RESEARCH ARTICLE

DNA metabarcoding of non-fungal eukaryotic diversity in air and snow of Livingston Island, South Shetland Islands, Antarctica

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Abstract

A major natural route of dispersal to Antarctica is often assumed to be atmospheric transport, although few studies have documented this in detail. Aerial dispersal to Antarctica is very challenging as the continent is geographically remote from other land areas and is isolated by the atmospheric circumpolar vortex. Detailed information about aerial routes by which microorganisms arrive and circulate in Antarctica is generally lacking, as few aerobiological studies have focused on eukaryotes and those that have predominantly relied on traditional morphological identification. Recent advances in molecular biology, such as DNA metabarcoding by high throughput sequencing (HTS), have provided a powerful new tool for the study of atmospheric biological diversity and can retrieve levels of diversity an order of magnitude higher than traditional methods. In this study, we used HTS to investigate the diversity of non-fungal eukaryotes present in the atmosphere and freshly precipitated snow on Livingston Island. In a total of 740 m^3 of air and 3.76 L of snow sampled, representatives of four kingdoms (Protozoa, Chromista, Viridiplantae and Animalia) and five phyla (Ciliophora, Ochrophyta, Chlorophyta, Magnoliophyta and Porifera) were found. The most diverse phylum was Chlorophyta, represented in our samples by 10 taxa, with *Trebouxia asymmetrica* Friedl & Gärtner the most abundant representative.

Keywords

High throughput sequencing; dispersal; Algae; Plants; Protozoa; Animalia

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Abbreviations

ASV: amplicon sequence variant
HTS: high throughput sequencing
ITS2: internal transcribed spacer 2
NCBI: National Center for Biotechnology Information, USA

To access the supplementary material, please visit the article landing page

Introduction

Livingston Island is the second largest of the South Shetland Islands, with an area of about 798 km^2 (Ivanov 2009). The archipelago forms part of the Maritime Antarctic region, a region that experienced the most rapid air temperature warming in Antarctica in the second half of the 20th century (Turner et al. 2009). Although that warming trend stopped at the opening of the 21st century (Turner et al. 2016), climate modelling predicts a return to the previous rapid warming rates through the remainder of this century and beyond (Bracegirdle et al. 2020). One of the consequences of this warming has been (and is predicted to be) an increase in the area of ice-free terrestrial habitats available through accelerated ice and snow melt (Convey & Peck 2019; Lee et al. 2020). This, in concert with the warmer environmental conditions, is predicted to lead to increased establishment of new biodiversity (including microorganisms) in the region, with potentially important consequential effects on Antarctic ecosystems (Amesbury et al. 2017; Robinson et al. 2018; Convey & Peck 2019; Câmara et al. 2020).

A major natural route of dispersal to Antarctica is often assumed to be via atmospheric transport, although few studies have documented this in detail (Marshall 1996; Hughes et al. 2004). However, aerobiological dispersal to Antarctica is very challenging as the continent is geographically remote from other land masses and is isolated by the atmospheric circumpolar vortex. Even if such transfer of

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viable propagules takes place, there are further challenges in achieving establishment, including arriving in the tiny area of exposed terrestrial habitat (ca. 0.2–0.4% of the continental area) and surviving the extreme climatic conditions (Hughes et al. 2006). Detailed information about the aerial routes by which microorganisms arrive and circulate in Antarctica is generally lacking (Bottos et al. 2013; Pearce et al. 2016; Archer et al. 2019; but see also Marshall 1996). Aerobiological studies have reported the presence of microbial cells, spores and fragments of organisms (e.g., bacteria, viruses, algae, fungi and plants) in the air column, some of which can only have originated beyond Antarctica (Marshall 1996; Marshall & Chalmers 1997; Marshall & Convey 1997; Pearce et al. 2010; Sundberg 2013; Rosa et al. 2020; Rosa et al. 2021), although propagule pressure remains unknown (Rosa et al. 2020).

Few aerobiological studies focused on eukaryotes have been carried out in Antarctica and those that have predominantly relied on traditional morphological identification or culturing techniques (Marshall 1996; Marshall & Chalmers 1997). However, many spores, pollen, encysted life forms, sterile organisms and microorganisms may be impossible to identify using traditional morphology observation and it is therefore likely that only a minor fraction of the diversity present in the atmosphere has been described. Recent advances in molecular biology, such as DNA metabarcoding by HTS, have, in combination with techniques for high-volume sampling of bioaerosol, provided a powerful new tool for the study of atmospheric biological diversity (Šantl-Temkiv et al. 2020). These approaches can retrieve diversity that is about an order of magnitude higher than traditional methods (Czechowski et al. 2017; Rippin et al. 2018), although it is important to recognize that identification or assignment of a sequence identity does not provide confirmation of the presence of a viable organism or propagule. Rosa et al. (2020, 2021) used HTS to assess fungal sequence diversity present in the atmosphere over the Keller Peninsula, on King George Island, South Shetland Islands and over Livingston Island, documenting the presence of a highly diverse fungal sequence assemblage potentially transported to and deposited in Antarctica. In this study, we applied HTS to the same air and snow samples as used by Rosa et al. (2020) to investigate the sequence diversity of non-fungal eukaryotes present in the atmosphere and freshly precipitated snow on Livingston Island, thereby complementing the already available data on fungi.

Materials and methods

The original samples and most of the analyses used here are those described by Rosa et al. (2020), and the following text notes differences and additions related to the current study.

Sampling

As described by Rosa et al. (2020), two air samples were collected in the summer of 2019 at Punta Polaca (62°40′16″S; 60°22′43″W), Hurd Peninsula, Livingston Island, South Shetland Islands, near the Spanish Juan Carlos I Antarctic Station (Fig. 1) with a high flow glass impinger, following methods described by Šantl-Temkiv et al. (2017, 2018). Water from the impinger was filtered using Sterivex filters at the station laboratories and then stored frozen at –20°C on station and during transport to Brazil by the Brazilian polar support vessel.

Two freshly deposited surface snow samples were collected on March 2019 at a remote site on the Hurd Peninsula (62°40′15″S; 60°22′40″W), using a sterilized spoon. Samples were immediately taken to the Juan Carlos I Antarctic Station and frozen at –20°C until transport to Brazil.

DNA extraction, amplification and sequencing

The total DNA was then extracted, amplified and sequenced as described by Rosa et al. (2020) and in the Supplementary material. It is important to note that the two air DNA extractions were combined to increase DNA yield. All raw sequences have been deposited in the NCBI database under the codes SRR12830238, SRR12830240 and SRR12830239, the same as used and deposited by Rosa et al. (2020). We selected the ITS2 of the nuclear ribosomal DNA (Chen et al. 2010; Richardson et al. 2015) as a barcode, as ITS2 has been widely used to identify a diverse range of eukaryote organisms including fungi, animals, protozoans, chromists and plants (Ruppert et al. 2009) and has proved effective in recent studies of Antarctic diversity (Câmara et al. 2020; Rosa et al. 2020).

Data analyses and ASV taxonomic identification

Sequence processing and bioinformatics analyses are described by Rosa et al. (2020) and in the Supplementary material. Taxonomic assignments of ASVs were determined using the QIIME 2 q2-feature-classifier plugin (Bokulich et al. 2018) with classify-sklearn against different databases, using a sequence similarity threshold of 97%. First, ASVs were classified against the PLANiTS2 database (Banchi et al. 2020). After this step, ASVs that remained unclassified were filtered and classify-sklearn classified against the UNITE Eukaryotes ITS database version 8.3 (Abarenkov et al. 2020). Finally, remaining unclassified ASVs were filtered and aligned using BLASTn (Camacho et al. 2009) with default parameters against the NCBI non-redundant nucleotide sequences (nt)

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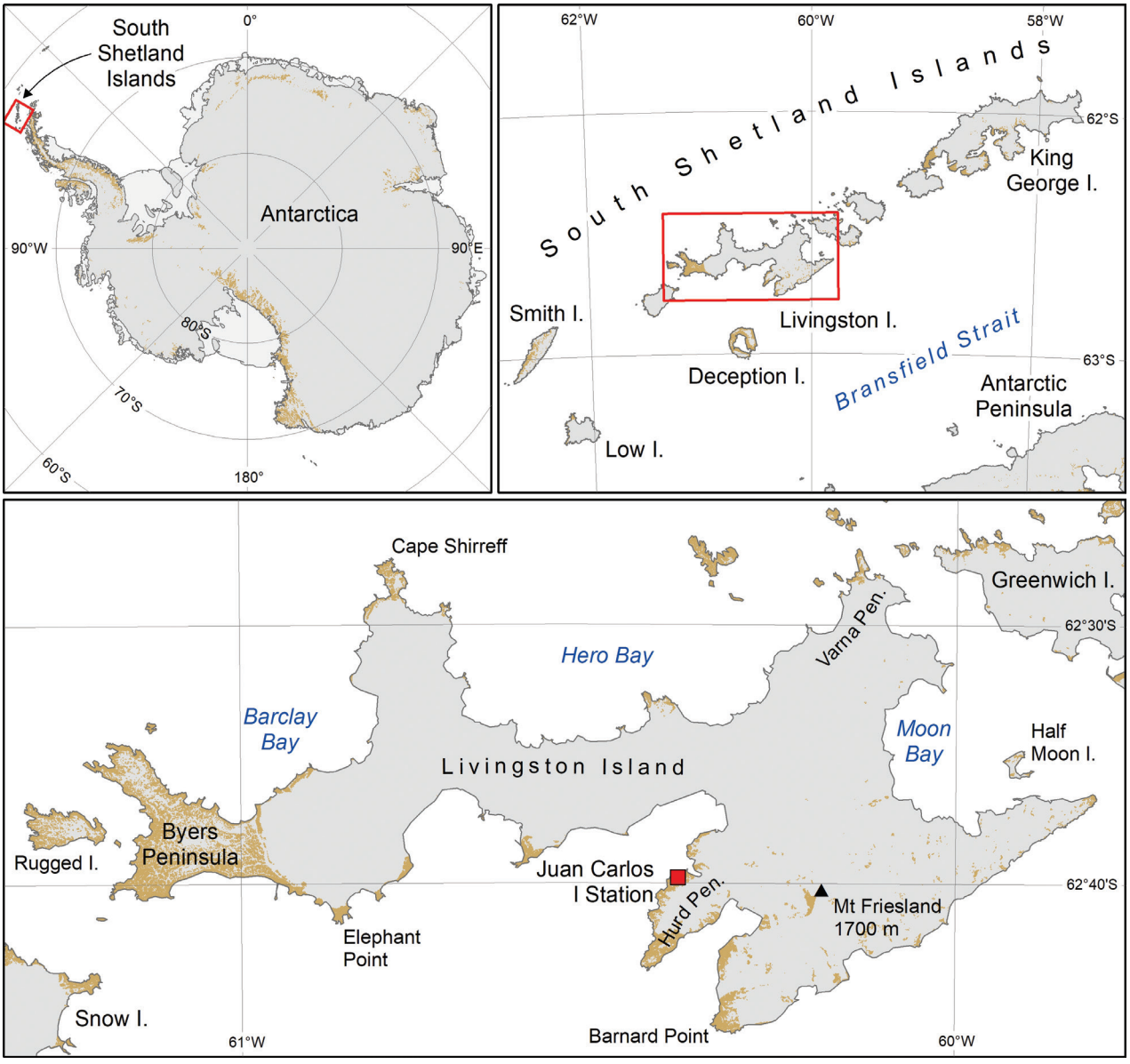

Figure 1 Location of Juan Carlos I Antarctic Station on Livingston Island, South Shetland Islands, Antarctica.

database (October 2021) using the following keywords: "ITS1," "ITS2," "internal transcribed spacer." For simplicity, we henceforth refer to the assigned ASVs as "taxa." For comparative purposes, we considered the number of reads as a proxy for abundance (Deiner et al. 2017; Hering et al. 2018; Câmara, Carvalho-Silva et al. 2021; Câmara, Convey et al. 2021; Carvalho-Silva et al. 2021; Rosa et al. 2021). Rarefaction curves were generated using the software PAST 3.26 (Hammer et al. 2001) and Venn diagrams were prepared as described by Bardou et al. (2014).

As the data presented here represent a wide range of organisms from many different taxonomic groups, there is no single classification system covering the entire range. We therefore decided to present the data according to two different systems, those of Ruggiero et al. (2015) and Adl et al. (2019). As all the databases consulted are based on ranks and the system by Adl et al. (2019) is rank-free, all the names retrieved were ranked and correlated with the Adl system manually.

Results

A total of 740 m^3 of air and 3.76 L of snow were sampled. A total of 751 670 paired-end DNA reads were generated in the sequencing run and 439 168 reads remained after

Table 1 Distribution of taxa among the analysed samples.

Classification A (Ruggiero et al. 2015)	Classification B (Adl et al. 2019)	Distribution[d]	Habitat[e]	Abundance (reads) Air	Snow[1]	Snow[2]
Kingdom Plantae						
Phylum Chlorophyta	Archaeplastida/Chloroplastida/Chlorophyta					
Chlamydomonas nivalis (F.A. Bauer) Wille	Archaeplastida/Chloroplastida/Chlorophyta/Chlorophyceae	W/A	S/F/T	0	71	0
Koliella longiseta (Vischer) Hindák [a]	Archaeplastida/Chloroplastida/Chlorophyta	E	F	0	491	0
Family Koliellaceae	Archaeplastida/Chloroplastida/Chlorophyta			0	35	0
Trebouxia australis Beck	Archaeplastida/Chloroplastida/Chlorophyta/Trebouxiophyceae		F	67	0	0
Trebouxia asymmetrica Friedl & Gärtner [a]	Archaeplastida/Chloroplastida/Chlorophyta/Trebouxiophyceae	E	T	0	3696	0
Trebouxia aff. *solaris* Voytsekhovich & Beck [b]	Archaeplastida/Chloroplastida/Chlorophyta/Trebouxiophyceae	E	P	0	148	0
Trebouxia potteri Ahmadjian ex Gärtner [b]	Archaeplastida/Chloroplastida/Chlorophyta/Trebouxiophyceae	E	T	0	98	0
Trebouxia sp.	Archaeplastida/Chloroplastida/Chlorophyta/Trebouxiophyceae			0	991	0
Phylum Tracheophyta	Archaeplastida/Streptophyta/Embryophyta					
Class Liliopsida	Archaeplastida/Streptophyta/Embryophyta	W	T	0	206	0
Citrus sp. [b]	Archaeplastida/Streptophyta/Embryophyta	W	T	2	0	0
Kingdom Protozoa		W	C	0	3	253
Kingdom Chromista						
Phylum Ciliophora	Sar/Alveolata/Ciliophora					
Vorticella sp.	Sar/Alveolata/Ciliophora/Intramacronucleata /CONTHREEP/Oligohymenophorea/Peritrichia /Sessilida/Vorticellidae	W	T/F	21	0	0
Phylum Ochrophyta	Sar/Stramenopiles/Gyrista/Ochrophyta			0	0	141
Desmarestia sp.	Sar/Stramenopiles/Gyrista/Ochrophyta/Chrysista /Chrysophyceae/Phaeophyceae/Desmarestiales	W/A	M	0	0	25
Fragilariopsis cylindrus (Grunow ex Cleve) Helmcke & Krieger	Sar/Stramenopiles/Gyrista/Ochrophyta/Diatomista/Bacillariophytina/Bacillariophyceae/Fragilariophycidae	W/A	M	0	0	72
Thalassiosira sp. [c]	Sar/Stramenopiles/Gyrista/Ochrophyta/Diatomista/Bacillariophytina/Thalassiosirophycidae	W/A	M/F	0	0	44
Thalassiosira punctigera (Castracane) Hasle[b]	Sar/Stramenopiles/Gyrista/Ochrophyta/Diatomista/Bacillariophytina /Thalassiosirophycidae	W	M	0	0	1
Kingdom Animalia						
Phylum Porifera	Opisthokonta/Holozoa/Metazoa/Porifera					
Order Poecilosclerida [c]	Opisthokonta/Holozoa/Metazoa/Porifera/Demospongiae/Heteroscleromorpha	W/A	M	0	1	0

[a]Previous Antarctic records only in metabarcoding studies. [b]First record from Antarctica. [c]Obtained from the NCBI GenBank database. [d]Widespread (W), Europe (E), Antarctica (A). [e]Snow (S), freshwater (F), terrestrial (T), marine (M), cosmopolitan (C), lichen photobiont (P).

quality filtering, representing 18 taxa (Table 1, Supplementary Table S1). The large majority of these—430748 reads—corresponded to fungi (see Rosa et al. 2020). Of the remaining reads, 6366 included representatives of four kingdoms (according to Ruggiero et al. 2015)—Protozoa, Chromista, Plantae and Animalia—and the five phyla Ciliophora, Ochrophyta, Chlorophyta, Tracheophyta and Porifera. The remaining 2054 sequences were not assigned to any group in the consulted databases (Supplementary Table S1). The most diverse phylum was Chlorophyta, represented by 10 taxa, with *Trebouxia asymmetrica* Friedl & Gärtner the most abundant representative. The sample Snow1 was the richest, containing 10 taxa. Snow2 had five taxa and the combined air sample three

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taxa. There were no shared taxa among the air and snow samples and only one taxon (Kingdom Protozoa) was shared between the two snow samples (Fig. 2).

The calculated rarefaction curves indicate that the sampling effort was sufficient to represent the sequence diversity present in each sample (Supplementary Fig. S1).

Discussion

The detection and assignment of a DNA sequence does not confirm the presence of viable organisms or propagules, whilst the assignment is also limited by sequence data available in publicly accessible databases. Taking this into consideration, the overall non-fungal eukaryotes DNA diversity was expected to be low, especially given the remoteness and extreme environmental conditions that characterize Antarctica.

We detected sequences of taxa affiliated to different taxonomic groups, including some organisms not previously recorded in Antarctica. Green algal sequences were abundant in snow, which may either result from their in-cloud presence or below-cloud aerosol scavenging. These algae may have arrived via long-distance transport, having been incorporated into cloud droplets or ice particles during cloud formation and subsequently wet-deposited.

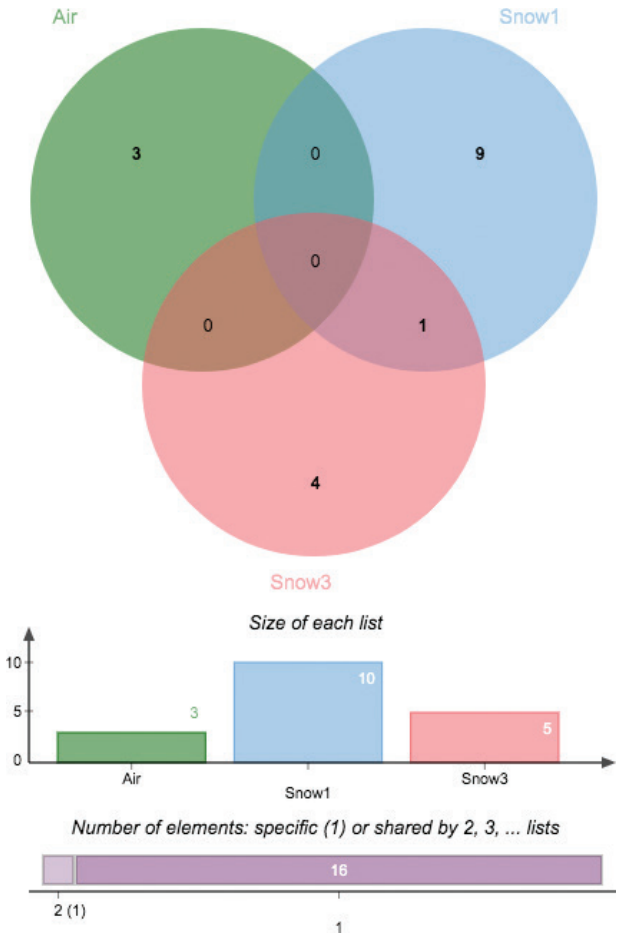

Figure 2 Venn diagram obtained from the three samples (Air, Snow1 and Snow2). Size indicates the number of ASVs (taxa) detected.

By far the most abundant taxa found in both air and snow samples were green algae affiliated to the genus *Trebouxia*, which includes common algae found both in free-living form and as lichen photobionts. The assigned sequences included species of *Trebouxia* that have not previously been reported from Antarctica (Table 1). Representatives of *Trebouxia* have been detected in rain (Dillon et al. 2020) and snow (Tesson & Šantl-Temkiv 2018) in temperate regions. Tesson & Šantl-Temkiv (2018) demonstrated a high ice-nucleation activity at sub-zero temperatures in *Trebouxia* strains isolated from snow, which, together with its presence in precipitations, may suggest a potential role in cloud formation. Snow-borne representatives of *Trebouxia* are able to withstand freezing, exhibit generalist ecological characteristics and are capable of establishing themselves in both simulated freshwater and brackish habitats (Seckbach 2002).

Among the Tracheophyta, only two Angiospermae (flowering plants) were found; the assignment of sequences to Liliopsida could refer to the native grass, *Deschampsia antarctica*, but also to numerous grasses whose pollen could be transported from South America (Câmara, Convey et al. 2021). Unfortunately, the available databases were not capable of resolving these sequences to lower taxonomic levels. The genus *Citrus* originates from Asia, but various species are widely cultivated worldwide. The detection of these sequences could indicate environmental contamination from food (citrus fruits) associated with the nearby Juan Carlos I Antarctic Station. The presence of Poecilosclerida, which includes some of the most common Antarctic sea sponges (Campos et al. 2007), is perhaps unsurprising in light of the proximity of the sampling locations to the coast. All the Ochrophyta sequences assigned are also common marine organisms. The DNA sequences that could not be assigned to any taxon might not be included in the consulted databases or they could come from new and as yet undescribed organisms.

The bioaerosol samples were obtained from more than 700 m^3 of air, providing an opportunity to detect rare taxa. While 171 fungal ASVs were detected in these samples (Rosa et al. 2020), a much smaller number of non-fungal eukaryotes was detected here. The results obtained in this study reflect the preliminary application of this approach in Antarctica: detecting more rare non-fungal eukaryotes may require an even greater sampling volume and period.

Future studies should include sampling at a larger scale, covering extended time periods and diverse meteorological conditions, to obtain more representative samples. This would give us a better grasp of the eukaryotic biodiversity in the atmosphere. Using atmospheric modelling approaches would help identify their likely sources.

The metabarcoding approach used here is a powerful tool to evaluate biological diversity, including exotic taxa. Other studies using the same approach have also detected a considerable number of sequences representing exotic organisms (Câmara et al. 2020; Carvalho-Silva et al. 2021; Câmara et al. 2022). It is noteworthy that studies of this type indicate that a greater diversity of material containing cells or DNA from biological organisms is reaching Antarctica than previously thought. This may be (partly) a result of local human activities (e.g., food found near Antarctic stations), highlighting the importance of the human footprint in Antarctica. The presence of diaspores that are very unlikely to have come from nearby stations also shows that long-distance dispersal plays an important role in the introduction of diaspores into Antarctica. Studies of this type do not allow the assessment of diaspore viability, but they do highlight the existence of this potential threat to the native Antarctic biodiversity.

Conclusions

The application of HTS-based DNA metabarcoding of a specific genetic marker is a promising tool to survey DNA present in both air and snow. Our data suggest the presence of propagules or fragments of numerous organisms in Antarctic air and snow, some of local origin and others from long distances. A more extensive application of this approach is now required to better understand the possible patterns of aerial dispersal and the origins of the organisms detected.

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Disclosure statement

The authors report no potential conflict of interest.

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