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Abstract: Antarctic ice-free areas are dominated by wind-dispersed organisms. However, which organisms arrive and circulate in Antarctica and how remain poorly understood. Due to their proximity to South America and less extreme conditions, the South Shetland Islands are likely to receive higher diaspore numbers. One possible consequence of climate change is that newcomers will be able to colonize ice-free areas, altering community compositions and impacting the native biota. We used DNA metabarcoding to identify non-fungal eukaryotic DNA present in the air that could potentially reach and circulate in Antarctica. Air was sampled near the Brazilian Comandante Ferraz Antarctic Station on King George Island between December 2019 and January 2020. Sequences representing a total of 35 taxa from 10 phyla and 3 kingdoms were assigned: Chromista (Ciliophora, Cercozoa, Haptophyta and Ochrophyta), Plantae (Chlorophyta, Bryophyta and Magnoliophyta) and Animalia (Mollusca, Arthropoda and Chordata). The most diverse group were the plants (26 taxa), followed by Chromista (6 taxa). The most abundant sequences represented the green algae *Chlamydomonas nivalis*. The two angiosperm sequences represent exotic taxa; *Folsomia* is also exotic and was recorded only on Deception Island. Metabarcoding revealed the presence of previously undocumented airborne diversity, suggesting that the Antarctic airspora includes propagules of both local and distant origin.

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Key words: Aerosol, airspora, climate change, colonization, dispersal, invasion

Introduction

The vegetation of ice-free areas in Antarctica is dominated by cryptogams and microorganisms, groups that are often dispersed in the air column, to a greater extent than almost any other region globally (Marshall & Chalmers 1997, Øvstedal & Smith 2001, Ruisi *et al.* 2007, Ochyra *et al.* 2008, Pearce *et al.* 2016, Sancho *et al.* 2017). Dispersal can take place in the form of whole organisms (especially in the case of unicellular species) or in the form of spores, viable fragments or specialized vegetative propagules. Together, these dispersing propagules constitute the diaspore or propagule rain (Sundberg 2013), which influences not only the species composition of ecosystems but also the atmosphere and climate itself, for example by acting as condensation nuclei for clouds (Després *et al.* 2012, Šantl-Temkiv *et al.* 2022). After deposition, they can start growing or become part of the soil propagule bank (Smith 1991, During 2001), in which they can potentially remain dormant until local conditions become favourable for germination. However, the mode and selectivity of atmospheric dispersal to and within Antarctica remain poorly documented and understood (Bottos *et al.* 2014, Pearce *et al.* 2016, Rosa *et al.* 2020, 2021).

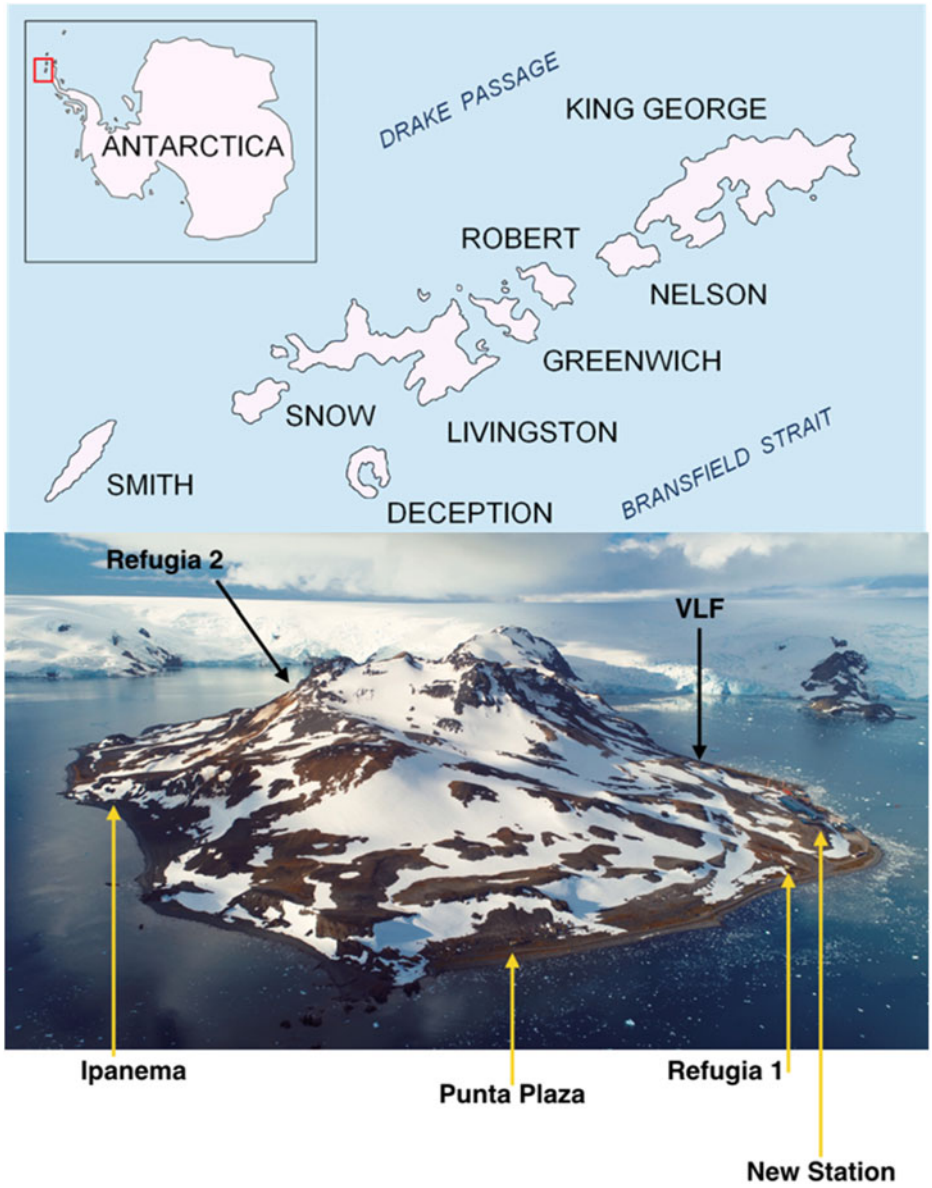

Figure 1. (Top) Map of the South Shetland Islands showing King George Island (obtained from Antarctic Places Commission of Bulgaria; apcbg.org). (Bottom) Map of Keller Peninsula showing Comandante Ferraz Antarctic Station and the various facilities around it, including Punta Plaza, the site where the air was sampled. Photograph by L.H. Rosa. VLF = very low frequency laboratory.

Antarctic vegetation is sensitive to the various components of climate change (Amesbury *et al.* 2017, Robinson *et al.* 2018, Convey & Peck 2019). Given that Antarctic organisms, such as bryophytes, can respond quickly to environmental and climatic changes (Sancho *et al.* 2007, Tuba *et al.* 2011), one expected consequence of future climate change is that new species arriving in the airspora will be able to colonize ice-free terrestrial areas, altering community composition, species abundance and the distributions of native taxa (Frahm & Klaus 2001, Sancho *et al.* 2007, Royles *et al.* 2013). This may have consequences for the availability of microhabitats for invertebrates such as nematodes and microarthropods, as well as for microorganisms such as bacteria, microalgae and fungi (Hogg *et al.* 2006, Bokhorst *et al.* 2008, Glime 2017, Câmara *et al.* 2021b, Carvalho-Silva *et al.* 2021, Rosa *et al.* 2021). The Antarctic Peninsula and the South Shetland Islands in particular experienced the fastest rates of warming in Antarctica in the second half of the twentieth century (Turner *et al.* 2009), although this trend paused early in the twenty-first century (Turner *et al.* 2016), with considerable effects on the lichen and bryophyte vegetation (Cannone *et al.* 2017, 2022, Sancho *et al.* 2017, 2019, Câmara *et al.* 2021b).

The South Shetland Islands, lying north-west of the tip of the Antarctic Peninsula, are likely to receive higher

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numbers of diaspores of both local and remote origin due to their less extreme climatic conditions, relatively well-developed native biodiversity and proximity to southern South America. The dispersal of biological propagules from South America to sub-Antarctic and Maritime Antarctic regions in the air column has been reported or inferred from microscopic aerobiological analyses as well as studies of snow and moss samples (Kappen & Straka 1988, Smith 1991, Marshall 1996). In addition, modelling the movement of air masses by backward trajectory analyses (Agostini *et al.* 2017, Biersma *et al.* 2018a) suggests that this part of Antarctica is more likely to receive diaspores originating from southern South America than it is to act as a source of propagules travelling in the reverse direction.

Before the advent of modern molecular biological techniques, few attempts were made to investigate the Antarctic airborne biodiversity (Chalmers *et al.* 1996, Marshall 1996, 1997, Marshall & Chalmers 1997, Marshall & Convey 1997). To assess the potential of airborne dispersal and colonization to act in synergy with climate change processes, dispersal data on both temporal and spatial scales are required, obtained using standardized methods and analysed with modern molecular tools (Pearce *et al.* 2016). Amongst the molecular tools available for species identification, DNA metabarcoding allows for the simultaneous identification of a large number of species present in environmental samples (eDNA) via the assignment of DNA sequence identities (Taberlet *et al.* 2012). Rosa *et al.* (2020, 2021) successfully used eDNA metabarcoding tools for the first time to investigate the presence of fungi in the air column over King George Island and Livingston Island in the South Shetland Islands. In the current study, we extended the application of the metabarcoding approach by identifying DNA sequences of diaspores or propagules of non-fungal eukaryotes in the airspora over King George Island.

Materials and methods

Sampling

Air samples were collected at Punta Plaza, ~1 km from the Brazilian Comandante Ferraz Antarctic Station (Fig. 1). As described by Rosa *et al.* (2021), air was collected using a polysulphone sterilized bottle filter (Nalgene, USA) fixed at 3 m above the ground and equipped with 0.22 µm sterilized membranes (47 mm diameter; Millipore, USA) coupled with a chemical duty pump (Millipore, USA). Three units (filter, membrane and pump) were operated in parallel. The sampling was performed using three membranes simultaneously for 5 successive days within a window of 20 days. A total of 12 membranes were produced between December 2019 and January 2020. The samples were defined as Sample 1 (air obtained 11–16 December 2019), Sample 2 (17–22 December 2019), Sample 3 (25–30 December 2019) and Sample 4 (1–6 January 2020). Membranes were added to previously sterilized filters inside a sterile laminar flow hood and kept in sterile bags until placed at the sampling site. After each sampling period, filters with membranes were immediately transported in sterile bags back to a laboratory at Comandante Ferraz Station. Membranes were removed from the filters inside a laminar flow hood for DNA extraction. All equipment was sterilized before being used (forceps, tubes, blades and tubes). Air flow was measured using a wind speed meter with a rotating vane sensor (Kimo LVB, Marne-la-Vallée, France) connected to the inlet pipe.

DNA extraction and data analyses

DNA from three membranes collected as replicates during each sampling interval was extracted together during the same DNA extraction in order to increase DNA yield. Total DNA was extracted using the DNeasy PowerWater Kit (Qiagen), following the manufacturer's instructions. Extracted DNA was used as a template for generating polymerase chain reaction (PCR) amplicons. The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification (Chen *et al.* 2010, Richardson *et al.* 2015). PCR amplicons were generated using the universal primers ITS3 and ITS4 (White *et al.* 1990). Library construction and DNA amplification were performed using the library kit Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2, following Illumina Metagenomic Sequencing Library Preparation Part #15,044,223 Rev. B protocol, and they were sequenced by Macrogen, Inc. (South Korea) using high-throughput paired-end sequencing (2 × 300 bp) on a MiSeq System (Illumina), using the MiSeq Reagent Kit v3 (600 cycles), 100K reads and following the manufacturer's protocol.

Raw fastq files were filtered using *BBDuk* version 38.87 (BBMap - Bushnell B.; sourceforge.net/projects/bbmap/) to remove Illumina adapters (Illumina artefacts and the PhiX Control v3 Library) and for quality read filtering (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 to *QIIME2* version 2021.4 (https://qiime2.org/) for bioinformatics analyses (Bolyen *et al.* 2019).

The *qiime2-dada2* plugin was used for filtering, dereplication, to turn paired-end fastq files into merged files, to remove chimeras and to create amplicon sequence variants (ASVs) with default parameters (Callahan *et al.* 2016). Taxonomic assignments of ASVs were determined using the *qiime2* feature classifier

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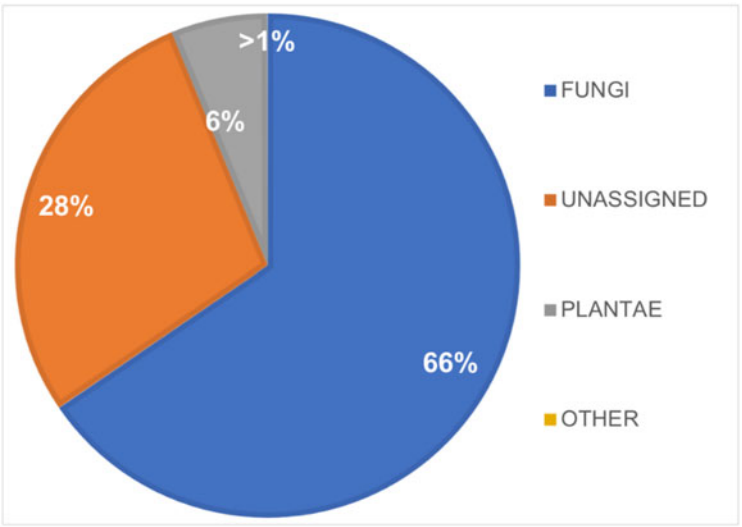

Figure 2. Distribution of DNA reads present in the four air samples analysed, including data both from our study and from Rosa *et al.* (2021) for Fungi. 'Other' includes Protozoa and Chromista.

(Bokulich *et al.* 2018) *classify-sklearn* against 1) the PLANiTS2 database (Banchi *et al.* 2020) and 2) the UNITE eukaryote ITS database version 8.3 (Abarenkov *et al.* 2020). The remaining unidentified sequences were classified against the National Center for Biotechnology Information (NCBI) non-redundant nucleotide sequences (nt) database (October 2021) using *BLASTn* (Camacho *et al.* 2009); the nt database was filtered using the following keywords: 'ITS1', 'ITS2', 'Internal transcribed spacer' and 'internal transcribed spacer'. The output files from *BLASTn* were imported into *MEGAN6* (Huson *et al.* 2016) for taxonomic assignments. The detected bryophyte sequences were compared with the sequence alignments used for phylogenetic analysis in Gama *et al.* (2016).

Classifications and systematic ranks for kingdoms and phyla follow Ruggiero *et al.* (2015). Here, we focus on four of the five kingdoms of eukaryotes defined by Ruggiero *et al.* (2015), namely Protozoa and Chromista (protist lineages), Plantae (including blue-green, red and green algae as well as land plants) and Animalia. The kingdom Fungi was studied by Rosa *et al.* (2021). For lower ranks and taxonomic authorities, we checked global databases for marine species (WoRMS Editorial Board 2021), algae (Guiry & Guiry 2023) and the Catalogue of Life (Roskov *et al.* 2020). Geographical

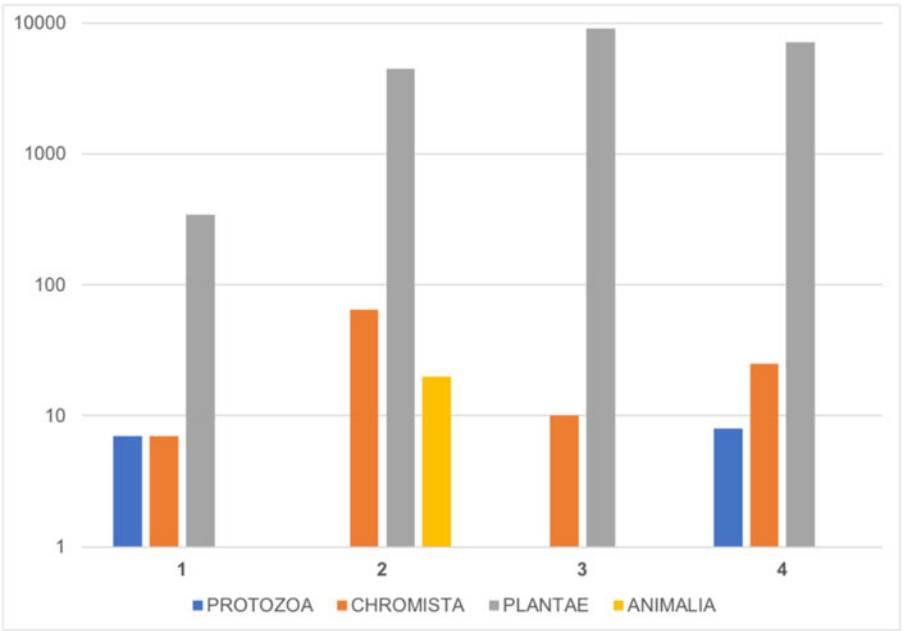

Figure 3. Bar graph showing DNA reads (*y*-axis) per sample (*x*-axis). Data are in logarithmic scale.

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Table I. Number of DNA reads of amplicon sequence variants (ASVs) from samples obtained from Antarctic air sampled over four different periods (taxonomic classification following Ruggiero *et al.* 2015).

DNA reads per sample				
Taxa	1	2	3	4
KINGDOM PROTOZOA	7	0	0	8
KINGDOM CHROMISTA				
Phylum Ciliophora				
Stylonychia lemnae Ammermann & Schlegel 1983[a]	0	0	1	0
Stylonychia sp.[b]	0	2	9	0
Phylum Cercozoa				
Amphorellopsis quinquealata (Laackmann) Balech 1971[a]	0	0	0	1
Amphorellopsis sp.	0	53	0	24
Phylum Haptophyta				
Phaeocystis globosa Scherffel[b]	7	0	0	0
Phylum Ochrophyta				
Thalassiosira sp.	0	10	0	0
KINGDOM PLANTAE				
Phylum Chlorophyta				
Chlamydomonadales	0	0	205	0
Chlamydomonas nivalis (F.A. Bauer) Wille	223	2186	8368	109
Sanguina nivaloides Procházková, Leya & Nedbalová[a,c]	2	0	0	1
Chlorellales				
Chloromonas alpina Wille[c]	0	0	43	0
Chloromonas nivalis (Chodat) Hoham & Mullet	0	0	7	0
Chloromonas pichinchae Wille[b]	0	242	0	0
Micractinium sp.	0	0	0	16
Prasiolales				
Desmococcus olivaceus (Persoon ex Acharius) J.R. Laundon[c]	0	0	0	13
Koliella longiseta (Vischer) Hindák[c]	0	33	294	69
Trebouxiales				
Trebouxiophyceae	0	0	0	9
Trebouxia sp.	0	60	28	0
Ulotrichales				
Chlorothrix sp.[c]	29	263	10	300
Monostroma angicava Kjellman[b]	81	1259	55	6436
Planophila sp.	0	0	1	1
Pseudothrix groenlandica (J. Agardh) Hanic & S. C. Lindstrom	0	192	36	0
Ulothrix sp.	0	37	0	91
Urospora sp.	8	64	06	50
Ulvales				
Ulvophyceae	0	0	0	14
Pseudendoclonium sp.	0	0	0	42
Umbraulva japonica (Holmes) Bae & I. K. Lee[b]	0	137	0	0
Phylum Bryophyta				
Campylopus incrassatus Kunze ex Müll. Hal.[b]	0	0	0	1
Campylopus introflexus (Hedw.) Brid.	0	0	0	9
Phylum Magnoliophyta				
Juncaginaceae				
Tetroncium magellanicum Willd.[b]	0	5	0	0
Polygonaceae				
Rumex graminifolius Rudolph ex Lamb.[b]	0	0	0	14
KINGDOM ANIMALIA				
Phylum Mollusca				
Class Bivalvia[a]	0	1	0	0
Phylum Arthropoda				
Folsomia sp.	0	19	0	0
Phylum Chordata				
Salpa thompsoni Foxton, 1961[a]	1	0	0	1

[a]Taxa assigned from blast search against the National Center for Biotechnology Information database.
[b]Taxa not previously recorded from Antarctica.
[c]Species previously detected only in DNA metabarcoding studies.

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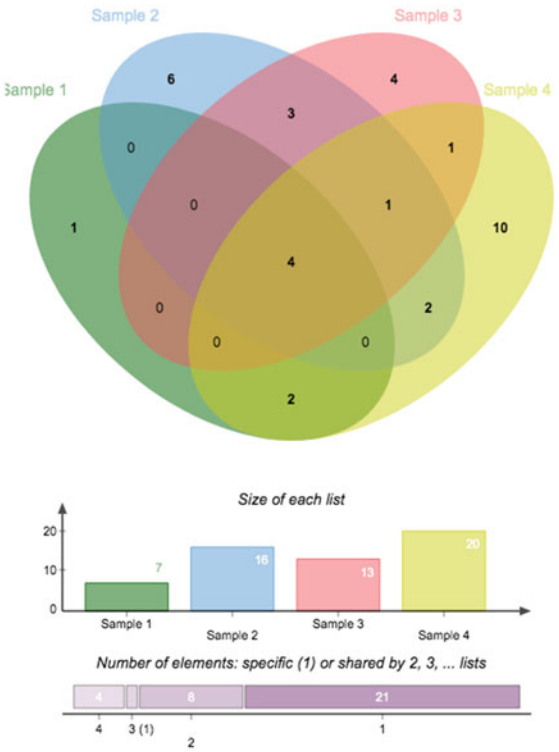

Figure 4. Venn diagram showing the numbers of taxa found in the four Antarctic air samples obtained over successive 5 day time periods.

distribution follows Petz (2005), Thompson *et al.* (2019), Guiry & Guiry (2023) and tropicos (www.tropicos.org). We used the number of reads as a proxy for abundance following the approaches of Deiner *et al.* (2017), Hering *et al.* (2018) and Rosa *et al.* (2020). Construction of Venn diagrams followed Bardou *et al.* (2014), and rarefaction curves were calculated using *PAST* ver. 4.09 (Hammer *et al.* 2001).

Results

The air sampled over the 20 day study period totalled 1697.76 m^3 in each continuous 5 day period. The calculated rarefaction curves for all detected taxa approached a plateau, indicating that the reads gave an accurate representation of the local sequence diversity in all four sampling intervals (Fig. S1). A total of 955 674 paired-end DNA reads were generated in the sequencing run, of which 342 174 remained after quality filtering. A total of 21 166 reads represented 35 taxa (Figs 2 & 3 & Table I) from 10 phyla within three of the four target kingdoms: Chromista (phyla Ciliophora, Cercozoa, Haptophyta and Ochrophyta), Plantae (phyla Chlorophyta, Bryophyta and Magnoliophyta) and Animalia (phyla Mollusca, Arthropoda and Chordata). Some of these assigned sequences could only be resolved at higher taxonomic levels. In addition, a small number of reads belonging to the kingdom Protozoa were found, which could not be further identified. The remaining reads were assigned as Fungi and have been reported by Rosa *et al.* (2021).

Amongst the different communities detected, the microalga *Chlamydomonas nivalis* was by far the most abundant taxon based on the number of sequence reads (10 886), followed by the macroalga *Monostroma angicava* (7831). The most diverse group was the plants, with 26 taxa, followed by Chromista, with six taxa. The numbers of taxa assigned in Samples 1–4 were 8, 16, 13 and 19, respectively. Only four taxa, the green algae *C. nivalis*, *Chlorothrix* sp., *M. angicava* and *Urospora* sp., were present in all samples across the entire sampling period (Fig. 4).

Discussion

At the outset, we recognize that assigning an identity to obtained eDNA sequences does not confirm the presence of a viable organism or propagule, as the assignments themselves rely heavily on the quality and completeness of available databases. As databases become more comprehensive over time, the outcomes of this type of study should become more specific and reliable.

As it can be seen in the Venn diagram (Fig. 4) and in Table I, the taxa composition changed over time, and only four taxa were found over the whole period, all of them being commonly found green algae: *C. nivalis* (also the most abundant in terms of DNA reads), *Chlorothrix* sp., *M. angicava* (also highly abundant) and *Urospora* sp. The reasons as to why this taxa composition varied and how it varied were not subjects of this study. A larger sampling area including a longer temporal sampling period would be necessary to answer these questions.

Amongst the assigned Chromista, representatives of the genus *Stylonychia*, which includes both terrestrial and brackish water species, were the only Ciliophora taxa detected. *Stylonychia* has previously been reported in Antarctica, but only *Stylonychia lanceolata* Ehrenberg, 1835 is a confirmed species (Thompson 2019), whilst the *Stylonychia mytilus* complex, to which *Stylonychia lemnae* belongs, is considered as taxonomically incomplete (see discussion in Thompson 2019). The only genus recognized in our dataset belonging to the phylum Cercozoa was *Amphorellopsis*, previously reported from the Atlantic sector of the Southern Ocean (*Amphorellopsis laackmannii*) by Petz (2005), and with *Amphorellopsis quinquealata* widely distributed in the Southern Ocean (Petz 2005). Amongst the Haptophyta, *Phaeocystis globosa* is well known for contributing to harmful algal blooms in

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temperate and polar regions. Despite the worldwide distribution of the genus (Guiry & Guiry 2023), this is the first report of *P. globosa* in Antarctica. However, *Phaeocystis antarctica* Karsten 1905 is one of the most abundant Antarctic planktonic species (Marchant *et al.* 2005), possibly illustrating the database limitations mentioned above. *Thalassiosira* was the only representative of Ochrophyta detected. This genus of centric diatoms is widespread in both freshwater and marine environments, and *Thalassiosira antarctica* is widespread, being commonly reported in both the South Shetland Islands and Wilkes Land (Cremer *et al.* 2003, Scott & Thomas 2005).

Amongst the assigned Chlorophyta, the majority of the green algal taxa detected have previously been reported in Antarctic eDNA studies (Câmara *et al.* 2021a,c, 2022a,b, Fonseca *et al.* 2022). The East Asian *Umbraulva japonica* (Kawai *et al.* 2021) is a species previously unreported in Antarctica. The presence of marine species is consistent with the proximity of the sampling site to the local shoreline. The most abundant green algal species detected was *C. nivalis*, a cosmopolitan species known to thrive in snow and one of several algal species responsible for the development of snow algal blooms (Davey *et al.* 2019, Procházková *et al.* 2019), reducing the local albedo (Cook *et al.* 2017). *Sanguina nivaloides*, a recently described polar-alpine species (Procházková *et al.* 2019), also causes similar blooms. Sequences assigned to this species were also reported from soils on Deception Island (Câmara *et al.* 2020), with the current report being the third such from the Antarctic. Although the presently available data suggest so, further studies are required to confirm whether the species *S. nivaloides* is truly bipolar in distribution.

Amongst the assigned Bryophyta, four species of the genus *Campylopus* are currently recorded in Antarctica (Ochyra *et al.* 2008). The genus is better represented on Maritime and sub-Antarctic islands, with three of the species restricted to the South Sandwich Islands and only one reported from the Antarctic continent (Convey *et al.* 2000). Of these, we detected only *Campylopus introflexus*, which is recorded from the South Sandwich Islands, the Falkland (Malvinas) Islands, Tierra del Fuego and South Georgia (Ochyra *et al.* 2008). It was also reported on Deception Island by Smith (1984, 1988), but this record was later excluded by Ochyra *et al.* (2008) due to misidentification. We also detected *Campylopus incrassatus*, but the sequences from the air samples actually belong to two different haplotypes according to the comparison with the data from Gama *et al.* (2016). Of these, one haplotype is identical to a specimen from Australia, which was earlier identified as *C. incrassatus*, a species reported on the South Sandwich Islands by Longton & Holdgate (1979) but excluded from Antarctica by Ochyra *et al.* (2008). The other sequence is closest to a haplotype so far detected in various areas of the native Southern Hemisphere distribution of *C. introflexus* (Chile, South Africa, Australia and New Zealand) as well as in North America and Europe, where the species was introduced (cf. analyses in Gama *et al.* 2016). Although further study is needed to infer the total distributions of each haplotype and the exact geographical origins of the detected sequences, the present data indicate two independent arrivals of diaspores of *C. introflexus* to Keller Peninsula.

Amongst the assigned angiosperms, the two sequences assigned represent exotic taxa for Antarctica. *Tetroncium magellanicum* occurs in Tierra del Fuego, the Falkland (Malvinas) Islands and Gough Island (Moore 1974). *Rumex graminifolius* (grassleaf sorrel) is a (sub-)Arctic species that is also present in northern Asia. The detection of their DNA could indicate that their pollen reached Antarctica in air currents, but other means of transport cannot be ruled out. Although the establishment of non-native angiosperms in Antarctica is very limited at present, Lityńska-Zajac *et al.* (2012) reported diaspores and plant remains in 78 samples of the clothing, gear and equipment of scientific expeditioners, including five fruit from two different species of *Rumex*. Carvalho-Silva *et al.* (2021) and Câmara *et al.* (2022b) also reported a high diversity of angiosperm DNA assignments in metabarcoding studies carried out on substrates from Deception Island in the South Shetland Islands and the Ellsworth Mountains in Continental Antarctica.

Amongst the assigned Animalia, the assignment of sequences to an unidentified bivalve and *Salpa thompsoni* could again relate to the close proximity of the air sampling site to the seashore. *S. thompsoni* is one of the most commonly found tunicates in Antarctica (Meunier 2020), and it is common to see large numbers of salps washed ashore in Admiralty Bay. According to Greenslade (1995), 11 native species of springtails occur in Maritime Antarctica. However, the genus *Folsomia* is not native to the region, although the species *Folsomia candida* Willem, 1902 (considered to be a non-native parthenogenetic species) has been recorded previously on Deception Island (Greenslade *et al.* 2012). The species has not been recorded elsewhere in Antarctica, including King George Island.

Conclusions

Using a DNA metabarcoding approach, our study of air samples taken over a 20 day period revealed a previously undocumented airborne diversity. The diversity of taxa detected here suggests that the Antarctic airspora includes propagules of both intra-

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(local) and inter-continental (distant) origin. Some species assigned from the sequences obtained, such as the two flowering plants, are unlikely to represent viable propagules. However, mosses and algae could potentially develop from either single cells or spores into new and viable organisms. Current rapid changes in environmental conditions in this region could act in synergy with the inwards transport of propagules of currently non-native taxa. Further detailed studies are required, across all seasons annually, covering larger geographical areas and analysing patterns of intraspecific genetic variation, to better understand the airborne transport of organisms to and around Antarctica.

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Author contributions

PEASC, TŠ-T, FLVB and LAdCR designed the study, prepared the logistics and performed the fieldwork. PEASC, FLVB and MC-S completed the laboratory work. OHBP, FLVB and FACL performed the bioinformatics assessments. MS analysed the bryophyte haplotypes. PEASC and LHR secured funds. All authors contributed to data interpretation and the development of the manuscript.

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Competing interests

The authors declare none.

Supplemental material

To view supplementary material for this article, please visit https://doi.org/10.1017/S095410202400004X.

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