Fine-scale phylogenetic diversity gradients support the Antarctic geothermal refugia hypothesis

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Abstract: The possible role of geothermal areas, such as volcanoes, in fostering biodiversity in Antarctica has received considerable recent attention. Under a geothermal refugia hypothesis, diverse life could be supported near or at geothermal sites, and we should see decreasing diversity and/or patterns of nestedness moving away from 'hotspots'. Although there is evidence that geothermal areas have played a role in the persistence of some terrestrial species through glacial periods in Antarctica, the spatial scales at which such refugia operate is not clear. We sampled sediment from a range of locations across volcanic Deception Island in the Maritime Antarctic and used eDNA metabarcoding approaches (targeting a region of the 28S marker) to assess patterns of diversity in relation to thermal gradients. We found that although colder sites harboured significantly greater taxonomic richness than warmer sites, phylogenetic diversity was lower at colder sites (i.e. taxa at colder sites tend to be more evolutionary close to each other). We infer that increased selective processes in low-temperature environments have reduced phylogenetic diversity, supporting a hypothesis of geothermal locations acting as refugia for diverse taxa, even on fine spatial scales, in cold-climate regions such as Antarctica.

Introduction

Despite geological evidence of Antarctic glaciers extending onto the continental shelf at the Last Glacial Maximum (Anderson *et al.* 2002), molecular and biological evidence indicates that diverse terrestrial life in Antarctica has persisted for millions of years (Convey *et al.* 2008, Green *et al.* 2011, Fraser *et al.* 2012), with relatively little colonization from outside of Antarctica (Convey *et al.* 2020).

There are several possible explanations for the persistence of terrestrial life in Antarctica through Pleistocene glaciations (Convey *et al.* 2009, 2020, Fraser *et al.* 2012). One is survival on ice-free nunataks - rocky patches poking through glaciers, particularly on mountain ridges and tops (Stevens & D'Haese 2014). Whereas some species can survive on nunataks, including some macrofauna (springtails: Stevens & D'Haese 2014, Collins *et al.* 2019), the extreme conditions of such refugia can support only a subset of Antarctic taxa (Sohlenius & Boström 2008, Convey *et al.* 2020). Another plausible explanation for persistence through glaciations is the presence of volcanic and

radiogenic geothermal areas (Fraser *et al.* 2014) that can create both ice-free areas and subglacial caves (Fraser *et al.* 2018).

Under the geothermal refugia hypothesis, we would expect species richness to be higher in or near warmer areas. The geothermal refugia hypothesis is well supported by modelling and species richness data (Fraser *et al.* 2014) and eDNA evidence of plants and animals in or around subglacial geothermal caves (Fraser *et al.* 2018). Furthermore, higher biodiversity associated with geothermally warmed areas has been reported in the South Sandwich Islands, particularly for floral diversity (Convey & Lewis Smith 2006). Until recently, however, the evolutionary relationships between species in purported refugia has been largely neglected; new work suggests than when ancient refugia are small, modern phylogenetic diversity is lower, indicating diversification out from the refugia (Barthelemy *et al.* 2021). Investigating these relationships can provide insights into the processes that structure Antarctic biodiversity.

Deception Island is an active volcano in Antarctica and is an ideal place to test the geothermal refugia hypothesis

on fine spatial scales, with numerous thermal gradients found on the island. Deception Island also experiences high levels of tourism (Dibbern 2010) and is the subject of considerable anthropogenic pressure (Carvalho-Silva *et al.* 2021). Studies have been examining biodiversity across the island using metabarcoding approaches (Rosa *et al.* 2020, Carvalho-Silva *et al.* 2021, de Souza *et al.* 2022) but have not tested the influence of thermal gradients around fumaroles. We explore the role of soil temperature in structuring eukaryotic communities on Deception Island to test the geothermal refugia hypothesis using DNA metabarcoding of eukaryotes on small spatial scales (metres).

We examine both taxonomic (the number of amplicon sequence variants (ASVs)/taxa present within a community) and phylogenetic diversity (the average evolutionary distance between taxa within a community). These diversity metrics can inform our understanding of the role of geothermal refugia in evolution, as taxonomic diversity helps to show the ecological structure of a community, whereas phylogenetic diversity can enable understanding of the evolutionary processes (e.g. diversification). For example, following glacial thawing, organisms may diversify out of geothermal refugia, and lineages that successfully diversify from initial founders of new areas would thus dominate the taxonomic structure of the community. Therefore, although a geothermal 'hotspot' may have similar levels of species richness to nearby 'coldspots', the phylogenetic diversities may be drastically different. In this contribution, we analyse this important issue in geothermal areas.

Methods

Sampling took place in February 2016 on Deception Island (62.95°S, 60.65°W), Maritime Antarctica. Approximately 30 ml of surface soil, at 0–4 cm depth, was collected for each sample using autoclave-sterilised steel spatulas and sterile Falcon tubes. Surface soil temperature was measured for each sample using a temperature probe (ATP Folding 115 mm Probe thermometer) to measure temperature in the soil surface layer (upper centimetres). Sampling locations were selected to be centred on geothermal hotspots such as fumaroles (where present), or - at sites where such hotspots were not clearly discernible - samples were haphazardly taken from areas of soils of different surface temperatures. Multiple samples were taken from each site, with 63 samples taken in total from nine sites: Crater Lake, Telefon Bay, Sea Lion Beach, Southeast Point, Obsidian Cove, Whalers Bay, Fumarole Bay, Stonethrow Ridge and Pendulum Cove (at the latter four sites, samples were taken of both beach and coastal vegetated sediments; Fig. 1). Samples were stored at -20°C (in transit) and then at -80°C.

DNA was extracted using MoBio PowerSoil kit (Qiagen), and DNA was amplified using universal 28S primer C1' (forward; D'Haese 2002) and C2 (reverse; Le *et al.* 1993). Primers were modified to include Ion Torrent sequencing barcodes. Sequencing was carried out on an Ion 318v2 chip using the Ion PGM Sequencing Kit on an Ion Torrent PGM DNA sequencer at the Waikato DNA Sequencing Facility; no DNA was detected via qubit fluorometry
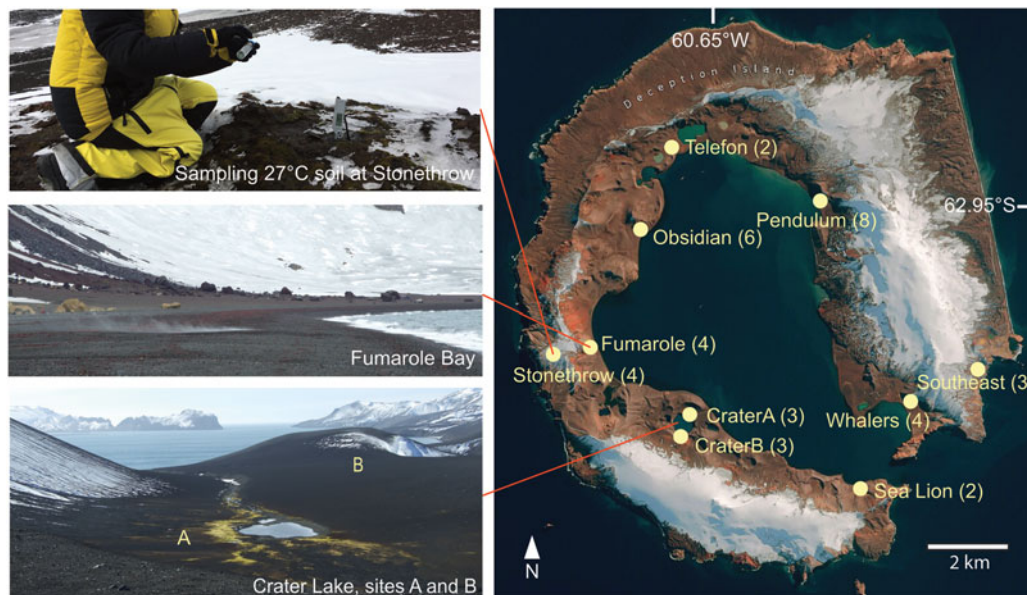

Figure 1. Sampling sites on Deception Island, Maritime Antarctica. Site names are abbreviated to exclude Bay, Cove, Ridge, etc. The numbers in parentheses indicate the number of samples for which data were obtained and analysed in this study.

(high-sensitivity assay) in DNA extraction controls, nor was amplification detected via gel electrophoresis in either DNA extraction or polymerase chain reaction (PCR) controls after library preparation; therefore, following practices at the time (Fraser *et al.* 2018, Monteiro *et al.* 2022), they were not sequenced.

Amplicon sequence analysis

Following DNA sequencing, libraries were demultiplexed using *cutadapt* (v. 4.4; Martin 2011) with a minimum length of 300 base pairs and with reads being flanked by the forward and reverse primers to ensure that the resultant libraries only represented full-length amplicons. Primer sequences were then trimmed, and the libraries were processed into ASVs using *DADA2* (Callahan *et al.* 2016). In *DADA2*, following developer guidance, we truncated reads at the first point of an expected error score < 2 and trimmed the first 15 bases off the reads followed by a truncation length of 285 bases. Error profiling and dereplication followed *DADA2* default settings; however, for ASV inference we explicitly modified the HOMOPOLYMER_GAP_PENALTY to -1 and BAND_SIZE to 32, following developer guidance for Ion Torrent sequencing data. Following ASV inference, we rarefied data at a value of 1700, which resulted in 4 (out of 43) samples being removed from further analyses.

Soil surface temperatures ranged from -1°C to 57.6°C (median: 4.5°C, standard deviation: ±14.6°C). Phylogenetic relationships between ASVs were inferred using *MAFFT* (v. 7.505; Katoh & Standley 2013) to align sequences, with a maximum of 10 iterations, and then *FastTree* (v. 2.1.11; Price *et al.* 2010) to generate a phylogeny using a generalized time-reversible (GTR) model. ASVs were classified using the Ribosomal Database Project (RDP) classifier in *DADA2* using the SILVA 138.1 database, with a minimum of 75 bootstraps required for taxonomic classification.

Analysis of data was conducted using *R* (v. 4.2.1; R Core Team 2020), and estimates of taxonomic richness were produced using the *estimate_richness* function in the *phyloseq R* package (McMurdie & Holmes 2013). Modified permutational multivariate analyses of variance (PERMANOVAs) were conducted using the *vegan* (Oksanen *et al.* 2010) *R* package using the *adonis2* function, with temperature treated as a continuous variable. Non-metric multidimensional scaling was performed using Bray-Curtis distance in the *phyloseq R* package. Selective processes were inferred using the *iCAMP.cm R* package, with the site as a meta-community group for randomizations (Ning *et al.* 2020). Null-model inference was performed with 1000 randomizations using the confidence significance index and beta mean nearest taxon distance (MNTD) as the

form of phylogenetic distance. Temperatures were binned into high (> 10°C), medium (4–10°C) and low (< 4°C) categories for *iCAMP.cm* analyses. Taxonomic classifications of ASVs were made using the *assignTaxonomy* function in the *phyloseq R* package, which utilizes the RDP naïve Bayes classifier (Wang *et al.* 2007) using the SILVA 138.1 LSURef_NR99 taxonomic database (Yilmaz *et al.* 2014).

Phylogenetic diversity within temperature classes was calculated as MNTD within communities using the *mntd* function in the *picante R* package (Kembel *et al.* 2010). This metric was chosen because it does not scale with species richness and thus is useful for comparing phylogenetic diversity across temperature classes.

Linear regressions were conducted using the *lm* function in base *R*, with the protected status of different sampling sites determined based on the Antarctic Specially Protected Areas (ASPA) 140 classifications from the Antarctic Protected Areas (APA) database.

Results

Sequencing summary

In total, 43 out of 62 samples were retained following sequencing. These samples had an average of 9745 reads (minimum: 3 reads, maximum: 22 785 reads). Following rarefaction, 39 samples were retained, with a total of 1902 ASVs (Fig. 1 indicates numbers of samples per site retained after quality control). Nineteen samples failed library preparation, either as a result of failed DNA extractions or PCR; therefore, these samples were not sequenced.

Taxonomic structure of communities

Although many ASVs could not be confidently assigned to taxonomic groups (1055 out 2484 ASVs at the class level, 575 at the order level, 341 at the family level and 354 at the genus level; due to taxonomic ambiguities, some taxa classified at lower levels remained unclassified at higher levels), we found that the dominant class across all temperature groups was Glissomonadida, a class of flagellated protists. High- and medium-temperature communities were more diverse at the class level (Fig. 2) and had higher abundances of plant and algal communities. Conversely, low-temperature communities had higher abundances of ciliates (subphylum: Intramacronucleata) and mobile animals (arachnids and tardigrades). Springtails (subclass: Collembola) and mosses (order: Bryales) were also found in low-temperature communities (13 and 19 samples, respectively) but were rarer in medium- (3 and 5 samples, respectively) and high-temperature (2 and 1 samples, respectively) communities.
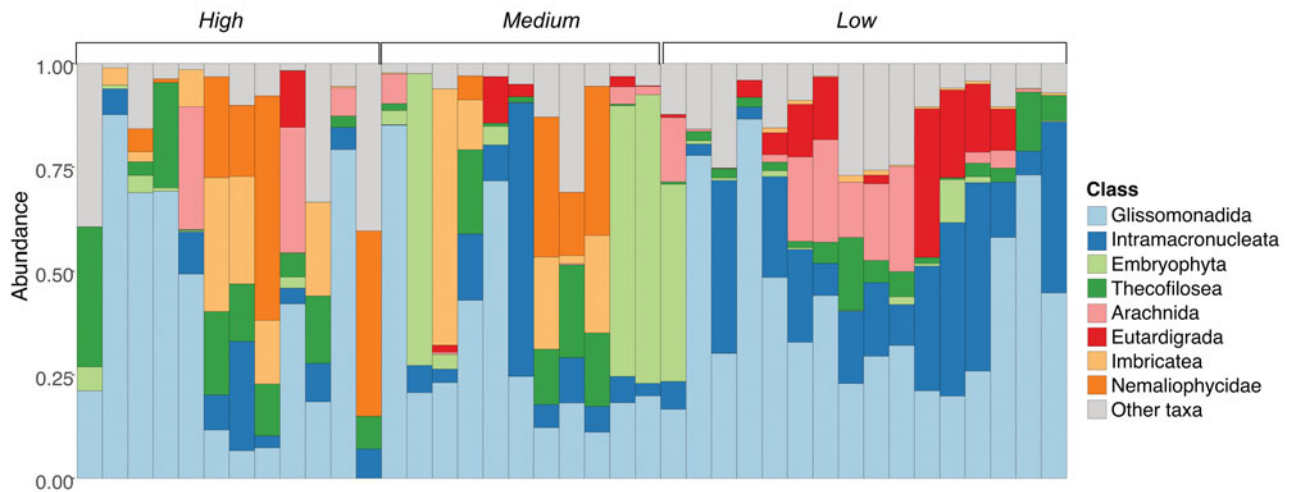
Figure 2. Stacked abundance bar plots of the taxonomic composition of samples (ordered in terms of temperature from high to low, with broad temperature classes specified).
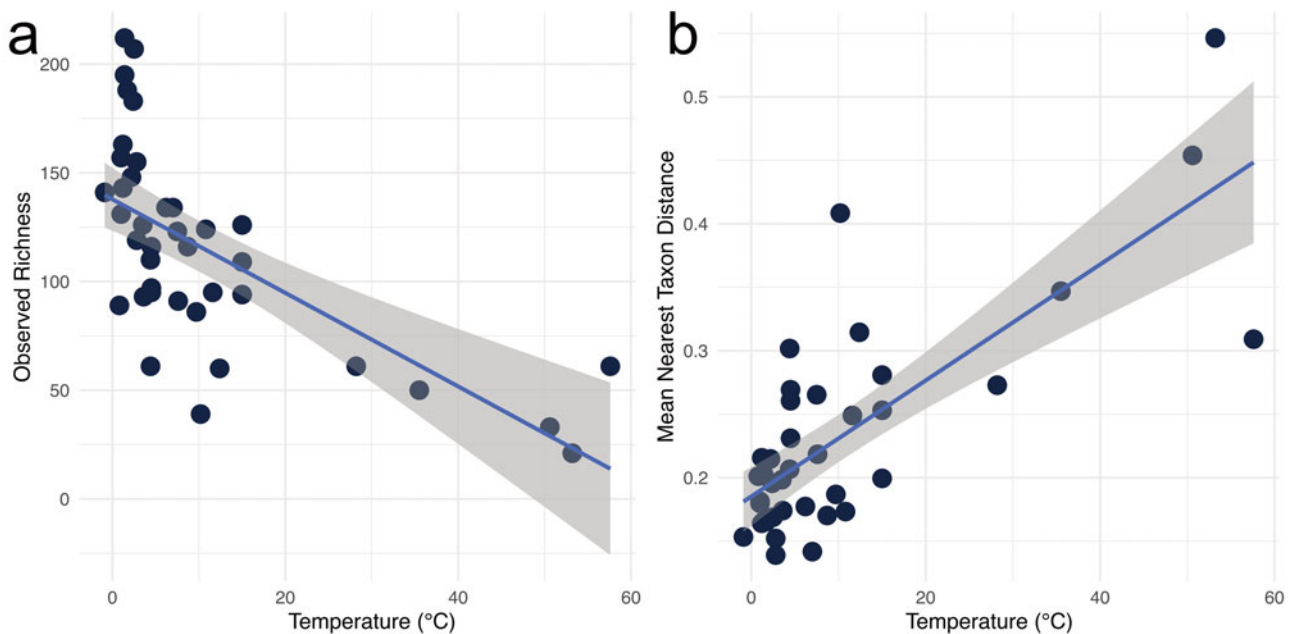

Figure 3. a. Taxonomic richness declines with temperature. **b.** Mean nearest taxon distance increases with temperature. Lines represent linear models, shading represents the 95% confidence intervals.

Table I. Summary output of the linear regression of taxonomic richness, temperature and protected status.

Coefficient	Estimate	Standard error	t value	Pr(> |t|)
Intercept (taxonomic richness)	152.06	8.75	17.37	0.0002
Temperature	-1.72	0.399	-5.399	< 0.0001
Protected area	-30.6	12.18	-2.52	0.017

Residual standard error: 33.59 on 36 degrees of freedom.
Multiple R^2: 0.52, adjusted R^2: 0.5.
F-statistic: 19.83 on 36 and 1 degrees of freedom, $P < 0.0001$.

Table II. Summary output of the linear regression of mean nearest taxon distance to temperature and protected area status.

Coefficient	Estimate	Standard error	t value	Pr(> |t|)
Intercept (mean nearest taxon distance)	0.19	0.011	16.18	< 0.0001
Temperature	0.005	0.001	7.15	< 0.0001
Protected area	-0.002	0.02	-0.079	0.94

Residual standard error: 0.06 on 36 degrees of freedom.
Multiple R^2: 0.58, adjusted R^2: 0.56.
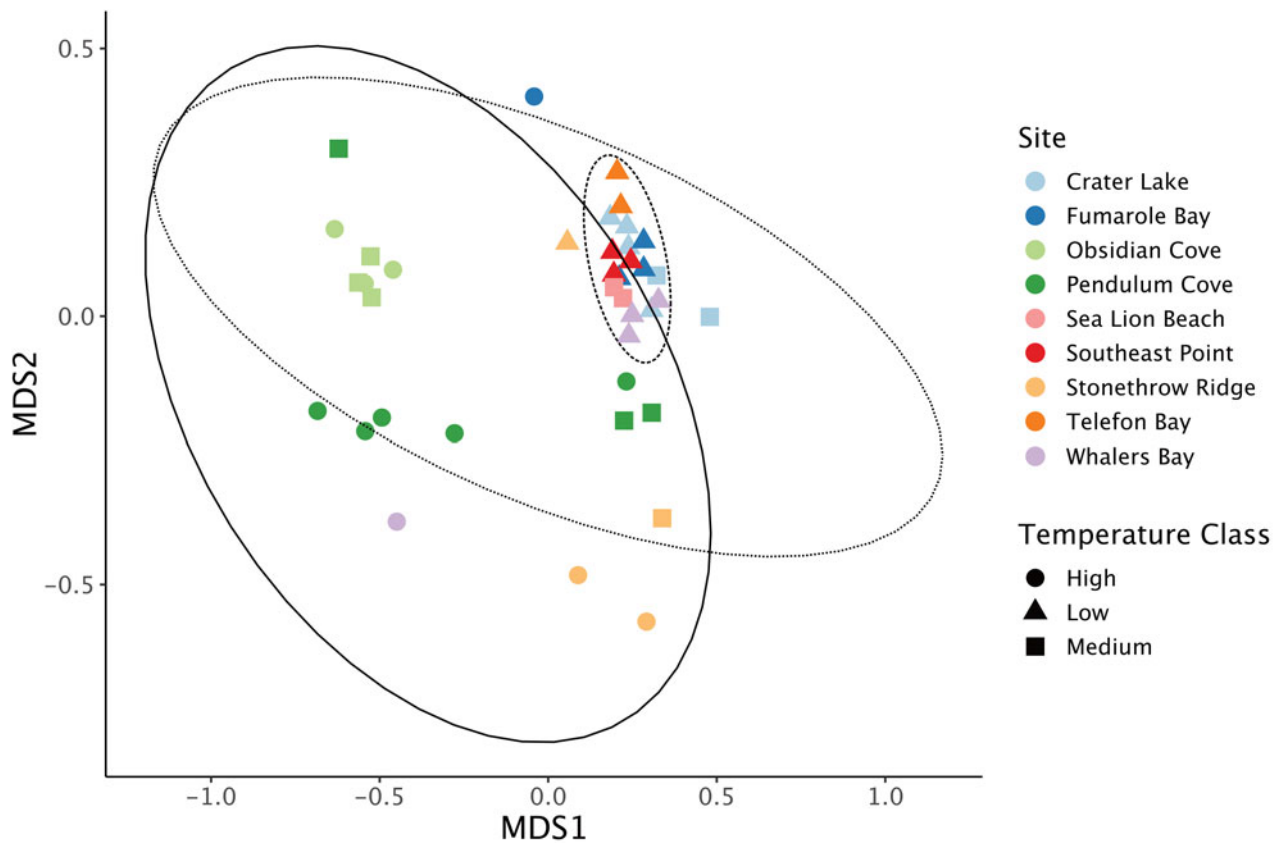F-statistic: 24.85 on 36 and 1 degrees of freedom, $P < 0.0001$.

Figure 4. Non-metric multidimensional scaling (nMDS) of community types on Deception Island, Antarctica. The dashed line indicates the ellipse for low-temperature communities, the dotted line indicates the ellipse for medium-temperature communities and the solid line indicates the ellipse for high-temperature communities. Ellipses were based on 95% confidence using the *stat_ellipse* function in *ggplot*. The stress value for this nMDS was 0.13.

Community diversity is associated with temperature and location

We observe that as temperature increases, the taxonomic richness of the community declines (Fig. 3a & Table I), whereas the MNTD increased with temperature (Fig. 3b & Table II). The protected status of the area was a significant variable for predicting species richness, with higher richness observed in protected areas (147 *vs* 95

ASVs), but this was non-significant with regards to MNTD.

Low-temperature samples typically clustered closely, regardless of the site of origin (Fig. 4), while medium- and high-temperature samples were much more dispersed and exhibited greater clustering based on sites. These results were supported by PERMANOVAs (Table III), which revealed that although both site of collection and zone (i.e. habitat type within zone) were significantly associated with community structure, temperature was also a significant factor.

Selective processes change with temperature

Null-model analyses to disentangle the relative contributions of deterministic and stochastic processes in shaping community assembly revealed that the influence of drift was greatest in high-temperature communities (Fig. 5). However, low temperatures made the greatest contribution to selection (specifically selection imposed by homogeneous conditions).
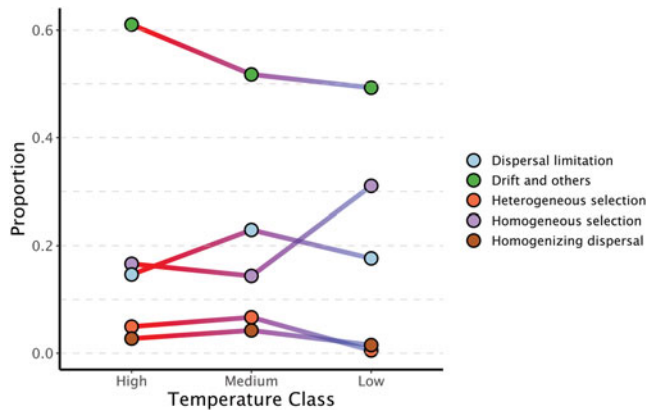
Table III. Permutational multivariate analysis of variance (PERMANOVA) results for amplicon sequence variants based on site, zone (subsite) and temperature at site of collection. Results are based on 999 permutations.

	Degrees of freedom	Sum of squares	R^2	F	$P (> F)$
Site	8	6.85	0.42	3.23	0.001
Zone	3	2.01	0.12	2.53	0.001
Temperature	1	0.44	0.03	1.66	0.016
Residuals	26	6.89	0.43	-	-
Total	38	16.19	1.00	-	-

Figure 5. Relative contributions of different ecological factors shaping community assembly across different temperature groups. Gradient colour lines are approximately indicative of changes in temperature.

Discussion

Our findings indicate that although cold zones on Deception Island, Antarctica, harbour significantly more taxa than geothermal hotspots on fine spatial scales, these cold zones are of comparatively low phylogenetic diversity (Fig. 3). Our data provide support for the geothermal refugia hypothesis through null-model analyses, revealing the increased selective processes in low-temperature environments and reduced phylogenetic diversity (Figs 3 & 5).

Under a geothermal refugia hypothesis, we would expect to see lower MNTD at lower temperatures as a result of recent diversification, whereas warmer sites would be expected to exhibit higher MNTD values. Following declines in ice and snow coverage, areas surrounding geothermal hotspots would become open to colonization, and some taxa from hotspots could diversify in these regions with reduced competition; by contrast, within-hotspot diversification would remain limited by competition. As a result, the hotspots would be highly phylogenetically diverse, as observed in other types of refugia (Mastrogianni *et al.* 2019), whereas 'coldspots' would exhibit low phylogenetic diversity. Our analyses supported this hypothesis, suggesting that low-temperature communities are typically more phylogenetically clustered than warmer communities. Such results are further supported by the high taxonomic richness of low-temperature communities, suggesting that diversification for cold-adapted taxa might be a reasonable explanation.

Joint consideration of both the phylogenetic structure and taxonomic richness of communities on Deception Island suggests that hotspots might be a source of diversity in relatively inhospitable environments. In Antarctica, where one of the dominant selective

pressures might be temperature (Bendia *et al.* 2021), warmer spots may act as reprieves from such harsh selective pressures (McGaughran *et al.* 2021). As a result, neutral processes dominate warmer environments, enabling greater diversity by virtue of reduced selection. Such warmer environments are also likely to persist through glacial cycles, and thus, even as the surrounding environments become increasingly inhospitable to life as ice cover increases, these hotspots remain isolated islands of diversity ready to be colonized when ice coverage decreases (Convey & Lewis Smith 2006, Fraser *et al.* 2014). Our results highlight the importance of considering the phylogenetic structure of communities when testing for refugia, as traditional approaches that focus on gradients of intraspecific diversity or species richness (Hewitt 1999, von Cräutlein *et al.* 2019) might not be adequate on small scales or in extreme environments. Similar results have also been observed when examining the phylogenetic structure of cold and dry communities in Norway, where post-colonization diversification results in higher levels of relatedness or neoendemism (Mienna *et al.* 2020).

Nevertheless, alternative explanations to the geothermal refugia hypothesis exist, and as a result further hypothesis testing is necessary. One possible alternative explanation is that the high levels of tourism to Deception Island relative to the rest of Antarctica might result in high levels of species invasions from warmer, inhabited regions (McCarthy *et al.* 2022). Such invaders may preferentially inhabit warmer zones (Hughes *et al.* 2015) and/or may be more likely to be deposited there by tourists, increasing the phylogenetic diversity of warm sites relative to colder zones. Future research should aim to better quantify the biodiversity in such locations and to explore the use of phylogenetic metrics alongside species richness to better understand the geothermal refugia hypothesis.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0954102024000099.

Data availability

DNA sequencing data, ASV tables and the metadata associated with this paper are available at doi.org/10.6084/m9.figshare.25293172.

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Competing interests

The authors declare none.

Author contributions

AQ and CIF conceived the study and collected samples. MM performed the laboratory work. WSP and CIMA analysed the data. WSP wrote the manuscript with input from all authors.

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