

Evidence of plant and animal communities at exposed and subglacial (cave) geothermal sites in Antarctica

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Abstract Geothermal areas, such as volcanoes, might have acted as glacial microrefugia for a wide range of species. The heavily glaciated but volcanically active Antarctic continent presents an ideal system for assessing this hypothesis. Ice-free terrain around volcanoes in Antarctica is, however, often restricted to small patches, whereas subglacial cave systems, formed by vented volcanic steam, can be extensive and interconnected. No observations of macrobiota have yet been made for subglacial geothermal environments in Antarctica, but these organisms are often patchily distributed and can be difficult to find. We carried out metabarcoding (eDNA) analyses of soil samples taken from exposed areas on three volcanoes in Victoria Land, and subglacial caves on Mount Erebus. We found evidence of numerous eukaryotic groups, including mosses, algae, arthropods, oligochaetes and nematodes, at both exposed and subglacial sites. Our findings support the notion that geothermal areas—including subglacial environments—can nurture biodiversity in glaciated regions.

Keywords Volcano · Polar · Environmental DNA eDNA · Refugia · Subglacial

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Introduction

Antarctica is a heavily glaciated continent with less than 0.3% of land currently exposed (Convey and Stevens 2007). These small patches of ice-free terrain are home to diverse life, including many eukaryotic invertebrate and plant species (Convey and Stevens 2007). Although Antarctic glaciers were more extensive during past ice ages, and are believed to have extended offshore at the LGM (Anderson et al. 2002), molecular evidence indicates that many terrestrial species survived on the continent throughout Pleistocene glaciations (reviewed by Convey et al. 2008; Fraser et al. 2012). Recently, geospatial analyses of species richness patterns have supported the hypothesis that geothermal areas, such as areas associated with active volcanoes, could have acted as glacial refugia for some terrestrial species in Antarctica (Fraser et al. 2014). Although ice-free terrain on active volcanoes in Antarctica is often restricted to small patches, subglacial cave systems, formed by vented volcanic steam, can be extensive and interconnected (Lyon and Giggenbach 1974). Together, these exposed and subglacial geothermal environments could represent significant refuges for terrestrial life on the heavily glaciated southern continent. Mount Erebus, an active volcano in Victoria Land, Antarctica, has some permanently ice-free geothermal areas close to the crater summit, as well as large subglacial geothermal cave systems that harbour prokaryotic (Herbold et al. 2014; Tebo et al. 2015) and fungal (Connell and Staudigel 2013) life. These subglacial environments are often tens of degrees warmer than outside air temperatures, could have liquid water, and have light near their mouths or where overlying ice is thin (Tebo et al. 2015). If geothermal environments in Antarctica, including subglacial geothermal caves, could indeed have housed invertebrates

and plants during past glacial periods, we hypothesise that diverse species should also live in these environments today. No direct observations of macroflora and macrofauna have yet been made for Antarctic subglacial geothermal environments, but thorough searches have not been made; research at the sites has been predominantly geological or microbiological, and many Antarctic eukaryotes are patchily distributed and not readily detectable by the casual eye (e.g. small organisms in and on the soil). We set out to address our hypothesis by conducting metagenomic analysis of soil samples taken from caves and ice chimneys, as well as exposed geothermal sites, on Antarctic volcanoes.

Methods

Soil samples were collected from geothermal sites on volcanic Mount Erebus (Fig. 1) (3794 m, 77°31' 47''S, 167°09'12''E), Mount Melbourne (2732 m, 74°21'0''S, 164°42'0''E) and Mount Rittman (2600 m, 73°28'20''S, 165°37'20''E). Sites were chosen to sample the full range of geothermal features including exposed heated soil (up to ~50 °C) which was open to local weather; ice 'hummocks' (up to around 40 °C) where a dome of unstable ice covered a fumarole, creating partial shade and some protection from

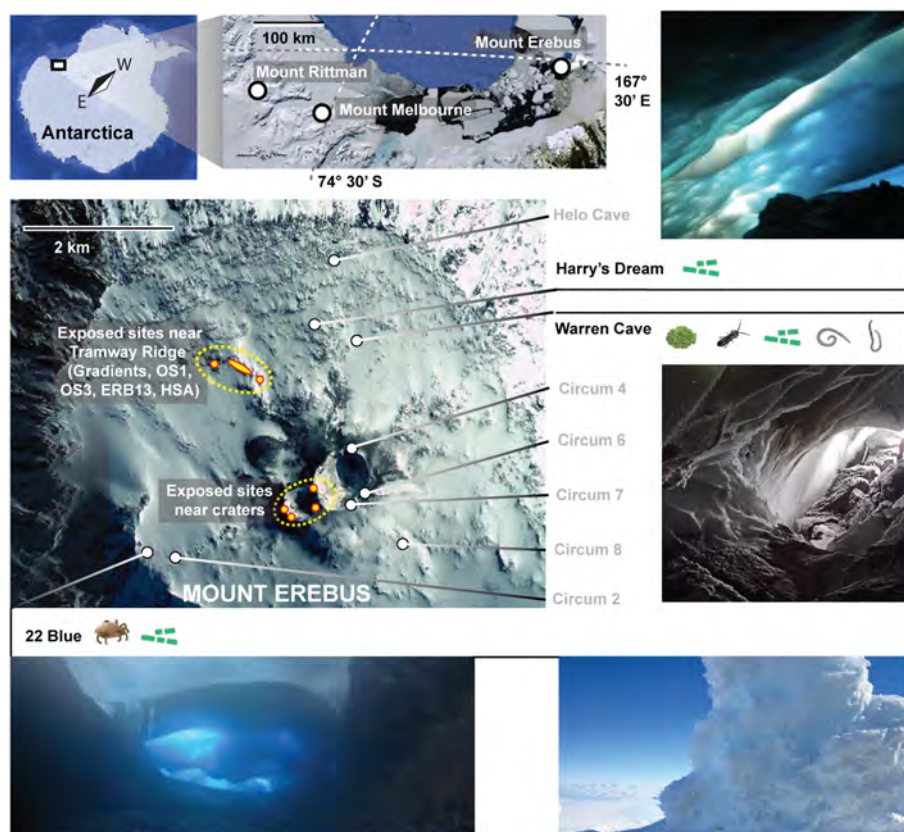
the elements; and subglacial chimneys/caves (up to around 25 °C) where there was extensive semi-permanent ice cover. For more information on sites, see electronic supplementary material (Online Resource 1).

The presence of eukaryotic DNA in samples was assessed using a metabarcoding approach involving mitochondrial COI and nuclear ribosomal 28S (via Ion Torrent sequencing), and nuclear ITS (via cloning); the latter were previously unpublished data that had been generated by earlier work focusing on fungi in the caves (Connell and Staudigel 2013). For detailed methods, see Online Resource 1.

Results

After filtering to remove low-quality reads (see below), we obtained 77,289 COI sequences and 455,329 28S sequences. Chao and Shen indices, calculated per sample, suggested some undersampling, particularly for COI, with a greater number of OTUs expected to be resolved with larger numbers of sequences. Sequence data are provided in Online Resource 2. Per sample, an average of 90% (COI) and 60% (28S) of raw reads were discarded on the basis of size, with 35–45% of the remaining reads discarded due to primer mismatch or high error rates (>1%).

Fig. 1 Site locations. For the enlarged map of the summit of Mount Erebus, exposed sites are circled by dashed ellipses, and subglacial sites are indicated by white dots. Sites listed as 'circum' were low ice hummocks; the site '22 Blue' was an ice chimney; all other subglacial sites were large caves. Subglacial sites from which non-fungal eukaryotic DNA was retrieved are labelled in black. Graphical illustrations show some of the Antarctic taxonomic groups (mosses, mites, springtails, green algae, nematodes, oligochaetes) represented in Harry's Dream, Warren Cave and 22 Blue. Photograph credits: Hubert Staudigel, Rebecca Williams



The majority (~53% for COI and ~70% for 28S) of sequences and OTUs were most closely matched to fungal sequences on GenBank. Many of these fungal sequences could not be identified to species or genus (Online Resource 2). Of those that were a good match for named species on GenBank, many corresponded to taxa previously identified in research focusing solely on the caves' fungal diversity (Connell and Staudigel 2013). We therefore here focused primarily on the non-fungal eukaryotes. The Ion Torrent analyses resulted in 16 non-fungal OTUs for COI, and 39 for 28S, across all sites sampled (Mount Erebus, Mount Rittman and Mount Melbourne). These OTUs matched diverse taxa in GenBank, including mosses (Bryophyta), animals (e.g. Nematoda, Oligochaeta, Arthropoda) and algae (predominantly Chlorophyta).

Non-fungal eukaryotes in volcanic ice caves

Of the nine subglacial sites analysed (Fig. 1), non-fungal eukaryotic DNA amplified for samples from Warren Cave, 22 Blue and Harry's Dream, with some sequences matching plant and invertebrate taxa (Table 1). 28S and ITS sequences obtained from Warren Cave samples matched a wide range of eukaryotic taxa including Collembola, Bryophyta, Oligochaeta and Nematoda known to occur in Antarctic soils. From the ice chimney "22 Blue", 28S and COI data supported the presence of Chlorophyta and Arthropoda. ITS data from Harry's Dream supported the presence of algae.

Some non-fungal OTUs from one subglacial site (Warren Cave) most closely matched taxa that are not naturally found in Antarctica (see Online Resource 2). As our focus in this research was on evidence for Antarctic taxa at the geothermal sites, the presence of which cannot be explained by human visits (see Online Resource 1), these human-associated OTUs are not considered further in our treatment of results.

A bryophyte OTU detected at exposed and subglacial sites on Mount Erebus, as well as at exposed sites on Mount Melbourne and Mount Rittman (see Table 1 and Online Resource 2), most closely matched *Campylopus australis* (99% identity); a closely related species, *Campylopus pyriformis*, is known from the summit of Mount Erebus (Skotnicki et al. 2001), but this species does not currently have complete analogous sequences available in GenBank for comparison. One ITS OTU most closely matched published sequences of Collembola (springtails). The only available ITS data from Antarctic springtail genera in GenBank had comparatively low coverage with our sequences, but much higher identity scores (e.g. *Desoria*, 23% coverage across two fragments totalling 228 bp, with 96 and 100% identity). Another arthropod sequence with no close matches on GenBank was also detected at

exposed and subglacial sites on Mount Erebus (Table 1 and Online Resource 2).

Discussion

Main findings and implications

Our results suggest that diverse invertebrates including nematodes, oligochaetes and arthropods, as well as plants such as mosses and green algae, occur at geothermal sites in Antarctica, and could inhabit subglacial cave systems on Mount Erebus.

Subglacial cave systems on Mount Erebus are extensive and can be interconnected (Giggenbach 1976). Subglacial geothermal caves have also been found on other volcanoes in Antarctica, including Mount Berlin and Mount Melbourne (Nelia Dunbar, *pers. comm.*), and are known from glaciated volcanoes in the Northern Hemisphere—for example, on Mt Rainier (Zimbelman et al. 2000) in the USA and Grímsvötn in Iceland (Stewart et al. 2008). Geothermal cave systems could therefore exist on many of Antarctica's volcanoes. There are more than 15 volcanoes in Antarctica that are either known to be currently active or show evidence of recent activity (Fraser et al. 2014), and new subglacial volcanoes continue to be discovered (Lough et al. 2013). Despite recent advances in our broad understanding of Antarctic biodiversity (Chown et al. 2015), we still know little about life in the continent's subglacial cave systems, which may harbour diverse and complex communities. Emerging molecular evidence for life in subglacial Lake Vostok, more than 3700 m below the surface of the East Antarctic Ice Sheet (Rogers et al. 2013; Shtarkman et al. 2013), and Lake Whillans, 800 m beneath the West Antarctic Ice Sheet (Christner et al. 2014), has helped us to begin to understand the broad importance of subglacial ecosystems for polar biodiversity. To date, biological studies of the cave systems in Antarctica have been limited to assessments of fungal and microbiological diversity (Connell and Staudigel 2013; Herbold et al. 2014; Tebo et al. 2015). Our results highlight the importance of investigating these cave systems in greater detail—despite the field challenges associated with such an endeavour—to confirm the presence of living macrobiota.

Our results are useful for indicating the presence of a wide range of taxa at geothermal sites, but almost certainly underestimate true biological diversity in these environments; most of the organisms have patchy distributions, and sampling was not spatially comprehensive enough to ensure that a representative sample was obtained for robust diversity assessments. Furthermore, although we used 'universal' primers (primers that are known to be capable

Table 1 List of operational taxonomic units from subglacial sites for which the closest match on GenBank was a non-fungal eukaryote

Antarctic taxa found in caves	Probable genus (where match >90%)	Gene	OTU (see Online Resource 2)	At subglacial sites?			Also at exposed geothermal sites?		
				Warren Cave	22 Blue	Harry's dream	Mount Erebus	Mount Melbourne	Mount Rittman
Stramenopiles	? (closest match 79% ident: Oomycetes)	COI	COI_OTU_15		×				
Animalia: Arthropoda	? (closest match 82% ident: Arachnida)	COI	COI_OTU_2		×		×		
Animalia: Arthropoda: Collembola	? (closest match 85% ident: <i>Homidia</i>)	ITS	ITS_OTU_1	×	N/A		N/A	N/A	N/A
Animalia: Nematoda	<i>Enchodelus</i>	28S	28S_OTU_1	×					
Animalia: Oligochaeta	<i>Achaeta</i>	28S	28S_OTU_3	×					
Animalia: Oligochaeta	<i>Bryodrilus</i>	28S	28S_OTU_2	×					
Bryophyta	<i>Campylopus</i>	28S	28S_OTU_4	×			×	×	×
Chlorophyta	<i>Coccomyxa</i>	28S	28S_OTU_8		×		×	×	×
Chlorophyta	<i>Chlorella</i>	28S	28S_OTU_9		×		×	×	×
Chlorophyta	<i>Parietochloris</i>	28S	28S_OTU_10		×		×	×	×
Chlorophyta	<i>Coccomyxa</i>	28S	28S_OTU_12		×		×	×	×
Chlorophyta	<i>Stichococcus</i>	28S	28S_OTU_13		×		×	×	×
Chlorophyta	<i>Elliptochloris</i>	28S	28S_OTU_14		×		×	×	×
Chlorophyta	<i>Chloroidium</i>	28S	28S_OTU_17		×				
Chlorophyta	? (closest match not identified to genus)	ITS	ITS_OTU_10	×	N/A	×	N/A	N/A	N/A
Chlorophyta	? (closest match not identified to genus)	ITS	ITS_OTU_11	×	N/A	×	N/A	N/A	N/A
Chlorophyta	? (closest match not identified to genus)	ITS	ITS_OTU_12		N/A	×	N/A	N/A	N/A
Chlorophyta	<i>Neocystis mucosa</i>	ITS	ITS_OTU_13		N/A	×	N/A	N/A	N/A
Chlorophyta	? (closest match 80% ident: <i>Coccomyxa</i>)	ITS	ITS_OTU_14		N/A	×	N/A	N/A	N/A

Crosses indicate presence in one or more samples from each location. Comparison with exposed sites was not possible for ITS sequences as data were only available from Warren Cave and Harry's Dream. For a full list of all OTUs recovered from the study see Online Resource 2

of amplifying genes from diverse eukaryotes), some taxa might not be represented in our data because of primer-template mismatch. For example, tardigrades and rotifers—invertebrates found in many regions of Antarctica (Convey et al. 2008)—were not detected at any sites in our study, but their non-detection does not demonstrate absence.

The diversity of OTUs detected at the geothermal sites in this study was roughly comparable to results from DNA analyses of soil from elsewhere in Antarctica, such as on Signy Island in the South Orkney Islands (Lawley et al. 2004: eight nematode OTUs, two arthropod OTUs, two chlorophyte OTUs and low numbers of other taxa), and in the Taylor and Wright Dry Valleys (Fell et al. 2006: three nematode OTUs, 14 chlorophyte OTUs and 15 ciliate OTUs). Each study, however, used different methods,

preventing in-depth comparisons of regional diversity. Our research nonetheless indicates that geothermal sites in Antarctica, including some subglacial geothermal locations, have diverse biological (particularly algal and fungal) communities, emphasising the important role of geothermal systems in supporting life and fostering diversity in polar and sub-polar regions (Convey and Lewis Smith 2006; Kornobis et al. 2010; Fraser et al. 2014).

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