



MICROBIOLOGY

Fungal community in the soil of Punta Hannequim, Antarctica, assessed by environmental DNA metabarcoding

MICHEL RODRIGO Z. PASSARINI, GABRIELA R.S. BENEGAS, GRECIA ANTONELLA NINA FLORES, GILENO V. LACERDA JÚNIOR, JÚLIA R. OTTONI, PAULO EDUARDO A.S. CÂMARA & LUIZ HENRIQUE ROSA

Abstract: The fungal community present in soil collected from Punta Hannequim on King George Island, Antarctica, was evaluated using DNA metabarcoding through high-throughput sequencing. A total of 123 fungal amplicon sequence variants (ASVs) were detected, belonging to the phyla Ascomycota, Basidiomycota, Chytridiomycota, Mortierellomycota, and Rozellomycota. The most dominant ASVs were from the genera *Trichoderma*, *Gorgomyces*, and *Neobulgaria*. In addition, many unassigned ASVs were identified and may represent previously unknown fungal diversity in Antarctic microbial ecology. The observed ASV richness rarefaction curve showed that all diversity of the sample was recovered. The taxonomic groups observed in the sampled region included endemic, cosmopolitan, and opportunistic plant pathogenic taxa. The results presented expand knowledge of the fungal community in the soil of King George Island, aiming to guide the development of selective culture media capable of recovering greater microbial diversity from this environment and consequently, broadening perspectives in the field of Antarctic mycology.

Key words: Cold microbial communities, Fungi, Antarctica, Metabarcoding, unassigned ASVs.

INTRODUCTION

The Antarctic continent is considered an extreme and unknown place. Environmental factors such as low temperatures, very strong winds, low humidity, high levels of UV radiation, and limited nutritional availability severely limit life in this environment (Duarte et al. 2018, Silva et al. 2018). Although these adverse conditions make life in Antarctica difficult, the microorganisms that inhabit these environments have undergone evolutionary adaptations that confer molecular modifications, making this community highly adapted to these inhospitable conditions (Ladygina et al. 2006, Rosa et al. 2020).

Fungi that inhabit Antarctic soils play an important ecological role, such as the decomposition of organic matter, and are dominant components of the biomass in this cold ecosystem (Wentzel et al. 2019), in addition to being able to influence the sequestration and balance of carbon in the soil (Větrovský et al. 2019). Fungi present in Antarctica may yet represent an unknown proportion of the diversity, and their true diversity may be much greater than currently estimated (Rosa et al. 2019). Environmental conditions on the Antarctic Peninsula are milder compared to the continental region, and the composition

of organic matter and nutrients is higher. This difference has provided distinct diversity and higher fungal abundance in this region, compared to the continental zone (Rosa et al. 2019).

The characterization of microbial communities present in diverse environments has been considered an important tool for the discovery of new taxonomic groups and understanding their ecological interactions, key points involved in the prosperity of these communities in little-known environments (Ottoni et al. 2022a, Gonçalves et al. 2023, Olmedo-Rojas et al. 2023, Zhang et al. 2022). Culture-independent approaches have been used in recent years as an efficient mechanism in the search for microbial communities in environmental samples, where culture-dependent tools have limitations, such as the inability to reproduce in culture medium the nutritional conditions that these communities need to develop outside the natural ecosystem

(Ottoni et al. 2022b). According to the panorama described above, in the present study, we evaluated fungal diversity in a soil sample from Punta Hannequim, King George Island, South Shetland Islands, using a DNA metabarcoding tool.

MATERIALS AND METHODS

Sampling

A soil sample was collected at Punta Hannequim, Admiralty Bay, King George Island, South Shetland Islands (Figure 1). A spatula cleaned with 70% ethanol was used to scrape the surface region of the collection point and to collect the soil, in triplicate. Three sterilized Falcon-type plastic tubes were used to store the collected soil. The tubes were stored at -20°C until laboratory processing. The samples were collected during the summer of OPERNTAR XL (2021-2022), on 12/27/2021.

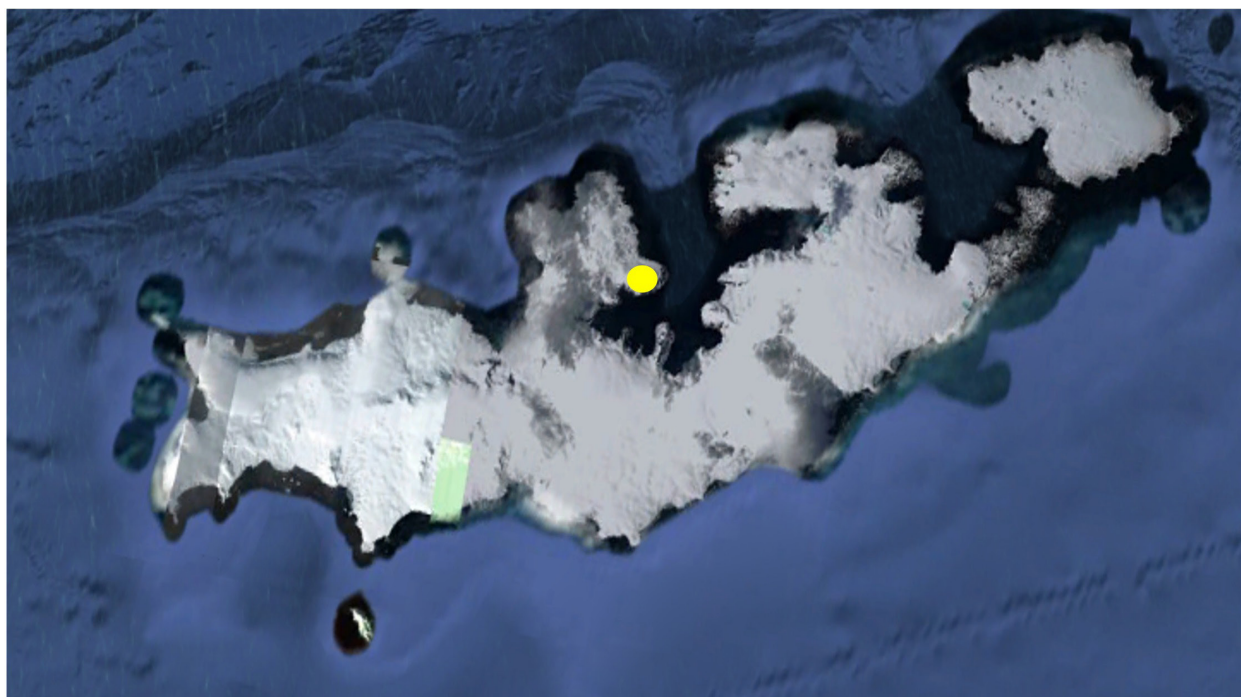


Figure 1. Map obtained from Google Earth (2023) of the location where the soil sample was collected. Punta Hannequim, Admiralty Bay, King George Island, South Shetland Islands (yellow dot indicates pickup location).

DNA Extraction, Sequencing, and Bioinformatics Analyses

All stages of DNA extraction, library construction, quality control, sequencing, and bioinformatics analysis were performed by a company specialized in genomic studies. About 0.5 g of the three soil samples was used for fungal DNA extraction using the DNeasy Powersoil Pro kit (Qiagen), according to the manufacturer's recommendations. The integrity of the extracted DNA was checked by electrophoresis on 0.8% agarose gel, followed by purity analysis on a NanoDrop 1000 (Thermo Fischer Scientific, Waltham, MA, USA), using absorbance ratios of 260/280 and 260/230. The ITS1-5F (Internal Transcribed Spacer) rRNA gene region was amplified using primers ITS5-1737F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2-2043R (5'-GCTGCGTTCTTCATCGATGC-3'). The PCR product was purified with the AMPure XP Beads kit (Beckman Coulter, Life Sciences), and ligation was performed in a PCR reaction using Index Nextera XT Index Primer 1 (N7xx) and Nextera XT Index Primer 2 (S5xx). Large-scale sequencing of amplicons was performed on the Illumina MiSeq platform (2 x 250 bp), in 2x250 bp runs.

The quality of the sequences was checked using the FASTQC v0.11.5 program (Andrews 2010). Sequences from the primers were removed using the tool Cutadapt v4.2 (Martin 2011). Microbiome analyses were performed using the DADA2 v1.24.0 tool (Callahan et al. 2016), comprising: removal of low-quality reads (Phred score <20), noise removal of chimeras (denoising) (consensus method), and grouping of representative sequences based on amplicon sequence variants (ASVs). The taxonomic classification was assigned using the UNITE fungal ITS database version 7.2 (Abarenkov et al. 2024). The analyses were performed in the R statistical environment (v. 4.2.1). The taxonomic table was analyzed in the Phyloseq package

(McMurdie & Holmes 2013) of R. Sequencing coverage was assessed by rarefaction analysis. The microbial composition was expressed in relative abundance for all taxonomic levels.

Chemical-physical analysis

The analysis was performed following the standard internal protocols, not informed by a specialized company. The parameters evaluated were carbon and nitrogen contents.

RESULTS AND DISCUSSION

Chemical-physical analysis

Chemical-physical analyses showed the presence of 2.52% and < 50.0 mg/kg related to the TOC parameters (total organic carbon) and total nitrogen, respectively.

Fungal richness

A total of 51.661 reads were recovered from soil samples, of which 38.117, 37.838, 27.940, 27.940, and 27.928 reads were about information input, filtered, denoised, denoisedR, merged, and nonchimeric, respectively. The observed ASV richness rarefaction curve showed that the sampling reached saturation, or its plateau, and all the diversity of the sample was recovered (Figure 2).

Metabarcoding analyses revealed the fungal profile of the soil sample, represented by five different phyla, including Ascomycota, Basidiomycota, Chytridiomycota, Mortierellomycota, and Rozellomycota (Figure 3a). The two dominant phyla were Ascomycota (~80%), with the most abundant classes *Leotiomyces* and *Sordariomyces* (Figure 3b), followed by Basidiomycota (~10%), with the most abundant class *Agaricomycetes*. A significant number of unassigned ASVs were observed in the analysis (Figure 3a). The most dominant ASV genera were *Trichoderma*, *Gorgomyces*, and *Neobulgaria* (Figure 3c).

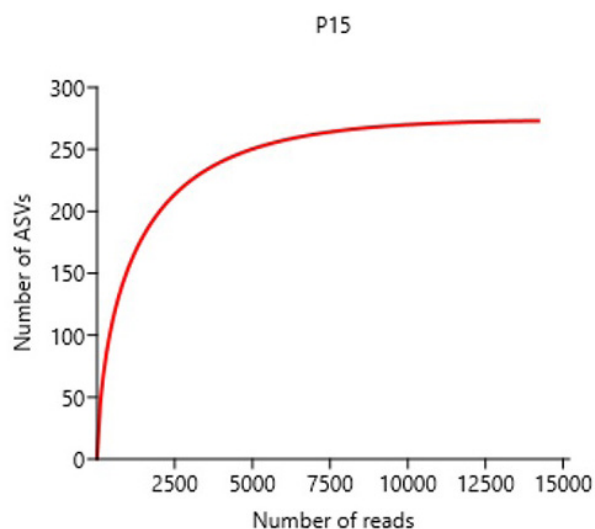


Figure 2. Rarefaction curves of fungal amplicon sequence variants (ASVs) obtained from a soil sample on Punta Hannequim, King George Island, South Shetland Islands, Antarctica, using the metabarcoding method. ASVs were calculated at 99% identity.

Fungal microbial communities in the soil perform fundamental ecological functions, including decomposers, pathogens, and symbionts. The most abundant genus in the present study was *Trichoderma* (Figure 3c), a taxon often reported from the Antarctic continent and one of the most common genera in this region (Menezes et al. 2019). Representatives from this genus and from *Penicillium* (also observed in the present study; however, in a very low abundance) are cosmopolitan mesophilic fungi (non-native microorganisms) and can be isolated from samples from different sources on this continent (Menezes et al. 2022). A low C: N ratio (~0.5) in the studied sample was observed, which may have influenced the low abundance of saprotrophs such as *Penicillium* and *Cladosporium*, important groups in the soil decomposition process (Newsham et al. 2016).

The second most abundant genus in the present study, *Gorgomyces* (Figure 3c) previously reported in a sample of lichens from the Antarctic continent (Beck et al. 2023, Park et al.

2015), but it is not commonly found in soil or other non-lichen samples recovered from the Antarctic continent. This suggests that it may be a genus associated with cold environments, as individuals of this group have been isolated from oak leaves collected from the forest floor under a snow layer, in Japan (Miyamoto et al. 2017), and associated with lichen in Svalbard, High Arctic (Zhang et al. 2015).

The third most abundant genus observed was *Neobulgaria* (Figure 3c), a taxon present in the sampled region and has already been found in coastal water from King George Island, South Shetlands, Antarctica (Garmendia et al. 2021), in a great abundance in sediment samples from lakes in the Fildes Peninsula, King George Island, Antarctica, as well as in other cold places such as permafrost (Siberia and Alaska) and lake (Alaska), (Ogaki et al. 2020), which demonstrates its dominance in extreme cold regions.

In the present study, a relatively large abundance of ASVs from the Orbiliales_gen_Incertae_sedis taxonomic group was observed (Figure 3c). Representatives of the Orbiliales order are known as nematode-trapping fungi, a unique and intriguing group of carnivorous microorganisms able to capture and digest nematodes through specialized structures and important agents for nematode regulation in the soil (Jiang et al. 2017). This group is scarcely reported in Antarctica, being previously mentioned only in the work of Gray et al. (1982).

Other abundant genera observed were *Pseudogymnoascus*, *Fusarium*, *Melanospora*, and *Verrucaria* from the phylum Ascomycota. Representatives of the genus *Pseudogymnoascus*, a psychrophilic fungus with ubiquitous distribution in Antarctica (Shi et al. 2021a), have already been well reported in substrates collected in this continent, including soil, plants, sediments, and lakes (Villanueva et al. 2021). This genus is

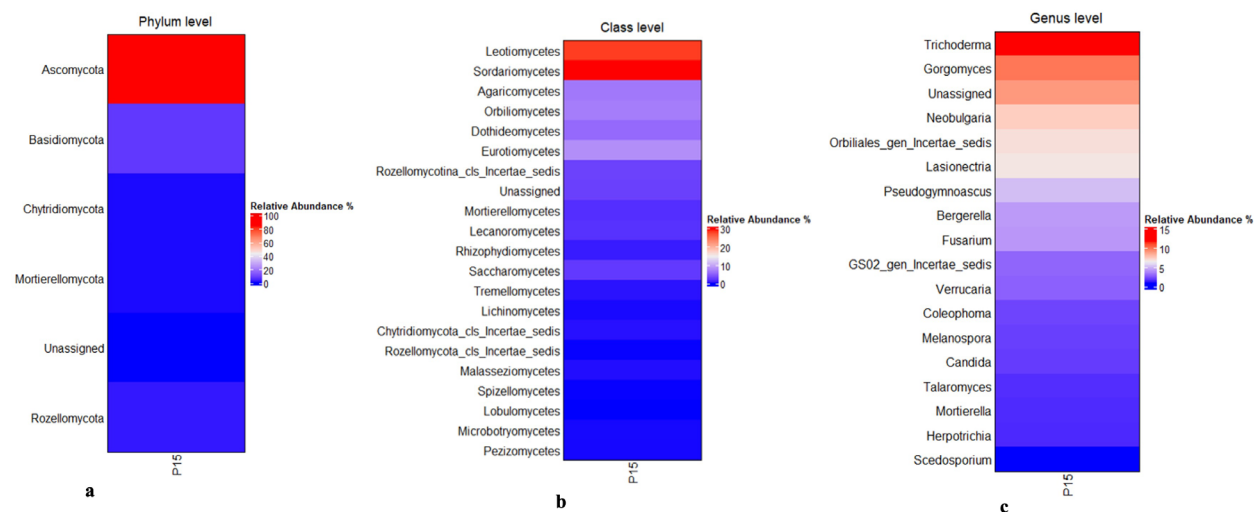


Figure 3. Relative abundance values of the phylum and hierarchical level of classes found in the soil sample.

considered a widely distributed group across various cold regions worldwide. Is the causal agent of white-nose syndrome (WNS), a lethal disease that affects bats in North America (Villanueva et al. 2021). Representatives of this genus have been increasingly investigated for their biotechnological properties, including antiparasitic activity against *Trypanosoma cruzi*, selective herbicidal activity (Gomes et al. 2018), antibacterial activities (Shi et al. 2021a), and cytotoxic activities against human cancer (Shi et al. 2021b).

Santiago et al. (2015) performed a study evaluating the diversity of fungi that inhabit the lichensphere of samples of *Usnea antarctica* and *U. aurantiaco-atra* collected in Elephant, King George, and Deception Island in the Antarctic Peninsula. The authors noted that one of the most abundant genera was *Fusarium*. Fungi of this genus are considered cosmopolitan and can be recovered from several substrates, including soil, plant debris, and other substrates in polar regions with extreme conditions (Santiago et al. 2015). Species of the *Fusarium* genus, found as endophytes in leaves of *Colobanthu quitensis*, have also been reported to be commonly associated with diseases in wild plants, justifying a great abundance of this genus in Punta

Hannequim (sampling site of the present study), Rei George Island, since there is a great diversity of mosses in this place, as well as these fungi can attack wood-based materials that were left behind by ancient explorers in the Antarctic continent (Rosa et al. 2010). According to Alves et al. (2019), some rock-associated Antarctic fungi, including *Fusarium* species, are reported to be endophytes. Thus, we can say that this taxon can be widely distributed throughout the entire Antarctic Peninsula.

Melanospora, a genus belonging to the order Melanosporales, is a group composed of a great majority of mycoparasites (*Fusarium* spp., as one of the hosts) (Bills et al. 2004), with pigmented ascospores (Marin-Felix et al. 2018). Because it is a mycoparasite of *Fusarium* species, its relative abundance may be related to the presence of *Fusarium* found in the present study. *Verrucaria* is a genus that inhabits rocks in Antarctica; therefore, the detection of this genus in the soil is probably associated with its free-living state as spores or other propagules (Newsham et al. 2016). Previous reports have already described the presence of species of this genus in lake and marine environments (Hospitaleche et al. 2011).

In the present work, several other genera associated with the collected soil were also

found; however, in a lower abundance (<1%) (Figure S1 – Supplementary Material). Some of these genera are endemic fungi of the Antarctic continent, characterized as psychrophilic, such as *Antarctomyces* (Rosa et al. 2019), the genus *Dactylospora*, a group of lichenicolous fungi possibly endemic and already reported in the Antarctic continent (Olech & Alstrup 1996), and the genus *Herpotrichia*, described as an endophyte of plants from cold ecosystems (Santiago et al. 2012). Other genera have already been reported in cold environments such as *Humicola*, a genus isolated from Signy Island, Antarctica (Weinstein et al. 2000); *Linnemannia*, a cold-adapted species isolated from early 19th century archaeological artifacts collected on Livingston Island, Antarctica (Menezes et al. 2022); *Microascus*, a genus of endophytic fungus associated with seaweed, collected on Deception Island (Teixeira et al. 2019); *Microdochium*, a genus of endophytic fungi collected from living plants on King George Island, Antarctica (Santiago et al. 2012); *Paranamyces* observed in analysis using metabarcoding in lakes in the South Shetland Islands (de Souza et al. 2021); *Sarocladium* and *Thelebolus* isolated from glacial soil samples collected after glacial retreat (Collins Glacier), in the Fildes Peninsula, King George Island (Santos et al. 2020); and *Xenopolyscytalum*, which was first reported as plant endophytes in the study by Zhang et al. (2013), isolated from bryophyte collected on King George Island, Antarctica.

Cold-tolerant cosmopolitan taxa that have adapted to the cold climate of Antarctica, including *Acremonium*, *Aspergillus* (Rosa et al. 2019), *Exophiala*, a genus of black yeast recovered from Sub-Antarctic Macquarie Island (Zhang et al. 2019), and *Filobasidium*, a genus of epiphytic yeasts already reported on the icy continent (Ferreira et al. 2019), were also found in our study. The genus *Chaetomium*, comprising

bryophyte-associated endophytic fungi, was isolated from the Fildes region, King George Island, maritime Antarctica (Zhang et al. 2013). *Cytospora*, a genus that causes plant diseases, has been observed in tree samples in Argentine Patagonia (Molina et al. 2020). We found unusual taxa present in polar soil communities, such as the genus *Cladophialophora*, previously reported in association with Antarctic rocks and widely distributed in rocks from the various islands of this continent (Alves et al. 2019) (Figure S1).

In the Basidiomycota phylum, some genera were observed in low abundance, including *Cortinarius*, an ectomycorrhiza isolated from the Chilean Antarctic (Torres et al. 2017). *Lycoperdon* (puffball mushroom) an edible fungus with a previous report of isolation from the Canadian Arctic environment (Bergin et al. 2021). *Malassezia* (yeast) was found in a study using Illumina MiSeq with brine samples collected from Antarctic permafrost in Northern Victoria Land (Borruso et al. 2018). However, we did not find reports of some genera of the same phylum from substrates collected in the Antarctic continent, including *Psathyrella* (Figure S1). We observed a certain abundance (~5%) of the genus *Bergerella*, a basidiomycete from the lichenicolous group, a genus not yet reported in association with any substrate collected in the Antarctic environment.

The genus *Mortierella* was the most abundant, representing the phylum Mortierellomycota. Representatives of this same genus have already been reported in the study with samples from Fildes Peninsula (glacial soil) (Santos et al. 2020) and Punta Plaza, Keller Peninsula (air), both from King George Island (Rosa et al. 2021). On the other hand, it was possible to observe a slightly higher abundance (~3.75%) of the enigmatic ASVs (Incertae sedis) belonging to the phylum Rozellomycota, which still do not have defined relationships, as well

as a high abundance (~11%) of unassigned ASVs, affiliated to the phyla Ascomycota and Chytridiomycota.

Interestingly, the results showed a great diversity of genera, including *Lasionectria*, *Chaetocapnodium*, *Conlarium*, *Coleophoma*, *Didymella* (cosmopolitan), *Epithamnia*, *Kazachstania*, *Lasiosphaeria*, *Phylliscum* (lichenized fungi), *Pseudopithomyces*, *Thelonectria*, *Xenodevriesia*, and *Yunnania*, representatives from Ascomycota phylum, and the genus *Geranomyces*, from Chytridiomycota phylum (Figure S1), which it was not possible to find reported or others studies about these taxonomic groups recovered and/or observed on the Antarctic continent, thus possibly being reported for the first time in this present study.

In this way, the results of the present study demonstrate that Punta Hannequim on King George, Antarctica, remains an unknown and potentially promising environment for the recovery of microbial taxa not yet described. It is likely that the great biological diversity found in Punta Hannequim, even considering the oligotrophic soil collected, commonly found in the Antarctic environment, can be observed by the activity of different birds that spawn in this place, the presence of moss carpet and other plants that grow in this place serves as a great nutritional source for the microbial communities that inhabit the soils of this inhospitable point of the Antarctic environment. Thus, the present work encourages future studies to develop differentiated culture media that allow the isolation of these taxonomic groups, which until then had not been recovered from substrate samples from Antarctica, and which were revealed using the Metabarcoding approach.

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SUPPLEMENTARY MATERIAL

Figure S1.

MICHEL RODRIGO Z. PASSARINI¹<https://orcid.org/0000-0002-8614-1896>**GABRIELA R.S. BENEGAS¹**<https://orcid.org/0000-0001-7446-6012>**GRÉCIA ANTONELLA NINA FLORES¹**<https://orcid.org/0009-0002-6919-6198>**GILENO V. LACERDA JÚNIOR²**<https://orcid.org/0000-0003-4493-0755>**JÚLIA R. OTTONI¹**<https://orcid.org/0000-0003-1229-7676>**PAULO EDUARDO A.S. CÂMARA³**<https://orcid.org/0000-0002-3944-996X>**LUIZ HENRIQUE ROSA⁴**<https://orcid.org/0000-0001-9749-5182>

¹Universidade Federal da Integração Latino-Americana (UNILA), Laboratório de Biotecnologia Ambiental, Avenida Tarquínio Joslin dos Santos, 1000, 85870-901 Foz do Iguaçu, PR, Brazil

²Empresa Brasileira de Pesquisa Agropecuária, Laboratório de Microbiologia Ambiental, Embrapa Meio Ambiente, Rodovia SP-340, Km 127,5, 13820-000 Jaguariúna, SP, Brazil

³Universidade de Brasília (UnB), Instituto de Ciências Biológicas (ICB), Departamento de Botânica, Bloco D, s/n, Campus Universitário Darcy Ribeiro, Asa Norte, 70910-900 Brasília, DF, Brazil

⁴Universidade Federal de Minas Gerais (UFMG), Departamento de Microbiologia, Avenida Presidente Antônio Carlos, 6627, Pampulha, 31270-901 Belo Horizonte, MG, Brazil

Correspondence to: **Michel Rodrigo Zambrano Passarini**E-mail: michel.passarini@unila.edu.br

Author contributions

GANF, GRSB, GVLJ, and MRZP carried out the analyses of the data; PEASC collected the samples; MRZP, JRO, and LHR reviewed the manuscript; JRO and MRZP wrote, reviewed, and edited the manuscript; all authors approved the final version of this manuscript.

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Data availability

The data used to support the findings of this study are available upon request.

