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RESEARCH ARTICLE

Investigating aerial diversity of non-fungal eukaryotes across a 40° latitudinal transect using DNA metabarcoding

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Abstract

The high number of bipolar and widespread bryophyte and microbial taxa in the Antarctic flora suggests the effective Long-Distance Dispersal (LDD) of spores and other propagules from lower latitudes and even the Northern Hemisphere to Antarctica and the sub-Antarctic regions. However, few studies have attempted to document the transfer mechanisms by which potential newcomers may arrive in Antarctica. Commonly suggested or assumed mechanisms include transport in air currents, adventitious transfer with migrating or vagrant birds, and with human assistance. In this study, we investigated the biodiversity present in the air along a 40° latitudinal transect, from the port city of Rio de Janeiro, Brazil (ca. 22°S) to Comandante Ferraz Station on King George Island (South Shetland Islands, ca. 62°S), maritime Antarctica, to shed light on the potential role of LDD in species distribution. Air samples were collected in October 2021 on the Brazilian polar support vessel *Ary Rongel* using air filters with membranes of 0.22 μm. Total DNA was extracted from the filters and the Internal Transcribed Spacer (ITS2) DNA sequence was used for metabarcoding. A total of 53 non-fungal taxa representing three kingdoms (most abundantly Viridiplantae) and six phyla (most abundantly Bryophyta and Magnoliophyta) were assigned from the sequences found. Aerial biodiversity was greater closer to the coast and generally decreased with increasing latitude, although a small increase was apparent in the South Shetland Islands. The taxa assigned are generally present in coastal biomes, although a small proportion of the assignments represented taxa of more distant origin, supporting the occurrence of LDD in the air column.

KEYWORDS

Antarctica, ITS, South America, South Atlantic, wind dispersal

INTRODUCTION

The Antarctic bryophyte, lichen, and microbial floras include many bipolar and/or globally widespread taxa (Ochyra et al., 2008; Pearce et al., 2016; Øvstedal & Lewis-Smith, 2001; Kleinteich et al., 2017). Three primary hypotheses have been proposed to explain bipolar distribution patterns. These are (i) vicariance (the fragmentation of previously existing wider distributions), (ii) stepping stone migration via lower latitude mountainous regions,

and (iii) direct long-distance dispersal (LDD) (Dodge, 1973; Lamb, 1970; Lindsay, 1977; Ochyra et al., 2008). The main routes proposed for such dispersal include (i) the American pathway, with dispersal via the Neotropics and Patagonia to sub-Antarctic South Georgia and maritime Antarctica, (ii) the African pathway via the East and Central African Mountains to sub-Antarctic islands such as Marion Island and the Kerguelen archipelago, and (iii) the Indomalayan-Malesian pathway from south-east Asia to south-east Australia, New Zealand, and associated islands (Ochyra et al., 2008). Molecular data have recently supported the bipolarity of several moss taxa (Biersma et al. 2017; overview in Câmara, Carvalho-Silva, Pinto, et al., 2021; Câmara, Carvalho-Silva, & Stech, 2021; Câmara, Convey, Rangel, et al., 2021; Câmara, De Souza, Pinto, et al., 2021).

Muñoz et al. (2004) showed the importance of air currents as an LDD mechanism in the Southern Hemisphere for non-flowering plant species distributions, demonstrating a pattern of diaspore transfer from South America towards Africa at mid latitudes. More recently, the application of newly available molecular tools to samples obtained on the mid-Atlantic Trindade Island (Câmara, Menezes, et al., 2022; Faria et al., 2021) has corroborated this hypothesis. Older Antarctic studies based on macroscopic observations of air, snow and moss carpets (e.g. Kappen & Straka, 1988; Smith 1991; Marshall, 1996; Marshall & Convey, 1997) suggested that Antarctica could receive biological propagules by means of aerial transfer from South America. Based on preliminary 'backtrack' analyses of air mass movements, Biersma et al. (2018, 2020), suggested that successful transfer to the northern Antarctic Peninsula from southern South America was likely to occur more frequently than movement in the opposite direction.

Parts of Antarctica have been strongly affected by climate change in recent decades, in particular the Antarctic Peninsula region (Convey & Peck 2019; Siegert et al., 2019; Turner et al., 2009), a trend predicted to continue (Lee et al., 2017). Warming in this region has led to widespread glacial retreat and snow melt (Cook et al., 2017; González-Herrero et al., 2022), increasing the ice-free area available for colonization. Due to its proximity to South America and to milder climate characteristics relative to most of Antarctica, the South Shetland Islands archipelago is particularly prone to the arrival – both natural and human-mediated – and establishment of non-native organisms, which likely will negatively impact the fragile native ecosystems and communities. However, despite recognition of the hypotheses described above, very little is known explicitly about the mechanisms by which potential colonizers arrive in Antarctica or their frequencies or numbers. The presence of viable non-native plant propagules in periglacial Antarctic habitats has been detected, but not the mechanism of their arrival (Cuba et al., 2012). More recently, modern molecular tools have been used to support analogous inferences of the presence of DNA sequences of a wide range of macro- and microscopic organisms not currently present in or previously recorded from Antarctica (e.g. Fraser et al., 2018, et al. 2020, 2021a, 2022a; Carvalho-Silva et al., 2021), although such studies cannot be used to demonstrate the arrival of viable biological material. Indeed, Câmara, Menezes, et al. (2022) documented that DNA sequences representing species from mainland South America could reach as far south as the Ellsworth Mountains (c. 80° S) at the base of the Antarctic Peninsula.

To date, few studies have used molecular data to investigate airborne organisms in Antarctica, with those that have mostly focused on microorganisms and fungi (Pearce et al., 2009, 2016; Rosa et al., 2020, 2021). No published studies have used molecular tools to investigate the possible movement and exchange of biodiversity in the air column across a latitudinal gradient in the South Atlantic region, from tropical South America to the Antarctic Peninsula. In this study, we investigated airborne biodiversity

along a 40° transect from the port city of Rio de Janeiro, Brazil (ca. 22°S) to Comandante Ferraz Station on King George Island (South Shetland Islands, ca. 62°S) to shed light on the role of LDD in species distribution and dispersal patterns present in this region.

MATERIALS AND METHODS

Air sampling

Air samples were collected in October 2021 with the support of the Brazilian polar support vessel *Ary Rongel*. Air samples were collected during the vessel's journey from Rio de Janeiro (Brazil) to King George Island. The route traversed was divided into five sampling areas, referred to as S1 – S5 (Table 1, Figure 1). Area S1 included the region from the city of Rio de Janeiro to the city of Rio Grande (both in Brazil), S2 the region from Rio Grande to a point in the vicinity of the Argentinian City Puerto Deseado, S3 the region from Puerto Deseado to the eastern entrance of the Magellan Strait and then Punta Arenas in Chile, S4 the transit through the Magellan Strait and the Chilean channels, and S5 the crossing of the Drake Passage to Admiralty Bay in King George Island. Air was sampled using a sterilized polysulfone bottle top filter (Nalgene, USA) equipped with 0.22 µm sterilized membranes (47 mm diameter; Millipore, USA) coupled with a chemical duty pump (Millipore, USA), as described by Rosa et al. (2021). Membranes were changed on average every three days and stored in sterile Petri dishes which were kept frozen on board the vessel until further processing.

DNA extraction and data analyses

At the end of each sampling period, the filter membranes were immediately transferred to sterile Petri dishes. In the molecular biology laboratory at Comandante Ferraz Station (Admiralty Bay, King George Island), the membranes were removed from the Petri dishes inside a sterile laminar flow hood before DNA extraction. All equipment used (forceps, blades and tubes) was sterilized before use. Total DNA was extracted using a modified SDS/CTAB extraction method (Goldenberger et al., 1995; Natarajan et al.,

TABLE 1 Dates, locations and coordinates of the air samples obtained.

Sample	Dates	Region	Coordinates
S1	October 13–16, 2021	Rio de Janeiro (BR) Rio Grande (BR)	22°53'51.6" S/43°10'8.6" W to 32°6'12.38" S/52°6'12.38" W
S2	October 17–21, 2021	Rio Grande (BR) Vicinity of Puerto Deseado (AR)	32°14'30" S/51°59'25" W to 48°20'39" S/63°58'60" W
S3	October 21–23, 2021	Vicinity of Puerto Deseado (AR) Magellan Strait entrance (CH)	48°20'39" S/63°58'60" W to 52°31'3" S/68°30'1" W
S4	October 25–26, 2021	Near Punta Arenas (CH) Drake Passage entrance (CH)	53°45'53.8" S/70°53'43.6" W to 55°5'8.72" S/67°1'9.85" W
S5	October 26–28, 2021	Drake Passage Admiralty Bay (King George Island)	55°31'28.89" S/66°28'55.4" W to 62°10'14.92" W/58°23'12.85" W

Abbreviations: AR, Argentina; BR, Brazil; CH, Chile.

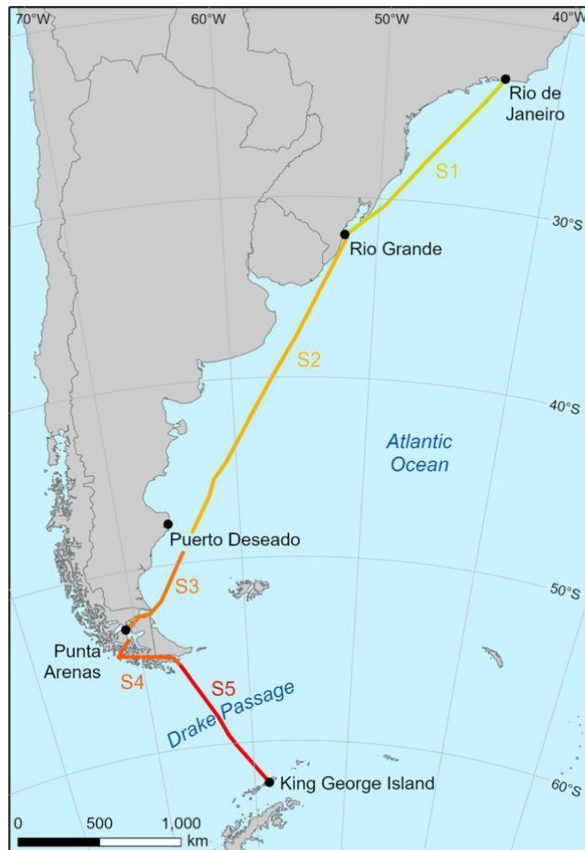


FIGURE 1 Air sampling regions. S1: City of Rio de Janeiro (RJ) to Rio Grande (RS). S2: Rio Grande to vicinity of Puerto Deseado. S3: Vicinity of Puerto Deseado to the eastern entrance of the Magellan Strait and then Punta Arenas. S4: Chilean channels from Punta Arenas to the Drake Passage. S5: Drake Passage to Admiralty Bay on King George Island. See also [Table 1](#).

2016; Zhou et al., 1996). Membranes were cut into small strips and added to plastic tubes, each containing 2 mL of SDS extraction buffer (0.1 M EDTA at pH8 and 2% SDS) and incubated at 55°C for 16 h. Then, 330 µL of 5 M NaCl and 330 µL of pre-heated CTAB 10% (55°C) were added, the solution was 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. The tubes were then centrifuged at 13000 rpm for 10 min and the supernatant transferred to a new tube. The extracted DNA was cleaned using the Genomic DNA purification Kit (QIAGEN, Carlsbad, USA), following the manufacturer's instructions. Extractions were carried out under strict sterile conditions to avoid 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). Extracted DNA was used as template for generating PCR amplicons. The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as DNA barcode for molecular species identification (Chen et al., 2010; Richardson et al., 2015; Câmara, Carvalho-Silva, Pinto, et al., 2021; Câmara, Carvalho-Silva, & Stech, 2021; Rosa et al., 2021). PCR amplicons generated using the universal primers ITS3 and ITS4 (White et al. 1990) were sequenced commercially by Macrogen Inc. (South Korea) using high-throughput paired-end sequencing (2×300bp) on a MiSeq System (Illumina), using the MiSeq Reagent Kit v3 (600 cycles) and following the manufacturer's protocol.

Three databases were used for identification of the amplicon sequences obtained, the PLANITS2 database (Banchi et al., 2020) for Plantae (version September 2020), the UNITE eukaryote ITS database version 8.3 (Abarenkov et al., 2020) for other Eukarya and, finally, sequences not assigned using these databases were classified against the NCBI non-redundant nucleotide sequences (nt) database (accessed October 2021); the nt database was filtered using the following keywords: “ITS1”, “ITS2”, “Internal transcribed spacer”, and “internal transcribed spacer”. Raw fastq files were filtered 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=r; 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, turning paired-end fastq files into merged, 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 the PLANITS2 database and trained with Naïve Bayes classifier with a confidence threshold of 98.5%. Remaining unclassified ASVs were filtered and aligned against the UNITE ITS database. Finally, the remaining unidentified sequences were classified against the filtered nt database using BLASTn (Camacho et al., 2009), and for taxonomic assignments MEGAN6 (Huson et al., 2016) was used.

Classification 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), Viridiplantae (including green algae as well as land plants), and Animalia. The kingdom Fungi was excluded and data on this group will be presented elsewhere. For lower ranks and taxonomic authorities, we checked global databases for marine species (WoRMS Editorial Board, 2021), algae (Guiry & Guiry, 2021), and the Catalogue of Life (Roskov et al., 2020). Geographic distribution follows Petz (2005), Thompson et al. (2019), Guiry and Guiry (2021), and Tropicos (www.tropicos.org). We used the number of reads as a proxy for abundance following the approach used by Deiner et al. (2017), Hering et al. (2018), Câmara et al. (2020, Câmara, de Menezes, et al., 2022; Câmara, Menezes, et al., 2022; Câmara, Bones, et al., 2022), and Rosa et al. (2020, 2021). Sequences were submitted to GenBank and accession numbers are given in Appendix 1.

Atmospheric modelling

Identification of the trajectories of air masses between continents was carried out using HYSPLIT models (Rolph et al. 2017; Stein et al., 2015, https://www.ready.noaa.gov/HYSPLIT_traj.php) with reanalysis from 1948 to the present. The following parameters were applied: 1-month file with a total run time of 24h starting at 11:00h on the last day of each month, 30m elevation, and geographical coordinates: October 13–16 (32°6′12.38″ S/52°6′12.38″ W); October 17–21 (48°20′39″ S/63°58′60″ W); October 21–23 (52°31′3″ S/68°30′1″ W); October 25–26 (55°5′8.72″ S/67°1′9.85″ W); October 26–28 (62°10′14.92″ W/58°23′12.85″ W). The trajectories of air masses are simulations generated using the basic concepts of Lagrangean mechanics. The models presented represent the possible routes of air masses to the collection site, considered as the last position of the ship during each interval in the route. Modelling using the Hysplit Platform has been widely used in studies linking meteorology and ecology (Parry et al., 2015, Rolph et al. 2017, Rivas Jr. et al., 2018).

RESULTS

The air sampled over the period averaged 350 m³ per day. The calculated rarefaction curves for all detected taxa approached a plateau, indicating that the reads gave an accurate representation of the sequence diversity in all five sampling intervals (Figure 2). A total of 742 518 paired-end DNA reads were generated in the sequencing run of which 689 646 ITS2 reads remained after quality filtering. A total of 42 894 of these reads were assigned to 53 eukaryotic taxa of the kingdoms Protozoa, Chromista, and Viridiplantae (Table 2, Figure 3). No reads belonging to kingdom Animalia were detected. A further 1170 reads represented unknown taxa, while the remaining reads represented fungi, which will be addressed separately.

Results obtained at the scale of the five sampling regions are summarized in Table 2. Sequences in region S1 represented a total of 17 taxa, from six phyla and three kingdoms: Chromista (Ciliophora, Haptophyta), Protozoa (Evosea), and Viridiplantae (Chlorophyta, Bryophyta, and Magnoliophyta). In region S2 nine taxa were assigned to the same three phyla of Viridiplantae (Chlorophyta, Bryophyta, and Magnoliophyta). In region S3 six taxa were assigned to two phyla of Viridiplantae (Chlorophyta and Magnoliophyta). In region S4 a total of five taxa were assigned to three phyla of two kingdoms: Chromista (Ciliophora) and Viridiplantae (Chlorophyta and Magnoliophyta). Finally, in region S5, a total of seven Viridiplantae taxa from the phyla Chlorophyta and Magnoliophyta were detected.

Very few of the assigned taxa below phylum level were recorded from more than one sampling region (Figure 3). The grass *Poa pratensis* L. was found in S2 and S3, the green algae order Mamiellales in S2, S3 and S5, the tree *Nothofagus antarctica* (Forster) Oerst. in S3, S4, and S5, and the green alga *Chrysochromulina simplex* Estep, P. G. Davis, P. E. Hargraves & Sieburth and tree *Quercus* sp. were present in S2, S4 and S5. No taxa were detected in all sampled regions. Sequence diversity and abundance decreased with latitude between regions S1 and S4, although there was a small increase in S5 (Figure 4).

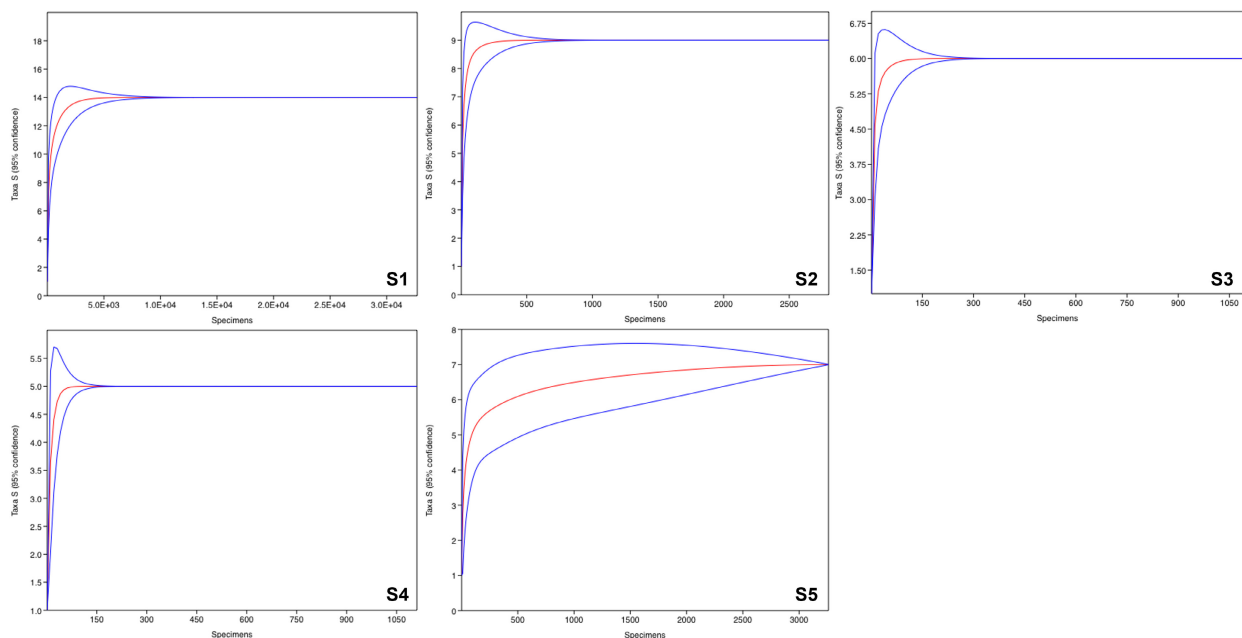


FIGURE 2 Rarefaction curves obtained from air samples from the five regions sampled (S1–S5); see also Table 1. Blue lines represent 95% confidence intervals.

TABLE 2 ASV abundances (DNA reads) from samples obtained from air sampled in five different regions between Rio de Janeiro and the South Shetland Islands (taxonomic classification follows Ruggiero et al., 2015).

REGION S1			
TAXON	Habitat	Distribution	Reads
KINGDOM CHROMISTA			
Phylum Ciliophora			
Order Sporadotrichida			66
<i>Stylonychia</i> sp.	M, T, B	An, As, Au, E	42
Phylum Haptophyta			
<i>Chrysochromulina simplex</i> Estep, P. G. Davis, P. E. Hargraves & Sieburth	M	NA, E, NZ	8
KINGDOM PROTOZOA			
Phylum Evosea			
<i>Protostelium mycophagum</i> L. S. Olive & Stoian	T	W	783
KINGDOM VIRIDIPLANTAE			
Phylum Chlorophyta			
Order Chlorellales			
<i>Apatococcus</i> sp.	F	Au, NZ, As, A, E	279
Phylum Bryophyta			
<i>Sanionia uncinata</i> (Hedw.) Loeske	T	Bi	24 039
<i>Sarmentypnum exannulatum</i> (Schimp.) Hedenäs			620
Phylum Magnoliophyta			
Fam. Asteraceae			
<i>Chorisiva</i> sp.	T	NAm, N	20
<i>Lactuca virosa</i> L.	T	N, NAm, A, As, E	24
Fam. Cannabaceae			
<i>Celtis iguanaea</i> (Jacq.) Sarg.	T	N	28
<i>Trema</i> sp.	T	N, A, As	472
Fam. Chloranthaceae			
<i>Hedyosmum</i> sp.	T	N, As	710
Fam. Euphorbiaceae			
<i>Euphorbia hirta</i> L.	T	NAm, A, As, Au	237
Fam. Fabaceae			
<i>Glycine max</i> (L.) Merr.	T	W	1285
Fam. Fagaceae			
<i>Quercus</i> sp.	T	W	87
Fam. Urticaceae			
<i>Urtica stachyoides</i> Webb & Berthel	T	NAm, A	130
Fam. Myrtaceae			
<i>Eucalyptus</i> sp.	T	W	5828
Total Reads			34 543
REGION S2			
KINGDOM VIRIDIPLANTAE			
Phylum Chlorophyta			
Order Mamiellales			
	M	W	599
Order Trebouxiales			
	A, F	W	

TABLE 2 (Continued)

REGION S1				
<i>Symbiochloris</i> sp.	T	Ar, As, Au, E, NA, NZ		510
Phylum Bryophyta				
<i>Bryoerythrophyllum recurvirostrum</i> (Hedw.) P.C.Chen	T	Bi		179
Phylum Magnoliophyta				
Fam. Amaranthaceae				
<i>Atriplex</i> sp.	T	E, N, NAm, A, Au, As		542
Fam. Fabaceae				
<i>Trifolium</i> sp.	T	W		201
Fam. Malvaceae				
<i>Malva</i> sp.	T	E, N, NAm, NZ, A, As, Au		84
Fam. Moraceae				
<i>Morus alba</i> L.	T	E, N, NAm, A, As, Au		577
Fam. Poaceae				
<i>Poa pratensis</i> L.	T	E, N, NAm, A, As, Au, A		98
Total Reads				2848
REGION S3				
KINGDOM VIRIDIPLANTAE				
Phylum Chlorophyta				
Class Trebouxiophyceae				
Order Mamiellales	M	W		226
Phylum Magnoliophyta				
Fam. Fagaceae				
<i>Nothofagus antarctica</i> (Forster) Oerst.	T	P		166
<i>Nothofagus pumilio</i> (Poepp. & Endl.) Krasser	T	P		392
Fam. Poaceae				
<i>Poa pratensis</i> L.	T	E, N, NAm, A, As, Au, A		34
Total Reads				1109
REGION S4				
KINGDOM CHROMISTA				
Phylum Ciliophora				
Fam. Oxytrichidae	M, F, T	W		65
KINGDOM VIRIDIPLANTAE				
Phylum Chlorophyta				
Order Prymnesiales				
<i>Chrysochromulina simplex</i> Estep, P. G. Davis, P. E. Hargraves & Sieburth	M	NA, NZ		103
Order Chlamydomonadales				
<i>Chlorosarcinopsis eremi</i> Chantanachat & Bold	T	E, NA, Me		650
Phylum Magnoliophyta				
Fam. Fagaceae				

TABLE 2 (Continued)

REGION S1				
<i>Nothofagus antarctica</i> (Forster) Oerst.	T	P		91
<i>Quercus</i> sp.	T	W		214
Total Reads				1123
REGION S5				
KINGDOM VIRIDIPLANTAE				
Phylum Chlorophyta				10
Class Trebouxiophyceae				
Order Chlamydomonadales	M, B, F, T	W		2
Order Mamiellales	M	W		66
Order Prymnesiales				
<i>Chrysochromulina simplex</i> Estep, P. G. Davis, P. E. Hargraves & Sieburth	M	NA, E, NZ		68
Order Trebouxiales				
<i>Trebouxia jamesii</i> (Hildreth & Ahmadjian) Gärtner	A, T, Ph	E		2525
Phylum Magnoliophyta				
Fam. Fagaceae				
<i>Nothofagus antarctica</i> (Forster) Oerst.	T	P		397
<i>Quercus</i> sp.	T/	W		203
Total Reads				3271

Abbreviations: Hab. A, aerial; M, Marine; F, Freshwater; B, Brackish; Ph, Phycobiont; T, Terrestrial. Distribution: A, Africa; An, Antarctica; Ar, Arctic; As, Asia; Au, Australia; Bi, Bipolar; E, Europe; Me, Middle East; N, Neotropical; NA, North Atlantic; NAm, North America; NZ, New Zealand; P, Patagonia; W, Widespread/Cosmopolitan.

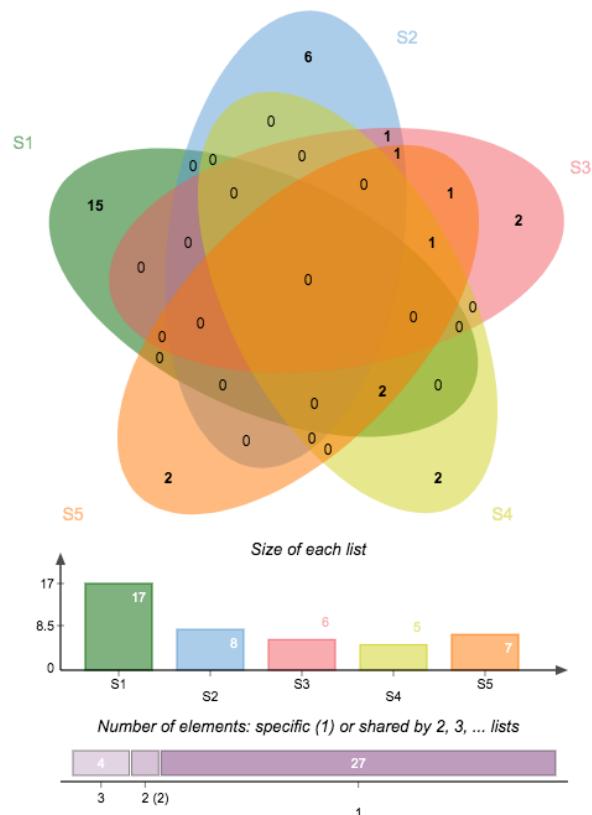


FIGURE 3 Venn diagram showing the numbers of taxa found in each of the five regions sampled.

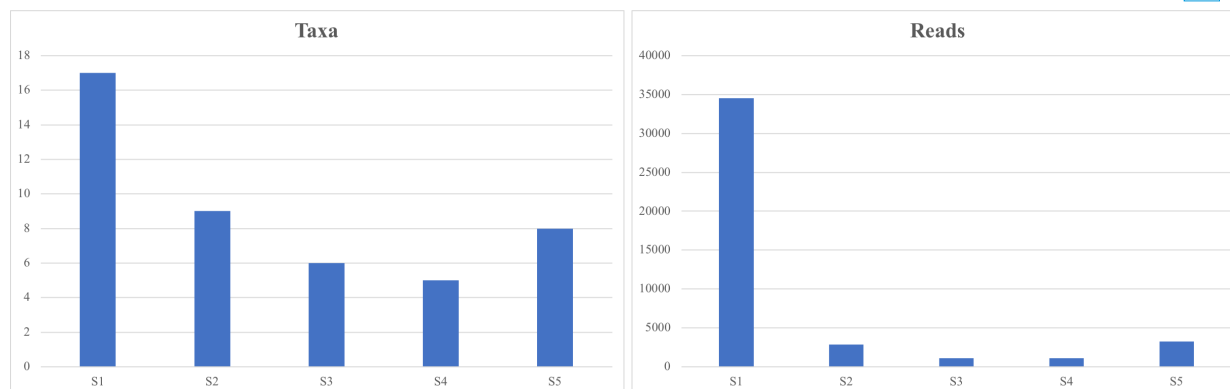


FIGURE 4 Taxon richness (left) and abundance based on number of reads (right) across the five sampling regions (S1–S5).

TABLE 3 Data description.

JOB ID	149411	146294	146942	147091	148961
Data	45 215	45 220	45 222	45 225	45 227
Altitude average (m)	500	250	500	250	250
Number of continental Trajectories	0	0	32	32	0
Number of maritime Trajectories	32	32	0	0	32

Region S1 recorded the greatest diversity (17 taxa) and abundance (34543 reads). The most abundant taxon was the moss *Sanionia uncinata* (Hedw.) Loeske, followed by *Eucalyptus* sp. and *Glycine max* (L.) Merr (soybean). Region S2 recorded nine taxa, with the order Mamiellales being the most abundant, followed by *Morus alba* L. Region S3 recorded six taxa, with the tree *Nothofagus pumilio* (Poepp. & Endl.) Krasser being most abundant, followed by Mamiellales. Region S4 recorded the lowest diversity with five taxa, of which the green alga *Chlorosarcinopsis eremi* Chantanachat & Bold was the most abundant, followed by *Quercus* sp. Finally, S5 recorded a small increase in diversity and abundance with seven taxa, of which the green alga *Trebouxia jamesii* (Hildreth & Ahmadjian) Gärtner was the most abundant, followed by *Nothofagus antarctica*.

The HYSPLIT quantitative analysis of trajectories and reanalysis of collection points revealed that, of the 160 possible trajectories, 64 originated in South America (Table 3). The results indicate the influence of air mass on the collection regions S2 and S3. The lack of models that point to the connection between the study regions is still a point of interest in atmospheric science; however, the altitude accuracy of the trajectory data shown in the colours of the trajectory lines in Figure 5, supports the hypothesis of continental origin of the material identified in the DNA Metabarcoding analyses, especially in sections S2 and S3. In the mathematical simulation carried out for the study region, the trajectories of the air masses were reconstructed over the regions of occurrence of the identified species, as shown in Figure 5.

DISCUSSION

In interpreting data obtained in a study such as this, it is important to note that assigning a DNA sequence identity does not confirm the presence of a viable organism or propagule, while the assignment accuracy itself relies heavily on

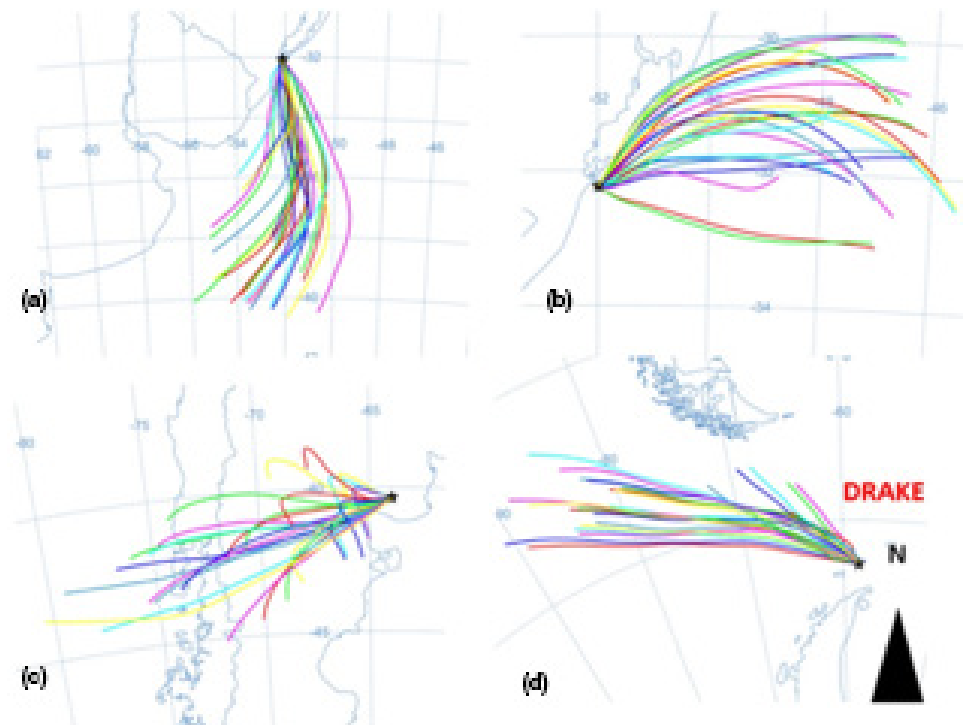


FIGURE 5 (a, b) 24-h analysis with predominance of oceanic air masses; (c) 24-h analysis with predominance of continental air masses (South America); (d) Drake air mass dynamics record (October 26–28). Colour lines represent different altitudes ranging from 0 to 550m high (see also Appendix 1).

the quality and completeness of available databases, and on the choice of molecular markers and primers. Apart from Fungi, which represented the majority of the obtained sequence reads and will be presented elsewhere, most non-fungal taxa represented Viridiplantae, excepting a small number of Chromista and one slime mould. This is not unexpected given our use of ITS2 as the reference molecular marker and universal ITS primers, along with the consulted databases. The use of markers such as 16S and Cox1 would allow identification of additional taxa not recognized in this study, such as Bacteria, Archaea, and Metazoa (Câmara, Carvalho-Silva, Pinto, et al., 2021). Assignments of a small fraction of sequence reads to unknown taxa may represent new and undescribed taxa or taxa absent from the consulted databases.

The highest diversity and abundance were obtained in region S1 (Figure 4). This region includes many larger centers of human population and commercial ports, such as Rio de Janeiro, Santos, and Paranaguá. Species linked with human activity were recorded in this region as well as representatives of the local biome found in this coastal region, with plants commonly found in the Brazilian Atlantic Rainforest. The considerable decrease in diversity and abundance in regions S2 and S3 likely reflects the increase in distance of the vessel's route from the South American coast, combined with the overall decrease in coastal regional terrestrial diversity with progression southwards towards Patagonia. The lowest diversity was detected in the Chilean channels (S4) linking Punta Arenas to the Drake Passage. In this sampling region the vessel was closer to the shore and its vegetation, but this was also the shortest sampling period. There was a small increase in diversity and abundance recorded in the final region S5, as the vessel crossed the Drake Passage and arrived at the South Shetland Islands.

Among the Chromista sequences assigned, some taxa could only be identified at higher taxonomic level (order and family), limiting the

inferences that can be made. Representatives of the genus *Stylonychia*, which includes both terrestrial and marine species, and Oxytrichidae (S4) were the only Ciliophora taxa detected. *Chrysochromulina simplex* is also a marine species, but has not previously been recorded from the South Atlantic. The only protozoan found was the slime mould, *Protostelium mycophagum*, which is considered a very common and widespread species that produces aerial spores (Landolt et al., 2008; Spiegel et al., 2004). It has been recorded from numerous different habitats globally, including sub-tropical Ascension Island in the mid-Atlantic.

Among the Viridiplantae, some of the green algae were also only identified at higher taxonomic level (class and order). Representatives of Trebouxiales, such as *Trebouxia jamesii*, are known to be present in the air column, and members of this genus have been detected both in rain (Dillon et al., 2020) and snow (Tesson & Šantl-Temkiv, 2018), and are associated with cloud nucleation. Other taxa such as *Apatococcus*, a freshwater genus, and *Symbiochloris*, a terrestrial genus, are common in the Southern Hemisphere. *Chlorosarcinopsis eremi* represents a terrestrial genus known from the Northern Hemisphere, suggesting that algal taxa may be more commonly present in the air column and transported over longer distances than generally realized. Marine taxa such as *Chrysochromulina simplex* have been recorded in southern regions around New Zealand, consistent with its presence in our southernmost sampling region S5, and may therefore also be more widespread than currently realized.

Among the three species of Bryophyta detected, *Sanionia uncinata* is a bipolar species distributed in northern boreal regions and in southern South America, the sub-Antarctic islands and the maritime Antarctic as well as high elevations of tropical and subtropical regions (Hedenäs, 2012; Ochyra et al., 2008; Ochyra & Matteri, 2001). Its occurrence in our southern samples might be expected, but its presence in region S1 is surprising, as its nearest known area of occurrence is in the central Andes (Hedenäs, 2002). This may indicate long-distance dispersal across its bipolar distribution, although contamination cannot be entirely ruled out. However, contamination seems very unlikely here as the samples from all regions were processed at the same time in the same laboratory, so any contamination would be expected to be present in all regions and not only S1. Three different databases were consulted in relation to this identification (Plantlts, Unite, NCBI), all matching this taxon. DNA sequences of *S. uncinata* obtained in the present study match those reported by Hedenäs (2012) as originating from Southern Hemisphere regions such as Tierra del Fuego, the South Orkney Islands, and Antarctica. *Sarmentypnum exannulatum* is a widespread species in the Arctic to temperate Northern Hemisphere, North Africa and tropical African mountains, Australia and New Zealand, and South America, and has been recorded from the region of Rio de Janeiro (Hedenäs, 2003). *Bryoerythrophyllum recurvirostrum* is a common and widespread bipolar species, distributed in Antarctica and the sub-Antarctic islands (Ochyra et al., 2008), as well as being reported from Tierra del Fuego (Matteri & Schiavone, 1991), South Africa (Magill, 1981), Tristan da Cunha (Dixon, 1960), and Australia/New Zealand (Streimann & Klazenga, 2002). No bryophyte sequences were assigned in regions S3–S5, despite the group being a major element of the flora of both the Magellanic sub-Antarctic region of southern South America and of the South Shetland Islands.

Flowering plant taxa related to human activity were commonly found during the early part of the vessel's route, closer to the Brazilian coast and its major cities, including lettuce (*Lactuca*), soybean (*Glycine max*), and the tree genus *Eucalyptus*. Brazil is the second largest soybean producer globally, including in the coastal states of Paraná and Santa Catarina. Species of *Eucalyptus* are grown in plantations covering about 33 000 000 ha in São

Paulo, Paraná, Santa Catarina, and Rio Grande do Sul states (Rodrigues et al., 2021). Native taxa such as *Hedyosmum* and *Trema* are present in the Brazilian Atlantic Rainforest. With progression further south into sampling region S2, *Morus alba* (mulberry) is a deciduous tree cultivated in tropical and sub-tropical countries (Sánchez, 2000). The genus *Atriplex*, the second most frequently assigned sequence in this region, is common in coastal habitats and its seeds are dispersed and flowers pollinated by wind (Čalasan et al., 2022). In regions S3 to S5, *Nothofagus* became more common, corresponding in regions S3 and S4 with its natural area of occurrence, but then extending across the Drake Passage to the South Shetland Islands. The presence of *Nothofagus* pollen in Antarctica has long been reported (e.g. Sein, 1961). DNA assigned to *N. pumilio* has been reported from glacial ice in the Antarctic Peninsula region (Câmara, Menezes, et al., 2022). Sequences of the grass *Poa pratensis* were recorded in regions S2 and S3 (also adjacent to areas where the species occurs widely, even if not originally native). This grass is invasive on various sub-Antarctic islands (Frenot et al., 2005) and was persistently established at Cierva Point in the western Antarctic Peninsula region until its eradication in the late 2010s (Pertierra et al., 2017). Sequences representing the cultivated but non-native Northern Hemisphere (temperate to tropical) tree genus *Quercus* were most abundant in region S4.

CONCLUSIONS

We present the first molecular study of non-fungal eukaryotic taxa present in the airspora along a 40° latitudinal transect from Brazil to the South Shetland Islands. Our data indicate a correlation between the diversity detected and distance from the coast as well as latitude. Airborne diversity generally decreased with increasing latitude. The range and potential sources of the taxa assigned suggest that the transport of eukaryotic DNA in the air column is more common than is generally acknowledged and provides further support for the occurrence of long-distance dispersal. In future studies, the use of different markers to assess the presence of a wider range of organisms is desirable.

AUTHOR CONTRIBUTIONS

Paulo Camara: Conceptualization (lead); data curation (equal); formal analysis (equal); funding acquisition (supporting); investigation (equal); project administration (equal); writing – original draft (lead). **Fabyano Lopes:** Formal analysis (lead); methodology (equal); software (lead); writing – original draft (supporting). **Fabio Bones:** Formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Luiz Rodrigues:** Investigation (equal); methodology (equal); resources (lead); writing – original draft (supporting). **Micheline Carvalho-Silva:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); validation (lead); writing – original draft (equal). **Michael Stech:** Conceptualization (equal); investigation (equal); writing – original draft (equal). **Peter Convey:** Conceptualization (equal); formal analysis (equal); investigation (equal); validation (equal); writing – original draft (equal). **Luiz Rosa:** Conceptualization (equal); funding acquisition (lead); writing – original draft (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Molecular data are deposited in NCBI GenBank.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.