UNIVERSIDADE FEDERAL DE MINAS GERAIS

Instituto de Ciências Biológicas

Programa de Pós-Graduação em Microbiologia

Láuren Machado Drumond de Souza

COMUNIDADE DE FUNGOS PRESENTES EM SEDIMENTOS DE LAGOS DA ANTÁRTICA: taxonomia, diversidade, ecologia e bioprospecção de enzimas, biossurfactantes, lipídios e metabólitos herbicidas

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Tese apresentada ao Programa de Pós-Graduação em Microbiologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito para obtenção do grau de Doutora em Microbiologia.

Orientador: Prof. Dr. Luiz Henrique Rosa

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UNIVERSIDADE FEDERAL DE MINAS GERAIS
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ATA DE DEFESA DE TESE

ATA DA DEFESA DE TESE DE **LÁUREN MACHADO DRUMOND DE SOUZA**

Nº REGISTRO: 2021696477

Às 09:00 horas do dia **26 de SETEMBRO de 2025**, reuniu-se no Instituto de Ciências Biológicas (ICB) da UFMG, a Comissão Examinadora composta pelos Drs. Nalu Teixeira de Aguiar Peres (Departamento de Microbiologia/ICB/UFMG), Alysson Wagner Fernandes Duarte (Universidade Federal de Alagoas), Denise de Oliveira Scoaris (Fundação Ezequiel Dias (FUNED-MG), Camila Rodrigues de Carvalho (FIOCRUZ - MG) e o Prof. Dr. Luiz Henrique Rosa (Orientador), para julgar o trabalho final **"Comunidade de fungos presentes em sedimentos de lagos da Antártica: taxonomia, diversidade, ecologia e bioprospecção de enzimas, biossurfactantes, lipídeos e metabólitos herbicidas"** da aluna **Láuren Machado Drumond de Souza**, requisito final para a obtenção do Grau de **DOUTORA EM CIÊNCIAS BIOLÓGICAS: MICROBIOLOGIA**. Abrindo a sessão, o Presidente da Comissão, Prof. Dr. Luiz Henrique Rosa, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra à candidata, para a apresentação de seu trabalho. Seguiu-se a arguição pelos Examinadores, com a respectiva defesa da candidata. Logo após, a Comissão se reuniu, sem a presença da candidata e do público, para julgamento e expedição de resultado final. A candidata foi considerada **APROVADA**. O resultado final foi comunicado publicamente à candidata pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora. A candidata tem 60 (sessenta) dias, a partir desta data, para entregar a versão final da tese ao Programa de Pós-graduação em Microbiologia da UFMG e requerer seu diploma.

Belo Horizonte, 26 de setembro de 2025

Membros da Banca:

Profa. Dra**.** Nalu Teixeira de Aguiar Peres

Prof. Dr. Alysson Wagner Fernandes Duarte

Dra. Denise de Oliveira Scoaris

Dra. Camila Rodrigues de Carvalho

De acordo:

Prof. Dr. Luiz Henrique Rosa

(Orientador)

Prof. Dr. Caio Tavares Fagundes

(Coordenador do Programa de Pós-graduação

em Microbiologia)

Documento assinado eletronicamente por **Luiz Henrique Rosa**, **Professor do Magistério Superior**, em 26/09/2025, às 11:50, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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Dedicatória

Aos meus pais, Clara e Sormane, que me ensinaram que o saber liberta e o amor sustenta.

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Em primeiro lugar, agradeço profundamente aos meus pais, que me deram todo o suporte, amor e incentivo para chegar até aqui. À minha avó, professora dedicada, que sempre foi uma inspiração na minha vida e, mesmo após sua partida, continua presente. Exatamente um ano depois de sua despedida, cheguei à Antártica e fui recebida por um arco-íris inesquecível, que interpretei como um sinal de que ela continuava comigo. Estendo ainda minha gratidão à minha família — tios e primos — que, de diferentes formas, sempre torceram por mim.

Ao meu esposo Bruno, que esteve ao meu lado em todos os momentos, me apoiando com paciência, amor e compreensão, mesmo diante das dificuldades que esse processo trouxe. Ele foi o melhor presente que a Antártica me deu, e a ele, meu companheiro em todas as batalhas, dedico uma parte essencial desta conquista.

Agradeço ainda aos meus cachorros, que me acompanharam nos dias mais difíceis e foram fonte de alegria, afeto e leveza em meio à tempestade.

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Ao meu orientador, pelo acompanhamento acadêmico, pelas oportunidades concedidas e pelas contribuições para esta pesquisa.

Aos companheiros do Laboratório de Microbiologia Polar e Conexões Tropicais e do LABLEV, pela parceria no dia a dia, pelas trocas científicas e pelas amizades construídas.

Um agradecimento especial à Elisa, que começou como minha aluna e se tornou uma grande amiga nesse percurso — sua amizade foi um dos maiores presentes desta jornada. Aos meus amigos de Itaúna, que sempre foram parte fundamental da minha vida, em especial à Celina, minha melhor amiga desde a infância, cuja presença constante e apoio incondicional me deram forças para seguir.

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À Antártica, cenário inesquecível que marcou minha trajetória científica e pessoal, por me mostrar a grandiosidade da vida.

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“O cosmos é tudo o que é, foi ou será. Nossas contemplações mais modestas do Cosmos nos comovem — há um arrepio na espinha, um nó na garganta, uma leve sensação, como uma lembrança distante de cair de uma grande altura.
Sabemos que estamos nos aproximando do maior dos mistérios.
O cosmos está dentro de nós.
O nitrogênio em nosso DNA, o cálcio em nossos dentes, o ferro em nosso sangue foram feitos no interior de estrelas em colapso. Somos feitos de matéria estelar.
Nossa espécie é jovem. Nossa curiosidade é insaciável. Estamos apenas começando.”

Carl Sagan

RESUMO

Este trabalho avaliou a diversidade e o potencial biotecnológico de comunidades de fungos presentes em sedimentos de lagos da Antártica por meio de abordagens dependente e independente de cultivo. Foram investigados os sedimentos dos lagos Florencia e Katerina, localizados na Ilha James Ross, e os lagos Jean e Antonia, situados na Ilha Deception. Nos estudos independentes de cultivo foram também incluídos os lagos Skua e Soto, ampliando a análise da diversidade microbiana na região. As abordagens utilizadas permitiram caracterizar a composição taxonômica, estrutura ecológica e potencial funcional das comunidades fúngicas nesses ambientes polares. Nos lagos Florencia e Katerina foram identificados 24 táxons cultiváveis, 16 provenientes de Florencia e oito de Katerina. Já nos lagos Jean e Antonia foram obtidos 139 isolados fúngicos, dos quais 84 foram oriundos do lago Jean e 55 do Antonia. Os fungos cultiváveis detectados foram majoritariamente membros do filo *Ascomycota*, seguido por *Basidiomycota* e *Mortierellomycota*. O gênero *Pseudogymnoascus* foi predominante em ambos os lagos, com destaque para *Pseudogymnoascus* sp. 1 (detectado no sedimento do Lago Jean) e *Pseudogymnoascus* sp. 3 (Lago Antonia). Nos sedimentos do Lago Florencia foram detectados táxons com menos relatos para Antártica, como *Leucosporidium muscorum*, *Mortierella antarctica* e *Glaciozyma martinii*, evidenciando um ambiente propício à ocorrência de leveduras psicrofílicas e espécies endêmicas. A comunidade obtida no sedimento do Lago Florencia foi mais diversa e rica, além de apresentar maior dominância, em comparação à do Lago Katerina. Já a comunidade presente no sedimento do Lago Jean apresentou maior riqueza, enquanto a comunidade presente no sedimento do Lago Antonia apresentou maior diversidade e dominância. No total, os isolados dos sedimentos dos lagos localizados na Ilha James Ross foram triados para produção de 11 enzimas de interesse industrial, das quais a enzima inulinase foi a mais detectada, seguida de protease**,** invertase**,** gelatinase e pectinase. Oito isolados dos gêneros *Pseudogymnoascus* e *Thelebolus* produziram biossurfactantes e 50 apresentaram corpos lipídicos intracelulares em suas hifas. Apenas o isolado *Penicillium palitans* UFMGCB 18874 foi capaz de apresentar atividade herbicida, onde seu extrato inibiu totalmente a germinação da planta alvo *Allium schoenoprasum*. Por meio de técnicas químicas, do extrato de *P. palitans* UFMGCB 18874 o metabólito (−)-palitantina foi identificado como ativo. Na abordagem independente de cultivo a partir da caracterização do DNA ambiental, por meio da técnica de *metabarcoding*, foram detectadas 218 sequências de amplicons variantes (ASVs), as quais se mostraram serem representantes dos filos *Ascomycota***,** *Basidiomycota***,** *Mortierellomycota***,** *Chytridiomycota***,**

Mucoromycota, bem como os filos crípticos/raros *Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota*, *Monoblepharomycota*, *Rozellomycota*, *Zoopagomycota* e membros do reino Straminopila (*Oomycota, Bacillariophyta*). As ASVs mais abundantes incluíram táxons não identificados (Fungal sp. 1), *Talaromyces rubicundus* e *Dactylonectria anthuriicola*, com registros inéditos para Antártica. Os sedimentos do Lago Florencia apresentaram os maiores índices de diversidade e riqueza, atribuídos à sua conectividade hidrológica, aporte de sedimentos e rica vegetação circundante. Já os sedimentos do Lago Antonia, por sua vez, exibiram a maior diversidade independente de cultivo entre os lagos da Ilha Deception, com 233.580 leituras, 106 táxons identificados. Em contraste, os sedimentos do Lago Jean apresentaram 76.496 leituras, 27 táxons, Fisher's $\alpha = 2,6$ e Margalef = 2,31, refletindo menor complexidade ecológica. Os sedimentos do Lago Soto apresentaram os menores índices de diversidade, compatíveis com um ecossistema em estágio inicial de formação, enquanto os do Lago Skua apresentram diversidade intermediária, influenciada pela vegetação e presença de aves. As comunidades independentes de cultivo foram compostas principalmente por fungos sapróbios, seguidos por simbiontes e patógenos, sugerindo que podem desempenhar funções ecológicas importantes na decomposição de matéria orgânica em ambientes frios. Foram ainda detectados diversos táxons sem registros prévios para Antártica, como *Sugiyamaella bullrunensis*, *Starmerella floris*, *Pseudosydowia indooroopillyensis*, *Chrysosporium vallenarense* e *Metschnikowia hawaiiana*, o que sugere a presença de uma diversidade críptica ou nichos ainda inexplorados. As diferenças geológicas observadas entre os lagos refletem variações ambientais locais, como profundidade, composição mineral, condutividade elétrica, pH, temperatura da água, aporte de matéria orgânica e tipo de rocha de cada bacia. Essa heterogeneidade pode influenciar a estrutura e a funções ecológicas das comunidades fúngicas residentes. A integração entre métodos dependentes e independentes de cultivo se mostroram primissoras para revelar a diversidade total presentes nos sedimentos lacustres da Antártica e o potencial funcional das comunidades, pois combina a caracterização ecológica com a prospecção biotecnológica de fungos adaptados a condições extremas. Este estudo reforça a importância dos sedimentos lacustres antárticos como *hotspots* de diversidade microbiana e como fontes promissoras de micro-organismos extremófilos com potenciais aplicações biotecnológicas, ao mesmo tempo em que contribui para o entendimento dos efeitos de variações ambientais e mudanças climáticas na estrutura das comunidades fúngicas polares.

Palavras-chaves: Antártica; lagos; fungos; metagenômica; metabólitos.

ABSTRACT

This study assessed the diversity and biotechnological potential of fungal communities present in sediments of Antarctic lakes through culture-dependent and culture-independent approaches. Sediments from Florencia and Katerina lakes, located on James Ross Island, and Jean and Antonia lakes, located on Deception Island, were investigated. In the culture-independent studies, Skua and Soto lakes were also included, broadening the analysis of microbial diversity in the region. The approaches used allowed the characterization of the taxonomic composition, ecological structure, and functional potential of fungal communities in these polar environments. In Florencia and Katerina lakes, 24 cultivable taxa were identified, 16 from Florencia and eight from Katerina. In Jean and Antonia lakes, 139 fungal isolates were obtained, of which 84 came from Jean Lake and 55 from Antonia. The cultivable fungi detected were mostly members of the phylum *Ascomycota*, followed by *Basidiomycota* and *Mortierellomycota*. The genus *Pseudogymnoascus* was predominant in both lakes, with emphasis on *Pseudogymnoascus* sp. 1 (detected in Jean Lake sediments) and *Pseudogymnoascus* sp. 3 (Antonia Lake). In Florencia Lake sediments, taxa with few records for Antarctica were detected, such as *Leucosporidium muscorum*, *Mortierella antarctica*, and *Glaciozyma martinii*, evidencing an environment favorable to psychrophilic yeasts and endemic species. The community obtained from Florencia Lake sediment was more diverse and richer, in addition to presenting greater dominance, compared to Katerina Lake. In contrast, the Jean Lake sediment community showed higher richness, while the Antonia Lake sediment community exhibited greater diversity and dominance. In total, isolates from sediments of lakes located on James Ross Island were screened for the production of 11 enzymes of industrial interest, among which inulinase was the most frequently detected, followed by protease, invertase, gelatinase, and pectinase. Eight isolates from the genera *Pseudogymnoascus* and *Thelebolus* produced biosurfactants, and 50 exhibited intracellular lipid bodies in their hyphae. Only the isolate *Penicillium palitans* UFMGCB 18874 displayed herbicidal activity, with its extract completely inhibiting germination of the target plant *Allium schoenoprasum*. Through chemical techniques, the metabolite (−)-palitantin was identified as the active compound in the extract of *P. palitans* UFMGCB 18874. In the culture-independent approach, based on environmental DNA characterization through metabarcoding, 218 amplicon sequence variants (ASVs) were detected, representing the phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Chytridiomycota*, and *Mucoromycota*, as well as cryptic/rare phyla such as *Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota*,

Monoblepharomycota, *Rozellomycota*, *Zoopagomycota*, and members of the kingdom Straminopila (*Oomycota*, *Bacillariophyta*). The most abundant ASVs included unidentified taxa (Fungal sp. 1), *Talaromyces rubicundus*, and *Dactylonectria anthuriicola*, representing first records for Antarctica. Florencia Lake sediments presented the highest diversity and richness indices, attributed to its hydrological connectivity, sediment input, and rich surrounding vegetation. Antonia Lake sediments, in turn, exhibited the greatest culture-independent diversity among Deception Island lakes, with 233,580 reads and 106 identified taxa. In contrast, Jean Lake sediments yielded 76,496 reads, 27 taxa, Fisher's $\alpha = 2.6$, and Margalef = 2.31, reflecting lower ecological complexity. Soto Lake sediments showed the lowest diversity indices, consistent with an ecosystem at an early formation stage, while Skua Lake sediments showed intermediate diversity, influenced by vegetation and bird presence. The culture-independent communities were composed mainly of saprobic fungi, followed by symbionts and pathogens, suggesting that they may play important ecological roles in organic matter decomposition in cold environments. Several taxa with no previous records for Antarctica were also detected, such as *Sugiyamaella bullrunensis*, *Starmerella floris*, *Pseudosydowia indooroopillyensis*, *Chrysosporium vallenarense*, and *Metschnikowia hawaiiana*, suggesting the presence of cryptic diversity or unexplored niches. Geological differences observed among the lakes reflect local environmental variations, such as depth, mineral composition, electrical conductivity, pH, water temperature, organic matter input, and basin lithology. This heterogeneity may influence the structure and ecological functions of the resident fungal communities. The integration of culture-dependent and culture-independent methods proved promising to reveal the total diversity present in Antarctic lake sediments and the functional potential of fungal communities, as it combines ecological characterization with biotechnological prospecting of fungi adapted to extreme conditions. This study reinforces the importance of Antarctic lake sediments as hotspots of microbial diversity and as promising sources of extremophilic microorganisms with biotechnological applications, while also contributing to the understanding of the effects of environmental variations and climate change on the structure of polar fungal communities.

Key-words: Antarctica; lakes; fungi; metagenomics; metabolites.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

%: Por cento

°C: Graus Celsius

µg mL^{-1}: Micrograma por mililitro

µg: Micrograma

µL mL^{-1}: Microlitro por mililitro

µL: Microlitro

ABI: Applied Biosystems

BLAST: Basic Local Alignment Search Tool

BLASTn: Nucleotide Basic Locus Alignment Search Tool

Cm: Centímetros

CTAB: Brometo de cetil trimetilamônio

D1/D2: Região da Subunidade Maior do rRNA

DMSO: Dimetilsulfóxido

DNA: Ácido desoxirribonucleico

dNTP: Desoxirribonucleotídeo trifosfato

DRBC: Ágar Dicloran Rosa Bengala

EDTA: Ácido etilenodiamino tetra-acético

g L^{-1}: Grama por litro

g: Grama

GYMP: Glucose-Yeasts Extract-Malt Extract-Fosfato de Potássio

h: Horas

HCl: Ácido clorídrico

ICB: Instituto de Ciências Biológicas

ITS: Região transcrita interna

ITS1-5.8S-ITS2: Região Transcrita Interna do Gene do rRNA

Km: Quilômetros

L: Litro

M: Molar

MEA: Meio extrato de malte

mg mL^{-1}: Miligrama por mililitro

mg: Miligrama

$MgCl_{2:}$ Cloreto de Magnésio

mL: Mililitro

MM: Meio mínimo

mm: Milímetros

NaCl: Cloreto de sódio

PCR: Reação em cadeia da polimerase

pH: Potencial Hidrogeniônico

pmol: Picomol

RNA: Ácido Ribonucléico

rpm: Rotações por minuto

TBE: Tris-borato

TE: Tampão Tris-EDTA

TRIS: 2-amino-2-hidroximetil-propano-1,3-diol

UFMG: Universidade Federal de Minas Gerais

UFMGCB: Coleção de Micro-organismos e Células da UFMG

UV: Ultravioleta

v/v: Volume por volume

V: Volts

YM: Ágar extrato de malte - Extrato de levedura

YNB: Yeast nitrogen base

SUMÁRIO

RELEVÂNCIA E JUSTIFICATIVA

A Antártica possui diferentes ecossistemas, os quais abrigam diferentes comunidades de seres vivos adaptados, principalmente, às baixas temperaturas (Rosa *et al*., 2019). Entretanto, com o potencial efeito do aquecimento global, a biodiversidade da região pode sofrer alterações significativas em sua composição (Comin & Justino, 2017). Dentro das comunidades biológicas residentes na Antártica, os micro-organismos são os mais abundantes de diversos, pois possuem diferentes mecanismos adaptativos para sobreviver em condições poliextremófilas. Entre os micro-organismos extremófilos da Antártica, os fungos, nosso alvo de estudo, também podem ser afetados, tornando ainda mais importante a realização de estudos acerca da sua diversidade nos diferentes ecossistemas antárticos.

Os lagos antárticos apresentam diversas condições limitantes à biota residente, como baixa disponibilidade de nutrientes e alta incidência de radiação ultravioleta, o que não impede o desenvolvimento de micro-organismos, incluindo fungos, nestes ecosistemas. Os fungos antárticos vêm sendo reportados como micro-organismos dominantes envolvidos na decomposição de diferentes fontes de matérias orgânicas originárias de plantas, líquens e animais nesses ambientes lacustres, uma vez que produzem diferentes enzimas degradativas em seus arsenais metabólicos (Ogaki *et al*., 2020a).

De acordo com Martorell *et al*. (2019), os micro-organismos psicrofílicos presentes na Antártica são fontes expressivas de enzimas ativas a baixas temperaturas, as quais podem ser até 10 vezes mais ativas em temperaturas baixas e moderadas quando comparadas às de mesofílicos e podem ser utilizadas em diversos processos industriais. Os biossurfactantes, surfactantes produzidos principalmente por micro-organismos, representam alternativas atraentes em comparação aos surfactantes sintéticos, uma vez que os sintéticos apresentam baixa biodegradabilidade, alta toxicidade ao meio ambiente e aos seres vivos e podem solubilizar xenobióticos hidrofóbicos (substâncias químicas poluentes) que afetam a qualidade da água potável em lagoas e riachos (Haigh, 1996; Palmer & Hatley, 2018). Os metabólitos secundários representam modelos de estruturas químicas que servem como protótipos para o desenvolvimento de fármacos de interesse na medicina e herbicidas e pesticidas com potencial uso na agricultura. Devido ao isolamento geográfico e por estarem sob constantes efeitos das condições extremas da Antártica, os fungos antárticos pode possuir vias metabólicas diferenciadas e úncias para produção desses metabólitos secundários bioativos (Rosa *et al.*, 2019).

Dessa forma, considerando a diversidade e ecologia dos fungos residentes nos diferentes ambientes da Antártica e, principalmente, sua capacidade de produção de diversos metabólitos, é importante que mais estudos sejam conduzidos nos sedimentos dos lagos antárticos para ampliar o conhecimento sobre taxonomia, diversidade, biogeografia das comunidades residentes, ecologia funcional e bioprospecção de enzimas, biosurfactantes, lipídios e metabólitos bioativos com potenciais aplicações biotecnológicas.

1. INTRODUÇÃO

1.1.Antártica

A Antártica é o continente mais frio do mundo (**Figura 1**), com temperatura média entre -25 ºC e -45 ºC, sendo que a mais baixa já registrada foi de -93,2 ºC (Brasil, 2006; Nasa, 2013), além de ser uma das regiões mais secas do planeta, com a predominância de ventos que podem chegar a 327 km/h (Bischoff, 1996). Cerca de 98% do continente é permanentemente coberto de gelo (espessura média de cerca de 2 km e espessura máxima de mais de 4 km) e, no inverno, a superfície do gelo, do continente, dos lagos e do mar é essencialmente desprovida de água disponível no estado líquido, apresentando características de um deserto frio, que duram até o degelo na primavera (Wynn-Williams, 1990; Bargagli, 2008).

Figura 1. Comparação do mesmo local, na Ilha Rei George, Antártica, em diferentes meses. Foto: Bruno dos Santos Moreira Rocha.

A abertura e o aprofundamento da passagem de Drake (canal de águas profundas entre a América do Sul e a Antártica Ocidental) permitiram o estabelecimento da corrente circumpolar antártica, um sistema de correntes oceânicas que contribui para o isolamento térmico da Antártica dos demais continentes (Rintoul, 2010). O continente antártico alcançou sua posição atual há cerca de 45 milhões de anos e foi geograficamente isolado dos outros continentes desde a separação da Península Antártica da América do Sul a aproximadamente 30 milhões de anos (Bargagli, 2008). Envolto pelo Oceano Antártico ou Austral, com uma superfície de aproximadamente 14 milhões de km^2, cerca de 1,6 vezes a área do Brasil, e coberto por uma camada de gelo maciço de aproximadamente 12 milhões de km^2, é o continente que circunda o Polo Sul até o paralelo de 60º S (Wilkins *et al*., 2013).

Os organismos que habitam a Antártica são submetidos a uma variedade de condições ambientais extremas, como grandes variações de temperaturas, verões com alta incidência de radiação solar, invernos com longos períodos de escuridão, salinidade elevada, dessecação, escassez de nutrientes, variação de pH e ciclos de congelamento e degelo (Gocheva *et al*., 2006; Gonçalves *et al*., 2012). Apesar de inóspito, o continente abriga organismos como aves, mamíferos, invertebrados marinhos e terrestres, plantas e macroalgas (Shivaji & Prasad, 2009) (**Figura 2**). Os micro-organismos antárticos são representados por fungos, bactérias, vírus e arqueias que apresentam destaque nos diferentes ecossistemas da região devido à sua elevada diversidade e capacidade adaptativa às condições extremas (Russell, 2006).

Figura 2. Exemplos de animais encontrados na Antártica. À esquerda, o pinguim-de-barbicha (*Pygoscelis antarcticus*), e à direita o lobo-marinho-antártico (*Arctocephalus gazella*). Fotos: Bruno dos Santos Moreira Rocha.

1.2.Fungos em sedimentos de lagos antárticos e seu potencial biotecnológico

O primeiro material fúngico coletado na Península Antártica, identificado como *Sclerotium antarcticum*, foi obtido durante a expedição Antártica Belga 1897-1899 (Bommer & Rousseau, 1905; Gamundi & Spinedi, 1987), mas desde então diferentes espécies já foram relatadas para o continente. Segundo Ruisi *et al*. (2007), a maioria dos fungos encontrados na Antártica se apresenta na forma anamórfica (estágio assexuado), o que pode implicar em ciclos de vida concluídos em intervalo de tempo mais curto, o que gera menos gastos metabólicos e, consequentemente, economia de energia, a qual é fundamental para sobrevivência nas condições extremas da Antártica. Apesar disso, existem exceções, como espécies do gênero *Thelebolus*, que foram relatadas no estágio teleomorfo (sexuada) na Antártica (de Hoog *et al*., 2004).

O elevado número de micro-organismos cosmopolitas encontrados nos diferentes ambientes antárticos sugere que a Antártica recebe constantemente diferentes propágulos microbianos de fora da região, especialmente das regiões mais próximas como América do

Sul e Oceania (Kappen, 1993). Segundo Ruisi *et al*. (2007), vetores como micropartículas suspensas nas correntes aéreas e animais são alguns dos responsáveis por essa dispersão a longa distância. Contudo, além da grande variedade de espécies cosmopolitas, também são encontradas espécies endêmicas na Antártica (Bridge & Spooner, 2012).

Segundo Kappen (1993), é possível que os micro-organismos antárticos apresentem uma combinação de mecanismos fisiológicos capazes de aumentar sua sobrevivência frente às condições extremas do ambiente, sugerindo capacidades adaptativas pré-existentes e que foram selecionados positivamente ao longo dos anos. Como exemplos desses mecanismos, já foram reportadas a produção de substâncias anticongelantes, capacidade de sobreviver à desidratação, alta tolerância ao frio e ao congelamento, tolerância à anóxia, alteração da composição lipídica da membrana, produção de pigmentos fotoprotetores e de enzimas adaptadas ao frio e presença de exopolissacarídeos capazes de evitar o congelamento celular (Duarte *et al*., 2018).

As vias metabólicas fúngicas são uma rica fonte de metabólitos bioativos, incluindo diferentes classes de moléculas secundárias como terpenóides, policetídeos, alcalóides, poliacetilenos e outras substâncias com diferentes atividades biológicas, e essas moléculas podem ser utilizadas como protótipos para desenvolver novos medicamentos e pesticidas (Rosa *et al*., 2019). Nos últimos anos, a capacidade de diferentes comunidades de fungos nos ambientes antárticos de produzir compostos para uso em processos biotecnológicos foi relatada, assim, a busca por fungos extremófilos na Antártica pode representar uma estratégia útil para encontrar novas vias metabólicas eucarióticas e, consequentemente, novos metabólitos bioativos (Santiago *et al*., 2012; Godinho *et al*., 2015; Rosa *et al*., 2019).

As bacias da maioria dos lagos antárticos são glaciais superprofundadas e os vales dos maiores riachos são vales glaciais (Michel *et al*., 2014). Materiais particulados e sólidos de áreas de captação são transportados pela água e depositados em sedimentos lacustres, contribuindo para os processos biogeoquímicos do lago (Alfonso *et al*., 2015); consequentemente, a comunidade microbiana pode ser afetada por essas mudanças constantes na composição dos habitats lacustres. Em geral, a composição e concentração de elementos nos sedimentos dos lagos dependem das propriedades das rochas-mãe e das condições climáticas na bacia hidrográfica (Alfonso *et al*., 2015).

Gonçalves *et al*. (2012) isolaram 128 fungos de água de lagos nas Ilhas Rei George e Deception e os gêneros mais frequentes foram *Pseudogymnoascus*, *Antarctomyces*, *Mortierella*, *Cladosporium*, *Penicillium*, *Phoma* e *Thelebolus*. Vaz *et al*. (2011) isolaram fungos de sedimentos lacustres em áreas de influência antrópica, relatando a presença das

espécies *Candida glaebosa, Nadsonia commutate* e *Cystobasidium laryngis*. Gonçalves *et al*. (2015) isolaram os seguintes fungos de sedimentos lacustres das Ilhas Rei George: *Helotiales* sp., *Schizophyllum commune*, *Annulohypoxylon* sp., *Cosmospora* sp., *Aspergillus* sp., *Pseudogymnoascus* sp., *Penicillium* sp., *Pleosporaceae* sp., *Sordariomycetidae* sp. e *Cadophora malorum*. Ogaki *et al*. (2020a) recuperaram 195 isolados de fungos de sedimentos de lagos de diferentes ilhas nas Shetland do Sul na Antártica marítima pertencentes aos filos *Ascomycota, Basidiomycota* e *Mortierellomycota*, sendo que alguns desses isolados foram capazes de produzir substâncias com atividades tripanocida, antifúngica e herbicida. Sedimentos de três lagos potencialmente impactados antropogenicamente na Ilha Rei George também foram avaliados por Ogaki *et al*. (2020b), que reportaram 63 isolados fúngicos, pertencentes aos filos *Ascomycota*, *Basidiomycota* e *Mortierellomycota*, incluindo isolados também produtores de metabólitos tripanocidas, antifúngicos e herbicidas.

1.3. Enzimas produzidas por micro-organismos de ambientes frios e suas aplicações biotecnológicas

As enzimas são proteínas globulares complexas que participam de uma reação sem serem consumidas na mesma, atingindo uma alta taxa de formação de produto e, devido à sua natureza específica, elas podem diferenciar produtos químicos com estruturas semelhantes e catalisar reações em uma ampla faixa de temperaturas (0-110 °C); em aplicações industriais, essas qualidades com uma enzima não tóxica e biodegradável podem resultar em produtos de alta qualidade e quantidade, menos subprodutos e procedimentos mais simples de purificação (Kulshrestha *et al*., 2013). Em geral, as enzimas biologicamente ativas podem ser obtidas de plantas, animais e micro-organismos; contudo, as enzimas microbianas têm sido geralmente mais estudadas por seu isolamento mais fácil em grandes quantidades, produção de baixo custo em pouco tempo, estabilidade em várias condições extremas, grande variedade de atividades catalíticas disponíveis, maiores rendimentos, possível facilidade de manipulação genética, fornecimento regular e rápido crescimento dos micro-organismos nos meios econômicos, produção relativamente mais conveniente e segura, e seus compostos são também mais controláveis e menos prejudiciais (Kasana & Gulati, 2011; Gopinath *et al*., 2017). Além disso, por serem secretadas no meio externo, elas são mais confiáveis em processos e aplicações industriais (Gopinath *et al*., 2017).

Considerando que mais de 80% da Terra é permanente ou periodicamente exposta a temperaturas abaixo de 5 °C (mares profundos, desertos frios e habitats glaciais) e que cerca de 10% da sua superfície é representada por habitats glaciais, os micro-organismos possuem

estratégias de adaptação para superar a influência direta e indireta do risco de vida em baixas temperaturas (Buzzini *et al*., 2012). Os micro-organismos que colonizaram os ambientes frios são referidos como psicrófilos, sendo sua temperatura ótima de sobrevivência e reprodução 15 °C e máxima de 20 °C (Ibrar *et al*., 2020).

Os ambientes psicrófilos, com alta radiação UV e ciclos de congelamento e descongelamento, têm efeito direto na integridade estrutural e no genoma de fungos, os quais têm mecanismo específico para sobreviver nesses ambientes (Ibrar *et al*., 2020). A síntese de enzimas psicrofílicas é uma das estratégias de adaptação mais investigadas a baixas temperaturas; e o aumento de sua atividade a baixas temperaturas é baseado em sua flexibilidade estrutural aprimorada, incluindo uma possível modificação do sítio ativo (Buzzini *et al*., 2012). O metabolismo enzimático dos psicrofílicos apresenta características marcantes, uma vez que lidam com um ambiente severo (Martorell *et al*., 2019), o que os torna extremamente relevantes no ramo científico e em processos industriais (Cavicchioli *et al*., 2011). Com relação a este último, as vantagens mais notáveis dessas enzimas são a redução nos custos de energia durante sua produção tecnológica (não exigiria o aquecimento caro dos reatores) por apresentarem atividades catalíticas mais altas e adaptações estruturais que garantem uma estrutura mais flexível do que as encontradas em mesofílicos e termofílicos (Siddiqui & Cavicchioli, 2006; Javed & Qazi, 2016). Segundo Sarmiento *et al*. (2015), as seguintes características são importantes adaptações para manter alta flexibilidade enzimática e atividade a baixas temperaturas:

- Diminuição da hidrofobicidade do núcleo;
- Aumento da hidrofobicidade da superfície;
- Mudanças nas composições de aminoácidos;
- Interações mais fracas entre proteínas;
- Diminuição das estruturas secundárias e oligomerização (complexos proteicos formados a partir da ligação de duas ou mais cadeias peptídicas).

As enzimas ativas a baixas temperaturas podem ser utilizadas na indústria de alimentos como, por exemplo, na redução da lactose, melhor extração do suco da fruta, amaciamento da carne, fermentação, produção de cerveja e vinho, fabricação de queijos (Gerday *et al*., 2000; Hoyoux *et al*., 2001; Javed & Qazi, 2016), indústria têxtil para o biopolimento de tecidos e lavagem de jeans (Gerday *et al*., 2000), produção de detergentes (Javed & Qazi, 2016), indústria de papel (Javed & Qazi, 2016), indústria farmacêutica (Novak

et al., 2013), aplicação ambiental em biorremediação (Margesin, 2007) e produção de cosméticos (Javed & Qazi, 2016), entre outros. Recentemente, de Souza (2022) avaliou a produção das enzimas celulase, protease, lipase, agarase, carragenase, invertase, amilase, esterase, pectinase, inulinase e gelatinase por fungos isolados de lagos da Antártica e observou que 97% de seus isolados produziram alguma delas. Além disso, as enzimas detectadas com mais frequência entre os isolados foram protease e lipase, e as menos frequentes foram celulase e pectinase, o que demonstra que os fungos de ambientes lacustres antárticos se apresentam como excelentes produtores de enzimas degradativas, mesmo que a baixas temperaturas.

1.4.Biossurfactantes e sua produção por micro-organismos extremófilos

Os biossurfactantes são, em sua maioria, uma classe de metabólitos que podem ser tanto primários quanto secundários e compreendem um grupo de moléculas anfipáticas (possuem porção hidrofílica e hidrofóbica) com estruturas distintas e sintetizadas por diferentes organismos (Sperb *et al*., 2015). Eles também são conhecidos como substâncias tensoativas (que influenciam a superfície de contato entre dois líquidos) estrutural e funcionalmente diversos, os quais reduzem a tensão superficial e interfacial entre as moléculas individuais nas respectivas superfícies e interfaces entre dois fluidos que não se misturam como, por exemplo, óleo/água (Bhardwaj *et al*., 2013; Varjani & Upasani, 2017). Os biossurfactantes são representados pelos glicolipídios, lipossacarídeos, fosfolipídios, ácidos graxos, lipídios neutros, lipopeptídeos e alguns polímeros como resultado de sua atividade surfactante com capacidade de interagir com moléculas polares (como a água) e apolares (gorduras); por isso, os biossurfactantes são importantes para o setor industrial em processos de detergência, emulsificação, lubrificação, capacidade de formação de espuma, capacidade de umectação (hidratação) e solubilização de substâncias apolares (Silva *et al*., 2021).

Embora altamente eficazes em muitas aplicações industriais e de remediação ambiental, os surfactantes sintéticos tendem a ser tóxicos e podem representar riscos ambientais e atividade biocida (Edwards *et al*., 2003). Além disso, os surfactantes sintéticos têm baixa biodegradabilidade e podem solubilizar xenobióticos hidrofóbicos (substâncias químicas poluentes), afetando a qualidade da água potável em lagoas e riachos (Palmer & Hatley, 2018). Já os surfactantes naturais (biossurfactantes), produzidos principalmente por micro-organismos, representam uma alternativa atraente em relação aos surfactantes convencionais sintéticos e veem sendo considerados uma tecnologia alternativa chave a ser

desenvolvida no século XXI (Santos *et al*., 2016). Os biossurfactantes possuem várias vantagens em relação aos seus similares sintéticos, incluindo maior biodegradabilidade, baixa toxicidade, propriedades de alta formação de espuma, produção sustentável e alta estabilidade em níveis extremos de pH, temperaturas e salinidade (Khademolhosseini *et al*., 2019).

Os fungos produzem uma boa quantidade de biossurfactantes, quando comparados às bactérias, e o motivo pode ser a presença de uma parede celular rígida (Bhardwaj *et al*., 2013). Entre os fungos, *Candida bombicola*, *Candida lipolytica*, *Candida ishiwadae*, *Candida batistae*, *Aspergillus ustus*, *Ustilago maydis* e *Trichosporon ashii* têm ganhado destaque por serem capazes de produzir, principalmente, biossurfactantes soforolipídios (glicolipídios) (Bhardwaj *et al*., 2013). Atualmente, um dos micro-organismos produtores de biossurfactantes mais valiosos no mercado é a levedura *Moesziomyces antarcticus* (anteriormente conhecido como *Candida antarctica*), a qual foi originalmente isolada do Lago Vanda no Vale Wright, Antártica (Goto *et al*., 1969). Além dessa levedura, espécies do gênero *Rhodotorula* são altamente representadas em ambientes polares e glaciais (por exemplo, *R. arctica*, *R. glacialis*, *R. psychrophenolica*, *R. psychrophila* e *R. mucilaginosa*) e são conhecidas por serem hiperprodutoras de biossurfactantes soforolipídios (Perfumo *et al*., 2018).

1.5.Produção de lipídios por fungos

Os lipídios, compostos hidrofóbicos essenciais aos organismos, como acilglicerídeos, ácidos graxos livres, esteróis e isoprenoides, são sintetizados a partir da acetil-CoA por meio de reações bioquímicas complexas (Passoth & Müller, 2025). Fungos oleaginosos podem acumular entre 20% e mais de 80% de sua biomassa como lipídios, convertendo fontes de carbono de baixo valor, como hidrolisado de lignocelulose, ácidos graxos voláteis ou glicerol bruto oriundo da produção de biodiesel (Athenaki *et al*., 2018). Segundo Passoth & Müller (2025), os lipídios possuem amplo potencial biotecnológico, sendo que ácidos graxos poli-insaturados, essenciais à nutrição humana, podem ser produzidos em grandes quantidades por espécies de zigomicetos ou leveduras geneticamente modificadas. Além disso, o metabolismo lipídico de fungos tem atraído crescente atenção devido ao seu potencial biotecnológico, incluindo a produção de glicolipídios com propriedades de biossurfactantes, lipoproteínas como alvos para novos antifúngicos e compostos lipídicos intracelulares com aplicações na produção sustentável de combustíveis e químicos (Athenaki *et al*., 2018; Passoth & Müller, 2025).

A produção de lipídios por micro-organismos está relacionada à acumulação de reservas de carbono durante o processo de crescimento, onde a composição e a quantidade destas substâncias são variáveis, influenciadas pela espécie produtora, estágio de desenvolvimento, condições ambientais e substrato de cultivo (Ratledge, 1997). Os ácidos graxos saturados e insaturados desempenham um papel importante na regulação do estresse que os fungos antárticos enfrentam frente às abaixas temperaturas, os quais auxiliam espécies psicrofílicas e adaptadas ao frio nos seus estágios de desenvolvimento, nutrição e colonização na região (Weet, 2012; de Carvalho & Caramujo, 2018; Gallardo *et al*., 2025). Neste contexto, espécies capazes de acumular altas concentrações de lipídios em suas células, tal como linhagens dos gêneros *Aspergillus*, *Cunninghamella*, *Fusarium*, *Mortierella*, *Mucor* e *Penicillium*, as quais são frequentemente encontradas na Antártica, são conhecidas como oleaginosas (Shapaval *et al*., 2014; Forfang *et al*., 2017; Athenaki *et al*., 2018; Zhang *et al*., 2022; Gallardo *et al*., 2025; Passoth & Müller, 2025) e podem representar fontes alternativas para uso de seus lipídios como uma fonte alternativa de energia.

1.6.Atividade herbicida/fitotóxica de fungos

Atualmente, o uso de metabólitos naturais tem despertado um interesse crescente na busca por novos mecanismos de ação de pesticidas, especialmente diante do aumento da resistência das pragas agrícolas às substâncias sintéticos disponíveis no mercado (Duke & Dayan, 2022). A redução da eficácia dos herbicidas comerciais em uso é agravada pela ausência de novos metabólitos com mecanismos de ação inéditos, já que nenhum herbicida com nova forma de atuação foi introduzido no mercado nos últimos 40 anos; fator que torna essa busca especialmente urgente e necessária (Duke & Dayan, 2022). Diante desse cenário, os metabólitos secundários produzidos por fungos extremófilos da Antártica, os quais para sobreviver em condições extremas podem expressar vias metabólicas únicas e podem ser fontes alternativas de moléculas bioativas (Rosa *et al*., 2019) e podem ser úteis para uso na agricultura (Gomes *et al*., 2018; da Silva *et al*., 2024). A partir do panorama reportado acima, este estudo teve como objetivo caracterizar a diversidade de fungos presentes em sedimentos de lagos da Antártica e avaliar seu potencial como produtores de enzimas, biossurfactantes e lipídios de interesse industrial, bem como metabólitos herbicidas de interesse na agricultura.

Gomes *et al*. (2018) avaliaram fungos isolados de solos da Antártica quanto à atividade herbicida sobre *Lactuca sativa* e *Allium schoenoprasum*, destacando *Penicillium tardochrysogenum* pela significativa ação fitotóxica observada. Godinho *et al.* (2015) avaliaram fungos de solos oligotróficos da Antártica Continental quanto à atividade herbicida

sobre *Lactuca sativa* (alface) e *Allium schoenoprasum* (cebolinha), obtendo resultado positivo para as espécies *Aspergillus sydowii, Penicillium allii-sativi, P. brevicompactum, P. chrysogenum* e *P. rubens*. Ogaki *et al*. (2020b) avaliaram fungos isolados de sedimentos marinhos da Antártica quanto à atividade herbicida sobre *Lactuca sativa* (alface) e *Allium schoenoprasum* (cebolinha), identificando a produção de metabólitos com efeito fitotóxico por isolados de *Penicillium allii-sativi, P. chrysogenum, P. palitans, P. solitum e Acremonium fusidioides.*

Metabólitos derivados de *Penicillium palitans*, isolado de sedimentos marinhos profundos na Antártica marítima, foram avaliados por Barreto *et al*. (2025) quanto às suas propriedades fitotóxicas. O fracionamento cromatográfico do extrato bruto em diclorometano resultou na obtenção de dois compostos estruturalmente relacionados: (−)-penienona e (−)-palitantina. Seus efeitos fitotóxicos foram testados em sementes de *Lactuca sativa* L. e *Agrostis stolonifera* L., com ambos os compostos demonstrando atividade exclusivamente contra a espécie monocotiledônea *A. stolonifera*. Na concentração de 1 mg mL^{-1}, a (−)-penienona inibiu completamente a germinação das sementes, enquanto a (−)-palitantina apresentou apenas efeitos moderados após sete dias de exposição. Em ensaios com *Lemna paucicostata*, a (−)-penienona apresentou IC_{50} de 57 μM, reduzindo significativamente o crescimento, enquanto a (−)-palitantina mostrou atividade consideravelmente menor (Barreto *et al*., 2025).

1.7. *Metabarcoding*: uso do DNA ambiental (eDNA) para estudos taxonômicos e ecológicos

A metagenômica constitui a análise funcional, baseada em sequência, dos genomas microbianos coletivos (microbioma) em um determinado ambiente ou nicho ambiental (Sleator *et al*., 2008). Considerando que a maioria dos micro-organismos de todos os ambientes da Terra ainda não foi cultivado, essa técnica independente de cultivo, que analisa todos os organismos em um nicho ambiental específico e evoluiu como um esforço para descobrir mais sobre a diversidade microbiana em ambientes naturais como o solo, água marinha e lacustre, sedimentos, plantas e tratos gastrointestinais de vertebrados e invertebrados (López-García & Moreira, 2008; Sleator *et al*., 2008). Esse método pode ser dividido em análise baseada na sequência (*metabarcoding*) e análise baseada na função dos micro-organismos independentes de cultivo (Gabor *et al*., 2007).

Rogers *et al*. (2013) realizaram análise metagenômica/metatranscriptômica da camada de gelo do Lago Vostok e 94% das espécies encontradas pertenciam aos domínios Bacteria,

6% a Eukarya e 2% a Archaea, sendo que os fungos (*Ascomycota* e *Basidiomycota*) foram os mais representativos de Eukarya. Baeza *et al*. (2017) utilizaram *metabarcoding* para avaliar a diversidade fúngica de oito ilhas do Arquipélago Shetland do Sul, duas ilhas na Península Antártica e a Geleira Union e detectaram 87 gêneros e 123 espécies, das quais foram detectadas espécies pertencentes a 37 gêneros de fungos não cultivados anteriormente na Antártica. Bacias lacustres permanentemente cobertas de gelo localizadas nos Vales Taylor e Miers foram analisadas por Rojas-Jimenez *et al*. (2017) utilizando análise independente de cultivo e detectaram os filos *Rozellomycota*, *Chytridiomycota*, *Ascomycota*, *Mortierellomycota*, *Blastocladiomycota* e *Basidiomycota*. A diversidade de fungos independentes de cultivo na Ilha Vega foi avaliada por Ogaki *et al*. (2021), que detectaram 640.902 leituras de DNA pertencentes aos filos *Ascomycota*, *Rozellomycota*, *Basidiomycota*, *Chytridiomycota* e *Mortierellomycota*, com *Pseudogymnoascus*, *Penicillium* e *Mortierella* como os gêneros mais abundantes. A diversidade de fungos em dois lagos nas Ilhas Shetland do Sul foi avaliada por de Souza *et al*. (2021) utilizando DNA *metabarcoding* em um experimento de microcosmo por dois anos, revelando 258.326 leituras de DNA distribuídas em 34 táxons de fungos dos filos *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Chytridiomycota* e *Rozellomycota*.

2. OBJETIVOS

2.1.Objetivo Geral

Caracterizar a diversidade de comunidades de fungos presentes em sedimentos de lagos da Antártica e avaliar seu potencial como produtores de enzimas, biossurfactantes e lipídios de interesse industrial, bem como metabólitos herbicidas de interesse na agricultura.

2.2.Objetivos Específicos

- Caracterizar as comunidades de fungos presentes em amostras de sedimentos de lagos de diferentes ilhas antárticas por meio de técnicas dependentes e independentes de cultivo;
- Depositar os fungos obtidos para preservação da biodiversidade *ex situ* na Coleção de Micro-organismos e Células da UFMG;
- Determinar, por meio de índices ecológicos, a diversidade das comunidades fúngicas dependentes e independentes de cultivo.
- Avaliar a capacidade dos fungos cultiváveis em produzir enzimas, biossurfactantes e lipídios de interesse industrial;
- Avaliar extratos a partir do crescimento dos fungos cultiváveis, realizar ensaios para determinação da atividade herbicida e isolar e identificar as substâncias bioativas.

3. RESULTADOS

Os resultados dessa tese foram apresentados seguindo a norma alternativa de acordo com as normas do Programa de Pós-Graduação em Microbiologia em formato de capítulos conforme listado abaixo:

- **Capítulo 1:** de Souza, L. M. D., Convey, P., Lirio, J. M., Rosa, L. H. **Diversity of freshwater fungi in polar and alpine lakes**. In: Freshwater Mycology: Perspectives of Fungal Dynamics in Freshwater Ecosystems. Elsevier, v. 1, p. 37-58, 2022. (https://doi.org/10.1016/B978-0-323-91232-7.00013-1);
- **Capítulo 2:** de Souza, L. M. D., Barreto, D. L. C., Coelho, L. C., Teixeira, E. A. A., Gonçalves, V. N., Ribeiro, J. P. M., Rabelo, N. G., Alves, S. E. O., da Silva, M. K., Martins, L. B. M., Cantrell, C. L., Duke, S. O., Rosa, L. H. **Fungal Biosurfactants: Applications in Agriculture and Environmental Bioremediation Processes**. In: Biosurfactants and Sustainability: From Biorefineries Production to Versatile Applications. John Wiley & Sons, Ltd., v. 1, p. 243-254, 2023. (https://doi.org/10.1002/9781119854395.ch12);
- **Capítulo 3:** de Souza, L. M. D., Lirio, J. M., Coria, S. H., Lopes, F. A. C., Convey, P., Carvalho-Silva, M., Oliveira, F. S., Rosa, C. A., Câmara, P. E. A. S., Rosa, L. H. **Diversity, distribution and ecology of fungal communities present in Antarctic lake sediments uncovered by DNA metabarcoding**. Scientific Reports, v. 12, p. 8407, 2022. (https://doi.org/10.1038/s41598-022-12290-6);
- **Capítulo 4:** de Souza, L. M. D., Ribeiro, J. P. M., Barreto, D. L. C., Teixeira, E. A. A., Carvalho, C. R., Lirio, J. M., Coria, S. H., Convey, P., Oliveira, F. S., Cantrell, C. L., Duke, S. O., Rosa, C. A., Rosa, L. H. **Diversity of culturable fungi in Antarctic lakes and their potential for producing compounds of biotechnological interest**. Submetido em 01/08/2025 para a revista *Extremophiles*.
- **Capítulo 5:** de Souza, L. M. D., Lirio, J. M., Coria, S. H., Carvalho-Silva, M., Câmara, P. E. A. S., Lopes, F. A. C., Oliveira, F. S., Convey, P., Rosa, L. H. **Fungal diversity in sediments of periglacial lakes of Deception Island, Maritime Antarctica assessed using culturing and DNA metabarcoding approaches**. Submetido em 14/08/2025 para a revista *Extremophiles*.

3.1.Diversity of freshwater fungi in polar and alpine lakes

Nesse capítulo abordamos que as regiões árticas, antárticas e alpinas são os principais elementos da criosfera, com as regiões polares compreendendo cerca de 14% da superfície da Terra. Esses ambientes diferem em vários aspectos. A Antártica experimenta condições climáticas muito mais severas em geral em comparação com as regiões árticas e alpinas. As áreas alpinas apresentam gradientes climáticos consideráveis em escala mais local, por exemplo, de áreas mais frias em altitudes mais elevadas a vales muito mais quentes. Nas regiões polares os lagos estão presentes tanto em áreas costeiras quanto interiores, variando amplamente em status trófico (ultra-oligotrófico a eutrófico) e osmótico (água de degelo quase pura a salmouras hipersalinas). Nas regiões alpinas os lagos estão presentes nas Montanhas Rochosas da América do Norte, nos Alpes Europeus, no Himalaia, no planalto tibetano na Ásia central e ocidental e nos Andes sul-americanos. Entre os micro-organismos presentes nos ecossistemas aquáticos polares e alpinos, os fungos são importantes organismos decompositores que desempenham papéis fundamentais na reciclagem de nutrientes. Os filos fúngicos comumente presentes nesses lagos incluem *Rozellomycota*, *Chytridiomycota*, *Ascomycota*, *Mortierellomycota*, *Blastocladiomycota*, *Basidiomycota* e *Mucoromycota*. As regiões polares e alpinas incluem ecossistemas frágeis e sensíveis, potencialmente vulneráveis a danos rápidos e consideráveis devido aos impactos das mudanças climáticas antropogênicas contemporâneas e outros impactos humanos diretos. No entanto, apesar da urgência desses grandes impactos antropogênicos diretos e indiretos, a diversidade e as funções ecológicas dos fungos em ambientes aquáticos polares e alpinos têm sido pouco estudadas.

CHAPTER

3

Diversity of freshwater fungi in polar and alpine lakes

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1 Polar and alpine regions

The term cryosphere was originally proposed by Polish scientist Antoni Boleslaw Dobrowolski, and collectively describes the portions of the Earth's surface where water exists in a frozen state, such as in snow cover, glaciers, ice sheets, continental water ice, sea ice, icebergs, permafrost and subterranean ice (Barry & Gan, 2011; Tsuji & Hoshino, 2019). The polar regions (Arctic and Antarctic) are the largest element of the cryosphere (Fig. 3.1), constituting about 14% of the Earth's surface (Tsuji & Hoshino, 2019). The Arctic has an area of about 7.1 million km^2 (4.8% of the Earth's surface) with its major components including the Arctic Ocean, which has up to approximately 10 m of ice covering the sea surface seasonally, Greenland (the world's largest island) and the northern fringes of the Northern Hemisphere continents, and has a mean annual temperature of around −18 °C (Barry et al., 2013; Tsuji & Hoshino, 2019). A 2009 study collated sea ice thickness records from submarines and ICESat observations between 1958 and 2008 (Kwok & Rothrock, 2009). Examining 42 years of submarine records (1958–2000) and five years of ICESat records (2003–2008), this study concluded that mean Arctic sea ice thickness declined from 3.64 m in 1980 to 1.89 m in 2008. In contrast, Antarctica is a large continental landmass overlying the geographic South Pole and has an area of approximately 14 million km^2, around 10% of the Earth's surface. It is the fifth largest continent, with about 99.6–99.8% of its area permanently covered by ice and snow. Mean monthly air temperatures in coastal areas range from 2–3 °C to −35 °C over the year, with those in inland continental regions ranging between −25 °C in summer and −70 °C in winter (Convey, 2017; Ravindra & Chaturvedi, 2011; Tsuji & Hoshino, 2019). In winter the surface of

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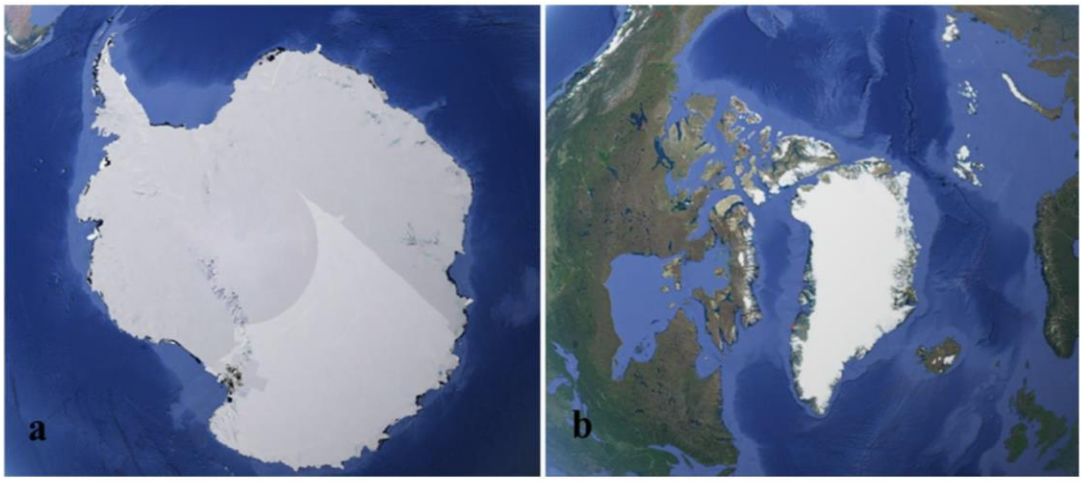

FIG. 3.1 The Earth's polar regions (A) Antarctica and (B) Arctic. *Source: Google Earth.*

the Southern Ocean surrounding Antarctica freezes, doubling the surface area of the continent and further increasing its isolation.

The word 'Arctic' derives from the ancient Greek word Arktikós, which means the land of the North and is related to Arktos, the Ursa Major constellation of stars that is used as a pointer to locate the North Star (Barry et al., 2013). With Antarctica encircling the geographic South Pole to around the latitude of the Polar Circle (~ 68° S), its name means 'opposite the Arctic' (anti-Arctic) (Bischoff, 1996). The Arctic includes the smallest of the global oceans, which is surrounded by a relatively narrow zone of island and continental tundra on the edge of the great northern continents, as well as Greenland, the world's largest island. With its continuous southward terrestrial connectivity, the Arctic has a comparatively rich terrestrial fauna and flora derived from the continents of Eurasia and North America, including many species that spread to lower latitudes during repeated cycles of Pleistocene glaciation (Meltofte et al., 2014). Extreme seasonality and permafrost, together with an abundance of inland water habitats ranging from shallow tundra ponds fed by small streams to large lakes and deep rivers, determine the hydrology, biodiversity and general characteristics of Arctic terrestrial ecosystems (Meltofte et al., 2014). The Arctic landscape is characterized by different types and sizes of aquatic systems, including rivers and streams and many types of standing water systems (small ponds and lakes, mires, bogs, spring areas, along melt water creeks and snow beds) (ACIA, 2005; Meltofte et al., 2014; Vincent & Laybourn-Parry, 2008; Wrona et al., 2006).

Although they are often referred as 'polar environments', the Arctic and Antarctic display many contrasts. Antarctic environments are subject to much more severe climatic conditions than are their Arctic equivalents at any given latitude (Convey, 1996). The higher altitude of the continent, currently covered by an average depth of around 2 km in its continental ice sheets, leads to colder temperatures. In addition, since the final breakup of the last elements of the supercontinent Gondwana, Antarctica has been isolated from all other continents for more than 25 million years, in particular following the formation of the Antarctic Circumpolar Current in the Southern Ocean (now the world's largest and most powerful

ocean current) and the atmospheric South Polar Vortex (Convey, Bowman, et al., 2018). At the Last Glacial Maximum in the late Pleistocene, Arctic ice sheets held almost double the amount of ice than did those in Antarctica, while today the large majority of the remaining Arctic ice sheet is only present in Greenland. As noted, in comparison to the Antarctic, the terrestrial Arctic is well connected to neighboring regions to the south (Convey, 2017; Miller & Whyte, 2011) and is at lower altitude and latitude as well as in parts strongly influenced by warm ocean currents. However, Arctic and Antarctic regions are subject to many extreme conditions such as low average temperatures, which affect microbial metabolism directly and indirectly through decrease in primary production, large daily variations in temperature and frequent freeze-thaw cycles (Convey, Coulson, Worland, & Sjöblom, 2018; Miller & Whyte, 2011; Yergeau & Kowalchuk, 2008). Terrestrial environments may also be nutrient-limited and water-limited, especially during winter when all available water is frozen (Miller & Whyte, 2011; Thomas et al., 2008). The polar regions receive lower maximum levels of incident solar radiations, relative to lower latitudes, compounded by the attenuating effects of snow and ice cover on habitats beneath. However, cumulative irradiance over the long summer daylength (24 h at latitudes above the polar circles) can be greater than at lower latitudes, while reflectance from extensive snow- and ice-covered surfaces can also serve to increase solar radiation in surrounding terrestrial habitats. Overall, the combination of irradiance and short summer active seasons limits total annual production in Arctic and Antarctic ecosystems, and it has a strong influence on the seasonality of photosynthesis, which ceases during the onset of winter darkness (Thomas et al., 2008; Vincent & Laybourn-Parry, 2008).

Alpine regions are characterized by climatic variability that encompasses cooler areas at higher altitudes and warmer areas located especially in sheltered valleys (Cattivelli, 2021). Climate change due to increased concentration of anthropogenic greenhouse gases has been studied in alpine regions (Auer et al., 2007; Beniston & Jungo, 2002; Brunetti et al., 2009; Haeberli & Beniston, 1998; OECD, 2007; Raible et al., 2006; Schär et al., 1998; Wagner et al., 2017). Future climate scenarios for the period 2021–2050 suggest drier summers and wetter winters, heavy rainfall events, long periods of drought, more days and nights with extreme temperatures and significant temperature increases (Cattivelli, 2021). However, while it is clear that alpine ecosystems are fragile and vulnerable to current climate change and other direct anthropogenic impacts, the biodiversity and ecological functions of microbes resident in their aquatic environments have been little studied (Cattivelli, 2021; Tasser, Sternbach, & Tappeiner, 2008).

Alpine lakes are primarily found in the Rocky Mountains of North America, the European Alps, the Himalayas, the Tibetan plateau in central and western Asia and the South American Andes (Burpee & Saros, 2020). Located in the center of the European continent and densely populated, the European Alps constitute a dominant feature of the European landscape. Characterized by extensive plains, deeply incised valleys and peaking at an altitude of more than 4800 m, the Alps are subject to strong topographical variability (Gobiet et al., 2014) and are among the most vulnerable areas in Europe (Wagner et al., 2017). Glaciers in the Bolivian Andes are an important water resource for Andean cities and mountain communities, but relatively few studies have evaluated changes in their extent in recent decades (Cook, Kougkoulos, Edwards, Dortch, & Hoffmann, 2016). The Chilean and Argentine Andes contain approximately 30,000 km^2 of glacial ice (about 93% of the total glacial area of South America) and approximately 16,000 glaciers; these areas are increasingly being used for mining purposes,

hydroelectric facilities and tourism activities (Wilson et al., 2018). Widespread glacial retreat is apparent in Andean glaciers, with those currently remaining at lower and tropical latitudes (in Ecuador, Colombia and Venezuela) almost inevitably to be lost along with their associated and likely unique biota in the next few years to decades, as with the few remaining central African glaciers. In the Himalayas, glaciers are also an important water resource. In their lower reaches they generally have extensive debris coverage and often terminate in glacial lakes (King, Bhattacharya, Bhambri, & Bolch, 2019). Contemporary deglaciation is leading to increasing numbers and extent of proglacial lakes globally, as illustrated in the European Alps, the Caucasus, Iceland, South America and across the Himalaya (Carrivick & Tweed, 2013), and even in milder parts of the Antarctic such as the South Shetland Islands (da Rosa et al., 2021).

In high altitude alpine flowing water organic matter largely originates in snow runoff and ice melt from the surrounding terrestrial environment. Watersheds are generally well-defined and relatively small. In these high-speed flow systems, most microbial activity occurs in biofilms, which are likely to host a diversity of fungi capable of degrading organic matter (Battin, Wille, Psenner, & Richter, 2004; Grossart et al., 2019). High altitude alpine lakes also contain a surprising richness of fungi belonging to the phylum *Chytridiomycota* and, therefore, may harbor a large proportion of phytoplankton parasites (Grossart et al., 2019; Ortiz-Álvarez, Triadó-Margarit, Camarero, Casamayor, & Catalan, 2018).

2 Characteristics of polar and alpine lakes

Lakes, lagoons, rivers and streams are found in many coastal locations and within the polar regions and host a wide variety of ecosystems, from dilute and ultra-oligotrophic meltwater to hypersaline brines (Miller & Whyte, 2011). Many polar aquatic habitats experience water temperatures close to 0 °C for much of the year, and many of the organisms present appear to be cold tolerant rather than cold-adapted, with the cold environmental conditions likely reducing their metabolism and growth rates (Vincent and Laybourn-Parry, 2008).

According to Miller and Whyte (2011), most polar lakes are characterized by prolonged, sometimes perennial, ice cover that is typically 3–6 m thick and may contain sand/rock flour and organic matter of aeolian origin. The lakes display a dynamic balance between the downward movement of contained sedimentary material as a result of melting during the summer and the upward movement of ice as a result of surface ablation and freezing at the bottom during the summer when solar radiation is continuous. Liquid water is present in the snow and ice profile during the summer melt period, especially when air temperatures are above zero and large volumes of melt water can be produced, forming channels that can connect and release concentrations of cryoconite and can transport nutrients and microorganisms into newly established proglacial lakes (Mindl et al., 2007; Mueller, Vincent, Pollard, & Fritsen, 2001). Thus, glacial meltwater has the potential to directly influence microbial community composition in these lakes (Mindl et al., 2007). Nutrient inputs to lakes in periglacial environments is strongly influenced by patterns of drainage flow from the glacier surface and through underground/underice channels (Mindl et al., 2007; Priscu et al., 2002).

In addition to their striking limnological characteristics, high latitude aquatic environments have broad global significance, acting as climate change sentinels, refuges for unique

species and communities, and, in the case of large Arctic rivers, as important sources of continental and organic materials for the different oceans of the planet (Margesin & Collins, 2019; Ogaki, Vieira, Lírio, Rosa, & Rosa, 2019; Quayle et al., 2003; Quayle, Peck, Peat, Ellis-Evans, & Harrigan, 2002; Vincent and Laybourn-Parry, 2008). An additional source of nutrients for glacial biological communities is glacial flour, which consists of clay and silt-sized rock particles generated by glacial erosion and solid material abrasion by glacier movement (Hodson, Mumford, Kohler, & Wynn, 2005; Hodson, Mumford, & Lister, 2004; Mindl et al., 2007).

Landscapes evolve over time and aquatic ecosystems, including lakes and lagoons, represent transitory habitats in polar regions that can undergo rapid changes, from their initial formation to the eventual filling of the basin by abiotic and biotic sediments (Vincent and Laybourn-Parry, 2008) and even catastrophic draining when ice damming valleys is lost during glacial recession. During the summer, liquid water with suspended glacial flour enters adjacent water bodies such as proglacial lakes or glacial streams (Mindl et al., 2007). Climate change has the potential to affect lake evolution through a variety of processes, especially in the polar regions, where even small changes in temperature can have profound impacts on landscape properties such as snow, permanent frost, glacial melt, hydrological inputs and soil and vegetation stability (Vincent and Laybourn-Parry, 2008).

Global circulation models generally predict that the most rapid and pronounced increases in temperature over the twenty-first century will occur at higher latitudes due to a variety of feedback processes that amplify warming in these regions (Beniston et al., 2018; Bibi et al., 2018; Huss et al., 2017; Margesin & Collins, 2019; Meehl et al., 2007; Turner et al., 2009; Vincent and Laybourn-Parry, 2008). High latitude lakes are already suffering marked impacts due to climate change, including the loss of perennial ice cover leading to increases in the duration of open water conditions and water temperatures, stronger water column stratification, and changes in the balance of inflow and outflow, in some cases leading to complete drainage or drying of lakes and swamps (Quayle et al., 2002, 2003; Vincent and Laybourn-Parry, 2008). In polar aquatic ecosystems, small climate-induced changes in the physical, chemical or biological environment can be amplified into large changes in limnological properties (Flocco, Mac Cormack, & Smalla, 2019; Ogaki et al., 2020; Quayle et al., 2002, 2003; Rosa et al., 2019), potentially threatening their structure and functioning.

While some polar aquatic habitats are characterized by very cold temperatures in summer, others can stay warm during winter. Shallow melt lakes and ponds can warm to 10 °C or more (Peck, Convey, & Barnes, 2006). The surface waters of northern lakes with high concentrations of dissolved organic matter that absorb light, can experience daytime heating and temperatures of up to 15 °C (Vincent and Laybourn-Parry, 2008).

Arctic lakes are classified according to the formation of the depression where the lake is located and of the bottom material. Many are formed by landscape erosion or recent glacial erosion, which can result in different shapes and depths. Ice masses often appear to determine the orientation of these lakes (Hasholt, 2012). Arctic catchments often contain large standing biomass of terrestrial vegetation, while those in the Antarctic generally have very limited occurrence of higher plants with vegetation limited to bryophytes and lichens, resulting in a much greater influence of allochthonous (external) sources of organic carbon on lakes in the Arctic compared to the Antarctic, where autochthonous (internal) processes are assumed to dominate (Vincent and Laybourn-Parry, 2008). Ice cover influences the light conditions and heat balance of lakes, and, consequently, lake biological systems (Hasholt, 2012; Quayle et al.,

2002, 2003). Depending on the bathymetry of the lake and the initial chemical composition of the water column, ice formation can also cause significant annual variation in chemical composition. After the disappearance of ice cover in spring and summer, heating by solar radiation and hot air advection can lead to surface temperatures of up to 10–15 °C, while the deeper parts remain at around 4 °C. Paleolimnological studies suggest that shorter durations of snow and ice cover are linked with higher primary production at sites throughout the Arctic (Hodgson & Smol, 2008), resulting from greater light availability for photosynthesis, increased wind-induced mixing and increased nutrient inputs from catchments in a warmer climate. Longer ice-free periods may alter some lakes from cold monomixis (one period of mixing a year) to dimixis (two periods of mixing a year) systems, potentially resulting in warmer waters, shifts in zooplankton communities and increased contaminant transfer to higher trophic levels (Vincent et al., 2011).

In glacial areas, lakes and streams often form on the margin between a glacier or ice cap and the surrounding landscape. On larger glaciers and ice caps they can also form in surface depressions such that they have no direct contact with the terrestrial environment. Such lakes can interact with the glacier or ice cap drainage system and can also cause severe flooding if bottom thaw removes the seal on the internal drainage system (Hasholt, 2012). Due to the Arctic's proximity to the northern temperate zone, Arctic freshwater ecosystems tend to host more diverse animal, plant and microbial communities, with more complex food webs than found in Antarctica. Native fish and other freshwater vertebrates are absent from lakes and streams in Antarctica (other than ducks on sub-Antarctic South Georgia and the Kerguelen archipelago), and many continental Antarctic lakes even lack zooplankton (Vincent and Laybourn-Parry, 2008). According to Vincent and Laybourn-Parry, 2008, the combination of regional similarities and contrasts make comparative limnological studies in the polar regions particularly attractive to address large scale subjects such as of the factors controlling the global biogeography of aquatic plants, animals and microorganisms, the limiting factors for organic production, the causes and consequences of the complexity of food webs and the responses of aquatic ecosystems to environmental changes.

Given the limited area of ice-free ground, it is perhaps surprising that the Antarctic continent and sub-Antarctic islands represent some of the most diverse and interesting lake districts on the planet (Hodgson, 2012). The lakes of the Antarctic Peninsula and Scotia Arc (maritime Antarctic) are typically shallow, transparent, receive high light and ultraviolet radiations, often ice-free for just a few weeks each year, cold and generally have low nutrient availability, characteristics that represent limiting factors for the resident microbiota (Ellis-Evans, 1996). Antarctic lakes generally experience temperatures below 10 °C and have winter ice cover between 1.5 and 6 m thick, forming a thermal barrier that prevents them from becoming frozen to the bottom (Hodgson, 2012).

In physicochemical terms, Antarctic lakes range from some of the purest continental waters in the world to hypersaline lakes with salt concentrations eight times that of seawater, preventing them from freezing, even during winter (Hodgson, 2012). The nutritional status of Antarctic lakes is typically oligotrophic, with eutrophy generally restricted to those that are directly influenced by visiting marine mammals, birds or even humans (Butler, 1999; de Souza et al., 2021; Gonçalves, Vaz, Rosa, & Rosa, 2012; Hodgson, 2012; Ogaki et al., 2019, 2020; Quayle & Convey, 2006). Typically, the freshwater bodies of Antarctica are in direct contact with rocks, soil and mud, and their catchments include vegetation such as *Deschampsia antarctica*,

Colobanthus quitensis (these flowering plants in the maritime Antarctic only), mosses, macroalgae and lichens (Ogaki et al., 2019). In addition, lakes have an interface with the air, called the extra-aquatic zone, and, consequently, represent important sites for the study of microbial diversity and ecology (Ogaki et al., 2019). Wingham, Siegert, Shepherd, and Muir (2006) suggest that many Antarctic sub-glacial lakes (at least c. 450 of these are now known to be present under the vast continental ice sheets) are interconnected, creating extensive subglacial drainage systems. Ice and snow cover result in low levels of photosynthetically available radiation annually in lakes but, when ice melts in summer, the high transparency of the water column can transmit so much light that it has an inhibiting effect on photosynthesis (Hodgson, 2012).

Most Antarctic lakes are located in coastal regions, such as the McMurdo Dry Valleys, Vestfold Hills, Larseman Hills, Bunger Hills, Schirmacher Oasis and Syowa Oasis, as well as along the Antarctic Peninsula and associated islands and archipelagoes (Brandão et al., 2017; de Souza et al., 2021; Gonçalves et al., 2012; Ogaki et al., 2019, 2020). Many coastal lakes were formed as a direct result of isostatic recovery after post-glacial ice retreat. Seawater trapped in this way can subsequently be washed out by freshwater inflow, but closed basin lakes typically become saline due to evaporative salt concentration over time (Hodgson et al., 2010; Hodgson, Vyverman, & Sabbe, 2001; Pickard, Adamson, & Heath, 1986; Verleyen, Hodgson, Sabbe, & Vyverman, 2004; Zwartz, Bird, Stone, & Lambeck, 1998). Antarctic lakes include some of the highest salinity water bodies on Earth (Vincent and Laybourn-Parry, 2008). Subglacial Lake Vostok represents a special example of a polar lake, composed of two types of ice, (i) the 3500 m thick overlying ice sheet which contains traces of nutrients of wind origin, including various acids, sea salts and mineral grains, and (ii) the ice below 3500 m comprising refrozen water from the lake itself that has accumulated at the bottom for approximately 420,000 years (Hodgson, 2012; Siegert et al., 2001).

Despite their biota being primarily microbial, what these Antarctic lake biota lack in size is more than compensated for in biomass and species diversity (Hodgson, 2012; Vincent, 2000). Given the remarkable diversity of life present in these lakes, despite the multiple extreme environmental challenges to life, there has been an ongoing debate about its evolutionary history, in particular whether its component species arrived on the continent more recently through dispersal from sub-Antarctic islands and nearby continents, such as South America, or they are endemic, surviving glacial advances in lacustrine refuges, and then recolonizing newly thawed areas. The latter hypothesis allows for a greater degree of endemism and the possibility that Antarctic lakes may contain remnant species even dating back to the final stages of break-up of Gondwana (Gibson et al., 2006; Hodgson, 2012; Verleyen et al., 2021; see Convey, Bowman, et al., 2018; Convey, Biersma, Casanova-Katny, & Maturana, 2020, for wider discussion of the general applicability of this hypothesis across both terrestrial and freshwater ecosystems in Antarctica).

In alpine regions, hydrological engines are powered by glaciers and snow, as their seasonal melt is strongly associated with surface and groundwater flows driven by freeze-thaw cycles, where discharge can be highly variable, with seasonal maxima during summer and winter minima, and large daily fluctuations in summer (Brighenti et al., 2019; Jansson, Hock, & Schneider, 2003; Malard, Tockner, & Ward, 1999; Smith, Hannah, Gurnell, & Petts, 2001; Ward, Malard, Tockner, & Uehlinger, 1999). Although rock glaciers currently contribute little to water flow in alpine stream networks, mountain rock glaciers store large amounts of trapped water in the form of ice, making them important water reservoirs in arid regions (Brighenti et al., 2019; Geiger, Daniels, Miller, & Nicholas, 2014; Jones, Harrison, Anderson, &

Betts, 2018; Krainer, Mostler, & Spötl, 2007; Rangecroft, Harrison, & Anderson, 2015). The source of water is considered the main influence on habitat conditions in alpine streams. Three main stream types were originally identified and described: (i) kryal (glacier-fed), (ii) krenal (groundwater-fed) and (iii) ritral (snow melt/precipitation-fed), each type being characterized by different water temperatures, channel shape and stability, discharge patterns, turbidity, electrical and hydrochemical conductivity (Brighenti et al., 2019; Brown, Hannah, & Milner, 2003; Milner, Brittain, Brown, & Hannah, 2010; Ward, 1994).

Alpine glaciers store airborne anthropogenic contaminants and transport them to inland waters through ice melt, including persistent organic pollutants (POPs) like industrial chemicals, pesticides and by-products from combustion processes and chemical reactions, black carbon or dark aerosol produced from the combustion of fuels and trace elements (Brighenti et al., 2019; Carling, Rupper, Fernandez, Tingey, & Harrison, 2017; Daly & Wania, 2005; Gabrielli, Cozzi, Torcini, Cescon, & Barbante, 2008; Hodson, 2014). The proximity of the European Alps to several highly industrialized regions makes them particularly sensitive to the entry and accumulation of POPs, but their release from glaciers has also been observed in the Himalayas and the Rocky Mountains (Brighenti et al., 2019; Miner et al., 2017) and their presence has been detected even in remote Antarctic ice (Bargagli, 2008).

Alpine aquatic environments are highly heterogeneous, even at small spatial scale (< 1 km), mainly due to variation in hydrological source contributions, including melting glaciers and snow, rain runoff, groundwater springs, among others (Hotaling, Finn, Joseph Giersch, Weisrock, & Jacobsen, 2017). Each source type results in a unique signature of flux, temperature, sediment load and chemistry, although individual sources rarely act in isolation, especially when seasonal melt is occurring (Brown et al., 2003; Füreder, Schütz, Wallinger, & Burger, 2001; Hotaling et al., 2017; Smith et al., 2001; Ward, 1994).

Microbial communities in aquatic environments have been widely recognized for their overall contribution to biodiversity, ecosystem processes and biogeochemistry (Battin, Besemer, Bengtsson, Romani, & Packmann, 2016; Hotaling et al., 2017; Zeglin, 2015). Alpine aquatic environments harbor substantial biodiversity and genetic resources due to the significant insularity of the habitat and environmental heterogeneity, and climate change is expected to impact the biodiversity of alpine rivers at different levels of biological resolution, from micro to macroscopic organisms and from genes to communities (Hotaling et al., 2017). Alpine lake ecosystems are particularly susceptible to global climate change through ice and permafrost melt, changing snow accumulation, reduction in the duration of ice cover and increasing water temperatures in summer, affecting biodiversity and functioning water cycles and microclimate (Holzapfel & Vinebrooke, 2005; Jiang et al., 2019; Parker, Vinebrooke, & Schindler, 2008). These changes can also lead to complex ecological changes in stream communities (microbial community, primary producers and invertebrates) and food webs, with an anticipated loss of biotic diversity (Brighenti et al., 2019).

3 Fungi in Arctic lakes

Meltwater may contain different viable or dormant microbial communities that are associated with the sediment layer (Belzile, Vincent, Gibson, & Hove, 2001; de Wit et al., 2003; Gaidos et al., 2004; Kriss, Mitskevich, Rozanova, & Osnitskaya, 1976; Miller & Whyte, 2011;

Priscu et al., 1998). Important among the different microbial groups present in polar lakes are the fungi, which include decomposing organisms capable of recycling nutrients in aquatic ecosystems and others with parasitic and mutualistic profiles. In the Arctic, the tundra, lacustrine systems and seas shelter thousands of species, many of them as yet undescribed (Meltofte et al., 2014). According to Dahlberg and Bültmann (2013), fungi are one of the most species-rich groups in the Arctic. Among them, lichenized fungi (known as lichens) are the best known in terms of geographic distribution and ecology. However, there is still surprisingly limited knowledge of these organisms. According to Dahlberg and Bültmann (2013), 4350 species of fungi have been reported in the Arctic, but their total richness in the region may exceed 13,000 species.

Lichenized fungi are the best known in the Arctic, with about 1750 species currently reported. They grow on various substrate surfaces and often make a remarkable and colorful contribution to Arctic phytogeography, which is particularly apparent in the High Arctic and in various types of lichen-dominated vegetation in the sub-Arctic (Dahlberg & Bültmann, 2013). Lichenized fungi are significant primary producers in the Arctic and their biomass contribution ranges from 2% in the Low Arctic to over 18% in High Arctic tundra, and 65% in areas with no vegetation (Dahlberg & Bültmann, 2013; Longton, 1988; Webber, 1974). Decomposition of dead organic matter and nutrient recycling in Arctic terrestrial systems is mainly driven by saprophytic fungi, with contributions from mycorrhizal taxa (Dahlberg & Bültmann, 2013; Ludley & Robinson, 2008; McMahon, Wallenstein, & Schimel, 2009).

The short favorable environmental conditions for growth and reproduction in the Arctic are believed to have selected for fungi with high longevity and slower population growth (Dahlberg & Bültmann, 2013; Gardes & Dahlberg, 1996). However, the population dynamics of fungi have not been studied in detail in this environment, and rather inferred from studies in boreal and temperate biomes (Dahlberg & Bültmann, 2013; Dahlberg & Mueller, 2011). Fungi present in the Arctic appear to have different physiological mechanisms to maintain mycelial activity, growth at low temperatures, water potential and, even when soils are frozen, fungal processes in this environment continue (Dahlberg & Bültmann, 2013; Robinson, 2001). Fungi contribute an order of magnitude greater contribution to Arctic soil microbial biomass compared to cohabiting bacteria. Fungal processes reach their annual peak under snow, where they can absorb carbon from the environment and grow in frozen soils at temperatures down to −2 °C (Dahlberg & Bültmann, 2013; McMahon et al., 2009; Schadt, Martin, Lipson, & Schmidt, 2003).

The effects of climate change on Arctic fungal diversity seem to be gradual although increase in intensity over time, leading to suggestions that their conservation status will not be strongly affected in upcoming decades but may change drastically in the longer term (Dahlberg & Bültmann, 2013). An assessment of the conservation status of Arctic fungi is feasible and mapping of rare and endemic species is necessary, recognizing that improved monitoring and functional research is needed for more accurate prediction of how fungal diversity and ecological functions could be affected by climate change (Dahlberg & Bültmann, 2013).

Zhang, Wang, Zhang, Liu, and Yu (2016) evaluated fungal diversity and distribution in water samples collected from four aquatic environments (stream, pond, meltwater and estuary) in the Ny Ålesund area of Svalbard (High Arctic), documenting a high diversity of 641 operational taxonomic units (OTUs). Of these, 200 were assigned to the *Ascomycota*, 196 to *Chytridiomycota*, 120 to *Basidiomycota*, 13 to *Glomeromycota*, 10 to *Zygomycota* (traditional) and

102 classified as 'unknown fungi'. The main orders characterized were *Helotiales*, *Eurotiales*, *Pleosporales*, *Chytridiales*, *Rhizophydiales*, *Leucosporidiales* and *Sporidiobolales*. The most common genera were *Penicillium*, *Rhodotorula*, *Epicoccum*, *Glaciozyma*, *Holtermanniella*, *Betamyces* and *Phoma*. Comeau, Vincent, Bernier, and Lovejoy (2016) reported *Chytridiomycota* as the most common phylum in Arctic aquatic environments, followed by *Ascomycota*, *Basidiomycota* and *Cryptomycota*. Perini, Gostinčar, and Gunde-Cimerman (2019) detected *Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Rozellomycota* in an Arctic lake located in Kongsfjorden, Svalbard, with *Chytridiomycota* predominating, followed by *Basidiomycota* most represented by the class *Agaricomycetes*; melt water from glaciers was also analyzed, showing the phyla *Basidiomycota* (*Agaricomycetes* and *Microbotryomycetes*), *Chytridiomycota* and *Ascomycota* (*Eurotiomycetes*) to be dominant.

4 Fungi in Antarctic lakes

Antarctica is generally seen as a remote, hostile region and a symbol of the last great wilderness, remaining little affected by human activities. Organisms that inhabit this continent are subjected to a variety of extreme environmental conditions, such as large variations in temperatures, summers with high levels of solar radiation, winters with long periods of darkness, high salinity, desiccation, nutrient scarcity, pH variation and freeze-thaw cycles (Convey, 2017; de Souza et al., 2021; Gocheva et al., 2006; Ogaki et al., 2020; Rosa et al., 2019). Despite being inhospitable, the continent is home to a diversity of biota including marine birds and mammals, marine and terrestrial invertebrates, plants and macroalgae (Clarke, Aronson, Crame, Gili, & Blake, 2004; Convey, 2017; Convey, Brandt, & Nicols, 2013; Ekltjnd, 1956; Quartino et al., 2017). The importance of microorganisms, represented by fungi, algae, bacteria, viruses and archaea stands out in the Antarctic environment, through their high diversity and great adaptive capacity to the extreme conditions of the region (Cavicchioli, 2015; Margesin & Collins, 2019; Perfumo, Banat, & Marchant, 2018; Rosa et al., 2019; Russell, 2006).

The high number of apparently cosmopolitan fungi found in Antarctica suggests that the continent constantly receives microbial propagules from beyond the region, especially from South America and Oceania, the closest adjacent regions (Archer et al., 2019; Bottos, Woo, Zawar-Reza, Pointing, & Cary, 2014; Kappen, 1993; Pearce et al., 2016; Rosa, Pinto, Convey, et al., 2020; Rosa, Pinto, Šantl-Temkiv, et al., 2020). Vectors such as microparticles suspended in the air column and animals are among the mechanisms responsible for such long-distance dispersal (Ruisi, Barreca, Selbmann, Zucconi, & Onofri, 2007). However, in addition to the wide variety of cosmopolitan species, endemic taxa are also found on the Antarctic continent (Bridge & Spooner, 2012; Rosa et al., 2019).

Most fungi found in Antarctica are in the anamorphic form (asexual stage), which can result in life cycles completed in a shorter time interval, and also generates lower metabolic costs and, consequently, savings in energy, which is critical for survival under extreme conditions (Ruisi et al., 2007). However, some, such as species of the genera *Thelebolus* and *Antarctomyces*, are present in the teleomorphic form (sexual stage) (de Hoog et al., 2004; de Menezes, Godinho, Porto, Gonçalves, & Rosa, 2017; Pegler, Spooner, & Smith, 1980).

The distribution of microorganisms in Antarctic lakes (Fig. 3.2) varies with lake type, physical and chemical environment, and local food webs, including inputs from terrestrial or marine vertebrate sources (Miller & Whyte, 2011). Since fungi are major decomposers,

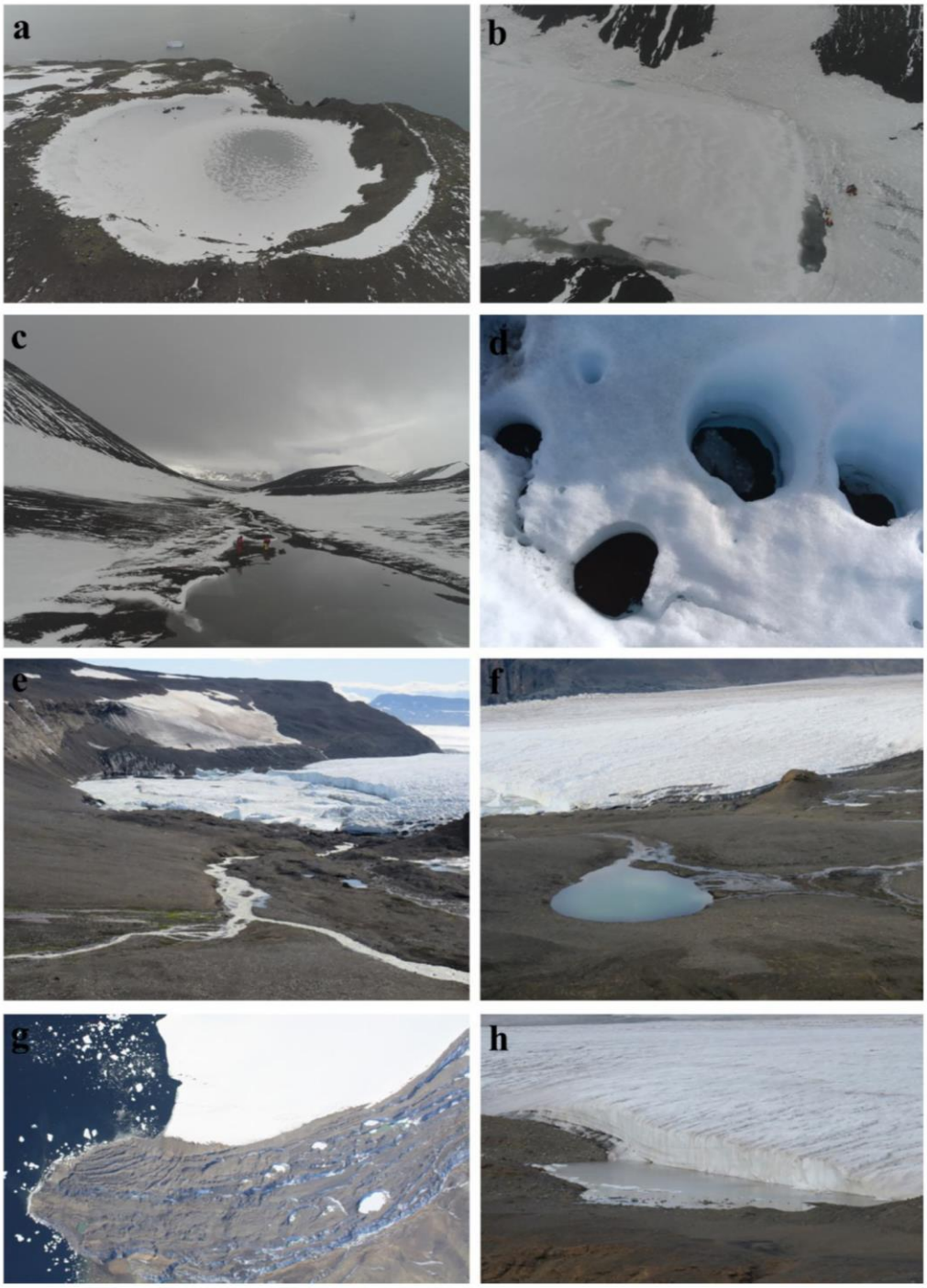

FIG. 3.2 Examples of different lakes in Antarctica (A) A maar, a volcanic crater lake formed by a phreatomagmatic eruption, with small ice-free portion, Penguin Island, (B) Soto lake, also a maar, in Deception Island, (C) Lagoon on Deception Island, (D) Cryoconite in James Ross Island, Haddington Ice Cap, (E) A Glacial lake dammed by Blancmange Glacier. Florencia Lake in Clearwater Mesa, James Ross Island, (F) A moraine dammed lake, Lake Cecilia in Clearwater Mesa, James Ross Island, (G) Thermokarst lakes at Cape Lamb, Vega Island and (H) Ice proximal lake, Lake Lilia in James Ross Island (Clearwater Mesa). *Photos: Luiz H. Rosa (A–C), Ricardo Suarez (D), Juan M. Lirio (E, F and H) and Fernando Calabozo (G).*

the main functional role of these organisms in continental aquatic ecosystems is the breakdown and mineralization of organic matter (Kuehn, 2016). Multiple studies have reported fungi from different lakes and regions in Antarctica, including Deception Island (Gonçalves et al., 2012; Stanley & Rose, 1967; Vaz et al., 2011), King George Island (de Souza et al., 2021; Gonçalves et al., 2012, 2015; Ogaki et al., 2020; Vaz et al., 2011), Signy Island (Ellis-Evans, 1985; McInnes, 2003; Willoughby, 1971), McMurdo Dry Valleys (Baublis, Wharton, Robert, & Volz, 1991; Brunati et al., 2009; Connell, Segee, Redman, Rodriguez, & Staudigel, 2018; de Hoog et al., 2004; Goto, Sugiyama, & Iizuka, 1969; Knox & Paterson, 1973; Kriss et al., 1976; Nagashima, Nishikawa, Matsumoto, & Tizuka, 1990; Rojas-Jimenez et al., 2017; Waguri, 1976; Waguri, Kawamura, & Tubaki, 1975), Vestfold Hills (Brunati et al., 2009; de Hoog et al., 2004), Larsemann Hills (Brunati et al., 2009; de Hoog et al., 2004), Skarvsnes (Tsuji et al., 2013) and Vostok Station (D'Elia, Veerapaneni, Theraisnathan, & Rogers, 2009; Rogers et al., 2013).

The main fungal phyla identified in continental water systems, including lake sediments, are *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Chytridiomycota* and *Rozellomycota* (de Souza et al., 2021; Monchy et al., 2011; Ogaki et al., 2019; Shearer et al., 2007; Wurzbacher, Kerr, & Grossart, 2011). In addition, some taxa of *Oomycetes* (Stramenopila) have also been reported (Gonçalves et al., 2012; Ogaki et al., 2019; Shearer et al., 2007). There are few detailed reports of fungal communities in lakes on the Antarctic continent, with most studies representing maritime Antarctica (Brunati et al., 2009; Ellis-Evans, 1996; Gonçalves et al., 2012; Ogaki et al., 2019). There is variation in freshwater microbial communities relating to the temperature gradient that occurs latitudinally on the continent (Ogaki et al., 2019). Brunati et al. (2009) analyzed the diversity of fungi and considered their potential for bioprospecting, isolating fungi from lakes in the Larsemann Hills (Lakes Manning and Reid), Vestfold Hills (Lakes Ace and Druzby) and McMurdo Dry Valleys (Lakes Fryxell and Hoare). Genera of psychrophilic fungi such as *Thelebolus* and *Pseudogymnoascus* were identified, as well as the cosmopolitan psychrotolerant genera such as *Acremonium*, *Aspergillus*, *Beauveria*, *Cladosporium*, *Curvularia*, *Debaryomyces* and *Penicillium*.

Fungal propagules representing the genera *Rhodotorula*, *Aureobasidium*, *Penicillium*, *Cystofilobasidium*, *Aspergillus*, *Cryptococcus* and *Pseudozyma* were detected in ice from the Lake Vostok ice core which was 3–5000 years old (D'Elia et al., 2009), as well as in deep glacial ice sections 1–2 million years old. These data indicated that this lake contains a mixture of heterotrophic psychrotolerant fungal species (D'Elia et al., 2009). Yeast species of the genera *Rhodotorula* and *Cryptococcus* have been found in the deep ancient glacial layers in the center of Lake Vostok.

The diversity and composition of the fungal communities of permanently ice-covered lacustrine basins located in the Taylor and Miers Valleys (Victoria Land; including two samples from Lake Bonney) were analyzed using a culture-independent approach (Rojas-Jimenez et al., 2017). *Rozellomycota* and *Chytridiomycota* dominated these communities and *Rozellomycota* were particularly dominant in Lakes Miers, Bonney West and Fryxell, while *Chytridiomycota* were dominant in Bonney East and Hoare (Rojas-Jimenez et al., 2017). In addition, members of the phyla *Ascomycota*, *Mortierellomycota*, *Blastocladiomycota* and *Basidiomycota* were also present; among them, *Basidiomycota* was dominant in the upper layer of Lake Hoare and the main genera identified were *Glaciozyma* and *Mrakia* (Ogaki et al., 2019; Rojas-Jimenez et al., 2017).

Rogers et al. (2013) used metagenomics/metatranscriptomics to analyze samples from the Lake Vostok ice core and found that only about 6% of the unique sequences obtained were closer to eukaryotes. Among them, taxa of the phyla *Ascomycota*, *Basidiomycota* and *Mucoromycota* were identified. Fungal diversity in two lakes in the South Shetland Islands was assessed by de Souza et al. (2021) with the use of DNA metabarcoding through high-throughput sequencing (HTS). In this study, a microcosm experiment was carried out for two consecutive years in lakes on Deception and King George Islands, aiming to capture potential decomposing fungi. The analyses revealed 258,326 DNA reads distributed in 34 taxa of fungi of the phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Chytridiomycota* and *Rozellomycota*. *Tetracladium marchlianum*, *Tetracladium* sp., and *Rozellomycota* sp. were the most commonly detected taxa. Among the taxa reported by de Souza et al. (2021), only six were detected in both lakes, including the most dominant *T. marchlianum* and *Tetracladium* sp., suggesting that these fungi are important in the breakdown of organic matter in Antarctic lakes, releasing carbon and nutrients to other aquatic organisms present in the complex lake food webs.

5 Fungi in alpine lakes

Knowledge of the distribution, biodiversity and the functional roles of microorganisms that inhabit alpine environments is essential for understanding ecosystem processes in a climate that is undergoing change (Elster, Margesin, Wagner, & Häggblom, 2017). The diversity of organisms occurring in alpine regions testifies to the long-term success of natural dispersal strategies, with polar and alpine habitats representing a vast source of hitherto unreported microbial diversity (Elster et al., 2017; Obbels et al., 2016; Sigurbjörnsdóttir & Vilhelmsson, 2016; Tytgat et al., 2016; Van Goethem, Makhalanyane, Valverde, Cary, & Cowan, 2016). Alpine regions, like the rest of the globe, feature a high genetic and functional diversity of prokaryotes, eukaryotes and viruses, which are present in all types of alpine habitats (Elster et al., 2017).

Different groups and species of fungi have been described associated with aquatic habitats in alpine regions. Buzzini, Turchetti, Diolaiuti, D'Agata, and Martini (2005) identified the following fungal genera in glaciers of the Italian Alps (Forni Glacier and Sforzellina Glacier): *Bullera*, *Bulleromyces*, *Candida*, *Cryptococcus*, *Dekkera*, *Filobasidium*, *Pichia*, *Rhodotorula*, *Trichosporon* and *Zygosaccharomyces*, with *Cryptococcus* and *Rhodotorula* being the most abundant. In the same environment, Turchetti et al. (2008) analyzed meltwater originating from the subglacial flow at the glacier snouts, obtaining 106 yeast isolates belonging to the species *Aureobasidium pullulans*, *Goffeauzyma gilvescens* (more than 50% of the total), *Solicoccozyma terricola*, *Mrakia gelida*, *Naganishia globosa*, *Rhodotorula glacialis*, *Phenolipheria psychrophenolica*, *Microstroma bacarum*, *Leucosporidium creatinivorum* and *Cystobasidium laryngis*. Lake Geneva is a large lake situated in the Alps, lying on the border between France and Switzerland at an altitude of 372 m (Anneville, Ginot, Druart, & Angeli, 2002), whose eukaryotic diversity was studied by Mangot et al. (2013). They identified fungi belonging to the phyla *Chytridiomycota*, *Rozellomycota*, *Ascomycota*, *Basidiomycota* and *Zygomycota* (traditional). Kammerlander et al. (2015) studied lakes originating from the Faselfadferner Glacier in the Austrian Central Alps, finding fungi belonging to the families *Agaricaceae*, *Tricholomataceae*, *Tulasnellaceae*, *Polyporaceae* and *Fomitopsidaceae*. In addition, they evaluated lakes sampled in the Khumbu Valley region

(Nepal), near Mount Everest, reporting taxa of *Coniochaetaceae*, *Cystofilobasidiales*, *Filobasidiales*, *Tremellales* and *Leucosporidiaceae*.

Sanyal, Antony, Samui, and Thamban (2018) evaluated heterotrophic microorganisms present in water from 22 cryoconite holes in a Himalayan glacier, finding representatives of the phylum *Basidiomycota* belonging to the genera *Bensingtonia*, *Curvibasidium*, *Leucosporidium*, *Mrakia* and *Rhodotorula*. Anupama et al. (2011) reported for the first time in the Himalayas the occurrence of the psychrophilic and halotolerant species *Thelebolus microsporus*, which was isolated from Lake Pangong. Sati, Arya, and Belwal (2009) reported a new species of aquatic hyphomycete, *Tetracladium nainitalense*, isolated as a root endophyte from riverine plants from Nainital, Kumaun Himalaya, India.

The occurrence of cultivable yeasts in glacial melt rivers of the Frías, Castaño Overo and Río Manso glaciers, located on Mount Tronador in the Nahuel Huapi National Park (Northwest Patagonia, Argentina), was investigated by de García, Brizzio, Libkind, Buzzini, and Van Broock (2007). They reported the genera *Candida*, *Cryptococcus*, *Holtermanniella*, *Dioszegia*, *Leucosporidiella*, *Mrakia*, *Rhodosporidium*, *Rhodotorula*, *Sporobolomyces* and *Udeniomyces*. Lake Nahuel Huapi is temperate, oligotrophic, of glacial origin, has high transparency, is surrounded by well-developed forests and is located in San Carlos de Bariloche, Nahuel Huapi National Park, in Patagonia, Argentina. Its fungal diversity was evaluated by Brandão et al. (2011). Thirty four yeast species were identified, belonging to the genera *Aureobasidium*, *Delphinella*, *Dioszegia*, *Rhodotorula*, *Naganishia*, *Holtermanniella*, *Filobasidium*, *Vishniacozyma*, *Cryptococcus*, *Cystofilobasidium*, *Rhodosporidium*, *Bullera*, *Guehomyces*, *Candida*, *Debaryomyces*, *Hanseniaspora* and *Pichia*. *Rhodotorula mucilaginosa* and *Vishniacozyma victoriae* were the most abundant species, representing 14.4% and 13.6% of the total isolates obtained, respectively.

6 Conclusions

Knowledge of the diversity and ecology of fungi present in cold environments has increased in recent years, contributing to understanding of the evolution of characteristics related to adaptation to extreme conditions. Understanding of the role and behavior of microorganisms in polar and alpine regions has focused on the sensitivity of these environments to global climatic changes, and in particular their influence on the diversity of these organisms. The study of microbial communities that develop at temperatures close to the freezing point of water is inherently interesting, and these ecosystems are clearly relevant for determining the limits of life in the biosphere, fungal influence on biogeochemical cycles, conducting evolutionary studies, and even astrobiological studies. The studies of fungi present in polar and alpine lake environments suggest that these fungal communities may have great ecological importance, playing a role in the primary decomposition of organic materials and actively participating in the cycling of essential nutrients and thereby maintaining the balance of micro- and macronutrients in lacustrine systems. In addition, the fungal communities of polar and alpine lakes represent interesting targets for studies relating to potential biotechnological applications, including the production of antibiotics, enzymes, biosurfactants, sunscreens and antifreezes.

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3.2. Fungal Biosurfactants: Applications in Agriculture and Environmental Bioremediation Processes

Nesse capítulo relatamos que os biossurfactantes produzidos por fungos têm ganhado atenção como alternativas sustentáveis aos surfactantes sintéticos, principalmente nas áreas de agricultura e biorremediação. No setor agrícola, essas substâncias atuam como adjuvantes em formulações de herbicidas e inseticidas, melhorando a dispersão, aderência e absorção dos princípios ativos. Isso permite não apenas maior eficiência na aplicação, mas também a redução da dose necessária. Estudos destacam, por exemplo, os sophorolipídios de *Starmerella bombicola* e *Rhodotorula babjevae*, que apresentaram ação contra diversos fitopatógenos de interesse agronômico.

Esses biossurfactantes também mostraram efeitos promissores como larvicidas, principalmente contra vetores de doenças como *Aedes aegypti*. Substâncias produzidas por leveduras como *Scheffersomyces stipitis* e *Wickerhamomyces anomalus* foram eficazes mesmo quando cultivados em resíduos agroindustriais, como bagaço de cana ou óleo de palma, o que reduz custos de produção e aumenta a viabilidade econômica para uso em larga escala.

Na área ambiental, os biossurfactantes demonstraram grande potencial na biorremediação de poluentes orgânicos e inorgânicos. Fungos filamentosos como *Aspergillus*, *Penicillium*, *Cladosporium* e *Fusarium* atuam na degradação de hidrocarbonetos, corantes, pesticidas e metais pesados, contribuindo para a restauração de solos, sedimentos e efluentes contaminados. Sua ação se dá principalmente por aumentar a solubilidade e a biodisponibilidade dos contaminantes, facilitando a degradação por microrganismos. Graças à sua baixa toxicidade, biodegradabilidade e eficiência, os biossurfactantes fúngicos representam uma solução promissora para desafios ambientais e agrícolas.

12

Fungal Biosurfactants

Applications in Agriculture and Environmental Bioremediation Processes

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12.1 Biosurfactants as Agrochemicals

Agriculture represents one of the most important economic sectors in the world, which contributes to food production and food security, as well as producton of fiber, biofuels, and feedstock for industrial processes [1]. In order to increase crop productivity, agrochemical products such as herbicides, fungicides, and insecticides are widely used to control pests; however, current agricultural methods follow unsustainable practices that result in a huge amount of pesticides contaminating air, soil, and water [2]. Many studies have reported the negative impacts caused by these synthetic compounds on the environment and on human health [3]. Recent biotechnological advances in the agricultural and medical sectors are being employed to discover sustainable, natural, and biodegradable compounds which are effective against different pests while having less impact on the environment [4].

Often used in crops, agricultural adjuvants such as surfactants are substances that facilitate the application of products in order to increase their efficiency. Such substances are added to the formulation of pesticides prior to their application partly due their capabilities to favorably modify the surface properties of liquids [5]. Most active ingredients present in pesticide formulations are insoluble or weakly soluble in water, making the addition of surfactants to the solution essential, as when sprayed, the pesticide will better adhere to the surface of its target and spread adequately, optimizing the biological effectiveness of the

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product [6]. According to Purwasena et al. [7], around 0.2 million tons per year of surfactants are used to protect crops in pesticide formulations. Among the surfactants used in agriculture, there are those called "green surfactants" or biosurfactants (Figure 12.1), which often can be derived from microorganisms such as bacteria and fungi. Additionally, biosurfactants have many advantages when compared to synthetic surfactants, being less toxic, more biodegradable and highly effective, making them ideal for use in conventional farming applications [8].

12.1.1 Biosurfactants as Herbicide Adjuvants

In agriculture, herbicides are extensively used to manage weeds. Herbicides represent about 60% of all pesticides used worldwide. For most herbicides to exert their toxic effects on the plant, uniform coverage of the treated parts is necessary, an objective facilitated by an adjuvant [9]. Adjuvants can also enhance absorption of the herbicide and increase rainfastness [10]. Currently, there are many compounds available on the market, including

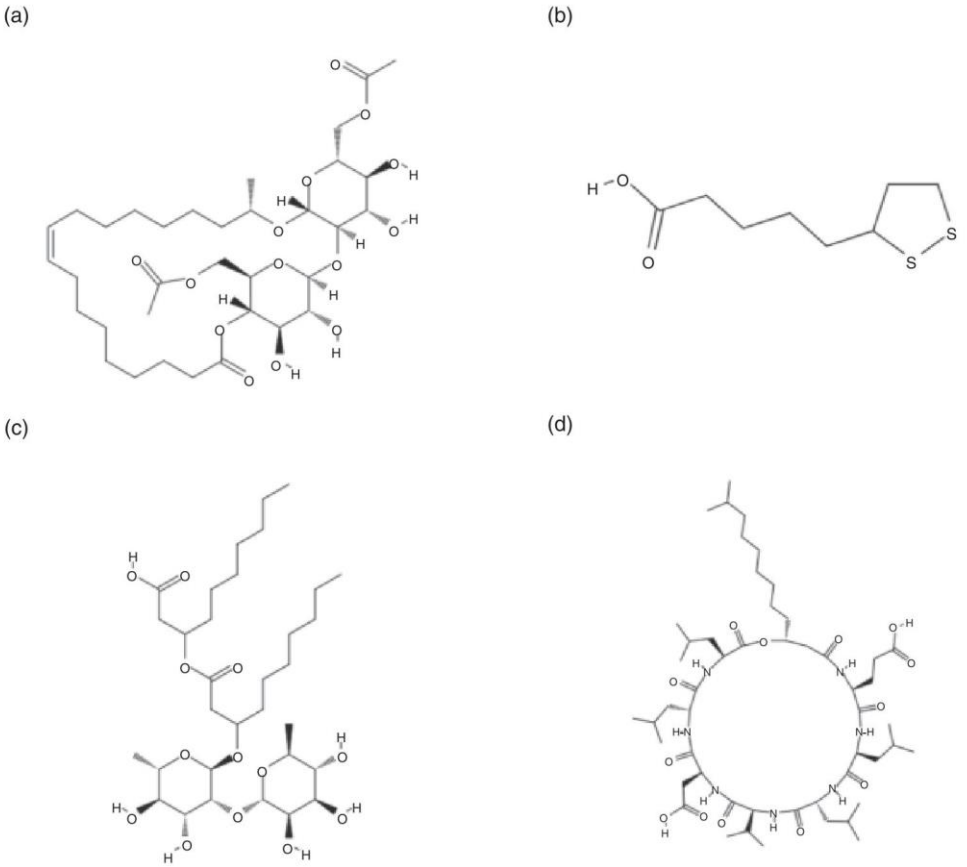

Figure 12.1 Example structural formulas of biosurfactants. (**a**) a lactonic sophorolipid; (**b**) lipoic acid; (**c**) a rhamnolipid 1; (**d**) a surfactin A. *Source:* MolView (https://molview.org).

surfactants, which are used to promote greater coverage of the leaves and increase the absorption of herbicides that also reduce the needed dose of the herbicide applied by more than 50% as compared to that used without the adjuvant [5].

Among the biosurfactants with potential for use as an adjuvant in agriculture are those produced by fungi. Vaughn et al. [11] reported sophorolipids produced by the yeast *Starmerella bombicola* as biosurfactants for post-emergence herbicides. In their study, lactone sophorolipids produced by *S. bombicola* (Sb) and non-lactone sophorolipids produced by *Candida kuoi* (Ck) were compared with a synthetic surfactant polyethoxylated tallow amine (POEA) that is used with some post-emergence commercial herbicides. *C. kuoi* sophorolipids formed longer-lasting and more stable emulsions with lemongrass oil (LGO) than Sb or T-15 (T-15 was chosen as the POEA for testing over T-5 and T-10, as it exhibited the best emulsifying abilities of the three synthetic surfactants), which is extremely important for the practical use of these compounds with lipophilic herbicides. Phytotoxicity (measured by reduced fresh and dry weight and visual damage three days after spraying) for *Senna obtusifolia* by the Ck/LGO and Sb/LGO mixtures was similar to a POEA/LGO mixture, while the visual damage to maize (*Zea mays* L.) was increased by the addition of all surfactants. When applied with the very water-soluble herbicide phosphinothricin, Ck and Sb enhanced reductions in *S. obtusifolia* fresh and dry weights and herbicide damage compared to phosphinothricin applied without surfactants. For corn, T-15 and Ck applied with PT resulted in the greatest reductions in fresh and dry weights and in HDR values. These results indicate that sophorolipids are excellent as natural surfactants for use with post-emergence herbicides.

12.1.2 Biosurfactants and Antifungal Activity

Some biosurfactants produced by fungi have antimicrobial activity against plant pathogens [8]. Most of these molecules produced by fungi have high biodegradability and low toxicity, which are important characteristics for large-scale, commercial use for plant protection in the context of sustained agriculture. In addition to antimicrobial activity, stimulation of local or systemic plant immunity can be involved in the efficiency of these molecules as biopesticides [12]. At least some of the biosurfactant antimicrobial activities might be attributed to the destabilization of cellular membranes, eventually resulting in their disfunction and rupture [13]. Different microorganisms, which produce biosurfactant molecules with antimicrobial activities, have been investigated to stem the worldwide evolution and spread of microbial antibiotic resistance [14], and may also be used against phytopathogens that have evolved resistance to antimicrobial pesticides. Biosurfactants produced by some yeast species have been assayed against some fungal plant pathogens (Table 12.1). An example is the sophorolipid biosurfactant produced by *Starmerella bombicola* that was assayed against *Phytophthora capsici*, *Phytophthora nicotianae*, *Phytophthora infestans*, *Pythium aphanidermatum* and *Pythium ultimum*, which at 2 mg mL^{-1} caused a 42% reduction of the damping off disease in pot trials [15]. Rhamnolipid, at the same concentration, reduced damping off by 33%. Another example is sophorolipids from *Rhodotorula babjevae* YS3 that showed promising activity against *Colletotrichum gloeosporioides*, a pathogen which causes post-harvest decay of apples, indicating that this yeast has a potential application as a food preservative; *R. babjevae* sophorolipid inhibited *C. gloeosporioides* (MIC = 62 μg mL^{-1}), it

Table 12.1 Biosurfactants produced by yeasts which were tested against fungal plants pathogens.

Biossurfactant class	Source	Plant pathogenic fungi	References
Sophorolipid	*Starmerella bombicola*	*Phytophthora capsici*	Yoo et al. [15]
Rhamnolipid		*Phytophthora nicotianae*	
		Phytophthora infestans	
		Pythium aphanidermatum	
		Pythium ultimum	
Sophorolipid	*Rhodotorula babjevae*	*Colletotrichum gloeosporioides*	Sen et al. [16]
		Fusarium verticilliodes	
		Fusarium oxysporum f. sp. *pisi*	
		Trichophyton rubrum	

also had a MIC of 125 μg mL^{-1} against *Fusarium verticilliodes* and *Fusarium oxysporum* f. sp. *pisi*. Unfortunately, it was only active at very high conentrations on two other fungi (MIC values of 1 mg mL^{-1} for *Trichophyton rubrum* and > 2 mg mL^{-1} for *Corynespora cassiicola*) [16].

12.1.3 Biosurfactants as Insecticidal Adjuvants

Biosurfactants have been used as adjuvants with insecticides, acting as emulsifying, dispersing, spreading, and wetting agents [17] and are considered to have less human toxicity, to be more environmentally safe, and a cheaper alternative to petroleum-based surfactants [18]. The insecticide activity of a biosurfactant is linked to several factors, such as inhibition of enzymes, destruction of biological membranes, drowning of pest insects, or removal of insects from the leaves [19].

Insect epicuticles are formed by a waxy layer, consisting of a mixture of lipids on the outer surface of the insect, which is the first physical barrier for the insecticide [20]. To overcome this barrier, surfactants can be employed to help in sticking the insecticide to the insect's surface and washing off lipids on its epicuticle, which could result in death either by dehydration or by increasing the infectivity of effectiveness of entomopathogenic microbes to the insect [21]. Biosurfactants, when when used to formulate a microbial biopesticide, often increase the dispersion of hydrophobic conidial formulations, helping the attachment and the penetration of the conidia into the insect's integument [21].

12.2 Insecticidal Biosurfactants for Use against Disease Vector Insects

Control of disease vector arthropod species harmful to human health with chemical insecticides is extensively used, despite its toxicity, which can cause dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine effects in humans

and animals, and its associated environmental impacts [22]. In addition, the increase in evolution and spread resistance of different insects to these insecticides has been another major problem with these pesticides in recent years [23]. For this reason, the search for alternatives for entomological control has been intensified, and interest in biomolecules produced by microorganisms with insecticidal activity has increased, among which biosurfactants stand out [24, 25].

Recent approaches point to some biosurfactants as pest biocontrol agents [26]. The larvicidal effect of biosurfactants is advantageous not only for possible applications in agricultural pest control, but also for significant disease vector insects [26, 27]. Tests that aim to determine the larvicidal activity from these biomolecules usually consider parameters such as the lowest concentrations used, exposure time, behavioral changes, and larval mortality rates [23, 28]. Studies on larvicidal application of biosurfactants are focused on controlling the larval stages of biological vectors of important diseases worldwide such as dengue fever, malaria, and filariasis transmitted by *Aedes*, *Anopheles* and *Culex* mosquitoes, respectively [29, 30]. According to Fernandes et al. [28], yeasts are prolific fungi that produce biosurfactants. Most of these fungi are known to be safe or GRAS (generally regarded as safe), which facilitates their application in the food and pharmaceutical industry. However, the volume of studies with surfactants produced by fungi is limited [23, 28].The yeast *Scheffersomyces stipitis* NRRL Y-7124 produces a glycolipid with strong activity against larvae in the third developmental stage of the *Aedes aegypti* mosquito, a period in which there is greater resistance to synthetic chemical larvicides [23]. The application of *S. stipitis* glycolipid at a concentration of 660 mg L^{-1} (LC50) was lethal to the larvae, possibly by interfering with the hydrostatic respiratory activity and integrity of their exoskeletons. The biosurfactant obtained from the fungus *Wickerhamomyces anomalus* CCMA 0358 exhibited stable larvicidal action against *A. aegypti* [28].

According to Mnif and Ghribi [24], the success of obtaining biosurfactants depends mainly on the development of processes that use low-cost raw materials, since this material represents between 10 and 30% of the total cost of the process. According to Fernandes et al. [28], *W. anomalous* produces more biosurfactants in the presence of palm and olive oil compared to residual cooking oil. The fungus *S. stipitis* was able to produce biosurfactants with larvicidal activity using sugarcane bagasse as a carbon source, a source widely available in countries such as Brazil and India [23]. These results prove the possibility of producing fungal biosurfactants with larvicidal activity with low-cost carbon sources, which allows for production of a product that is more economically competitive with synthetic insecticides.

The interesting physicochemical properties of biosurfactants seem to be effective for larvicidal application in the control of disease vectors, either by direct interaction with larvae cells or by acting on the surface tension of aquatic environments, necessary for the control of humidity and gas exchange during larval development [31, 32]. Due to the great diversity of biosurfactants produced by fungi, whose structures vary according to the carbon source, there is still great unexplored potential [33]. Although the work on surfactants generated by fungi is still an ongoing field of investigation, future studies in this area can contribute to possible pesticide alternatives to commercially available synthetic insecticides as a safer and more viable ecotoxicological option that might also be used in integrated insecticide resistance management strategies [23, 28, 32].

12.3 Fungal Biosurfactants in Bioremediation Processes

In recent decades the rapid development of agriculture and industries has resulted in the contamination of the environment by several recalcitrant pollutants, including heavy metals, polychlorinated biphenyls (PCBs), plastics, and some agrochemicals [34]. In addition, human activities such as mining, final disposal of effluents containing toxic metals and metal chelates from steel mills, battery industries, and thermoelectric plants have resulted in water quality deterioration, generating serious environmental problems [34]. Although there are some physicochemical techniques for removing contamination, such as by oils (chemical method), excavation and dumping, separation, stabilization, and heat treatment, these methods are expensive, are not ecologically sound and reduce soil fertility [34, 35]. Considering that the presence of these recalcitrant compounds in the environment is of great concern due to their toxicity and non-biodegradable nature, processes that use biological systems to reduce, eliminate, contain or transform contaminants present in soils, sediments, water and air are of great relevance [34, 36]. To overcome the limitations of physicochemical processes, bioremediation can be a promising, less costly, environmentally friendly, and more reliable technology for the removal of environmental pollutants [34, 35]. The bioremediation process involves the production of energy in a redox reaction within microbial cells, including respiration and other biological functions necessary for the maintenance and multiplication of the cells, and a delivery system is usually required that provides one or more of the following items: an energy source (electron donor), an electron acceptor and nutrients [36].

Among the different bioremediation processes, biosurfactants have been used in the remediation of oil pollution because they have advantages such as biodegradability and low toxicity; in addition, the way these hydrocarbons are degraded by microorganisms reduces damage to the ecosystem [37]. Microorganisms that degrade hydrocarbons typically produce a variety of extracellular biosurfactants, which emulsify compounds, increase water solubility, and make compounds more accessible to these microorganisms [37, 38].

Microbial communities have been shown to function well for bioremediation, considering that most indigenous microorganisms have the ability to successfully carry out environmental restoration through immobilization or chemical transformation of contaminants [39, 40]. Different microorganisms use contaminants as carbon sources for their growth and reproduction, carrying out their decomposition and transforming them into simpler, less toxic compounds [41]. Fungi are saprobic microorganisms reported for their high tolerance to toxic environments, and their hyphae have an additive advantage over unicellular microorganisms, because a complex matrix is formed around them where different enzymes capable of catalyzing the degradation of substrates are formed [40, 42].

Some species of fungi play important roles in the bioremediation of contaminants such as persistent organic pollutants, textile dyes, charcoal, pharmaceuticals and personal care products, polycyclic aromatic hydrocarbons, and pesticides [41, 43, 44]. Different studies report the use of fungi from different groups – including *Aspergillus*, *Penicillium* and alkalophilic fungi for bioremediation of textile dyes, effluents from the sugar industry, chemicals used in kraft pulp mills, and effluents from leather tanning, indicating the different substrate choices of these fungi [41, 45–47]. Substantial removal of gasoline and diesel contaminants from soil by short-term incubation of *Aspergillus niger* and *Phanerochaete*

chrysosporium with petroleum hydrocarbons has been shown in conjunction with elimination of total organic carbon which aids in the bioremediation process [41, 48–50].

The term "environmental friendliness" combined with the ability to solubilize hydrophobic compounds may explain why biosurfactants have been recognized as excellent agents to improve the bioremediation of pollutant-contaminated environments [51, 52]. First, biosurfactants tend to interact with poorly soluble contaminants and improve their transfer to the aqueous phase, which allows the mobilization of recalcitrant pollutants incorporated into the soil matrix and their subsequent removal [52, 53]. The presence of biosurfactants can also lead to a potential increase in the efficiency of biodegradation, since these molecules act as mediators capable of increasing the mass transfer rate, making hydrophobic pollutants more bioavailable to microorganisms [52, 54, 55]. Alternatively, biosurfactants can also induce changes in cell membrane properties, resulting in greater microbial adhesion, an important mechanism when two immiscible phases (oil and water) are present and direct substrate absorption is plausible [52, 56, 57]. Another notable environmental application of biosurfactants is based on their ability to complex heavy metal ions, which can improve their removal or extraction through biological treatment [52, 58, 59].

Regarding fungi, more than 100 genera are known to play complex roles in the biodegradation of hydrocarbons [60], and fungi belonging to the *Cladosporium* and *Aspergillus* genera are among those known to participate in the degradation of aliphatic hydrocarbons, while representatives of *Cunninghamella*, *Penicillium*, *Fusarium*, *Aspergillus*, and *Mucor* have been shown to participate in the degradation of more recalcitrant aromatic hydrocarbons [61, 62]. Although the pathways of hydrocarbon degradation by bacteria have been well studied, knowledge of the enzymatic mechanisms and genetic pathways associated with hydrocarbon degradation in fungi is much more limited [62, 63].

Some fungi may also play a critical and complementary role in facilitating the bioavailability of hydrocarbons to other microbial communities (as for other fungi and/or bacteria) by synthesizing biosurfactants [62]. This hypothesis is supported by culture-based studies that detected increased degradation of polyaromatic hydrocarbons when different fungi were added [62, 64–66]. Biosurfactant-producing fungi belong mainly to the genera *Candida*, *Pseudozyma*, and *Rhodotorula* for yeasts and *Cunninghamella*, *Fusarium*, *Phoma*, *Cladophialophora*, *Exophiala*, *Aspergillus*, and *Penicillium* for filamentous fungi [16, 62, 67–70].

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3.3.Diversity, distribution and ecology of fungal communities present in Antarctic lake sediments uncovered by DNA metabarcoding

Nesse artigo avaliamos a diversidade fúngica em sedimentos obtidos de quatro lagos nas Ilhas Shetland do Sul e na Ilha James Ross, Antártica, utilizando a técnica de DNA *metabarcoding*. Detectamos 218 sequências de *amplicons* variantes (ASVs) dominadas pelos filos *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota* e *Chytridiomycota*. Além disso, foram detectados os filos raros/crípticos *Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota*, *Monoblepharomycota*, *Rozellomycota* e *Zoopagomycota*, bem como Straminopila, semelhante a um fungo, pertencente aos filos *Bacillariophyta* e *Oomycota*. As assembleias fúngicas foram dominadas por táxons fúngicos desconhecidos (Fungal sp. 1 e Fungal sp. 2), seguidos por *Talaromyces rubicundus* e *Dactylonectria anthuriicola*. Em geral, apresentaram alta diversidade, riqueza e dominância moderada. Sequências representando fungos saprofíticos, patogênicos e simbióticos foram detectadas, incluindo o fungo fitopatogênico *D. anthuriicola*, abundante no relativamente jovem Lago Soto, na Ilha Deception. Os sedimentos lacustres estudados continham o DNA de comunidades fúngicas ricas, diversas e complexas, incluindo fungos comumente relatados na Antártida e outros táxons considerados raros. No entanto, como o estudo se baseou no uso de DNA ambiental, o que não confirma a presença de organismos ativos ou viáveis, estudos adicionais utilizando outras abordagens, como o sequenciamento *shotgun*, são necessários para elucidar a ecologia dos fungos nesses sedimentos lacustres antárticos.

O material suplementar deste artigo encontra-se no Anexo 7.1.

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Diversity, distribution and ecology of fungal communities present in Antarctic lake sediments uncovered by DNA metabarcoding

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We assessed fungal diversity in sediments obtained from four lakes in the South Shetland Islands and James Ross Island, Antarctica, using DNA metabarcoding. We detected 218 amplicon sequence variants (ASVs) dominated by the phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota* and *Chytridiomycota*. In addition, the rare phyla *Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota*, *Monoblepharomycota*, *Rozellomycota* and *Zoopagomycota* as well as fungal-like Straminopila belonging to the phyla *Bacillariophyta* and *Oomycota* were detected. The fungal assemblages were dominated by unknown fungal taxa (Fungal sp. 1 and Fungal sp. 2), followed by *Talaromyces rubicundus* and *Dactylonectria anthuriicola*. In general, they displayed high diversity, richness and moderate dominance. Sequences representing saprophytic, pathogenic and symbiotic fungi were detected, including the phytopathogenic fungus *D. anthuriicola* that was abundant, in the relatively young Soto Lake on Deception Island. The lake sediments studied contained the DNA of rich, diverse and complex fungal communities, including both fungi commonly reported in Antarctica and other taxa considered to be rare. However, as the study was based on the use of environmental DNA, which does not unequivocally confirm the presence of active or viable organisms, further studies using other approaches such as shotgun sequencing are required to elucidate the ecology of fungi in these Antarctic lake sediments.

Antarctica represents one of the most extreme regions of the planet, including polyextremophilic environments and habitats that combine cold, dry and ultra-oligotrophic conditions, and offers unique opportunities to discover and study extremophilic organisms[1]. The large majority of Antarctica (>99.6% of its area) is permanently covered by ice and snow. Nevertheless, terrestrial and freshwater ecosystems are present that provide habitats occupied by organisms living in some of the most extreme conditions on the planet[2]. Antarctic lakes offer multiple extreme environmental challenges, including low temperatures, high salinity, pH variation, seasonally high UV radiation and low nutrient availability[1]. Various types of lakes are present, of different size, depth, trophic status and age, whose ecosystems are dominated by microorganisms adapted to the extreme conditions[3].

Lakes present in the maritime Antarctic are typically shallow systems (<10 m deep), transparent allowing penetration of high levels of light and UV radiation, with low temperatures and low available nutrients[4]. However, the complex geology of the different islands in maritime Antarctica results in variable lake chemistry, which may favor the dominance of different microorganisms able to survive in these ecosystems and play a major role in the transfer of inorganic and organic material and energy[5]. The freshwater aquatic zones of Antarctica, including lakes, are in direct or indirect contact with air, rocks, soil and animals, and are bordered by vegetation;

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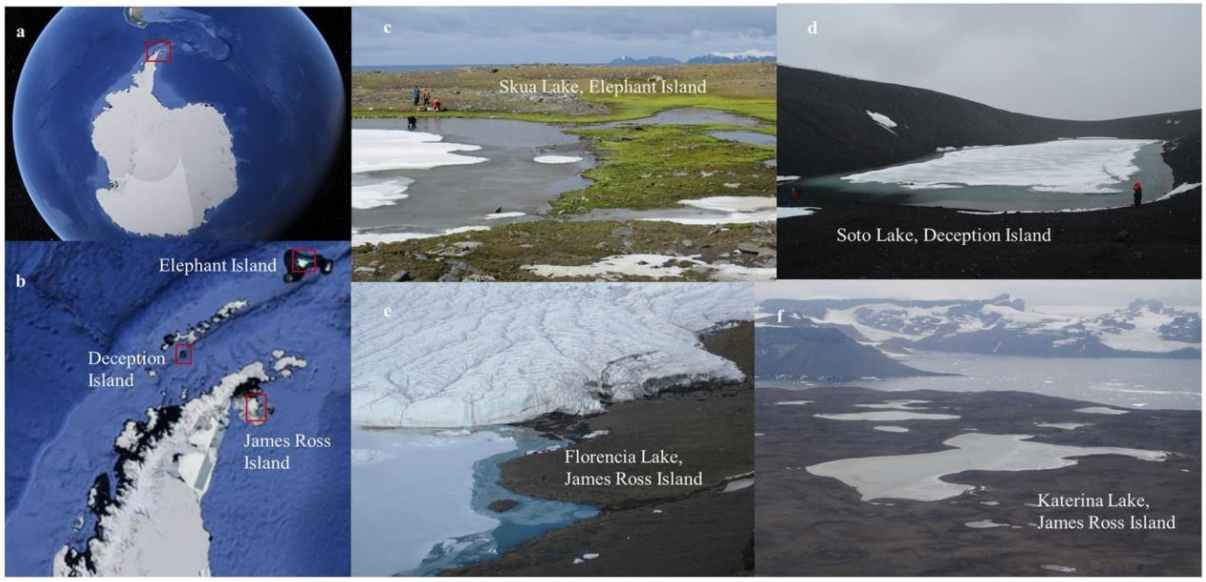

Figure 1. Satellite images (**a**) and (**b**) (obtained in Google Earth Pro, 2019). (**a**) Antarctica with the Antarctic Peninsula inside the red rectangle, (**b**) Elephant, Deception and James Ross Islands inside the red rectangle, (**c**) Skua Lake, Elephant Island (61°13′18.2″S; 55°21′54.3″W), (**d**) Soto Lake, Deception Island (62°58′44.6" S; 60°33′20.2" W), (**e**) Florencia (64°01′27.8" S; 57°40′00.6" W) and (**f**) Katerina Lakes (64°01′18.6" S; 57°43′26.2" W), James Ross Island. Photos (**c**) and (**e**) taken by Juan M. Lirio, photo (**f**) taken by Matej Roman and photo (**d**) taken by Luiz H. Rosa.

consequently, Antarctic lakes represent important sites for the study of microbial diversity and ecology[6]. Antarctic lake sediments can provide regional climatic archives and also shelter unique microbial communities, including bacteria, cyanobacteria, viruses, protists and fungi[7,8].

Various Antarctic fungal communities have been characterized, including those in Antarctic lakes, which face various extreme conditions including low temperatures, high salinity, pH variation, seasonally high UV radiation and low nutrient availability[1]. To date, most fungal diversity studies in Antarctic lakes have applied traditional culturing methods[1,3,8–11], which do not reveal the full diversity of the resident mycobiota. The recent application of approaches using metabarcoding have highlighted that fungal diversity in Antarctic lakes can potentially be considerably greater than previously appreciated[13–15].

With this background, we hypothesized that metabarcoding approaches using high throughput sequencing (HTS) may detected cryptic fungal assemblages present in sediments of Antarctic lakes and contribute to better understanding of the complex resident fungal ecological networks involving saprophytic, mutualistic and parasitic taxa living under different extreme environmental conditions. We studied fungal richness, diversity and ecology in sediments sampled from four Antarctic lakes in the South Shetland Islands, north-west of the Antarctic Peninsula and James Ross Island to the east of the tip of the Antarctic Peninsula. We used a metabarcoding approach to assess and characterize fungal DNA sequence diversity present in sediment samples obtained from these four lakes.

Methods

Study sites and sediment sampling. The four Antarctic lakes sampled were Skua Lake (Elephant Island), Soto Lake (Deception Island), both in the South Shetland Islands, and Florencia Lake and Katerina Lake (James Ross Island) (Fig. 1). Skua Lake is the northern-most of these lakes. It is an open basin, up to 1 m deep, fed by glacial meltwater from the Elephant Island Ice Cap. The lake occupies a depression on metamorphic rocks formed by glacial erosion. Soto Lake is a shallow crater depression formed by a phreatomagmatic eruption (maar). Its precise depth has not been measured, but is likely to be several meters. The lake is in a closed basin, fed by precipitation and its basement is formed by volcanic rock of basaltic composition. Florencia Lake is located on a sector of Clearwater Mesa known as Foreland on James Ross Island. It is an open basin, more than 10 m deep, directly abutting Blancmange Glacier. Its basement is formed by basal till deposits composed mainly of volcanic hyaloclastite breccias. Katerina Lake is located on top of Clearwater Mesa, lying on a relatively flat surface formed by a basaltic lava flow. It is an open basin, up to 1 m depth, and is fed by snowmelt. Each of these lakes displays different geological characteristics that may influence the resident mycobiota.

Sediments were collected from Skua Lake and Soto Lake during the austral summer of 2016/17 and from Katerina Lake and Florencia Lake in the austral summer of 2020/21 (Fig. 1; Table 1). Samples from all lakes were collected in triplicate using PVC pipes (60 mm diameter × 50 mm length), disinfected to avoid contamination as described by Ogaki et al.[8]. Sediments were sampled from three different points (each separated by approximately 50 m) in the littoral region of each lake at a depth of 20–50 cm. Approximately 500 g of each sediment sample

Lake geological characteristics	Lake			
	Skua	Soto	Katerina	Florencia
Location	61° 13′ 18.2" S 55° 21′ 54.3" W	62° 59′ 05.4" S 60° 39′18.0" W	64° 01′ 25.5" S 57° 43′ 03.6" W	64° 01′ 24.0" S 57° 40′ 03.1" W
Altitude (meters above sea level)	59	13	250	25
Total area (m^2)	6009	18,190	126,951	132,000
Perimeter (m)	400	630	2085	1854
Distance to coastline (m)	500	220	1,337	1,429
Depth (m)	0.4	>3	1	>10
Lake shape	Elongate (E–W)	Rectangular	Irregular	Circular
Sediment chemical parameters				
pH in H_2O	6.1	8.8	8.4	8.5
Exchangeable P—mg dm^{3-1}	218.4	19.5	120.5	9.3
Sum of exchangeable bases Ca + K + Mg (SB)—$cmol_c/dm^3$	0.73	3.51	5.53	7.59
Percentage of base saturation (PBS)—%	35.6	78.0	94.4	79.3
H + Al–potential acidity—$cmol_c$ dm^{3-1}	1.32	0.99	0.33	1.98
Cation exchange capacity at pH 7 (CEC)—$cmol_c$ dm^{3-1}	2.05	4.50	5.86	9.57
Total organic carbon (TOC)—dag/kg	0.07	0.08	0.54	0.38
Micronutrient Fe—mg dm^{3-1}	84.5	231.2	252.2	44.0
Micronutrient Mn–mg dm^{3-1}	2.3	11.1	54.2	63.2
Resident fungal diversity indices				
Number of DNA reads	33,429	71,983	54,939	46,813
Number of taxa	50	31	51	171
Fisher's-α (diversity)	21.40	10.27	22.1	559.7
Margalef (richness)	9.25	5.66	9.44	32.09
Simpson's (dominance)	0.76	0.78	0.79	0.92

Table 1. Lake locations, characteristics, sediment physicochemical data and diversity indices of fungal assemblages obtained from Skua Lake (Elephant Island), Soto Lake (Deception Island), Katerina Lake and Florencia Lake (James Ross Island).

was immediately sub-sampled, sealed, placed in sterile Whirl-pack bags (Nasco, Ft. Atkinson, WI) and frozen at −20 °C until processing in the laboratory at the Federal University of Minas Gerais, Brazil. There, the sampled was gradually thawed at 4 °C for 24 h before carrying out DNA extraction. In all DNA extraction steps, we proceeded under strict control conditions within a laminar flow hood to recover the fungal DNA and avoid contaminations.

Sediment chemical analysis. Sediment chemical analyses were performed following Embrapa[16]. pH was determined using a 1:2.5 sediment:deionized water ratio. Potential acidity (H + Al) was extracted with 0.5 mol L^{-1} $Ca(OAc)_2$ buffered to pH 7.0 and quantified by titration with 0.0606 mol L^{-1} NaOH. Exchangeable Ca^{2+}, Mg^{2+} and Al^{3+} were extracted with 1 mol L^{-1} KCl, and K^+ and P^+ were extracted with Melich[16]. The element levels in the extracts were determined by ICP (Al^{3+}), flame emission (Na^+, K^+) and photocolorimetry (P) by the ascorbic acid method. Total organic carbon (TOC) was quantified by wet oxidation using the Walkley–Black method. All analyses were performed in triplicate. Total cation exchange capacity (CEC) was calculated as the sum of the bases (Ca^{2+}, Mg^{2+}, K^+, Al^{3+}) and potential acidity ($H^+ + Al^{3+}$). Chemical analyses of all sediment samples were performed in triplicate.

DNA extraction, Illumina library construction and sequencing. Three replicate sub-samples were taken from the center of each core section under strict contamination control conditions. Total DNA was extracted from these using the FastDNA Spin Kit for Soil (MPBIO, Ohio, USA), following the manufacturer's instructions. DNA quality was analyzed by agarose gel electrophoresis (1% agarose in 1 × Trisborate-EDTA) and then quantified using the Quanti-iT™ Pico Green dsDNA Assay (Invitrogen). The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification of Fungi[17,18] using the universal primers ITS3 and ITS4[19]. Library construction and DNA amplification were performed using the Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2, following Illumina 16S Metagenomic Sequencing Library Preparation protocol (Part #15044223, Rev. B). Paired-end sequencing (2 × 300 bp) was performed on a MiSeq platform (Illumina) by Macrogen Inc. (South Korea). All quality control to avoid contamination of DNA extraction, PCR, sequencing were carried out and analyzed by Macrogen Inc.

Data analysis and fungal identification. Quality analysis was carried out using BBDuk v. 38.87 in BBmap software[20] 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 = rl; trimq = 20; ftm = 5;

maq = 20. The remaining sequences were imported to QIIME2 version 2021.4 (https://qiime2.org/) for bioinformatics analyses[21]. The qiime2-dada2 plugin was used for filtering, dereplication, turn paired-end fastq files into merged, and remove chimeras, using default parameters[22]. Taxonomic assignments were determined for amplicon sequence variants (ASVs) in three steps. First, ASVs were classified using the qiime2-feature-classifier[23] classify-sklearn against the UNITE Eukaryotes ITS database version 8.3[24]. Second, remaining unclassified ASVs were filtered and aligned against the filtered NCBI non-redundant nucleotide sequences (nt) database (October 2021) using BLASTn[25] with default parameters; the nt database was filtered using the following keywords: "ITS1", "ITS2", "Internal transcribed spacer", and "internal transcribed spacer". Third, output files from BLASTn[25] were imported to MEGAN6[26] and taxonomic assignments were performed using the "megan-nucl-Jan2021.db" mapping file with default parameters and trained with Naive Bayes classifier and a confidence threshold of 98.5%. Taxonomic profiles were plotted using the Krona[27]. The heatmaps of ASV abundance and clustering analysis were created using Heatmapper[28]; clustering analysis was performed using the following parameters: Average Linkage, Spearman Rank Correlation and Z-score among samples for each ASV.

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 classification followed Kirk et al.[34], Tedersoo et al.[35], MycoBank (http://www.mycobank.org) and the Index Fungorum (http://www.indexfungorum.org).

Fungal diversity and ecology. The relative abundances of the ASVs were used to quantify the fungal taxa present in the total sediments sampled, where fungal ASVs with relative abundance > 10% were considered dominant, those between 1 and 10% as intermediate and those with < 1% as minor (rare) components of the fungal community[36]. The numbers of reads were used to quantify taxon diversity, richness and dominance, using the following indices: (i) Fisher's α, (ii) Margalef's and (iii) Simpson's, respectively. In addition, species accumulation curves were obtained using the Mao Tao index. All results were obtained with 95% confidence, and bootstrap values were calculated from 1000 replicates using the PAST computer program 1.90[37]. Functional assignments of fungal ASVs at species and generic levels were prepared using FunGuild[38], which can be accessed at http://www.funguild.org/.

Results

Fungal taxonomy. A total of 207,164 DNA reads were detected in the sediments from the four lakes, representing 218 ASVs (see Supplementary Table S1 online). The Mao Tao rarefaction curves of the fungal assemblages detected in all four lake sediments are shown in Supplementary Fig. S1 online. The curves did not reach asymptote, indicating that further fungal sequence diversity is likely to be present in these sediments. Supplementary Figs. S2 and S3 show the abundances of fungal and allied organism ASVs at different hierarchical levels, which varied across the four lakes. Unknown fungi dominated the sequence assemblages, followed by the phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota* and *Chytridiomycota*. The lake sediment assemblages were dominated by Fungal sp. 1 (Skua, Katerina and Florencia Lakes), *Talaromyces rubicundus* (Soto and Katerina Lakes) and *Dactylonectria anthuriicola* (Soto Lake), in rank order, which displayed the highest relative abundances. However, richness (215 ASVs) of the fungal assemblages detected primarily comprised taxa of intermediate and rare relative abundance, including those of fungal phyla rarely (*Rozellomycota*, *Zoopagomycota*) or unreported (*Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota*, *Monoblepharomycota*) from Antarctica as well as members of the allied kingdom Straminopila (phylum *Oomycota*), which have also been infrequently detected in Antarctica in studies using traditional isolation methods. Ninety-five ASVs (47.57% of the total detected) could only be assigned to higher taxonomic levels (phylum, class, order or family) and may represent taxa not currently included in the available sequence databases or be new Antarctic records and/or previously undescribed taxa.

Influence of physical and chemical properties of lacustrine sediments on the contained fungal communities. The fungal assemblages present in the lake sediments showed variation in their ecological diversity indices but, in general, despite the extreme physicochemical characteristics, they displayed high diversity and richness indices and moderate dominance (Table 1). The sediment fungal assemblage of Florencia Lake displayed the highest number of taxa and diversity indices, followed by those from Katerina, Skua and Soto Lakes.

The four lakes had sediments with similar chemical attributes, with low values for most of the elements. The sediment of Skua Lake differed from the other lakes in having slightly higher acidity (lower pH value and higher H + Al), higher exchangeable P content, and very low values for sum of bases and CEC. It was the only lake with dystrophic sediments (PBS < 50%). In contrast, sediments from Katerina Lake and Florencia Lake had the largest nutrient reserve in the form of exchangeable elements, mainly Ca, K and Mg, with the highest values of base saturation and CEC observed. The total organic carbon content was very low in sediments from all four lakes (< 1 dag kg^{-1}). Of the total fungal ASVs detected, eight were present in all four lake sediments (Fig. 2; Supplementary Table S2). Taxa distribution varied across the four lakes, with sediments in each lake hosting some specific fungal taxa, in particular Florencia Lake with 116 fungal ASVs. The most abundant fungal ASV overall (Fungal sp. 1) was present in all four lake sediments. Beyond the eight cosmopolitan fungal ASVs that were shared amongst all lakes, no other ASVs were shared among Skua Lake (Elephant Island) and Soto Lake (Deception Island). Although both Katerina Lake and Florencia Lake are in relatively close proximity on Clearwater Mesa, James Ross Island (see Supplementary Fig. S4 online), the fungal assemblages detected in each (51 and 171 ASVs,

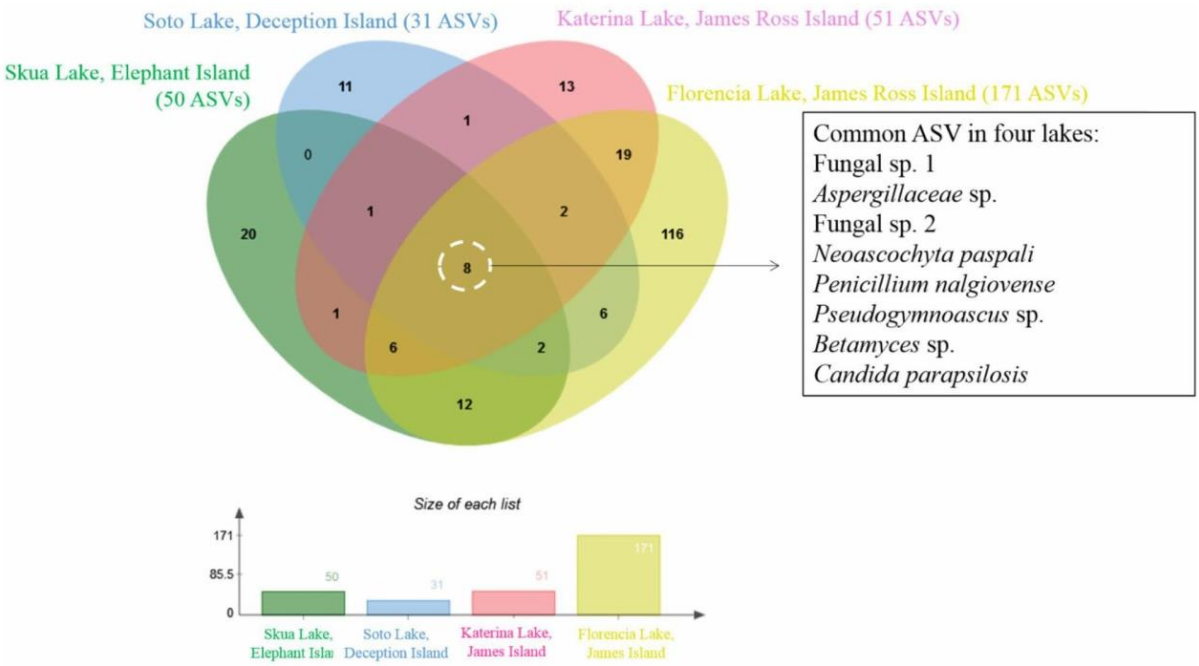

Figure 2. Venn diagram showing the distribution of fungal amplicon sequence variants (ASVs) among the sediment samples obtained from Skua Lake (Elephant Island), Soto Lake (Deception Island), Katerina Lake and Florencia Lake (James Ross Island).

respectively) shared only 19 fungal taxa and displayed distinct ecological diversity indices. Functional ecology assignments of the ASVs detected at generic level are shown in Supplementary Table S3 online, and indicated that the fungal communities present in the sediments of all four lakes were dominated by saprotrophic, plant and animal pathogenic and symbiotic taxa.

Discussion

Taxonomy and occurrence. The availability of studies of fungal diversity in Antarctic lake sediments has increased in recent years, but still remains limited[8,12]. Most previous studies of Antarctic lake fungal communities have used traditional culture-dependent methods[1,3,5,8,39]. However, recent applications of metabarcoding approaches have detected the presence of DNA sequences of different fungal taxa in Antarctic lake sediments[14,15]. The current study similarly confirmed the presence of high fungal sequence richness and diversity in sediments from four lakes sampled in the South Shetland Islands and James Ross Island. The study detected both the commonly encountered phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota*, *Chytridiomycota*, *Rozellomycota* and *Zoopagomycota* (cf. reports of da Silva et al.[40], Ogaki et al.[14] and Rosa et al.[15]), and the more rarely detected or unreported *Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota* and *Monoblepharomycota*, as well as members of the fungal-like kingdom Straminopila in the phylum *Oomycota*.

ASVs assigned to Fungal sp. 1, *T. rubicundus* and *D. anthuriicola* were the most abundant fungal taxa detected in the lake sediments. The most abundant ASV was Fungal sp. 1 (24.757%), suggesting a dominance of possibly undescribed fungi present in Antarctic lake sediment ecosystems. This unidentified dominant ASV provides an example of a sequence not currently included in the available sequence databases used in our study. *Talaromyces*, the teleomorphic form of *Penicillium*[41], includes species pathogenic to humans, but also producers of antibiotics and enzymes potentially useful in biotechnological processes[42]. *Talaromyces rubicundus* has been reported to produce various extrolites such as islandicin chromophore, mitorubrin, mitorubrinic acid and rubratoxins[42]. In Antarctica, representatives of *Talaromyces* and *Penicillium* have been detected in different habitats and substrates including soil, marine and lake sediments, freshwater and seawater, plants, macroalgae, rocks, snow, ice[43] and air[36]. *Talaromyces rubicundus* (anamorphic form of *Penicillium rubicundum*) was originally isolated from cultivated soil in Georgia, USA, and displays mesophilic characteristics and an optimal growth temperature of 25°C[44]. The genus *Dactylonectria* includes 14 species, some of which are recognized as soil-borne plant pathogens[45]. *Dactylonectria anthuriicola* (synonym of *Ilyonectria anthoriicola*) was isolated from the roots of *Anthurium* sp. in the Netherlands[46]. Recently, Parkinson et al.[47] reported that *D. anthuriicola* caused significant root rot on avocado trees in Australia. Our study represents the first records of both *T. rubicundus* and *D. anthuriicola* in Antarctica.

Ninety-five ASVs, representing almost the half of the total community, were identified at phylum, class, order or family levels. These fungi may represent taxa not currently included in the available sequence databases or be undescribed taxa. However, as our study focused on fungal diversity assigned from environmental DNA samples, further specific taxonomic studies are required to elucidate if the apparently high richness of undescribed taxa does indeed represent previously unrecognized fungal species present in Antarctica.

Diversity and ecology. Our fungal sequence diversity data can be compared with studies using both traditional culturing methods and the increasing number of recent metabarcoding studies of Antarctic lakes (see Supplementary Table S5 online). The diversity indices of fungal sequence assemblages detected in the sediments of the four lakes studied here were greater than those reported in culture-based studies but comparable with those using metabarcoding. Ogaki et al.[12] used culture-dependent methods to characterize the same sediments from Skua and Soto Lakes, identifying only 13 and 6 fungal taxa, respectively, mostly at genus and species levels, contrasting with our data obtained using metabarcoding.

The four lakes also differed in their diversity indices, with Soto Lake and Florencia Lake displaying the lowest and highest diversity indices, respectively. No significant correlation was observed between the diversity indices and the sediment physicochemical parameters of the lakes. Considerable differences in the perimeters and depths of these two lakes may be correlated with their sediment accumulation and, hence, DNA deposition into the sediments, underlying the different levels of diversity detected.

Soto Lake is not surrounded by a well-developed community of mosses or lichens and is characterized by low nutrient availability. It represents an ecosystem still in formation and displayed the lowest diversity indices. Skua Lake showed intermediate diversity indices, which may be due to the rich terrestrial vegetation and presence of nesting skuas and giant petrels in its surroundings. Florencia Lake showed the highest diversity and richness indices, which were strongly divergent with those in Katerina Lake, despite their close proximity (separated by approximately 2050 m) (see Supplementary Fig. S4 online). However, these two lakes have geological differences and are surrounded by extensive moss and lichen communities. Florencia Lake has the greatest water inflow of the four studied lakes, being fed mainly by meltwater that carries significant quantities of clastic sediment, as well as receiving water from other lacustrine basin lakes with which it is connected. These are fed by snowmelt and are surrounded by a rich flora of mosses and microbial mats that provide organic matter to the lake. The local gradients in the Florencia Basin also encourage fluvial erosion of the moss carpets and the transport of fragments towards Florencia Lake. In contrast, Katerina Lake is fed by snowmelt and linked with other small lakes also fed by snowmelt through small ephemeral streams with low transport capacity, although it is also surrounded by a relatively rich terrestrial flora of mosses and lichens. The range of potential sources of organic matter input to Florencia Lake perhaps underlies this lake having much higher fungal diversity indices than Katerina Lake.

The chemical attributes of lake sediments reflect their geological contexts and the biological communities in their catchments. These attributes and the low TOC values suggest a mainly mineral composition of the sediments in the studied lakes. The lakes associated with basic igneous rocks, of basaltic composition (Soto, Katerina and Florencia) had higher SB values and other indices (PBS, CEC). These rocks contain minerals rich in Ca, K and Mg, which form a major constituent of the sediments in these lakes. Geochemical studies (personal communication Dr. Siliva Coria—Instituto Antártico Argentino) of Florencia Lake sediments have identified minerals such as plagioclase, potassium feldspar, piroxenes, zeolites and forsterite, that are rich in Ca, K, Na, Fe and Mg.

In contrast, Skua Lake is located in an area of metamorphic rocks that have greater content of aluminosilicate minerals, such as mica, quartz and garnet, with lower base content. Metamorphic rocks are commonly associated with more acidic and less fertile soils than rocks of basic composition[48]. Skua Lake is surrounded by rich vegetation and receives input of excrement from surrounding breeding birds, which leads to higher values of exchangeable P[49]. The sediment samples examined in this study were collected in the littoral regions of each lake at 20–50 cm depth, meaning that there could be a mix of present-day and past fungal communities dating from years to centuries ago contained in the sediments.

The fungal genera detected in the sediments display different ecological roles including saprophytes, mutualists, symbionts and parasites, as more generally reported for Antarctic fungi[43]. Among the genera with known ecological roles, saprophytes dominated the fungal communities present in all four lake sediments, followed by plant and animal pathogens and symbionts. Studies that have addressed the functional ecological roles of fungi in different Antarctic environments and habitats report saprophytes as the dominant functional group, followed by plant and animal pathogens and symbionts. The same functional ecological profile detected here has also been reported in studies sampling fungi in the air[36,50], soil[51], fresh water[13] and rocks[52] in Antarctica. Similar functional profiles were reported in metabarcoding studies of lake sediments reported by Ogaki et al.[14] from Vega Island (located close to James Ross Island) and Rosa et al.[15] in Hope Bay at the north-eastern tip of the Antarctic Peninsula. However, in sediment samples obtained from Soto Lake (Deception Island), the recognized phytopathogenic species *D. anthuriicola* dominated the sequence assemblage, although the species has not previously been recorded in Antarctica. Fungi inhabiting polar environments commonly display the capability to degrade organic matter at low temperature and release compounds containing carbon, nitrogen and other elements to other organisms[53], suggesting that the saprophytic fungi detected in the four lake sediments studied here might host a complex fungal community that plays a vital role in the decomposition of organic matter under extreme conditions.

Conclusions

Application of a metabarcoding approach revealed that sediments from the four lakes studied contained DNA potentially representing rich, diverse and complex fungal communities. These included both known fungi commonly reported in Antarctica (members of the phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota* and *Mucoromycota*), as well as others considered rare or not previously reported in Antarctica (*Chytridiomycota*, *Rozellomycota*, *Zoopagomycota*, *Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota*, *Monoblepharomycota*), and the presence of the fungal-like Straminopila. Antarctic lake sediments accumulate over long periods of time, and our data indicate that they may be considered a hotspot of fungal diversity, potentially including new and/or previously unreported species. The dominance of sequences of a small number of saprophytic and phytopathogenic

taxa was notable. This metabarcoding study was based on the use of environmental DNA and further studies for instance using shotgun sequencing are now required to elucidate the ecology of Antarctic lake sediment fungi.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

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Author contributions

L.M.D.S., L.H.R., J.M.L., S.H.C. and P.E.A.S.C. conceived the study. L.M.D.S. and L.H.R. performed DNA extraction from lake sediments. F.A.C.L. performed the metabarcoding analysis. F.S.O. performed the sediment physicochemical analysis. L.M.D.S., L.H.R., J.M.L., S.H.C., P.E.A.S.C., F.A.C.L., P.C., F.S.O., M.C.S. and C.A.R. analyzed the results and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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3.4.Diversity of culturable fungi in Antarctic lakes and their potential for producing compounds of biotechnological interest

Neste artigo avaliamos a diversidade e o potencial biotecnológico de fungos cultiváveis de sedimentos dos lagos Florencia e Katerina, Ilha James Ross, Antártica Marítima. Um total de 24 táxons foi identificado: 16 do lago Florencia e oito do lago Katerina. O filo dominante foi *Ascomycota*, seguido por *Mortierellomycota* e *Basidiomycota*. Os principais gêneros incluíram *Cladosporium*, *Dactylaria*, *Glaciozyma*, *Graphium*, *Leucosporidium*, *Mortierella*, *Penicillium*, *Pseudeurotium*, *Pseudogymnoascus*, *Tetracladium* e *Thelebolus*. *Pseudogymnoascus* sp. 1 e espécies de *Thelebolus* foram os mais frequentes. O lago Florencia apresentou maior riqueza taxonômica e diversidade em comparação ao lago Katerina. Dos táxons identificados, 12 foram exclusivos de Florencia, quatro de Katerina e quatro foram compartilhados. Cinquenta e sete isolados foram avaliados quanto à produção de 11 enzimas de relevância industrial; as enzimas mais comuns foram inulinase, protease, invertase, e as menos comuns foram gelatinase e pectinase. Oito isolados (*Pseudogymnoascus* e *Thelebolus*) produziram biossurfactantes, e 50 apresentaram corpos lipídicos intracelulares. Um isolado de *Penicillium palitans* inibiu completamente a germinação de sementes de Allium schoenoprasum, e análises de RMN confirmaram o (−)-palitantina como o composto ativo. Esses resultados confirmam que sedimentos de lagos antárticos abrigam fungos diversos com potencial para produção de enzimas, biossurfactantes, lipídios e metabólitos bioativos, reforçando o valor do estudo de fungos extremofílicos como fonte de bioprodutos no contexto de ecossistemas frágeis afetados pelas mudanças climáticas.

Diversity of culturable fungi in Antarctic lakes and their potential for producing compounds of biotechnological interest

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Abstract

We evaluated the diversity and biotechnological potential of culturable fungi from sediments of Florencia and Katerina lakes, James Ross Island, maritime Antarctica. A total of 24 taxa were identified: 16 from Florencia and eight from Katerina. *Ascomycota* was the dominant phylum, followed by *Mortierellomycota* and *Basidiomycota*. The main genera included *Cladosporium*, *Dactylaria*, *Glaciozyma*, *Graphium*, *Leucosporidium*, *Mortierella*, *Penicillium*, *Pseudeurotium*, *Pseudogymnoascus*, *Tetracladium*, and *Thelebolus*. *Pseudogymnoascus* sp. 1 and *Thelebolus* species were the most frequent. Florencia Lake showed greater taxonomic richness and diversity than Katerina Lake. Of all taxa, 12 were exclusive to Florencia, four to Katerina, and four were shared. Fifty-seven isolates were screened for the production of 11 industrially relevant enzymes; inulinase was the most common, followed by protease, invertase, gelatinase and pectinase. Eight isolates (*Pseudogymnoascus* and *Thelebolus*) produced biosurfactants and 50 contained intracellular lipid bodies. A *Penicillium palitans* isolate fully inhibited germination of *Allium schoenoprasum* seeds, and NMR analysis confirmed (-)-palitantin as the active compound. These results confirm that Antarctic lake sediments harbor diverse fungi with potential for producing enzymes, biosurfactants, lipids and bioactive metabolites, reinforcing the value of studying extremophilic fungi as a source of bioproducts in the context of fragile ecosystems affected by climate change.

Keywords: Antarctica; enzymes; extremophiles; biosurfactants; lipids; phytotoxins.

Introduction

Antarctica is a polyextremophilic region where organisms are subjected to multiple extreme environmental conditions, such as wide temperature variations, high solar radiation in summer, long periods of darkness in winter, high salinity, desiccation, nutrient scarcity, pH variation and repeated freeze-thaw cycles (Gocheva et al. 2006; Gonçalves et al. 2012). Antarctic microorganisms possess various physiological and biochemical mechanisms that enhance their survival in extreme environmental conditions, resulting from evolutionary adaptation over time (Kappen 1993). The ability to produce psychrophilic enzymes is a widely studied adaptive strategy to low temperatures. The increased activity of these enzymes at low temperatures stems from improved structural flexibility, potentially involving modification of the active site (Buzzini et al. 2012). Enzymes active at low temperatures have found application in the food industry for lactose reduction, improved fruit juice extraction, meat tenderization, fermentation processes, beverage production and cheese manufacturing (Gerday et al. 2000; Hoyoux et al. 2001; Javed and Qazi 2016). They are also utilized in various other industrial contexts, including the textile industry for biopolishing fabrics (Gerday et al. 2000), detergent production, the paper industry, cosmetics production (Javed and Qazi 2016), the pharmaceutical industry (Novak et al. 2013) and in environmental bioremediation (Margesin 2007).

Psychrophilic and psychrotolerant microbes can survive inhospitable conditions by synthesizing various compounds, including biosurfactants, which prevent cellular freezing (de Lemos et al. 2023). Biosurfactants produced by microorganisms are an attractive alternative to conventional, synthetic surfactants and are considered a key alternative technology for development in the 21st century (Santos et al. 2016). Biosurfactants offer various advantages over their synthetic counterparts, including greater biodegradability, lower toxicity, high foaming properties, sustainable production and high stability at extreme pH, temperature and

salinity levels (Khademolhosseini et al. 2019). Fungi produce a greater quantity of biosurfactants compared to bacteria, possibly due to the presence of a rigid cell wall (Bhardwaj et al. 2013).

Saturated and unsaturated fatty acids play an important role in regulating the stress that Antarctic fungi faces at low temperatures, aiding in their development, nutrition and colonization (Weet 2012; de Carvalho and Caramujo 2018; Gallardo et al. 2025). Species capable of accumulating high concentrations of lipids in their cells, such as strains of the genera *Aspergillus, Cunninghamella, Fusarium, Mortierella, Mucor* and *Penicillium*, which are frequently found in Antarctica, are known as oleaginous (Shapaval et al. 2014; Forfang et al. 2017; Athenaki et al. 2018; Zhang et al. 2022; Gallardo et al. 2025; Passoth and Müller 2025) and may provide alternative sources for lipids as an alternative energy source.

The use of natural metabolites has sparked growing interest in the search for new mechanisms of pesticide action, especially given the increasing resistance of agricultural pests to synthetic pesticides currently available (Duke and Dayan 2022). The reduced effectiveness of commercial herbicides is a particularly acute problem in the absence of new herbicides with novel mechanisms of action. No herbicide with a new mode of action has been introduced to the market in the last 40 years, making the search for new herbicides particularly urgent (Duke and Dayan 2022). Secondary metabolites produced by extremophilic fungi from Antarctica, which can contribute to unique metabolic pathways enabling survival under extreme conditions, can provide alternative sources of novel bioactive molecules (Rosa et al. 2019) with potential agricultural applications (Gomes et al. 2018; da Silva et al. 2024). With this background, this study aimed to characterize the diversity of culturable fungi present in sediments from two maritime Antarctic lakes and evaluate their potential as producers of enzymes, biosurfactants, lipids of industrial interest and herbicide metabolites of agricultural interest.

Materials and Methods

Sample Collection

Sediment samples (three replicates per lake) were obtained from Lake Florencia and Lake Katerina (James Ross Island, north-east Antarctic Peninsula) in February 2020 (Fig. 1), After collection they were stored at -20 °C until processing in the Laboratory of Polar Microbiology and Tropical Connections at UFMG.

Sediment chemical analysis

Sediment chemical analyses were performed following EMBRAPA (2017) procedures. pH was determined using a 1:2.5 sediment:deionized water ratio. Potential acidity (H + Al) was extracted with 0.5 mol L^{-1} $Ca(OAc)_2$ buffered to pH 7.0 and quantified by titration with 0.0606 mol L^{-1} NaOH. Exchangeable Ca^{2+}, Mg^{2+} and Al^{3+} were extracted with 1 mol L^{-1} KCl, and K^{+} and P^{+} were extracted with Melich reagent. The element levels in the extracts were determined by ICP (Al^{3+}), flame emission (Na^{+}, K^{+}) and photocolorimetry (P) by the ascorbic acid method. Total organic carbon (TOC) was quantified by wet oxidation using the Walkley–Black method. Total cation exchange capacity (CEC) was calculated as the sum of the bases (Ca^{2+}, Mg^{2+}, K^{+}, Al^{3+}) and potential acidity (H^{+} + Al^{3+}). All analyses were performed in triplicate.

Isolation of fungi

Lake Florencia is an ice contact lake, at least 10 m deep, fed by ice melt water, and characterized by very low temperature and oligotrophic status with low organic matter. Lake Katerina is up to 1 m deep, located on the top of a basaltic mesa, fed by snow melt, has slightly higher temperatures and considerably more organic matter content than Lake

Florencia. The sediment samples were thawed and kept at a temperature of 4 ºC until processing and isolation of the fungi, for which the following solid culture media (2% agar w/v) were used: 1) Dicloran Rose Bengal Agar (0.5% peptone, 1% glucose, 0.1% KH_2PO_4, 0.05% $MgSO_4$ $7H_2O$, 0.0002% dicloran, 0.0025% rose bengal, 0.01% chloramphenicol); 2) Minimal Medium (0.025% peptone, 0.5% glucose, 0.698% K_2HPO_4, 0.544% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.11% $MgSO_4$ $7H_2O$, 0.01% chloramphenicol); 3) Malt Extract Medium (bacteriological peptone 1 g L^{-1}, malt extract 20 g L^{-1}, glucose 20 g L^{-1} and agar 20 g L^{-1}); and 4) Dicloran Glycerol DG18 Agar (peptic digestion of animal tissue 5 g L^{-1}, dextrose 10 g L^{-1}, monopotassium phosphate 1 g L^{-1}, magnesium sulfate 0.5 g L^{-1}, chloramphenicol 0.1 g L^{-1}, dichloran 0.002 g L^{-1}, glycerol 18 g L^{-1}).

Samples from each lake were processed in triplicate, with 1 g of subsampled sediment resuspended in 1 mL of 0.85% saline solution. From this, 100 μL were plated on four culture media. Plates were incubated at 15 °C for 60 d. Fungal colonies obtained were quantified as colony-forming units, and each isolate was purified in Petri dishes containing Malt Extract solid culture medium. Replicates of the purified fungi were incubated at 15 °C for 7-21 d and preserved. They were then deposited in the Microorganisms and Cells collection of the Institute of Biological Sciences (ICB) of the Federal University of Minas Gerais (UFMG) under the code UFMGCB.

Fungal identification

DNA extraction was carried out as described by Rosa et al. (2009). For filamentous fungi, the internal transcribed spacer (ITS) region was amplified using the universal primers ITS1 and ITS4 (White et al. 1990). Amplification of the ITS region was performed as described by Rosa et al. (2009). In addition, partial amplification of RNA polymerase II (RPB2) was performed using primers RBP2 5F and RPB2 7R as described by Malkus et al. (2006) and

amplification of DNA replication licensing factor (MCM7) using primers MCM7-709for and MCM7-1348rev (Schmitt et al. 2009; Villanueva et al. 2021). Yeasts were grouped and identified following the methods of Lachance et al. (1999). Yeast molecular identities were confirmed by sequencing the D1-D2 variable domains of the large subunit rRNA gene using the primers NL1 and NL4. Fungi with query coverage and identity ≥ 99% were considered to represent the same taxon. Representative consensus sequences of the fungal taxa were deposited in the NCBI GenBank database. To achieve species-rank identification based on ITS, RPB2, NL and MCM7 data, the consensus sequence was aligned with all sequences from related species retrieved from the GenBank database using BLAST. Fungal classification followed Kirk et al. (2011) and the databases MycoBank (http:// www. mycob ank. org) and the Index Fungorum (http://www.indexfungorum.org).

Fungal diversity

To quantify species diversity, richness and dominance, Fisher's α, Margalef's and Simpson's indices were used, respectively. Species accumulation curves were obtained using the Mao Tao index. For the construction of these curves, the density of each taxon obtained was used. All results were obtained with 95% confidence, and bootstrap values were calculated from 1000 replicates using the PAST computer program 1.90 (Hammer et al. 2001). A Venn diagram was prepared as described by Bardou et al. (2014) to illustrate the similarities in fungal community composition obtained from the two lakes.

Enzymatic activity

The filamentous fungi and yeasts obtained were initially grown on malt extract agar (MEA) at 15 °C for 7 and 2 d, respectively. Then, for filamentous fungi, one plug (0.5 mm in diameter) of agar culture medium including fungal growth was transferred to solid culture media

containing the specific substrate for each enzyme. For yeasts, a needle loop from each colony was inoculated directly into the solid medium containing the specific substrate for each enzyme. The isolates were incubated for 7 d in Petri dishes at 15 °C. All assays were performed in duplicate. The presence and activity of cellulase, protease, lipase, agarase, carrageenase, invertase, amylase, esterase, pectinase, inulinase and gelatinase were evaluated as described below. Enzymatic activity was determined as described by Hankin and Anagnostakis (1975), by assessing the relationship between the diameter of the degradation halo and the diameter of the colony, expressed as the enzymatic index (EI), using the formula EI = diameter of the degradation halo/colony diameter. Cellulase: Yeast extract-Malt extract (YM) (1:10) media plus carboxymethylcellulose (5 g L^{-1}) replacing glucose. Petri dishes were flooded with a solution of Congo red (2.5 g L^{-1} in 0.1 M Tris HCl buffer, pH 8) for 15 min, which was then discarded, and the plates were flooded with 1 M NaCl for 15 min, which was also discarded. Positive cellulase activity was defined as a clear halo around the colony on a red background (Martorell et al. 2017). Protease: Sabouraud dextrose agar (65 g L^{-1}) plus skimmed milk (10 g L^{-1}). A positive reaction was detected as a clear halo (produced by casein degradation) around the colony in the opaque medium (Brizzio et al. 2007; Duarte et al. 2013). Lipase: 1 g L^{-1} peptone, 0.5 g L^{-1} yeast extract, 15 g L^{-1} agar, 31.25 mL L^{-1} olive oil as a carbon source and rhodamine B solution 0.01% v/v (10 mL L^{-1}) in absolute ethanol. Positive activity was detected by the presence of an orange fluorescent halo under UV light at 350 nm (interaction of rhodamine B with fatty acids released by the enzymatic hydrolysis of olive oil) (Duarte et al. 2013). Agarase: YM supplemented with 1.5% agar, the enzyme activity being determined by the addition of lugol (Furbino et al. 2018). Carrageenase: YM supplemented with 1.5% carrageenan (Sigma Aldrich, USA). Enzyme activity was determined by the addition of lugol (Furbino et al. 2018). Amylase: starch agar (6.7 g L^{-1} Yeast Nitrogen Base (YNB) (Difco), 2 g L^{-1} soluble starch and 20 g L^{-1} agar). Enzyme

activity was determined by the addition of lugol (Brizzio et al. 2007; Carrasco et al. 2012). For agarase, carrageenase and amylase activities, lugol was used to reveal the enzymatic halo formation. Invertase: YM with 2% sucrose and 0.003% bromocresol green. The appearance of a yellow halo was indicative of positive activity (Troncoso et al. 2017). Esterase: bacteriological peptone (10 g L^{-1}), NaCl (5 g L^{-1}), $CaCl_2$ $2H_2O$ (4 g L^{-1}) and Tween 80 (10 g L^{-1}). Positive activity was indicated by a white precipitate around the colony (Martorell et al. 2017). Pectinase: YM (1:10), pH 7.0, 10 g L^{-1} pectin. The plates were flooded with 10 g L^{-1} of CTAB (cetyltrimethylammonium bromide) and positive activity was indicated by a clear halo (Martorell et al. 2017; Poveda et al. 2018). Inulinase: inulin agar (10 g L^{-1} inulin, 2 g L^{-1} yeast extract, 5 g L^{-1} peptone, 0.5 g L^{-1} $MgSO_4$, NaCl 0.5 g L^{-1}, $CaCl_2$ 0.15 g L^{-1} and 20 g L^{-1} agar (pH 6.0)). Enzyme activity was determined by the addition of lugol (Martinez et al. 2016). Gelatinase: YM was prepared with 160 g L^{-1} of gelatin as a gelling agent in place of agar. Liquefaction of the medium around the colony indicated positive activity (Troncoso et al. 2017).

Biosurfactant production

The emulsifying activity of fungi was assessed using an adaptation of the biosurfactant methodology of Sena et al. (2018). Isolates were pre-cultured in MEA (0.1% peptone and 2% agar, glucose and malt extract) at 15 °C for 7 d. For filamentous fungi, 5 fragments (5 mm) of mycelia were inoculated into 125 mL Erlenmeyer flasks containing 50 mL of sterilized liquid culture medium, prepared with 40 g L^{-1} of sunflower oil (Soya) and 10 g L^{-1} yeast extract (Merck, US). The flasks were maintained under agitation at 100 rpm at 20 °C for 9 d. For yeasts, a pre-inoculum was prepared in which a loop of the colony grown in MEA was transferred to Falcon-type tubes with 10 mL of YM broth (glucose 10 g L^{-1}, peptone 5 g L^{-1}, yeast extract 3 g L^{-1} and malt extract 3 g L^{-1}), which were kept under agitation at 100 rpm, at

20 ºC for 48 h. The solution was then standardized using spectrophotometry (absorbance between 0.4 and 0.5 at a wavelength of 660 nm) and then 0.5 mL (5% of the pre-inoculum in the YM broth) of this solution was transferred to Erlenmeyer flasks containing 50 mL of the same medium with sunflower oil as used for filamentous fungi, under the same incubation conditions. After 9 d, the liquids were centrifuged at 7,197×g for 8 min at 20 °C in 50 mL conical tubes to separate the supernatant from the mycelia, with the supernatant used to determine the Emulsification Index ($EI_{24\%}$) following Cooper and Goldberg (1987). Fifteen mL glass test tubes with lids were filled with an aliquot of 4 mL of supernatant and 6 mL of kerosene solvent (Aciflex), then vortexed at maximum speed (3,000 rpm) for 2 min and left to rest at room temperature for 24 h. Then, the emulsifying capacity of the fungal supernatants was analyzed by calculating the Emulsification Index, by dividing the height of the emulsion layer (Hemulsion) by the total height (Htotal) of the mixture (mm) multiplied by 100 ($EI_{24\%}$ = Hemulsion/ Htotal × 100). Tests were carried out in triplicate, with the final values defined from the average of the triplicate results, with good producers of emulsifying surfactant substances being considered those isolates that exhibited an average index ≥ 50% (Willumsen and Karlson 1996). For this test, 1% SDS (Sodium Dodecyl Sulfate) industrial surfactant (Kasvi, Brazil) was used as a positive control, while the inoculum-free liquid culture medium was used as a negative control.

Intracellular lipid production

Fungal isolates were precultured on malt extract agar (1 g L^{-1} bacteriological peptone, 20 g L^{-1} malt extract, 20 g L^{-1} glucose, and 20 g L^{-1} agar) at 15 °C for 7 d. For filamentous fungi, a 5-mm diameter fragment with mycelial growth was inoculated into 2-mL tubes containing 1 mL of malt extract liquid culture medium (1 g L^{-1} bacteriological peptone, 20 g L^{-1} malt extract, and 20 g L^{-1} glucose) and incubated at 15 °C for 9 d. The resulting content was then ground

using FastPrep-24™ 5G (MP Biomedicals), programmed to operate at 4.0 m s^{-1} for 15 s. For microscopic evaluation of intracellular lipid accumulation, a solution of Nile Red dye (Sigma, USA) was prepared immediately before use. One milligram of dye was dissolved in 10 mL of acetone, and 40 μL of this solution was added to a tube containing 1 mL of the crushed fungal culture.

After dye addition, tubes were kept protected from light at room temperature for 30 min (Kamoun et al. 2018). Then, 20 μL of the solution was inoculated on a slide and covered with a coverslip for microscopic observation. For yeasts, the methodology was adapted from Filippucci et al. (2016). One loop was inoculated into 2 mL tubes containing 1 mL of GMY liquid medium (40 g L^{-1} glucose, 3 g L^{-1} yeast extract, 8 g L^{-1} KH_2PO_4, and 0.5 g L^{-1} $MgSO_4$ $7H_2O$), which were incubated for 9 d at 15 °C. Subsequently, the cell concentration in the solution was standardized in a spectrophotometer, with absorbance adjusted to 0.3 at a wavelength of 600 nm. For microscopic evaluation, 40 μL of the aforementioned Nile Red dye solution was added to 1 mL of yeast cultures, which remained protected from light at room temperature for 5 min. Then, 20 μL of the solution was placed on a slide and covered with a coverslip.

The yeast, *Yarrowia lipolytica* QU21, obtained from the Collection of Microorganisms and Cells of UFMG, served as a positive control under identical culture conditions (Poli et al. 2014). *Yarrowia lipolytica* (synonym: *Candida lipolytica*) is a widely utilized model for lipid accumulation studies and holds significant biotechnological relevance for sustainable alternatives (Bankar et al. 2009; Beopoulos et al. 2009; Poli et al. 2014). All fungi were observed using a Nikon C2+ Confocal Microscope with a 60X objective lens and immersion oil. All images were recorded during fluorescence emission. ImageJ (http://imagej.nih.gov/), a free software, was used to estimate the fluorescence intensity emitted by each isolate and the percentage of the cellular area occupied by lipid particles. This was achieved by capturing and

processing cellular images, as described by Filippucci et al. (2016). Thus, the amount of intracellular lipids was estimated by correlating the intensity of the fluorescence emitted by each isolate with the percentage of the cellular area occupied by lipid particles, using the following formula: PELI = IFE × ATPL/AC, where PELI is the estimated production of intracellular lipids, IFE is the intensity of the emitted fluorescence, ATPL is the total area of lipid particles, and AC is the cellular area.

Fungal cultivation and preparation of extracts for biological assays

To obtain fungal extracts, each isolate was inoculated separately into five 90 × 15 mm Petri dishes containing 20 mL MEA medium and incubated for 15 d in a BOD incubator at 15 ºC. Subsequently, the contents of the plates (culture medium with mycelial growth) were fragmented into small pieces, placed in 125 mL Erlenmeyer flasks, frozen at -20 ºC for 24 h, and then subjected to lyophilization (LíoTop Lyophilizer – K105) for 5 d. Then, 100 mL of Dichloromethane P.A. Neon was added to each Erlenmeyer flask, and left to stand for 7 d at room temperature. The supernatant from each container (dichloromethane phase) was then filtered through a filter paper (50 × 50 – 80 g) and transferred to previously weighed scintillation vials (Rosa et al. 2013). After drying in a fume hood, the extract was stored at 4 ºC until the bioassay was performed.

Determination of herbicidal activity

Herbicidal activity determination assay was performed as described by Dayan et al. (2000) and Gomes et al. (2018). The fungal extracts were tested against seeds of lettuce (*Lactuca sativa*) and chives (*Allium schoenoprasum*), model species of dicotyledon and monocotyledon, respectively. The seeds were first disinfected with 70% ethanol for one minute and 2.5% sodium hypochlorite for 7.5 min, washed in sterilized distilled water four

times and dried in a laminar flow hood on sterilized filter paper. The assay was performed in duplicate, using 24-well plates with a filter paper disc in each well. To the respective wells, 400 μL of the herbicide glyphosate (Sigma, USA) at a concentration of 3 mg mL^{-1} were added as a positive control, 400 μL of sterilized distilled water as a negative control, 400 μL of sterile distilled water with diluted acetone (10%) as a solvent control, and 400 μL of the crude extracts diluted in acetone at 1 mg mL^{-1}. Then, five lettuce seeds or five chive seeds were placed in all wells. The plates were sealed and stored in a biological oxygen demand incubator under continuous illumination at 26 ºC. Results were evaluated qualitatively after 10 d incubation for *L. sativa* and 14 d for *A. schoenoprasum*, assigning a scale from 0 (all seeds germinated) to 5 (no seeds germinated). The extracts that inhibited seed germination by 100% were then subjected to assay to establish their minimum inhibitory concentration (MIC). Serial dilutions of the extracts, from 1000 to 3.9 μg mL^{-1} were made, using the same controls as mentioned above.

Results

Fungal Identification

A total of 57 fungal isolates, representing the phyla *Ascomycota*, *Mortierellomycota* and *Basidiomycota* in rank order of abundance, were identified. These comprised 20 taxa: 16 (66.6%) from Lake Florencia and 8 (33.3%) from Lake Katerina. Fungal identifications, based on molecular techniques are given in Table 1. The most abundant taxa were *Leucosporidium muscorum*, *Mortierella antarctica*, *Penicillium palitans*, *Pseudeurotium ovale*, *Pseudeurotium* sp. 1, *Pseudogymnoascus* sp. 1, *Pseudogymnoascus* sp. 2 (present in both lakes), *Thelebolus ellipsoideus* and *T. globosus*.

Diversity of fungal assemblages

The sampling effort to obtain fungal assemblages from the sediments of the two lakes was assessed using rarefaction curves (Fig. 2), with both reaching asymptote. The Fisher-α, Margalef and Simpson indices for the fungal assemblages of the two lakes are presented in Table 2. The assemblage obtained from the sediment of Lake Florencia was more diverse and richer, and also showed greater dominance, compared to that of Lake Katerina. Of the 24 taxa identified, 12 were exclusive to Lake Florencia, four to Lake Katerina and four were shared between the two lakes (Fig. 3).

Evaluation of enzymatic activities

Fifty-five isolates produced at least one of the enzymes studied (Suppl. Table 1). The most frequently detected enzymes were protease, cellulase and inulinase (49% each). The least produced was pectinase (1.8%), followed by invertase and gelatinase (20% each). In summary, 11 isolates (20%) produced invertase, 19 (34.5%) amylase, 27 (49%) protease and 27 (49%) cellulase. *Penicillium palitans* UFMGCB 18874 was the best cellulase producer, with an EI of 2.45 ± 0.44. Additionally, 12 isolates (21.8%) produced esterase while only one isolate (1.8%), *P. palitans* UFMGCB 18874, produced pectinase. Twenty-two isolates (40%) produced agarase, with *Pseudogymnoascus* sp. 1 UFMGCB 18915 being the best producer (IE = 2.02 ± 0.15). Twenty-two isolates (40%) were carrageenanase best producers. Twenty-seven isolates (49%) were inulinase producers, amongst which *Pseudogymnoascus* sp. 1 UFMGCB 18909 was the best producer (IE = 2.25 ± 0.08). Additionally, 23 isolates (41.8%) produced lipase and 11 isolates (20%) produced gelatinase.

Evaluation of biosurfactant production

Fifty-five of the 57 fungal isolates were evaluated for their ability to produce biosurfactants using the emulsification index test (Suppl. Table 2). Isolates with an average emulsification

higher than 50% were classified as good producers, following the protocol of Willumsen and Karlson (1996). Eight isolates (14.3%) demonstrated surfactant production; seven of these were members of the genus *Pseudogymnoascus*. *Thelebolus globosus* generated an $E_{24\%}$ of 57.01%, while *Pseudogymnoascus* sp. UFMGCB 18909 was the best producer, with an emulsification index of 68.86%.

Evaluation of intracellular lipid production

Intracellular lipid bodies in fungi were visualized at low temperature (Suppl. Figure 1). ImageJ analysis revealed that estimated intracellular lipid production varied among isolates, with values ranging from 0.03 to 22.24 (Suppl. Table 3). The isolates exhibiting the highest intracellular lipid production were *L. muscorum* UFMGCB 18934 and *L. muscorum* UFMGCB 18932.

Evaluation of phytotoxic activity

Of the isolates tested, only *P. palitans* UFMGCB 18874 exhibited phytotoxic activity, demonstrating 100% inhibition against *A. schoenoprasum* seeds. The one-dimensional NMR (^{1}H and ^{13}C) spectra of the active crude extract revealed its near chemical purity. Comparison of ^{1}H and ^{13}C NMR data along with the HRMS data between *P. palitans* UFMGCB 18874 and authentic (-)-palitantin (Fig. 4a) confirmed the presence of this compound as the primary constituent in this extract (Fig. 4b). This compound recently isolated from a strain of *Penicillium palitans* recovered from deep-sea Antarctic marine sediment (Barreto et al. 2025).

Discussion

Identification and diversity of fungi

Studies of the diversity of cultivable fungi in Antarctic lakes have increased in recent years (Ellis-Evans 1985, 1996; Vincent 2000; Gonçalves et al. 2012; Ogaki et al. 2020a; de Souza et al. 2023; Teixeira et al. 2024). However, further investigation is required given the large number of lakes in Antarctica, and their general lack of study (Hawes et al. 2023).

Ascomycota was the dominant phylum in this study, including genera known to have cosmopolitan cold-adapted and Antarctic endemic species. Species of *Pseudogymnoascus*, an ascomycete genus, were detected in both lakes, although were not identified to species level. Representatives of this genus are common in the polar regions and are often particularly abundant in Antarctica (Rosa et al. 2019). The genus has been previously reported from Antarctica in studies of marine invertebrates (Godinho et al. 2019), lake sediments (Ogaki et al. 2020a), soils (Gomes et al. 2018), rocks (Alves et al. 2019) and marine sediments (Ogaki et al. 2020b).

Members of the genus *Thelebolus*, known to possess psychrophilic adaptations to tolerate the chemical stresses of ornithogenically-influenced soils, was detected in both lakes, suggesting that these isolates may have originated from avian vectors (de Hoog et al. 2004; Ogaki et al. 2020a). The genus *Penicillium*, with isolates obtained from both lakes, has a global distribution and considerable ecological and economic importance due to its primary role in decomposing organic materials (Frisvad and Samson 2004; Rastegari et al. 2020). Members of this cosmopolitan genus have been reported from various Antarctic substrates, including Antarctic lakes (Rosa et al. 2019).

The ascomycete genus *Tetracladium*, detected only in Lake Katerina , includes aquatic hyphomycetes and important decomposer taxa. Members have been reported from various polar ecosystems (Anderson and Marvanová 2020). In Antarctica, the genus has been reported in lakes (Ogaki et al. 2020c; de Souza et al. 2022). The genus *Cladosporium* was isolated from both lakes studied here. Members of this genus *Cladosporium* are found globally in air,

soil and aquatic substrates (Ogórek et al. 2012) and have been reported from Antarctic lakes (Gonçalves et al. 2012).

Pseudeurotium ovale was isolated from Lake Florencia. In Antarctica, *Pseudeurotium* species have been reported from various substrates, including the active layer of ice-free oases in continental Antarctica (Kochkina et al. 2014), associated with sponges (Henríquez et al. 2014), soil (Arenz and Blanchette 2009), wood (Arenz et al. 2006) and lake sediments (Ogaki et al. 2020a). The genus *Dactylaria* was isolated from Lake Katerina and is considered to be a nematode-predatory fungus (Drechsler 1950). In Antarctica, it has been reported in association with the flowering plants *Deschampsia antarctica* and *Colobanthus quitensis* and the moss *Brachythecium austrosalebrosum* (Gray and Smith 1984).

The phylum *Basidiomycota* was second most frequently obtained amongst the isolates in this study. Several species of the genus *Leucosporidium*, detected here only in Lake Florencia, have been reported from polar regions (Summerbell 1983), including *L. muscorum* from Antarctic lake water and sediments (Brandão et al. 2017; Ogaki et al. 2020a). The yeast, *Glaciozyma martinii*, was also isolated only from Lake Florencia and is considered rare in Antarctica (Sannino et al. 2023). It has previously been reported from Antarctic lake sediments by Ogaki et al. (2020a). *Mortierella antarctica* (*Mortierellomycota*) is an endemic Antarctic species also isolated solely from Lake Florencia. Other member of *Mortierella* have been previously reported from the Antarctic continent, including in lakes (de Souza et al. 2022).

Detection of enzymes, biosurfactants and lipids

Our data confirm the enzymatic and other biotechnological potential of the Antarctic fungi as reported by Krishnan et al. (2011, 2016), Martinez et al. (2016), Martorell et al. (2017, 2019), Furbino et al. (2018), Poveda et al. (2018), Tsuji (2018), Zucconi et al. (2020), and Nikitin et

al. (2022). In the present study, we detected the production of significative amount of cellulase by *P. palitans* UFMGCB 18874, consistent with data reported by da Silva et al. (2024).

Penicillium palitans is a ubiquitous mesophilic fungus that has been isolated from food (Frisvad et al. 2004) and has also been reported from Antarctic terrestrial habitats including mosses and soils (da Silva et al. 2024). *Penicillium* species seem to display a functional ecology capability in the biodegradation of environmental organic matter in Antarctica by their high capacity to produce a wide range of exoenzymes (Rastegari et al. 2020; da Silva et al. 2024, Teixeira et al. 2024). The genus *Pseudogymnoascus* was a good producer of the enzymes inulinase and agarase. de Souza et al. (2023) and Teixeira et al. (2024) highlighted the ability of members of this genus recovered from Antarctica to produce enzymes at low temperatures.

Studies have investigated the potential of various psychrotolerant microorganisms for biosurfactant production. Several of the fungal isolates found here demonstrated considerable biosurfactant production. This capacity may be related to their adaptation to cold habitats, where biosurfactants can emulsify and solubilize water-insoluble substances, thereby facilitating growth on relevant substrates (Francy et al. 1991). A crucial element of microorganism survival strategy is their ability to colonize or modify environments suitable for growth. In this context, biosurfactants may play a fundamental role by influencing the cell wall, which allows adhesion to or detachment from a given surface according to environmental demands (Rosenberg and Ron 1999). Studies have also highlighted that microorganisms capable of surviving in cold, extreme, environments may provide reservoirs of valuable biosurfactants and lipids (Perfumo et al. 2018). A notable example is the yeast, *Moesziomyces antarcticus* (formerly *Candida antarctica*), originally reported from Lake Vanda in Victoria Land, continental Antarctica (Sugiyama et al. 1967), which is currently one

of the most sought-after producers of biosurfactants in the global market (Perfumo et al. 2018).

Pseudogymnoascus and *Thelebolus* taxa isolated in this study exhibited high emulsification rates. As noted above, members of *Pseudogymnoascus* are widely reported in the polar regions, with some species potentially endemic to Antarctica, although their ecological roles are not fully understood (Rosa et al. 2019). The genus *Thelebolus* includes one of the biosurfactant-producing isolates identified in this study, *T. ellipsoideus*, which is an Antarctic endemic and psychrophilic species (Rosa et al. 2019).

Intracellular lipid production

Lipid production by microorganisms is a mechanism for the accumulation of carbon reserves during the growth process, which its composition and quantities are variable, influenced by the species considered, developmental stage, environmental conditions and cultivation substrate (Ratledge 1997). Fungi capable to accumulate intracellular lipids represent a potentially source for the food, cosmetics, textiles and fuel production industries (Tasselli et al. 2019). Yeasts of the genus *Leucosporidium* are known to be psychrophilic/psychrotolerant and acidophilic/acidotolerant, and are therefore polyextremophilic/extremotolerant (Buzzini et al. 2018). As noted above, *Leucosporidium muscorum*, identified in the current study, is reported to produce multiple enzymes (Freire et al. 2021; de Souza et al. 2023). However, this appears to be the first report of *L. muscorum* as an excellent lipid producer.

Evaluation of phytotoxic activity

Secondary metabolites produced by fungi display a wide structural diversity and spectra of bioactivity and represent an alternative for the development of new, efficient natural herbicides (Dayan and Duke 2014). *Penicillium* species represent a promising source of

physiologically active compounds, including antibiotics, hormones and mycotoxins. They are recognized for their ability to produce a wide variety of secondary metabolites of various chemical classes, such as alkaloids, diketopiperazines, benzodiazepines, quinolines, quinazolines and polyketides (Kozlovskii et al. 2013).

Other *Penicillium* species from Antarctica are known to produce metabolites with phytotoxic properties. For instance, Ogaki et al. (2020c) evaluated fungi isolated from Antarctic marine sediments and detected phytotoxic metabolites produced by *P. allii-sativi*, *P. chrysogenum*, *P. palitans* and *P. solitum* produced In this study, only *P. palitans* UFMGCB 18874 exhibited phytotoxic activity, producing the phytotoxic compound, (-) palitantin, a white crystalline heptacetate metabolite, which was first isolated in 1936 from culture filtrates of the same species (Birkinshaw and Raistrick 1936; Demetriadou et al. 1988). (-) palitantin has attracted research interest due to its unique biological activities. Derivatives of (-) palitantin have primarily been isolated from fungi of various genera, including *Penicillium* and *Aspergillus* (Yu et al. 2022). Consistent with our results, metabolites derived from *P. palitans* isolated from deep-sea sediments in the maritime Antarctic, included (-) palitantin Barreto et al. (2025).

Conclusions

The results obtained in this study demonstrate that sediments from Antarctic lakes host a significant reservoir of fungal diversity, in which psychrophilic taxa were dominant, alongside cosmopolitan cold-adapted taxa. Some of the fungal taxa obtained stand out as potential candidate sources of metabolites of biotechnological and agricultural interest. Since Antarctic lakes represent fragile ecosystems threatened by regional climate change, our results reinforce the importance of research on the diversity of extremophilic fungi in Antarctica and the evaluation of their potential for producing novel bioproducts.

Author Contributions Statement

L.M.D.S. and L.H.R. conceived the study. J.M.L. and S.C. collected the samples. F.S.O. performed the samples physicochemical analysis. L.M.D.S. J.P.M.R., E.A.A., C.R.C. determined the enzymes, lipids, and biossufactant activities. D.L.C.B., C.C., S.O.D. characterized the phytotoxic compound. C.A.R. identified the yeasts. P.C. analyzed the results. L.M.D.S., L.H.R., F.S.O., J.M.L., S.C., J.P.M.R., E.A.A., C.R.C., D.L.C.B., C.C., S.O.D., C.A.R., and P.C. analyzed the results and wrote the manuscript. All authors read and approved of the final manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are available in the NCBI repository, which can be accessed at https://www.ncbi.nlm.nih.gov/.

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Figuras e Tabelas

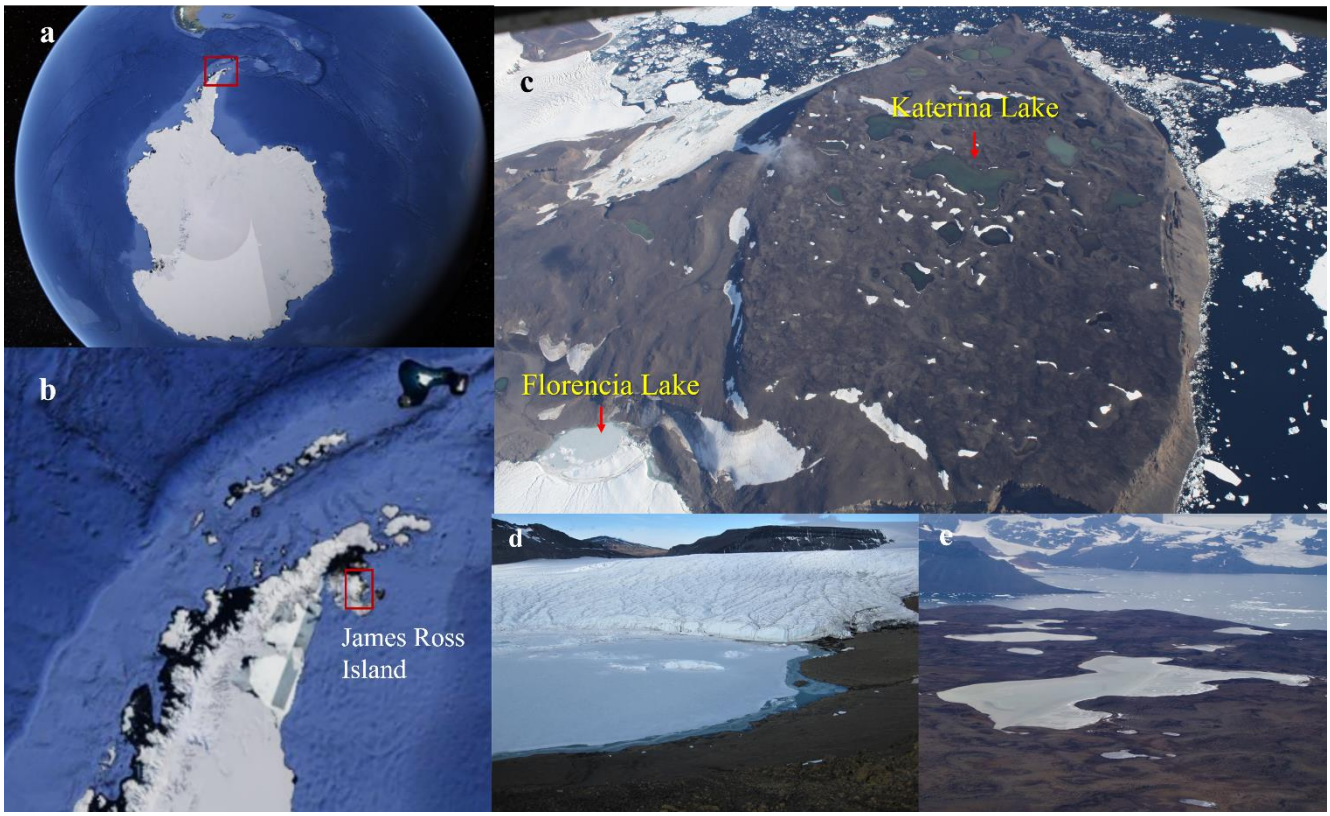

Fig. 1 Satellite images **a** and **b** (obtained from Google Earth Pro, 2019). (**a**) Antarctica with the northern Antarctic Peninsula inside the red rectangle, (**b**) James Ross Island inside the red rectangle, (**c**) Clearwater Mesa, James Ross Island. (**d**) Lake Florencia (64°01′24.0″ S; 57°40′03.1″ W) and (**e**) Lake Katerina (64°01′25.5″ S; 57°43′03.6″ W). Photo **c** by Fernando Calabozo, photo **d** by Silvia Coria and photo **e** by Matej Roman.

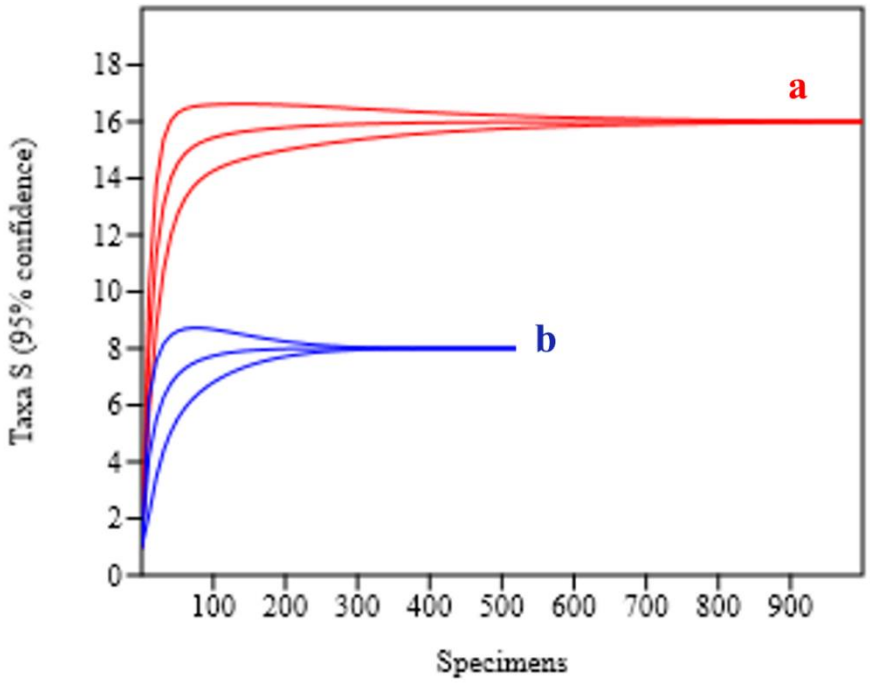

Fig. 2 Rarefaction curves, with 95% confidence limits, of fungal assemblages obtained from sediments from (**a**) Lake Florencia and (**b**) Lake Katerina.

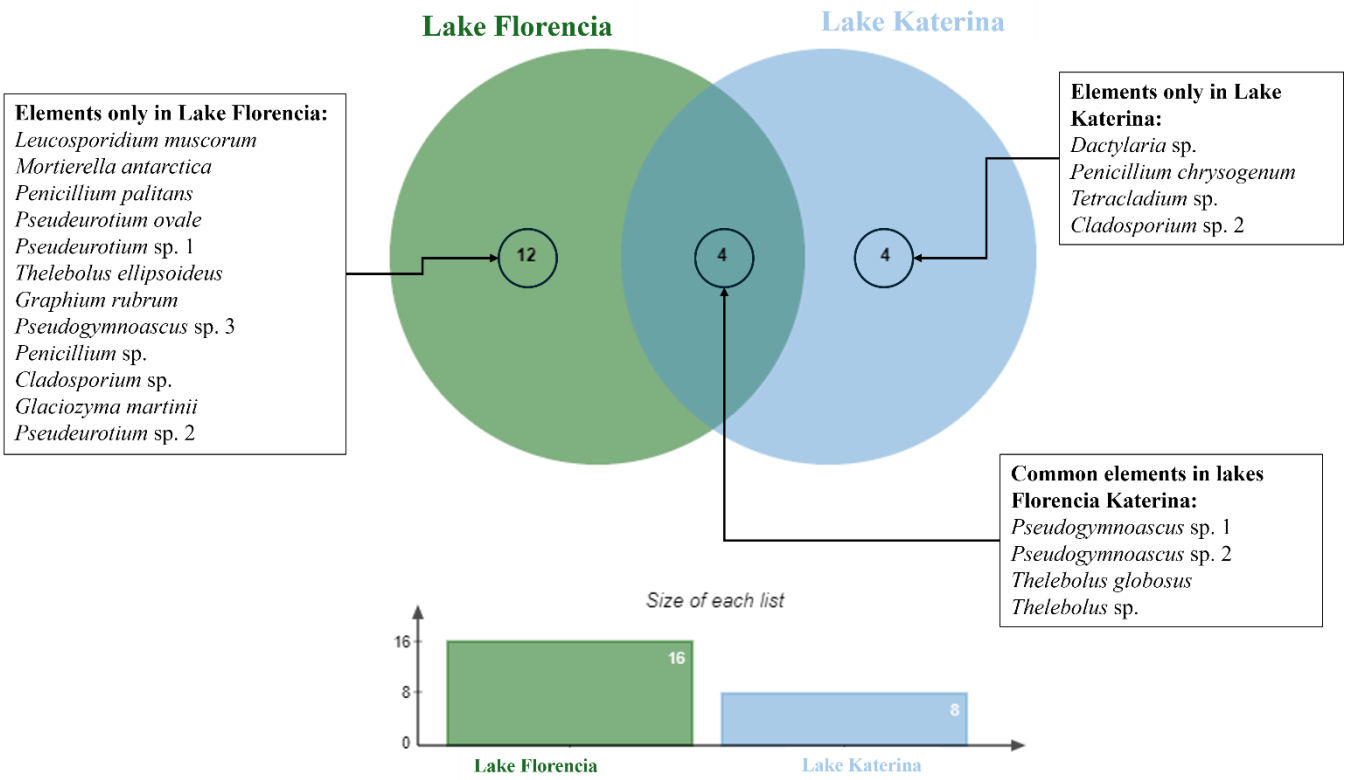

Fig. 3 Venn diagram comparing the fungal assemblages obtained in sediments from Lake Florencia and Lake Katerina.

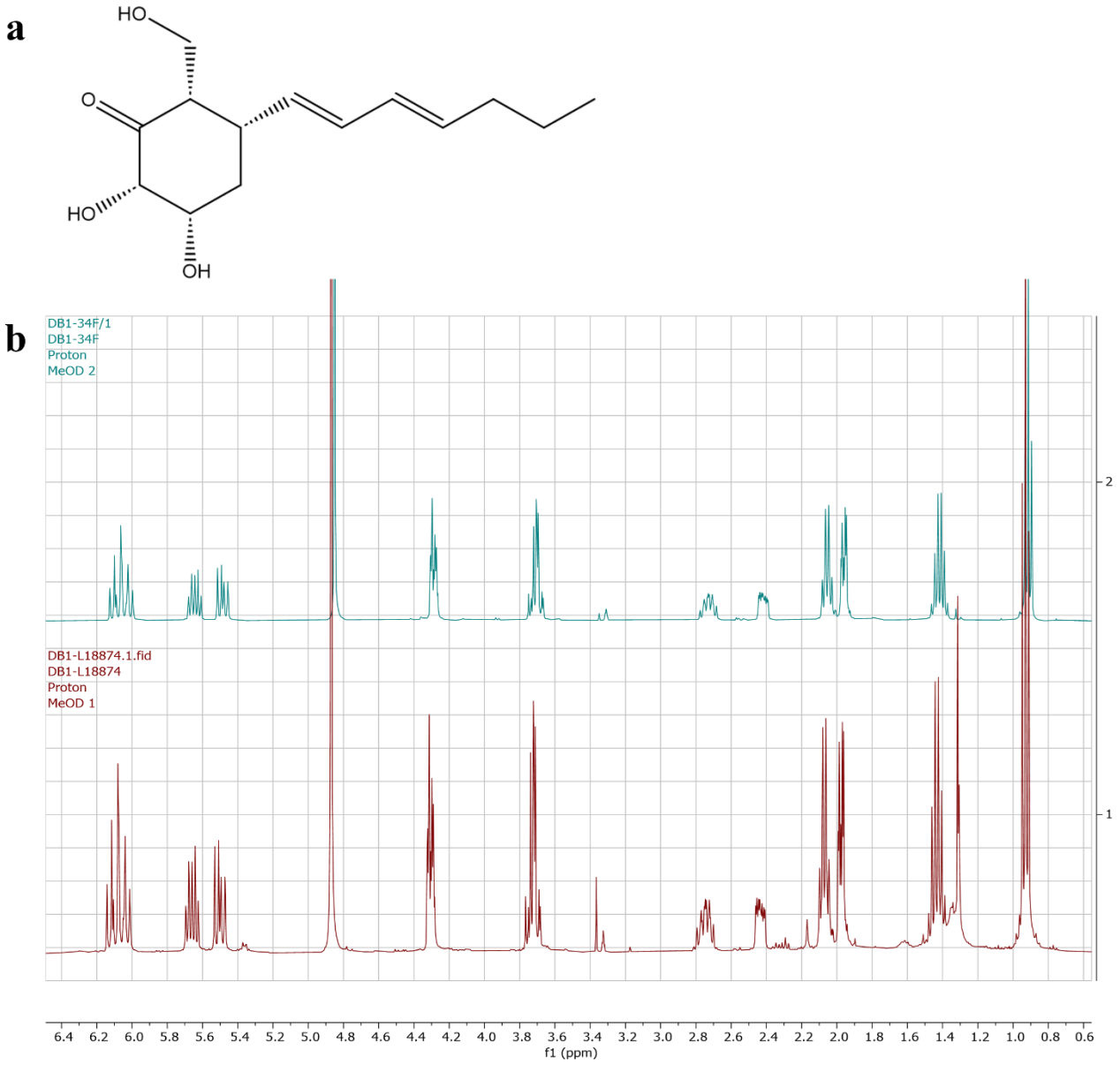

Fig. 4. (**a**) chemical structure of (-)-palitantin and (**b**) ^{1}H NMR spectrum of the crude extract UFMGCB 18874 (bottom) and the ^{1}H NMR spectrum for (−)-palitantin in MeOD-d_6 (400 MHz).

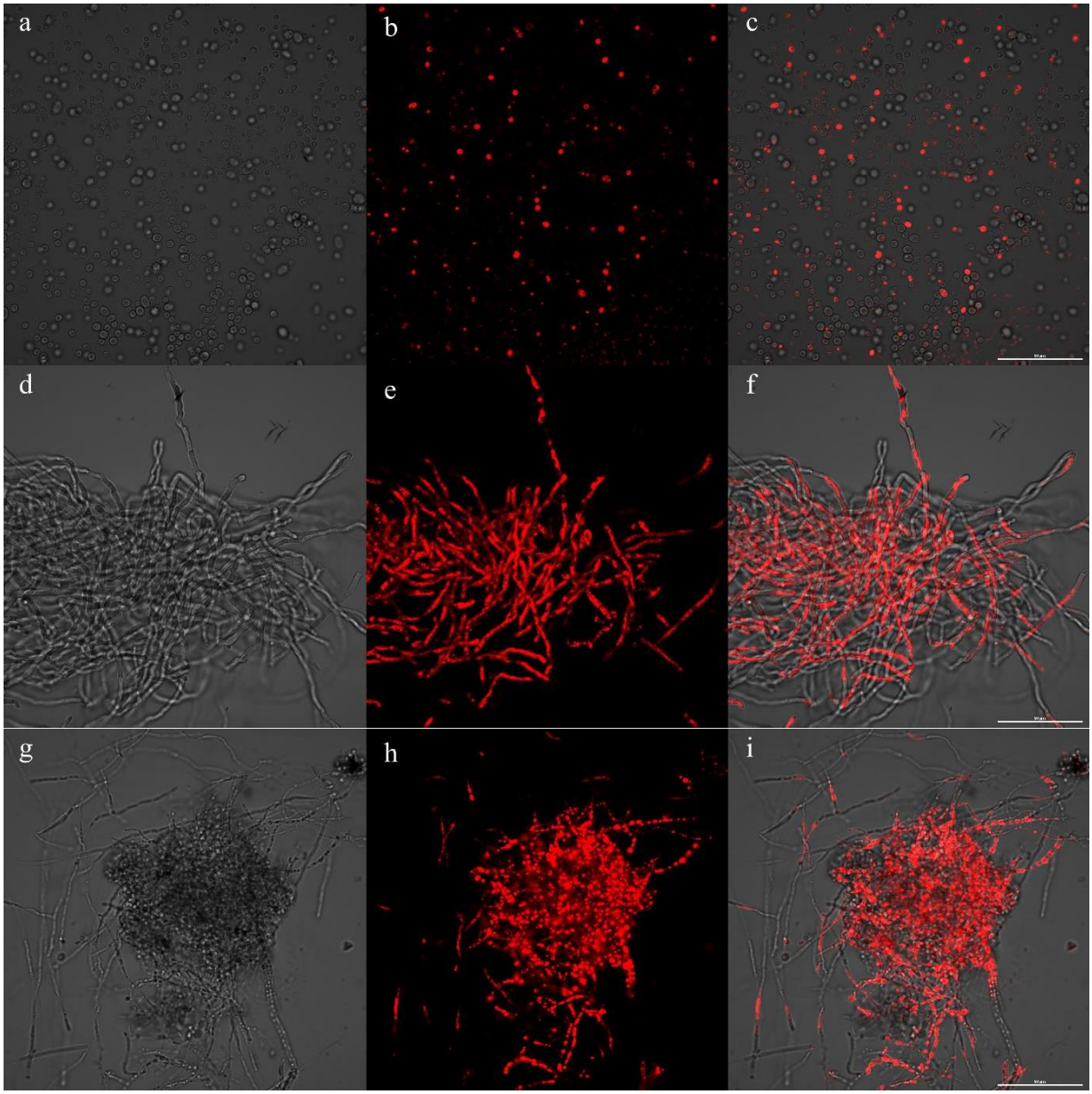

Figure S1. Intracellular lipid production observed by confocal microscopy. Photographs (**a**), (**b**) and (**c**) show the yeast, *Yarrowia lipolytica* QU21, used as a positive control. Photographs (**d**), (**e**) and (**f**) show the mycelium of the Antarctic *Thelebolus* sp. UFMGCB 18892. Photographs (**g**), (**h**) and (**i**) show the Antarctic fungus *Pseudogymnoascus* sp. 1 UFMGCB 18886. Images display bright-field visualization, fluorescence-field visualization and an overlay of bright-field and fluorescence-field, recorded at 60× magnification. Photos: Camila Carvalho.

Table 1. Identification of Antarctic fungi isolated from sediments obtained from Lake Florencia and Lake Katerina based on molecular sequencing of rDNA regions and Basic Local Alignment Search Tool (BLASTn) search.

Lake	UFMGCB[a] code	Top results from BLASTn search (GenBank accession number)	Density (CFU g^{-1})	Identity (%)	Query cover (%)	N° of bp[e]	Proposed taxon (GenBank accession number of sequences)
Florencia	18930	*Leucosporidium muscorum* (KY108280)[c]	>300	100	100	462	*Leucosporidium muscorum* (PV801138)
	18896	*Mortierella antarctica* (NR111580)[b]	>300	99	100	412	*Mortierella antarctica* (PV818020)
	18874	*Penicillium palitans* (KU904360)[b,d]	>300	100	100	689	*Penicillium palitans* (PV805467)
	18890	*Pseudeurotium ovale* (MH857368)[b]	>300	99	100	366	*Pseudeurotium ovale* (PV818018)
	18895	*Pseudeurotium bakeri* (NG069753)[b,c]	>300	100	95	437	*Pseudeurotium* sp. 1 (PV818019)
	18916	*Geomyces destructans* (EU884921)[b]	>300	99	100	447	*Pseudogymnoascus* sp. 1 (PV818025)
	18877	*Pseudogymnoascus verrucosus* (KJ755525)[b]	>300	99	100	472	*Pseudogymnoascus* sp. 2 (PV818014)
	18881	*Thelebolus ellipsoideus* (NR160216)[b]	>300	100	100	440	*Thelebolus ellipsoideus* (PV818017)
	18903	*Thelebolus globosus* (NG067263)[b,c]	>300	100	100	410	*Thelebolus globosus* (PV818023)
	18884	*Graphium rubrum* (NR145268)[b]	260	100	100	518	*Graphium rubrum* (PV818035)
	18897	*Pseudogymnoascus griseus* (MN432492)[b,e]	230	93	100	478	*Pseudogymnoascus* sp. 3 (PV818021)
	18922	*Penicillium goetzii* (MT558933)[b]	210	100	100	458	*Penicillium* sp. (PV818036)
	18925	*Cladosporium maltirimosum* (OQ186147)[b]	150	100	100	481	*Cladosporium* sp. (PV818032)
	18923	*Thelebolus globosus* (MH862951)[b]	140	100	100	486	*Thelebolus* sp. (PV818037)
	18927	*Glaciozyma martinii* (KY103469)[b]	100	100	100	497	*Glaciozyma martinii* (PV818027)
	18919	*Pseudeurotium ovale* (MH857368)[b]	30	99	100	384	*Pseudeurotium* sp. 2 (PV818034)
Katerina	18901	*Pseudogymnoascus verrucosus* (OR485038)[b]	>300	99	100	447	*Pseudogymnoascus* sp. 2 (PV818045)
	18883	*Dactylaria dimorphospora* (NR164282)[b]	95	92	96	400	*Dactylaria* sp. (PV818038)
	18880	*Thelebolus globosus* (NG067263)[b,c]	40	100	100	472	*Thelebolus globosus* (PV817921)
	18882	*Penicillium chrysogenum* (JX996668)[b,d]	30	100	100	578	*Penicillium chrysogenum* (PV805468)

	18892	*Thelebolus globosus* (MH862951)[b]	30	100	100	445	*Thelebolus* sp. (PV818039)
	18894	*Tetracladium fraxineum* (ON922528)[b]	25	93	93	446	*Tetracladium* sp. (PV818043)
	18918	*Cladosporium phyllactiniicola* (MH863929)[b]	10	100	100	482	*Cladosporium* sp. 2 (PV818044)
	18875	*Geomyces destructans* (EU884921)[b]	10	99	100	455	*Pseudogymnoascus* sp. 1 (PV818042)

[a]UFMGCB = Collection of Microorganisms and Cells of the Federal University of Minas Gerais; bp = base pairs. Taxa subject to BLAST analysis based on [b]ITS, [c]Domain D1-D2, [d]RPB2, and [e]MCM7.

Table 2. Location, geology, fungal assemblage diversity and physicochemical parameters of sediments obtained from Lake Florencia and Lake Katerina, James Ross Island, Antarctica.

Physical characteristics	**Lake Florencia**	**Lake Katerina**
Location	64°01′24.0″ S 57°40′03.1″ W	64°01′25.5″ S 57°43′03.6″ W
Altitude (meters above sea level)	25	250
Total area (m^2)	132,000	126,951
Perimeter (m)	1,854	2,085
Distance from the coast (m)	1,429	1,337
Depth (m)	> 10	1
Shape	Circular	Irregular
Type of geological substrate	Glacial deposit	Basaltic lava
Diversity		
Density (CFU cm^{-1})	>300	>300
Number of taxa	16	08
Fisher-α	2.14	1.33
Margalef	1.82	1.11
Simpson	0.93	0.65
Physicochemical parameters		
pH in H_2O	8.03	8.15
Electrical conductivity (µS cm^{-1})	104.1	508.5
Exchangeable P – mg dm^{3-1}	9.3	120.5
Sum of exchangeable bases Ca+K+Mg (SB) – $cmol_c$ dm^{3-1}	7.59	5.53
Percentage of base saturation (PBS) - %	79.3	94.4
H+Al - potential acidity - $cmol_c$ dm^{3-1}	1.98	0.33
Cation exchange capacity at pH 7 (CEC) - $cmol_c$ dm^{3-1}	9.57	5.86
Total organic carbon (TOC) – dag kg^{-1}	0.38	0.54
Micronutrient Fe – mg dm^{3-1}	44.0	252.2

Exchangeable P – mg dm^{3-1}	63.2	54.2

Table S1. Enzyme production and activity of fungal isolates obtained from sediments of Lake Florencia and Lake Katerina, James Ross Island, northieast Antarctic Peninsula.

		Enzymatic index										
		Mean ± standard deviation of enzyme index									LIP	GEL
Fungi	**UFMGCB**	**INV**	**AMI**	**PRO**	**CEL**	**EST**	**PEC**	**AGA**	**CAR**	**INU**		
Cladosporium sp. 1	18925	-	-	1.03 ± 0.01	1.02 ± 0.00	-	-	-	-	-	+	-
Cladosporium sp. 2	18918	-	1.38 ± 0.33	-	-	1.29 ± 0.09	-	-	-	-	-	-
Dactylaria sp.	18883	-	-	-	-	-	-	-	-	-	-	-
Dactylaria sp.	18908	-	-	-	1.22 ± 0.00	-	-	1.22 ± 0.00	1.15 ± 0.03	-	-	-
Dactylaria sp.	18924	-	-	-	-	-	-	-	-	-	+	-
Glaciozyma martinii	18927	1.11 ± 0.08	-	1.27 ± 0.02	-	1.11 ± 0.00	-	-	-	1.13 ± 0.01	-	-
Graphium rubrum	18884	-	-	-	1.40 ± 0.18	-	-	1.11 ± 0.05	1.07 ± 0.05	1.12 ± 0.01	-	-
Leucosporidium muscorum	18930	1.19 ± 0.13	-	1.39 ± 0.05	-	1.13 ± 0.02	-	-	-	1.13 ± 0.04	+	+
L. muscorum	18932	1.21 ± 0.11	-	1.32 ± 0.24	-	1.18 ± 0.05	-	-	-	1.04 ± 0.01	+	+
L. muscorum	18934	1.08 ± 0.04	-	-	-	1.38 ± 0.20	-	-	-	1.04 ± 0.01	+	+
Mortierella antarctica	18887	-	-	-	-	-	-	-	-	-	+	-
M. antarctica	18889	-	1.04 ± 0.00	1.17 ± 0.03	-	-	-	-	-	-	-	-
M. antarctica	18896	-	-	-	-	-	-	-	-	-	-	-
M. antarctica	18910	-	-	-	-	-	-	-	-	-	+	-
M. antarctica	18917	1.09 ± 0.04	-	-	-	-	-	-	-	1.22 ± 0.07	-	-
M. antarctica	18920	-	-	-	-	-	-	-	-	-	-	-
Penicillium chrysogenum	18882	-	1.14 ± 0.01	1.50 ± 0.08	-	-	-	-	-	-	+	-
Penicillium palitans	18874	1.23 ± 0.08	1.36 ± 0.04	1.91 ± 0.06	**2.45 ± 0.44**	-	1.19 ± 0.07	1.14 ± 0.01	1.16 ± 0.13	1.22 ± 0.20	+	-
Penicillium sp.	18922	1.15 ± 0.05	1.07 ± 0.01	1.08 ± 0.05	-	-	-	-	-	-	+	-
Pseudeurotium ovale	18890	-	-	-	1.11 ± 0.02	-	-	-	-	-	-	-

Pseudeurotium sp. 1	18906	-	1.09 ± 0.07	-	1.10 ± 0.05	-	-	-	-	-	-	-
Pseudeurotium sp. 2	18895	-	-	-	1.29 ± 0.06	-	-	-	-	1.12 ± 0.02	-	-
Pseudeurotium sp. 2	18899	-	-	-	-	-	-	-	-	-	-	-
Pseudeurotium sp. 2	18905	-	1.15 ± 0.05	-	1.13 ± 0.06	-	-	-	-	-	+	-
Pseudeurotium sp. 2	18911	-	-	-	1.54 ± 0.01	-	-	-	-	-	-	-
Pseudeurotium sp. 2	18921	-	1.11 ± 0.04	-	1.87 ± 0.45	-	-	-	-	-	-	-
Pseudeurotium sp. 3	18919	-	-	-	1.45 ± 0.20	-	-	-	-	-	-	-
Pseudogymnoascus sp. 1	18873	-	1.04 ± 0.00	1.11 ± 0.03	1.11 ± 0.05	1.04 ± 0.02	-	1.06 ± 0.03	1.12 ± 0.00	1.07 ± 0.03	-	-
Pseudogymnoascus sp. 1	18875	-	1.20 ± 0.02	-	1.11 ± 0.02	1.04 ± 0.01	-	1.06 ± 0.01	1.04 ± 0.00	1.19 ± 0.07	+	-
Pseudogymnoascus sp. 1	18878	-	1.11 ± 0.01	1.07 ± 0.00	1.09 ± 0.01	-	-	1.17 ± 0.01	1.07 ± 0.00	1.17 ± 0.01	-	-
Pseudogymnoascus sp. 1	18879	-	1.19 ± 0.07	1.09 ± 0.00	1.14 ± 0.06	-	-	1.15 ± 0.03	1.15 ± 0.03	1.07 ± 0.04	-	-
Pseudogymnoascus sp. 1	18885	1.14 ± 0.07	1.04 ± 0.01	1.10 ± 0.03	1.10 ± 0.04	-	-	1.02 ± 0.00	1.04 ± 0.02	1.04 ± 0.00	-	+
Pseudogymnoascus sp. 1	18886	-	1.06 ± 0.01	1.10 ± 0.02	1.67 ± 0.15	1.14 ± 0.01	-	1.02 ± 0.00	1.05 ± 0.01	1.09 ± 0.06	-	-
Pseudogymnoascus sp. 1	18898	-	-	1.06 ± 0.02	1.09 ± 0.03	-	-	1.14 ± 0.03	1.32 ± 0.14	1.08 ± 0.02	+	-
Pseudogymnoascus sp. 1	18900	1.16 ±0.07	1.28 ± 0.06	1.07 ± 0.04	1.11 ± 0.03	1.25 ± 0.03	-	1.09 ± 0.01	1.06 ± 0.02	1.02 ± 0.00	+	+
Pseudogymnoascus sp. 1	18907	-	-	1.00 ± 0.02	1.08 ± 0.03	-	-	1.12 ± 0.07	1.15 ± 0.09	1.19 ± 0.13	+	-
***Pseudogymnoascus* sp. 1**	18909	-	-	-	1.56 ± 0.09	1.43 ± 0.03	-	1.60 ± 0.13	1.54 ± 0.09	**2.25 ± 0.08**	+	-
Pseudogymnoascus sp. 1	18913	-	-	-	1.14 ± 0.03	-	-	1.09 ± 0.00	1.07 ± 0.00	1.08 ± 0.02	-	-
Pseudogymnoascus sp. 1	18914	-	1.06 ± 0.00	1.06 ± 0.00	1.11 ± 0.05	1.15 ± 0.06	-	1.22 ± 0.16	1.16 ± 0.07	1.22 ± 0.01	+	+
Pseudogymnoascus sp. 1	18915	-	-	1.46 ± 0.17	-	1.68 ± 0.15	-	**2.02 ± 0.15**	1.47 ± 0.13	1.89 ± 0.09	-	-
Pseudogymnoascus sp. 1	18916	-	1.32 ± 0.19	1.05 ± 0.00	1.26 ± 0.11	-	-	1.48 ± 0.27	1.31 ± 0.11	1.23 ± 0.21	-	-
Pseudogymnoascus sp. 2	18877	-	1.40 ± 0.12	-	1.38 ± 0.24	-	-	1.15 ± 0.02	1.12 ± 0.07	1.22 ± 0.13	-	-
Pseudogymnoascus sp. 2	18901	-	-	1.08 ± 0.03	-	-	-	1.08 ± 0.01	1.35 ± 0.08	1.06 ± 0.01	-	-
Pseudogymnoascus sp. 2	18936	-	1.17 ± 0.10	1.06 ± 0.01	1.13 ± 0.01	-	-	1.13 ± 0.03	1.22 ± 0.00	1.12 ± 0.05	-	-
Pseudogymnoascus sp. 3	18897	1.08 ± 0.02	-	1.11 ± 0.03	-	-	-	1.08 ± 0.07	1.19 ± 0.11	1.12 ± 0.03	+	-
Pseudogymnoascus sp. 3	18926	-	-	1.13 ± 0.02	1.03 ± 0.02	-	-	1.27 ± 0.06	0.84 ± 0.09	1.26 ± 0.07	-	-
Tetracladium sp.	18894	-	-	-	-	-	-	-	-	-	-	-
Tetracladium sp.	18912	-	-	-	-	-	-	-	-	-	-	-
Thelebolus ellipsoideus	18876	-	-	1.05 ± 0.04	-	-	-	-	-	-	+	+

T. ellipsoideus	18881	-	-	1.13 ± 0.08	-	-	-	-	-	-	+	+
T. globosus	18880	1.17 ± 0.09	-	-	-	-	-	-	-	-	-	-
T. globosus	18891	-	-	1.13 ± 0.07	-	-	-	-	-	-	+	+
T. globosus	18903	-	-	1.05 ± 0.00	-	-	-	-	-	-	+	+
Thelebolus sp.	18892	-	-	-	-	-	-	-	-	-	-	-
Thelebolus sp.	18923	-	-	-	-	-	-	-	-	-	+	+

UFMGCB = Collection of Microorganisms and Cells, Federal University of Minas Gerais, MIE = mean enzyme index, INV = invertase, AMI = amylase, PRO = protease, CEL = cellulase, EST = esterase, PEC = pectinase, AGA = agarase, CAR = carrageenanase, INU = inulinase, LIP = lipase, GEL = gelatinase. + = positive, – = negative. Bold: Good producers (≥ 2). Bold highlighted = best producer (2.45 ± 0.44).

Table S2. Results obtained in the emulsification index test for biosurfactant production.

Lake	UFMGCB[a]	Fungal taxon	$E_{24\%}$*[c]
Florencia	18909	*Pseudogymnoascus* sp. 1	68.86 ± 1.53
	18886	*Pseudogymnoascus* sp. 1	65.57 ± 1.71
	18897	*Pseudogymnoascus* sp. 3	63.19 ± 0.70
	18877	*Pseudogymnoascus* sp. 2	59.81 ± 6.3
	18913	*Pseudogymnoascus* sp. 1	58.96 ± 0.55
	18891	*Thelebolus globosus*	57.02 ± 2.51
	18873	*Pseudogymnoascus* sp. 1	53.57 ± 4.14
Katerina	18875	*Pseudogymnoascus* sp. 1	60.12 ± 3.29
	Negative control	Broth culture medium extract	5.21 ± 1.48
	Positive control	Sodium dodecyl sulfate 1%	73.57 ± 2.65

UFMGCB = deposit code in the UFMG Collection of Microorganisms and Cells, $EI_{24\%}$ = Emulsification index (%) with the standard deviation given, NC = negative control (inoculum-free culture medium), SDS 1% = positive control (sodium dodecyl sulfate 1%). *Values are the means of triplicates.

Table S3. Intracellular lipid production by fungal isolates.

Fungal taxon	UFMGCB[a]	Estimated production of intracellular lipids
Leucosporidium muscorum	18934	22.24
L. muscorum	18932	6.97
Pseudeurotium sp. 2	18921	3.31
L. muscorum	18930	3.03
Thelebolus ellipsoideus	18876	2.23
Glaciozyma martinii	18927	2.10
Penicillium palitans	18874	1.73
Mortierella antarctica	18889	1.3
Pseudogymnoascus sp. 1	18886	1.13
Pseudogymnoascus sp. 1	18878	1.12
Pseudeurotium sp. 2	18911	1.00
Pseudogymnoascus sp. 1	18914	0.97
Pseudogymnoascus sp. 1	18913	0.92
Dactylaria sp.	18908	0.79
Pseudogymnoascus sp. 1	18898	0.77
Thelebolus globosus	18891	0.73
M. antarctica	18910	0.73
Pseudogymnoascus sp. 2	18877	0.61
Pseudogymnoascus sp. 1	18916	0.59
Pseudogymnoascus sp. 2	18901	0.56
Dactylaria sp.	18883	0.53
M. antarctica	18896	0.51
Thelebolus sp.	18923	0.46
T. globosus	18880	0.45
Thelebolus sp.	18892	0.45
Cladosporium sp. 1	18925	0.45
Pseudogymnoascus sp. 3	18897	0.44
T. globosus	18903	0.44
Pseudeurotium sp. 2	18899	0.43
M. antarctica	18887	0.4
Tetracladium sp.	18894	0.36
Pseudeurotium sp. 1	18906	0.31
Pseudeurotium sp. 2	18895	0.27
Pseudogymnoascus sp. 1	18873	0.25
Pseudogymnoascus sp. 1	18885	0.25
Pseudogymnoascus sp. 1	18915	0.24
Dactylaria sp.	18924	0.24
Pseudogymnoascus sp. 1	18879	0.23
Graphium rubrum	18884	0.23
Pseudogymnoascus sp. 1	18900	0.22

Pseudogymnoascus sp. 1	18907	0.22
Penicillium sp.	18922	0.21
Pseudogymnoascus sp. 1	18875	0.20
Pseudeurotium ovale	18890	0.20
Dactylaria sp.	18904	0.20
Penicillium chrysogenum	18882	0.19
Pseudeurotium sp.2	18905	0.16
Pseudogymnoascus sp. 1	18909	0.11
T. ellipsoideus	18881	0.10
Pseudogymnoascus sp. 3	18926	0.10
Pseudogymnoascus sp. 2	18936	0.10
Tetracladium sp.	18912	0.09
Cladosporium sp. 2	18918	0.06
Pseudeurotium sp. 3	18919	0.03
Yarrowia lipolytica QU21	Control	0.12

[a]UFMGCB = UFMG Collection of Microorganisms and Cells.

3.5. Fungal diversity in sediments of periglacial lakes of Deception Island, Maritime Antarctica assessed using culturing and DNA metabarcoding approaches

A Ilha Deception, no arquipélago das Ilhas Shetland do Sul (Antártica Marítima), abriga ecossistemas lacustres jovens influenciados pela atividade vulcânica. Este estudo avaliou a diversidade de fungos cultiváveis e independentes de cultivo em sedimentos dos lagos Jean e Antonia. A partir de 139 isolados, foram identificados 13 táxons cultiváveis: nove no lago Jean e oito no lago Antonia, com *Ascomycota* como filo dominante, seguido por *Basidiomycota* e *Mortierellomycota*. *Pseudogymnoascus* foi o fungo cultivável mais abundante. O lago Jean apresentou maior riqueza, enquanto Antonia apresentou maior diversidade e dominância. As análises de metabarcoding resultaram em 310.076 leituras, representando 112 táxons atribuídos (106 de Antonia e 27 de Jean), dominados por *Ascomycota*, *Basidiomycota* e *Mortierellomycota*, mas incluindo também *Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota*, *Chytridiomycota*, *Monoblepharomycota*, *Mucoromycota*, *Rozellomycota* e *Zoopagomycota*. *Betamyces* sp. e Fungal sp. foram os mais abundantes em ambos os lagos. Ambas as abordagens revelaram a dominância de *Ascomycota*, *Basidiomycota* e *Mortierellomycota*, em contraste com outros sistemas antárticos frequentemente dominados por fungos cosmopolitas. Fungos psicrófilos/endêmicos foram altamente dominantes entre os táxons cultiváveis e moderadamente representados nos dados de metabarcoding, sugerindo que esses lagos abrigam organismos pioneiros adaptados a condições poliextremófilas, funcionando como reservatórios de fungos específicos da Antártica. A combinação de cultivo e metabarcoding proporcionou uma caracterização mais completa da diversidade fúngica nesses ecossistemas.

Fungal diversity in sediments of periglacial lakes of Deception Island, Maritime Antarctica assessed using culturing and DNA metabarcoding approaches

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Abstract

Deception Island, in the South Shetland Islands (Maritime Antarctic), hosts young lake ecosystems influenced by volcanic activity. This study assessed culture-dependent and culture-independent fungal diversity in sediments from Jean and Antonia lakes. From 139 isolates, 13 culturable taxa were identified: nine from Jean and seven from Antonia, with *Ascomycota* dominant, followed by *Basidiomycota* and *Mortierellomycota*. *Pseudogymnoascus* were the most abundant culturable fungi. Jean Lake showed higher richness, while Antonia had greater diversity and dominance. Metabarcoding yielded 310,076 reads, representing 112 assigned taxa (106 from Antonia, 27 from Jean), dominated by *Ascomycota*, *Basidiomycota*, and *Mortierellomycota*, but also including *Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota*, *Chytridiomycota*, *Monoblepharomycota*, *Mucoromycota*, *Rozellomycota*, and *Zoopagomycota*. *Betamyces* sp. and Fungal sp. were most abundant in both lakes. Both approaches revealed dominance of *Ascomycota*, *Basidiomycota*, and *Mortierellomycota*, contrasting with other Antarctic systems often dominated by cosmopolitan fungi. Psychrophilic/endemic fungi were highly dominant among culturable taxa and moderately so in metabarcoding data, suggesting that these lakes harbor pioneer organisms adapted to polyextremophilic conditions, serving as reservoirs for Antarctic-specific fungi. Combining cultivation and metabarcoding provided a more complete characterization of fungal diversity in these ecosystems.

Keywords: Antarctic; taxonomy; extremophiles; fungi; metabarcoding.

Introduction

Deception Island, located in the South Shetland Islands close to the Antarctic Peninsula, is a large, active, Quaternary volcano that has erupted multiple times over recorded history

(Smellie 2001; Ramos et al. 2017). Between 1967 and 1970, these eruptions devastated much of the island's land surface and vegetation; however, extensive recolonization of more stable surfaces has occurred since 1970, while geothermally heated areas (heated ground, fumaroles) on the island host specific floras exceptional for the Antarctic (Smith 2005). Approximately 57% of Deception Island is currently glaciated, with 47 km² being ice-free. The island's climate is typical of the Maritime Antarctic, cold-oceanic, characterized by frequent summer rainfall, a moderate annual temperature range and high relative humidity (80-90%) (Ramos et al. 2017).

The lake ecosystems of Deception Island vary in origin, morphology and geological characteristics, of which Jean and Antonia Lakes are notable examples. Jean Lake is located in a circular remnant eruption crater, with no outflow, and is fed by two main sources, one to the south originating from a glacier (forming an alluvial fan) and the other fed by snowmelt (forming a shallow drainage channel and alluvial fan). The deepest part of the crater is composed of clay that has been carried by water flow, with moss patches and lichens being frequent in the crater surroundings. Higher elevations are characterized by alluvial fans, which are predominantly composed of ash and dark, reddish, lapilli. Antonia Lake, situated on a rock glacier, exhibits significant fluctuations in depth, and is drained by small underground efflux. Antonia Lake is fed by several small inflows of ice- and snow-melt, some draining from a penguin colony, and moss patches are frequent. The lakebed consists of a clay layer approximately 15 cm thick overlying pyroclasts, and accumulates reddish and black pyroclasts originating from the glacier's surface.

The importance and diversity of fungal communities in Antarctic lakes has long been recognized (Ellis-Evans 1985, 1996; Ogaki et al. 2020a; de Souza et al. 2023; Teixeira et al. 2024). These communities comprise taxa with diverse ecological functions, including those crucial for nutrient cycling under extreme conditions. Their contribution to the primary

decomposition of organic materials is essential for balancing micro- and macronutrients in lake systems (Ogaki et al. 2019, 2020b). This study set out to characterize the diversity of the fungal communities present in sediments of these two lakes on Deception Island using both traditional culturing and DNA metabarcoding approaches.

Materials and methods

Sampling

Sediment samples from Jean and Antonia Lakes, Deception Island (South Shetland Islands, Maritime Antarctica), were collected in February 2023 (Fig. 1; Table 1). Samples from both lakes were collected directly into Whirl-pak bags (Nasco, Ft. Atkinson, WI) in triplicate close to the edge of each lake using a sterilized spoon. All samples were frozen at -20 °C until processing. Additionally, physicochemical parameter of both lakes was measured *in situ* three times over the field period. Sediment temperature was measured using a Digital Thermometer thermocouple with a temperature range of -50 °C to + 300 °C, an accuracy of ± 1 °C, and a resolution of 0.1 °C; temperature, conductivity, pH and total dissolved solids (TDS) of lake water were measured using a Hanna Hi981289 portable tester (Hanna Instruments, Woonsocket, RI, USA).

Sediment chemical analyses

Sediment chemical analyses were performed following EMBRAPA (2017). pH was determined using a 1:2.5 sediment:deionized water ratio. Potential acidity (H + Al) was extracted with 0.5 mol $Ca(OAc)_2$ buffered to pH 7.0 and quantified by titration with 0.0606 mol L^{-1} NaOH. Exchangeable Ca^{2+}, Mg^{2+} and Al^{3+} were extracted with 1 mol L^{-1} KCl, and K^{+} and P^{+} were extracted with Melich. The element levels in the extracts were determined by ICP (Al^{3+}), flame emission (Na^{+}, K^{+}) and photocolorimetry (P) by the ascorbic acid method. Total

organic carbon (TOC) was quantified by wet oxidation using the Walkley–Black method. Total cation exchange capacity (CEC) was calculated as the sum of the bases (Ca^{2+}, Mg^{2+}, K^{+}, Al^{3+}) and potential acidity ($H^{+} + Al^{3+}$). All analyses were performed in triplicate.

Culturable fungal isolation

The samples were thawed and kept at 4 ºC for 24 h before processing and isolation of fungi. The following solid culture media were used (2% agar m/v): 1- Dicloran Rose Bengal Agar (0.5% peptone, 1% glucose, 0.1% KH_2PO_4, 0.05% $MgSO_4$ $7H_2O$, 0.0002% dicloran, 0.0025% rose bengal, 0.01% chloramphenicol); 2- minimum medium (0.025% peptone, 0.5% glucose, 0.698% K_2HPO_4, 0.544% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.11% $MgSO_4$ $7H_2O$, 0.01% chloramphenicol); 3- malt extract medium (bacteriological peptone 1 g L^{-1}, malt extract 20 g L^{-1}, glucose 20 g L^{-1} and agar 20 g L^{-1}); 4- Dicloran Glycerol DG18 Agar (peptic digestion of animal tissue 5 g L^{-1}, dextrose 10 g L^{-1}, monopotassium phosphate 1 g L^{-1}, magnesium sulfate 0.5 g L^{-1}, chloramphenicol 0.1 g L^{-1}, dichloran 0.002 g L^{-1}, glycerol 18 g L^{-1}).

Samples from each lake were processed in triplicate. One gram of subsampled sediment was resuspended in 1 mL of 0.85% saline, from which 100 µL were plated on the different culture media. The plates were incubated at 15 °C for 60 d. The fungal colonies obtained were quantified in colony-forming units, and each isolate was purified in Petri dishes containing the malt extract solid culture medium. The purified fungi were incubated at 15 °C for 7-21 d and preserved in glycerol (15%). They were then deposited in the Microorganisms and Cells Collection of the Institute of Biological Sciences (ICB) of the Federal University of Minas Gerais (UFMG) under the code UFMGCB.

Culturable fungal identification

Fungal cultures were grown on malt extract medium at 15 °C for 14 days, and their mycelia were used for DNA extraction, as described by Rosa et al. (2009). For filamentous fungi, the internal transcribed spacer (ITS) region was amplified using the universal primers ITS1 and ITS4 (White 1990), as described by Rosa et al. (2009). In addition, partial amplification of RNA polymerase II (RPB2) was performed using primers RBP2 5F and RPB2 7R as described by Malkus et al. (2006). Yeasts were grouped and identified following Lachance et al. (1999). Yeast identities were confirmed by sequencing the D1-D2 variable domains of the large subunit rRNA gene using the primers NL1 and NL4. Fungi with query coverage and identity ≥ 99% were considered to represent the same taxon. Representative consensus sequences of the fungal taxa were deposited in the NCBI GenBank database. To achieve species-rank identification based on ITS, RPB2 and NL data, the consensus sequence was aligned with all sequences from related species retrieved from the GenBank database using BLAST. Fungal classification followed Kirk et al. (2011) and the databases MycoBank (http:// www. mycob ank. org) and Index Fungorum (http://www.indexfungorum.org).

Uncultured fungal identification using metabarcoding

Three replicate sub-samples (500 mg) were taken from the sediment samples under strict contamination control conditions. Total DNA was extracted from these using the FastDNA Spin Kit for Soil (MPBIO, Ohio, USA), following the manufacturer's instructions. DNA quality was analyzed by agarose gel electrophoresis (1% agarose in 1 × Trisborate-EDTA) and then quantified using the Quanti-iT™ Pico Green dsDNA Assay (Invitrogen). The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification of fungi (Chen et al. 2010; Richardson et al. 2015) using the universal primers ITS3 and ITS4 (White 1990). Library construction and DNA amplification were performed using the Herculase II Fusion DNA Polymerase Nextera

XT Index Kit V2, following Illumina 16S Metagenomic Sequencing Library Preparation protocol (Part #15044223, Rev. B). Paired-end sequencing (2 × 300 bp) was performed on a MiSeq platform (Illumina) by Macrogen Inc. (South Korea). All quality controls to avoid contamination during DNA extraction, PCR and sequencing were carried out by Macrogen Inc.

Quality analysis was carried out 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 = rl; trimq = 20; ftm = 5; maq = 20. The remaining sequences were imported to QIIME2 version 2021.4 (https://qiime2.org/) for bioinformatics analyses (Bolyen et al. 2019). The qiime2-dada2 plugin was used for filtering, dereplication, turning paired-end fastq files into merged, and removing chimeras, using default parameters (Callahan et al. 2016). Taxonomic assignments were determined for amplicon sequence variants (ASVs) in three steps. First, ASVs were classified using the qiime2-feature-classifier (Bokulich et al. 2018) classify-sklearn against the UNITE Eukaryotes ITS database version 8.3 (Abarenkov et al. 2020). Second, remaining unclassified ASVs were filtered and aligned against the filtered NCBI non-redundant nucleotide sequences (nt) database (October 2024) using BLASTn (Camacho et al. 2009) with default parameters; the nt database was filtered using the following keywords: “ITS1”, “ITS2”, “Internal transcribed spacer” and “internal transcribed spacer”. Third, output files from BLASTn (Camacho et al. 2009) were imported to MEGAN6 (Huson et al. 2016) and taxonomic assignments were performed using the “megan-nucl-Jan2024.db” mapping file with default parameters and trained with Naive Bayes classifier and a confidence threshold of 99%.

Many factors, including extraction, PCR and primer bias, can affect the number of reads obtained (Medinger et al. 2010), and thus lead to misinterpretation of absolute abundance

(Weber and Pawlowski 2013). However, Giner et al. (2016) concluded that such biases did not affect the proportionality between reads and cell abundance, implying that more reads are linked with higher abundance (Deiner et al. 2017; Hering et al. 2018). Therefore, for comparative purposes, we used the number of reads as a proxy for relative abundance. Fungal classification followed Kirk et al. (2011), Tedersoo et al. (2018), MycoBank (http://www.mycobank.org) and the Index Fungorum (http://www.indexfungorum.org).

Fungal diversity

To estimate taxonomic diversity indices and abundance for culturable fungi, the colony-forming units per gram (CFU g^{-1}) of each fungal taxon was used. For uncultured fungi, relative abundances of the ASVs assigned in the metabarcoding analyses were used to quantify the fungal taxa present in the sediments sampled, where fungal ASVs with relative abundance > 10% were considered dominant, those between 1 and 10% as intermediate and those with < 1% as minor (rare) components of the fungal community (Rosa et al. 2021). The numbers of reads were used as a proxy to quantify taxon diversity, richness and dominance. Species accumulation curves were obtained using the Mao Tao index. Diversity, richness and dominance of taxa were assessed using the indices Fisher's α, Margalef's and Simpson's indices, respectively. All diversity results were obtained with 95% confidence, and bootstrap values of 1,000 replicates obtained using PAST v1.9 software (Hammer 2001).

Results

Culturable fungal diversity

A total of 139 fungal isolates were obtained from the lake sediment samples, 84 (60.4%) from Jean Lake and 55 (39.6%) from Antonia Lake. These isolates were identified to represent 13 distinct taxa belonging to the phyla *Ascomycota*, *Basidiomycota* and *Mortierellomycota*

(Suppl. Table 1). *Ascomycota* was the most abundant phylum, represented by 120 isolates (86.3%), followed by *Basidiomycota* with 14 isolates (10.1%) and *Mortierellomycota* with eight isolates (5.7%). Among the fungi obtained from Jean Lake sediment, *Pseudogymnoascus* sp. 1 was the most abundant taxon (52 isolates), while *Pseudogymnoascus* sp. 3 (17 isolates) was the most abundant taxon obtained from Antonia Lake sediments.

Uncultured fungal diversity assessed through metabarcoding

Sediments from Jean Lake and Antonia Lake contained high diversity and abundance of fungal taxa based on environmental DNA (eDNA) analysis. A total of 310,076 DNA reads were obtained, assigned to 112 ASVs (106 from Lake Antonia and 27 from Lake Jean) (Suppl. Table 2). Taxa representing the phyla *Ascomycota*, *Basidiomycota* and *Mortierellomycota* dominated the assigned assemblages; representatives of the phyla *Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota*, *Chytridiomycota*, *Monoblepharomycota*, *Mucoromycota*, *Rozellomycota* and *Zoopagomycota* were also detected. *Betamyces* sp. (*Chytridiomycota*) and Fungal sp. were the most abundant assigned taxa in both lakes and the only taxa assessed as dominant. Twelve taxa exhibited intermediate relative abundance, while the majority of taxa (98) were minor components of the community. In addition, the metabarcoding assignments indicated the detection of the taxa *Sugiyamaella bullrunensis*, *Starmerella floris*, *S. meliponinorum*, *Pseudosydowia indooroopillyensis*, *Chrysosporium vallenarense*, *Heydenia alpina* and *Metschnikowia hawaiiana*, which were not previously recorded from Antarctica.

Comparison of culturable and eDNA assigned fungal communities

The Mao-Tao rarefaction curves for both culturable and eDNA-assigned fungal communities (Fig. 2) approached asymptote, indicating that the sampling effort was sufficient to characterize the communities present in the sampled lake sediments. Table 1 presents the geological data and physicochemical characteristics of the sediments from the two lakes, as well as the Fisher's-α, Margalef's and Simpson's indices for their respective culturable and eDNA-assigned fungal communities. The culturable community in Jean Lake sediment exhibited greater richness, while that in Antonia Lake displayed greater diversity and dominance. In contrast, the eDNA-assigned assemblage present in Antonia Lake showed the highest diversity and richness indices, and low dominance, when compared with that of Jean Lake.

The physical and chemical analyses indicated that the primary distinguishing factor between the lake sediments was their grain size. Jean Lake sediment contained a higher proportion of silt (76%), while Antonia Lake sediment contained less silt (43%) and more sand (52%), the latter more than double the proportion present in Jean Lake (21%). In both lake sediments, the clay content was minimal, not exceeding 5%. This suggests a sedimentary origin linked to predominantly physical periglacial processes, particularly cryoclasts, with limited chemical mineral neoformation. The dominant textures were silty loam in Jean Lake and sandy loam in Antonia Lake. The higher silt proportion in Jean Lake may be related to its larger specific surface area, which favors the adsorption of chemical elements and may explain the slightly greater availability of these elements in this environment. Both lakes were hypereutrophic, with base saturation (BS) close to or equal to 100%, resulting in high cation exchange capacity (CEC), both potential and effective. CEC values were slightly higher in Jean Lake. Sediment pH was similar in both lakes and in equilibrium with the lake water pH. High sodium levels likely result from the primary dissolution of minerals from surrounding volcanic rocks and/or sea spray from the neighboring coast.

The Venn diagram (Fig. 3) illustrates the distribution of assigned taxa between the two lakes. Amongst the culturable community, five taxa were unique to Jean Lake, four to Antonia Lake and four shared (Fig. 3a). Of the eDNA-assigned taxa, only six taxa were unique to Jean Lake, contrasting with 84 unique to Antonia Lake, and 21 taxa were shared (Fig. 3b). However, comparing the cultivable and eDNA-assigned fungal communities (Fig. 3c), only *Penicillium* sp. and *Oidiodendron truncatum* were shared between the Antonia Lake eDNA assemblage and the culturable Jean Lake assemblage.

Discussion

Culturable fungal diversity

Lakes have been recognized as important reservoir of fungal diversity in the Maritime Antarctic, where a complex ecological web exists even under the prevailing extreme conditions (Ogaki et al. 2020a, 2020b). Our data indicate that fungal communities in sediments from Jean Lake and Antonia Lake on Deception Island are dominated by representatives of the genus *Pseudogymnoascus* (formerly *Geomyces*), a genus frequently reported in Antarctic environments (Rosa et al. 2019). *Pseudogymnoascus* species are known to be keratinophilic, psychrophilic, halotolerant and capable of utilizing cellulose (Hayes 2012). Gomes et al. (2024) recently note that some *Pseudogymnoascus* strains obtained from various substrates and habitats in Maritime Antarctica may possess potential virulence *in vitro* and *in vivo* towards experimental animal hosts, particularly those that are immunocompromised. Furthermore, Gomes et al. (2024) also highlighted that, as the Antarctic environment provides a reservoir for a diversity of representatives of *Pseudogymnoascus*, increasing temperatures in Maritime Antarctica as the global climate warms could activate dormant genes or biochemical pathways, select for virulent species and/or strains, and facilitate their dissemination within and beyond the region.

The genus *Antarctomyces* includes only two known species, *A. psychrotrophicus* and *A. pellizariae*, both psychrophilic and endemic to Antarctica (Stchigel et al. 2001; de Menezes et al. 2017). *Antarctomyces psychrotrophicus* is widely distributed in Maritime Antarctica and has been identified in various habitats, including soils (Stchigel et al. 2001; Gomes et al. 2018), plants (Rosa et al. 2009; Coelho et al. 2021), lake water (Gonçalves et al. 2012) and lake sediments (Ogaki et al. 2020a). *Mortierella* appears to be ubiquitous in Antarctica, reported in soil (Newsham et al. 2018; Gomes et al. 2018), associated with plants (Gonçalves et al. 2015), snow (de Menezes et al. 2019) and lacustrine environments (Ogaki et al. 2020a, 2020b; de Souza et al. 2023).

The genus *Holtermanniella* is commonly reported in temperate and cold regions. Two of the five known species (*H. wattica* and *H. nyarrowii*) were originally collected in Antarctica (Wuczkowski et al. 2011). The genus *Penicillium* has a global distribution and some members are of significant economic and societal importance due to their ecological functions as decomposers of organic matter through the production of hydrolytic enzymes, abilities to cause severe rots as pre- and post-harvest pathogens in food crops, and the production of a wide variety of mycotoxins and antibiotics (Rastegari et al. 2020). *Periconia* is a filamentous fungus that has also attracted interest due to its production of bioactive metabolites with antimicrobial, antiviral, cytotoxic and anti-inflammatory activities (Azhari and Supratman 2021). In Antarctica, the genus has been reported in lakes (Ogaki et al. 2020b) and on rock surfaces (de Menezes et al. 2021).

Entomortierella is the genus with the greatest morphological and ecological diversity within the family *Mortierellaceae*. The fungal family includes species associated with insects, arthropods and worms, which are present in soil, decaying plant matter and roots (Telagathoti et al. 2022). *Nadsonia* is a rare yeast genus found primarily in tree exudates and soils (Smith 2011). In Antarctica, it has been reported from soil (Martorell et al. 2017), including in

association with penguin guano, and from marine sediment (Vaz et al. 2011). Its presence in sediment from Antonia Lake may be linked to the guano produced in the neighboring penguin colony.

Fungal diversity assessed through eDNA metabarcoding

DNA metabarcoding of eDNA samples commonly reveals the potential presence of much higher cryptic fungal diversity than detected in culture-based approaches in different Antarctic ecosystems/habitats, including communities in Maritime Antarctic lakes (de Souza et al. 2021; Rosa et al. 2021; de Souza et al. 2022; Gonçalves et al. 2022). The occurrence and dominance of *Ascomycota* in both lakes studied here, albeit with varying relative abundances and species dominance patterns, aligns with previous studies identifying this phylum as one of the most abundant and diverse in Antarctic ecosystems (Rosa et al. 2020, 2021, 2022; de Souza et al. 2021, 2022; Ogaki et al. 2021; Gonçalves et al. 2022, 2024).

Different fungal taxa were detected in the two studied lakes; however, *Betamyces* sp. (*Chytridiomycota*) was dominant, which may indicate that this zoosporic fungus plays an important ecological role in the studied lakes and, by implication, others in the region. The *Chytridiomycota* are a freshwater zoosporic fungal group that has rarely been reported in Antarctic ecosystems (Rosa et al. 2021). The genus *Betamyces* includes only one known species (*Betamyces americae-meridionalis*), reported from pollen baits in the Paraná River, Buenos Aires, Argentina, and from soil in Costa Rica (Letcher et al. 2012). However, Gonçalves et al. (2022) reported *Betamyces* sp. as a dominant fungus in an eDNA study of lake sediments sampled on James Ross Island, Antarctica. The predominance of an aquatic taxon such as *Betamyces* sp. in Antonia Lake may indicate that the lake has a more hydrologically stable environment, creating favorable conditions for the development of truly aquatic fungi.

Zhang et al. (2020) highlight environmental heterogeneity as a primary structuring factor for microbial communities in Antarctic aquatic ecosystems. Within the eDNA-assigned fungal communities in the sediments of the two studied lakes, a relatively small number of taxa were of intermediate dominance. The high number of DNA reads assigned to *A. pellizariae*, a psychrophilic filamentous fungus previously isolated from Antarctic snow (de Menezes et al. 2017), reinforces its adaptability to cold, mineral-rich, sediments, such as those in Antonia Lake. Conversely, the dominance of *Cyberlindnera* sp. in Jean Lake, a yeast commonly associated with less extreme environments, suggests a less specialized mycobiome or one influenced by external deposition. Additionally, the presence of genera such as *Pseudogymnoascus* and *Penicillium*, frequently found in cold soils and cryogenic environments, is consistent with previous reports from the Antarctic Peninsula (Tosi et al. 2002; Gomes et al. 2018; Rosa et al. 2021; de Souza et al. 2022), reinforcing the suggestion that many of the detected fungi represent widely distributed lineages adapted to cold.

Comparison of culturable and eDNA-assigned fungal diversity

The culturable fungal diversity indices of communities obtained from the sediments of Jean Lake and Antonia Lake were similar, although the number of fungal isolates was higher in the former, and the dominance was lower in the latter. These diversity differences may be due to the geological and physicochemical characteristics of Jean Lake, which has a less extremophilic environment compared to Antonia Lake, including higher water and sediment temperatures, more favorable sediment pH, and experiences the influence of marine spray. In contrast, the eDNA-assigned fungal community of Antonia Lake displayed the highest diversity indices, despite the lake's more extreme environmental conditions. Antonia Lake exhibited lower potential for nutrient exchange in its sediment, higher electrical conductivity values, and lower water and sediment temperatures. These characteristics favor the occurrence

of species adapted to more extreme environments. Antonia Lake also has greater total area, perimeter and depth than Jean Lake. These characteristics may underlie greater accumulation of fungal propagules over time. The differences detected in the diversity and composition of sediment fungal communities between the two lakes may reflect the sensitivity of fungi to environmental variations in polar ecosystems.

Notably, the culturable and eDNA-assigned fungal assemblages shared few taxa between the two studied lakes. Only *Penicillium* sp. and *O. truncatum* were shared between the Lake Antonia eDNA-assigned assemblage and the culturable Jean Lake assemblage. Despite utilizing multiple culture media to maximise recovery of a broad fungal spectrum, we obtained low culturable fungal diversity from the lake sediments. This suggests that inclusion of a greater range of physicochemical characteristics in different culture media might be necessary to recover greater culturable fungal diversity from these sediments. In contrast, metabarcoding suggested that the lake sediments may contain approximately double the fungal diversity than indicated by the traditional culturing approach, with the important caveat that metabarcoding does not confirm the presence of a viable organism.

Conclusions

The sediments of Jean Lake and Antonia Lake on Deception Island host moderate fungal species richness and high abundance. Unlike other Antarctic ecosystems that are often dominated by cold-adapted, widely distributed fungi, both the culturable and eDNA-assigned communities of the sediments were dominated by the phyla *Ascomycota*, *Basidiomycota* and *Mortierellomycota*, reinforcing the dominance of these fungal groups in Antarctic ecosystems. Psychrophilic/endemic fungi, such as taxa representing the genera *Pseudogymnoascus*, *Antarctomyces* and *Mortierella*, displayed high dominance in the culturing part of the study and moderate dominance in the metabarcoding analysis. This may indicate that the lakes of

Deception Island harbor pioneer organisms adapted to the region's polyextremophilic conditions and represent a natural reservoir for fungi unique to the Antarctic region. The metabarcoding assignments indicated the detection of several taxa not previously recorded from Antarctica. Acknowledging the limitations of such assignments, this may indicate recent dispersal events of these organisms to Deception Island, the presence of highly specialized niches, or of cryptic, under-sampled diversity. The simultaneous use of cultivation and DNA metabarcoding approaches in the study provided an efficient methodology for more completely characterizing the diversity of fungal communities present in Antarctic lake ecosystems.

Author Contributions Statement

L.M.D.S. and L.H.R. conceived the study. J.M.L. and S.C. collected the samples. F.S.O. performed the samples physicochemical analysis. F.A.C.L., M.C.S. and P.E.A.S.C. analyzed the bioinformatic data. P.C. analyzed the results. L.M.D.S., L.H.R., F.S.O., J.M.L., S.C., F.A.C.L., M.C.S. and P.E.A.S.C. and P.C. analyzed the results and wrote the manuscript. All authors read and approved of the final manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are available in the NCBI repository, which can be accessed at https://www.ncbi.nlm.nih.gov/.

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Figuras e Tabelas

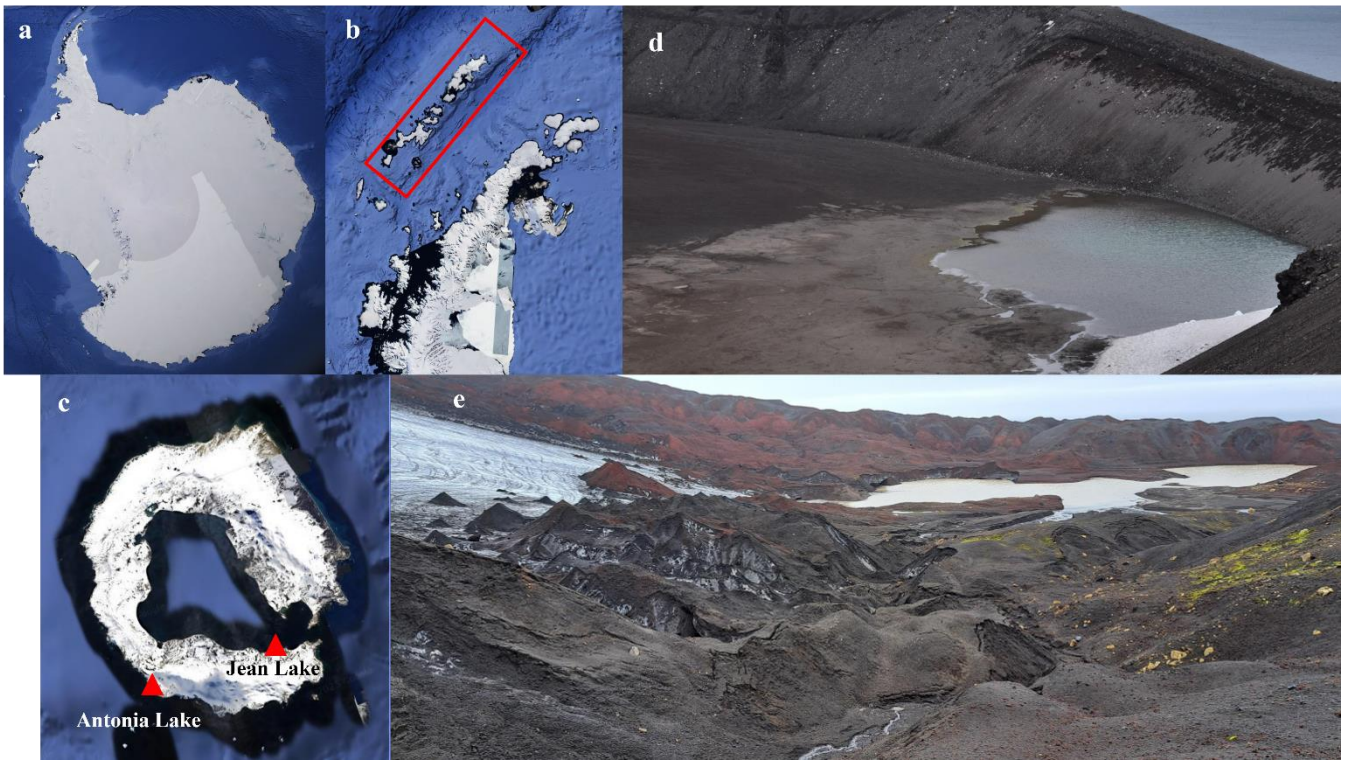


Fig. 1 Locations of Jean Lake and Antonia Lake on Deception Island, South Shetland Islands. (**a**) Antarctica, (**b**) South Shetland Islands, (**c**) Deception Island (images obtained from Google Earth) and photographs of (**d**) Jean Lake (62° 59' 49.04" S 60° 35' 57.07" W) and (**e**) Antonia Lake (62° 59' 41.60" S 60° 43' 5.10" W). Photo credit: Juan Manuel Lirio.

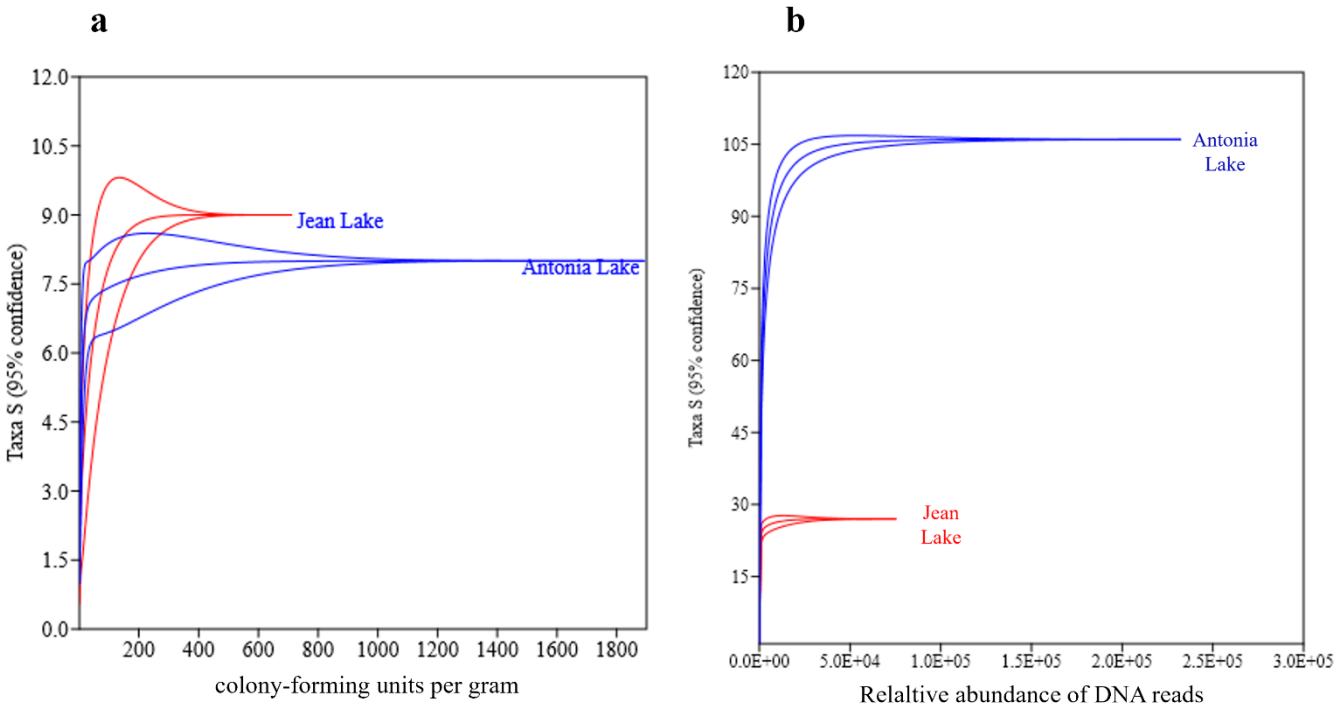
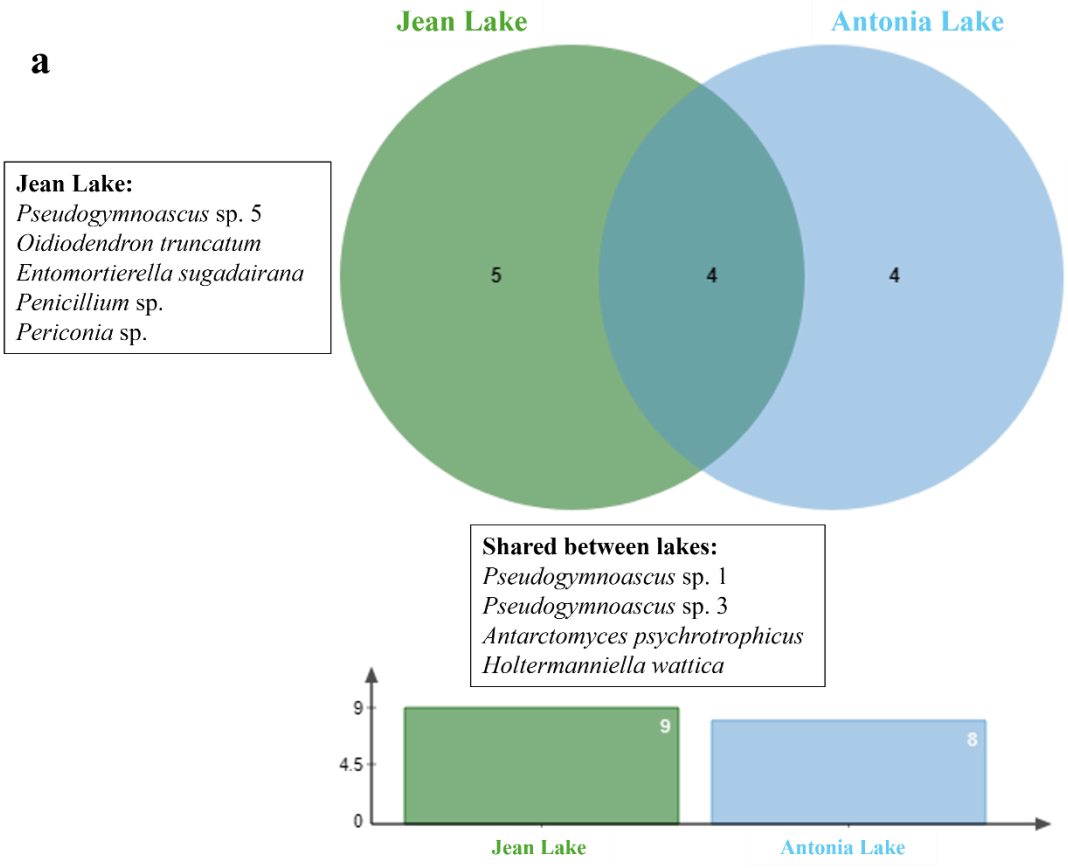
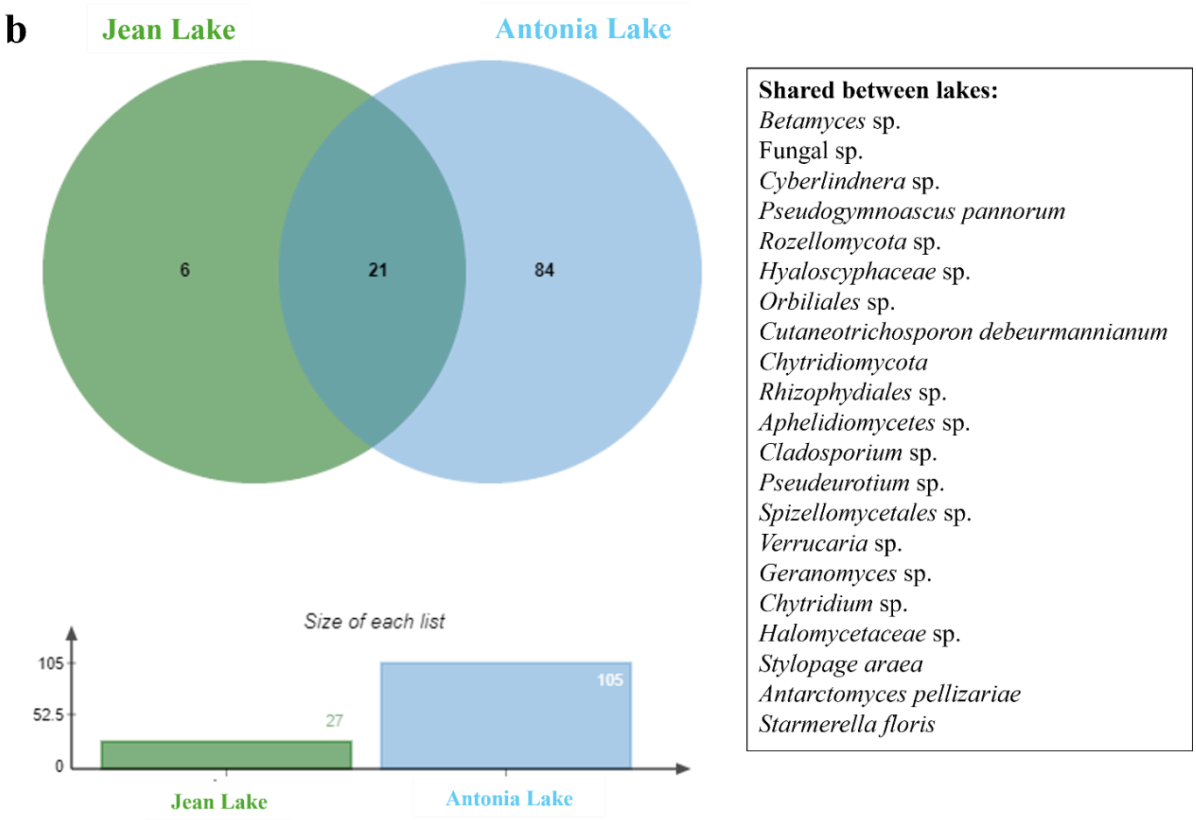


Fig. 2 Rarefaction curves (Mao Tau index) of (**a**) culturable and (**b**) eDNA-assigned fungal communities present in the sediments of Jean Lake (red) and Antonia Lake (blue).

a


Jean Lake
Antonia Lake
5
4
4
Jean Lake:
Pseudogymnoascus sp. 5
Oidiodendron truncatum
Entomortierella sugadairana
Penicillium sp.
Periconia sp.
Antonia Lake:
Mortierella sp.
Pseudogymnoascus sp. 2
Pseudogymnoascus sp. 4
Nadsonia commutata
Shared between lakes:
Pseudogymnoascus sp. 1
Pseudogymnoascus sp. 3
Antarctomyces psychrotrophicus
Holtermanniella wattica
9
4.5
0
9
8
Jean Lake
Antonia Lake

b


Jean Lake
Antonia Lake
6
21
84
Shared between lakes:
Betamyces sp.
Fungal sp.
Cyberlindnera sp.
Pseudogymnoascus pannorum
Rozellomycota sp.
Hyaloscyphaceae sp.
Orbiliales sp.
Cutaneotrichosporon debeurmannianum
Chytridiomycota
Rhizophydiales sp.
Aphelidiomycetes sp.
Cladosporium sp.
Pseudeurotium sp.
Spizellomycetales sp.
Verrucaria sp.
Geranomyces sp.
Chytridium sp.
Halomycetaceae sp.
Stylopage araea
Antarctomyces pellizariae
Starmerella floris
Size of each list
105
52.5
0
27
105
Jean Lake
Antonia Lake

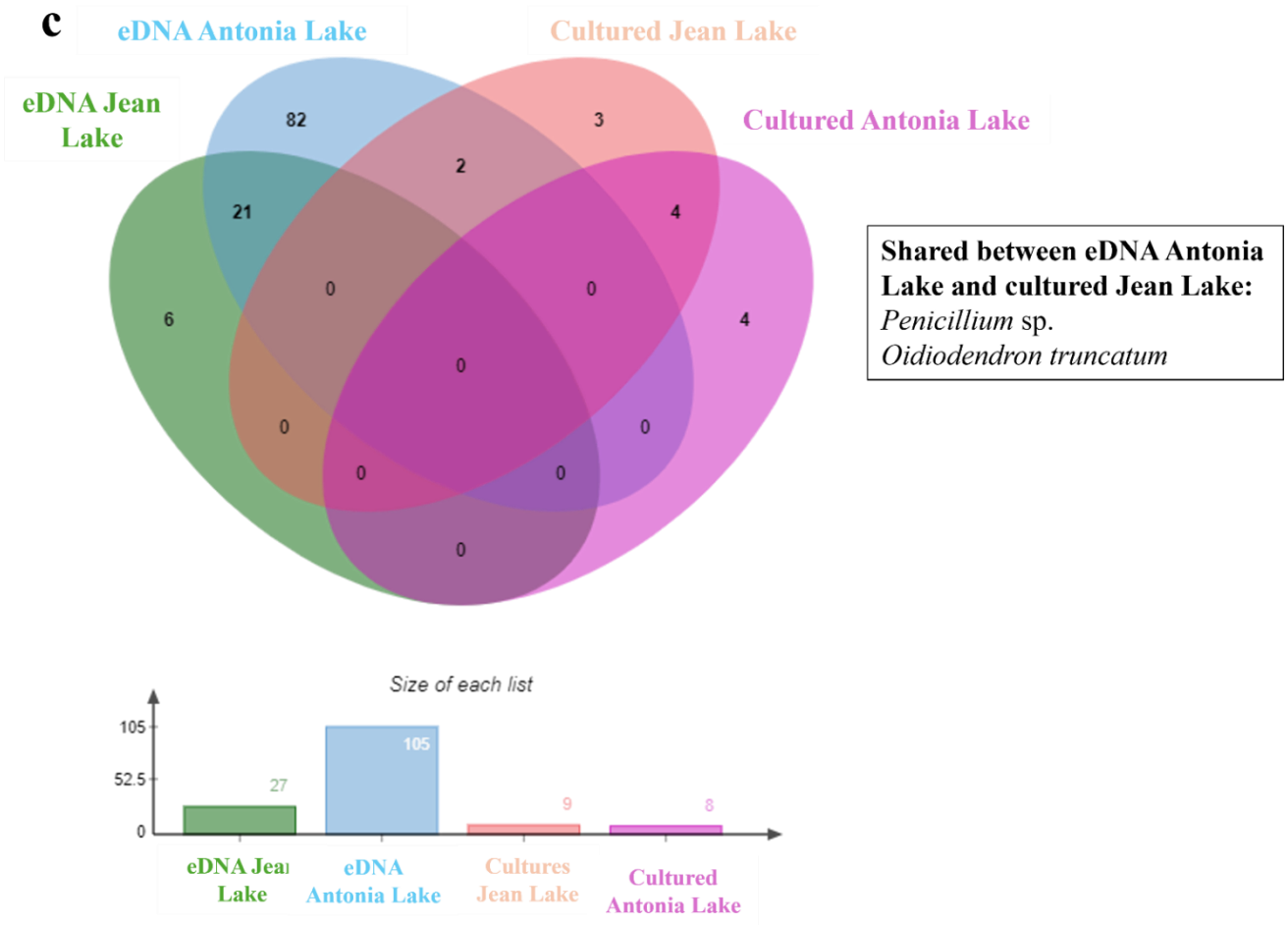


Fig. 3 Venn diagram illustrating the culturable and eDNA-assigned fungal taxa present in sediment samples from Jean Lake and Antonia Lake, Deception Island. Comparison of (**a**) culturable assemblages, (**b**) eDNA-assigned assemblages, and (**c**) between culturable and eDNA-assigned assemblages.

Table 1 Geological characteristics and diversity indices of the sediment fungal communities of Lake Jean and Lake Antonia on Deception Island, South Shetland Islands.

	Lake	
Geological characteristics	**Jean**	**Antonia**
Location	62° 59' 49.04" S 60° 35' 57.07" W	62° 59' 41.60" S 60° 43' 5.10" W
Altitude (meters above sea level)	36	39

Total area (m^2)	3.959	5.773
Perimeter (m)	326	409
Depth (m)	> 2	1 - 10
Form	Crescent moon	Elongated
Lake water pH	8.30	8.30
Conductivity ($\mu S\ cm^{-1}$)	39.2	138
Water temperature (ºC)	7.4	4.1
Sediment temperature (ºC)	6.0	2.8
Sediment physicochemical characteristics		
Texture	Silty loam	Sandy loam
pH (in H_2O)	7.3	6.8
SB ($cmol_C\ dm^{3-1}$)	9.62	8.45
BS (%)	100.0	96.2
CEC (p) ($cmol_C\ dm^{3-1}$)	9.62	8.78
CEC (e) ($cmol_C\ dm^{3-1}$)	9.62	8.45
TOC ($dag\ kg^{-1}$)	0.23	0.23
Na satutration O (%)	25.3	23.7
Culturable fungal diversity		
Total density ($CFUC\ g^{-1}$)	>300	>300
Number of taxa assigned	9	8
Number of fungal isolates	84	55
Fisher's-α	2.56	2.57
Margalef's	1.81	1.75
Simpson's	0.56	0.79
eDNA-assigned fungal diversity		
Total DNA reads	76,496	233,580
Number of taxa	27	106
Fisher's-α	2.63	10.6
Margalef's	2.31	8.494
Simpson's	0.82	0.78

SB = sun of bases, BS = base saturation, CEC = cation exchange capacity, TOC = total organic carbon, $CFU\ g^{-1}$ = colony-forming units per gram.

Suppl. Table 1 Culturable fungi isolated from sediments of Jean Lake and Antonia Lake identified through sequencing of rDNA regions and analysis using the Basic Local Alignment Search Tool (BLASTn).

Lake	Top results on BLASTn (GenBank accession number)	UFMGCB[a]	Number of isolates	Density (CFU g^{-1})	Identity (%)	Query cover (%)	Base pairs analyzed	Proposed taxon (GenBank accession number of sequences)
Jean	*Pseudogymnoascus verrucosus* (OR485038)[b]	20432	4	230	99	100	447	*Pseudogymnoascus* sp. 1 (PV801173)
	Pseudogymnoascus verrucosus (OR485038)[b]	20303	2	>300	99	100	447	*Pseudogymnoascus* sp. 1 (PV801173)
	Pseudogymnoascus verrucosus (OR485038)[b]	20351	3	>300	99	100	460	*Pseudogymnoascus* sp. 1 (PV801175)
	Pseudogymnoascus verrucosus (OR485038)[b]	20369	2	110	99	100	445	*Pseudogymnoascus* sp. 1 (PV801176)
	Pseudogymnoascus verrucosus (OR485038)[b]	20305	7	>300	99	100	442	*Pseudogymnoascus* sp. 1 (PV801177)
	Pseudogymnoascus verrucosus (OR485038)[b]	20418	6	>300	99	100	449	*Pseudogymnoascus* sp. 1 (PV801178)
	Pseudogymnoascus verrucosus (OR485038)[b]	20421	11	>300	99	100	448	*Pseudogymnoascus* sp. 1 (PV801179)
	Pseudogymnoascus verrucosus (OR485038)[b]	20324	2	>300	99	100	449	*Pseudogymnoascus* sp. 1 (PV801180)
	Pseudogymnoascus verrucosus (OR485038)[b]	20355	1	100	99	100	433	*Pseudogymnoascus* sp. 1 (PV801181)
	Pseudogymnoascus verrucosus	20409	2	20	99	100	448	*Pseudogymnoascus* sp. 1

Lake	Top results on BLASTn (GenBank accession number)	UFMGCB[a]	Number of isolates	Density (CFU g^{-1})	Identity (%)	Query cover (%)	Base pairs analyzed	Proposed taxon (GenBank accession number of sequences)
	(OR485038)[b]							(PV801182)
	Pseudogymnoascus verrucosus (OR485038)[b]	20337	1	40	100	100	446	*Pseudogymnoascus* sp. 1 (PV801183)
	Pseudogymnoascus verrucosus (OR485038)[b]	20366	1	90	100	100	458	*Pseudogymnoascus* sp. 1 (PV801184)
	Pseudogymnoascus verrucosus (OR485038)[b]	20368	1	230	99	100	446	*Pseudogymnoascus* sp. 1 (PV801185)
	Pseudogymnoascus verrucosus (OR485038)[b]	20403	4	170	99	100	440	*Pseudogymnoascus* sp. 1 (PV801186)
	Pseudogymnoascus verrucosus (OR485038)[b]	20350	1	130	99	100	457	*Pseudogymnoascus* sp. 1 (PV801187)
	Pseudogymnoascus verrucosus (OR485038)[b]	20356	4	>300	99	100	446	*Pseudogymnoascus* sp. 1 (PV801188)
	Pseudogymnoascus verrucosus (OR485038)[b]	20383	1	20	99	100	450	*Pseudogymnoascus* sp. 1 (PV801189)
	Pseudogymnoascus verrucosus (OR485038)[b]	20420	1	100	100	100	446	*Pseudogymnoascus* sp. 1 (PV801190)
	Pseudogymnoascus lanuginosus (MN417286)[b]	20348	15	>300	100	99	445	*Pseudogymnoascus* sp. 3 (PV801191)
	Pseudogymnoascus lanuginosus (MN417286)[b]	20423	1	140	100	100	447	*Pseudogymnoascus* sp. 3 (PV801192)

Lake	Top results on BLASTn (GenBank accession number)	UFMGCB[a]	Number of isolates	Density (CFU g^{-1})	Identity (%)	Query cover (%)	Base pairs analyzed	Proposed taxon (GenBank accession number of sequences)
	Pseudogymnoascus lanuginosus (MN417286)[b]	20319	1	10	100	98	449	*Pseudogymnoascus* sp. 3 (PV801193)
	Pseudogymnoascus lanuginosus (MN417286)[b]	20385	4	>300	100	99	486	*Pseudogymnoascus* sp. 3 (PV801194)
	Pseudogymnoascus wildlifiparkensis (OR680529)[b]	20340	2	20	99	100	485	*Pseudogymnoascus* sp. 5 (PV801195)
	Pseudogymnoascus wildlifiparkensis (OR680529)[b]	20344	1	10	99	99	450	*Pseudogymnoascus* sp. 5 (PV801196)
	Entomortierella sugadairana (NR182448)[b]	20331	1	10	99	99	514	*Entomortierella sugadairana* (PV801197)
	Antarctomyces psychrotrophicus (MH874317)[b]	20328	1	10	100	100	492	*Antarctomyces psychrotrophicus* (PV801198)
	Penicillium rubens (NR111815)[b]	20390	1	10	100	100	461	*Penicillium* sp. (PV801199)
	Periconia submersa (NR_158842)[b]	20410	1	10	96	97	412	*Periconia* sp. (PV801200)
	Oidiodendron truncatum (NR111036)[b]	20349	3	30	99	100	486	*Oidiodendron truncatum* (PV801201)
	Holtermanniella wattica (NG058307)[c]	20414	1	10	100	100	519	*Holtermanniella wattica* (PV801210)
Antonia	*Pseudogymnoascus verrucosus* (OR485038)[b]	20394	3	>300	99	100	408	*Pseudogymnoascus* sp. 1 (PV801159)

Lake	Top results on BLASTn (GenBank accession number)	UFMGCB[a]	Number of isolates	Density (CFU g^{-1})	Identity (%)	Query cover (%)	Base pairs analyzed	Proposed taxon (GenBank accession number of sequences)
	Pseudogymnoascus verrucosus (OR485038)[b]	20377	8	>300	99	100	447	*Pseudogymnoascus* sp. 1 (PV801160)
	Pseudogymnoascus verrucosus (OR485038)[b]	20375	1	210	99	100	450	*Pseudogymnoascus* sp. 1 (PV801161)
	Pseudogymnoascus appendiculatus (OR485252)[b]	20376	1	250	98	100	436	*Pseudogymnoascus* sp. 2 (PV801162)
	Pseudogymnoascus lanuginosus (MN417286)[b]	20481	11	>300	100	99	442	*Pseudogymnoascus* sp. 3 (PV801163)
	Pseudogymnoascus lanuginosus (MN417286)[b]	20483	2	120	100	100	445	*Pseudogymnoascus* sp. 3 (PV801164)
	Pseudogymnoascus lanuginosus (MN417286)[b]	20458	1	50	100	99	480	*Pseudogymnoascus* sp. 3 (PV801165)
	Pseudogymnoascus lanuginosus (MN417286)[b]	20428	3	>300	100	98	446	*Pseudogymnoascus* sp. 3 (PV801166)
	Pseudogymnoascus roseus (OR485022)[b]	20400	1	130	98	100	447	*Pseudogymnoascus* sp. 4 (PV801167)
	Antarctomyces psychrotrophicus (MH874317)[b]	20353	3	>300	100	100	433	*Antarctomyces psychrotrophicus* (PV801168)
	Antarctomyces psychrotrophicus (MH874317)[b]	20457	1	190	100	100	475	*Antarctomyces psychrotrophicus* (PV801169)
	*Mortierella elongatul*a	20461	1	>300	95	100	504	*Mortierella sp.* (PV801170)

Lake	Top results on BLASTn (GenBank accession number)	UFMGCB[a]	Number of isolates	Density (CFU g^{-1})	Identity (%)	Query cover (%)	Base pairs analyzed	Proposed taxon (GenBank accession number of sequences)
	(MH859811)[b]							
	*Mortierella elongatul*a (MH859811)[b]	20449	3	>300	96	100	556	*Mortierella sp.* (PV801171)
	*Mortierella elongatul*a (MH859811)[b]	20474	1	>300	95	100	535	*Mortierella sp.* (PV801172)
	Nadsonia commutata (NG055213)[c]	20469	1	10	100	100	504	*Nadsonia commutata* (PV801202)
	Holtermanniella wattica (NG058307)[c]	20413	1	50	100	100	517	*Holtermanniella wattica* (PV801203)
	Holtermanniella wattica (NG058307)[c]	20415	1	30	100	100	520	*Holtermanniella wattica* (PV801204)
	Holtermanniella wattica (NG058307)[c]	20467	2	70	100	100	487	*Holtermanniella wattica* (PV801205)
	Holtermanniella wattica (NG058307)[c]	20477	4	270	100	100	486	*Holtermanniella wattica* (PV801206)
	Holtermanniella wattica (NG058307)[c]	20468	1	10	100	100	525	*Holtermanniella wattica* (PV801207)
	Holtermanniella wattica (NG058307)[c]	20360	2	80	100	100	535	*Holtermanniella wattica* (PV801208)
	Holtermanniella wattica (NG058307)[c]	20372	1	10	100	100	534	*Holtermanniella wattica* (PV801209)

[a]UFMGCB = Collection of Microorganisms and Cells of the Federal University of Minas Gerais; bp = base pairs. Taxa were subjected to BLAST analysis based on [b]ITS, [c]Domain D1-D2, and [d]RPB2.

Suppl. Table 2 Relative abundances of fungal amplicon sequence variants assigned from lake sediment samples obtained from Jean Lake and Antonia Lake, Deception Island, Maritime Antarctica.

Database	Phylum	Taxa	Number of DNA reads			Relative abundance (%)
			Jean Lake	Antonia Lake	Total	
Unite	*Aphelidiomycota*	*Aphelidiomycetes* sp.	1,022	249	1,271	0.4099
	Ascomycota	*Cyberlindnera* sp.	14,693	42	14,735	4.7521
		Antarctomyces pellizariae	10	12,548	12,558	4.0500
		Pseudogymnoascus pannorum	8,571	3,764	12,335	3.9781
		Meyerozyma guilliermondii	0	5,168	5,168	1.6667
		Hyaloscyphaceae sp.	1,993	1,617	3,610	1.1642
		Penicillium sp.	0	3,512	3,512	1.1326
		Pseudeurotium sp.	594	2,267	2,861	0.9227
		Chrysosporium vallenarense	0	2,603	2,603	0.8395
		Acremonium biseptum	0	2,476	2,476	0.7985
		Starmerella floris	7	1,856	1,863	0.6008
		Orbiliales sp.	1,469	193	1,662	0.5360
		Chaetothyriales sp.	1,648	0	1,648	0.5315
		Arthrodermataceae sp.	0	1,427	1,427	0.4602
		Thelebolus sp.	0	1,211	1,211	0.3905
		Candida orthopsilosis	0	993	993	0.3202
		Pseudosydowia indooroopillyensis	0	898	898	0.2896
		Myriolecis sp.	0	856	856	0.2761
		Nadsonia sp.	0	782	782	0.2522
		Cladosporium sp.	628	69	697	0.2248
		Saccharomycetales sp.	0	606	606	0.1954
		Coleophoma sp.	0	576	576	0.1858
		Verrucaria sp.	417	106	523	0.1687
		Aureobasidium pullulans	0	284	284	0.0916

Starmerella apicola	0	284	284	0.0916
Blastobotrys sp.	0	274	274	0.0884
Pleosporales sp.	0	236	236	0.0761
Neobulgaria sp.	233	0	233	0.0751
Candida haemuloni	0	227	227	0.0732
Helotiales sp.	0	226	226	0.0729
Pseudeurotiaceae sp.	0	213	213	0.0687
Pyronemataceae sp.	0	192	192	0.0619
Bryostigma muscigenum	0	180	180	0.0581
Oidiodendron truncatum	0	175	175	0.0564
Starmerella etchellsii	0	165	165	0.0532
Heydenia alpina	0	135	135	0.0435
Metschnikowia hawaiiana	0	128	128	0.0413
Sugiyamaella bullrunensis	0	99	99	0.0319
Rhizoscyphus sp.	0	98	98	0.0316
Sordariales sp.	0	95	95	0.0306
Saccharomyces bayanus	0	89	89	0.0287
Chrysosporium ovalisporum	0	87	87	0.0281
Aureobasidium sp.	0	86	86	0.0277
Wickerhamomyces anomalus	0	80	80	0.0258
Lachancea thermotolerans	0	52	52	0.0168
Acaulium caviariforme	0	47	47	0.0152
Starmerella meliponinorum	0	45	45	0.0145
Lecanorales sp.	0	42	42	0.0135
Nectriaceae sp.	0	32	32	0.0103
Wickerhamiella versatilis	0	32	32	0.0103
Microdochium panattonianum	30	0	30	0.0097
Starmerella stellata	0	21	21	0.0068
Knufia petricola	0	15	15	0.0048
Onygenaceae sp.	0	14	14	0.0045
Wickerhamomyces spegazzinii	0	8	8	0.0026

	Trimmatothelopsis smaragdula	0	7	7	0.0023
Basidiobolomycota	*Basidiobolales* sp.	0	448	448	0.1445
Basidiomycota	*Cutaneotrichosporon debeurmannianum*	1,431	54	1,485	0.4789
	Filobasidiella sp.	0	1,386	1,386	0.4470
	Cryptococcus watticus	0	1,294	1,294	0.4173
	Malassezia restricta	334	0	334	0.1077
	Microbotryomycetes sp.	285	0	285	0.0919
	Papiliotrema laurentii	0	211	211	0.0680
	Cantharellales sp.	0	153	153	0.0493
	Hannaella sp.	0	68	68	0.0219
	Dioszegia butyracea	0	48	48	0.0155
	Moniliella fonsecae	0	36	36	0.0116
	Camptobasidiaceae sp.	0	33	33	0.0106
	Moniliella sp.	0	29	29	0.0094
	Glaciozyma martinii	0	27	27	0.0087
	Cryolevonia giraudoae	0	21	21	0.0068
	Malassezia globosa	0	21	21	0.0068
	Trechispora sp.	0	19	19	0.0061
	Moniliella acetoabutans	0	13	13	0.0042
	Serendipita sp.	0	12	12	0.0039
Blastocladiomycota	*Blastocladiomycota* sp.	0	1,475	1,475	0.4757
	Blastocladiales sp.	0	85	85	0.0274
Chytridiomycota	*Betamyces* sp.	10,338	100,887	111,225	35.8702
	Rhizophydiales sp.	1,034	3,757	4,791	1.5451
	Geranomyces sp.	308	59	367	0.1184
	Chytridium sp.	197	137	334	0.1077
	Lobulomycetales sp.	0	331	331	0.1067
	Halomycetaceae sp.	135	25	160	0.0516
Monoblepharomycota	*Monoblepharis* sp.	367	0	367	0.1184
	Monoblepharidales sp.	0	89	89	0.0287
Mortierellomycota	*Mortierella turficola*	0	10,112	10,112	3.2611

		Mortierellaceae sp.	0	144	144	0.0464
		Mortierella sarnyensis	0	138	138	0.0445
		Mortierella polycephala	0	104	104	0.0335
		Linnemannia amoeboidea	0	52	52	0.0168
		Linnemannia sclerotiella	0	24	24	0.0077
	Mucoromycota	*Pirella circinans*	0	6	6	0.0019
	Rozellomycota	*Rozellomycota* sp.	4,262	4,555	8,817	2.8435
	Zoopagomycota	*Acaulopage dichotoma*	0	39	39	0.0126
GenBank	*Ascomycota*	*Ascomycota*	0	11,793	11,793	3.8033
		Neolectales sp.	0	329	329	0.1061
		Glomerellales sp.	0	224	224	0.0722
		Arthonia phaeobaea	0	20	20	0.0065
		Saccharomycetes sp.	0	17	17	0.0055
		Gyalidea fritzei	0	15	15	0.0048
	Basidiomycota	*Polyporales* sp.	0	172	172	0.0555
		Agaricales sp.	0	42	42	0.0135
		Basidiomycota	0	43	43	0.0139
	Chytridiomycota	*Chytridiomycota*	1,283	3,012	4,295	1.3851
		Spizellomycetales sp.	589	1,327	1,916	0.6179
		Rhizophydiales sp.	0	107	107	0.0345
	Cryptomycota	*Cryptomycota*	0	121	121	0.0390
	Mucoromycota	*Mortierellales* sp.	0	125	125	0.0403
		Archaeosporales sp.	0	36	36	0.0116
	Zoopagomycota	*Zoopagomycota* sp.	0	3,159	3,159	1.0188
		Stylopage araea	84	1,316	1,400	0.4515
	Fungi	Fungal sp.	24,534	38,343	62,877	20.2779

Green indicates dominant, blue intermediate, and orange minor relative abundance (see Methods).

4. DISCUSSÃO INTEGRADA

Comunidades cultiváveis dos lagos

Estudos acerca da diversidade de fungos cultiváveis presentes em lagos da Antártica vêm aumentando nos últimos anos (Ellis-Evans, 1985, 1996; Vincent, 2000; Golçalves *et al*., 2012; Ogaki *et al*., 2020a, 2020b, de Souza *et al*., 2023; Teixeira *et al*., 2024), mas ainda necessitam de mais aprofundamento visto ao grande número de lagos presentes na Antártica. A análise dos fungos cultiváveis revelou uma diversidade notável nos sedimentos dos quatro lagos estudados, com predominância de fungos cosmopolitas e adaptados a ambientes frios. *Pseudogymnoascus* foi o gênero mais representativo, particularmente nos sedimentos dos lagos Jean e Antonia (78,4% dos isolados), mas também amplamente presente nos lagos Florencia e Katerina, o que destaca seu papel ecológico recorrente na Antártica (Rosa *et al*., 2019). Esses fungos psicrofílicos apresentam notável capacidade de degradação de matéria orgânica e tolerância a condições extremas, sendo comumente encontrados em solos, rochas, sedimentos e organismos antárticos (Hayes, 2012; Rosa *et al*., 2019).

Além de *Pseudogymnoascus*, os gêneros *Penicillium*, *Thelebolus*, *Cladosporium* e *Tetracladium* foram encontrados em múltiplos lagos, evidenciando um núcleo de fungos cultiváveis amplamente distribuídos nos ambientes lacustres antárticos. A presença de *Antarctomyces* nos sedimentos dos lagos Jean e Antonia, gênero endêmico da Antártica, reforça o papel exclusivo da micobiota polar e sua especialização a nichos criogênicos. O isolamento de *Glaciozyma martinii* e *Leucosporidium muscorum* apenas a partir dos sedimentos do Lago Florencia sugere um microambiente mais favorável à ocorrência de leveduras psicrofílicas raras. Esses organismos possuem características poliextremofílicas e alto potencial para produção de enzimas friotolerantes, como lipases e inulinases (Duarte *et al*., 2016; Kachalkin *et al*., 2023).

Os sedimentos dos lagos Jean e Antonia, apesar de apresentarem menor diversidade cultivável em relação à Katerina, forneceram isolados de gêneros como *Mortierella*, *Graphium* e *Holtermanniella*, os quais são reportados como degradadores de polímeros complexos, produção de metabólitos bioativos e adaptação a ambientes oligotróficos (Wuczkowski *et al*., 2011; Ozimek & Hanaka, 2020; Elkhateeb & Ghoson, 2022). *Dactylaria*, isolado dos sedimentos dos lagos Katerina e Jean, é conhecido por seu potencial predador e bioherbicida, indicando múltiplas funções ecológicas e aplicações possíveis (Gray & Lewis Smith, 1984; Jin *et al*., 2022).

Nos sedimentos do Lago Florencia observou-se a produção de biossurfactantes por táxons como *Pseudogymnoascus* e *Thelebolus*, além de produção de lipídios intracelulares por *Leucosporidium muscorum*, constituindo o primeiro relato dessa capacidade para essa espécie. Além disso, *Penicillium palitans* isolado de Florencia produziu o metabólito fitotóxico (−)-palitantina, com atividade herbicida já relatada anteriormente (Barreto *et al*., 2025). Por outro lado, nenhum dos isolados obtidos dos lagos Jean e Antonia apresentou atividade herbicida detectável, o que reforça a influência das condições locais sobre a expressão metabólica fúngica e indica a importância de expandir a triagem funcional.

Comunidades de fungos independentes de cultivo presentes nos sedimentos dos lagos antárticos

As análises de *metabarcoding* revelaram uma diversidade fúngica expressiva nos sedimentos dos lagos Florencia, Katerina, Jean, Antonia, Soto e Skua, evidenciando que as comunidades microbianas presentes nestes ambientes são ricas, complexas e amplamente estruturadas provavelmente por fatores ambientais locais. Foram detectados filos amplamente relatados na Antártica, como *Ascomycota*, *Basidiomycota*, *Mortierellomycota* e *Mucoromycota* (de Souza *et al*., 2022), bem como aqueles considerados crípticos/raros ou inéditos na região, como *Chytridiomycota*, *Rozellomycota*, *Zoopagomycota*, *Blastocladiomycota*, *Aphelidiomycota* e *Monoblepharomycota*. A presença de ASVs não identificados em nível de gênero ou espécie reforça o papel dos sedimentos antárticos como *hotspots* de diversidade fúngica ainda pouco descrita.

Os dados de diversidade mostraram que os sedimentos do Lago Florencia apresentou os maiores índices de riqueza e equitatividade entre os lagos analisados. Esse resultado pode ser atribuído ao maior aporte hídrico, à conectividade hidrológica com outros lagos da bacia e à presença abundante de musgos e tapetes microbianos em suas margens, que fornecem matéria orgânica e favorecem a deposição de sedimentos ricos em DNA ambiental. Em contraste, os sedimentos do Lago Soto apresentaram os menores índices de diversidade, sendo um ecossistema jovem, pouco colonizado e carente de vegetação terrestre ao redor.

As comunidades independentes de cultivo dos lagos também mostraram diferenças marcantes. Nos sedimentos do Lago Jean a dominância de leveduras, como do gênero *Cyberlindnera*, pode refletir maior influência de deposição aérea ou menor especialização ecológica. Já nos sedimentos do Lago Antoniaa dominância de *Betamyces* sp.

(*Chytridiomycota*), um fungo aquático típico, sugere um sistema lacustre mais estável hidrologicamente, profundo e com maior heterogeneidade química e térmica, o que favorece a especialização de nichos microbianos (Grossart *et al*., 2019). *Antarctomyces pellizariae* também foi dominante no sedimento do Lago Antonia, reforçando sua associação a sedimentos frios e mineralizados (de Menezes *et al*., 2017).

A técnica de metabarcoding também permitiu ainda o primeiro registro antártico de diversos táxons, como *Sugiyamaella bullrunensis*, *Starmerella floris*, *Pseudosydowia indooroopillyensis*, *Chrysosporium vallenarense* e *Metschnikowia hawaiiana*. Esses achados sugerem rotas de colonização recentes, dispersão passiva ou a presença de nichos subexplorados, além de reforçar a necessidade de estudos contínuos para mapear a diversidade oculta desses ambientes.

Do ponto de vista funcional, as comunidades fúngicas independentes de cultivo foram majoritariamente compostas por sapróbios, seguidos por simbiontes e patógenos. Essa distribuição funcional é coerente com padrões já descritos em solo, água, rochas e ar da Antártica (Arenz & Blanchette, 2009; Ogórek *et al*., 2012; Brandão *et al*., 2017; Gomes *et al*., 2018; Alves *et al*., 2019; Rosa *et al*., 2020). Esses fungos exercem papel essencial na ciclagem de nutrientes sob condições extremas, especialmente na decomposição de matéria orgânica em baixas temperaturas e na liberação de compostos essenciais à biota microbiana.

Comparação integrada entre abordagens e lagos

A comparação entre dados das comunidades cultiváveis e independentes de cultivo revela que essas abordagens são complementares. Enquanto os métodos de cultivo permitem isolar linhagens com potencial biotecnológico direto - produção de enzimas, biossurfactantes, lipídios e metabólitos bioativos - a independente de cultivo fornece um panorama muito mais abrangente da diversidade fúngica a partir do DNA ambiental presente, incluindo táxons e grupos ainda não descritos.

Os sedimentos dos lagos Florencia e Antonia se destacaram por apresentarem elevada diversidade e riqueza tanto em ASVs quanto em táxons cultiváveis, embora com perfis distintos: Florencia mais conectado e sujeito a aporte sedimentar constante; Antonia mais estável e quimicamente estratificado. Katerina e Jean, por sua vez, apresentaram características que favoreceram a recuperação de isolados cultiváveis, ainda que com menor diversidade de independentes de cultivo.

Fatores como profundidade, condutividade elétrica, composição mineral dos sedimentos, cobertura vegetal, presença de aves, tipo de rocha da bacia e regime de degelo parecem serem fundamentais para a estruturação das comunidades fúngicas. Em particular, os baixos teores de matéria orgânica total não impediram a formação de comunidades diversificadas, o que sugere que a qualidade da matéria orgânica e a complexidade microambiental exercem papel mais determinante do que a quantidade absoluta.

De forma geral, os dados demonstram que os sedimentos dos lagos antárticos, mesmo geograficamente próximos, abrigam comunidades fúngicas distintas e funcionalmente relevantes. A micobiota desses ambientes permanece amplamente subexplorada e representa uma fonte valiosa de organismos com papel ecológico-chave e potencial para aplicações biotecnológicas em ambientes extremos.

5. CONCLUSÕES GLOBAIS

Os resultados obtidos neste trabalho demonstram que os sedimentos lacustres da Antártica constituem reservatórios altamente diversos de comunidades fúngicas, tanto em termos de táxons cultiváveis quanto de sequências ambientais detectadas pela técnica de *metabarcoding*. As análises revelaram a presença de fungos com ampla distribuição geográfica e reconhecida adaptabilidade a condições extremas, como *Pseudogymnoascus*, *Penicillium* e *Thelebolus*, bem como a ocorrência de linhagens raras, endêmicas ou ainda não relatadas na Antártica, ampliando significativamente o conhecimento sobre a micobiota antártica.

A abordagem integrada entre isolamento de fungos cultiváveis e independentes de cultivo permitiu não apenas caracterizar a diversidade fúngica com maior profundidade, mas também evidenciar diferenças estruturais nas comunidades entre os lagos estudados, relacionadas a variáveis físico-químicas, geológicas e hidrológicas. Destaca-se que os sedimentos dos lagos Florencia e Antonia apresentaram maior riqueza e diversidade, enquanto os dos lagos Katerina e Jean revelaram perfis distintos possivelmente associados à menor profundidade, conectividade hídrica e composição mineral dos sedimentos.

Enquanto os métodos tradicionais de cultivo permitiram a identificação de linhagens com potencial biotecnológico direto - como produtoras de enzimas, lipídios, biossurfactantes e metabólitos com atividade herbicida – o *metabarcoding* revelou uma complexidade microbiana muito mais ampla, incluindo a detecção de táxons ainda não descritos, com implicações para a bioprospecção e o entendimento da ecologia funcional em ambientes polares.

A ausência de correlação direta entre riqueza fúngica e teor de matéria orgânica nos sedimentos, associada à detecção de comunidades fúngicas estruturadas por características ambientais locais, reforça a relevância de abordagens integradas e multiescalares para a compreensão da diversidade e dinâmica dos fungos em ecossistemas extremos.

Diante da fragilidade dos ambientes antárticos frente às mudanças climáticas e da relevância científica e biotecnológica das linhagens detectadas, este estudo ressalta a importância de ampliar os esforços de monitoramento, isolamento e caracterização de fungos da Antártica. A micobiota desses ambientes permanece subexplorada e representa uma fonte valiosa de informação ecológica, evolutiva e aplicada, com grande potencial para contribuir em áreas como saúde, agricultura e energia.

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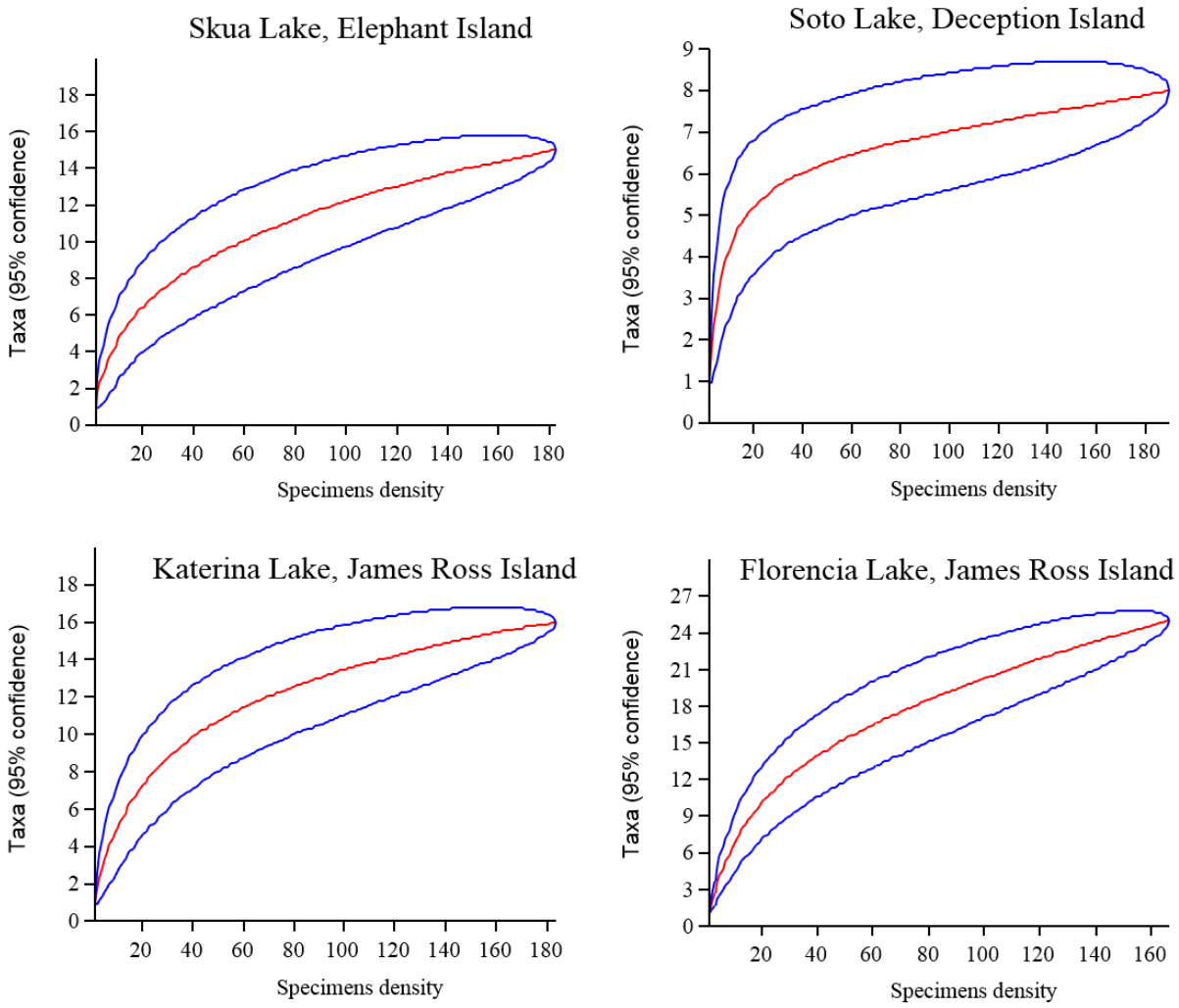
7. ANEXOS

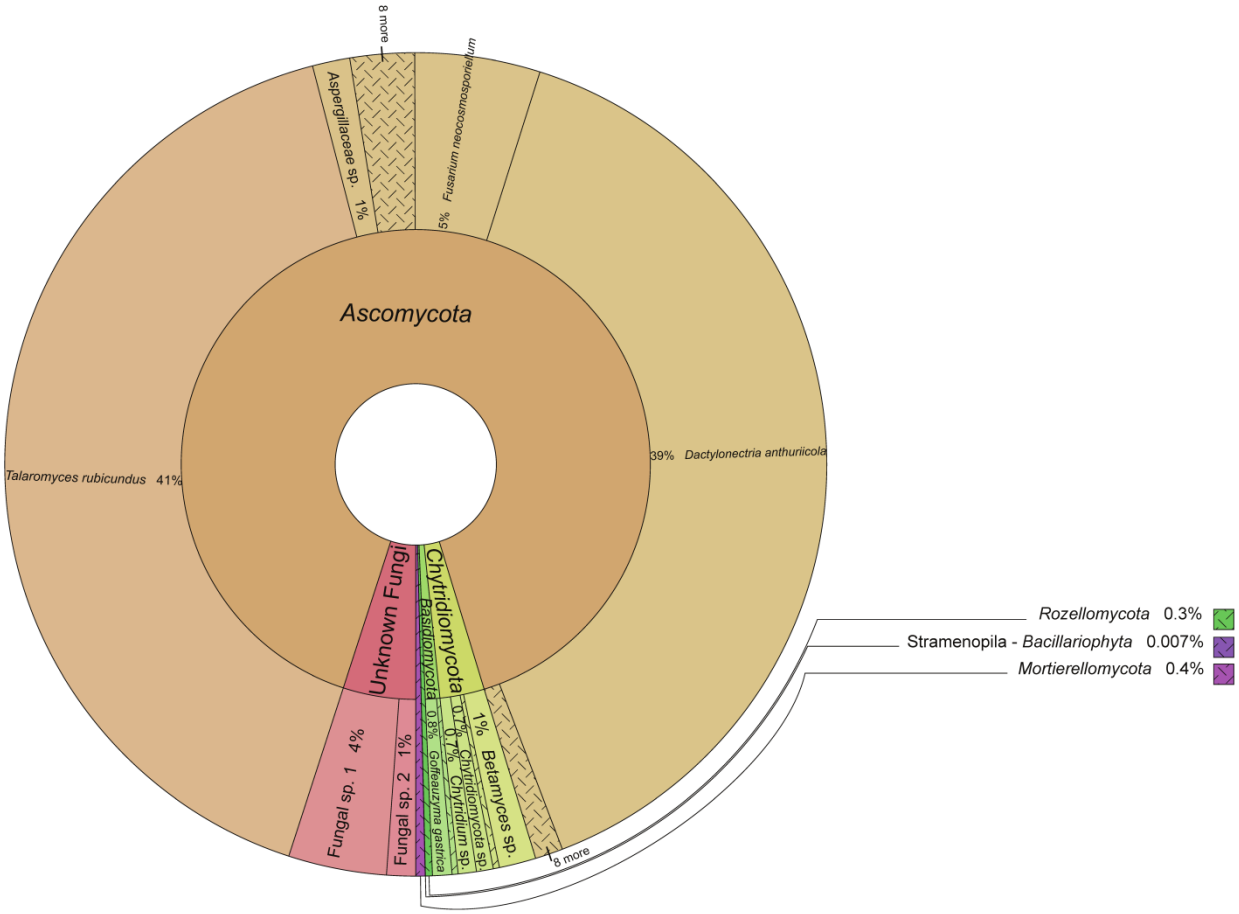
7.1.Material suplementar do Capítulo 03



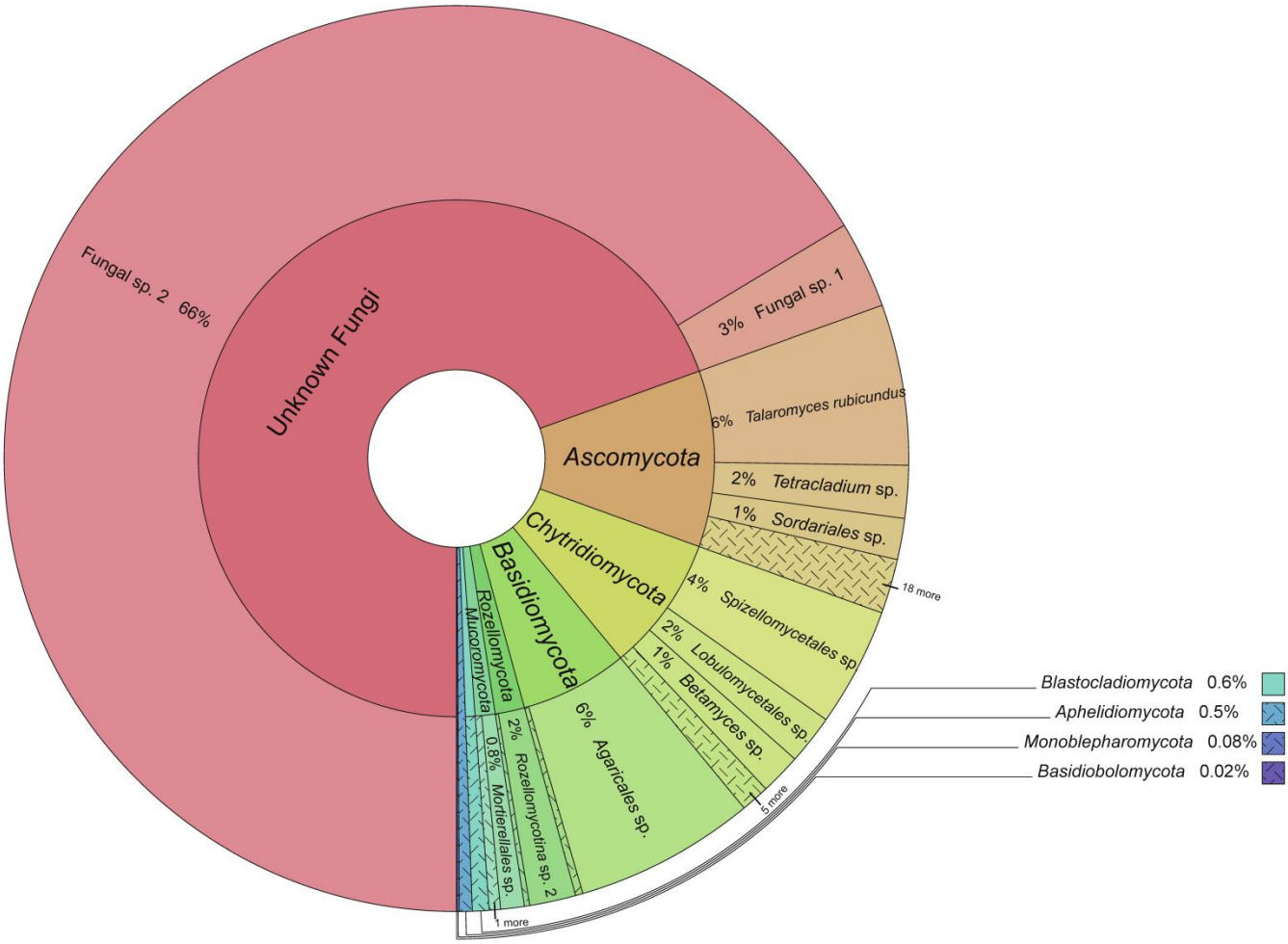
Supplementary Figure S1. Rarefaction curves, with 95% confidence limits, of fungal amplicon sequence variants (ASVs) obtained from the sediments of Skua Lake (Elephant Island), Soto Lake (Deception Island), Katerina Lake and Florencia Lake (James Ross Island).


Camptobasidiaceae sp. 22%
0.8% Mrakia sp.
0.7% Mrakia psychrophila
1 more
3% Glaciozyma sp.
3% Glaciozyma martinii
14% Leucosporidiales sp. 2
13% Phenoliferia psychrophila
1% Tremellomycetes sp. 2
0.7% Mrakia niccombsii
9 more
4% Monoblepharidales sp.
Monoblepharomycota
Fungal sp. 2 20%
Unknown Fungi
Fungal sp. 1 3%
Aspergillaceae sp. 3%
Penicillium nalgiovense 1%
Antarctomyces sp. 0.7%
Penicillium sp. 2%
Neoascochyta paspali 2%
Pseudogymnoascus sp. 0.7%
Ciliophora sp. 1%
Pseudeurotium sp. 1%
Chytridiomycota 0.8%
Glaciozyma antarctica 0.7%
7 more
Ascomycota
Basidiomycota
Mucoromycota 0.06%
Zoopagomycota 0.007%

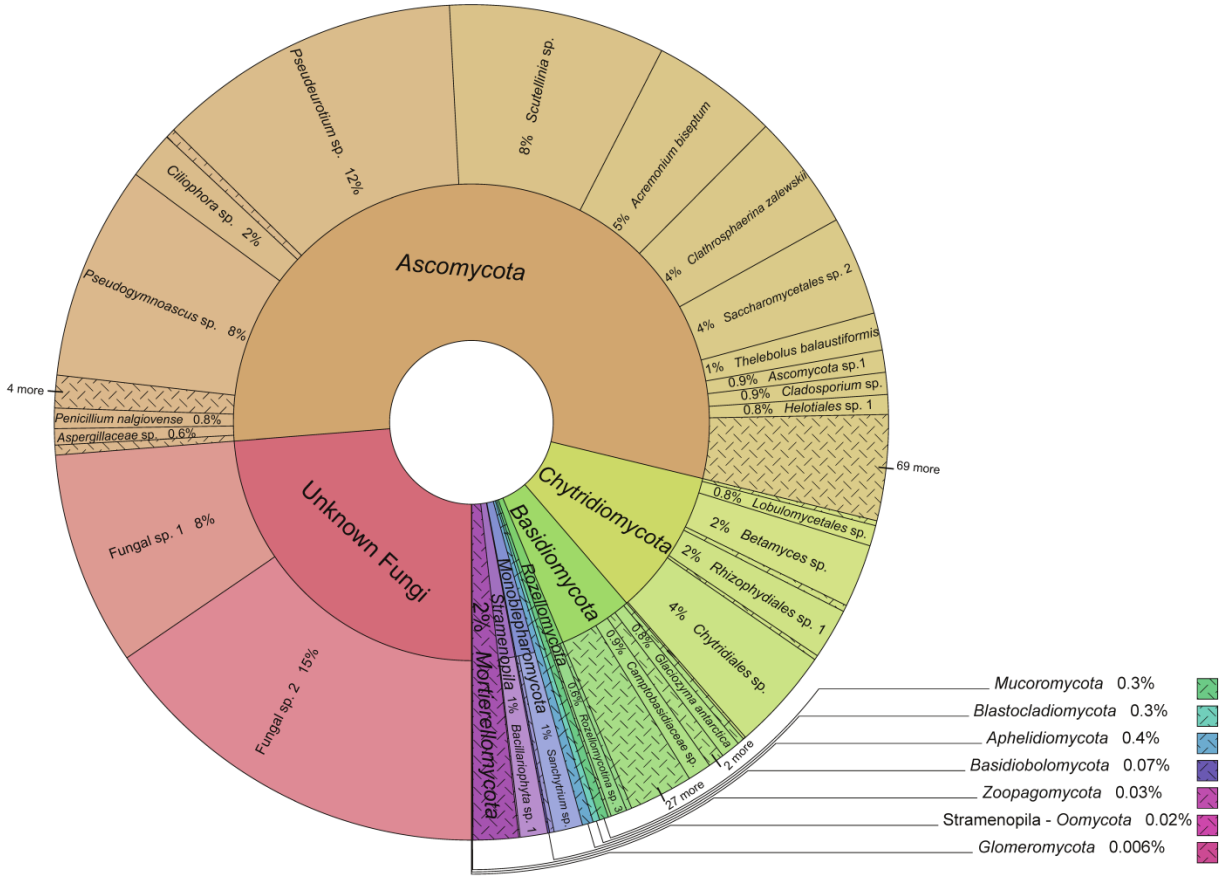
(**a**)


8 more
Aspergillaceae sp. 1%
5% Fusarium neocosmosporiellum
Ascomycota
39% Dactylonectria anthuriicola
Talaromyces rubicundus 41%
Unknown Fungi
Chytridiomycota
Basidiomycota
1% Betamyces sp.
0.7% Chytridiomycota sp. 1
0.7% Chytridium sp.
0.8% Gorfieuzyma gastrica
Fungal sp. 1 4%
Fungal sp. 2 1%
8 more
Rozellomycota 0.3%
Stramenopila - Bacillariophyta 0.007%
Mortierellomycota 0.4%

(b)


Fungal sp. 2 66%
Unknown Fungi
3% Fungal sp. 1
6% Talaromyces rubicundus
2% Tetracladium sp.
1% Sordariales sp.
18 more
Ascomycota
Chytridiomycota
Basidiomycota
Rozellomycota
Mucoromycota
4% Spizellomycetales sp.
2% Lobulomycetales sp.
1% Betamyces sp.
6% Agaricales sp.
2% Rozellomycotina sp. 2
0.8% Mortierellales sp.
1 more
5 more
Blastocladiomycota 0.6%
Aphelidiomycota 0.5%
Monoblepharomycota 0.08%
Basidiobolomycota 0.02%

(**c**)

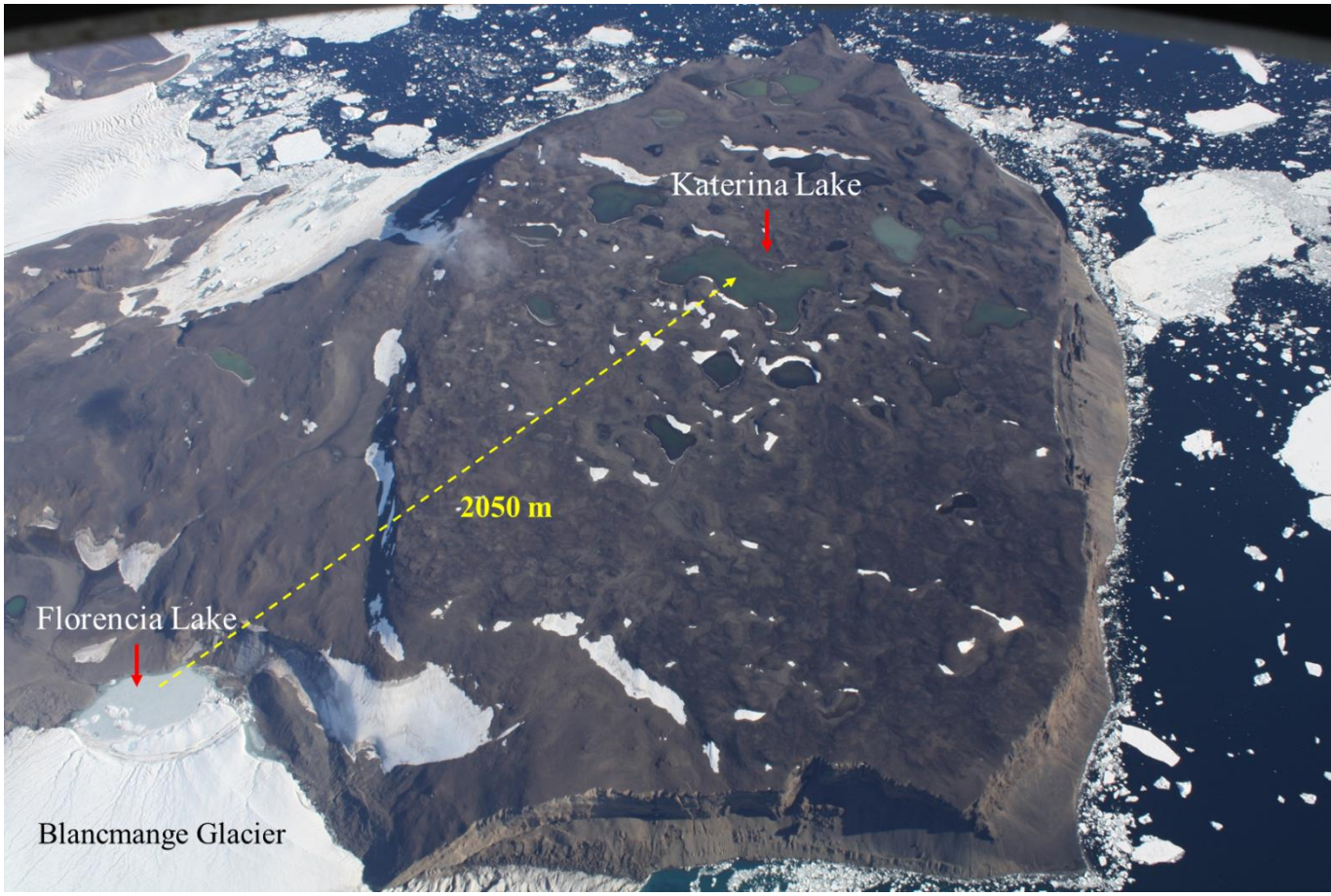


(**d**)

Supplementary Fig. S2. Krona chart showing the abundances of different fungal taxonomic levels detected in sediment samples from (**a**) Skua Lake (Elephant Island), (**b**) Soto Lake, Deception Island, (**c**) Katerina Lake, and (**d**) Florencia Lake, James Ross Island.


Aphelidiomycota
Ascomycota
Basidiomycota
Blastocladiomycota
Chytridiomycota
Glomeromycota
Monoblepharomycota
Mortierellomycota
Mucoromycota
Rozellomycota
Zoopagomycota
Unknown fungi
Stramenopila
Florencia Lake, James Ross Island
Skua Lake, Elephant Island
Soto Lake, Deception Island
Katerina Lake, James Ross Island
−1.5
−0.5
0.5
1.5
Row Z−Score

Supplementary Fig. S3. Heat map of fungal assemblage relative abundances. The color intensities range from red (highest relative abundance) to green (lowest relative abundance). These values represent percentages of DNA fungal reads in Skua Lake (Elephant Island), Soto Lake (Deception Island), Katerina Lake and Florencia Lake (James Ross Island). The heatmap of ASV abundance was performed using the following parameters: Average Linkage, Spearman Rank Correlation, and Z-score among samples for each ASV.



Supplementary Figure S4. Mesa Clearwater plateau showing the distance between Florencia and Katerina lakes at James Ross Island, Antarctica. Photo taken by Juan M. Lírio.

Supplementary Table S1. Relative abundances of the fungal amplicon sequence variants (ASVs) detected in lake sediment samples obtained from Skua Lake (Elephant Island), Soto Lake (Deception Island), Katerina Lake and Florencia Lake (James Ross Island).

				Relative abundance (%) of fungal ASV in Lake/Island				
Source	**Kingdom**	**Phylum**	**Fungal amplicon sequence variant**	**Skua/ Elephant**	**Soto/ Deception**	**Katerina/ James Ross**	**Florencia/ James Ross**	**Total**
UNITE	Fungi	*Ascomycota*	*Talaromyces rubicundus*	0.000**	40.961	5.735	0.000	15.754
			Dactylonectria anthuriicola	0.000	39.311	0.000	0.017	13.663
			Pseudeurotium sp.	1.130	0.000	0.000	11.814	2.852
			Pseudogymnoascus sp.	0.694	0.207	0.049	8.302	2.073
			Scutellinia sp.	0.000	0.000	0.000	8.380	1.894
			Fusarium neocosmosporiellum	0.000	4.925	0.000	0.073	1.728
			Aspergillaceae sp.	2.996	1.470	0.522	0.622	1.273
			Clathrosphaerina zalewskii	0.000	0.390	0.000	4.478	1.148
			Acremonium biseptum	0.000	0.000	0.000	4.928	1.113
			Penicillium nalgiovense	1.051	0.529	0.365	0.790	0.629
			Ciliophora sp.	1.008	0.000	0.043	1.854	0.593
			Tetracladium sp.	0.000	0.000	1.873	0.358	0.578
			Neoascochyta paspali	1.513	0.406	0.137	0.542	0.544
			Thelebolus balaustiformis	0.000	0.000	0.000	1.437	0.325
			Penicillium sp.	1.556	0.000	0.174	0.105	0.321
			Cladosporium sp.	0.000	0.343	0.000	0.850	0.311
			Antarctomyces sp.	0.730	0.000	0.194	0.080	0.187
			Helotiales sp. 1	0.000	0.000	0.000	0.765	0.173
			Nectriaceae sp.	0.000	0.000	0.086	0.537	0.144

	Didymosphaeriaceae sp.	0.136	0.224	0.000	0.115	0.126
	Verrucaria sp.	0.000	0.000	0.000	0.551	0.124
	Candida parapsilosis	0.015	0.235	0.042	0.117	0.122
	Trichoderma sp.	0.000	0.197	0.000	0.188	0.111
	Gliocephalotrichum cylindrosporum	0.000	0.282	0.000	0.000	0.098
	Cheilymenia theleboloides	0.000	0.245	0.000	0.000	0.085
	Lipomyces yarrowii	0.000	0.000	0.000	0.275	0.062
	Didymellaceae sp.	0.000	0.000	0.000	0.205	0.046
	Aspergillus ellipticus	0.000	0.114	0.000	0.000	0.040
	Rhinocladiella sp.	0.000	0.000	0.149	0.000	0.040
	Talaromyces clemensii	0.000	0.114	0.000	0.000	0.040
	Sporocadaceae sp.	0.000	0.104	0.000	0.000	0.036
	Clonostachys rosea	0.000	0.097	0.000	0.000	0.034
	Trichoderma spirale	0.000	0.000	0.000	0.147	0.033
	Periconia epilithographicola	0.000	0.000	0.000	0.143	0.032
	Chaetothyriales sp. 1	0.000	0.000	0.007	0.126	0.030
	Gorgomyces honrubiae	0.000	0.000	0.000	0.131	0.030
	Xylonomycetes sp.	0.000	0.000	0.000	0.117	0.027
	Chaetomiaceae sp.	0.000	0.000	0.000	0.116	0.026
	Fusarium sp.	0.000	0.072	0.000	0.000	0.025
	Tolypocladium sp.	0.000	0.000	0.032	0.065	0.023
	Pyrenochaeta sp.	0.000	0.065	0.000	0.000	0.023
	Saccharomyces sp.	0.096	0.000	0.000	0.022	0.020
	Herpotrichiellaceae sp. 1	0.000	0.000	0.000	0.083	0.019
	Verrucaria alpicola	0.000	0.000	0.000	0.083	0.019
	Helotiaceae sp.	0.000	0.000	0.012	0.062	0.017
	Microdochium phragmitis	0.000	0.000	0.000	0.070	0.016
	Oidiodendron truncatum	0.000	0.000	0.000	0.067	0.015

	Protomyces inouyei	0.093	0.000	0.000	0.000	0.015
	Orbiliaceae sp.	0.000	0.000	0.053	0.000	0.014
	Orbiliales sp. 1	0.000	0.000	0.000	0.060	0.014
	Leohumicola sp.	0.000	0.000	0.000	0.060	0.014
	Alatospora acuminata	0.000	0.000	0.000	0.058	0.013
	Sordariomycetes sp.	0.000	0.000	0.000	0.050	0.011
	Sarocladium sp.	0.000	0.000	0.000	0.042	0.010
	Sporormiaceae sp.	0.000	0.000	0.000	0.041	0.009
	Cyberlindnera jadinii	0.000	0.000	0.000	0.040	0.009
	Toxicocladosporium sp.	0.000	0.000	0.034	0.000	0.009
	Aspergillus thermomutatus	0.000	0.000	0.000	0.039	0.009
	Leotiomycetes sp. 1	0.000	0.000	0.000	0.039	0.009
	Penicillium coffeae	0.053	0.000	0.000	0.000	0.009
	Penicillium mallochii	0.000	0.000	0.000	0.037	0.008
	Porpidia sp.	0.000	0.000	0.000	0.036	0.008
	Stagonospora trichophoricola	0.000	0.000	0.000	0.035	0.008
	Lipomyces kononenkoae	0.000	0.000	0.000	0.035	0.008
	Pyricularia oryzae	0.019	0.000	0.016	0.000	0.007
	Beauveria sp.	0.000	0.000	0.000	0.032	0.007
	Bionectriaceae sp.	0.000	0.000	0.000	0.032	0.007
	Neonectria candida	0.000	0.000	0.000	0.032	0.007
	Cladophialophora sp.	0.000	0.000	0.000	0.029	0.007
	Lipomyces starkeyi	0.000	0.000	0.000	0.028	0.006
	Archaeorhizomyces sp.	0.000	0.000	0.000	0.027	0.006
	Leptosphaeria sclerotioides	0.000	0.000	0.000	0.027	0.006
	Cladophialophora minutissima	0.000	0.000	0.000	0.026	0.006

	Dothideales sp.	0.000	0.000	0.000	0.025	0.006
	Coniosporium sp.	0.000	0.000	0.000	0.024	0.005
	Coniochaeta sp.	0.000	0.000	0.000	0.023	0.005
	Saccharomycopsis fibuligera	0.000	0.000	0.000	0.022	0.005
	Helotiales sp. 2	0.000	0.000	0.000	0.020	0.004
	Clavicipitaceae sp. 1	0.000	0.000	0.000	0.019	0.004
	Herpotrichiellaceae sp. 2	0.000	0.000	0.000	0.018	0.004
	Annulatascaceae sp.	0.000	0.000	0.000	0.017	0.004
	Capnodiales sp.	0.000	0.000	0.000	0.017	0.004
	Pseudeurotiaceae sp.	0.000	0.000	0.000	0.017	0.004
	Diatrypaceae sp.	0.023	0.000	0.000	0.000	0.004
	Candida sp.	0.000	0.000	0.000	0.016	0.004
	Buellia russa	0.022	0.000	0.000	0.000	0.004
	Hyaloscypha sp.	0.000	0.000	0.000	0.015	0.003
	Debaryomyces sp.	0.000	0.000	0.000	0.013	0.003
	Gyoerffyella entomobryoides	0.000	0.000	0.000	0.013	0.003
	Pleosporaceae sp.	0.000	0.000	0.000	0.013	0.003
	Hypocreales sp.	0.000	0.000	0.000	0.012	0.003
	Periconia byssoides	0.000	0.000	0.000	0.012	0.003
	Pezicula sp.	0.000	0.000	0.000	0.011	0.003
	Chloridium sp.	0.000	0.000	0.000	0.010	0.002
	Cenococcum geophilum	0.000	0.000	0.000	0.010	0.002
	Wickerhamomyces anomalus	0.000	0.000	0.000	0.009	0.002
	Iodophanus carneus	0.000	0.000	0.000	0.009	0.002
	Phialocephala sp.	0.000	0.000	0.000	0.009	0.002
	Clavicipitaceae sp. 2	0.000	0.000	0.000	0.007	0.002
	Dothideomycetes sp. 1	0.000	0.000	0.000	0.005	0.001

		Galactomyces reessii	0.000	0.000	0.000	0.002	0.000
	Basidiomycota	*Camptobasidiaceae* sp.	22.120	0.000	0.000	0.918	3.777
		Leucosporidiales sp. 1	13.957	0.000	0.000	0.000	2.252
		Phenoliferia psychrophila	13.445	0.000	0.000	0.000	2.170
		Glaciozyma sp.	3.019	0.000	0.000	0.068	0.503
		Glaciozyma martinii	2.537	0.000	0.000	0.050	0.421
		Glaciozyma antarctica	0.655	0.000	0.064	0.751	0.292
		Goffeauzyma gastrica	0.000	0.763	0.000	0.000	0.265
		Mrakia sp.	0.768	0.000	0.000	0.409	0.216
		Tremellomycetes sp. 1	1.128	0.000	0.000	0.000	0.182
		Mrakia psychrophila	0.713	0.000	0.000	0.167	0.153
		Mrakia niccombsii	0.677	0.000	0.000	0.000	0.109
		Cutaneotrichosporon debeurmannianum	0.004	0.235	0.000	0.029	0.089
		Malassezia restricta	0.086	0.000	0.008	0.313	0.087
		Leucosporidiales sp. 2	0.496	0.000	0.000	0.000	0.080
		Dioszegia fristingensis	0.483	0.000	0.000	0.000	0.078
		Microbotryomycetes sp.	0.000	0.000	0.015	0.315	0.075
		Leucosporidium creatinivorum	0.000	0.000	0.000	0.281	0.063
		Vishniacozyma victoriae	0.351	0.000	0.000	0.000	0.057
		Holocotylon sp.	0.349	0.000	0.000	0.000	0.056
		Glaciozyma watsonii	0.000	0.000	0.000	0.243	0.055
		Solicoccozyma terricola	0.148	0.000	0.000	0.097	0.046
		Holtermanniella takashimae	0.266	0.000	0.000	0.000	0.043
		Tremellomycetes sp. 2	0.235	0.000	0.000	0.000	0.038
		Psathyrellaceae sp.	0.000	0.000	0.000	0.112	0.025
		Wallemia tropicalis	0.000	0.000	0.000	0.087	0.020
		Phanerochaete citri	0.073	0.000	0.000	0.034	0.019

	Malassezia globosa	0.000	0.000	0.000	0.068	0.015
	Agaricomycetes sp. 1	0.000	0.000	0.000	0.067	0.015
	Coprinopsis sp.	0.000	0.000	0.049	0.000	0.013
	Porostereum spadiceum	0.000	0.000	0.000	0.052	0.012
	Dioszegia sp.	0.062	0.000	0.000	0.000	0.010
	Malassezia arunalokei	0.000	0.000	0.000	0.040	0.009
	Naganishia friedmannii	0.000	0.000	0.000	0.029	0.007
	Malassezia sympodialis	0.023	0.000	0.000	0.012	0.006
	Genolevuria amylolytica	0.000	0.000	0.000	0.023	0.005
	Bullera penniseticola	0.000	0.000	0.000	0.022	0.005
	Clavaria sp.	0.000	0.000	0.000	0.016	0.004
	Renatobasidium sp.	0.000	0.000	0.000	0.014	0.003
	Acaromyces ingoldii	0.000	0.000	0.000	0.013	0.003
	Rhodotorula pacifica	0.000	0.000	0.010	0.000	0.003
	Tremellales sp.	0.013	0.000	0.000	0.000	0.002
	Trametes hirsuta	0.000	0.000	0.000	0.009	0.002
	Filobasidiales sp.	0.009	0.000	0.000	0.000	0.001
	Piskurozyma sp.	0.005	0.000	0.000	0.000	0.001
Mortierellomycota	*Mortierellales* sp. 1	0.000	0.367	0.000	0.000	0.127
	Mortierella antarctica	0.000	0.000	0.000	0.503	0.114
	Mortierella sp. 1	0.000	0.000	0.000	0.489	0.110
	Mortierella minutissima	0.000	0.000	0.000	0.335	0.076
	Mortierella sp. 2	0.000	0.000	0.000	0.156	0.035
	Mortierella horticola	0.000	0.000	0.000	0.088	0.020
	Mortierella pseudozygospora	0.000	0.000	0.000	0.050	0.011
	Mortierella humilis	0.000	0.000	0.000	0.050	0.011
	Mortierella basiparvispora	0.000	0.000	0.000	0.036	0.008
	Mortierella alpina	0.000	0.000	0.000	0.029	0.007

			Mortierella gemmifera	0.000	0.000	0.000	0.018	0.004
		Chytridiomycota	*Betamyces* sp.	0.373	1.446	1.464	2.444	1.503
			Spizellomycetales sp. 1	0.000	0.000	4.221	0.000	1.119
			Lobulomycetales sp.	0.000	0.000	1.763	0.798	0.648
			Chytridium sp.	0.000	0.715	0.000	0.099	0.271
			Rhizophydiales sp. 1	0.354	0.000	0.172	0.215	0.151
			Lobulomycetes sp.	0.000	0.000	0.342	0.035	0.098
			Rhizophydiales sp. 2	0.036	0.240	0.007	0.000	0.091
			Chytridiales sp. 1	0.000	0.000	0.028	0.000	0.007
		Monoblepharomycota	*Monoblepharidales* sp.	3.566	0.000	0.000	0.097	0.597
			Sanchytrium sp.	0.000	0.000	0.081	1.112	0.273
			Sanchytriaceae sp.	0.000	0.000	0.000	0.051	0.011
			Monoblepharis sp.	0.000	0.000	0.000	0.027	0.006
		Rozellomycota	*Rozellomycotina* sp. 1	0.000	0.275	1.653	0.209	0.581
			Rozellomycotina sp. 2	0.000	0.000	0.000	0.625	0.141
			Rozellomycotina sp. 3	0.000	0.000	0.186	0.000	0.049
			Rozellomycotina sp. 4	0.000	0.000	0.000	0.117	0.027
		Mucoromycota	*Pirella circinans*	0.000	0.000	0.000	0.012	0.003
			Endogonomycetes sp.	0.000	0.000	0.000	0.004	0.001
		Zoopagomycota	*Zoopagales* sp.	0.000	0.000	0.000	0.004	0.001
			Acaulopage sp.	0.000	0.000	0.000	0.003	0.001
		Aphelidiomycota	*Aphelidiomycetes* sp.	0.000	0.000	0.458	0.420	0.216
		Blastocladiomycota	*Paraphysoderma* sp.	0.000	0.000	0.596	0.000	0.158
		Basidiobolomycota	*Basidiobolales* sp.	0.000	0.000	0.021	0.074	0.022
		Glomeromycota	*Scutellospora* sp.	0.000	0.000	0.000	0.006	0.001
	Straminopila	*Oomycota*	*Saprolegniaceae* sp.	0.000	0.000	0.000	0.020	0.004
BLAST	Fungi	Unknown	Fungal sp. 1	20.205	1.136	66.391	15.467	24.757
			Fungal sp. 2	2.614	3.862	3.118	8.282	4.462
		Ascomycota	*Saccharomycetales* sp. 1	0.000	0.000	0.000	3.842	0.868

		Sordariales sp.	0.000	0.000	1.469	0.027	0.396
		Ascomycota sp.1	0.000	0.000	0.000	0.860	0.194
		Eurotiales sp.	0.000	0.000	0.000	0.243	0.055
		Ascomycota sp. 2	0.000	0.000	0.000	0.102	0.023
		Neolectales sp.	0.000	0.000	0.000	0.091	0.021
		Saccharomycetales sp. 2	0.000	0.000	0.000	0.043	0.010
		Orbiliales sp. 2	0.036	0.000	0.000	0.000	0.006
		Letiomyceta sp.	0.000	0.000	0.000	0.020	0.004
		Chaetothyriales sp. 2	0.000	0.000	0.016	0.000	0.004
		Leotiomycetes sp. 2	0.000	0.000	0.000	0.018	0.004
		Dothideomycetes sp. 2	0.000	0.000	0.011	0.003	0.004
		Leptodiscella sp.	0.000	0.000	0.007	0.000	0.002
	Basidiomycota	*Agaricales* sp.	0.000	0.000	6.461	0.200	1.759
		Polyporales sp.	0.000	0.000	0.000	0.451	0.102
		Basidiomycota sp.	0.000	0.000	0.095	0.062	0.039
		Boletales sp.	0.000	0.000	0.000	0.058	0.013
		Gomphales sp.	0.000	0.000	0.025	0.000	0.007
		Trechisporales sp.	0.000	0.000	0.000	0.017	0.004
		Agaricomycetes sp. 2	0.000	0.000	0.000	0.008	0.002
	Chytridiomycota	*Chytridiales* sp. 2	0.000	0.000	0.000	3.982	0.900
		Rhizophydiales sp. 3	0.000	0.000	0.138	1.964	0.481
		Chytridiomycota sp.	0.000	0.663	0.061	0.176	0.286
		Rhizophydiales sp. 4	0.000	0.000	0.272	0.000	0.072
		Spizellomycetales sp. 2	0.000	0.000	0.027	0.192	0.051
	Mucoromycota	*Mortierellales* sp. 2	0.000	0.000	0.847	0.261	0.284
		Cryptomycota sp.	0.000	0.000	0.403	0.021	0.112
		Glomerales sp.	0.000	0.000	0.000	0.041	0.009
		Archaeosporales sp.	0.056	0.000	0.000	0.000	0.009
		Glomeromycotina sp.	0.000	0.000	0.000	0.011	0.003
	Blastocladiomycota	*Blastocladiales* sp.	0.000	0.000	0.000	0.260	0.059

		Zoopagomycota	*Entomophthoromycota* sp.	0.007	0.000	0.000	0.027	0.007
	Stramenopila	*Bacillariophyta*	*Bacillariophyta* sp. 1	0.000	0.000	0.000	1.063	0.240
			Bacillariophyceae sp.	0.000	0.000	0.000	0.056	0.013
			Bacillariophyta sp. 2	0.000	0.007	0.000	0.000	0.002
Total				**100**	**100**	**100**	**100**	**100**

Green indicates dominant, blue intermediate, and orange minor relative abundance (see Methods).

Supplementary Table S2. Shared fungal amplicon sequence variants (ASVs) detected in sediment samples of Skua Lake (Elephant Island), Soto Lake (Deception Island), Katerina and Florencia lakes (James Ross Island) at Antarctic Peninsula.

Lake/Island													
Skua/Elephant	**Soto/Deception**	**Katerina/James Ross**	**Florencia/James Ross**	**Skua/Elephant and Katerina/James Ross**	**Skua/Elephant and Florencia/James Ross**	**Soto/Deception and Katerina/James Ross**	**Soto/Deception and Florencia/James Ross**	**Katerina and Florencia/James Ross**	**Skua/Elephant, Soto/Deception and |Katerina/James Ross**	**Skua/Elephant, Soto/Deception and Florencia/James Ross**	**Skua/Elephant, Katerina and Florencia/James Ross**	**Soto/Deception, Katerina and Florencia/James Ross**	**Skua/Elephant, Soto/Deception, Katerina and Florencia/James Ross**
Leucosporidiales sp. 1	*Goffeauzyma gastrica*	*Spizellomycetales* sp. 1	*Scutellinia* sp.	*Pyricularia oryzae*	*Camptobasidiacea e* sp.	*Talaromyces rubicundus*	*Dactylonectria anthuriicola*	*Agaricales* sp.	*Rhizophydiale s* sp. 2	*Didymosphaeriaceae* sp.	*Penicillium* sp.	*Chytridiomycot a* sp.	Fungal sp. 1
Phenoliferia psychrophila	*Mortierellales* sp. 1	*Paraphysoderma* sp.	*Acremonium biseptum*		*Monoblepharidale s* sp.		*Fusarium neocosmosporiellum*	*Tetracladium* sp.		*Cutaneotrichosporon debeurmannianum*	*Ciliophora* sp.	*Rozellomycotin a* sp. 1	*Aspergillaceae* sp.
Tremellomycetes sp. 1	*Gliocephalotrichum cylindrosporum*	*Rhizophydiales* sp. 4	*Chytridiales* sp. 2		*Glaciozyma* sp.		*Chytridium* sp.	*Lobulomycetales* sp.			*Antarctomyces* sp.		Fungal sp. 2
Mrakia niccombsii	*Cheilymenia theleboloides*	*Rozellomycotina* sp. 3	*Saccharomycetales* sp. 1		*Glaciozyma martinii*		*Clathrosphaerina zalewskii*	*Sordariales* sp.			*Glaciozyma antarctica*		*Neoascochyta paspali*
Leucosporidiales sp. 2	*Aspergillus ellipticus*	*Rhinocladiella* sp.	*Thelebolus balaustiformis*		*Pseudeurotium* sp.		*Cladosporium* sp.	*Mortierellales* sp. 2			*Rhizophydiales* sp. 1		*Penicillium nalgiovense*
Dioszegia fristingensis	*Talaromyces clemensii*	*Orbiliaceae* sp.	*Bacillariophyta* sp. 1		*Mrakia* sp.		*Trichoderma* sp.	*Aphelidiomycete s* sp.			*Malassezia restricta*		*Pseudogymnoasc us* sp.
Vishniacozyma victoriae	*Sporocadaceae* sp.	*Coprinopsis* sp.	*Ascomycota* sp.1		*Mrakia psychrophila*			*Cryptomycota* sp.					*Betamyces* sp.
Holocotylon sp.	*Clonostachys rosea*	*Toxicocladosporium* sp.	*Helotiales* sp. 1		*Solicoccozyma terricola*			*Lobulomycetes* sp.					*Candida parapsilosis*
Holtermanniella takashimae	*Fusarium* sp.	*Chytridiales* sp. 1	*Rozellomycotina* sp. 2		*Saccharomyces* sp.			*Rhizophydiales* sp. 3					
Tremellomycetes sp. 2	*Pyrenochaeta* sp.	*Gomphales* sp.	*Verrucaria* sp.		*Phanerochaete citri*			*Basidiomycota* sp.					
Protomyces inouyei	*Bacillariophyta* sp. 2	*Chaetothyriales* sp. 2	*Mortierella antarctica*		*Malassezia sympodialis*			*Nectriaceae* sp.					
Dioszegia sp.		*Rhodotorula pacifica*	*Mortierella* sp. 1		*Entomophthoromycota* sp.			*Sanchytrium* sp.					
Archaeosporales sp.		*Leptodiscella* sp.	*Polyporales* sp.					*Tolypocladium* sp.					
Penicillium coffeae			*Mortierella minutissima*					*Spizellomycetale s* sp. 2					
Orbiliales sp. 2			*Leucosporidium creatinivorum*					*Basidiobolales* sp.					
Diatrypaceae sp.			*Lipomyces yarrowii*					*Microbotryomyc etes* sp.					
Buellia russa			*Blastocladiales* sp.					*Helotiaceae* sp.					
Tremellales sp.			*Glaciozyma watsonii*					*Dothideomycetes* sp. 2					
Filobasidiales sp.			*Eurotiales* sp.					*Chaetothyriales* sp. 1					
Piskurozyma sp.			*Didymellaceae* sp.										
			Mortierella sp. 2										
			Trichoderma spirale										
			Periconia epilithographicola										
			Gorgomyces honrubiae										
			Xylonomycetes sp.										
			Rozellomycotina sp. 4										
			Chaetomiaceae sp.										
			Psathyrellaceae sp.										
			Ascomycota sp. 2										

Lake/Island													
Skua/Elephant	Soto/Deception	Katerina/James Ross	Florencia/James Ross	Skua/Elephant and Katerina/James Ross	Skua/Elephant and Florencia/James Ross	Soto/Deception and Katerina/James Ross	Soto/Deception and Florencia/James Ross	Katerina and Florencia/James Ross	Skua/Elephant, Soto/Deception and |Katerina/James Ross	Skua/Elephant, Soto/Deception and Florencia/James Ross	Skua/Elephant, Katerina and Florencia/James Ross	Soto/Deception, Katerina and Florencia/James Ross	Skua/Elephant, Soto/Deception, Katerina and Florencia/James Ross
			Neolectales sp.										
			Mortierella horticola										
			Wallemia tropicalis										
			Herpotrichiellaceae sp. 1										
			Verrucaria alpicola										
			Microdochium phragmitis										
			Malassezia globosa										
			Oidiodendron truncatum										
			Agaricomycetes sp. 1										
			Orbiliales sp. 1										
			Leohumicola sp.										
			Alatospora acuminata										
			Boletales sp.										
			Bacillariophyceae sp.										
			Porostereum spadiceum										
			Sanchytriaceae sp.										
			Mortierella pseudozygospora										
			Sordariomycetes sp.										
			Mortierella humilis										
			Saccharomycetales sp. 2										
			Sarocladium sp.										
			Sporormiaceae sp.										
			Glomerales sp.										
			Cyberlindnera jadinii										
			Malassezia arunalokei										
			Aspergillus thermomutatus										
			Leotiomycetes sp. 1										
			Penicillium mallochii										
			Mortierella basiparvispora										
			Porpidia sp.										
			Stagonospora trichophoricola										

Lake/Island													
Skua/Elephant	Soto/Deception	Katerina/James Ross	Florencia/James Ross	Skua/Elephant and Katerina/James Ross	Skua/Elephant and Florencia/James Ross	Soto/Deception and Katerina/James Ross	Soto/Deception and Florencia/James Ross	Katerina and Florencia/James Ross	Skua/Elephant, Soto/Deception and |Katerina/James Ross	Skua/Elephant, Soto/Deception and Florencia/James Ross	Skua/Elephant, Katerina and Florencia/James Ross	Soto/Deception, Katerina and Florencia/James Ross	Skua/Elephant, Soto/Deception, Katerina and Florencia/James Ross
			Lipomyces kononenkoae											
			Beauveria sp.											
			Bionectriaceae sp.											
			Neonectria candida											
			Cladophialophora sp.											
			Naganishia friedmannii											
			Mortierella alpina											
			Lipomyces starkeyi											
			Monoblepharis sp.											
			Archaeorhizomyces sp.											
			Leptosphaeria sclerotioides											
			Cladophialophora minutissima											
			Dothideales sp.											
			Coniosporium sp.											
			Coniochaeta sp.											
			Genolevuria amylolytica											
			Saccharomycopsis fibuligera											
			Bullera penniseticola											
			Helotiales sp. 2											
			Saprolegniaceae sp.											
			Letiomyceta sp.											
			Clavicipitaceae sp. 1											
			Herpotrichiellaceae sp. 2											
			Mortierella gemmifera											
			Leotiomycetes sp. 2											
			Annulatascaceae sp.											
			Capnodiales sp.											
			Pseudeurotiaceae sp.											
			Trechisporales sp.											
			Candida sp.											
			Clavaria sp.											

Lake/Island													
Skua/Elephant	Soto/Deception	Katerina/James Ross	Florencia/James Ross	Skua/Elephant and Katerina/James Ross	Skua/Elephant and Florencia/James Ross	Soto/Deception and Katerina/James Ross	Soto/Deception and Florencia/James Ross	Katerina and Florencia/James Ross	Skua/Elephant, Soto/Deception and |Katerina/James Ross	Skua/Elephant, Soto/Deception and Florencia/James Ross	Skua/Elephant, Katerina and Florencia/James Ross	Soto/Deception, Katerina and Florencia/James Ross	Skua/Elephant, Soto/Deception, Katerina and Florencia/James Ross
			Hyaloscypha sp.											
			Renatobasidium sp.											
			Debaryomyces sp.											
			Gyoerffyella entomobryoides											
			Acaromyces ingoldii											
			Pleosporaceae sp.											
			Hypocreales sp.											
			Periconia byssoides											
			Pirella circinans											
			Pezicula sp.											
			Glomeromycotina sp.											
			Chloridium sp.											
			Cenococcum geophilum											
			Wickerhamomyces anomalus											
			Iodophanus carneus											
			Phialocephala sp.											
			Trametes hirsuta											
			Agaricomycetes sp. 2											
			Clavicipitaceae sp. 2											
			Scutellospora sp.											
			Dothideomycetes sp. 1											
			Zoopagales sp.											
			Endogonomycetes sp.											
			Acaulopage sp.											
			Galactomyces reessii											

Supplementary Table S3. Ecological profiles obtained from the FUNGuild database and specific citations at generic level of the fungi detected in sediment samples obtained from Skua Lake (Elephant Island), Soto Lake (Deception Island), Katerina Lake and Florencia Lake (James Ross Island).

Genus	Skua Lake, Elephant Island	Soto Lake, Deception Island	Katerina Lake, James Ross Island	Florencia Lake, James Ross Island	Total	Trophic mode	Guild*
Acaromyces	0.000	0.000	0.000	0.013	0.003	Symbiotic- Pathogenic	Endophyte-Acaropathogenic[48,49]
Acaulopage	0.000	0.000	0.000	0.003	0.001	Pathogenic	Animal Pathogen[35]
Acremonium	0.000	0.000	0.000	4.928	1.113	Pathogenic-Saprotrophic-Symbiotic	Animal Pathogen- Endophyte-Fungal[35] Parasite-Plant Pathogen-Wood[35] Saprotrophic[35]
Alatospora	0.000	0.000	0.000	0.058	0.013	Saprotrophic	Undefined Saprotrophic[35]
Antarctomyces	0.730	0.000	0.194	0.080	0.187	Saprotrophic	Undefined Saprotrophic[35]
Archaeorhizomyces	0.000	0.000	0.000	0.027	0.006	Saprotrophic	Soil Saprotrophic[50]
Aspergillus	0.000	0.057	0.000	0.019	0.024	Pathogenic-Saprotrophic	Animal Pathogen-Undefined Saprotrophic[35]
Beauveria	0.000	0.000	0.000	0.032	0.007	Pathogenic	Animal Pathogen[35]
Betamyces	0.373	1.446	1.464	2.444	1.503	Saprotrophic	Freshwater and Soil Saprotrophic[51]

Buellia	0.022	0.000	0.000	0.000	0.004	Symbiotic	Lichenized[35]
Bullera	0.000	0.000	0.000	0.022	0.005	Symbiotic	Endophyte[52]
Candida	0.007	0.117	0.021	0.066	0.063	Pathogenic	Animal Pathogen[35]
Cenococcum	0.000	0.000	0.000	0.010	0.002	Symbiotic	Ectomycorrhizal[35]
Cheilymenia	0.000	0.245	0.000	0.000	0.085	Saprotrophic	Dung Saprotrophic-Undefined Saprotrophic[35]
Chloridium	0.000	0.000	0.000	0.010	0.002	Pathogenic-Saprotrophic-Symbiotic	Ectomycorrhizal-Endophyte-Plant Pathogen-Wood Saprotrophic[35]
Chytridium	0.000	0.715	0.000	0.099	0.271	Pathogenic	Plant Pathogen[35]
Ciliophora	1.008	0.000	0.043	1.854	0.593	Symbiotic	Endophyte[53]
Cladophialophora	0.000	0.000	0.000	0.028	0.006	Saprotrophic	Undefined Saprotrophic[35]
Cladosporium	0.000	0.343	0.000	0.850	0.311	Symbiotic	Endophyte[35]
Clathrosphaerina	0.000	0.390	0.000	4.478	1.148	Saprotrophic	Undefined Saprotrophic[35]
Clavaria	0.000	0.000	0.000	0.016	0.004	Saprotrophic	Undefined Saprotrophic[35]
Clonostachys	0.000	0.097	0.000	0.000	0.034	Pathogenic	Plant Pathogen[35]
Coniochaeta	0.000	0.000	0.000	0.023	0.005	Pathogenic-Saprotrophic-Symbiotic	Animal Pathogen-Dung Saprotrophic-Endophyte-Lichen Parasite-Plant Pathogen-Undefined Saprotrophic[35]

Coniosporium	0.000	0.000	0.000	0.024	0.005	Saprotrophic	Undefined Saprotrophic[54]
Coprinopsis	0.000	0.000	0.049	0.000	0.013	Saprotrophic	Undefined Saprotrophic[55]
Cutaneotrichosporon	0.004	0.235	0.000	0.029	0.089	Saprotrophic	Undefined Saprotrophic[56]
Cyberlindnera	0.000	0.000	0.000	0.040	0.009	Symbiotic	Insect Symbiont[57]
Dactylonectria	0.000	39.311	0.000	0.017	13.663	Saprotrophic-Pathogenic	Soil Saprotrophic- Plant Pathogen[58]
Debaryomyces	0.000	0.000	0.000	0.013	0.003	Saprotrophic	Undefined Saprotrophic[35]
Dioszegia	0.272	0.000	0.000	0.000	0.044	Saprotrophic	Undefined Saprotrophic[35]
Fusarium	0.000	2.499	0.000	0.037	0.876	Pathogenic-Saprotrophic-Symbiotic	Animal Pathogen-Endophyte-Lichen[35] Parasite-Plant Pathogen-Soil[35] Saprotrophic-Wood Saprotrophic[35]
Galactomyces	0.000	0.000	0.000	0.002	0.000	Pathogenic	Plant Pathogen[35]
Genolevuria	0.000	0.000	0.000	0.023	0.005	Saprotrophic	Undefined Saprotrophic[59]
Glaciozyma	1.553	0.000	0.016	0.278	0.318	Saprotrophic	Undefined Saprotrophic[60]
Gliocephalotrichum	0.000	0.282	0.000	0.000	0.098	Saprotrophic	Undefined Saprotrophic[35]
Goffeauzyma	0.000	0.763	0.000	0.000	0.265	Saprotrophic	Soil-Acid rock drainage-Acidic water[61]
Gorgomyces	0.000	0.000	0.000	0.131	0.030	Pathogenic	Nematophagous[48]
Gyoerffyella	0.000	0.000	0.000	0.013	0.003	Saprotrophic	Undefined Saprotrophic[35]

Holocotylon	0.349	0.000	0.000	0.000	0.056	Saprotrophic	Undefined Saprotrophic[35]
Holtermanniella	0.266	0.000	0.000	0.000	0.043	Symbiotic	Plant Symbiotic[62]
Hyaloscypha	0.000	0.000	0.000	0.015	0.003	Saprotrophic	Undefined Saprotrophic[35]
Iodophanus	0.000	0.000	0.000	0.009	0.002	Saprotrophic	Undefined Saprotrophic[35]
Leohumicola	0.000	0.000	0.000	0.060	0.014	Saprotrophic	Undefined Saprotrophic[35]
Leptodiscella	0.000	0.000	0.007	0.000	0.002	Saprotrophic	Undefined Saprotrophic[35]
Leptosphaeria	0.000	0.000	0.000	0.027	0.006	Pathogenic	Plant Pathogen[35]
Leucosporidium	0.000	0.000	0.000	0.281	0.063	Saprotrophic	Soil Saprotrophic-Undefined Saprotrophic[35]
Lipomyces	0.000	0.000	0.000	0.113	0.025	Saprotrophic	Soil Saprotrophic-Insect frass[63]
Malassezia	0.027	0.000	0.002	0.108	0.029	Pathogenic	Animal Pathogen[35]
Microdochium	0.000	0.000	0.000	0.070	0.016	Pathogenic-Symbiotic	Endophyte-Plant Pathogen[35]
Monoblepharis	0.000	0.000	0.000	0.027	0.006	Saprotrophic	Undefined Saprotrophic[35]
Mortierella	0.000	0.000	0.000	0.175	0.040	Saprotrophic-Symbiotic	Endophyte-Litter Saprotrophic-Soil[35] Saprotrophic-Undefined Saprotrophic[35]
Mrakia	0.719	0.000	0.000	0.192	0.159	Saprotrophic	Soil Saprotrophic-Undefined Saprotrophic[35]
Naganishia	0.000	0.000	0.000	0.029	0.007	Saprotrophic	Soil-Flowers[64,65]

Neoascochyta	1.513	0.406	0.137	0.542	0.544	Pathogenic	Plant Pathogen[66]
Neonectria	0.000	0.000	0.000	0.032	0.007	Pathogenic	Plant Pathogen[35]
Oidiodendron	0.000	0.000	0.000	0.067	0.015	Pathogenic-Symbiotic	Ericoid Mycorrhizal[35]
Paraphysoderma	0.000	0.000	0.596	0.000	0.158	Pathogenic	Plant Pathogen[35]
Penicillium	0.665	0.132	0.135	0.233	0.242	Saprotrophic	Dung Saprotrophic-Undefined[35] Saprotrophic-Wood Saprotrophic[35]
Periconia	0.000	0.000	0.000	0.077	0.017	Pathogenic-Saprotrophic-Symbiotic	Endophyte-Plant Pathogen-Wood Saprotrophic[35]
Pezicula	0.000	0.000	0.000	0.011	0.003	Saprotrophic-Symbiotic-Pathogenic	Wood Saprotrophic-Endophyte-Plant Pathogen[67]
Phanerochaete	0.073	0.000	0.000	0.034	0.019	Saprotrophic	Wood Saprotrophic[35]
Phenoliferia	13.445	0.000	0.000	0.000	2.170	Symbiotic-Saprotrophic	Plant-Cold habitats[68]
Phialocephala	0.000	0.000	0.000	0.009	0.002	Symbiotic	Endophyte[35]
Pirella	0.000	0.000	0.000	0.012	0.003	Saprotrophic	Undefined Saprotrophic[35]
Piskurozyma	0.005	0.000	0.000	0.000	0.001	Saprotrophic	Soil Saprotrophic[69]
Porostereum	0.000	0.000	0.000	0.052	0.012	Saprotrophic	Undefined Saprotrophic[35]
Porpidia	0.000	0.000	0.000	0.036	0.008	Symbiotic	Lichenized[35]

Protomyces	0.093	0.000	0.000	0.000	0.015	Pathogenic	Plant Pathogen[35]
Pseudeurotium	1.130	0.000	0.000	11.814	2.852	Saprotrophic	Undefined Saprotrophic[35]
Pseudogymnoascus	0.694	0.207	0.049	8.302	2.073	Pathogenic-Saprotrophic-Symbiotic	Animal pathogen-Soil Saprotrophic[35]
Pyrenochaeta	0.000	0.065	0.000	0.000	0.023	Pathogenic-Symbiotic-Saprotrophic	Plant Pathogen- Endophyte -Soil Saprotrophic[35][70]
Pyricularia	0.019	0.000	0.016	0.000	0.007	Pathogenic	Plant Pathogen[35]
Renatobasidium	0.000	0.000	0.000	0.014	0.003	Saprotrophic	Undefined Saprotrophic[35]
Rhinocladiella	0.000	0.000	0.149	0.000	0.040	Pathogenic-Saprotrophic	Plant Pathogen- Wood Saprotrophic[48]
Rhodotorula	0.000	0.000	0.010	0.000	0.003	Pathogenic-Saprotrophic	Animal Endosymbiont-Animal Pathogen Endophyte-Plant Pathogen-Undefined Saprotroph[35]
Saccharomyces	0.096	0.000	0.000	0.022	0.020	Saprotrophic	Undefined Saprotrophic[35]
Saccharomycopsis	0.000	0.000	0.000	0.022	0.005	Saprotrophic	Undefined Saprotrophic[71]
Sanchytrium	0.000	0.000	0.081	1.112	0.273	Pathogenic	Algae Pathogen[72]
Sarocladium	0.000	0.000	0.000	0.042	0.010	Saprotrophic	Undefined Saprotrophic[35]
Scutellinia	0.000	0.000	0.000	8.380	1.894	Saprotrophic	Undefined Saprotrophic[35]

Scutellospora	0.000	0.000	0.000	0.006	0.001	Symbiotic	Arbuscular Mycorrhizal[35]
Solicoccozyma	0.148	0.000	0.000	0.097	0.046	Saprotrophic	Soil Saprotrophic[61]
Stagonospora	0.000	0.000	0.000	0.035	0.008	Pathogenic	Plant Pathogen[35]
Talaromyces	0.000	20.537	2.867	0.000	7.897	Saprotrophic	Undefined Saprotrophic[35]
Tetracladium	0.000	0.000	1.873	0.358	0.578	Saprotrophic	Undefined Saprotrophic[35]
Thelebolus	0.000	0.000	0.000	1.437	0.325	Saprotrophic-Symbiotic	Dung Saprotrophic-Endophyte-Undefine Saprotrophic[35]
Tolypocladium	0.000	0.000	0.032	0.065	0.023	Pathogenic-Symbiotic	Animal Pathogen-Clavicipitaceous Endophyte-Fungal Parasite[35]
Toxicocladosporium	0.000	0.000	0.034	0.000	0.009	Pathogenic-Symbiotic	Endophyte-Plant Pathogen[35]
Trametes	0.000	0.000	0.000	0.009	0.002	Saprotrophic	Wood Saprotrophic[35]
Trichoderma	0.000	0.099	0.000	0.167	0.072	Pathogenic-Saprotrophic-Symbiotic	Animal Pathogen-Endophyte-Epiphyte-Fungal Parasite-Plant Pathogen-Wood Saprotrophic[35]
Verrucaria	0.000	0.000	0.000	0.317	0.072	Symbiotic	Lichenized[73]
Vishniacozyma	0.351	0.000	0.000	0.000	0.057	Pathogenic-Saprotrophic-Symbiotic	Animal Pathogen-Endophyte-Epiphyte-Undefined Saprotrophic[35]
Wallemia	0.000	0.000	0.000	0.087	0.020	Saprotrophic	Undefined Saprotrophic[35]

Wickerhamomyces	0.000	0.000	0.000	0.009	0.002	Saprotrophic	Undefined Saprotrophic[35]

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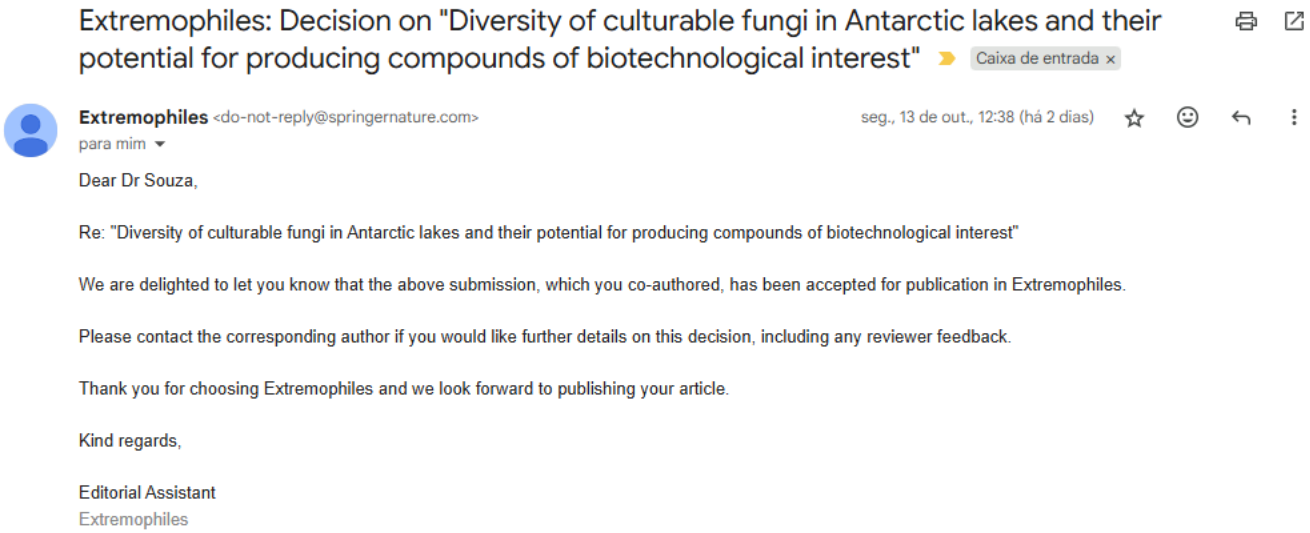
Supplementary Table S5. Comparison of diversity indices obtained in studies of Antarctic lake sediment fungal diversity assessed by DNA metabarcoding and traditional culturing methods.

Region	Lake	Diversity indices					Citation
		Number of taxa	Number of DNA reads[a] or isolates[b]	Fisher's-α (diversity)	Margalef's (richness)	Simpson's (dominance)	
Elephant Island	Skua	50	33,429[a]	21.40	9.25	0.76	Current study
Deception Island	Soto	31	71,983[a]	10.27	5.66	0.78	
James Ross	Katerina	51	54,939[a]	22.1	9.44	0.79	
James Ross	Florencia	171	46,813[a]	559.7	32.09	0.92	
Elephant Island	Skua	13	64[b]	0.79	1.11	1.22	Ogaki et al.[Erro! onte de referência não encontrada.]
Deception Island	Soto	6	20[b]	0.61	0.47	0.54	
	Deception B	0	0[b]	0	0	0	Ogaki et al.[Erro! onte de referência não encontrada.]
	Kroner	3	9[b]	0.60	0.29	0.39	
Fildes Peninsula, King George Island	North	41	122[b]	5.25	4.22	0.94	Ogaki et al.[Erro! onte de referência não encontrada.]
	Central	15	43[b]	2.06	1.75	0.86	
	South	22	95[b]	2.51	2.17	0.8	
Admiralty Bay, King George	Punta Hennequin	8	33[b]	0.81	0.72	0.78	

Island	Wanda A	10	20[b]	0.81	1.16	1.37	Ogaki et al.**Erro! onte de referência não encontrada.**
	Wanda B	5	10[b]	0.63	0.59	0.70	
Penguin Island	Petrel	15	39[b]	0.82	1.58	1.81	Ogaki et al.**Erro! onte de referência não encontrada.**
Hope Bay	Boeckella (top)	114	36,471[a]	41.7	17.66	0.89	Rosa et al.**Erro! Fonte e referência não encontrada.**
	Boeckella (middle)	55	26,582[a]	14.74	8.44	0.88	
	Boeckella (base)	146	30,088[a]	61.44	22.67	0.89	
Vega Island	Copépodo	161	223,679[a]	16.97	12.99	0.81	Ogaki et al.**Erro! onte de referência não encontrada.**
	Esmeralda	224	640,902[a]	21.77	21.77	0.74	
	Pan Negro	116	243,518[a]	11.66	9.27	0.66	
Fildes Peninsula, King George Island	North	41	122[b]	5.25	4.22	0.94	Ogaki et al.**Erro! onte de referência não encontrada.**
	Central	15	43[b]	2.06	1.75	0.86	
	South	22	95[b]	2.51	2.17	0.8	
Admiralty Bay, King George Island	Punta Hennequin	8	33[b]	0.81	0.72	0.78	Ogaki et al.**Erro! onte de referência não encontrada.**
	Wanda A	10	20[b]	0.81	1.16	1.37	
	Wanda B	5	10[b]	0.63	0.59	0.70	

Penguin Island	Petrel Lake	15	39[b]	0.82	1.58	1.81	Ogaki et al.Erro! onte de referência não encontrada.
Deception Island	Deception A	6	20[b]	0.61	0.47	0.54	Ogaki et al.Erro! onte de referência não encontrada.
	Deception B	0	0[b]	0	0	0	
	Kroner	3	9[b]	0.60	0.29	0.39	

7.2.Aceite do Capítulo 04 pela revista *Extremophiles*



Extremophiles: Decision on "Diversity of culturable fungi in Antarctic lakes and their potential for producing compounds of biotechnological interest" Caixa de entrada ×

Extremophiles <do-not-reply@springernature.com> seg., 13 de out., 12:38 (há 2 dias)
para mim

Dear Dr Souza,

Re: "Diversity of culturable fungi in Antarctic lakes and their potential for producing compounds of biotechnological interest"

We are delighted to let you know that the above submission, which you co-authored, has been accepted for publication in Extremophiles.

Please contact the corresponding author if you would like further details on this decision, including any reviewer feedback.

Thank you for choosing Extremophiles and we look forward to publishing your article.

Kind regards,

Editorial Assistant
Extremophiles

7.3.Submissão do Capítulo 05 para a revista *Extremophiles*

Extremophiles - Receipt of Manuscript 'Fungal diversity in...' Caixa de entrada ×

Extremophiles <theresa.ancheta@springernature.com> qui., 14 de ago., 13:06 (há 11 dias)
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Dear Dr Souza,

Please note that you are listed as a co-author on the manuscript "Fungal diversity in sediments of periglacial lakes of Deception Island, Maritime Antarctica assessed using culturing and DNA metabarcoding approaches", which was submitted to Extremophiles on 14 August 2025 UTC.

If you have any queries related to this manuscript please contact the corresponding author, who is solely responsible for communicating with the journal.

Kind regards,

Editorial Assistant
Extremophiles

7.4.Relação da produção científica

Artigos publicados como primeira autora

de Souza, L. M. D., Lirio, J. M., Coria, S. H. *et al.* Diversity, distribution and ecology of fungal communities present in Antarctic lake sediments uncovered by DNA metabarcoding. *Sci Rep* 12, 8407 (2022). https://doi.org/10.1038/s41598-022-12290-6

de Souza, L.M.D., Teixeira, E. A. A., da Costa Coelho, L. *et al.* Cryptic fungal diversity revealed by DNA metabarcoding in historic wooden structures at Whalers Bay, Deception Island, maritime Antarctic. *Braz J Microbiol* 54, 213–222 (2023). https://doi.org/10.1007/s42770-022-00869-0

Artigos submetidos como primeira autora

de Souza, L. M. D., Ribeiro, J. P. M., Barreto, D. L. C., Teixeira, E. A. A., Carvalho, C. R., Lirio, J. M., Coria, S. H., Convey, P., Oliveira, F. S., Cantrell, C. L., Duke, S. O., Rosa, C. A., Rosa, L. H. Diversity of culturable fungi in Antarctic lakes and their potential for producing compounds of biotechnological interest. Submetido em 01/08/2025 para a revista *Extremophiles*.

de Souza, L. M. D., Lirio, J. M., Coria, S. H., Carvalho-Silva, M., Câmara, P. E. A. S., Lopes, F. A. C., Oliveira, F. S., Convey, P., Rosa, L. H. Fungal diversity in sediments of periglacial lakes of Deception Island, Maritime Antarctica assessed using culturing and DNA metabarcoding approaches. Submetido em 14/08/2025 para a revista *Extremophiles*.

Artigos publicados em colaboração

da Silva, M. K., **de Souza, L. M. D.**, Vieira, R. et al. Fungal and fungal-like diversity in marine sediments from the maritime Antarctic assessed using DNA metabarcoding. *Sci Rep* **12**, 21044 (2022). https://doi.org/10.1038/s41598-022-25310-2

Gonçalves, V. N., **de Souza, L. M. D.**, Lirio, J. M. et al. Diversity and ecology of fungal assemblages present in lake sediments at Clearwater Mesa, James Ross Island, Antarctica, assessed using metabarcoding of environmental DNA. *Fungal Biology*, v. **126**, n. 10, p. 640-647 (2022). https://doi.org/10.1016/j.funbio.2022.08.002

Santos, A. R. O., Souza, G. F., Barros, K. O., Alvarenga, F. B., Lopes, M. R., **de Souza, L. M. D.** et al. *Spathaspora brunopereirae* sp. nov. and *Spathaspora domphillipsii* sp. nov., two d-xylose-fermenting ascosporogenous yeasts from Amazonian Forest biomes. International Journal of Systematic and Evolutionary Microbiology 73(3), 005752 (2023). https://doi.org/10.1099/ijsem.0.005752

Câmara, P. E. A. S., Lopes, F. A., Bones, F. L. V., Carvalho-Silva, M., Convey, P., **de Souza, L. M. D.** et al. Life in ruins: DNA metabarcoding contributes to the history of Whalers Bay wooden structures at Deception Island, South Shetland Islands. Antarctic Science 36(5):281-289 (2024). https://doi.org/10.1017/S0954102024000245

Teixeira, E. A. A., **de Souza, L. M. D.**, Vieira, R. et al. Enzymes and biosurfactants of industrial interest produced by culturable fungi present in sediments of Boeckella Lake, Hope Bay, north-east Antarctic Peninsula. Extremophiles 28, 30 (2024). https://doi.org/10.1007/s00792-024-01345-3

Teixeira, E. A. A., **de Souza, L. M. D.**, de Carvalho, C.R. et al. Fungal diversity in Antarctic lignocellulosic substrates and their production of enzymes and lipids with potential industrial applications. Blue Biotechnol. 2, 11 (2025). https://doi.org/10.1186/s44315-025-00035-9

Costa, K., Lopes, M. R., Barros, K. O., Souza, G. F., Santos, A. R. O., **de Souza, L. M. D.** et al. *Spathaspora cesarfonsecae* sp. nov., a xylose-fermenting yeast isolated from gut of passalid beetles and rotting wood in the Amazonian rainforest biome. International Journal of Systematic and Evolutionary Microbiology, 75(8), 006871 (2025). https://doi.org/10.1099/ijsem.0.006871

Capítulos de livros publicados

de Souza, L. M. D., Teixeira, E. A. A., Rosa, L. H. Enzimas com potencial biotecnológico produzidas por fungos da Antártica. In: Andrés Zarankin; Luiz Henrique Rosa; Rosa Maria Esteves Arantes; Fernanda Codevilla Soares. (Org.). Antártica em Minas Gerais - Avanços científicos nas áreas de medicina/fisiologia, microbiologia e arqueologia do Polo Sul e sua importância para o Brasil. 1ed. Belo Horizonte: Imprensa Universitária da UFMG, v. 1, p. 197-211 (2021).

de Souza, L. M. D., Gonçalves, V. N., Menezes, G. C. A., Teixeira, E. A. A., Rosa, L. H. Biossurfactantes produzidos por fungos da Antártica. In: Andrés Zarankin; Luiz Henrique Rosa; Rosa Maria Esteves Arantes; Fernanda Codevilla Soares. (Org.). Antártica em Minas Gerais - Avanços científicos nas áreas de medicina/fisiologia, microbiologia e arqueologia do Polo Sul e sua importância para o Brasil. 1ed. Belo Horizonte: Imprensa Universitária da UFMG, 2021, v. 1, p. 213-224.

de Souza, L. M. D. et al. Diversity of freshwater fungi in polar and alpine lakes. In: Freshwater Mycology. *Elsevier*, p. 37-58 (2022). https://doi.org/10.1016/B978-0-323-91232-7.00013-1

de Souza, L. M. D. et al. Fungal Biosurfactants: Applications in Agriculture and Environmental Bioremediation Processes. Biosurfactants and Sustainability: From Biorefineries Production to Versatile Applications. *Wiley*, p. 243-254 (2023). https://doi.org/10.1002/9781119854395.ch12

Barreto, D. L. C., de Carvalho, C. R., Ribeiro, V. P., **de Souza, L. M. D.** et al. Evaluating the Efficacy of Endophytic Fungal Compounds as Biopesticides in Agriculture. In: Abd-Elsalam, K.A., Hashem, A.H. (eds) Fungal Endophytes Volume II. Springer, Singapore (2025). https://doi.org/10.1007/978-981-97-8804-0_8

Orientações e colaborações

- **Orientação Júlia de Paula Muzetti Ribeiro.** Detecção de biossurfactantes e lipídios produzidos por fungos isolados de sedimentos de lagos da Ilha James Ross, Antártica. 2022. Trabalho de Conclusão de Curso. (Graduação em Abi - Ciências Biológicas) - Universidade Federal de Minas Gerais.

- **Orientação Rayane Raniele Silva Bezerra.** Produção de metabólitos com atividade herbicida por fungos antárticos e seu potencial para aplicações sustentáveis – Revisão da literatura. 2024. Trabalho de Conclusão de Curso. (Graduação em Abi - Ciências Biológicas) - Universidade Federal de Minas Gerais.

- **Colaboração Elisa Amorim Amâncio Teixeira.** Bioprospecção de enzimas e biossurfactantes de interesse industrial produzidos por fungos presentes em sedimentos do

Lago Boeckella, Baía Esperança, nordeste da Península Antártica. Dissertação de mestrado em Microbiologia. Universidade Federal de Minas Gerais.