



DNA Metabarcoding to Assess the Diversity of Airborne Fungi Present over Keller Peninsula, King George Island, Antarctica

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Abstract

We assessed fungal diversity present in air samples obtained from King George Island, Antarctica, using DNA metabarcoding through high-throughput sequencing. We detected 186 fungal amplicon sequence variants (ASVs) dominated by the phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota*, and *Chytridiomycota*. *Fungi* sp. 1, *Agaricomycetes* sp. 1, *Mortierella parvispora*, *Mortierella* sp. 2, *Penicillium* sp., *Pseudogymnoascus roseus*, *Microdochium lycopodium*, *Mortierella gamsii*, *Arrhenia* sp., *Cladosporium* sp., *Mortierella fimbriocystis*, *Moniliella pollinis*, *Omphalina* sp., *Mortierella antarctica*, and *Pseudogymnoascus appendiculatus* were the most dominant ASVs. In addition, several ASVs could only be identified at higher taxonomic levels and may represent previously unknown fungi and/or new records for Antarctica. The fungi detected in the air displayed high indices of diversity, richness, and dominance. The airborne fungal diversity included saprophytic, mutualistic, and plant and animal opportunistic pathogenic taxa. The diversity of taxa detected reinforces the hypothesis that the Antarctic airspora includes fungal propagules of both intra- and inter-continental origin. If regional Antarctic environmental conditions ameliorate further in concert with climate warming, these fungi might be able to reactivate and colonize different Antarctic ecosystems, with as yet unknown consequences for ecosystem function in Antarctica. Further aeromycological studies are necessary to understand how and from where these fungi arrive and move within Antarctica and if environmental changes will encourage the development of non-native fungal species in Antarctica.

Keywords Antarctic Peninsula · Ecology · Fungi · Metabarcoding · Taxonomy

Introduction

The pristine environments of Antarctica offer unique opportunities to study how biological diversity disperses and

colonizes habitats under extreme conditions. Among the barriers that isolate Antarctica from other Southern Hemisphere landmasses (such as South America, Africa and Oceania) are the atmospheric circumpolar vortex (resulting in the consistently strong westerly winds flowing around the continent) and the continent's extreme environmental conditions including cold temperatures and typically oligotrophic conditions [1–3]. Nonetheless, over time, Antarctic ecosystems receive a rain of microbial particles from other parts of the world [4–8], the so-called diaspore rain [9]. However, how viruses, bacteria, microalgae, and fungi, as well as plant propagules, arrive and circulate in Antarctica remains poorly understood [8, 10, 11].

Fungi occur in virtually all terrestrial ecosystems of Antarctica. Many fungi have small and light spores and other propagules that in principle can be easily dispersed by air currents globally, which may arrive in Antarctica. Among the fungal diversity currently known from Antarctica, globally cosmopolitan taxa often appear to dominate in many

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ecosystems and are to adapt to and function well under the environmental challenges, while few fungi are considered to be either endemic or true psychrophiles [12, 13]. A number of aerobiological studies have been carried out in Antarctica in recent decades, which reported the arrival of various microbial groups, including fungi [3–5, 14–16]. However, the majority of these studies only used traditional culturing techniques and, thus, are likely to have detected only a minority of the microbes present in the airspora.

In the second half of the Twentieth Century the Antarctica Peninsula was one the three most rapidly warming regions globally, a trend which, while recently paused [17], is predicted to resume in the remainder of the twenty-first century [18–20]. The rapid changes in physical and chemical environmental conditions raise concerns of accelerating colonization of Antarctica by non-native species, particularly through anthropogenic assistance [19]. In the present study, we assessed fungal diversity present in air samples obtained over one continuous month on the Keller Peninsula, King George Island, South Shetland Islands, maritime Antarctic, using DNA metabarcoding through high-throughput sequencing (HTS).

Material and Methods

Air Sampling

Air samples were collected at Punta Plaza, Keller Peninsula, King George Island, South Shetland Islands, close to the Brazilian Antarctic Station Comandante Ferraz (Fig. 1). Air was collected using a polysulfone sterilized bottle top filter (Nalgene, USA) equipped with 0.22- μ m sterilized membranes (47-mm diameter; Millipore, USA) coupled with chemical duty pump (Millipore, USA). Three systems (filter, membranes, and pump) were operated in parallel, and the sampling was performed using three membranes simultaneously for 5 days in a row during 20 days, totaling 12 membranes from December of 2019 to January of 2020. The temporal samples were defined as sample 1 (air obtained in December 11–16, 2019), sample 2 (air obtained in December 17–22, 2019), sample 3 (air obtained in December 25–30, 2019), and sample 4 (air obtained in January 1–06, 2020). Membranes were added to previously sterilized filters inside a sterile laminar flow hood and kept in sterile bags until placed on the experiment site. After each experiment, filters with membranes were transported in sterile bags immediately back to the microbiology laboratory of Comandante Ferraz Antarctic Station, then inside a laminar flow hood, membranes were removed from the filters and DNA extractions were performed. All equipment used (e.g. forceps, tubes, blades, and tubes) were sterilized before use.

DNA Extraction, Data Analyses, and Fungi Identification

Three membranes from each sampling interval were processed together into the same DNA extraction in order to increase DNA yield. Total DNA was extracted from environmental samples using the DNeasy PowerWater Sterivex Kit, following the manufacturer's instructions. Extracted DNA was used as template for generating PCR amplicons. The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification [21, 22]. PCR amplicons were generated using the universal primers ITS3 and ITS4 [23] and were sequenced by high-throughput sequencing at Macrogen Inc. (South Korea) paired-end sequencing (2×300 bp) on MiSeq System (Illumina), using the MiSeq Reagent Kit v3 (600 cycles) following the manufacturer's protocol.

Raw fastq files were filtered using BBDuk version 38.34 (BBMap – Bushnell B. – sourceforge.net/projects/bbmap/) to remove Illumina adapters, known Illumina artifacts, and the PhiX Control v3 Library. Quality read filtering was carried out using Sickle version 1.33 -q 30 -l 50 [24], to trim ends 3' or 5' with low Phred quality score, and sequences shorter than 50 bp were also discarded. The remaining sequences were imported to QIIME2 version 2019.10 (<https://qiime2.org/>) for bioinformatics analyses [25]. The qiime2-dada2 plugin is a complete pipeline that was used for filtering, dereplication, turn paired-end fastq files into merged, and remove chimeras [26]. Taxonomic assignments were determined for amplicon sequence variants (ASVs) using the qiime2-feature-classifier [27] classify-sklearn against the UNITE fungal ITS database version 7.2 [28] and trained with Naive Bayes classifier and a confidence threshold of 98.5%.

Many factors, including extraction, PCR, and primer bias, can affect the number of reads obtained [29], and thus lead to misinterpretation of absolute abundance [30]. However, Giner et al. [31] concluded that such biases did not affect the proportionality between reads and cell abundance, implying that more reads are linked with higher abundance [32, 33]. Therefore, for comparative purposes, we used the number of reads as a proxy for relative abundance.

Fungal Diversity and Distribution

To quantify species diversity, richness, and dominance, we used the following indices: (i) Fisher's α , (ii) Margalef's, and (iii) Simpson's, respectively. The relative abundances of the amplicon sequence variants (ASVs) were used to quantify the fungal taxa present in the air sampled, where fungal ASVs with relative abundance $> 10\%$ were considered dominant, ASVs with $< 10\%$ $> 1\%$ as intermediate, and ASVs with $< 1\%$ as minor (rare) components of the fungal community. All of the results were obtained with 95% confidence, and

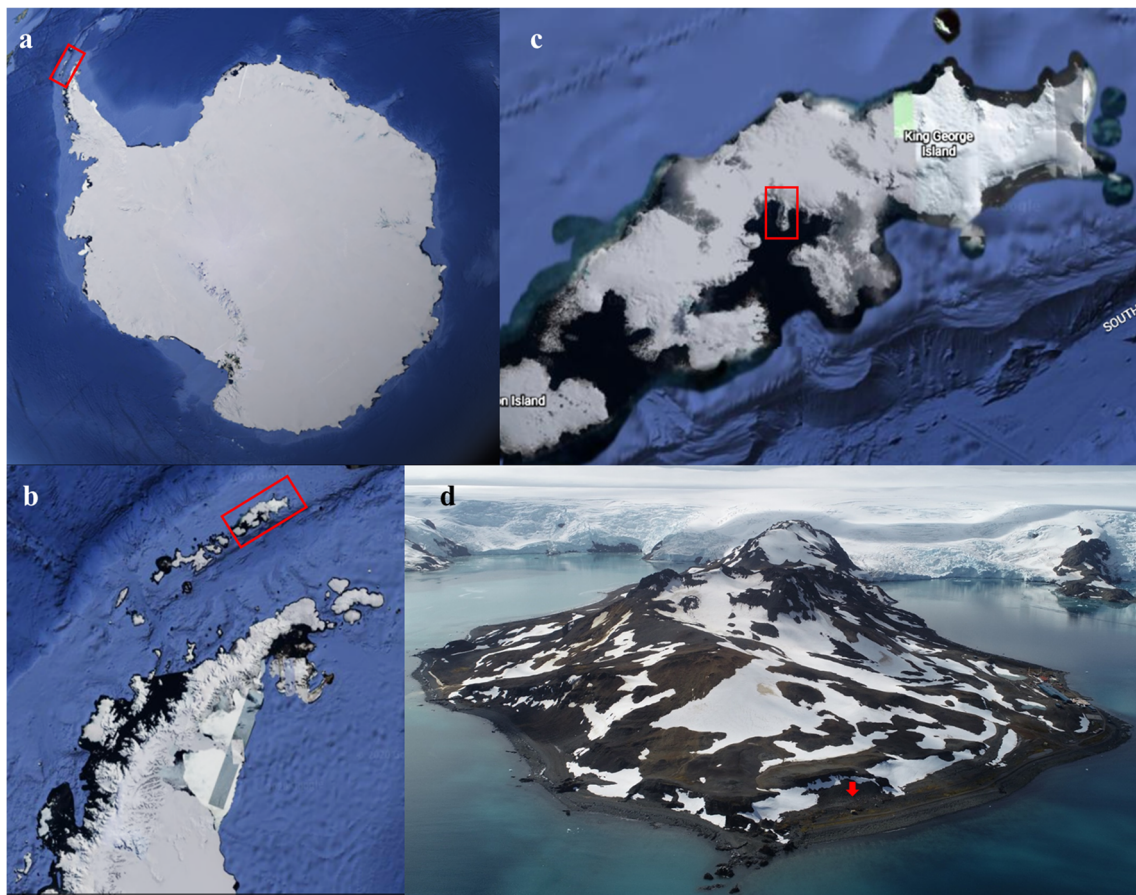


Fig. 1 Satellite images **a**, **b**, and **c** (obtained in Google Earth Pro, 2019) and the site where the air was sampled. **a** Antarctic Continent with the Antarctic Peninsula inside the red rectangle. **b** Antarctic Peninsula with the South Shetland Islands archipelago inside the red rectangle. **c** King

George Island with the Keller Peninsula inside the red rectangle. **d** Keller Peninsula, Punta Plaza (red arrow) at 62° 5' 23.695" S, 58° 24' 24.162" W. Photo (**d**) by L.H. Rosa

bootstrap values were calculated from 1000 iterations. Taxon accumulation curves were obtained using the Mao Tao index. All diversity index calculations were performed using PAST, version 1.90 [34]. Venn diagrams were prepared as described by Bardou et al. [35] to compare the fungal assemblages present in the air samples.

Results

Fungal Taxonomy

In the complete air sampled over the 20-day study period (total of 1697.76 m³ or 424.44 m³ in each continuous 5-day period) on the Keller Peninsula, King George Island, we detected 186 fungal ASVs (Supplementary Table 1; Supplementary Table 2). The ASVs were dominated, in rank order, by the phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota*, and *Chytridiomycota*. *Ascomycota* and *Basidiomycota* were detected in all samples; however, *Chytridiomycota* was detected only in sample 3 and

Mucoromycota in sample 4 (Fig. 2a). At class level, *Mallasseziomycetes*, *Agaricomycetes*, *Saccharomycetes*, *Leotiomycetes*, *Eurotiomycetes*, and *Dothideomycetes* displayed broad distribution across all samples; in contrast, *Endogonomycetes* and *Exobasidiomycetes* were detected only in sample 4 (Fig. 2b). Of the total of 187,423 reads, 19,460 (10.38%) could only be assigned as Fungi sp. and may therefore represent currently unknown taxa. Similarly, a number of ASVs could only be identified to higher taxonomic levels (phylum, class, order, family) and again may represent new species and/or new records for Antarctica, or the unclassified ASVs might be an artefact due to the small amplicon size. The airborne fungal community included 14 dominant fungal taxa, 43 intermediate, and 130 minor. Fungi sp. 1, *Agaricomycetes* sp. 1, *Mortierella parvispora*, *Mortierella* sp. 2, *Penicillium* sp., *Pseudogymnoascus roseus*, *Microdochium lycopodium*, *Mortierella gamsii*, *Arrhenia* sp., *Cladosporium* sp., *Mortierella fimbriocystis*, *Moniliella pollinis*, *Omphalina* sp., *Mortierella antarctica*, and *Pseudogymnoascus appendiculatus* were the dominant fungal taxa, in rank order.

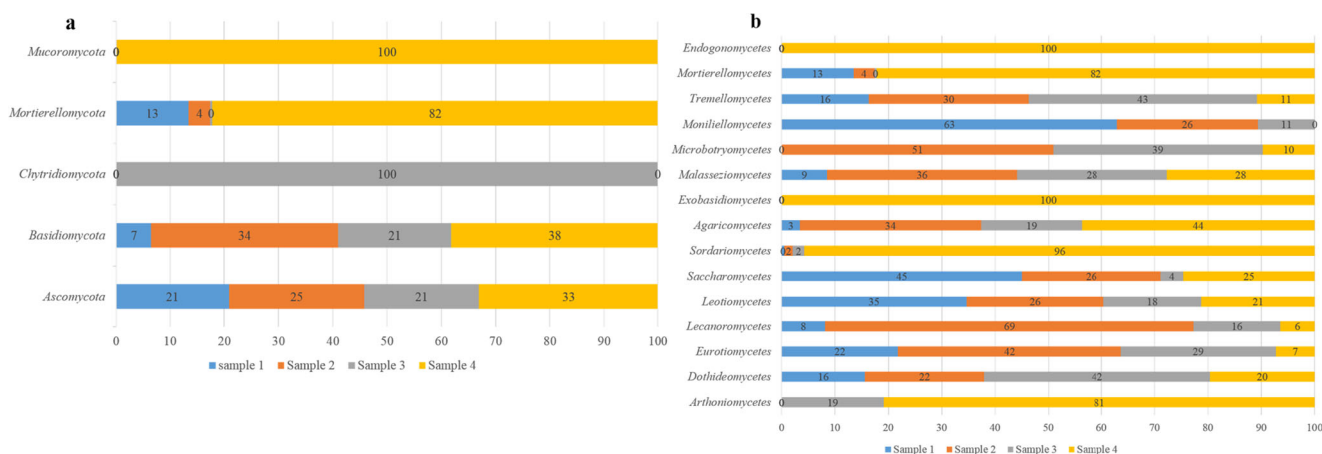


Fig. 2 Relative abundance values of **a** phylum and **b** class hierarchical level of the fungal amplicon sequence variants (ASVs) present in the four air samples collected on the Keller Peninsula, King George Island, South Shetland Islands, Antarctica

Fungal Diversity

The Mao Tao rarefaction curves of the fungal assemblages detected in the different samples reached asymptote (Fig. 3), indicating that the data provided a good description of the diversity present. The total fungal community detected in the air displayed high indices of diversity (Fisher α), richness (Margalef), and dominance (Simpson). However, the indices varied among the different samples, and the diversity and richness were higher in the first and final samples (Table 1). Of the total fungal ASVs detected, only 20 (10.75%) were detected in all samples, with these including known *Ascomycota* airborne fungi such as *Aspergillus*, *Cladosporium*, and *Penicillium* taxa (Fig. 4).

Discussion

Fungal Taxonomy and Diversity

The fungal component of the airspora circulating in Antarctica is poorly characterized, with the few previous studies based only on traditional morphological methods [36]. Marshall [14]

Table 1 Ecological indices of the fungal DNA recovered from air samples obtained on the Keller Peninsula, King George Island, South Shetland Islands

| Ecological indices | Air samples | | | | Total |
|----------------------|-------------|--------|--------|--------|---------|
| | 1 | 2 | 3 | 4 | |
| Number of reads | 32,551 | 47,161 | 43,223 | 64,488 | 187,423 |
| Number of fungal ASV | 46 | 58 | 101 | 133 | 186 |
| Fisher α | 5.27 | 6.65 | 12.52 | 16.16 | 20.5 |
| Margalef | 4.33 | 5.4 | 9.5 | 12 | 15.32 |
| Simpson | 0.78 | 0.84 | 0.87 | 0.78 | 0.90 |

ASV amplicon sequence variant

characterized airborne fungal spores over a period of 13.6 months on Signy Island (South Orkney Islands, also in the maritime Antarctic), reporting *Epicoccum* spp. and *Cladosporium* spp. to be dominant. Duncan et al. [37] sampled the air inside the historic explorers' huts on Ross Island and found viable fungal propagules dominated by *Cladosporium cladosporioides*, *Pseudeurotium desertorum*, *Pseudogymnoascus* sp., and *Antarctomyces psychrotrophicus*. Archer et al. [3] assessed the microbial cultured diversity in air in one of the Victoria Land Dry Valleys and detected basidiomycetous yeasts and unclassified fungi as dominant taxa. However, studies of fungal diversity using metabarcoding approaches remain scarce. Recently, Rosa et al. [36] used this approach to study the airspora fungi present in air and snow on Livingston Island, South Shetland Islands, detecting a rich fungal diversity mostly including taxa that are not usually detected in culture approaches.

In our study, application of metabarcoding revealed the presence of high fungal diversity in the air sampled, dominated by members of the phyla *Ascomycota* and *Basidiomycota*, but also with dominant members of *Mortierellomycota*, *Mucoromycota*, and *Chytridiomycota* that are typically uncommon in the air. The detection of DNA of *Ascomycota* and *Basidiomycota* taxa in all four samples (across one whole month of air sampling) suggests that these fungal groups are the most dominant in the Antarctic Peninsula region as proposed by several studies [13]. In contrast, those belonging to *Chytridiomycota* and *Mucoromycota* were detected only in one sample, suggesting that they are uncommon fungi in the air. Fungi included in the classes *Malasseziomycetes*, *Agaricomycetes*, *Saccharomycetes*, *Leotiomycetes*, *Eurotiomycetes*, and *Dothideomycetes*, which occurred in all samples, include taxa with different ecological characteristics such as cosmopolitan, cold-tolerant taxa, and endemic [13]. The detection of the DNA of a rich and diverse fungal community in the air samples obtained here is consistent with the hypothesis proposed by Rosa et al. [9] that many of these

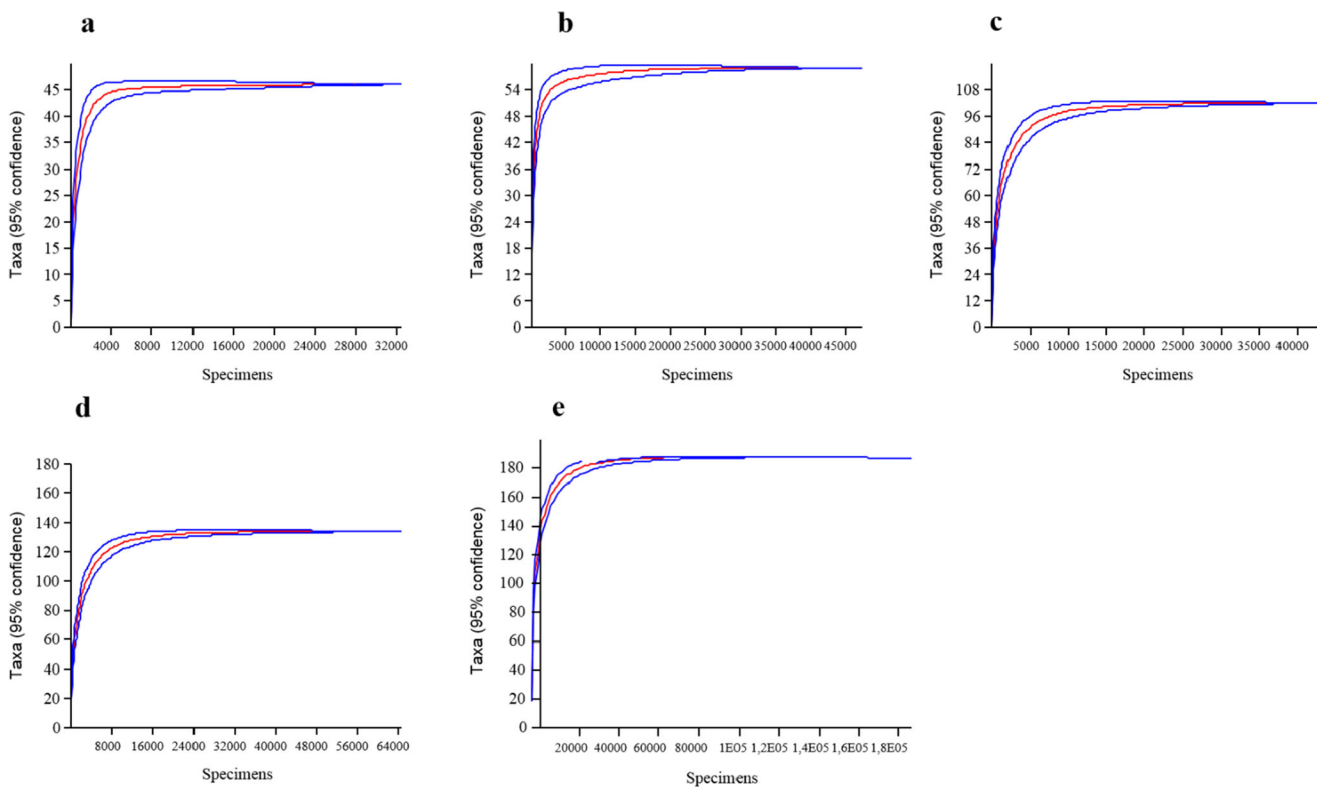


Fig. 3 Rarefaction curves of fungal amplicon sequence variants (ASV) obtained in weekly air samples on the Keller Peninsula, King George Island, South Shetland Islands, Antarctica. Fungal ASVs sampled in (a) sample 1, (b) sample 2, (c) sample 3, (d) sample 4, and (e) total. Blue lines represent 95% confidence limits

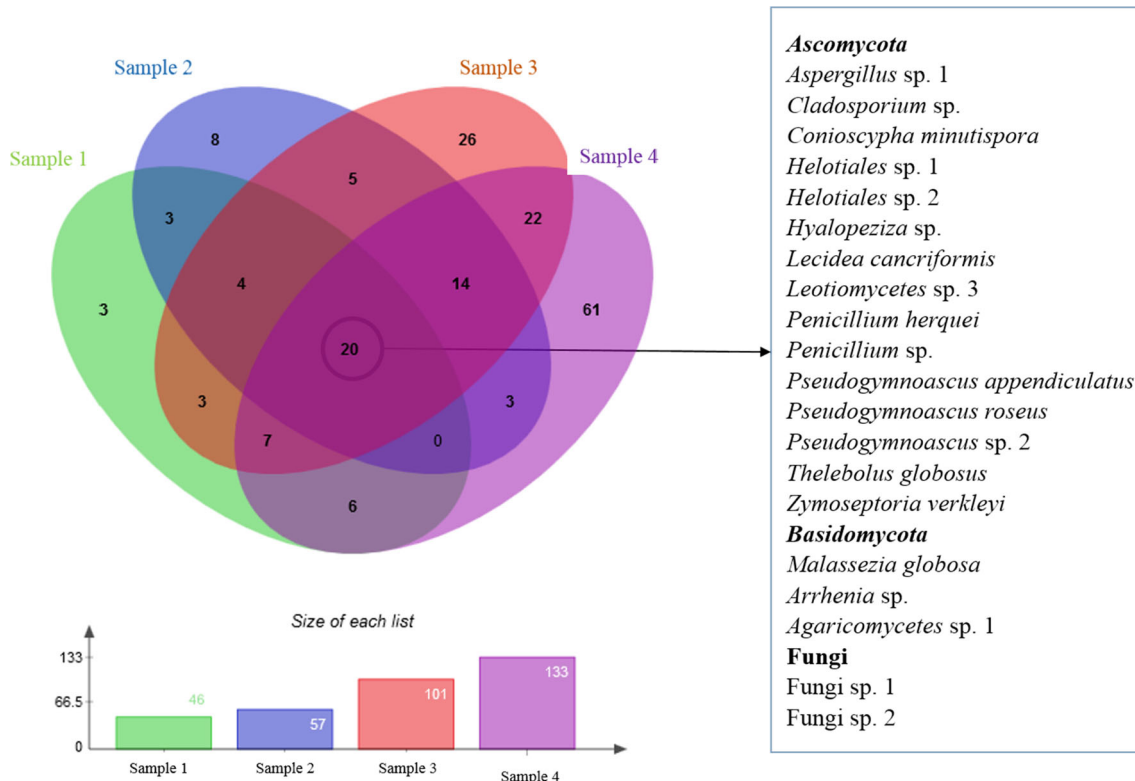


Fig. 4 Venn diagram showing the fungal amplicon sequence variants (ASVs) detected in air sampled on the Keller Peninsula, King George Island, South Shetland Islands, highlighting those detected in all samples

fungi might have mechanisms enabling survival during transport into and within the Antarctic atmosphere.

The dominant fungi detected in the air were mainly represented not only by known airborne taxa such as *Penicillium* and *Cladosporium* but also by *Microdochium lycopodium*, *Pseudogymnoascus roseus*, and *P. appendiculatus*, which are rarely recorded as airborne species. In addition, we detected many other fungi in the air sampled, mainly as intermediate and minor components. Many of the taxa could only be assigned to higher taxonomic levels, suggesting that the real fungal diversity present in Antarctica (and elsewhere) is still poorly known.

The genus *Microdochium* includes about 20 known species [38], some described as plant pathogens of cereals and grasses in cold regions [39, 40]. On the Antarctic Peninsula, *M. phragmitis* has been reported in freshwater lakes [41] and *M. nivale* as an endophyte of *Colobanthus quitensis* [42]. *Microdochium lycopodium* (previously known as *Monographella lycopodina*) was originally recovered from living and recently dead stems and leaves of *Spinulum annotinum* (syn. *Lycopodium annotinum*) and has a known distribution in boreal Central Europe (Austria, Germany) [43]. However, Carvalho et al. [44] also reported *M. lycopodium* as an endophyte of the moss *Polytrichastrum alpinum* on the Antarctic Peninsula.

Pseudogymnoascus (syn. *Geomyces*) is a fungal genus well known from cold environments, including Arctic, alpine, temperate, and Antarctic ecosystems [13, 45–47]. Cultivable *Pseudogymnoascus* taxa have been detected in soils [45, 48–50], associated with plants [44, 51, 52] and macroalgae [53, 54], in freshwater lakes [41], and associated with lichens [55]. *Cladosporium* and *Penicillium* are fungal genera previously reported in the global airspora. In Antarctica, *Cladosporium* species have been described as dominant in association with plants and soil [13]. Representatives of *Penicillium* appear to be ubiquitous in Antarctica and have been reported in many studies of different habitats such as soils [50, 56, 57], permafrost [58, 59], associated with macroalgae [60], snow [6], and ice [7]. The DNA of both genera was detected in the air and snow of Livingston Island by Rosa et al. [8], again supporting their broad distribution and dominance in Antarctica. The dominance of *Pseudogymnoascus*, *Cladosporium*, and *Penicillium* in air sampled on King George Island is similar to the results described by Rosa et al. [8] from air sampled on Livingston Island. Together, these studies suggest the importance of aerial transport of fungal spores and/or propagules at different scales both globally and regionally within Antarctica.

Conclusions

Using a metabarcoding approach, this study of the fungal airspora sampled over a 20-day period on Keller Peninsula,

King George Island, revealed a rich airborne fungal diversity, much of which would be unlikely to be detected using traditional culture methods. Among the fungi dominant within the airspora, we detected representatives of the known cosmopolitan genera *Cladosporium* and *Penicillium*, commonly present in temperate and tropical environments, which have high dispersal capabilities. However, apparently psychrotolerant fungi were also detected, such as members of the genus *Pseudogymnoascus*, which are widely reported from different Antarctic and other cold habitats. The majority of fungal taxa detected were of intermediate and minor abundance, including taxa of saprophytes, mutualists, parasites, and opportunistic fungi already reported in culture-based studies in Antarctica. The diversity of taxa detected reinforces the hypothesis that the Antarctic airspora includes fungal propagules of both intra- (local) and inter-continental (distant) origin, such as from South America, Africa, and Oceania. It is possible that aeri-ally dispersed propagules of non-native fungi, often undetectable by culture methods, may remain in dormant but viable forms in Antarctica. As environmental conditions become less severe with ongoing climate change particularly in the Antarctic Peninsula region, these propagules may become able to grow and colonize different ecosystems in the region, with unknown impacts. Further aeromycological studies across Antarctica are required in order to understand how and from where fungi arrive and disperse within the continent.

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