protection in encysted snow algae (Procházková et al., 2020). RSA bloom-forming taxa are therefore likely to be better adapted to high-irradiance conditions found in surface snow layers on north-facing aspects. Additionally, on higher-elevation snow, where air temperatures are below 0 °C, the radiative forcing of algal cells within the snowpack may be sufficient

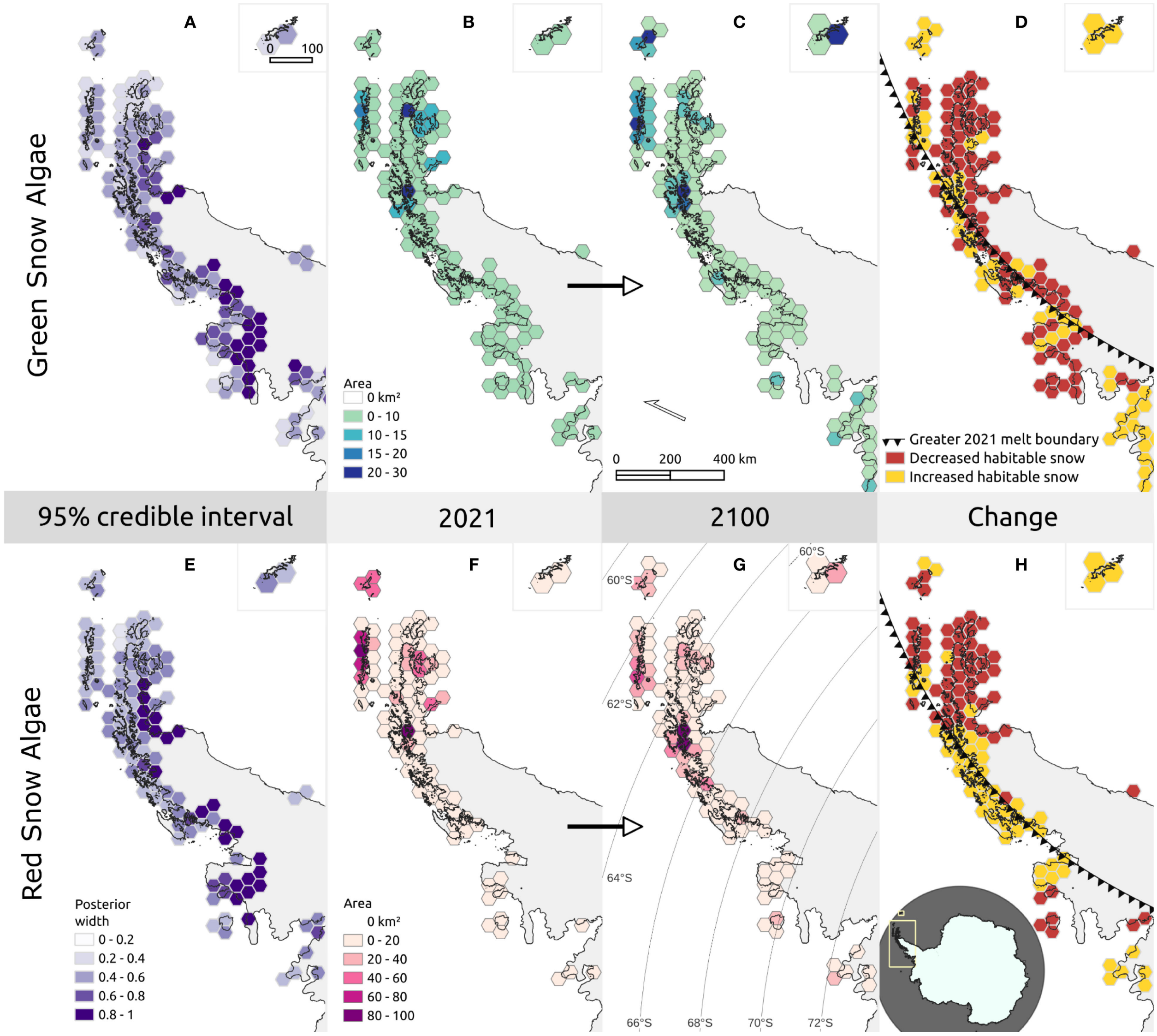

FIGURE 5
Habitable snow area changes across the Antarctic Peninsula. 50 km hexagonal grids showing the sum of area of habitable snow for green snow algae (GSA – **(B, C)**) and red snow algae (RSA – **(F, G)**) under 2021 **(B, F)** and predicted 2100 **(C, G)** climate conditions. Habitable area is cumulative from 1st November to 21st January (2021 or 2100). Maps **(A)** and **(E)** show the length of the 95% credible interval for areas that meet the models' threshold criteria. Maps **(D)** and **(H)** show whether habitable area increased or decreased between 2021 and 2100 within each 50 km grid cell. The triangle marker line is the boundary of greater melt in 2021 than in 2100, where melt was greater to the east of the line and less to the west. Map insets are of the South Orkney Islands and are a different scale (also depicting a 50 km hexagonal grid). The map overview in h shows the extent of the main map views of the Antarctic Peninsula and of the South Orkney Islands inset in yellow.

to initiate localized melting around the cell (Dial et al., 2018; Halbach et al., 2022). In this instance, direct irradiance may play a larger role in bloom formation than our modelled snow melt, which did not factor albedo into its prediction. Habitable areas for RSA were predicted at higher elevations relative to GSA (Figure 6E), possibly because this enhanced albedo-mediated melt was captured within the RSA training data set. Future work could incorporate albedo and melt effects relating to algal presence into an improved melt model to disentangle the effects of radiative heat transfer and aspect upon snow melt and habitat suitability.

The presence of multimodal distributions in the response curves for days of snow melt, melt depth and aspect, suggested heterogenous responses and preferences to variables within GSA and RSA communities (Figure 4). The clustering of training images in similar months was not responsible for the multimodality. Instead, snow melt-based predictor variables exhibited considerable spatial variation across the sampling range, resulting in an even distribution of inputs. The GSA response for days of snow melt shows an even distribution between an initial preference peak for short seasons of melt (mean ~30 days) and a second preference for longer seasons (mean ~110 days). Due to a lack of snow depth information, the model was unable to predict the total melt out of snowpacks. Shorter melt season lengths therefore reflected snow patches exposed to fewer days above melting temperature, rather than shorter seasons due to melt-out of the snow.

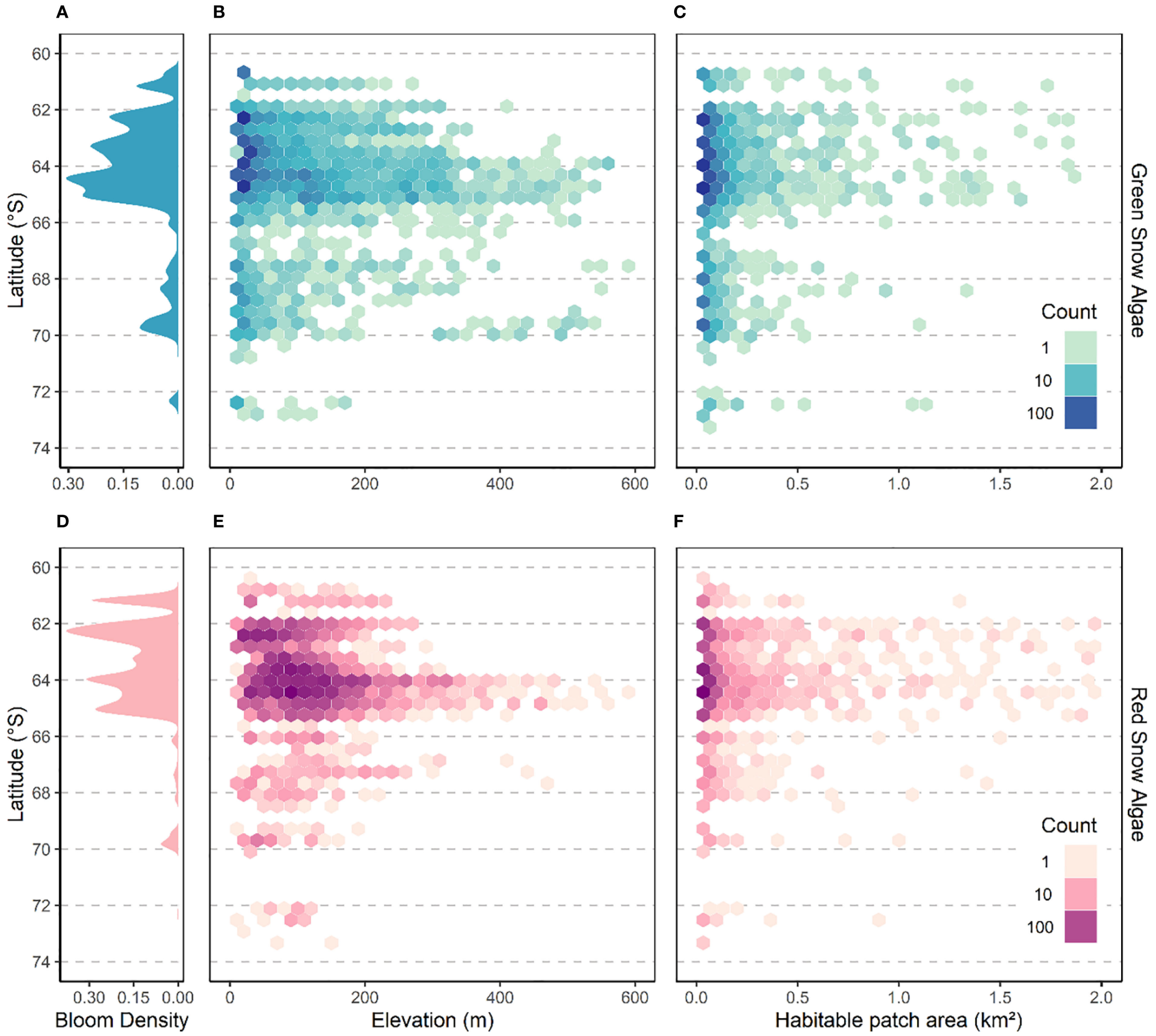

FIGURE 6
Latitude ((**A**) GSA, (**D**) RSA), elevation ((**B**) GSA, (**E**) RSA) and habitable patch (a contiguous habitable area) area ((**C**) GSA, (**F**) RSA) distribution of habitable areas for all blooms on the Antarctic Peninsula under 2021 conditions between 1st November and 21st January. Plots a and d show the latitudinal probability density distribution of pixels determined as habitable from model threshold values. Plots (**B, E**) show 2-dimensional density distribution of the elevation of each habitable pixel versus latitude. Plots c and f show 2-dimensional density distribution of the area of individual patches of habitable snow.

The long-season peak is indicative of GSA communities that require melt over longer periods of time to form. The late-summer timing of most of the WorldView images used (Table 1) points to these blooms having occurred in long-lasting snowpacks which have experienced melt throughout the summer.

In contrast, a response peak at c. 30 days of snow melt for GSA (Figure 4) reflects occurrence in snowpacks with much shorter melting seasons, or in shaded aspects. In such cases opportunistic species that can capitalize on the short melting season are more likely to be the drivers of bloom formation. Field observations indicate that certain snow algae, such as *Hydrurus* spp. and *Raphidonema* spp., can grow rapidly to take advantage of saturated snow at the melting edge of snowpacks (Remias et al., 2013; Kol, 1972). These often manifest as smaller, more localized blooms, forming along snowpack margins and melt out zones (Luo et al., 2020; Remias et al., 2013). Increased detection of blooms in high resolution WorldView imagery similarly suggests that small blooms are more frequent than previously reported for the region (Gray et al., 2021).

The response curves for melt intensity and a preference for terrestrial snow packs highlighted a general preference of GSA for higher melt environments than RSA. This agrees with the concept of opportunistic GSA taking advantage of rapidly melting patches towards the end of the season, as well as supporting the hypothesis of GSA occurring in patches with longer melt seasons preferring saturated layers below the snowpack surface (Hoham and Remias, 2020). One exception among recorded red blooms in Antarctica is the occurrence of blooms dominated by the ciliated red chlorophyte *Chlainomonas* spp (Luo et al.,

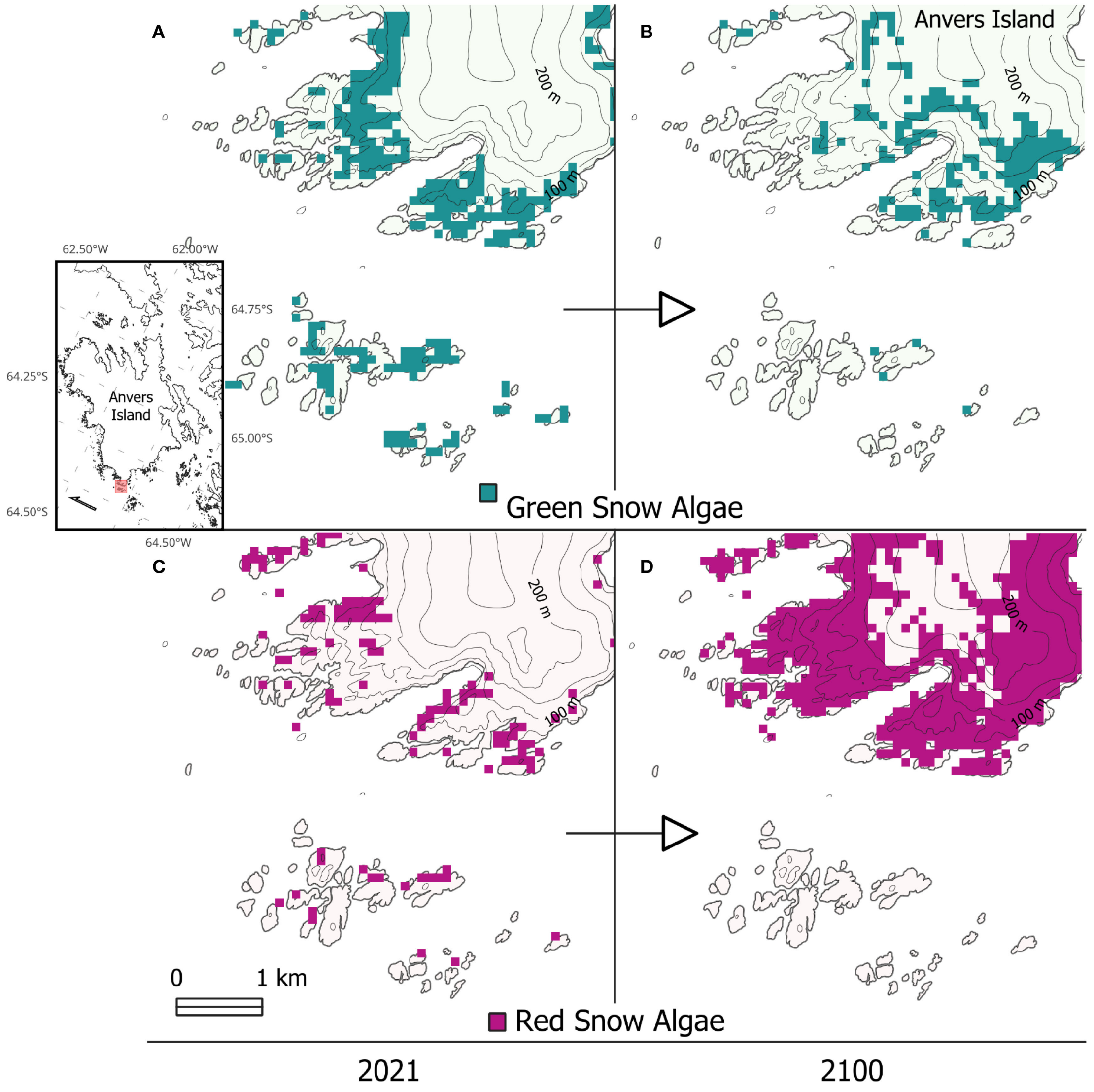

FIGURE 7
A close-up view of the vicinity of Anvers Island showing habitable snow for GSA **(A, B)** and RSA **(C, D)**, for model runs using 2021 **(A, C)** and 2100 **(B, D)** conditions. Contours show 50 m intervals. Inset shows Anvers Island, with the extent of **(A–D)**. shown in red.

2020; Ling and Seppelt, 1993). *Chlainomonas* spp. elsewhere in the world are known to prefer wet snow conditions (Remias et al., 2016; van Hees et al., 2023). The secondary peak in the melt response curve for RSA might therefore reflect the presence of *Chlainomonas*-like species blooms in the observation dataset with a preference for more saturated snow conditions.

Multimodal distributions in the variable response curves highlight the heterogeneous community nature of the GSA and RSA bloom categories. The model is simplified to look at algal blooms as either green or red, though in reality there is a significant variety of communities, with many distinct species comprising a bloom regardless of its dominant color (Davey et al., 2019; Soto et al., 2023, 2020). The presence of multimodal distributions for days of snow melt, cumulative snow melt, and aspect suggest species- or group-specific responses within GSA or RSA are present. Determining how our modelled ecological niche reflects actual habitat niches for different snow algal species and functional groups remains a challenge for the future. These will require additional ecological and physiological studies both *in situ* and in the laboratory (Chevrollier et al., 2022; Halbach et al., 2022).

4.2 Distribution of habitable snow

Across the Antarctic Peninsula region, modelled habitable snow was generally on north-facing, low-lying and coastal slopes, though it was also present on other slope aspects. Seasonally snow-covered rocky outcrops provide suitable habitats for both GSA and RSA, whereas RSA had a greater probability of occurrence on snow-covered Antarctic glaciers than GSA (e.g. Chen et al., 2023). Exceptions to the latter observation were found on the lower latitude and low-lying ice caps of Signy, King George, Nelson, and Robert Islands, where cumulative snow melt and days of snow melt variables also provided habitable snow for GSA blooms. This is consistent with *in situ* and remote sensing observations, with GSA observed on Robert Island ice cap in 2023 and Robert Island and Nelson Island ice caps within Sentinel 2 imagery (Gray et al., 2020).

The 3.4 km² maximum snow algal area observed over a 463 km² area within WorldView imagery was almost double the area of GSA previously reported (1.95 km²) using coarser resolution Sentinel 2 imagery over the entire Antarctic Peninsula (35,000 km² area) (Gray et al., 2020). This highlights the benefits of using high-resolution satellites, such as WorldView, to complete this type of ecological study, both in terms of their ability to classify red blooms and in detecting smaller blooming areas with greater confidence.

The modelled extent of habitable snow was significantly greater than the observed bloom area. This is to be expected as the model parameters tested do not capture the full array of environmental factors which constrain habitat suitability for the algae, for instance omitting nutrient availability or precipitation. This relationship also suggests that stochastic processes of dispersal and recruitment, including mechanisms such as wind, birds, and precipitation (Marshall and Chalmers, 1997; Procházková et al., 2019; Gupta, 2021; Tesson and Šantl-Temkiv, 2018) may also limit the formation of new blooms. The elevation range of habitable snow was primarily influenced by the most important predictor, days of snow melt, as it defined snowpacks that were in a melting state long enough for blooms to form (see Figure 4 for the shape of that relationship). Therefore, in the case of larger, high elevation habitable areas within our model, snow may be suitable for bloom formation, but algae have yet to disperse upwards or establish at higher elevations. Alternatively, blooms may be present in higher elevation snowpacks, but only cross thresholds for remote-sensed detection during extreme summer seasons, for example within the 2023 Robert Island image used for training (Supplementary Figure S1A).

4.3 Climate change and extreme weather event effects upon habitable snow

The positive temperature anomaly in 2021 coupled with the modelled habitable snow area for 2021 *versus* 2100 predictions, highlights the ecological effects of extreme summer weather events and interannual climate variability experienced in the Antarctic Peninsula region. The 2021 anomaly was caused by a combination of low pressure drawing warmer northerly winds over the Peninsula and low sea ice concentrations in the Bellingshausen Sea region (Clem et al., 2022). Sea ice acts as a barrier to exchange of heat and moisture from the sea into the atmosphere, and low sea ice extent has been shown to negatively influence snow accumulation in the western Antarctic Peninsula region (Porter et al., 2016; Thomas et al., 2017). Sea ice in the Bellingshausen Sea has been in decline over the past century (Porter et al., 2016; Turner et al., 2022). Should this trend continue, this may lead to less snow cover and fewer low-lying snow patch habitats persisting through the summer.

Our model indicated that habitable snow could expand southwards in range with climatic warming and indeed the zero-degree isotherm is predicted to increase in latitude over the next 80 years (González-Herrero et al., 2024). Summer temperatures in the west and north-east of the Antarctic Peninsula also experience significant non-seasonal climate variability and positively correlate with the phase of the Southern Annular Mode (SAM) (Clem et al., 2016). The SAM was in a positive phase during the 2021 summer, where strengthening and a poleward shift of westerly winds lead to warmer temperatures and contribute to anomalously high melt during the summer (Clem et al., 2016). Climate reconstructions suggest an increasing positive trend in summer SAM phase throughout the last century (Fogt and Marshall, 2020). Though there is significant uncertainty as to how this trend will continue to affect the Antarctic climate (Fogt and Marshall, 2020), it does seem to be forced by anthropogenic climate change (King et al., 2023). Our modelling work, with supporting evidence from remote-sensed observations from Robert and Nelson Island ice caps, as well as King George Island (Chen et al., 2023), indicates that extreme weather events such as in 2021 and 2023 enabled widespread blooms to form at higher elevations on snow-covered glaciers, and indeed we saw a preference for RSA forming on snow covered glacier/permanent snow surfaces. Research in the Arctic, particularly in Greenland, has shown that algal growth on snow and ice can contribute significantly to the darkening and enhanced melt of glaciers and ice sheets (Cook et al., 2020; Halbach et al., 2022, Williamson et al., 2019). More heatwaves on the Antarctic Peninsula, coupled with recent observations of ice algal species in Antarctica (Thomson et al., 2025), may, therefore, point to future increases in biological darkening and enhanced melt of glaciers and ice caps in the region.

Precipitation patterns across the Antarctica Peninsula are predicted to change significantly with warming in the next century, with summer rainfall and extreme snowfall events becoming increasingly common across the region (Turner et al., 2019; Vignon et al., 2021). The effect on habitable range and snow algal bloom formation is uncertain. However, seasonal field measurements of bloom development have shown strong correlation between summer snowfall events and the retardation of bloom formation (Onuma et al., 2022). In addition, anecdotal field observations have reported fewer blooms and the wash-out of existing blooms following rain events in Antarctica and elsewhere (Stibal et al., 2017). Such weather events may introduce new selection pressures - for instance favoring opportunist species - and destabilize bloom formation processes, with unknown consequences for longer-term bloom re-occurrence (Soto et al., 2023). Such feedback mechanisms and uncertain trends represent unknown factors in the future habitable range for snow algae and remain difficult processes to integrate into forecasting models.

4.4 Model advantages, limitations, and future considerations

This model aimed to capture the conditions that allow the formation of snow algal blooms on the Antarctic Peninsula by parameterizing variables relating to water availability, sunlight, and nutrient supply. The use of high spatial resolution data enabled us to capture habitat suitability in landscape features where snow persists throughout the summer, such as hollows, valleys and below cliffs, potential refugia for snow algae on low-lying islands. This was evident in Figure 3, where river valleys in the low-lying southern reaches of the Coppermine Peninsula and Harmony Point had a greater probability of occurrence than the surrounding area and were indeed filled with snow and GSA/RSA in the WorldView images. The improvement comes from the use of a sun view factor to normalize the net solar radiation component of the snow melt model, producing longer melt periods but with less snow melt over shaded landforms. This fine scale was not captured within the 100 m posterior analysis, however, as the influence of smaller topographic features upon melt was averaged over the coarser model resolution.

Our simplified snow melt modelling approach was limited by the omission of feedbacks between snow algal presence and albedo. Snow algal cells absorb solar radiation (Chevrollier et al., 2022; Halbach et al., 2022), meaning that melt would be greater than our model predictions where algal blooms were present. Since the net solar radiation dataset we used was based upon a fixed albedo over the study region, this is a systematic uncertainty and does not affect the spatial interpretation presented here. However, algal presence may decrease the time taken for a snowpack to melt completely or increase the area and magnitude of melt by increasing the amount of solar energy absorbed by the snow (Cook et al., 2020; Halbach et al., 2022). A second limitation derived from the simplification of our model runs, which omitted snow accumulation and depth information as well as longwave radiation. Moreover, terrestrial snow patches will experience greater heat transfer from surrounding or underlying rock versus snow on glacial surfaces, causing enhanced melt that was not characterized within our melt model. Biases resulting from these limitations were partially addressed within the scope of the BART model by the inclusion of specific GSA and RSA absence training data, within areas that experienced high modelled melt. Ultimately, future work should aim to improve melt model accuracy, incorporating regional climate data, locally defined lapse rate and photon transport models to estimate short and longwave radiation distribution. Over smaller areas where baseline snow depth information is present, this would greatly improve the spatial precision of habitable snow estimation by the BART model.

The use of remotely sensed data for model training allowed us to capture a large sample size that encompassed a wide range of geographic and climatic conditions, and it enabled the use of observed absences rather than generated pseudo-absences. However, this approach also introduces detection limitations that may influence model output. GSA can bloom beneath fresh snow or be overlain by red RAS, obscuring GSA from satellite view (Gray et al., 2020). Because most of our training scenes were late-season, to allow seasonal snow to melt and expose the underlying blooms (Gray et al., 2020), our training dataset likely underrepresents early-season and sub-surface GSA and is biased toward lower snowpack margins. Although random selection of training points and one early-season training image helped to mitigate this detection bias, it likely persists, causing posterior estimates to underestimate the elevation and extent of GSA habitat suitability, especially for predictions over shorter time intervals. Mechanistically, this bias (i) underestimates early-season and sub-surface GSA, and (ii) biases low-elevation/coastal patches downward. This issue is less pronounced for RSA, which typically blooms at the snow surface (Hoham and Remias, 2020; Gray et al., 2021; Onuma et al., 2022), though transient burial by summer snowfall may have reduced the observable RSA extent in some training scenes. Finally, although we attempted to include data from multiple points in the growth season, remaining gaps may contribute to multimodality in some responses. For example, in Figure 7 the 2100 map shows GSA not extending to sea level, and at low elevations the "days of snow melt" predictor is ~50, coinciding with a trough in its partial response. These uncertainties could be reduced by expanding the training set to earlier-season scenes and adding drone or *in situ* time series.

A key assumption of the model is that the absence of a detectable bloom infers non-habitable conditions. In reality, the occurrence of blooms is thought to be determined by stochastic processes of dispersal and seeding success, as well as the availability of a suitable environment (Soto et al., 2023; Onuma et al., 2022). Absence data can be the result of dispersal or settlement barriers in otherwise habitable snow. The inclusion of these points in training the model can lead to the underestimation of habitable range, and lower confidence in predictors (summarized in Table 3). However, the stochastic nature of these absences means the effects on the model output are small. One area of exception is the reduced probability of dispersal inland and to higher elevations as animal-vector and aeolian input become less likely (Bokhorst et al., 2019). This may create a systematic bias and underestimation of habitable snow inland and at higher elevations. The model design can also lead to the conflation of factors determining the absence of blooms (Table 3). The conflation of physical and snow chemistry factors in determining snow algae absence highlights the need for integrated snow chemistry and biology sampling in the development of such models, though achieving comparable scales of sampling to remote sensing approaches remains a challenge in extreme environments.

Incorporating nutrients within the model proved difficult due to a lack of direct nutrient observations that could be mapped at scale. Distances to animal colonies or the coastline were used as proxies for nutrient availability within the model, as isotope analysis has previously shown snow algae to utilize guano as a nutrient source (Gray et al., 2020; Bokhorst et al., 2019). The modelled relationship between occurrence probability and distance to animal colonies or the coast (shown in Figure 4) suggested that blooms within the training dataset were close to both potential nutrient sources, though both variables were assigned low importance. Proximity alone failed to account for actual nutrient concentrations within snow melt and we acknowledge that there are gaps within animal colony records. Moreover, mineral dust within the snow or nitrogen fixation by cyanobacteria may also provide nutrients, unrelated to marine or animal inputs (McCutcheon et al., 2021; Murakami et al., 2022; Phillips-Lander et al., 2020).

Using the model to predict future habitable areas was predominantly based upon predicted air temperature for the region, which in turn fed into the snow melt model for 2100. Improvements could therefore be made by including more complex temporal feedback

TABLE 3 Mechanisms, effects, and outcomes of different forms of true and false detection from remote sensing observation in the model.

Detection result	Description	Likelihood/effect	Outcome
True positive	Detected by remote sensing – true habitable site	n/a	Increased accuracy model
True Negative - habitat suitability	No bloom detected – not habitable snow	n/a	Increased accuracy model
True negative – false inference	No bloom detected – not habitable snow – but determinant is an unmeasured factor (e.g. nutrients, chemistry, biological factor)	Uncertain – effect on overall mapping minor – effect on predictor scores more significant – a conflating effect	Underestimate of habitable range - Reduced confidence scores in measured predictors (noise)
False positive - detection	Detected by remote sensing – not snow algae (mineral/dust/marine algae)	Rare – often detectable or deducible – minimal effect	Overestimation of habitable area
False positive - habitat suitability	Detected by remote sensing – not habitable snow (displaced bloom)	Rare – semi-systematic – minimal effect as physical displacement small	Minor spatial mismatch – underestimation of habitable range - in particular elevation
False negative – detection	No bloom detected – but bloom present - under snow/vertical slope/weak signal	Common - semi systematic as often within snow above exposed bloom - minimal effect	Underestimation of habitable range
False negative – detection	No bloom detected – but bloom present previously - melted out	Common - mitigated by inclusion of early season and late season imagery – uncertain effect	Underestimation of habitable range at lower elevations at season end
False negative - habitat suitability	No bloom detected – but habitable snow	Common – governed by stochastic factors of occurrence (dispersal and seeding) – minor effect overall – though possibly systematic at higher elevations and inland (lower dispersal probability), or where GSA blooms under surface snow cover.	Potential underestimation of habitat range inland/higher elevations/GSA

effects into the model, such as changes to the wider ecosystem and precipitation patterns in the region. Shifting animal colonies and the increase in vegetation in the Antarctic Peninsula, for example, may influence vectors of dispersal, re-seeding processes from soils, and nutrient dynamics for snow algae (Archer et al., 2019; Newsham et al., 2016; Robinson, 2022). In return, the predicted increase in habitable range for snow algae may result in increased organic input to Antarctic habitats, with the potential to drive successional processes in newly exposed soils and ice surfaces (Garrido-Benavent et al., 2020).

Crucially, this model aims to map the prevalence of physical conditions that allow snow algal blooms to form, rather than predicting occurrence. Likewise, it does not aim to model seasonal growth, though aspects of seasonal bloom development are captured in the frequency of bloom detection and the probability of presence across the season in the model output. Rather, this model is a tool that expands our previous work mapping snow algal blooms in Antarctica, upscaling RSA bloom extent estimates, exploring bloom niche requirements, and providing a method to explore seasonal, interannual, and long-term patterns in distribution.

5 Conclusions

We utilized a Bayesian additive regression tree model to explore large-scale distribution controls on snow algal blooms across the Antarctic Peninsula. This modelling approach provides a means to gap-fill the discontinuous observations made by field surveys, satellites or drones as well as enabling future distribution changes to be explored. Modelling indicated that the primary controls on distribution were the number of days of snow melt and aspect, with most habitable snow being north of 66° S, the relatively warmer conditions there providing larger patches of habitable area, and patches at higher elevations that were suitable for bloom formation. Future work should refine the model, using smaller areas for training with better-defined snow accumulation information, adding albedo feedback mechanisms into the snow melt model component and incorporating occurrence information weighted by remotely estimated cell density. Moreover, running the model over the continental Antarctic coastline, much of which exists close to 66° S, or specific ice bodies, would provide greater insight into the extent of Antarctica's cryospheric biology.

The impacts of long-term climate change on bloom distribution were obscured by the anomalous extreme summer temperatures and melt rates experienced during the beginning of the 2021 summer, which exceeded predicted 2100 temperatures in the north and east of the Antarctic Peninsula. However, we determined that there was an increase in the number, size and elevation of habitable areas in regions that had experienced warming, though we also observed significant losses of habitable areas on smaller islands where summer snow is unlikely to persist through the summer under warmer conditions. The higher elevation areas in which our model detected habitable snow were predominantly glaciers or island icecaps and indicate the potential for Arctic-style glacial blooms to become established on the Antarctica Peninsula, changing the landscape of snow algae on the continent and increasing glacial melt rates.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NERC EDS UK Polar Data Centre, https://doi.org/10.5285/3c636579-0389-4ba1-bf3d-d53f32892079.

Author contributions

AG: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. AT: Conceptualization, Data curation, Formal Analysis, Investigation, Project administration, Writing – original draft, Writing – review & editing. CC: Funding acquisition, Project administration, Resources, Writing – original draft, Writing – review & editing. PC: Conceptualization, Funding acquisition, Project administration, Resources, Writing – original draft, Writing – review & editing. PF: Funding acquisition, Writing – original draft, Writing – review & editing. AS: Writing – original draft, Writing – review & editing. LP: Writing – original draft, Writing – review & editing, Funding acquisition, Resources. MD: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, 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.

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References

Ahmadi, K., Mahmoodi, S., Pal, S. C., Saha, A., Chowdhuri, I., Kolyaie, S., et al. (2023). Modeling tree species richness patterns and their environmental drivers across Hyrcanian mountain forests. *Ecol. Inf.* 77, 102226. doi: 10.1016/j.ecoinf.2023.102226

Allouche, O., Tsoar, A., and Kadmon, R. (2006). Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *J. Appl. Ecol.* 43, 1223–1232. doi: 10.1111/j.1365-2664.2006.01214.x

Archer, S. D. J., Lee, K. C., Caruso, T., Maki, T., Lee, C. K., Cary, S. C., et al. (2019). Airborne microbial transport limitation to isolated Antarctic soil habitats. *Nat. Microbiol.* 4, 925–932. doi: 10.1038/s41564-019-0370-4

Benning, L. G., Anesio, A. M., Lutz, S., and Tranter, M. (2014). Biological impact on Greenland's albedo. *Nat. Geosci.* 7, 691–691. doi: 10.1038/ngeo2260

Bokhorst, S., Convey, P., and Aerts, R. (2019). Nitrogen inputs by marine vertebrates drive abundance and richness in antarctic terrestrial ecosystems. *Curr. Biol.* 29, 1721–1727.e3. doi: 10.1016/j.cub.2019.04.038

Bunting, P., and Clewley, D. (2022). *Atmospheric and Radiometric Correction of Satellite Imagery (ARCSI)*. Available at: http://remotesensing.info/arcsi/ (Accessed August 2022).

Burton-Johnson, A., Black, M., Fretwell, P. T., and Kaluza-Gilbert, J. (2016). An automated methodology for differentiating rock from snow, clouds and sea in Antarctica from Landsat 8 imagery: a new rock outcrop map and area estimation for the entire Antarctic continent. *Cryosphere* 10, 1665–1677. doi: 10.5194/tc-10-1665-2016

Carlson, C. J. (2020). embarcadero: Species distribution modelling with Bayesian additive regression trees in r. *Methods Ecol. Evol.* 11, 850–858. doi: 10.1111/2041-210X.13389

Chen, X.-Y., Li, S.-L., Zhang, C., and Liu, D.-Y. (2023). Snow algal blooms in Antarctic King George Island in 2017–2022 and their future trend based on CMIP6 projection. *Adv. Climate Change Res.* 14, 732–745. doi: 10.1016/j.accre.2023.09.013

Chevrollier, L.-A., Cook, J. M., Halbach, L., Jakobsen, H., Benning, L. G., Anesio, A. M., et al. (2022). Light absorption and albedo reduction by pigmented microalgae on snow and ice. *J. Glaciology*, 69 (274), 333–341. doi: 10.1017/jog.2022.64

Chipman, H. A., George, E. I., and McCulloch, R. E. (2010). BART: Bayesian additive regression trees. *Ann. Appl. Stat* 4, 266–298. doi: 10.1214/09-AOAS285

Chown, S. L., and Convey, P. (2016). Antarctic entomology. *Annu. Rev. Entomology* 61, 119–137. doi: 10.1146/annurev-ento-010715-023537

Clem, K. R., Raphael, M. N., Adusumilli, S., Baiman, R., Banwell, A. F., Barreira, S., et al. (2022). State of the climate in 2021 Antarctica and the Southern ocean. *Bull. Am. Meteorological Soc.* 103 (Suppl. 1), S307–S340. doi: 10.1175/BAMS-D-22-0078.1

Clem, K. R., Renwick, J. A., McGregor, J., and Fogt, R. L. (2016). The relative influence of ENSO and SAM on Antarctic Peninsula climate. *J. Geophysical Research: Atmospheres* 121, 9324–9341. doi: 10.1002/2016JD025305

Colesie, C., Walshaw, C. V., Sancho, L. G., Davey, M. P., and Gray, A. (2023). Antarctica's vegetation in a changing climate. *WIREs Climate Change* 14, e810. doi: 10.1002/wcc.810

Cook, J. M., Tedstone, A. J., Williamson, C., McCutcheon, J., Hodson, A. J., Dayal, A., et al. (2020). Glacier algae accelerate melt rates on the south-western Greenland Ice Sheet. *The Cryosphere*. 14 (1), 309–330. doi: 10.5194/tc-14-309-2020

Davey, M. P., Norman, L., Sterk, P., Huete-Ortega, M., Bunbury, F., Loh, B. K. W., et al. (2019). Snow algae communities in Antarctica: metabolic and taxonomic composition. *New Phytol.* 222, 1242–1255. doi: 10.1111/nph.15701

de Menezes, G. C. A., Amorim, S. S., Gonçalves, V. N., Godinho, V. M., Simões, J. C., Rosa, C. A., et al. (2019). Diversity, distribution, and ecology of fungi in the seasonal snow of Antarctica. *Microorganisms* 7, 445. doi: 10.3390/microorganisms7100445

Dial, R. J., Ganey, G. Q., and Skiles, S. M. (2018). "What color should glacier algae be? An ecological role for red carbon in the cryosphere," in *FEMS Microbiology Ecology*, vol. 94. (Oxford, United Kingdom: Oxford University Press (OUP). doi: 10.1093/femsec/fiy007

Dong, Y., Zhao, J., Li, C., and Liao, M. (2022). Gapless-REMA100: A gapless 100-m reference elevation model of Antarctica with voids filled by multi-source DEMs. *ISPRS J. Photogrammetry Remote Sens.* 186, 70–82. doi: 10.1016/j.isprsjprs.2022.01.024

Engstrom, C. B., Williamson, S. N., Gamon, J. A., and Quarmby, L. M. (2022). Seasonal development and radiative forcing of red snow algal blooms on two glaciers in British Columbia, Canada, summer 2020. *Remote Sens. Environ.* 280, 113164. doi: 10.1016/j.rse.2022.113164

Fogt, R. L., and Marshall, G. J. (2020). The Southern Annular Mode: Variability, trends, and climate impacts across the Southern Hemisphere. *WIREs Climate Change* 11, e652. doi: 10.1002/wcc.652

Fretwell, P. T., and Trathan, P. N. (2021). Discovery of new colonies by Sentinel-2 reveals good and bad news for emperor penguins. *Remote Sens Ecol. Conserv.* 7 (1), 139–153. doi: 10.1002/rse2.176

Garrido-Benavent, I., Pérez-Ortega, S., Durán, J., Ascaso, C., Pointing, S. B., Rodríguez-Cielos, R., et al. (2020). Differential colonization and succession of microbial communities in rock and soil substrates on a maritime antarctic glacier forefield. *Front. Microbiol.* 11. doi: 10.3389/fmicb.2020.00126

Gerrish, L., Fretwell, P., and Cooper, P. (2020). *High resolution vector polygons of the Antarctic coastline - VERSION 7.2* (Cambridge, United Kingdom: UK Polar Data Centre, Natural Environment Research Council, UK Research & Innovation). doi: 10.5285/065b9abc-1b5a-4fc6-aa57-9052428aa6ca

González-Herrero, S., Navarro, F., Pertierra, L. R., Oliva, M., Dadic, R., Peck, L., et al. (2024). Southward migration of the zero-degree isotherm latitude over the Southern Ocean and the Antarctic Peninsula: Cryospheric, biotic and societal implications. *Sci Total Environ.* 912, 168473. doi: 10.1016/j.scitotenv.2023.168473

Gray, A., Krolikowski, M., Fretwell, P., Convey, P., Peck, L. S., Mendelova, M., et al. (2020). Remote sensing reveals Antarctic green snow algae as important terrestrial carbon sink. *Nat. Commun.* 11, 1–9. doi: 10.1038/s41467-020-16018-w

Gray, A., Krolikowski, M., Fretwell, P., Convey, P., Peck, L. S., Mendelova, M., et al. (2021). Remote sensing phenology of antarctic green and red snow algae using worldView satellites. *Front. Plant Sci* 12. doi: 10.3389/FPLS.2021.671981/BIBTEX

Groos, A., Mayer, C., Smiraglia, C., Diolaiuti, G., and Lambrecht, A. (2017). A first attempt to model region-wide glacier surface mass balances in the Karakoram: Findings and future challenges. *Geografia Fisica e Dinamica Quaternaria* 40 (2), 137–159. doi: 10.4461/GFDQ2017.40.10

Gupta, P. (2021). First report on diversity of Cyanoprokaryotes and Algae on Feather of Snow Petrel (Pagodroma nivea Forster) in Broknes Peninsula of Larsemann Hills, East Antarctica. *Int. J. Biol. Innov.* 03, 373–381. doi: 10.46505/IJBI.2021.3219

Halbach, L., Chevrollier, L.-A., Doting, E. L., Cook, J. M., Jensen, M. B., Benning, L. G., et al. (2022). Pigment signatures of algal communities and their implications for glacier surface darkening. *Sci. Rep.* 12, 17643. doi: 10.1038/s41598-022-22271-4

Harris, C. M., Lorenz, K., Fishpool, L. D. C., Lascelles, B., Cooper, J., Coria, N. R., et al. (2015). *Important Bird Areas in Antarctica 2015*. Cambridge: BirdLife International and Environmental Research & Assessment Ltd. doi: 10.13140/RG.2.1.1554.2884

Hashim, M., Misbari, S., Reba, N. M., Abdul-Wahab, M. F., Pour, A. B., Said, M. F. M., et al. (2016). "Mapping snow-algae in Antarctic Peninsula with multi-temporal satellite remote sensing data," in *International Geoscience and Remote Sensing Symposium* (New York, USA: IGARSS), 5221–5224. doi: 10.1109/IGARSS.2016.7730360

Hersbach, H., Bell, B., Berrisford, P., Hirahara, S., Horányi, A., Muñoz-Sabater, J., et al. (2020). The ERA5 global reanalysis. *Q.J.R. Meteorol. Soc* 146, 1999–2049. doi: 10.1002/qj.3803

Hodson, A. J., Anesio, A. M., Tranter, M., Fountain, A. G., Osborn, M., Priscu, J. C., et al. (2008). Glacial ecosystems. *Ecol. Monogr.* 78, 41–67. doi: 10.1890/07-0187.1

Hoham, R. W., and Remias, D. (2020). Snow and glacial algae: A review1. *J. Phycology* 56, 264–282. doi: 10.1111/jpy.12952

Howat, I. M., Porter, C., Smith, B. E., Noh, M.-J., and Morin, P. (2019). The reference elevation model of Antarctica. *Cryosphere* 13, 665–674. doi: 10.5194/tc-13-665-2019

Humphries, G. R. W., Naveen, R., Schwaller, M., Che-Castaldo, C., McDowall, P., Schrimpf, M., et al. (2017). Mapping Application for Penguin Populations and Projected Dynamics (MAPPPD): Data and tools for dynamic management and decision support. *Polar Rec* 53, 160–166. doi: 10.1017/S0032247417000055

Huovinen, P., Ramírez, J., and Gómez, I. (2018). Remote sensing of albedo-reducing snow algae and impurities in the Maritime Antarctica. *ISPRS J. Photogrammetry Remote Sens.* 146, 507–517. doi: 10.1016/j.isprsjprs.2018.10.015

Jawak, S. D., Luis, A. J., Fretwell, P. T., Convey, P., and Durairajan, U. A. (2019). Semiautomated detection and mapping of vegetation distribution in the Antarctic environment using spatial-spectral characteristics of WorldView-2 imagery. *Remote Sens.* 11, 1909. doi: 10.3390/rs11161909

Khan, A. L., Dierssen, H. M., Scambos, T. A., Höfer, J., and Cordero, R. R. (2021). Spectral characterization, radiative forcing and pigment content of coastal Antarctic snow algae: Approaches to spectrally discriminate red and green communities and their impact on snowmelt. *Cryosphere* 15, 133–148. doi: 10.5194/tc-15-133-2021

King, J., Anchukaitis, K. J., Allen, K., Vance, T., and Hessl, A. (2023). Trends and variability in the Southern Annular Mode over the Common Era. *Nat. Commun.* 14, 2324. doi: 10.1038/s41467-023-37643-1

Kol, E. (1972). Snow algae from signy island (South orkney islands,Antarctica). *Annales Historico-Naturales Musei Nationalis Hungarici* 64, 63–70.

LaRue, M., Salas, L., Nur, N., Ainley, D., Stammerjohn, S., Pennycook, J., et al. (2021). Insights from the first global population estimate of Weddell seals in Antarctica. *Sci Adv.* 7, eabh3674. doi: 10.1126/sciadv.abh3674

Lavoie, D., Denman, K., and Michel, C. (2005). Modeling ice algal growth and decline in a seasonally ice-covered region of the Arctic (Resolute Passage, Canadian Archipelago). *J. Geophysical Res.* 110, C11009. doi: 10.1029/2005JC002922

Lee, J. R., Raymond, B., Bracegirdle, T. J., Chadès, I., Fuller, R. A., Shaw, J. D., et al. (2017). Climate change drives expansion of Antarctic ice-free habitat. *Nature* 547, 49–54. doi: 10.1038/nature22996

Ling, H. U., and Seppelt, R. D. (1993). Snow algae of the Windmill Islands, continental Antarctica. 2. Chloromonas rubroleosa sp. nov. (Volvocales, Chlorophyta), an alga of red snow. *Eur. J. Phycology* 28, 77–84. doi: 10.1080/09670269300650131

Lopez, A. (2016). *CMIP5 daily data on single levels*. Reading, United Kingdom: Copernicus Climate Change Service (C3S) through the Climate Data Store (CDS), ECMWF. doi: 10.24381/CDS.D3513DBF

Luo, W., Ding, H., Li, H., Ji, Z., Huang, K., Zhao, W., et al. (2020). Molecular diversity of the microbial community in coloured snow from the Fildes Peninsula (King George Island, Maritime Antarctica). *Polar Biol.* 43, 1391–1405. doi: 10.1007/s00300-020-02716-0

Marshall, W. A., and Chalmers, M. O. (1997). Airborne dispersal of Antarctic terrestrial algae and cyanobacteria. *Ecography* 20, 585–594. doi: 10.1111/j.1600-0587.1997.tb00427.x

Martin, P. J., and Peel, D. A. (1978). The spatial distribution of 10 m temperatures in the antarctic peninsula. *J. Glaciology* 20, 311–317. doi: 10.3189/S0022143000013861

Matsuoka, K., Skoglund, A., Roth, G., De Pomereu, J., Griffiths, H., Headland, R., et al. (2018). *QuAntarctica*. Tromsø, Norway: Norwegian Polar Institute. doi: 10.21334/NPOLAR.2018.8516E961

McCutcheon, J., Lutz, S., Williamson, C., Cook, J. M., Tedstone, A. J., Vanderstraeten, A., et al. (2021). Mineral phosphorus drives glacier algal blooms on the Greenland Ice Sheet. *Nat. Commun.* 12, 570. doi: 10.1038/s41467-020-20627-w

Murakami, T., Takeuchi, N., Mori, H., Hirose, Y., Edwards, A., Irvine-Fynn, T., et al. (2022). Metagenomics reveals global-scale contrasts in nitrogen cycling and cyanobacterial light-harvesting mechanisms in glacier cryoconite. *Microbiome* 10, 50. doi: 10.1186/s40168-022-01238-7

Newsham, K. K., Hopkins, D. W., Carvalhais, L. C., Fretwell, P. T., Rushton, S. P., O'Donnell, A. G., et al. (2016). Relationship between soil fungal diversity and temperature in the maritime Antarctic. *Nat. Climate Change* 6, 182–186. doi: 10.1038/nclimate2806

Onuma, Y., Yoshimura, K., and Takeuchi, N. (2022). Global simulation of snow algal blooming by coupling a land surface and newly developed snow algae models. *J. Geophysical Res.* 127, e2021JG006339. doi: 10.1029/2021JG006339

Phillips-Lander, C. M., Harrold, Z., Hausrath, E. M., Lanzirotti, A., Newville, M., Adcock, C. T., et al. (2020). Snow algae preferentially grow on fe-containing minerals and contribute to the formation of fe phases. *Geomicrobiology J.* 37, 572–581. doi: 10.1080/01490451.2020.1739176

Porter, S. E., Parkinson, C. L., and Mosley-Thompson, E. (2016). Bellingshausen Sea ice extent recorded in an Antarctic Peninsula ice core. *J. Geophysical Res.* 121, 13,886–13,900. doi: 10.1002/2016JD025626

Poursanidis, D., Kougioumoutzis, K., Minasidis, V., Chartosia, N., Kletou, D., and Kalogirou, S. (2022). Uncertainty in marine species distribution modelling: trying to locate invasion hotspots for pterois miles in the Eastern mediterranean sea. *J. Mar. Sci Eng.* 10, 729. doi: 10.3390/jmse10060729

Procházková, L., Leya, T., Křížková, H., and Nedbalová, L. (2019). Sanguina nivaloides and Sanguina aurantia gen. et spp. nov. (Chlorophyta): the taxonomy, phylogeny, biogeography and ecology of two newly recognised algae causing red and orange snow. *FEMS Microbiol. Ecol.* 95, fiz064. doi: 10.1093/femsec/fiz064

Procházková, L., Remias, D., Bilger, W., Křížková, H., Řezanka, T., and Nedbalová, L. (2020). Cysts of the snow alga chloromonas krienitzii (Chlorophyceae) show increased tolerance to ultraviolet radiation and elevated visible light. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.617250

Pugh, P. J. A., and Convey, P. (2008). Surviving out in the cold: Antarctic endemic invertebrates and their refugia. *J. Biogeography* 35, 2176–2186. doi: 10.1111/j.1365-2699.2008.01953.x

Remias, D., Jost, S., Boenigk, J., Wastian, J., and Lütz, C. (2013). Hydrurus-related golden algae (Chrysophyceae) cause yellow snow in polar summer snowfields. *Phycological Res.* 61, 277–285. doi: 10.1111/pre.12025

Remias, D., Pichrtová, M., Pangratz, M., Lütz, C., and Holzinger, A. (2016). Ecophysiology, secondary pigments and ultrastructure of Chlainomonas sp. (Chlorophyta) from the European Alps compared with Chlamydomonas nivalis forming red snow. *FEMS Microbiol. Ecol.* 92, fiw030. doi: 10.1093/femsec/fiw030

Robinson, S. A. (2022). Climate change and extreme events are changing the biology of Polar Regions. *Global Change Biol.* 28, 5861–5864. doi: 10.1111/gcb.16309

Robledano, A., Picard, G., Arnaud, L., Larue, F., and Ollivier, I. (2022). "Modelling surface temperature and radiation budget of snow-covered complex terrain," in *The Cryosphere*, vol. 16. (Göttingen, Germany: Copernicus GmbH), 559–579. doi: 10.5194/tc-16-559-2022

Roussel, L., Dumont, M., Gascoin, S., Monteiro, D., Bavay, M., Nabat, P., et al. (2024). Snowmelt duration controls red algal blooms in the snow of the European Alps. *Proc. Natl. Acad. Sci. United States America* 121 41, e2400362121. doi: 10.1073/pnas.2400362121

Schwaller, M. R., Lynch, H. J., Tarroux, A., and Prehn, B. (2018). A continent-wide search for Antarctic petrel breeding sites with satellite remote sensing. *Remote Sens. Environ.* 210, 444–451. doi: 10.1016/j.rse.2018.02.071

Siegert, M. J., Bentley, M. J., Atkinson, A., Bracegirdle, T. J., Convey, P., Davies, B., et al. (2023). Antarctic extreme events. *Front. Environ. Sci* 11. doi: 10.3389/fenvs.2023.1229283

Soto, D. F., Fuentes, R., Huovinen, P., and Gómez, I. (2020). Microbial composition and photosynthesis in Antarctic snow algae communities: Integrating metabarcoding and pulse amplitude modulation fluorometry. *Algal Res.* 45, 101738. doi: 10.1016/j.algal.2019.101738

Soto, D. F., Gómez, I., and Huovinen, P. (2023). Antarctic snow algae: unraveling the processes underlying microbial community assembly during blooms formation. *Microbiome* 11, 200. doi: 10.1186/s40168-023-01643-6

Stibal, M., Box, J. E., Cameron, K. A., Langen, P. L., Yallop, M. L., Mottram, R. H., et al. (2017). Algae drive enhanced darkening of bare ice on the Greenland ice sheet. *Geophysical Res. Lett.* 44, 11,463–11,471. doi: 10.1002/2017GL075958

Sun, X., Wu, W., Li, X., Xu, X., and Li, J. (2021). Vegetation abundance and health mapping over Southwestern Antarctica based on worldView-2 data and a modified spectral mixture analysis. *Remote Sens. 13 Page* 166 13, 166–166. doi: 10.3390/RS13020166

Tesson, S. V. M., and Šantl-Temkiv, T. (2018). Ice nucleation activity and aeolian dispersal success in airborne and aquatic microalgae. *Front. Microbiol.* 9. doi: 10.3389/fmicb.2018.02681

Thomas, E. R., van Wessem, J. M., Roberts, J., Isaksson, E., Schlosser, E., Fudge, T. J., et al. (2017). Regional Antarctic snow accumulation over the past 1000 years. *Climate Past* 13, 1491–1513. doi: 10.5194/cp-13-1491-2017

Thomson, A. I., Gray, A., Colesie, C., Thomas, N., Moulton, H., Convey, P., et al. (2025). Surface darkening by abundant and diverse algae on an Antarctic ice cap. *Nat. Commun.* 16, 2647. doi: 10.1038/s41467-025-57725-6

Turner, J., Holmes, C., Caton Harrison, T., Phillips, T., Jena, B., Reeves-Francois, T., et al. (2022). Record low Antarctic sea ice cover in February 2022. *Geophysical Res. Lett.* 49, e2022GL098904. doi: 10.1029/2022GL098904

Turner, J., Phillips, T., Thamban, M., Rahaman, W., Marshall, G. J., Wille, J. D., et al. (2019). The dominant role of extreme precipitation events in antarctic snowfall variability. *Geophysical Res. Lett.* 46, 3502–3511. doi: 10.1029/2018GL081517

van Hees, D., Hanneman, C., Paradis, S., Camara, A. G., Matsumoto, M., Hamilton, T., et al. (2023). Patchy and Pink: Dynamics of a Chlainomonas sp. (Chlamydomonadales, chlorophyta) algal bloom on Bagley Lake, North Cascades, WA. *FEMS Microbiol. Ecol.* 99, fiad106. doi: 10.1093/femsec/fiad106

Vignon, É., Roussel, M.-L., Gorodetskaya, I. V., Genthon, C., and Berne, A. (2021). Present and future of rainfall in Antarctica. *Geophysical Res. Lett.* 48, e2020GL092281. doi: 10.1029/2020GL092281

Vimercati, L., Solon, A. J., Krinsky, A., Arán, P., Porazinska, D. L., Darcy, J. L., et al. (2019). Nieves penitentes are a new habitat for snow algae in one of the most extreme high-elevation environments on Earth. *Arctic Antarctic Alpine Res.* 51, 190–200. doi: 10.1080/15230430.2019.1618115

Vyverman, W., Verleyen, E., Wilmotte, A., Hodgson, D. A., Willems, A., Peeters, K., et al. (2010). Evidence for widespread endemism among Antarctic micro-organisms. *Polar Sci* 4, 103–113. doi: 10.1016/j.polar.2010.03.006

Walshaw, C. V., Gray, A., Fretwell, P. T., Convey, P., Davey, M. P., Johnson, J. S., et al. (2024). A satellite-derived baseline of photosynthetic life across Antarctica. *Nat. Geosci.* 17, 755–762. doi: 10.1038/s41561-024-01492-4

Williamson, C. J., Cameron, K. A., Cook, J. M., Zarsky, J. D., and Stibal Edwards, M. A. (2019). Glacier algae: A dark past and a darker future. *Front. Microbiol.* 10, Article 524. doi: 10.3389/fmicb.2019.00524

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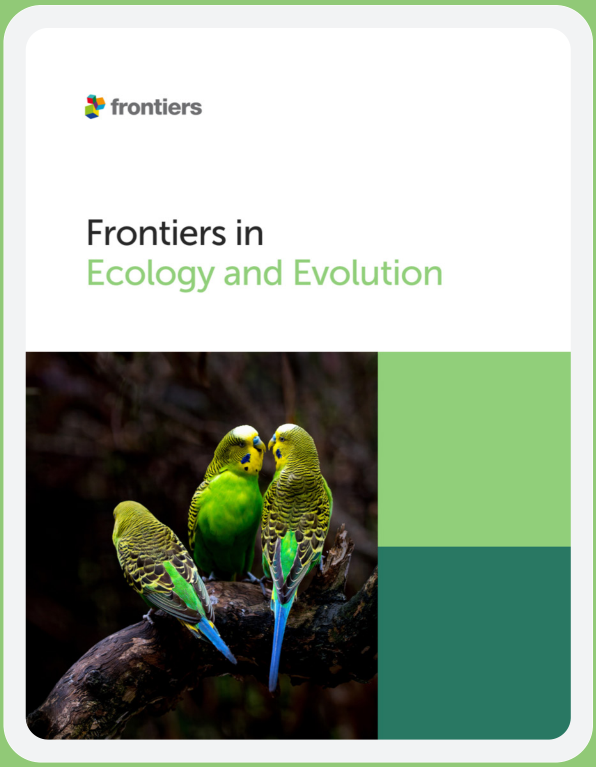

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