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Life in ruins: DNA metabarcoding contributes to the history of Whalers Bay wooden structures at Deception Island, South Shetland Islands

PAULO E.A.S. CÂMARA [1,2], FABYANO A. LOPES [3], FÁBIO L.V. BONES[2], MICHELINE CARVALHO-SILVA [1], PETER CONVEY [4,5], LÁUREN DE SOUZA[6], LÍVIA COELHO[6] and LUIZ ROSA[6]

[1]*Botany Department, University of Brasília, Campus Darcy Ribeiro, Brasília, Brazil*
[2]*Federal University of Santa Catarina, Florianópolis, Brazil*
[3]*Federal University of Tocantins, Porto Nacional, Brazil*
[4]*British Antarctic Survey, NERC, Cambridge, UK*
[5]*University of Johannesburg, Department of Zoology, Johannesburg, South Africa*
[6]*Federal University of Minas Gerais, Belo Horizonte, MG, Brazil*
paducamara@gmail.com

Abstract: Deception Island is an Antarctic Specially Managed Area that houses historically important sites such as the remains of historical wooden buildings. The impacts of fungal communities on wood in polar historical sites have been investigated, but little is known of the impacts of other eukaryote groups. In the current study we used high-throughput sequencing to investigate the diversity of non-fungal eukaryotic organisms present in wood samples from Whalers Bay. Four sites were sampled, and DNA sequences representing three kingdoms (Chromista, Protozoa and Viridiplantae) and four phyla (Ciliophora, Perclozoa, Chlorophyta and Magnoliophyta) were identified, representing a total of 43 taxa. Biscoe House Annex hosted the richest diversity, with 20 taxa, followed by the whaling boat, Biscoe House and the Hunting Lodge, with 16, 15 and 12 taxa, respectively. The most frequently detected sequences were assigned to the ciliate group Sporadotrichida, some of which are known to play a role in cellulose degradation. Among the Chlorophyta, the sequences detected included common taxa previously recorded, but the flowering plant data represented only exotic taxa, probably associated with human activity or airborne transfer. The use of high-throughput sequencing provided valuable data on communities associated with anthropogenically sourced and now decaying wood in Antarctica.

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Keywords: eDNA, HSM 71, HTS, ITS, Maritime Antarctic

Introduction

Deception Island (62°57'S, 60°38'W), located in the South Shetland Islands archipelago, is one of few active volcanic locations in Antarctica today (Fig. 1). Due to its unique geothermally associated flora, exceptional aesthetic and scientific value and historical importance, compounded by exposure to multiple contemporary sources of human impact from both national research operations and the tourism industry, the entire island has been designated as Antarctic Specially Managed Area (ASMA) 4, and it also contains two Antarctic Specially Protected Areas (ASPAs 140 and 145). The area also includes two Historic Sites or Monuments (HSM 71 and 76), an important reminder that human activity in Antarctic marine exploitation dates back to the early 1820s (Held & Blanchette 2017).

The sealing industry was very active in the South Shetland Islands during the nineteenth century after the archipelago's discovery in the early 1820s, followed by the whaling industry in the early twentieth century. The first shore-based whaling station on the island was established in 1911 - the Aktieselskabet Hecktor Whaling Station at Whalers Bay - which operated until 1931 when it was no longer commercially viable. In 1944, as part of a military operation towards the end of the Second World War, the UK constructed its Base B. In the post-war years, now operated by the Falkland Islands Dependencies Survey, that base was expanded to include a small aerodrome and associated hangar. Base B included some buildings from the preceding whaling station but also expanded its footprint further with new buildings. Active aircraft operations took place from 1955 to 1957 and again from 1959 to 1969. Base B was decommissioned in 1969, at least in part because many of its structures were heavily damaged during volcanic eruptions in 1967 and 1969 (Smith 1984, Morales *et al.* 2017). The area was partially cleared by the UK in 1990–1992, but many structures dating from both the whaling and research eras remain, with many of these

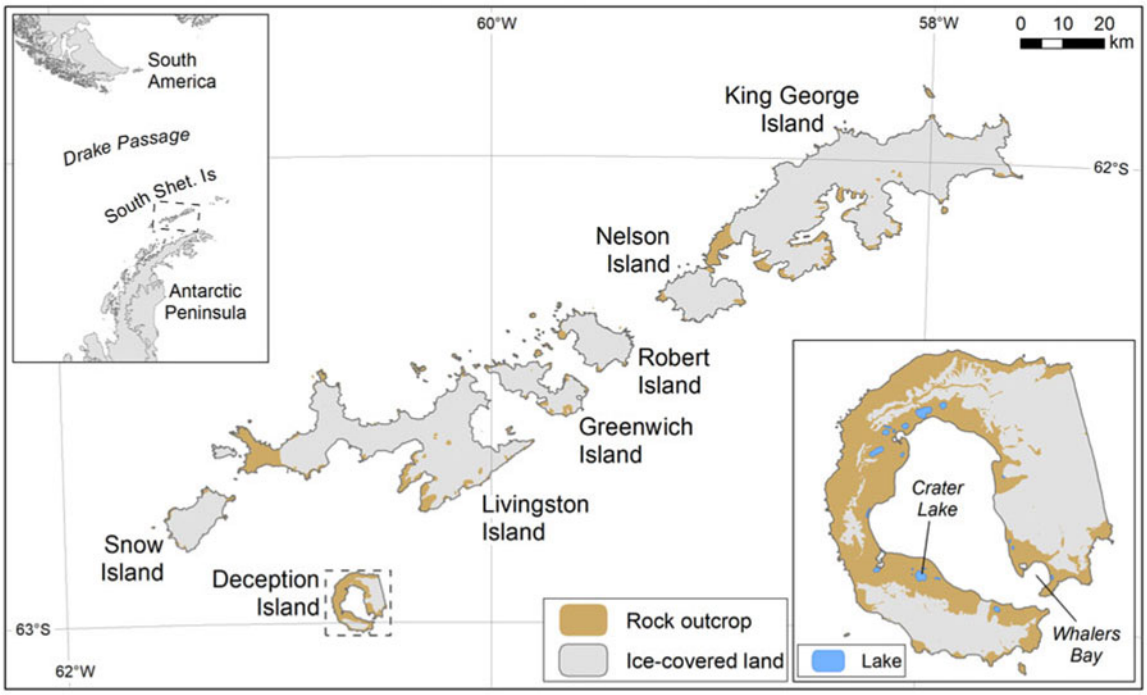

Figure 1. Location of Deception Island in the South Shetland Islands, Maritime Antarctica, where the samples were obtained.

Figure 2. Whalers Bay ruins where the wood samples were obtained. **a.** Whaling boat, **b.** Hunting Lodge, **c.** Biscoe House and **d.** Biscoe House Annex. Photographs: L.H. Rosa.

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Table I. Taxa present in the four sampled sites based on the assignment of amplicon sequence variants. Numbers indicate the DNA reads present in each sample.

Taxa	Biscoe House	Whaling boat	Biscoe House Annex	Hunting Lodge
Kingdom Chromista				
Phylum Ciliophora				
Acropisthiidae	0	18	0	0
Fuscheria sp.	0	0	27	0
Homalogastra sp.	0	0	235	0
Hymenostomatida	0	326	0	0
Oxytrichidae	0	33	278	0
Spirotrichea	0	88	0	20
Sporadotrichida	39	2052	2921	799
Trithigmostoma sp.	0	13	0	0
Kingdom Protozoa				
Phylum Perclozoa				
Allovahlkampfia sp.	0	22	371	551
Neovahlkampfia damariscottae	0	0	0	0
Kingdom Viridiplantae				
Phylum Chlorophyta	0	0	0	81
Trebouxiophyceae	0	20	0	22
Chlamydomonadales	23	183	39	39
Chlamydomonas nivalis	0	0	29	0
Chloromonas sp.	0	48	0	0
Chloromonas fonticola	0	0	15	0
Chlorellales				
Chlorella pituita	0	0	117	0
Chlorella vulgaris	0	0	2238	0
Dictyosphaerium minutum	0	0	288	0
Pseudochlorella pyrenoidosa	0	0	0	71
Trebouxiales				
Coccomyxa sp.	428	0	421	43
Coccomyxa antarctica	0	0	0	99
Chloroidium engadinense	15	0	0	0
Lobosphaera incisa	30	0	0	0
Trebouxia sp.	1873	96	0	388
Trebouxia flava	138	0	0	0
Trebouxia jamesii 'lethariі'	0	0	0	1222
Trebouxia potteri	278	0	0	0
Prasiolales				
Elliptochloris reniformis	110	0	0	0
Stichococcus bacillaris	62	0	101	32
Stichococcus mirabilis	16	32	0	0
Prasiola delicata	0	17	0	0
Stichococcus sp.	0	18	1169	334
Raphidonema nivale	0	0	31	0
Sphaeropleales				
Gloeocystis polydermatica	65	0	0	0
Neocystis mucosa	241	0	742	0
Ulotrichales				
Planophila sp.	0	0	0	29
Ulvales				
Kornmannia sp.	0	0	576	13
Pseudendoclonium submarinum	0	0	0	15
Phylum Magnoliophyta				
Apiaceae				
Petroselinum crispum	19	43	0	19
Fabaceae				
Glycine soja	07	30	0	0
Myrtaceae				
Eucalyptus fulgens	0	19	0	0
Rosaceae				
Prunus sp.	0	0	18	0
Total	*3344*	*3058*	*9646*	*3777*

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being constructed from or incorporating wood imported to the island (Fig. 2). The Whalers Bay site has been under the protection of the Antarctic Treaty since being designated as HSM 71 in 1995.

Whalers Bay is one of the most visited and popular sites in Antarctica by both national operator staff and the Antarctic tourism industry. Two national operators (Argentina and Spain) currently operate summer-only research stations on the island, with Chile and the UK previously operating year-round stations before the late 1960s' eruptions. Research and logistical support vessels from multiple national operators also routinely visit the island and land personnel in most summers. A total of ~160 000 tourists visited Whalers Bay between 2010 and 2019 (www.iaato.org; Carvalho-Silva *et al.* 2021), the majority of whom visited and explored the ruins of HSM 71. Human impacts on Whalers Bay local terrestrial ecosystems, in the context of the presence of non-native species anthropogenically transferred to the island (Greenslade *et al.* 2012, Hughes *et al.* 2015) or of environmental DNA (eDNA) sequences assigned to exotic species (Rosa *et al.* 2020, Câmara *et al*. 2021b, Carvalho-Silva *et al.* 2021), have received some research attention. Many of the wooden structures in Whalers Bay are > 100 years old and are increasingly deteriorating. The effects of decomposer fungal communities on the wood of many Antarctic and Arctic historical sites have been investigated, including on Deception Island (Held *et al.* 2011, Held & Blanchette 2017, Blanchette *et al.* 2021). However, little to nothing is known regarding the impacts of other organism groups that may have become established on these exotic wood habitats, such as microalgae and protozoans, as well as regarding the presence of pollen and spores.

Many organisms of these types are difficult to identify conclusively, requiring the development of appropriate culture methodologies, and some may be encysted. Recently developed molecular tools such as DNA metabarcoding using high-throughput sequencing (HTS; Taberlet *et al.* 2012) are increasingly being applied to infer the presence of active or dormant life forms, propagules, pollen and detritus of plants (Fahner *et al.* 2016). These methodologies use the total and even sometimes the degraded DNA extracted from environmental samples (e.g. water, soil or air). The methodology has the potential to increase by a factor of ~11 the number of taxa detected when compared with classical morphological approaches (Rippin *et al.* 2018), and they can reveal the presence of DNA of taxa not detectable through traditional surveys. In the current study, we used HTS of eDNA to investigate the diversity of non-fungal eukaryotic organisms present in wood samples obtained from various sites in HSM 71 Whalers Bay on Deception Island.

Methods

Study sites and sampling

Four sites were selected for sampling: Biscoe House, an abandoned whaling boat, the Biscoe House Annex and the Hunting Lodge (Fig. 2), all located in close proximity to each other. Three small samples (~1 cm^3) of wood from each site were obtained during the Antarctic summer (December 2016) and placed into individual sterilized Whirl-Pak bags (Sigma-Aldrich, USA), which were sealed and kept at -20°C for 3 weeks until being processed at the Microbiology Laboratory at the Federal University of Minas Gerais, Brazil. Data from each sample were obtained separately but are presented together in Table I.

DNA extraction, amplification and sequencing

Total DNA was extracted using a modified sodium dodecyl sulphate (SDS) extraction method (Goldenberger *et al.* 1995, Zhou *et al.* 1996, Natarajan *et al.* 2016). Wood fragments of ~1 cm^3 were added to plastic tubes each containing 2 ml of SDS extraction buffer (0.1 M ethylenediaminetetraacetic acid (EDTA) at pH 8 and 2% SDS) and ground with sterilized iron beads for 3 min before being incubated at 55°C for 16 h. Then, 330 µl of 5 M NaCl and 330 µl of pre-heated cetrimonium bromide (CTAB) 10% (55°C) were added; the solution was then vortexed, spun down and incubated at 55°C for 10 min. The solution was then transferred to a new tube, and 600 µl of chloroform was added and vortexed at maximum speed for 1 min. After that, the tubes were centrifuged at 13 000 rpm for 10 min and the supernatant transferred to a new tube.

The extracted DNA was cleaned using the Genomic DNA Purification Kit (QIAGEN, USA) following the manufacturer's instructions. Extractions were carried out under strict sterile conditions to avoid contamination (de Menezes *et al.* 2020). Samples were manipulated inside a sterile flow hood, and all equipment (forceps, spatula, etc.) was previously disinfected. ddH_2O samples were used as blanks to ensure the absence of contamination.

DNA quality was analysed using agarose gel electrophoresis (1% agarose in 1 × Trisborate-EDTA) and then quantified using the Quanti- iT™ Pico Green dsDNA Assay (Invitrogen, USA). The internal transcribed spacer 2 region (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification (Chen *et al.* 2010, Richardson *et al.* 2015, Hadi *et al.* 2016, Ruppert *et al.* 2019, Câmara *et al.* 2021a,b, 2022, Carvalho-Silva *et al.* 2021) using the universal primers ITS3 and ITS4 (White *et al.* 1990). Library construction and DNA amplification were performed using the Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2, following the

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Illumina 16S Metagenomic Sequencing Library Preparation Part #15044223 Rev. B protocol. Paired-end sequencing (2 × 300 bp) was performed commercially on a MiSeq System (Illumina, USA) by Macrogen, Inc. (South Korea), including negative controls.

Data analyses and taxa identification

Quality analysis was carried out using *BBDuk* v. 38.87 in *BBmap* software (Bushnell 2014) with the following parameters: Illumina adapters removing (Illumina artefacts and the PhiX Control v3 Library); ktrim = l; k = 23; mink = 11; hdist = 1; minlen = 50; tpe; tbo; qtrim = rl; trimq = 20; ftm = 5; maq = 20. The remaining sequences were imported into *QIIME2* version 2021.4 (https://qiime2.org/) for bioinformatics analyses (Bolyen *et al.* 2019). The *qiime2-dada2* plugin was used for filtering, dereplication, turning paired-end fastq files into merged files, removing chimeras and creating amplicon sequence variants (ASVs) with default parameters (Callahan *et al.* 2016). Taxonomic assignments of ASVs were determined using the *qiime2-feature-classifier* (Bokulich *et al.* 2018) *classify-sklearn* against different databases; the sequence similarity threshold was set at 97%. Firstly, ASVs were classified against the PLANiTS2 database (Banchi *et al.* 2020). After this step, ASVs that were not classified were filtered and *classify-sklearn* classified against the UNITE Eukaryotes ITS database version 8.3 (Abarenkov *et al.* 2020). Finally, any remaining unclassified ASVs were filtered and aligned against the filtered National Center for Biotechnology Information (NCBI) non-redundant nucleotide sequences (nt) database (October 2021) using *BLASTn* (Camacho *et al.* 2009) with default parameters; the nt database was filtered with the following keywords: 'ITS1', 'ITS2', 'internal transcribed spacer' and 'internal transcribed spacer'. Taxonomic assignments were performed using *MEGAN6* (Huson *et al.* 2016).

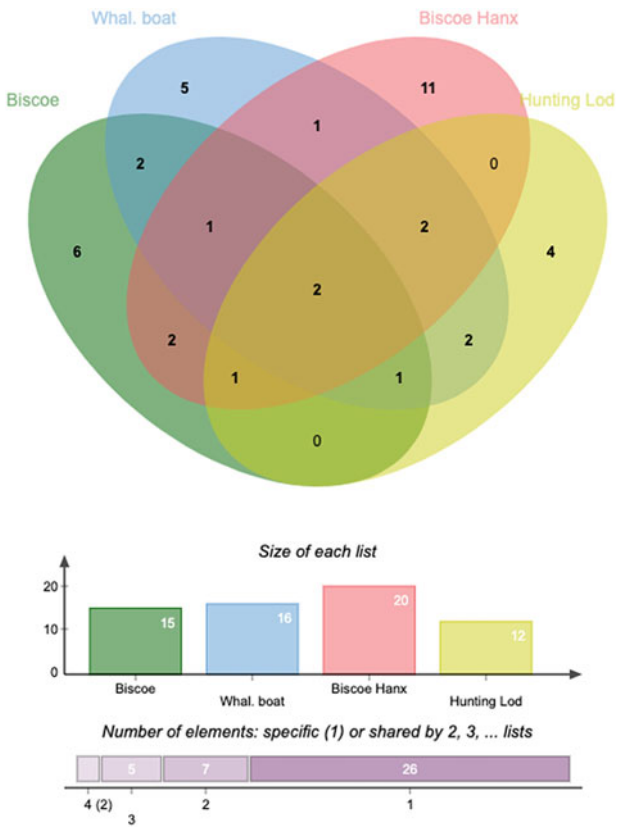

Figure 3. Venn diagram showing the taxa distribution and shared species among the different samples. Biscoe = Biscoe House; Biscoe HAnx = Biscoe House Annex; Hunting Lod = Hunting Lodge; Whal. Boat = Whaling boat.

For comparative purposes, the number of reads can be used as a proxy for relative abundance (Giner *et al.* 2016, Deiner *et al.* 2017, Câmara *et al.* 2021a,b, 2022). Classifications and systematic ranks for kingdoms and phyla followed Ruggiero *et al.* (2015). Venn diagrams were prepared as described by Bardou *et al.* (2014).

Results

A total of 2 327 468 reads were obtained, of which 2 218 794 remained after quality control (for detailed data, see Supplemental Table 1). A total of 19 820 reads were DNA sequences representing three kingdoms (Chromista, Protozoa and Viridiplantae) and four phyla (Ciliophora, Perclozoa, Chlorophyta and Magnoliophyta). The remaining reads corresponded to fungi (de Souza *et al.* 2022). All calculated rarefaction curves (Supplemental Fig. 1) reached a plateau, indicating that the sampling effort was sufficient to represent the taxa analysed in all sampled sites. Sequences representing a total of 43 taxa were detected (Table I). Biscoe House Annex hosted the greatest diversity, with 20 taxa, followed by the whaling boat, Biscoe House and the Hunting Lodge, with 16, 15 and 12 taxa, respectively. Representatives of the ciliate order Sporadotrichida were the most frequently detected sequences, this being the only ciliate taxa present at all sites, followed by the green algal genus *Trebouxia*, which was present in three of the four sites. The Venn diagram illustrates that only two taxa were detected at all sites (Sporadotrichida and Chlamydomonadales; Fig. 3).

Non-metric multidimensional scaling and cluster analysis did not identify any differences between sampled structures, which were in close proximity, of similar material and subject to the same environmental conditions. Data are available upon request.

Discussion

Among the Chromista detected, although giving the greatest taxonomic resolution of sequence assignments,

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none were assigned to species level. *Fuscheria* is a genus of approximately six species and is globally distributed, inhabiting terrestrial, fresh, brackish and marine environments (Petz *et al*. 1995). Two terrestrial species - *Fuscheria lacustres* and *Fuscheria terricola* - have been reported from the Antarctic continent near Casey Station (ATCM 2013, Thompson *et al*. 2019), and the Maritime Antarctic species *Fuscheria marina* has been reported from the Weddell Sea (Petz *et al.* 1995). The genus *Homalogastra* includes one species record (*Homalogastra setosa*) from Antarctica (Thompson *et al.* 2019). *Trithigmostoma* is a cosmopolitan genus with five species inhabiting fresh, marine and brackish water. It has been reported from Antarctica from soils in Wilkes Land (Petz & Foissner 1997). There are no previous records of these genera from Deception Island.

All other taxa were assigned to higher taxonomic levels. Some of these include taxa with wide geographical distributions, as also reported by Câmara *et al.* (2021a). The very abundant order Sporadotrichida is a highly diverse group present in marine, freshwater and terrestrial habitats, containing four families and 83 genera, some of which are very common, such as *Halteria*, *Oxytricha* and *Cyrtohymena* (Parr *et al.* 2014), which are all recorded from Antarctica (Thompson *et al.* 2019, Câmara *et al.* 2021a,b,c). As sequences were assigned only at the order level, and considering that this order contains many very common species, this may be suggestive of the presence of new and as yet undescribed species present on the wood sampled. Ciliates are known to feed on bacteria, which are likely to be present in decaying wood. Protists are also known to play an important role in cellulose degradation (Peterson *et al.* 2015). However, functional roles cannot be inferred directly in the current study. Ciliates in the class Spirotrichea and family Acropisthiidae are known to occur in both marine and terrestrial environments in Antarctica (De Broyer *et al.* 2020). The ciliate groups assigned here are widespread and have been previously recorded in Antarctica, and our data confirm their presence in decaying wood of anthropogenic origin.

Among the Protozoa, the genus *Allovahlkampfia* includes free-living amoebae that feed primarily on bacteria. It has previously been recorded from Deception and King George islands (Câmara *et al.* 2021b), and representatives are known to be capable of causing disease in humans (Mohamed *et al.* 2016, Tolba *et al.* 2016). In contrast, *Neovahlkampfia* is a genus that includes only one described marine species that has not been previously recorded from Antarctica. The taxonomy of these genera remains poorly known (Jonckheere *et al.* 2011). However, as with the ciliates, bacterivorous organisms are expected to be found on decaying wood.

The assigned Viridiplantae sequences include some very common taxa (genera *Trebouxia*, *Chlamydomonas* and *Prasiola*) previously recorded from multiple locations in Antarctica, including Deception Island (e.g. Câmara *et al.* 2021a,b, Fonseca *et al.* 2022). The close proximity of the Whalers Bay sampling locations to the sea as well as to snow and ice could underlie the taxon composition found. Representatives of *Trebouxia* are very common and widespread in Antarctica (Câmara *et al.* 2021a,b), and some members are also photobionts in lichens found growing on the wood surface as well as on other natural substrata (de Souza *et al*. 2022).

In contrast with the algae, the flowering plant (Magnoliophyta) sequence assignments all represented exotic taxa not native to Antarctica, and the presence of their DNA is probably indicative of either association with human activity or the airborne transfer of pollen. *Petroselinum crispum* (parsley) is a plant originally in the Mediterranean region but now widely cultivated around the planet and used for culinary purposes. *Glycine soja* (soybean), originally from Asia, is today one of the most important crop plants on the planet and is widely used as food (milk, tofu, oil, etc.). *Eucalyptus fulgens* (green scent bark) is a tree species endemic to Australia; however, this assignment could be an artefact arising from the quality or completeness of the database consulted, as the genus *Eucalyptus* includes > 700 species, many widely cultivated in the Americas, Europe, Asia and Africa for timber, cellulose or oil. The ruined wooden structures on Deception Island were primarily constructed of pine (*Pinus* sp.) and spruce (*Picea* sp.; Held *et al*. 2011), genera that were not detected in the current study. However, even today many wooden materials are imported to Antarctica (Osyczka *et al.* 2012), and ~47% of the garbage found in Antarctica comprises or includes wood (Anfuso *et al.* 2020). Lityńska-Zając *et al.* (2012) have also shown that many wood fragments arrive in Antarctica accidentally. Finally, the genus *Prunus* includes some of the most widely consumed food fruits, including cherries, almonds, plums, apricots and peaches, again suggestive of human influence.

Although we recognize that the possibility exists of pollen or plant propagules reaching Deception Island independently, taking into consideration the high number of visitors to the island (Roura 2012, Carvalho-Silva *et al.* 2021) and the ruderal nature of all of the flowering plant taxa assigned, we believe that there is a strong likelihood of the detection of flowering plant sequences here being a consequence of human activity. Carvalho-Silva *et al.* (2021), in a study of eDNA from Whalers Bay soil samples, similarly reported assignments of many taxa potentially linked with human activities, including foodstuffs.

It is always important to note that assignment of a DNA sequence from eDNA samples does not confirm the presence of the organism itself or viable parts of it.

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The identification of exotic eDNA highlights the need for protecting these ecosystems from inadvertent and irreversible genetic contamination. The method used here provided valuable and novel data on communities living on exotic decaying wood in Antarctica in addition to the better-studied Fungi and Bacteria.

Conclusions

Historical wooden ruins in Antarctica host a very diverse and largely unknown assembly of organisms, including some not previously reported from the region. DNA metabarcoding using HTS is a useful tool for detecting species that would be otherwise difficult or impossible to identify by morphology alone, including organisms that may be encysted or that cannot at present be grown in culture. The assignment of identities based on DNA sequences alone does not confirm the presence of living organisms or viable propagules, and the assignments themselves rely heavily on the quality of consulted databases, which has been increasing substantially in recent years. In addition, no single DNA marker is effective for all groups of organisms, and further studies using a wider range of markers, backed by traditional direct observation and culturing methods, will ultimately be required to provide a more thorough description of the diversity present. The data presented here will be useful in the development of conservation policies and in studies on colonization processes and human influence in Antarctica.

Author contributions

PEASC, MC-S, LdS and LR performed the fieldwork, PEASC, FAL and FLVB and performed the bioinformatic and data analysis, MC-S analysed the flowering plant data and PEASC analysed the non-flowering plant data. PEASC, PC and LR conceived the paper and its experimental design and provided funds. All of the authors participated in writing the paper.

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Competing interests

The authors declare no conflicts of interest.

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References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, H.R. & Kõljalg, U. 2020. UNITE QIIME Release for Eukaryotes 2. Version 4: 2020. Retrieved from https://doi.org/10.15156/BIO/786388

Anfuso, G., Bolívar-Anillo, H.J., Asensio-Montesinos, F., Portantiolo Manzolli, R., Portz, L. & Villate Daza, D.A. 2020. Beach litter distribution in Admiralty Bay, King George Island, Antarctica. *Marine Pollution Bulletin*, **160**, 10.1016/j.marpolbul.2020.111657.

ATCM. 2013. Management Plan for Antarctic Specially Protected Area No 135. Retrieved from https://documents.ats.aq/recatt/att192_e.pdf

Banchi, E., Ametrano, C.G., Greco, S., Stanković, D., Muggia, L. & Pallavicini, A. 2020. PLANiTS: a curated sequence reference dataset for plant ITS DNA metabarcoding. *Database: The Journal of Biological Databases and Curation*, **2020**, 10.1093/database/baz155.

Bardou, P., Mariette, J., Escudié, F., Djemiel, C. & Klopp, C. 2014. *Jvenn*: an interactive Venn diagram viewer. *BMC Bioinformatics*, **15**, 10.1186/1471-2105-15-293.

Blanchette, R.A., Held, B.W., Jurgens, J., Stear, A. & Dupont, C. 2021. Fungi attacking historic wood of Fort Conger and the Peary Huts in the High Arctic. *PLoS ONE*, **16**, 10.1371/journal.pone.0246049.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., *et al*. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with *QIIME* 2's *q2-feature-classifier* plugin. *Microbiome*, **6**, 10.1186/s40168-018-0470-z.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander H., *et al*. 2019. Reproducible, interactive, scalable and extensible microbiome data science using *QIIME* 2. *Nature Biotechnology*, **37**, 10.1038/s41587-019-0209-9.

Bushnell, B. 2014. *BBMap*: a fast, accurate, splice-aware aligner. Berkeley, CA: Lawrence Berkeley National Lab (LBNL). Retrieved from sourceforge.net/projects/bbmap

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. 2016. *DADA*2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, **13**, 10.1038/nmeth.3869.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K. & Madden, T.L. 2009. *BLAST*+: architecture and applications. *BMC Bioinformatics*, **10**, 10.1186/1471-2105-10-421.

Câmara, P.E.A.S., de Souza, L.M.D., Pinto, O., Convey, P., Amorim, E.T., Carvalho-Silva, M. & Rosa, L.H. 2021a. Periphyton diversity in two different Antarctic lakes assessed using metabarcoding. *Antarctic Science*, **33**, 10.1017/S0954102021000316.

Câmara, P.E.A.S., Carvalho-Silva, M., Pinto, O.H.B., Amorin, E.T., Henriques, D.K., da Silva, T.H., *et al*. 2021b. Diversity and ecology of Chlorophyta (Viridiplantae) assemblages in protected and non-protected sites in Deception Island (Antarctica, South Shetland Islands) assessed using an NGS approach. *Microbial Ecology*, **81**, 10.1007/s00248-020-01584-9.

Câmara, P.E.A.S., Convey, P., Rangel, S.B., Konrath, M., Barreto, C.C., Pinto, O.H.B., *et al*. 2021c. The largest moss carpet transplant in Antarctica and its bryosphere cryptic biodiversity. *Extremophiles*, **25**, 10.1007/s00792-021-01235-y.

Câmara, P.E.A.S., de Menezes, G.C.A., Oliveira, F.S., Souza, C.D., Amorim, E.T., Shaefer, C.E.G.R., *et al*. 2022. Diversity of Viridiplantae DNA present on rock surfaces in the Ellsworth Mountains, Continental Antarctica. *Polar Biology*, **45**, 10.1007/s00300-022-03021-8.

Carvalho-Silva, M., Rosa, L.H., Pinto, O., Da Silva, T., Henriques, D.K., Convey, P. & Câmara, P.E.A.S. 2021. Exploring the plant environmental DNA diversity in soil from two sites on Deception Island (Antarctica, South Shetland Islands) using metabarcoding. *Antarctic Science*, **33**, 10.1017/S0954102021000274.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., *et al*. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS One*, **5**, 10.1371/journal.pone.0008613.

De Broyer, C., Clarke, A., Koubbi, P., Pakhomov, E., Scott, F., Vanden Berghe, E. & Danis, B., eds. 2020. Register of Antarctic Marine Species. Spirotrichea. Retrieved from http://www.marinespecies.org/RAMS/aphia.php?p=taxdetails&id=1348

de Menezes, G.C.A., Porto, B.A., Amorim, S.S., Zani, C.L., de Almeida Alves, T.M., Sales Junior, P.A., *et al*. 2020. Fungi in glacial ice of Antarctica: diversity, distribution and bioprospecting of bioactive compounds. *Extremophiles*, **24**, 10.1007/s00792-020-01161-5.

De Souza, L.M.D., Teixeira, E.A.A., Coelho, L.C., Lopes, F.A.C., Convey, P., Carvalho-Silva, M., *et al*. 2022. Cryptic fungal diversity in historic wooden structures at Whalers Bay, Deception Island, Maritime Antarctic, revealed using DNA metabarcoding. *Brazilian Journal of Microbiology*, **54**, 10.1007/s42770-022-00869-0.

Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., *et al*. 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Molecular Ecology*, **26**, 10.1111/mec.14350.

Fahner, N.A., Shokralla, S., Baird, D.J. & Hajibabaei, M. 2016. Large-scale monitoring of plants through environmental DNA metabarcoding of soil: recovery, resolution, and annotation of four DNA markers. *PLoS One*, **11**, 10.1371/journal.pone.0157505.

Fonseca, B.M., Câmara, P.E.A.S., Ogaki, M.B., Pinto, O.H.B., Lirio, J.M., Coria, S.H., *et al*. 2022. Green algae (Viridiplantae) in sediments from three lakes on Vega Island, Antarctica, assessed using DNA metabarcoding. *Molecular Biology Reports*, **49**, 10.1007/s11033-021-06857-1.

Giner, C.R., Forn, I., Romac, S., Logares, R., de Vargas, C. & Massana, R. 2016. Environmental sequencing provides reasonable estimates of the relative abundance of specific picoeukaryotes. *Applied Environmental Microbiology*, **82**, 10.1128/AEM.00560-16.

Goldenberger, D., Perschil, I., Ritzler, M. & Alwegg, M. 1995. A simple 'universal' DNA extraction procedure using SDS and Proteinase K is compatible with direct PCR amplification. *PCR Methods and Applications*, **4**, 10.1101/gr.4.6.368.

Greenslade, P., Potapov, M., Russell, D. & Convey, P. 2012. Global Collembola on Deception Island. *Journal of Insect Science*, **12**, 10.1673/031.012.11101.

Hadi, S.I.I.A., Santana, H., Brunale, P.P.M., Gomes, T.G., Oliveira, M.D., Matthiensen, A., *et al*. 2016. DNA barcoding green microalgae isolated from Neotropical inland waters. *PLoS ONE*, **11**, 10. 1371/journal.pone.0149284.

Held, B.W. & Blanchette, R.A. 2017. Deception Island, Antarctica, harbors a diverse assemblage of wood decay fungi. *Fungal Biology*, **121**, 10.1016/j.funbio.2016.11.009.

Held, B.W., Arenz, B.E. & Blanchette, R.A. 2011 Factors influencing deterioration of historic structures at Deception Island, Antarctica. *In* Barr, S. & Chaplin, P., *eds*, *Polar settlements – location, techniques and conservation*. ICOMOS Monuments and Sites. Oslo, Norway: International Polar Heritage Committee, 35–43.

Hughes, K.A., Pertierra, L.R., Molina-Montenegro, M.A. & Convey, P. 2015. Biological invasions in terrestrial Antarctica: what is the current status and can we respond? *Biodiversity and Conservation*, **24**, 10.1007/s10531-015-0896-6.

Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., *et al*. 2016. *MEGAN* community edition - interactive exploration and analysis of large-scale microbiome sequencing data. *PLoS Computational Biology*, **12**, 10.1371/journal.pcbi.1004957.

Jonckheere, J.F., Murase, J. & Opperdoes, F.R. 2011. A new thermophilic heterolobosean amoeba, *Fumarolamoeba ceborucoi*, gen. nov., sp. nov., isolated near a fumarole at a volcano in Mexico. *Acta Protozoologica*, **50**, 10.4467/16890027AP.11.005.0005.

Lityńska-Zając, M., Chwedorzewska, K., Olech, M., Korczak-Abshire, M. & Augustyniuk-Kram, A. 2012. Diaspores and phyto-remains accidentally transported to the Antarctic station during three expeditions. *Biodiversity and Conservation*, **21**, 10.1007/s10531-012-0371-6.

Mohamed, M.E., Huseein, E.A., Farrag, H.M., Mostafa, F.A.A. & Hassan, A.T. 2016. *Allovahlkampfia spelaea* is a potential environmental host for pathogenic bacteria. *Journal of Bacteriology and Parasitology*, **7**, 10.4172/2155-9597.1000255.

Morales, J.V., Almendros, J. & Carmona, E. 2017. Detection of long-duration tremors at Deception Island volcano, Antarctica. *Journal of Volcanology and Geothermal Research*, **347**, 10.1016/j.jvolgeores.2017.09.016.

Natarajan, V.P., Zhang, X., Morono, Y., Inagaki, F. & Wang, F. 2016. A modified SDS-Based DNA extraction method for high quality environmental DNA from seafloor environments. *Frontiers in Microbiology*, **7**, 10.3389/fmicb.2016.00986.

Osyczka, P., Mleczko, P., Karasiński, D. & Chlebicki, A. 2012. Timber transported to Antarctica: a potential and undesirable carrier for alien fungi and insects. *Biological Invasions*, **14**, 10.1007/s10530-011-9991-0.

Parr, C.S., Wilson, N., Leary, P., Schulz, K.S., Lans, K., Walley, L., *et al*. 2014. The Encyclopedia of Life v2: providing global access to knowledge about life on Earth. *Biodiversity Data Journal*, **2**, 10.3897/BDJ.2.e1079.

Peterson, B.F., Stewart, H.L. & Scharf, M.E. 2015. Quantification of symbiotic contributions to lower termite lignocellulose digestion using antimicrobial treatments. *Insect Biochemical Molecular Biology*, **59**, 10.1016/j.ibmb.2015.02.009.

Petz, W. & Foissner, W. 1997. Morphology and infraciliature of some soil ciliates (Protozoa, Ciliophora) from continental Antarctica, with notes on the morphogenesis of *Sterkiella histriomuscorum*. *Polar Record*, **33**, 10.1017/S0032247400025407.

Petz, W., Song, W. & Wilbert, N. 1995. Taxonomy and ecology of the ciliate fauna (Protozoa, Ciliophora) in the endopagial and pelagial of the Weddell Sea, Antarctica. *Stapfia*, **40**, 1–223.

Richardson, R.T., Lin, C., Sponsler, D.B., Quijia, J.O., Goodell, K. & Johnson, R.M. 2015. Application of ITS2 metabarcoding to determine the provenance of pollen collected by honey bees in an agroecosystem. *Applications in Plant Sciences*, **3**, 10.3732/apps.1400066.

Rippin, M., Borchhardt, N., Williams, L., Colesie, C., Jung, P., Büdel, B., *et al*. 2018. Genus richness of microalgae and cyanobacteria in biological soil crusts from Svalbard and Livingston Island: morphological versus molecular approaches. *Polar Biology*, **41**, 10.1007/s00300-018-2252-2.

Rosa, L.H., da Silva, T.H., Ogaki, M.B., Pinto, O.H.B., Stech, M., Convey, P., *et al*. 2020. DNA metabarcoding uncovers fungal diversity in soils of protected and non-protected areas on Deception Island, Antarctica. *Scientific Reports*, **10**, 10.1038/s41598-020-78934-7.

Roura, M. 2012. Being there: examining the behavior of Antarctic tourists through their blogs. *Polar Research*, **201**, 10.3402/polar.v31i0.10905.

Ruggiero, M.A., Gordon, D.P., Orrell, T.M., Bailly, N., Bourgoin, T., Brusca, R.C., *et al*. 2015. Correction: a higher level classification of all living organisms. *PLoS ONE*, **10**, 10.1371/journal.pone.0130114.

Ruppert, K., Kline, R.J. & Rahman, M.S. 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: a systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, **17**, 10.1016/j.gecco.2019.e00547.

Smith, R.I.L 1984. Colonization and recovery by cryptogams following recent volcanic activity on Deception Island, South Shetland Islands. *British Antarctic Survey Bulletin*, **62**, 25–51.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C. & Willerslev, E. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, **21**, 10.1111/j.1365-294X.2012.05470.x.

Thompson, A., Powell, G. & Adams, B. 2019. Provisional checklist of terrestrial heterotrophic protists from Antarctica. *Antarctic Science*, **31**, 10.1017/S0954102019000361.

Tolba, M.E.M., Huseein, E.A.M., Farrag, H.M.M., Mohamed, H.E.D., Kobayashi, S., Suzuki, J., *et al*. 2016. *Allovahlkampfia spelaea* causing keratitis in Humans. *PLoS Neglected Tropical Diseases*, **10**, 10.1371/journal.pntd.0004841.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J., *eds*, *PCR protocols: a guide to methods and applications*. Molecular Biology Reports. London: Academic Press, 515–322.

Zhou, J., Bruns, M.A. & Tiedje, J.M. 1996. DNA recovery from soils of diverse composition. *Applied and Environmental Microbiology*, **62**, 10.1128/aem.62.2.316-322.1996.