Universidade Federal de Minas Gerais
Programa de Pós-Graduação em Microbiologia

Fungos presentes em sedimentos marinhos e lacustres da Antártica: taxonomia, diversidade e bioprospecção de metabólitos bioativos

Mayara Baptistucci Ogaki

Belo Horizonte, MG

2019

Universidade Federal de Minas Gerais
Programa de Pós-Graduação em Microbiologia

Fungos presentes em sedimentos marinhos e lacustres da Antártica: taxonomia, diversidade e bioprospecção de metabólitos bioativos

Tese apresentada ao programa de Pós-Graduação em Microbiologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais.

Orientador:

Dr. Luiz Henrique Rosa
Departamento de Microbiologia/ICB, UFMG

Belo Horizonte, MG

2019

Colaboradores:

Dr. Carlos Augusto Rosa
Departamento de Microbiologia/ICB, UFMG

Dr. Carlos Leomar Zani
Dra. Tânia Maria de Almeida Alves
Centro de Pesquisas René Rachou, FIOCRUZ/MG

Dra. Rosemary Vieira
Dr. Arthur Ayres
Universidade Federal Fluminense, RJ

Dr. Juan Manoel Lírio
Instituto Antartico Argentino, Buenos Aires, Argentina

043 Ogaki, Mayara Baptistucci.
Fungos presentes em sedimentos marinhos e lacustres da Antártica: taxonomia, diversidade e bioprospecção de metabólitos bioativos [manuscrito] / Mayara Baptistucci Ogaki. – 2019.
212 f. : il. ; 29,5 cm.

Orientador: Dr. Luiz Henrique Rosa.
Tese (doutorado) – Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Microbiologia.

1. Microbiologia. 2. Regiões Antárticas. 3. Fungos. 4. Bioprospecção. 5. Biodiversidade. I. Rosa, Luiz Henrique. II. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas. III. Título.

CDU: 579

Ficha Catalográfica elaborada por Fabiane C. M. Reis – CRB6/ 2680

UNIVERSIDADE FEDERAL DE MINAS GERAIS

INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM MICROBIOLOGIA

ATA DA DEFESA DE TESE DE MAYARA BAPTISTUCCI OGAKI
Nº REGISTRO: 2015744775

Às 14 horas do dia 30 de agosto de 2019, reuniu-se, no Instituto de Ciências Biológicas da UFMG, a Comissão Examinadora composta pelos Drs. Betânia Barros Cota, Denise de Oliveira Scoaris, Nalu Teixeira de Aguiar Peres, Viviane Souza Alves e o Prof. Dr. Luiz Henrique Rosa – Orientador, para julgar o trabalho final, da aluna **Mayara Baptistucci Ogaki**, requisito final para a obtenção do Grau de **DOUTORA EM CIÊNCIAS BIOLÓGICAS: MICROBIOLOGIA**. Abrindo a sessão, o Presidente da Comissão, Prof. Flávio Guimarães da Fonseca - Coordenador do Programa, 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. Belo Horizonte, 30 de agosto de 2019. O candidato 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.

Profa. Dra. Betania Barros Cota _____

Profa. Dra. Denise de Oliveira Scoaris _____

Profa. Dra. Nalu Teixeira de Aguiar Peres _____

Profa. Dra. Viviane Souza Alves _____

Prof. Dr. Luiz Henrique Rosa (Orientador) _____

_____
Prof. Flávio Guimarães da Fonseca
Coordenador

Dedicatória

Aos meus maiores apoios, “minha família e amigos”.

.

Agradecimentos

Agradeço a todo corpo docente e funcionários do programa de pós-graduação em Microbiologia e ao apoio financeiro do CNPq e CAPES pela concessão de minha bolsa de estudos e pelos subsídios que permitiram a concretização desta tese.

Ao meu orientador Luiz Henrique Rosa pela acolhida, oportunidades, ajuda e compreensão a mim oferecidas durante todo o tempo que permaneci em sua equipe e também por ter proporcionado a maior experiência profissional por mim vivenciada.

As minhas amigas de doutorado, Thamar e Mariana, pelo respeito, amor e cuidado que recebo diariamente.

A todas as amigas do laboratório pela benevolência, suporte emocional e intelectual nos dias de angústia e também nos de comemoração à vida.

Aos meus professores Wagner e Marcus por me fazerem notar a beleza da vida mesmo sob tantas dificuldades humanas. Somente suportei as perdas e aguentei as dores graças à cada palavra despretensiosa de luz e carinho.

Aos meus amigos paranaenses Bruna, Eddie, Paloma, Paula, Sara, Renato, Larissa, Angela e Guilherme por todas as risadas, o aconchego e as palavras de incentivo que deram acalento as minhas lamúrias e foram em grande parte responsáveis pela minha permanência na carreira acadêmica até hoje. Aos queridos Olímpio, Diogo, Diego, Laís, Clayton, Marina, Bruno e Rafael por todo abraço fraterno e pela insistência para que eu buscasse ser uma pessoa melhor, mais feliz e atenta a enxergar as dores do mundo com outros olhos.

Aos meus pais Maria e Valter, ao meu irmão William e a minha avó Adelaide pelo amor incondicional que sempre me permitiu sonhar e ser capaz mesmo quando eu não acreditava ser possível continuar.

Não te rendas, ainda estás a tempo
de alcançar e começar de novo,
aceitar as tuas sombras
enterrar os teus medos,
largar o lastro,
retomar o voo.

Não te rendas que a vida é isso,
continuar a viagem,
perseguir os teus sonhos,
destravar os tempos,
arrumar os escombros,
e destapar o céu.

Mario Benedetti

SUMÁRIO

LISTA DE ABREVIATURAS ..... **IV**

LISTA DE FIGURAS ..... **V**

RESUMO ..... **VI**

ABSTRACT ..... **VIII**

1. RELEVÂNCIA E JUSTIFICATIVA ..... **X**

2. INTRODUÇÃO **1**

2.1 Antártica **1**

2.2 Micobioma antártico e estratégias adaptativas **3**

2.3 Fungos em ambientes aquáticos **5**

2.3.1 Fungos de ambientes marinhos da Antártica **7**

2.3.2 Fungos em ambientes lacustres da antártica **9**

2.4 Bioprospecção de metabólitos bioativos de fungos extremófilos **11**

3. OBJETIVOS **18**

3.1 Objetivo Geral **18**

3.2 Objetivos Específicos **18**

4. MATERIAIS E MÉTODOS **19**

4.1 Fungos em sedimentos marinhos **19**

4.1.1 Coleta e processamento das amostras de sedimento marinho **19**

4.2 Fungos em sedimentos lacustres **20**

4.2.1 Coleta e processamento das amostras de sedimentos lacustres **20**

4.2.2 Isolamento de fungos em sedimentos lacustres **23**

4.3 Preservação dos fungos **24**

4.4 Identificação dos fungos **24**

4.4.1 Identificação de fungos filamentosos **24**

4.4.1.1 Extração de DNA total 25

4.4.1.2 Amplificação utilizando o iniciador $(GTG)_5$ 25

4.4.1.3 Amplificação da região ITS 26
4.4.1.4 Amplificação parcial do gene da β-tubulina 26
4.4.1.5 Amplificação da RNA polimerase II 27
4.4.1.6 Caracterização morfofisiológica das espécies do gênero Penicillium 27
4.4.2 Identificação de leveduras 28
4.4.2.1 Extração de DNA total 28
4.4.2.2 Amplificação do domínio D1/D2 28
4.4.3 Purificação dos amplicons 29
4.4.4 Sequenciamento 29
4.4.4.1 Reações de sequenciamento 29
4.4.4.2 Precipitação da reação de sequenciamento 30
4.4.4.3 Análise computacional das sequências e análise filogenética 30
4.5 Avaliação da diversidade da comunidade fúngica 31
4.6 Cultivo dos fungos e preparo dos extratos para os ensaios biológicos 32
4.6.1 Preparo dos extratos de fungos marinhos 32
4.6.2 Preparo dos extratos de fungos lacustres 33
4.7 Ensaios biológicos 33
4.7.1 Ensaio antimicrobiano contra *Cladosporium sphaerospermum* 33
4.7.2 Ensaio *in vitro* com a forma amastigota-like de *Leishmania* (*Leishmania*) *amazonenses* 34
4.7.3 Ensaio *in vitro* com formas tripomastigotas e formas amastigotas intracelulares de *Trypanosoma cruzi* e ensaio citotóxico *in vitro* de extratos tripanossomicidas 35
4.7.4 Manutenção e ensaios esquizonticidas *in vitro* de formas sanguíneas de *Plasmodium falciparum* 36
4.7.5 Triagem da atividade antiviral de extratos de plantas e fungos contra o vírus da dengue sorotipo 2 (DENV2) 37
4.7.6 Determinação de atividade herbicida 38
4.7.7 Determinação da atividade nematicida 40

5. CAPÍTULOS 41

5.1 Capítulo 1: "Diversity and ecology of fungal assemblages present in lakes of Antarctica." 42

5.2 Capítulo 2: "Sub-antarctic and Antarctic marine ecosystems: An unexplored ecosystem of fungal diversity." 71

5.3 Capítulo 3. "Cultivable fungi present in deep-sea sediments of Antarctica: Taxonomy, diversity, and bioprospection of bioactive compounds." 93

5.4 Capítulo 4. "Diversity, ecology, and bioprospection of fungi in lakes under anthropogenic effects in Antarctic Peninsula" 122

5.5 Capítulo 5. "Diversity and bioprospection of cultivable fungal community in sediments of lakes at Antarctic Peninsula." 156

6. DISCUSSÃO INTEGRADA 189

7. CONCLUSÕES 195

8. REFERÊNCIAS BIBLIOGRÁFICAS 197

9. PRODUÇÃO ACADÊMICA 210

10. ANEXOS 212

LISTA DE ABREVIATURAS

UFC-Unidade formadora de colônia

UFC g^{-1}-Unidades formadoras de colônias por grama de sedimento

CTAB-Brometo de cetil trimetilamonio

DMSO-Dimetilsulfóxido

rDNA-Ácido desoxirribonucleico ribossomal

rRNA-Ácido ribonucleico ribossomal

dNTP-Desoxirribonucleotídeos fosfatados

EDTA-ácido etilenodiamino tetra-acético

UTOs-Unidades taxonômicas operacionais

RMN-Ressonância Magnética Nuclear H^1

M-molar

µmol μL^{-1}-micromol por microlitro

µmol s^{-1} m^{-2}-micromol metros por segundo

mg mL^{-1}-miligramas por mililitro

mg L^{-1}-miligramas por litro

min-minutos

s-segundos

h-horas

rpm-rotação por minuto

ºC-graus Celsius

pH-potencial hidrogeniôco

p/v-peso por volume

LISTA DE FIGURAS

Figura 1. Estruturas químicas de moléculas bioativas isoladas de fungos extremófilos. A – micosporinas. B – asperelinas (A e B). C – geomicinas (A, B e C). D – citocalasinas. ... 14

Figura 2. Estrutura química da molécula bioativa cefalosporina C produzida pelo fungo marinho *Acremonium chrysogenum*. ... 15

Figura 3. Imagens das áreas de coleta das amostras de sedimento lacustre durante a OA XXXIV. Ilha Rei George, Península Fildes: A – Lago Fildes Norte; B – Lago Fildes Central; C – Lago Fildes Sul. ... 21

Figura 4. Imagens das áreas de coleta das amostras de sedimento lacustre durante OA XXXV. Ilha Rei George, Baia do Almirantado: A – Lago Punta Hannequin; E – Lago Wanda A; F – Lago Wanda B. Ilha Deception, Creater Lake: B – Lago Deception A, C – Lago Deception B; Ilha Deception, Whalers Bay: D – Lago Kroner. Ilha Elefante, Refúgio Emilio Goeldi, BR: G – Lago Elefante. Ilha Pinguim: H – Lago Pinguim. ... 22

Figura 5. Esquema de seccionamento dos testemunhos de sedimento lacustre. A imagem à esquerda representa os testemunhos ao serem seccionados em Topo, Meio e Base; à direita a obtenção das três alíquotas A, B e C de cada secção para fins de isolamento de fungos. ... 23

Figura 6. Modelo da disposição dos extratos e controles na placa de 24 poços utilizada nos ensaios herbicidas. ... 39

RESUMO

Alguns ecossistemas, tais como o mar profundo, lagoas hipersalinas, substratos vulcânicos, desertos e regiões polares possuem condições físico-químicas consideradas desfavoráveis à vida e são caracterizados pela presença de comunidades microbianas denominadas extremófilas, as quais podem ser compostas principalmente por vírus, bactérias, arqueias e fungos. Nos ecossistemas extremos, os fungos são de grande importância ecológica na ciclagem de nutrientes e se destacam por sua alta plasticidade genética e metabólica, as quais ao longo de sua evolução estiveram condicionadas a grandes variações de salinidade, temperatura, radiação, disponibildade de água, pressão, acidez, entre outras. Na Antártica, a pressão seletiva conferida pelas condições climáticas, principalmente as baixas temperaturas e pouca disponibilidade de água, pode ter selecionado fungos com distintas estratégias de sobrevivência, entre elas a produção de substâncias anticongelantes, modificações na composição de membrana celular e também produção de metabólitos primários e secundários diferenciados. Considerando a diversidade e potencial produção de biomoléculas apresentada por fungos extremófilos, estudos básicos e aplicados que envolvam esses micro-organismos são de grande interesse, pelo fato de representarem uma parcela da biodiversiade mundial e também uma rica fonte de substâncias com potencial aplicação em processos biotecnológicos. Desta forma, o presente estudo teve como objetivo caracterizar as comunidades de fungos presentes em sedimentos marinhos e lacustres da Antártica a fim de avaliá-los como potenciais produtores de substâncias bioativas. Nos diferentes substratos avaliados foram obtidos 486 isolados fúngicos pertencentes a 111 unidades taxonômicas operacionais (UTOs). Nas amostras de sedimento marinho das Ilhas Shetlands dos Sul, a maioria dos táxons pertenceu ao gênero *Penicillium*, com abundância das espécies *P. allii-sativi*, *P. Palitans*, *P. soliutm* e *P. chrysogenum*, bem como as espécies *Pseudoagymnoascus verrucosus* e *Acremonium fusidioides*. Quanto à prospecção de metabólitos bioativos produzidos por estes fungos marinhos, todos os extratos apresentaram atividadade nematicida. Os extratos de 12 táxons foram capazes de inibir o crescimento do fungo fitopatogênico *Cladosporium sphaerospermum*, entre os quais os extratos de *P. solitum* e *P. chrysogenum* foram os mais ativos. Os extratos de 30 isolados marinhos (96.8%) apresentaram atividade herbicida, destes 58% apresentaram máxima inibição da germinação de *Allium schoenoprasum* e/ou *Lactuca sativa*, dentre os quais *P. allii-sativi*, *P. chrysogenum*, *P. Solitum*, *P. palitans* e *A. fusidioides* foram os mais ativos. Diferentes isolados de *Penicillium* spp. também se mostraram potencias

produtores de substâncias com atividade antiplasmodial e tripanossomicida. Os oito extratos dos fungos marinhos mais ativos foram caracterizados quimicamente por meio de seus espectros de Ressonância Magnética Nuclear H^1 (RMN H^1), indicando a presença de moléculas aromáticas, as quais podem ser as responsáveis pela expressivas atividades biológicas. As comunidades de fungos presentes em sedimentos lacustres foram analisadas a partir de lagos impactados pela atividade antrópica e lagos não impactados, ambos amostrados no arquipélago das Ilhas Shetlands dos Sul. Nas amostras de sedimento de lagos impactados pela atividade antrópica, 63 UTOs foram caracterizadas e os táxons *Pseudeurotium hygrophilum*, *Pseudogymnoascusus verrucosus* e *Neobulgaria* sp. ocorreram com maior frequência. No sedimento do Lago Central, próximo das estações antárticas, maiores concentrações de metais e menores valores de diversidade e riqueza fúngica foram detectados, ao contrário do Lago Norte, longe das estações. Os extratos de 40 isolados (15.4%) mostraram pelo menos uma atividade biológica contra *T. cruzi*, *C. sphaerospermum* ou herbicida, com destaque aos extratos de diferentes isolados de *P. hygrophilum*, os quais mostraram atividade seletiva para os alvos citados. A partir das amostras de sedimento de lagos não impactados, foram identificadas 42 UTOs, entre as quais os táxons endêmicos e/ou psicrófilos *Thelebolus globosus*, *Vishniacozyma victoriae*, *Antarctomyces psychrotrophicus* e *P. verrucosus* foram os mais frequentes. Entre os extratos produzidos por estes fungos, 60 (30,8%) apresentaram pelo menos uma atividade biológica contra *Leishmania amazonensis, Plamodium falciparum*, *T. cruzi*, *C. sphaerospermum* ou herbicida e nematicida; contudo, as atividades apresentadas não foram espécie dependente. Apesar das diferentes condições desfavoráveis à vida nos sedimentos marinhos e lacustres da Antártica, as comunidades fúngicas se mostraram ricas e diversas, com elevada dominância de espécies cosmopolitas. Os dados obtidos contribuem para um maior conhecimento das comunidades fúngicas presentes nos sedimentos marinhos e lacustres, considerados oligotróficos, pois muitas espécies foram relatas pela primeira vez. Para os lagos próximos as estações antárticas e afetados pelo turismo na região, o aumento de atividades antropogênicas pode afetar a diversidade e a composição da micota presente, e esta pode representar um modelo útil para o estudo do impacto antrópico na Antártica. Por fim, fungos obtidos a partir sedimentos representam uma fonte rica de metabólitos bioativos, os quais podem ser explorados como protótipos para novos fármacos e herbicidas menos tóxicos para a agricultura.

ABSTRACT

Some ecosystems, such as deep sea, hypersaline lagoons, volcanic substrates, deserts and polar regions, have physicochemical conditions considered unfavorable to life and are characterized by the presence of microbial communities known as extremophiles, which may be composed mainly of viruses, bacteria, archaea and fungi. In extreme ecosystems, fungi have ecological importance in nutrient cycling, and due to their high genetic and metabolic plasticity, which throughout its evolution have been conditioned by large variations in salinity, temperature, radiation, water availability, pressure, acidity, etc. In Antarctica, the selective pressure provided by weather conditions, especially low temperatures and poor water availability, may have selected fungi with different survival strategies, including antifreeze production, changes in cell membrane composition and production of differentiated primary and secondary metabolites. Considering the diversity and potential production of biomolecules presented by extremophile fungi, basic and applied studies involving these microorganisms are of great interest because they represent a portion of the world's biodiversity and also a rich source of substances with potential application in biotechnological processes. Thus, the present study aimed to characterize the fungal communities present in Antarctic marine and lake sediments in order to evaluate them as potential producers of bioactive substances. In the different substrates evaluated were obtained 486 fungal isolates belonging to 111 operational taxonomic units (OTUs). In the Southern Shetlands marine sediment samples, most taxa belonged to the *Penicillium* genus, with abundance of *P. allii-sativi*, *P. palitans*, *P. solitum* and *P. chrysogenum*, as well as *Pseudoagymnoascus verrucosus* and *Acremonium fusidioides* species. Regarding the prospecting of bioactive metabolites produced by these marine fungi, the extracts of 12 taxa were able to inhibit the growth of the phytopathogenic fungus *Cladosporium sphaerospermum*, among which the extracts of *P. solitum* and *P. chrysogenum* were the most active. All extracts have nematicidal activity. The extracts of 30 isolates (96.8%) presented herbicidal activity, of which 58% showed maximum inhibition of *Allium schoenoprasum* and/or *Lactuca sativa* germination, among which *P. allii-sativi*, *P. chrysogenum*, *P. solitum*, *P. palitans* and *A. fusidioides*. were the most active. Different isolates of *Penicillium* spp. Potential producers of substances with antiplasmodial and trypanosomicidal activity were also shown. The eight extracts of the most active marine fungi were chemically characterized by their H^1 Nuclear Magnetic Resonance (H^1 NMR) spectra, indicating the presence of aromatic molecules, which may be responsible for the

expressive biological activities. The fungal communities present in lake sediments were analyzed from lakes impacted by anthropic activity and non-impacted lakes, both sampled in the archipelago of the South Shetlands. In the sediment samples from lakes impacted by anthropic activity, 63 OTUs were characterized and taxa *Pseudeurotium hygrophilum*, *Pseudogymnoascusus verrucosus* and *Neobulgaria* sp. occurred more frequently. In the Central Lake sediment, near the Antarctic stations, higher metal concentrations and lower diversity and fungal richness values were detected, unlike the North Lake, far from the stations. The extracts of 40 isolates (15.4%) showed at least one biological activity against *T. cruzi*, *C. sphaerospermum* or herbicide, with emphasis on extracts of different *P. hygrophilum* isolates, which showed selective activity for the mentioned targets. From sediment samples from non-impacted lakes, 42 OTUs were identified, among which the endemic and/or psychophilic taxa *Thelebolus globosus*, *Vishniacozyma victoriae*, *Antarctomyces psychrotrophicus* and *P. verrucosus* were the most frequent. Among the extracts produced by these fungi, 60 (30.8%) showed at least one biological activity against *Leishmania amazonensis*, *Plamodium falciparum*, *T. cruzi*, *C. sphaerospermum* or herbicide; However, the activities presented were not species dependent. Despite the different unfavorable conditions for life in Antarctic marine and lake sediments, the fungal communities were rich and diverse, with high dominance of cosmopolitan species. The data obtained contribute to a better knowledge of the fungal communities present in marine and lake sediments, considered oligotrophic, since many species were reported for the first time. For lakes near the Antarctic stations and affected by tourism in the region, increased anthropogenic activities may affect the diversity and composition of the present mycota, and this may represent a useful model for the study of anthropic impact in Antarctica. Finally, fungi obtained from sediments represent a rich source of bioactive metabolites, which can be exploited as prototypes for new drugs and herbicides less toxic to agriculture.

1. RELEVÂNCIA E JUSTIFICATIVA

Os fungos desempenham um papel vital nos ecossistemas como sapróbios, mutualistas e parasitas de plantas e animais (DIX & WEBSTER, 1995). Várias espécies possuem fácil dispersão e por isso são capazes de colonizar diferentes substratos em condições abióticas variáveis, como as encontradas na Antártica (RUISI *et al*., 2007; ROSA *et al*., 2019a). A Antártica, bem como outras regiões extremas do planeta, possui inúmeras adversidades ambientais como baixas temperaturas e disponibilidade de água, ciclos de congelamento e descongelamento e alta incidência de radiação ultravioleta (RUISI *et al*., 2007; STERFLINGER *et al*., 2012). Fungos presentes em ambientes extremos, como os da Antártica, são capazes de sobreviver em temperaturas inferiores a 0 °C e possuem diferentes estratégias adaptativas que permitem sua sobrevivência (ROBINSON *et al*., 2000). Estudos sobre a taxonomia, diversidade e biotecnologia de fungos antárticos ainda são escassos e os substratos/habitats mais estudados até o momento foram os solos, plantas líquens e a macroalgas (ROSA *et al*., 2019a). Pouco se sabe ainda a composição das comunidades de fungos presentes nos sedimentos marinhos e lacustres da Antártica.

Nas profundezas do ambiente marinho, a ausência de luz solar, baixas temperaturas, alta pressão hidrostática e condições anóxicas fazem do fundo oceânico um abrigo para micro-organismos únicos e possivelmente desconhecidos, principalmente sedimentos marinhos da Antártica, que ainda permanecem praticamente inexplorados (NAGANO *et al*., 2010). Fungos que habitam esses locais de deposição podem apresentar grande variabilidade metabólica, uma vez que lidam com intempéries que divergem muito dos ambientes mesófilos temperados e tropicais do restante do planeta. Até o momento apenas os estudos publicados por Vaz *et al*., (2011), Gonçalves *et al*., (2013; 2015), Laich *et al*. (2013), López García *et al*., (2001) e Wentzel *et a*l., (2019) abordaram a presença de fungos nos sedimentos marinhos da Antártica (ROSA et al., 2019b).

Em substratos lacustres da Antártica, principalmente nas áreas livres de gelo (Antártica Marítima), os fungos presentes sofrem influência direta dos limitantes abióticos. Como mecanismos de tolerância, microrganismos adaptados ao frio podem produzir substâncias bioativas potenciais de interesse agro-industrial, como enzimas ativas a baixas temperaturas (FELLER & GERDAY, 2003), crioprotetores como trealose, exopolissacarídeos e proteínas anticongelantes (PHADTARE, 2004; XIAO *et al*., 2010; TSUJI *et al*., 2013, 2016). Além disso, fungos isolados de lagos já foram investigados como produtores de metabólitos

secundários com atividade biológica contra patógenos da área médica e células tumorais (BRUNATI *et al*., 2009; GONÇALVES *et al*., 2015).

Considerando a plasticidade metabólica e as estratégias adaptativas dos fungos antárticos, pesquisas de relevância caracterizando a micobiota residente na região têm recebido atenção nas últimas décadas, devido a sua importância ecológica e potencial utilização em processos biotecnológicos (ROSA *et al*., 2019a). Nesse contexto, esta tese apresenta a identificação e caracterização das comunidades de fungos presentes em sedimentos marinhos e lacustres da Antártica, a fim de contribuir com conhecimentos sobre taxonomia, filogenia, diversidade, ecologia e biogeografia de comunidades pouco ou ainda não caracterizadas pela ciência. Além disso, os fungos obtidos foram avaliados quanto à capacidade de produzir substâncias bioativas, as quais podem representar protótipos para o desenvolvimento de fármacos para uso na medicina e pesticidas menos tóxicos para utilização na agricultura.

2. INTRODUÇÃO

2.1 Antártica

A Antártica é um continente com cerca de 14 milhões de km^2 e coberta por uma camada de gelo maciço de aproximadamente 12 milhões de km^2 de extensão (WYNN-WILLIAMS, 1990; WILKINS *et al*., 2013). Os ventos catabáticos, provenientes das massas de ar frias e densas que se acumulam nos planaltos polares, fluem em direção ao mar e tendem a isolar a Antártica da influência térmica dos demais continentes (WYNN-WILLIAMS, 1990).

A Antártica é isolada dos outros continentes do mundo pelo Oceano Antártico e pela corrente circumpolar da Antártica (SMITH, 1991; WYNN-WILLIAMS, 1990). O continente antártico é considerado o lugar mais frio do planeta, sendo a temperatura mais baixa já registrada no continente de - 89,4 °C e a mais alta de 15 °C. O continente apresenta duas estações do ano: inverno e verão. Ao todo são seis meses com períodos de luz no verão e seis meses de completa escuridão no inverno. A temperatura média registrada no inverno é de - 34,4 °C, sendo a temperatura no interior do continente muito menor que da costa (NASA, 2018).

Existe uma delimitação no continente em três zonas biogeográficas, Antártica subantártica, Antártica marítima e Antártica continental, sendo os ecossistemas terrestres e as características climáticas das três zonas muito distintos. Segundo Convey (2006), a zona subantártica inclui uma série de ilhas isoladas situadas em altas latitudes no Oceano Antártico, sendo o clima subantártico fortemente influenciado pelo oceano circundante, o que restringe sua variação de temperatura (as temperaturas médias mensais são positivas em 6 a 12 meses do ano). A Antártica marítima inclui a costa ocidental da Península Antártica, incluindo os arquipélagos das Shetland do Sul, Orkney do Sul e Sandwich do Sul, e as ilhas isoladas de Bouvet e de Pedro I. Como o subantártico, o clima da região marítima também sofre influência oceânica, especialmente durante o período do verão austral, após a perda do gelo marinho acumulado no inverno (as temperaturas do ar são positivas, mas < 2 °C, por 1 a 4 meses do ano no verão, enquanto no inverno as médias permanecem negativas) (CONVEY, 2006). Já a Antártica continental é muito maior em extensão, com grandes áreas desérticas, sendo as temperaturas médias registradas as mais extremas do planeta. Na costa a temperatura média do ar pode chegar a ser positiva, enquanto no interior, apenas negativa (CONVEY, 2006).

O continente antártico é considerado um habitat extremo e constitui um dos ambientes mais inóspitos da Terra. Na perspectiva terrestre e aquática, o isolamento faz do continente um local onde a riqueza de espécies é baixa, sendo alguns ecossistemas continentais antárticos um dos mais simples da Terra, como p.ex. algumas comunidades endolíticas restritas a espécies de algas, fungos e bactérias (CHOWN, 2006). As temperaturas extremas, a baixa umidade, e a alta radiação incidente com longos períodos de escuridão completa, desencadearam múltiplos mecanismos de adaptação em diferentes espécies presentes na Antártica (COWAN & TOW, 2004).

Anualmente, ciclos de congelamento e degelo ocorrem naturalmente devido às variações sazonais de temperaturas, que chegam as mais baixas já registradas durante o extremo frio do inverno. Sendo assim, a cobertura de gelo do mar da Antártida sofre variações bruscas durante o ano, pois em fevereiro possui a extensão de 3 milhões de km^2 e no fim de setembro atinge cerca de 18 milhões de km^2 (NASA, 2015). Essa expansão, por sua vez, altera radicalmente o clima da Antártica, tornando-a o exemplo mais extremo de sazonalidade do planeta, tanto no mar como em terra, devido à transição da seca continental para a marítima rodeada de gelo (PHILLPOT, 1985).

Nos períodos mais frios (inverno no Hemisfério Sul) a superfície do gelo, dos continentes, dos lagos e dos mares antárticos, é essencialmente desprovida de água livre, adotando características de um deserto frio, até o início do degelo na primavera. Segundo Wynn-Williams (1990), a camada de gelo do planalto polar também não permite a ocorrência de água livre, pois é permanente durante o ano. Portanto, a água disponível se localiza em riachos, lagoas, lagos ou em saliências onde houve degelo de geleiras que fluíram do platô para os vales com menor altitude em relação ao mar. Existem também grandes áreas de solo e rocha expostos, algumas cadeias montanhosas e vales costeiros, onde a neve e o gelo derretem, mas raramente sublimam devido à influência dos ventos catabáticos (WYNN-WILLIAMS, 1990).

Além do estresse hídrico e térmico, a Antártica também representa um ambiente de estresse osmótico, pois com o congelamento da água formam-se microrregiões de soluções salinas concentradas a temperaturas abaixo de zero, abaixo do ponto de congelamento da água (-30 °C, mesmo na Antártica marítima). Este fenômeno é mais perceptível no gelo do mar, mas também ocorre em menor escala no gelo de água continental e no solo. Diferentes micro-habitats transitórios são criados nas superfícies do gelo sazonalmente e esses podem ser

colonizados por micro-organismos e, além disso, a formação de regiões super-resfriadas salinas também selecionam espécies psicrotolerantes e halotolerantes (WYNN-WILLIAMS, 1990). O processo de congelamento no inverno e degelo no verão também ocasiona uma transição frequente de ambientes anóxicos para aerados, assim algumas espécies de micro-organismos antárticos também respondem à ausência de oxigênio, bem como a baixa disponibilidade de nutrientes (WYNN-WILLIAMS, 1990).

O isolamento geográfico do continente e as condições extremas distinguem a Antártica dos demais ecossistemas, tornando-a um habitat natural para ocorrência de pequenos invertebrados, poucas plantas, espécies de aves e mamíferos, e de micro-organismos (SHIVAJI & PRASAD, 2009). A comunidade microbiana da Antártica é proveniente de propágulos carreados pelo ar atmosférico de outras regiões, já que milhões de toneladas de poeira contendo microrganismos viáveis, elementos traço e materiais orgânicos são transportados entre continentes a cada ano (CHOI *et al*., 1997; GARRISON *et al*., 2003). Para os fungos mais especificamente, a rota de dispersão mais importante é o transporte aéreo. Na Antártica, onde há limitações devido aos baixos níveis de partículas no ar (SATTLER & STORRIE-LOMBARDI, 2009), a dispersão também pode ocorrer pela dispersão local por pássaros, peixes e mamíferos, pela circulação oceânica e da água de degelo, entre outros vetores (VINCENT, 2000).

2.2 Micobioma antártico e estratégias adaptativas

Fungos vêm sendo caracterizados na Antártica desde o século XIX. O primeiro material coletado na Península Antártica, reconhecido como *Sclerotium antarcticum*, foi obtido durante a expedição Antártica Belga 1897-1899 (BOMMER & ROUSSEAU, 1905; GAMUNDI & SPINEDI, 1987). Desde então, diferentes espécies já foram caracterizadas, as quais pertencem aos filos *Ascomycota*, *Basidiomycota* e seus anamorfos, *Chytridiomycota*, *Glomeromycota*, *Mucoromycota* e *Mortierellomycota* (BRIDGE & SPOONER, 2012; ROSA *et al*., 2019a).

Os fungos desempenham um papel essencial nos ecossistemas antárticos, os quais atuam como sapróbios e agem na biodegradação da matéria orgânica e na ciclagem de nutrientes, e também atuam como mutualistas e parasitas associados a plantas e animais presentes na Antártica (ROSA *et al*., 2019a). Além disso, os fungos antárticos apresentam diferentes morfologias, ciclos reprodutivos e formas de dispersão (RUSSELL, 1990; RUISI *et*

al., 2007). Propágulos fúngicos (esporos e fragmentos de hifas) são de fácil dispersão e por isso os fungos são capazes de colonizar e habitar diferentes substratos tais como solos, materiais lignocelulósicos, águas continentais e marinhas, neve (de MENEZES *et al*., 2017), bem como superfície e interior de plantas (endofíticos) e animais (DIX & WEBSTER, 1995; ARENZ *et al*., 2006; ROSA *et al*., 2009).

Alguns ambientes da Antártica recebem propágulos microbianos de outras regiões como da porção austral da América do Sul, o que pode explicar a ocorrência de muitas espécies de fungos cosmopolitas na região (RUISI *et al*., 2007). Muitos dessas espécies podem estar presentes no ambiente Antártico como propágulos transitórios ou dormentes, associados a hospedeiros específicos, ou em outros substratos, podendo se adaptar a estilos de vida alternativos (BRIDGE, 2007). Entretanto existem espécies capazes de crescer e se reproduzir mesmo em baixas temperaturas que parecem ocorrer apenas no ecossistema antártico, e por isso, consideradas endêmicas (BRIDGE & SPOONER, 2012), como por exemplo as espécies *Mortierella antarctica* (ZYCHA *et al*., 1969), *Thelebolus globosus* (de HOOG *et al*., 2006), *Antarctomyces psychrotrophicus* (STCHIGEL *et al*., 2001) e *Antarctomyces pellizariae* (de MENEZES *et al*., 2017).

De modo geral, fungos psicrófilos têm temperatura ótima de crescimento de 15 a 20 °C, mas também são capazes de crescer em temperaturas ≤ 0 °C (MORITA, 1975; BRUNATI *et al*., 2009), os quais são prevalentes em habitats permanentemente frios, como nas regiões polares e em elevadas altitudes ou no mar profundo. Já os psicrotolerantes crescem em diferentes temperaturas, mesmo com taxas de crescimento ótimo de 15 °C a acima de 20 °C e predominantes em ambientes com baixas temperaturas periódicas (SHIVAJI & PRASAD, 2009). De acordo com Robison et al. (2001), fungos psicrófilos foram selecionados pelos ambientes frios por suas diferentes características fisiológicas como a produção crioprotetores como açúcares, polióis, ácidos graxos, proteínas extracelulares e enzimas ativas pelo frio que protegem as células do congelamento intracelular. Leveduras psicrófilas produtoras de substâncias criprotetoras já foram descritas na Antártica, como *Glaciozyma antarctica* (HOSHINO & MATSUMOTO, 2012) que é conhecida por sua capacidade de formar colônias no gelo a partida da produção de proteínas anticongelantes (LEE *et al*., 2010) e polissacarídeos extracelulares (TSUJI *et al*., 2013).

Na Antártica, fungos tolerantes ao frio (psicrófilos e psicrotolerantes) podem apresentar vias metabólicas diferenciadas ou únicas, e desta forma representar uma fonte de

diversidade química, o que os torna interessantes à comunidade científica no âmbito biotecnológico (ROSA *et al*., 2019a). Várias espécies de fungos da Antártica são descritas como adaptadas às baixas temperaturas, aos ciclos de congelamento e descongelamento, à baixa disponibilidade de água, de carbono orgânico e outros nutrientes, bem como, à dessecação e à alta radiação ultravioleta (COWAN & TOW, 2004; RUISI *et al*., 2007; STERFLINGER *et al*., 2012). Além disso, os taxa que estão presentes em solos, rochas e ambientes marinhos ainda lidam com o estresse osmótico provocado pela hipersalinidade desses locais (STERFLINGER *et al*., 2012).

2.3 Fungos em ambientes aquáticos

O ambiente aquático da Antártica compreende diferentes habitats como lagoas temporárias ou perenes, lagos salinos e de água continental, córregos de degelo, lagos formados pelo derretimento de gelo, fontes termais, mares e oceanos (OGAKI *et al*., 2019; ROSA et al., 2019b) . Tais áreas são colonizadas por fungos que habitam permanentemente a água durante todo seu ciclo de vida ou se originaram dela, bem como por espécies que estão presentes na água transitoriamente (DIX & WEBSTER, 1995). As zonas aquáticas estão em contato direto com rochas, solo e lama, e podem ser margeadas por vegetação, além da sua interface com o ar (zonas extra-aquáticas). A maioria dos fungos terrestres dispersam seus esporos e esses são depositados nas águas, carreados pelo ar, pela precipitação ou por inundações (DIX & WEBSTER, 1995). Já os fungos presentes em águas continentais e marinhas produzem esporos adaptados à dispersão em condições aquáticas, além disso, tais habitats possuem numerosos organismos que podem ser fonte de nutrientes para os fungos parasitas e/ou sapróbios (RAGHUKUMAR, 2017).

As espécies fúngicas adaptadas para completar seus ciclos de vida em habitats aquáticos e não são encontrados fora desses ambientes são consideradas indígenas ou residentes; já aquelas lixiviadas ou dispersas pelo vento e que se encontram transitoriamente no sistema são chamados de transitórias (SHEARER *et al*., 2007). As espécies transitórias podem ser imigrantes ou migratórias; as imigrantes normalmente têm um habitat extra-aquático e por dispersão, seus propágulos se depositaram na água, enquanto às migratórias, não regularmente, mas sim casualmente, alternam periodicamente entre habitats aquáticos e extra-aquáticos (DIX & WEBSTER, 1995).

Fungos aquáticos podem atuar como decompositores, mutualistas ou parasitas (WURZBACHER *et al*., 2011). De acordo com Dix & Webster (1995) os fungos aquáticos residentes são totalmente adaptados à vida na água e sua maioria é capaz de esporular na água e manter sua biomassa em níveis constantes à medida que os substratos e nutrientes se tornam disponíveis. Já os transitórios, por sua vez, ao adentrar no ambiente aquático podem chegar ativos, mas incapazes de esporular e colonizar novos substratos, bem como diminuir sua atividade em decorrência das mudanças na disponibilidade de oxigênio em relação ao seu habitat de origem, da perda de nutrientes pela lixiviação ou da competição com organismos melhor adaptados.

Os principais grupos taxonômicos já relatados em habitats aquáticos são *Ascomycota*, *Basidiomycota* e seus anamorfos, *Chytridiomycota* e os não fúngicos *Saprolegniales* na classe *Oomycetes*. Segundo SHEARER *et al*. (2007) cerca de 3 mil espécies de fungos e 138 espécies de Saprolegniales foram relatadas a partir de habitats aquáticos. O maior número de taxa compreende aos ascomicetos, incluindo taxa mitospóricos e os quitridiomicetos.

Muitas espécies fúngicas têm sido descobertas em substratos aquáticos como sedimentos oceânicos de profundidade (EDCOMB *et al*., 2011; RÉDOU *et al*., 2015; GONÇALVES *et al*., 2013), água do mar aberto (BASS *et al*., 2007), lagos de água continental (TSUJI *et al*., 2013; GONÇALVES *et al*., 2012) e salinos (STANLEY & ROSE, 1967) assim como ambientes extremófilos como fontes hidrotermais (LE CALVEZ *et al*., 2009), lagos subglaciais congelados, mares e gelo da Antártica e do Ártico (GUNDE-CIMERMAN *et al*., 2003; BUTINAR *et al*., 2006; D'ELIA *et al*., 2009; ROGERS *et al*., 2013; GONÇALVES *et al*., 2017). Além de seu papel ecológico, a micobiota aquática tem sido alvo de pesquisas, principalmente, devido a sua variabilidade metabólica, a qual é responsável pela produção de metabólitos secundários de interesse médico, agro-industrial e também na área biorremediação, como decompositores de poluentes em águas contaminadas (GROSSART & ROJAS-JIMENEZ, 2016).

Na Antártica, alguns autores têm realizado pesquisas envolvendo fungos isolados de água de lagos, sedimentos marinhos, água em mar aberto, invertebrados e macroalgas em diferentes regiões da Antártica marítima e continental (BRUNATI *et al*., 2009; DUARTE *et al*., 2013; GONÇALVES *et al*., 2015, 2017; CUI *et al*., 2016; FURBINO *et al*., 2018). Tais estudos demonstraram que esses organismos apresentam substâncias com atividades biológicas de interesse médico e agroindustrial.

2.3.1 Fungos de ambientes marinhos da Antártica

O sedimento marinho compreende um depósito de partículas de rocha e solo carreadas de áreas terrestres para o oceano, por meio dos ventos, gelo, rios e correntes marítimas, produtos de vulcanismo submarino, precipitados químicos da água do mar e materiais do espaço (meteoritos), bem como de matéria orgânica proveniente de restos de organismos marinhos e vegetais, que se acumulam no fundo do mar (ONI *et al*., 2015). Os propágulos microbianos presentes no sedimento são responsáveis pela reciclagem da matéria orgânica. Segundo Hedges & Keil (1995), a composição da matéria orgânica dispersa no sedimento depende diretamente do tamanho da fonte, da profundidade da água e das taxas de sedimentação.

Nos sedimentos de superfície, a matéria orgânica é facilmente degradada pelos microrganismos (COWIE & HEDGES, 1994; WAKEHAM *et al*., 1997), enquanto que a matéria orgânica menos reativa se acumula e fica enterrada no sedimento profundo (ZONNEVELD *et al*., 2010), assim os micro-organismos presentes nas camadas basais de sedimento requerem adaptações especiais para a utilização dessa matéria orgânica menos reativa e escalas de tempo mais longas para degradação dos nutrientes (MIDDELBURG, 1989; BIDDLE *et al*., 2006; INAGAKI *et al*., 2006). Devido a essas estratégias adaptativas, pesquisas sobre a microbiota de sedimento profundo, em geral, têm sido realizadas a fim de investigar mecanismos moleculares e regulatórios de microrganismos do fundo oceânico, bem como produtos dos seus metabolismos, como proteínas, enzimas e outras substâncias, que podem ser de grande interesse na área de biotecnologia (SATYANARAYANA *et al*., 2005).

Os ecossistemas marinhos representam um ambiente praticamente desconhecido em relação as suas comunidades microbianas, onde cerca de um milhão de micro-organismos (bactérias, arquéias, vírus e fungos) são encontrados por mililitro de água (GLÖCKNER *et al*., 2012). Nos ambientes marinhos, os micro-organismos são responsáveis pela ciclagem biogeoquímica de carbono, nitrogênio, fósforo, sílica, ferro e outros oligoelementos (NELSON *et al*., 1996; MOORE *et al*., 2002; MOREL & PRICE, 2003; VOSS *et al*., 2013).

Em particular, os fungos se destacam devido a sua alta plasticidade e capacidade de produzir diferentes enzimas ou outros metabólitos como estratégias ecológicas. Os fungos marinhos são capazes de colonizar uma ampla variedade de substratos que variam de madeira e algas a sedimentos, lamas, solos, areia, corais, tubos calcários de moluscos, folhas

decompostas de mangues, gramíneas entre-marés e animais vivos, como os que crescem nos tubos digestórios de crustáceos (HYDE *et al*., 1998). Esses materiais quando caem na coluna d'água podem transportar propágulos dormentes de fungos terrestres ou de água continental que são passivamente lavados no meio marinho (JONES *et al*., 2015; RAGHKUMAR, 2017).

Os ambientes marinhos abrangem colunas de água costeira, mar aberto e sedimento presente no fundo oceânico (RÉDOU *et al*., 2015). A distribuição de fungos nesses ambientes não é uniforme. As áreas costeiras sustentam diversas comunidades fúngicas de formas filamentosas e leveduriformes (RAGHKUMAR, 2017). Por outro lado, o mar aberto é considerado um deserto fúngico (KOHLMEYER & KOHLMEYER, 1979), trata-se de substrato oligotrófico onde os nutrientes são escassos e as enzimas secretadas pelos fungos são provavelmente perdidas pela rápida difusão na coluna de água (KOHLMEYER *et al*., 2004). A difícil obtenção de formas cultiváveis explica a preponderância de estudos baseados em sequências, onde estruturas leveduriformes ascomicéticas e basidiomicéticas são relatadas como mais abundantes (BASS *et al*., 2007; KOHLMEYER & KOHLMEYER, 1979).

O fundo oceânico, por sua vez, corresponde a 2/3 da superfície da Terra e abriga micro-organismos adaptados a sobreviver em condições impostas pela grande profundidade (NAGANO *et al*., 2010; RÉDOU *et al*., 2015). Com habitats hidrotermais, anóxicos e de baixas temperaturas, o mar profundo é reconhecido como um ambiente em sua maior parte estável, frio e escuro, caracterizado pela ausência de luz solar, baixas temperaturas, alta pressão hidrostática e baixa disponibilidade de nutrientes (NAGANO *et al*., 2010; RHAGHUKUMAR *et al*., 2010). As comunidades microbianas do fundo oceânico são responsáveis por um teor de carbono celular total de aproximadamente 3×10^{17} g (WHITMAN *et al*., 1998). Tais comunidades compreendem bactérias, arqueias, protistas e fungos (SNELGROVE *et al*., 1997). Grande parte dos estudos têm abordado procariotos em amostras marinhas de profundidade (DELONG & PACE, 2001; SOGIN *et al*., 2006; HONGXIANG *et al*., 2008; LUNA *et al*., 2009; SASS & PARKES, 2011), porém pouco se sabe a respeito dos fungos em amostras profundas.

Os fungos habitantes de sedimentos marinhos profundos foram isolados pela primeira vez em amostras coletadas no Oceano Atlântico a 4.450 m de profundidade (ROTH *et al*. 1964). Estudos da comunidade fúngica de sedimentos marinhos profundos, até o momento, mostram uma baixa diversidade de indivíduos, sendo a maioria baseada em estudos de fungos não cultiváveis (RAGHUKUMAR *et al*., 2004; NAGANO *et al*., 2010; EDGCOMB *et al*.,

2011; SINGH *et al*., 2012; RÉDOU *et al*., 2015). A fim de avaliar a diversidade de fungos de sedimentos marinhos profundos, Singh *et al*. (2012) coletaram testemunhos de 5000 m de profundidade na Bacia do Oceano Índico Central e identificaram gêneros como *Rhodotorula*, *Aspergillus*, *Candida* e *Nectria*. Rédou *et al*. (2015) investigaram comunidades de fungos de testemunhos de aproximadamente 2000 m do fundo do mar, da bacia de *Canterbury* (Nova Zelândia), onde foram isolados 21 gêneros de fungos pertencentes aos filos Ascomycota e Basidiomycota. Dentre eles, os gêneros *Exophiala*, *Fusarium*, *Penicillium*, *Rhodotorula* e *Meyerozyma* foram detectados tanto por métodos dependentes de cultivo quanto independentes, como a metagenômica.

Quando se trata de sedimentos marinhos antárticos, há poucos relatos na literatura sobre a presença de fungos. López-Gacía *et al*. (2001) avaliaram a diversidade de eucariotos em amostras de sedimento no limite frontal da Antártida ao longo da passagem Drake, onde apenas um táxon fúngico foi encontrado em amostras de mais de 3.000 m de profundidade e cujo gênero não foi identificado. Gonçalves *et al*. (2013) obtiveram vários isolados de *Penicillium solitum* de sedimentos marinhos de 100, 500, 700 e 1.100 m na Baía do Almirantado da Ilha Rei George, os fungos obtidos apresentaram perfis de atividade amilásica e esterásica. Fungos marinhos da Antártica precisam se adaptar e obter diferentes capacidades metabólicas que divergem das intempéries apresentadas pelo ambiente terrestre. Nesse sentido, estudos envolvendo diversidade e ecologia de fungos marinhos, estudos de prospecção de metabólitos bioativos também se tornam promissores, já que esses fungos podem constituir uma fonte praticamente inexplorada, a partir da qual novos protótipos de fármacos podem ser descobertos.

2.3.2 Fungos em ambientes lacustres da antártica

Há escassez de relatos sobre micro-organismos em lagos de água continental no continente antártico e a maior parte é voltada para as áreas da Antártica marítima. Quanto à diversidade da microbiota dos lagos, existe uma variação nas comunidades de micro-organismos de acordo com o gradiente de temperatura que ocorre latitudinalmente do hemisfério norte até o sul (HYDE *et al*., 2016), bem como quanto a composição físico-química da água que influencia o desenvolvimento de determinados grupos de micro-organismos. Na Antártica, segundo Ellis-Evans (1996), o índice de estado trófico, o qual avalia a qualidade de água pelo grau de trofia, é maior no extremo norte do continente e reduz

consideravelemnte rumo ao sul; há também uma grande variedade de micro-organismos que ocorrem em toda a extensão latitudinal da Antártica.

As áreas ao norte, que compreendem a Península Antártica e as Ilhas Shetland do Sul na Antártica marítima, possuem temperaturas mais elevadas durante o ano em relação ao restante do continente antártico (NEWSHAM *et al*., 2015) e isso consequentemente influencia nas comunidades microbianas dos corpos d'água presentes na região. As mudanças climáticas que vem modificando ambientes em todo o globo também tiveram contribuição no aumento ds temperaturas médias da Península Antártica, o que, consequentemente, de acordo com Ellis-Evans (1996), pode ter refletido na atividade microbiana lacustre em determinados períodos do ano. Estudos mostram que nos últimos 40 a 50 anos, na Península Antártica, houveram alterações rápidas de temperatura (cerca de 2 °C) e, mesmo com a permanência das baixas temperaturas ao longo de todo o ano, os lagos da região responderam mais rapidamente às mudanças climáticas em comparação a outras áreas da Antártica (QUAYLE *et al*., 2002).

Em grande parte da Península Antártica, sobretudo nas áreas livres de gelo, os lagos são as principais zonas de escoamento para a água de derretimento das geleiras e/ou da neve acumulada, portanto os ciclos de descongelamento, que ocorrem durante o verão, podem ser responsáveis pela formação de pequenos riachos e lagos, os quais se alimentam dessas águas de derretimento fruto do aumento das temperaturas (OGAKI *et al*., 2019). Solutos e materiais particulados das áreas de captação são carreados pela água e depositados nos sedimentos dos lagos, os quais podem contribuir com os processos biogeoquímicos lacustres (ALFONSO *et al*., 2015). Na maioria dos lagos da Antártica, a ciclagem de nutrientes ocorre devido à atividade de comunidades bentônicas de cianobactérias e bactérias, bem como outros micro-organismos, como os fungos (ELLIS-EVANS, 1996; GONÇALVES *et al*. 2012).

Os fungos que habitam substratos lacustres da Antártica sofrem influência direta dos processos de congelamento e degelo ao longo do ano, da alta radiação ultravioleta no verão versus períodos de escuridão no inverno (OGAKI *et al*., 2019). A baixa diversidade florística das margens, a ausência de peixes, e a baixa biomassa de zooplâncton, características dos lagos antárticos (LAYBOURN-PARRY *et al*., 1997), também limitam a disponibilidade de nutrientes, já que a maioria dos fungos atuam na decomposição de matéria orgânica, ou como endofíticos e parasitas (MCINNES 2003; WURZBACHER *et al*., 2011; KOIVUSAARI *et al*., 2019). Entre os substratos lacustres da Antártica em que já foram identificados fungos, estão consórcio microbiano marginal (BRUNATI *et al*., 2009), solo marginal e sedimentos (TSUJI

et al., 2013; GONÇALVES *et al*., 2015) e água (GONÇALVES, *et al*., 2012). Brunati *et al*. (2009) isolaram fungos associados a consórcios microbianos das margens de lagos da região de Larsemann Hills, Vestfold Hills e McMurdo Dry Valleys. Gêneros de fungos psicrófilos como *Thelebolus* e *Pseudogymnoascus* foram identificados, bem como os cosmopolitas *Acremonium*, *Aspergillus*, *Beauveria*, *Cladosporium*, *Curvularia* e *Penicillium*.

Na área de Skarvsnes, Antártica, Tsuji *et al*. (2013) isolaram 71 fungos de sedimentos lacustres dos gêneros *Dioszegia*, *Leucosporidium* (*Glaciozyma*), *Mrakia*, *Pseudogymnoascus* e *Thelebolus*. Dentre os fungos obtidos, *Glaciozyma antarctica* e *Rhodotorula glacialis* demonstraram atividade anticongelante. Na Antártica Marítima, Ilhas Shetland do Sul, Gonçalves *et al*. (2012) isolaram 128 fungos de águas de lagos das Ilhas Rei George e Deception, sendo os táxons mais frequentemente isolados foram aqueles dos gêneros, *Cadophora*, *Cladosporium*, *Mortierella*, *Penicillium*, *Pseudogymnoascus* e *Thelebolus*, bem como o endêmico *Antarctomyces*. Em amostras de sedimento de lago, ainda nas Ilhas Shetland do Sul, Gonçalves *et al*. (2015) também identificaram espécies de *Aspergillus*, *Cosmospora*, *Penicillium* e *Pseudogymnoascus*.

Fatores abióticos e bióticos exercem grande pressão seletiva nesses fungos e estes por sua vez podem ser selecionados por sua composição de ácidos graxos na membrana celular, produção de substâncias anti-congelantes intra e extracelulares (trealose, açúcares crioprotetores, proteínas, polióis) e capacidade de produzir enzimas frio-ativas (ROBINSON *et al*., 2000). Essas substâncias fúngicas, bem como outros metabólitos secundários, podem ter diferentes aplicações, sendo de grande interesse industrial, médico e na agricultura.

2.4 Bioprospecção de metabólitos bioativos de fungos extremófilos

Metabólitos secundários ou produtos naturais são substâncais orgânicas de baixo peso molecular com estruturas químicas complexas e diversas funções fisiológicas (BÉRDY, 2005; NIU & TAN, 2013; JOYCE *et al*., 2011). O metabolismo secundário evoluiu na natureza em resposta às necessidades e desafios do ambiente natural, onde se espera que a produção de metabólitos secundários forneça alguma vantagem competitiva ao produtor, por exemplo, eliminar nutrientes ou organismos competidores (DEMAIN & VAISHNAV, 2011; JOYCE *et al*., 2011). Estudos de bioprospecção de metabólitos bioativos têm focado micro-organismos pouco caracterizados e capazes de sobreviver em condições adversas, como por exemplo,

espécies de bactérias e fungos extremófilos (FERRER *et al*., 2007; PETTIT, 2011; PASCALE *et al*., 2012).

Os metabólitos produzidos por fungos incluem terpenos, policetídeos, alcalóides, poliacetilenos, quinonas, antibióticos, pigmentos entre outras sbustâncias bioativas (CHRISTENSEN, 1989; BUZZINI *et al*., 2012; NIU & TAN, 2013; GODINHO *et al*., 2015). Muitos desses metabólitos têm aplicações agrícolas e médicas, sendo importantes em processos biotecnológicos como protótipos estruturais para o desenvolvimento de novos antimicrobianos, antioxidantes, imunomoduladores, anticancerígenos, bem como herbicidas menos tóxicos para a agricultura (BUZZINI *et al*., 2012; SANTIAGO *et al*., 2012; GODINHO *et al*., 2015).

Os fungos extremófilos vivem em condições extremas e limitantes de sobrevivência o que os torna metabolicamente distintos de outros organismos mesófilos (ZHANG *et al*., 2018). Muitos desses fungos já foram identificados por produzirem diferentes metabólitos secundários (produtos naturais) com diferentes atividades biológicas. Alguns exemplos de substâncias já caracterizadas produzidas por fungos extremófilos são as: micosporinas, asperelinas, geomicinas e citochalasinas. Exemplods de estruturas químicas das respectivas moléculas bioativas citadas estão representadas na **Figura 1**.

As micosporinas ou aminoácidos do tipo micosporina (MAAs) são metabólitos secundários que podem ser usados na produção de protetores solares pela indústria cosmética e farmacêutica (CARRETO & CARIGNAN, 2011). As moléculas de micosporina contêm um anel central de ciclohexenona ou ciclohexenimina (item A, **Figura 1**) que se acredita absorver a luz UV e acomodar os radicais livres (que as agrega função antioxidante) (BANDARANAYAKE, 1998; CARRETO & CARIGNAN, 2011). Alguns exemplos de micosporinas como a micosporina-glutaminol-glicosídeo e a micosporina-glutamicol-glicosídeo (item A, **Figura 1**) são produzidas pela levedura negra halotolerante *Aureobasidium pullans* isolada de ambiente hipersalino do ártico (KOGEJ *et al*., 2006). As asperelinas, por sua vez, são substâncias fúngicas bioativas conhecidas como peptaibóis, uma classe de peptídeos lineares ou cíclicos constituídos por cadeias de 4-21 resíduos de aminoácidos, onde há um ácido α-aminoisobutírico ou α metil alanina, terminando em um álcool C-terminal (item B, **Figura 1**) (CHUGH & WALLACE, 2001; REN *et al*., 2009). As cadeias anfipáticas dos peptaibóis formam canais iônicos dependentes de voltagem nas membranas celulares, levando a lise das células alvo (CHUGH & WALLACE, 2001). Como

exemplos de estrutura química das asperelinas estão as A e B obtidas do fungo marinho *Trichoderma asperellum* isolado de sedimento da Ilha Pinguim na Antártica, representadas no item B, **Figura 1** (REN *et al*., 2009 e 2013).

Segundo Li *et al*. (2008), as geomicinas são derivados de ácido astérico com atividade antimicrobiana, como por exemplo as geomicinas: A, B e C, obtidas do fungo da Antártica *Pseudogymnoascus* sp. (item C, **Figura 1**) que em ensaios biológicos as moléculas já apresentaram atividade antimicrobiana contra algumas cepas de referência de *Aspergillus fumigatus*, *Escherichia coli*, *Staphylococcus aureus* e/ou *Streptococcus pneumoniae* (NCBI, 2019a). Já as citocalasinas são um grupo diverso de peptídeos não ribossomais e policetídeos com uma gama de funções biológicas, incluindo atividade antitumoral que já foi descrita para as moléculas Z24 e Z25 (item D, **Figura 1**) obtidas do fungo do Ártico *Eutypella* sp. D-1 (LIU *et al*., 2014).

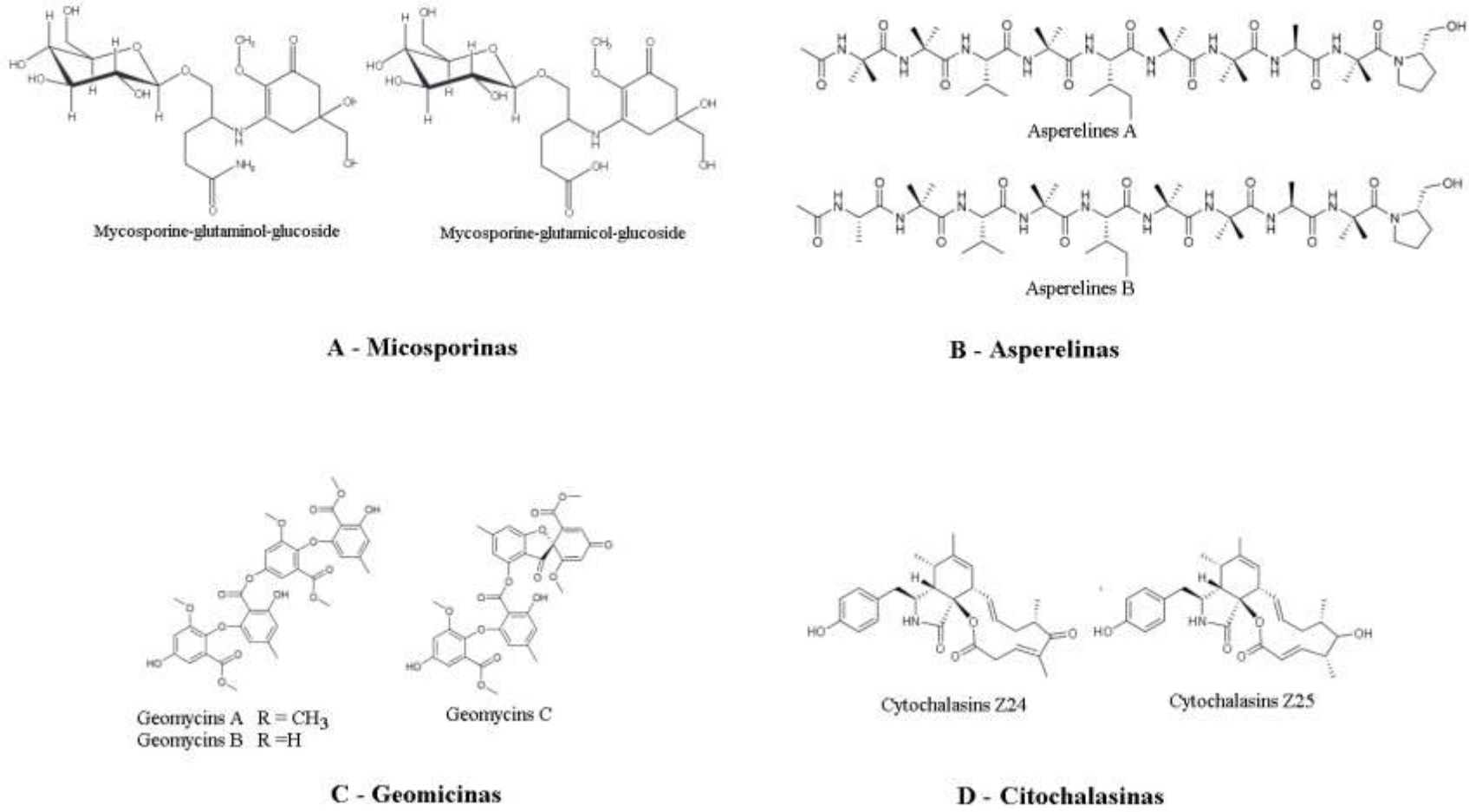


Figura 1. Estruturas químicas de moléculas bioativas isoladas de fungos extremófilos. A – micosporinas. B – asperelinas (A e B). C – geomicinas (A, B e C). D – citocalasinas.

Ambientes marinhos abrigam fungos associados com algas e que coabitam com invertebrados, especialmente corais e esponjas, bem como aqueles presentes em detritos e em sedimentos marinhos (RHAGHUKUMAR, 2008). Fungos marinhos são diversificados, e já têm sido caracterizados quimicamente quanto sua produção de metabólitos secundários com potencial antibacteriano, antifúngico, antiviral, antioxidante, citotóxico, antitumoral e anti-inflamatório (MAYER *et al*., 2013). A cefalosporina C (**Figura 2**) produzida por *Acremonium chrysogenum* foi o primeiro metabólito bioativo isolado de um fungo marinho (BUGNI & IRELAND, 2004); é um antibiótico da classe das cefalosporinas que atua na síntese da parede celular de bactérias, catalisando a transpeptidação de peptidoglicano (NCBI, 2019b). Depois dessa descoberta, outras substâncias com atividades biológicas foram caracterizadas a partir de espécies dos gêneros *Aspergillus*, *Cephalosporium*, *Fusarium* e *Penicillium* bem conhecidos como produtores (MANIMEGALAI *et al*., 2013).

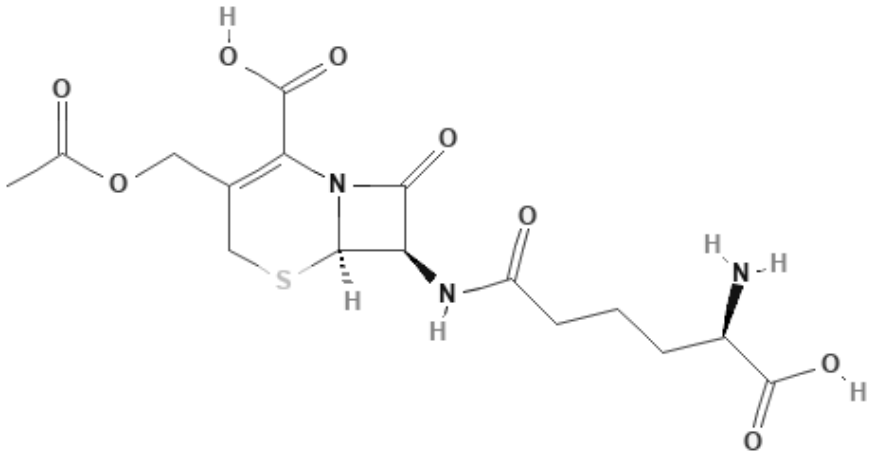


Figura 2. Estrutura química da molécula bioativa cefalosporina C produzida pelo fungo marinho *Acremonium chrysogenum*.

Mais de 4 mil metabólitos de fungos foram relatados até o momento (TIWARI *et al*., 2014). Com respeito aos fungos marinhos, de 5 a 7 mil espécies já foram avaliadas quimicamente e diversos metabólitos biológicos foram detectados para essas espécies marinhas, incluindo alcalóides, macrolídeos, quinonas, peptídeos cíclicos, xantonas, terpenóides, isoprenóides e outros aromáticos (ZHANG & KIM, 2012; LIANG *et al*., 2014; HASAN *et al*., 2015). Entretanto, poucas espécies da Antártica foram quimicamente investigadas até o momento.

Extratos de fungos provenientes de amostras de solo, da porção continental da Antártica, foram avaliados por Godinho *et al*. (2015) quanto às atividades antiviral, antibacteriana, antifúngica, antiparasitária, antitumoral, herbicida e inseticida. Uma espécie de *Aspergillus* e quatro de *Penicillium* apresentaram atividade antimicrobiana, antitumoral e herbicida, e a maioria dos extratos, analisados por ressonância magnética nuclear protônica (RMN ^{1}H), indicou a presença de bioativos protonados em regiões aromáticas e olefínicas. Gonçalves *et al*. (2015) também avaliaram extratos etanólicos de fungos antárticos recuperados de sedimento marinho e lacustre, solo e rizospfera de gramíneas, cujas análises espectrais por RMN 1H do extrato de *Purpureocillium lilacinum* isolado de solo indicou a presença de metabólitos bioativos aromáticos capazes de inibir em 100% o crescimento de formas intracelulares amastigotas de *Trypanossoma cruzi*, *Paracoccidioides brasiliensis* e *S. aureus*, importantes patógenos clínicos.

Em ambientes marinhos da Antártica estudos prospectaram fungos associados a hospedeiros vegetais e animais, os quais avaliaram extratos frente a diferentes ensaios biológicos. Godinho *et al*. (2013) mostraram alta e seletiva atividade antifúngica e/ou tripanosomicida de extratos de *Penicillium chrysogenum* recuperado da macroalga endêmica da Antártica *Palmaria decipiens*. Furbino *et al*. (2014) obtiveram fungos associados aos talos das macroalgas *Monostroma hariotii* e *Porphyra endiviifolia* coletadas nas regiões infralitorais das Ilhas Elephant, Rei George e Deception na Península Antártica. Ao todo, dos 50 táxons de fungos algícolas encontrados, quatro apresentaram atividade antifúngica contra patógenos clínicos e da agricultura e *Penicillium steckii* foi capaz de produzir extrato com atividade antiviral contra o vírus da febre amarela.

Fungos isolados de invertebrados marinhos também já foram avaliados quanto à produção de substâncias bioativas. Contudo, existem apenas dois trabalhos na literatura para a região da Antártica, os quais foram obtidos de espojas marinhas (HENRÍQUEZ *et al*., 2014) e do microcrustáceo *Euphausia superba* (CUI *et al*., 2016). Henriquez *et al*. (2014) obtiveram isolados recuperados de 11 táxons de esponjas marinhas na Ilha Fildes, também na Península Antártica. Dentre os extratos fúngicos obtidos, os provenientes dos gêneros *Cladosporium*, *Epicoccum*, *Penicillium* e *Pseudogymnoascus* apresentaram atividade antibacteriana contra as bactérias *Staphylococcus aureus* e *Pseudomonas aeruginosa*. Além disso, o gênero *Pseudogymnoascus* também apresentou efeito positivo na inibição de formação de galhas em amostras de batata, sendo de interesse também como potencial precursor de substâncias anti-

fitopatógenas. Cui *et al*. (2016) isolaram fungos associados a espécie de krill antártico – *Euphausia superba* capazes de inibir o crescimento de bactérias patogênicas aquáticas. Nesse mesmo trabalho os autores isolaram sete substâncias (ácido trimetilbenzóico, citreoroseína, pinselina, citrinina, dihidrocitrinona, penicitrinona e quinolactacina) da espécie *Penicillum citrinum* que apresentaram citotoxicidade moderada a forte contra células tumorais A549, K562 e MCF-7.

Fungos extremófilos presentes na água dos lagos antárticos foram capazes de produzir moléculas bioativas. Brunati *et al*. (2009) isolaram dois metabólitos bioativos (bis-antraquinonas: *rugulosin* e *skyrin*) produzidos por *P*. *chrysogenum* e frações de duas espécies de *Aspergillus* presentes de consórcio microbiano de lagos, tais compostos apresentaram atividade antimicrobiana e capacidade citotóxica em células de carcinoma humano, respectivamente. Tais micro-organismos tornam-se interessantes às pesquisas científicas em estudos de diversidade e ecologia e evolução microbiana e também de biotecnologia, como potenciais produtores de biomoléculas protótipos de novos fármacos.

3. OBJETIVOS

3.1 Objetivo Geral

Caracterizar a comunidade de fungos cultiváveis presentes em amostras de sedimentos marinhos e lacustres da Antártica e avaliar seu potencial como produtores de metabólitos bioativos de interesse na medicina e na agricultura.

3.2 Objetivos Específicos

- Coletar sedimentos lacustres e marinhos de diferentes regiões e profundidades da Península Antártica para o isolamento dos fungos extremófilos;
- Identificar todos os fungos por meio de técnicas morfo-fisiológicas e de biologia molecular;
- Contribuir para a preservação *ex situ* dos fungos obtidos por meio da montagem de uma coleção de culturas temática de fungos antárticos;
- Caracterizar a diversidade das comunidades de fungos encontradas;
- Preparar extratos a partir do cultivo dos fungos obtidos e avaliar as atividades:
    - Antifúngica contra *Cladosporium sphaerospermum*,
    - Antiparasitária contra formas amastigotas de *Leishmania amazonesis*,
    - Antiaparasitária contra formas tripomastigotas de *Trypanossoma cruzi*,
    - Antiparasitária contra *Plasmodium falciparum*,
    - Antiviral frente ao vírus da dengue do sorotipo 2,
    - Herbicida frente a modelos de plantas mono e dicotiledôneas,
    - Nematicida contra *Caenorhabditis elegans*.

4. MATERIAIS E MÉTODOS

4.1 Fungos em sedimentos marinhos

4.1.1 Coleta e processamento das amostras de sedimento marinho

Os testemunhos de sedimento foram obtidos por meio de um amostrador *Gravity-Corer* em diferentes sítios marinhos das Ilhas Shetland do Sul, Antártica, durante a Operação Antártica XXXV no verão austral de 2014 e 2015. Os testemunhos de 150 e 250 m foram coletados na Baía Maxwell, o de 550 m no mar das Ilhas Shetlands do Sul e o mais profundo de 1463 m no Oceano Antártico. Assim que coletados, os testemunhos foram fracionados em secções de 10 cm, as quais foram armazenadas a - 20 °C e encaminhadas para o Brasil até serem processados. A fração mais basal dos testemunhos coletados foi usada neste trabalho. Um descongelamento gradual a 4 °C 24 h antes foi realizado antes do isolamento e três alíquotas A, B e C foram obtidas das partes centrais de cada secção para evitar contaminações, essas posteriormente foram usadas para os inóculos.

4.1.2 Isolamento de fungos em sedimento marinho

Para o isolamento dos fungos em sedimento foram usados os meios de cultura: Ágar Batata Dextrose (BDA), Ágar Sabouraud (ASd), Ágar fubá (CMA), Ágar Czapek-dox (CZA) e Ágar extrato de malte (MEA) na proporção de 1:5 para simular condições baixas de nutrientes de sedimentos profundos como descrito por Damare *et al*., 2006 e Redou *et al*., 2015. Além dos meios diluídos, o inóculo foi realizado nos meios Ágar marinho 2% (p / v) de glicose (AM) e Ágar Sabouraud 4% (p / v) de glicose (AS), todos os meios contendo 100 $mg.mL^{-1}$ de cloranfenicol (Sigma/EUA) prevenindo o crescimento de bactérias. Tendo em vista o baixo número de isolados obtidos por uma única técnica de isolamento, três técnicas foram realizadas:

(a) Técnica 1: Foram adicionados 1 g de cada alíquota (A, B e C) a 1 mL de água marinha – sal marinho 30 g L^{-1} (Sigma, EUA) em microtubos, esses foram homogeneizados e 100 μL de cada suspensão foram plaqueados;

(b) Técnica 2: Plaquelamento do sedimento puro em forma de esfregaço;

(c) Técnica 3: Enriquecimento usando 1 g de cada alíquota adicionados a 5 mL de meio líquido (os mesmos meios citados acima, porém sem adição de ágar) sob agitação 120 rpm a 10 °C por 7 dias, após o período de incubação, 100 μL de cada solução foi plaqueada.

Os inóculos em duplicata foram incubados a 10 °C e o crescimento de colônias fúngicas foi observado durante 30 dias. As colônias foram quantificadas (UFC g^{-1}), considerando cada técnica de isolamento, e purificadas em meio AM. Características macromorfológicas (cor e textura de colônia, tipo de borda e taxa de crescimento radial) foram usadas para agrupamento e posterior identificação molecular.

4.2 Fungos em sedimentos lacustres

4.2.1 Coleta e processamento das amostras de sedimentos lacustres

Duas coletas de sedimento lacustre foram realizadas em colaboração com a equipe dos Professores Rosemery Vieira e Arthur Ayres, ambos da Universidade Federal Fluminense, RJ. A primeira coleta foi realizada durante a Operação Antártica XXXIV (OA XXXIV) no verão de 2016. As amostras de sedimentos lacustres foram coletadas em três lagos da Península Fildes Ilha Rei George, Península Antártica (**Figura 3**). A segunda coleta foi realizada durante a Operação Antártica XXXV durante o verão de 2016 em oito lagos de diferentes ilhas na Península Antártica (**Figura 4**).

Figura 3. Imagens das áreas de coleta das amostras de sedimento lacustre durante a OA XXXIV. Ilha Rei George, Península Fildes: A – Lago Fildes Norte; B – Lago Fildes Central; C – Lago Fildes Sul.

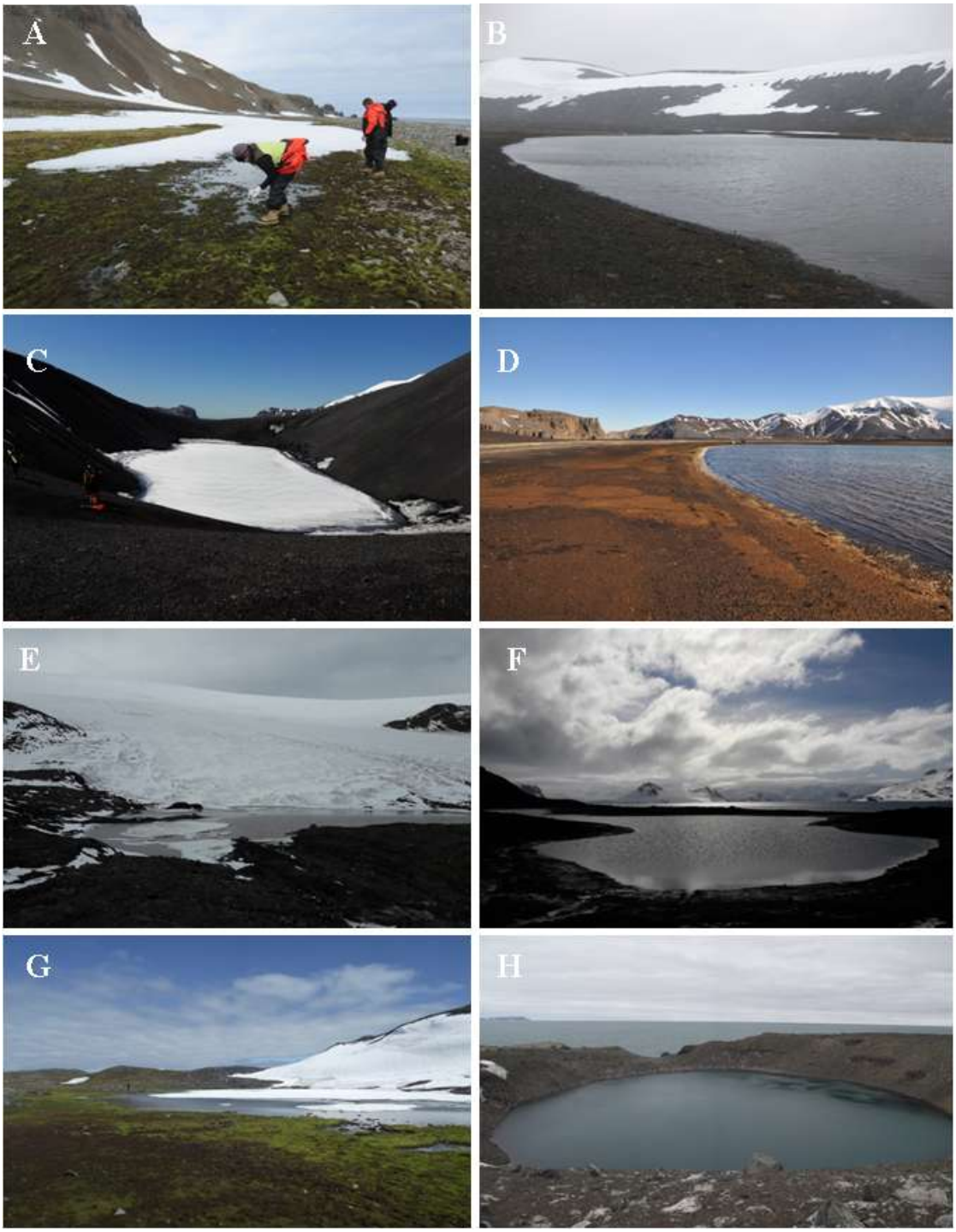


Figura 4. Imagens das áreas de coleta das amostras de sedimento lacustre durante OA XXXV. Ilha Rei George, Baia do Almirantado: A – Lago Punta Hannequin; E – Lago Wanda B; F – Lago Wanda A. Ilha Deception, Creater Lake: B – Lago Deception A, C – Lago Deception B; Ilha Deception, Whalers Bay: D – Lago Kroner. Ilha Elefante, Refúgio Emilio Goeldi, BR: G – Lago Elefante. Ilha Pinguim: H – Lago Pinguim.

Todos os testemunhos lacustres foram coletados manualmente usando canos de PVC de 60 mm de diâmetro por 50 cm de altura devidamente desinfestados para evitar contaminações. Foram realizadas lavagens com solução de hipoclorito de sódio 1,5 % por 10 min, com água destilada e solução de etanol 70 %, posteriormente a secagem ocorreu em fluxo laminar sob luz UV e os canos foram mantidos em sacos esterilizados até o momento do uso. Os testemunhos obtidos foram seccionados em frações de 5 cm referentes ao topo, meio e base (**Figura 5**), dependendo do tamanho total do testemunho obtido. Alíquotas de cada secção foram retiradas em triplicata A, B e C edevidamente acondicionadas a - 20 °C até a realização dos cultivos no Brasil.

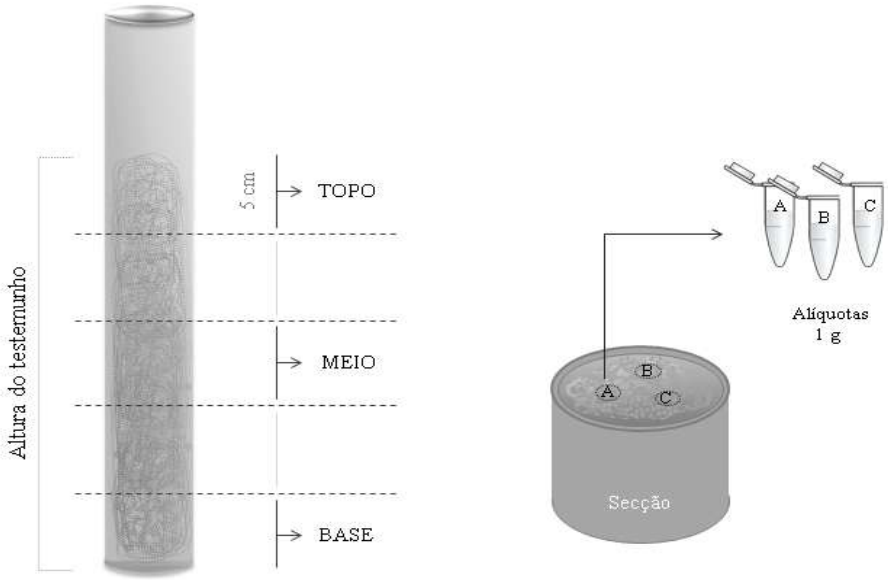


Figura 5. Esquema de seccionamento dos testemunhos de sedimento lacustre. A imagem à esquerda representa os testemunhos ao serem seccionados em Topo, Meio e Base; à direita a obtenção das três alíquotas A, B e C de cada secção para fins de isolamento de fungos.

4.2.2 Isolamento de fungos em sedimentos lacustres

A fim de isolar fungos presentes nas amostras de sedimento lacustre, 1 g de sedimento foi ressuspendido em 1 mL de salina 0,9%, de tal suspensão 100 µL foram plaqueados nos meios: i) DRBC – Ágar Dicloran Rosa Bengala, composição: Peptona 0,5 %, glicose 1 %, KH_2PO_4 0,1 %, $MgSO_4 \cdot 7H_2O$ 0,05 %, dicloran 0,0002 %, rosa begala 0.0025 % e ágar 2 % (Oxoid, USA); ii) MEA – Ágar Extrato de Malte, composição: Extrato de malte 5 %, ágar 2%; iii) MM – Meio mínimo, composição: Peptona 0,025 %, glicose 0,5 %, K_2HPO_4 0,698 %,

KH_2PO_4 0,544 %, $(NH_4)_2SO_4$ 0,1%, $MgSO_4$. 7 H_2 O 0,11 %, ágar 2 %. Todos os meios foram suplementados com 100 $mg.mL^{-1}$ de cloranfenicol (Sigma/EUA) a fim de evitar o crescimento de colônias bacterianas. As placas de Petri contendo os meios de cultura foram incubadas a 10 °C e o crescimento de colônias monitorado por 60 dias. As colônias obtidas foram quantificadas em unidade formadora de colônias por grama (UFC g^{-1}) e purificadas em novas placas para preservação com os mesmos meios de isolamento, com exceção dos isolados obtidos do meio DRBC, cuja preservação foi feita no meio APG – Ágar peptona glicose de composição semelhante, sem acréscimo dos antifúngicos (Peptona 0,5%, glicose 1%, KH_2PO_4 0,1%, $MgSO_4 \cdot 7H_2$ O 0,05%, ágar 2%). Características macromorfológicas (cor e textura de colônia, tipo de borda e taxa de crescimento radial) foram usadas para agrupamento e posterior identificação molecular.

4.3 Preservação dos fungos

Para a preservação de fungos, discos de 3 mm diâmetro de ágar contendo micélio ativo das culturas puras dos fungos filamentosos foram adicionadas em frascos de vidro contendo água destilada esterilizada a temperatura ambiente (CASTELLANI, 1967) e em glicerol 15% a -80 °C, ambos os ensaios em duplicata. As culturas puras das leveduras foram inoculadas em meio GYMP (glicose 2%, extrato de levedura 0,5%, extrato de malte 1% e de fosfato de sódio 0,2%) e, após o período de 48-72 h, foi retirada uma alíquota de 1 mL deste caldo com a levedura para criotubos esterilizados e acrescido 20% de glicerol e depositadas no ultrafreezer a -80 °C. Todos os fungos obtidos foram depositados na Coleção de Micro-organismos e Células do Departamento de Microbiologia do Instituto de Ciências Biológicas da UFMG, com o código UFMGCB.

4.4 Identificação dos fungos

4.4.1 Identificação de fungos filamentosos

As colônias foram agrupadas por meio de análises macro-morfológicas quanto à coloração, formato, borda, relevo e coloração. Posteriormente, análises moleculares foram realizadas a fim de identificar a espécie fúngica. O agrupamento molecular foi realizado por meio da técnica de PCR microsatélite (PCR-MST), também chamada “DNA *fingerprint*”, usando como iniciador o oligonucleotídeo sintético $(GTG)_5$. Em seguida, um indivíduo de cada grupo foi submetido à identificação por meio da amplificação da região transcrita interna

(ITS1-5.8S-ITS2) do gene do rRNA, a amplificação parcial do gene da β-tubulina e/ou gene RNA polimerase II. A escolha da região a ser amplificada foi determinada de acordo com os gêneros encontrados por meio da amplificação da região ITS (para alguns táxons identifica apenas em nível de gênero).

4.4.1.1 Extração de DNA total

A extração de DNA total seguiu a metodologia descrita por Rosa *et al*. (2009). O cultivo dos fungos filamentosos foi realizado no meio de isolamento de cada morfotipo encontrado e incubado por 7 a 14 dias. Após o período de incubação, uma porção do micélio foi adicionada em microtubos de 2 mL acrescidos de 400 µL de tampão de lise (Tris-HCl 0,05 M, EDTA 0,005 M, NaCl 0,1 M e SDS 1%) que foram mantidos em repouso a -20 ºC por no mínimo 30 min. Aos tubos contendo micélio foi adicionado de duas a três pérolas de aço inoxidável e os mesmos foram submetidos à trituração com auxílio de microdesmembranador sob intensa agitação. Após homogeneização foram adicionados 162 µL de CTAB (Tris 2M, NaCl 8,2%, EDTA 2M e CTAB 0,2%) e os tubos foram incubados a 65 ºC por 40 min. Em seguida, foram acrescidos 570 µL de clorofórmio/álcool isoamílico (24:1), os tubos foram homogeneizados e mantidos em gelo por 30 min, e posteriormente centrifugados a 14.000 rpm por 10 min. O sobrenadante foi transferido para um novo tubo com adição de 10% de seu volume de uma solução de acetato de sódio 3M, a suspensão foi homogeneizada e incubada a 0 ºC por 30 min, e em seguida centrifugada a 14.000 rpm por 10 min. O sobrenadante foi transferido para um novo tubo acrescido de 50% de seu volume de isopropanol (Merck). A suspensão foi homogeneizada e centrifugada a 14.000 rpm por 5 min. O sobrenadante foi desprezado, e o sedimento homogeneizado com etanol 70% (Merck) v/v. As amostras foram secas a 37 ºC por 30 min e acrescidas de 50 µL de tampão TE/Tris-EDTA (Tris-HCl 0,01 M e EDTA 0,001 M) e incubadas a 65 ºC por 60 min para hidratação do DNA, em seguida foram armazenadas em freezer a -20 ºC.

4.4.1.2 Amplificação utilizando o iniciador $(GTG)_5$

O iniciador $(GTG)_5$ foi utilizado para amplificar regiões microssatélites por meio de PCR (Reação em Cadeia da Polimerase), segundo a metodologia descrita por Lieckfeldt *et al*. (1993). A PCR foi conduzida em microtubos com volume final de 25µL contendo: 1 a 5 µL de DNA (dependendo da concentração de DNA, variável de 50-500 ng μL^{-1}); 2 µL do iniciador $(GTG)_5$ 10 µmol/µL (MWG Biotech); 2,5 µL de tampão de PCR 5X (Fermentas); 1,5 µL de

$MgCl_2$ 25mM; 1 μL de dNTP 10 mM; 0,2 μL de Taq DNA polimerase 5U (Fermentas) e o volume final completado com água ultrapura esterilizada. As reações de PCR foram realizadas em termociclador *PCR* Mastercycler (Eppendorf), nas seguintes condições: desnaturação inicial a 94 ºC por 5 min, seguida por 40 ciclos de desnaturação a 94 ºC por 15 s, pareamento a 55 ºC por 1 min e extensão a 72 ºC por 90 s, e uma extensão final a 72 ºC por 6 min. A observação dos produtos da PCR (amplicons) foi por eletroforese em gel de agarose 1,5%, em tampão TBE 0,5X (54 g de tris base, 27,5 g de ácido bórico, 20 mL de EDTA 0,5M, pH 8), esses foram submetidos a 80 V por 1h e 30 min e corados com uma solução de agente intercalante (GelRed), e os géis visualizados sob luz ultravioleta e fotodocumentados (Vilber Lourmat, França).

4.4.1.3 Amplificação da região ITS

A amplificação da região transcrita interna (ITS1-5.8S-ITS2) do gene do rRNA foi realizada segundo a metodologia de White *et al*. (1990), utilizando os iniciadores universais ITS1 (TCCGTAGGTGAACCTGCGG) e ITS4 (TCCTCCGCTTATTGATATGC). A PCR foi realizada com volume final de 50 μL contendo: 1 a 5 μL de DNA (dependendo da concentração de DNA, variável de 50-500 ng/μL); 1 μL de cada iniciador ITS1 e ITS4 10 μmol μL^{-1} (MWG Biotech); 5 μL de tampão de PCR 5X (Fermentas); 2 μL de $MgCl_2$ 25mM; 2 μL de dNTP 10 mM; 0,2 μL de Taq DNA polimerase 5U (Fermentas) e o volume final completado com água esterilizada. As reações da PCR foram realizadas utilizando o termociclador *PCR* Mastercycler (Eppendorf), nas seguintes condições: desnaturação inicial a 94 ºC por 5 min, seguido por 35 ciclos de desnaturação a 94 ºC por 1 min, pareamento a 55 ºC por 1 min, e extensão a 72 ºC por 1 min, e uma extensão final a 72 ºC por 5 min. Os *amplicons* foram analisados por eletroforese em gel de agarose 1%, em tampão TBE 0,5X onde foram submetidos a 120 V por 20 min, e em seguida corados com solução de GelRed, e os géis visualizados sob luz ultravioleta e fotodocumentados (Vilber Lourmat, França).

4.4.1.4 Amplificação parcial do gene da β-tubulina

A amplificação parcial do gene da β-tubulina foi realizada utilizando os iniciadores BT2a (GGTAACCAAATCGGTGCTGCTTTC) e BT2b 15 (ACCCTCAGTGTAGTGACCCTTGGC), segundo Glass & Donaldson (1995). A PCR foi realizada com volume final de 50 μL contendo: 1 a 5 μL de DNA (dependendo da concentração de DNA, variável de 50-500 ng μL^{-1}); 1 μL de cada iniciador BT2a e BT2b 10

μmol/μL (MWG Biotech), 5,0 μL de tampão de PCR 5X 20 (Fermentas), 2,0 μL de MgCl2 25 mM, 2,0 μL de dNTP 10 mM, 0,2 μL de Taq DNA polimerase 5U (Fermentas) e o volume final completado com água esterilizada. As reações de PCR foram realizadas utilizando o termociclador *PCR* Mastercycler (Eppendorf), nas seguintes condições: desnaturação inicial a 94 ºC por 5 min, seguido por 35 ciclos de desnaturação a 94 ºC por 1 min, pareamento a 59 ºC por 1 min e extensão a 72 ºC por 90 s e uma extensão final a 72 ºC por 7 min. Os *amplicons* foram analisados por eletroforese em gel de agarose 1%, em tampão TBE 0,5X, onde foram submetidos a 120 V por 20 min, corados com solução de GelRed e os géis foram visualizados sob luz ultravioleta e fotodocumentados (Vilber Lourmat, França).

4.4.1.5 Amplificação da RNA polimerase II

Na amplificação parcial da RNA polimerase II (RPB2) foram utilizados os iniciadores RBP2 5F (GAYGAYMGWGATCAYTTYGG) e RPB2 7R (CCCATRGCTTGYTTRCCCAT) conforme descrito por Malkus *et al*. (2006). A PCR foi realizada em um volume final de 50 μL contendo de 1,0 a 5,0 μL de DNA (de modo que a reação contenha entre 50-500 ng μL^{-1}), 1,0 μL de cada iniciador RBP2 5F e RPB2 7R a 10 μmol (Invitrogen), 5,0 μL de tampão de PCR 10X (Fermentas), 3,0 μL de $MgCl_2$ mM, 2,0 μL de dNTP 10 mM, 0,2 μL de Taq DNA polimerase 1.25U (Fermentas) e o volume final completado com água de injeção esterilizada. As reações de PCR foram realizadas utilizando o termociclador *PCR* Mastercycler (Eppendorf). O programa consistiu de uma desnaturação inicial a 94 ºC por 3 min, seguido por 35 ciclos de 20 segundos de desnaturação a 94 ºC, 55 segundos de pareamento a 55 ºC e 1 minuto de extensão a 72 ºC e uma extensão final por 10 min a 72 ºC. Os amplicons foram analisados por eletroforese em gel de agarose 1%, em tampão 15 TBE 0,5X, onde foram submetidos a 120 V por 20 min, corados com solução de GelRed, e os géis visualizados sob luz ultravioleta e fotodocumentados (Vilber Lourmat, França).

4.4.1.6 Caracterização morfofisiológica das espécies do gênero Penicillium

Após a análise das sequências de espécies pertencentes ao gênero *Penicillium*, aquelas que permaneceram com dúvidas sobre as posições taxonômicas, mesmo após o sequenciamento de mais de uma região, foram submetidas à caracterização dos parâmetros macroscópicos. Dentre os parâmetros morfofisiológicos foram analisados: cor da colônia, textura, cor reversa, tipo de borda, esporulação e produção de pigmentos solúveis amarelos e diâmetros da colônia em meio YES (4,0 extrato de levedura; 20 sacarose; 1,0 KH_2PO_4; 0,5

$MgSO_4$; 15 ágar em g L^{-1}) e CYA (1,0 K_2HPO_4; 10 mL concentrado de Czapek; 5 extrato de levedura; 30 sacarose 15 ágar em g. L^{-1}) a 25 e 30 °C, respectivamente, por 14 dias (HOUBRAKEN *et al*., 2012). As cores seguiram a especificação proposta por Kornerup e Wanscher (1984).

4.4.2 Identificação de leveduras

As culturas de leveduras obtidas foram fotografadas e agrupadas de acordo com as características macromorfológicas como coloração da colônia e aspecto da colônia (mucóide, cremosa e rugosa). Os grupos formados foram submetidos à análise de perfis moleculares, por meio da técnica de PCR microsatélite (PCR-MST) idem ao item 4.2.2. Um isolado levedura de cada grupo molecular formado foi selecionado para sequenciamento dos domínios D1/D2 da subunidade maior do DNA ribossomal utilizando os iniciadores ITS1/NL4.

4.4.2.1 Extração de DNA total

Uma alçada do cultivo foi ressuspendida em 100 µL de tampão de lise em tubos de 0,6 mL e incubada a 65 °C por no mínimo 30 min. Em seguida, 200 µL de clorofórmio: álcool isoamílico 24:1 foram adicionados e os tubos foram homogeneizados e centrifugados a 14000 rpm por 10 min. O sobrenadante foi transferido para um novo tubo acrescido de isopropanol (Merck) v/v e mantido em repouso por 15 min para precipitação do DNA. Em seguida os tubos foram centrifugados a 14000 rpm por 10 min, 200 µL de etanol 70% (Merck) v/v foram adicionados e os tubos foram centrifugados novamente nas mesmas condições. O sobrenadante contendo etanol foi desprezado e as amostras foram secas por 60 min, acrescidas de 50 µL de tampão TE e incubadas a 65 ºC por 60 min para hidratação do DNA, em seguida foram armazenadas em freezer a -20 ºC. As concentrações de DNA tanto de filamentosos quanto de leveduras foram diluídas na concentração de 50 a 500 ng.μL^{-1} para posterior utilização.

4.4.2.2 Amplificação do domínio D1/D2

De cada um dos perfis moleculares de leveduras obtidos, um isolado foi selecionado e submetido ao seqüenciamento da região D1/D2 da subunidade maior (26S) do gene do rRNA como descrito por Lachance *et al*. (1999). Para a reação de PCR foram utilizados os iniciadores ITS1 (5’-TCCGTAGGTGAACCTGCGG-3’) e NL4 (5’GGTCCGTGTTTCAAGACGG3’) que abrangem a região dos espaçadores transcritos

internos (ITS1-5.8S-ITS2) do gene do RNA ribossomal, e o domínio D1/D2. A reação foi realizada em um volume final de 50 µL contendo 5µL de tampão de PCR 10X (MBI Fermentas), 3µL de $MgCl_2$ 25 mM (MBI Fermentas), 1µL de dNTP 2,5 mM (Invitrogen, USA), 1µL do primer NL1 10 pmol (Invitrogen, USA), 1µL do primer NL4 10 pmol (Invitrogen, USA), 0,2µL de taq DNA polimerase 1,25U (MBI Fermentas) e 1µL de DNA. O volume da reação foi ajustado com a adição de água deionizada. A reação foi realizada em um termociclador *PCR* Mastercycler (Eppendorf) nas seguintes condições: desnaturação inicial a 95 °C por dois min, 35 ciclos de desnaturação a 95 °C por 15 segundos, anelamento a 54 °C por 25 segundos e extensão a 72 °C por 20 segundos, seguidos de extensão final a 72 °C por 10 min. Os *amplicons* foram analisados por eletroforese em gel de agarose 1%, em tampão TBE 0,5X onde foram submetidos a 120 V por 20 min, e em seguida corados com solução de GelRed, e os géis visualizados sob luz ultravioleta e fotodocumentados (Vilber Lourmat, França).

4.4.3 Purificação dos amplicons

Aos produtos de PCR gerados (volume de 47 µL) foram adicionados 11,25 µL de EDTA 125 mM e 141 µL de etanol absoluto (Merk). Em seguida os tubos foram homogeneizados por inversão e deixados à temperatura ambiente por 30 min. Após o repouso, foram centrifugados a 14.000 rpm por 30 min. O sobrenadante foi descartado, e o sedimento tratado com 120 µL de etanol 70%, homogeneizado e centrifugado a 14.000 rpm por 15 min. O sobrenadante foi descartado novamente e o restante do etanol foi evaporado a 37 ºC por 30 min. O DNA foi ressuspendido em 10 µL de água esterilizada, e mensurado em NanoDrop ND 1000 (NanoDrop Thecnologies) e armazenado a -20 °C até o momento da reação de sequenciamento.

4.4.4 Sequenciamento

4.4.4.1 Reações de sequenciamento

O sequenciamento foi realizado utilizando-se o Kit BigDye Terminator v3.1 (Applied Biosystem) em combinação com o sistema de sequenciamento automatizado ABI 3730xl (Applied Biosystem) do Laboratório de Parasitologia Celular e Molecular (LPCM) da FIOCRUZ/Instituto René Rachou. A reação de PCR foi realizada em microplacas de 96 poços (Applied 5 Biosystems, EUA) em um volume final de 10 µL, contendo: 1 µL do iniciador (5 ρmol), 1 µL de tampão (presente no kit de sequenciamento), 1 µL de Big Dye, 1 µL de DNA

(concentração do DNA entre 10 - 20 ng) e o restante de água de ultrapura esterilizada. O programa consistiu de uma desnaturação inicial a 36 °C por 1 min, 36 ciclos de anelamento a 96 °C por 15 s, seguido por 15 s de extensão a 50 ºC e 4 min de extensão final a 60 ºC. Em seguida, os produtos das reações foram transferidos para uma placa de sequenciamento de 96 poços para serem precipitados.

4.4.4.2 Precipitação da reação de sequenciamento

Para precipitação das reações de sequenciamento, 1,0 µL de EDTA 125 mM, 1,0 µL de acetato de sódio 3M e 50 µL de etanol absoluto (Merck) gelado, foram adicionados em cada poço da placa de 96 poços. A placa foi submetida à agitação em vórtex e incubada por 15 min à temperatura ambiente. Após período de incubação, a placa foi centrifugada por 45 min a 4.000 20 rpm a 4 ºC. O sobrenadante foi descartado por inversão e em seguida, foram adicionados 100 µL de etanol 70% (Merck) gelado. Então, a placa foi novamente centrifugada por 15 min a 4.000 rpm a 4 ºC e o sobrenadante descartado por inversão. Para remoção do excesso de etanol, foi retirado o selante e a placa foi invertida sobre um papel absorvente e submetida a um spin (pulso até a centrífuga atingir 900 rpm). Após esta etapa, a placa foi mantida em repouso, sem o selante, durante 20 min em temperatura ambiente para evaporação do etanol residual. Na sequência, o DNA das amostras precipitado em cada poço foi então ressuspendido em 10 µL de Formamida HI DI (Applied Biosystems, EUA). A placa foi armazenada a 4 ºC, protegida da luz, até injeção das amostras no sistema automatizado ABI 3730 (Applied Biosystems, EUA).

4.4.4.3 Análise computacional das sequências e análise filogenética

As sequências de DNA foram comparadas com as sequências de espécies de fungos referências depositadas no GenBank, incluindo "*type species*", pertencentes às coleções de culturas internacionais, utilizando o programa BLASTn (Basic Local Alignment Serch Tool - versão 2.215 do BLAST 2.0) disponível no portal NCBI (http://www.ncbi.nlm.nih.gov/blast/), desenvolvido pelo National Center For Biotechnology.

Fungos com sequências de valor E = 0, cobertura e identidade ≥ 99%, e similaridade quando analisadas filogeneticamente com o programa MEGA 6 (*Molecular Evolutionary Genetics Analysis*) (TAMURA *et al*., 2013) foram considerados como pertencentes à mesma espécie. Já fungos com sequências de valor E ≠ 0, cobertura e identidade ≤ 98% foram identificados em nível de espécie, gênero ou níveis hierárquicos mais altos após a análise

filogenética. Para alguns táxons o termo ‘cf.’ (latim “*for confer”* = comparado com) foi utilizado para indicar a espécie a qual se assemelha, mas apresenta pequenas diferenças com a espécie referência.

Para identificação molecular foram utilizadas as sequências com ≥ 350 pares de bases. As árvores filogenéticas foram construídas utilizando o algoritmo de *Neighbor-joining*. O modelo *Maximum composite likelihood* foi utilizado para estimar a distância evolucionária. Uma análise de *bootstrap* foi realizada com 1000 repetições utilizando os programas inclusos no MEGA 6. Informações sobre os níveis hierárquicos utilizados na taxonomia dos fungos foram obtidos no *MycoBank* (http://www.mycobank.org/), *Index Fungorum* (http://www.indexfungorum.org/) e no *Dictionary of the Fungi* (KIRK *et al*., 2008).

4.5 Avaliação da diversidade da comunidade fúngica

Para determinar a frequência de cada táxon em comparação com o total de táxons das comunidades fúngicas nas áreas de coleta de sedimento lacustre foi realizado o cálculo da abundância relativa de acordo a fórmula: Abundância relativa do táxon A (%) = N° de isolados do táxon A x 100 / total de isolados de todos os táxons.

Para avaliar espécies compartilhadas ou exclusivas dentro das comunidades fúngicas das amostras de sedimento da Antártica foi utilizado o diagrama de *Venn*, de acordo com Bardou *et al*. (2014). O diagrama de *Venn* permite a visualização de espécies compartilhadas ou exclusivas nos locais amostrados por meio de um gráfico, este indica as sobreposições das formas geométricas como os elementos compartilhados (táxons) entre os pontos de coleta (BARDOU *et al*., 2014).

Para determinar a diversidade ecológica das comunidades fúngicas das amostras de sedimento foram usados os índices de (a) Fisher-α (diversidade), (b) Margalef (riqueza) e (c) Simpson (dominância). O índice de diversidade de Fisher-α é adequado para frequências em que diferentes espécies ocorrem de forma aleatória onde, comumente, algumas espécies são tão raras que sua chance de inclusão é pequena (FISHER *et al*., 1943). O cálculo do índice é baseado na fórmula S = a*ln (1+n / a), onde *S* é o número de táxons presente na amostra, *n* é o número de indivíduos e *a* representa o índice de Fisher-α. O índice de Margalef estima a riqueza de espécies de uma comunidade com base na distribuição numérica dos indivíduos pertencentes às diferentes espécies em função do número total de indivíduos existentes na amostra. Sua fórmula é dada por S = (n-1) / ln (N), onde *n* é o número de táxons encontrados e *N* representa o número de indivíduos. Quanto mais alto o valor de *S* maior a riqueza de

espécies do ponto amostrado. O índice de Simpson estabelece a relação de dominância dentro das comunidades, esse mensura a probabilidade de dois indivíduos, selecionados ao acaso na amostra, pertencer à mesma espécie. O cálculo considera o número de espécies presentes no ponto de coleta, bem como a abundância de cada espécie, e é dado pela fórmula $D = \Sigma (n / N)^2$, onde *n* é o número total de organismos de uma mesma espécie e *N* o número total de organismos de todas as espécies. O valor estimado de *D* pode variar de 0 a 1, sendo que 0 representa o máximo de diversidade e 1 o mínimo de diversidade. Sendo assim, uma comunidade de espécies com maior diversidade terá uma menor dominância.

Para avaliar a similaridade entre as comunidades das amostras de sedimento, bem como entre os locais amostrados, foram utilizados o índice de Bray-Curtis (*B*) e o coeficiente de Sorensen (*QS*). O índice de Bray-Curtis (*B*) é utilizado para quantificar a dissimilaridade composicional entre dois locais diferentes, com base nas contagens de cada ponto de coleta (BRAY; CURTIS, 1957). O índice Bray-Curtis (*B*) varia de 0 a 1, sendo que 0 significa que as comunidades não compartilham nenhuma espécie e 1 que compartilham todas as espécies na mesma frequência. O coeficiente de Sorensen (*QS*) é utilizado para avaliar a similaridade de duas amostras, é representado pela fórmula QS = 2C / (A+B), onde A e B representam o número de espécies nas amostras A e B, respectivamente, e C o número de espécies compartilhadas pelas duas amostras. A dissimilaridade (Bray-Curtis) está diretamente relacionada ao índice de similaridade (Soresen) entre os mesmos locais amostrados.

Uma curva de rarefação ou de acumulação de espécies foi traçada utilizando o índice de Mao Tau, o qual interpola valores entre zero e o número de amostras analisadas, e calcula a riqueza esperada e o intervalo de confiança. Este cálculo permite uma comparação estatística direta entre a riqueza e os conjuntos de dados (COLWELL *et al*., 2004). Todos os índices foram calculados utilizando o programa computacional PAST 1.90 (HAMMER *et al*., 2001). Todos os resultados foram obtidos com 95% de confiança, e os valores de *bootstrap* calculados a partir de 1000 repetições.

4.6 Cultivo dos fungos e preparo dos extratos para os ensaios biológicos

4.6.1 Preparo dos extratos de fungos marinhos

Os fungos obtidos foram crescidos em placas de Petri (90 x 15mm) a 15 ºC contendo Ágar marinho (Difco) suplementado com 2 % de glicose. Após 15 dias de crescimento, o meio de cultura com o crescimento micelial foi transferido para erlenmeyers de 250 mL, contendo cerca de 100 mL de Acetato de etila P.A. (Synth). Os frascos foram incubados a 4 ºC

por 72 h e o sobrenadante (fase acetanólica) foi obtido por filtração com auxílio de papel filtro e transferido para frascos de cintilação de 10 mL. Todos os extratos acetanólicos brutos obtidos foram secos em centrífuga a vácuo Savant RVT 400 (Thermo Scientific, EUA) com temperatura inferior a 35 – 40 ºC. Os extratos secos foram solubilizados em dimetilsulfóxido (DMSO, Sigma), a uma concentração de 100 mg mL^{-1} para deposição na extratoteca do Laboratório de Microbiologia Polar e Conexões Tropicais, UFMG e também em acetona a 10 mg mL^{-1} para o ensaio herbicida.

4.6.2 Preparo dos extratos de fungos lacustres

Os fungos obtidos foram crescidos em placas de Petri (90 x 15mm) a 15 ºC contendo seu meio de isolamento sem acréscimo de cloranfenicol, com exceção dos fungos isolados em meio DRBC, estes foram plaqueados em meio APG – Ágar peptona glicose (Peptona 0,5%, glicose 1%, KH_2PO_4 0,1%, $MgSO_4 \cdot 7H_2O$ 0,05%), muito semelhante à composição basal do meio original de isolamento, porém sem acréscimo de antifúngico. Após 15 dias de crescimento, o meio de cultura com o crescimento micelial foi transferido para tubos cônicos de 50 mL, contendo 35 mL de etanol P.A. (Vetec). Os tubos foram incubados a 4 ºC por 72 h e o sobrenadante (fase etanólica) foi obtido por filtração com auxílio de papel filtro e transferido para frascos de 10 mL e microtubos de 1,5 mL. Todos os extratos etanólicos brutos obtidos foram secos em centrífuga a vácuo Savant RVT 400 (Thermo Scientific, EUA) com temperatura inferior a 35 – 40 ºC. Os extratos secos foram solubilizados em dimetilsulfóxido (DMSO, Sigma), a uma concentração de 100 mg mL^{-1} para deposição na extratoteca do Laboratório de Microbiologia Polar e Conexões Tropicais, UFMG e também em acetona a 10 mg.mL^{-1} para o ensaio herbicida.

4.7 Ensaios biológicos

Os extratos obtidos a partir dos fungos foram testados para a avaliação de possíveis atividades antifúngicas, antiparasitárias, antiviral e herbicida.

4.7.1 Ensaio antimicrobiano contra *Cladosporium sphaerospermum*

Para o ensaio contra *C. sphaerospermum* CCT 1740, este foi previamente crescido em BDA a 25 ºC por 7 a 10 dias. Para o preparo da solução uma alçada bem carregada de esporos foi suspensa em solução salina esterilizada 0,85%. A suspensão resultante foi homogeneizada em agitador tipo vórtex durante 15 segundos. A densidade celular da suspensão foi

padronizada em espectrofotômetro (Bioespectro SP-22) para 86–88% de transmitância a 620 nm, o que corresponde a 10^6 esporos mL^{-1} (NCCLS M38 - A, v. 22, n° 16). Posteriormente, a suspensão obtida foi diluída 50 vezes em meio de cultura RPMI1640 (INLAB Diagnóstica) para utilização no ensaio.

Os extratos foram testados na concentração de 20 mg mL^{-1}, e todos os testes foram realizados em duplicata. Em cada poço utilizado para teste foram inoculados 25 µL do extrato (dissolvidos em DMSO e diluídos em água deionizada autoclavada para a concentração de 1 mg mL^{-1}), 25 µL do meio de cultura e 50 µL do inóculo. Como controle positivo foi utilizado Benomyl a 1,16 µg mL^{-1}. Ao final, o volume de cada poço foi de 100 µL e as concentrações de DMSO 0,1% e extrato 250 µg mL^{-1}. Ao final do processo as placas foram colocadas em agitador (Uniscience $OS^{-1}0$) por 20 min a 200 rpm. Posteriormente, foram incubadas a 25 ºC por 48 h. A leitura foi realizada em leitor de microplaca VERSA*max* (Molecular Devices) pelo programa Softmax® Pro 5 (Molecular Devices), com a absorbância de 620 nm. A absorbância dos poços testes foi comparada com a absorbância do controle de micro-organismo. Arbitrariamente foram considerados ativos os extratos com valor de inibição maior ou igual que 60%.

4.7.2 Ensaio *in vitro* com a forma amastigota-like de *Leishmania* (*Leishmania*) *amazonenses*

A manutenção das células axênicas de *L*. (*L*.) *amazonensis* e o ensaio com amastigotas-*like* foram realizados pela pesquisadora Daniela Nabak do Instituto René Rachou – Fiocruz, MG. A atividade leishmanicida seguiu os protocolos estabelecidos por (CALLAHAN *et al*., 1997), onde promastigotas de *Leishmania amazonensis* (linhagem IFLA/BR/196/PH-8) foram obtidas a partir de lesões de hamsters infectados experimentalmente. Culturas axênicas da forma amastigota-*like* de *L*. (*L*.) *amazonensis*, previamente caracterizadas por meio de eletroforese de isoenzimas e depositadas na Coleção de Leishmania do Centro de Referência em Tipagem de Leishmania do Instituto Oswaldo Cruz, foram utilizadas para o ensaio.

Os parasitas foram incubados durante 9 dias a 26 ºC em meio Schneider pH 7,2. As formas promastigotas foram então estimuladas para se diferenciarem em formas semelhantes a amastigotas, para isso, a temperatura de incubação foi de 32 ºC, além da redução do pH do meio para 6,0. Após 7 dias nestas condições, 90% dos parasitas estavam sob as formas amastigotas. A concentração do parasita foi ajustada para 1 x 10^8 células mL^{-1}, e 90 µL foram

adicionados a cada poço de placas de 96 poços, seguidos de 10 μL das soluções contendo as amostras e controle da droga (0,2 mg mL^{-1} de Anfotericina B). As placas foram incubadas a 32ºC por 72 h eo número de parasitas estimado peloensaio colorimétrico baseado em MTT (metil tiazolil tetrazólio). Os resultados foram calculados a partir das absorbâncias medidas utilizando a seguinte equação:

$$[1 – (\text{Abs experimento} / \text{Ab scontrole})] \times 100$$

A equação matemática expressa o percentual de morte parasitária em relação aos controles sem fármaco. Todas as amostras foram testadas em duplicata e os experimentos repetidos no mínimo uma vez.

4.7.3 Ensaio *in vitro* com formas tripomastigotas e formas amastigotas intracelulares de *Trypanosoma cruzi* e ensaio citotóxico *in vitro* de extratos tripanossomicidas

Os ensaios in vitro com formas amastigotas e tripomastigotas de *Trypanosoma cruzi* forma realizados pelo pesquisador Policarpo Sales do Instituto René Rachou – Fiocruz e baseado no descrito anteriormente por Buckner *et al*. (1996) e modificado por Romanha *et al*. (2010), usando *T. cruzi* (cepa de *Tulahuen*) expressando o gene da β-galactosidase de *Escherichia coli*.

As formas tripomastigotas infecciosas são obtidas através da cultura em monocamadas de fibroblastos L929 de camundongo em meio RPMI-1640 (pH 7,2 - 7,4), sem vermelho de fenol, contendo 10% de soro fetal bovino e 2 mM de glutamina. O ensaio foi realizado em microplaca de cultura de tecidos com 96 poços, em cada poço 4.000 células L929 foram adicionadas a 80 μL de meio suplementado e as placas são incubadas *overnight*. Após o período de incubação foram adicionados às células 4 x 10^3 tripomastigotas em 20 μL de meio, seguida de incubação por 2 h. Para estabelecer a infecção, o meio contendo parasitas que não penetraram nas células foi substituído por 200 μL de meio fresco e a placa foi incubada por mais 48 h. Em seguida, o meio foi substituído pelos extratos a 20 μg mL^{-1} em meio fresco (200 μL) e a placa foi incubada por 96 h a 37 °C. Após esse período, 50 μL de 500 μM de vermelho de clorofenol β-D-galactopiranosídeo em 0,5% Nonidet P40 foi adicionado a cada poço, seguido de incubação por 18 h a 37 °C. A leitura da absorbância foi feita a 570 nm. São utilizados controles com células não infectadas, células infectadas não tratadas, células infectadas tratadas com benzonidazol a 3,8 μM (controlepositivo) ou DMSO a 1%. Os resultados são expressos como a percentagem de inibição do crescimento do *T. cruzi* em células testadas com composto em comparação às células infectadas e às células não tratadas.

As duplicatas são executadas em duas placas diferentes. Foram considerados ativos, os extratos que inibiram o crescimento do parasita com porcentagem maior que 40%.

Os extratos que se apresentaram ativos foram testados in vitro para determinação da toxicidade celular (Ensaio citotóxico) em células L929 não infectadas utilizando o corante alamarBlue® (ROMANHA *et al*., 2010). As células foram expostas a extratos com concentrações crescentes a partir do valor de IC50 para *T. cruzi*. Após 96 h de incubação com os compostos, o alamarBlue® foi adicionado e a absorbância foi medida em 570 e 600 nm após 4 - 6 h. A viabilidade celular foi expressa como a percentagem de diferença na redução entre células tratadas e não tratadas. Os valores de IC_{50} foram calculados por interpolação linear e o índice de seletividade (SI), foi determinado com base na razão entre o valor de IC_{50} na célula hospedeira dividido pelo valor de IC_{50} do parasita. Os quadruplicados foram executados na mesma placa, e os experimentos foram repetidos pelo menos uma vez.

4.7.4 Manutenção e ensaios esquizonticidas *in vitro* de formas sanguíneas de *Plasmodium falciparum*

A manutenção do parasito *Plasmodium falciparum* e o ensaio esquizonticidas in vitro foram realizados pelas pesquisadoras Isabela Ceravolo e Patrícia Pereira do Instituto René Rachou – Fiocruz, MG. O ensaio realizado utilizou o fluoróforo SYBR, um intercalante de DNA, conforme Smilkestein *et al*. (2004) com algumas modificações. Para a realização deste ensaio, a cultura de parasitos foi ajustada para 0,5 % de parasitemia e 2 % de hematócrito, e mantida em uma placa de 96 poços de fundo em U (180 µL/poço). Após este ajuste, 180 µL da suspensão de parasitos foi adicionada aos poços de uma placa de 96 poços contendo 20 µL da solução dos extratos, avaliados em triplicatas, e incubada a 37 °C por 48 h. O sobrenadante foi retirado, e 150 µL de PBS acrescentado a cada poço da placa que foi centrifugada por 5 min a 700 xg. Após a retirada do sobrenadante, 100 µL de tampão de lise (0,2 µL de SYBR/mL em tampão de lise -Tris 20mM pH 7,5; EDTA 5mM; saponina 0,008% p/v e triton X-100 0,08% v/v) foram acrescentados a cada poço, os conteúdos foram bem homogeneizados e transferidos para uma placa de 96 poços de fundo chato, contendo 100 µL de PBS/poço. A placa foi incubada por 30 min a temperatura ambiente e sob abrigo da luz. A leitura da fluorescência foi realizada no leitor Synergy H4 (Biotek, Winooski, VT, EUA) em um comprimento de onda de excitação e de emissão de 485 e 535 nm, respectivamente, e com um ganho de 100. Para análise, a leitura do *background* dos poços das hemácias normais foi subtraída da contagem da fluorescência. Quanto maior a fluorescência, maior a quantidade de

DNA onde foi incorporado o SYBR, maior o número de parasitos viáveis, e menor a atividade da droga sobre o parasito. A amostra foi considerada promissora quando, a 20 μg mL^{-1}, apresentou uma porcentagem média de inibição de crescimento do parasito ≥ 40% quando comparada ao controle sem adição de substância-teste em, pelo menos, dois ensaios independentes.

4.7.5 Triagem da atividade antiviral de extratos de fungos contra o vírus da dengue sorotipo 2 (DENV2)

Os ensaios antivirais foram realizados pelo aluno de doutorado Emerson de Castro Barbosa sob a orientação dos professores Dr. Carlos Leomar Zani e Dra. Jaquelline Germanode Oliveira no Instituto René Rachou – Fiocruz, MG. Inicialmente, os extratos na concentração de 20 mg mL^{-1} em solução aquosa com 90% de dimetilsulfóxido (DMSO) foram depositados em placas de 96 poços (1 μL/poço) e armazenados a 4°C até o momento do ensaio. Para os ensaios de atividade antiviral contra Dengue vírus sorotipo 2 (DENV-2), 7 x 10^3 células BHK-21/poço foram adicionadas à placa de 96 poços de fundo chato em meio DMEM suplementado com 5 % de soro fetal bovino (SFB) e 100 U mL^{-1} de penicilina, 100 μg mL^{-1} de estreptomicina e 0,25 μg mL^{-1} de anfotericina B (todos Gibco, Thermo Sci, EUA) e incubadas por 24 h em estufa a 37°C com atmosfera de 5% de CO_2.

O ensaio é realizado após confirmação ao microscópio óptico da monocamada celular com 80 % de confluência. Para o tratamento das células, os extratos são diluídos diretamente na placa de 96 poços e transferidos simultaneamente com a suspensão viral de DENV-2 (m.o.i. = 2), para as placas contendo as células denominadas aqui de placas espelhos (duplicatas). A concentração de cada extrato no ensaio foi de 25 μg mL^{-1} e o volume de meio por poço foi de 200 μL. As colunas 1 e 12 das placas de 96 poços foram reservadas para os controles: a) células não tratadas e não infectadas sem DMSO; b) células não tratadas e não infectadas com 0,25 % de DMSO; iii)células não tratadas e infectadas sem DMSO; c) células não tratadas e infectadas com 0,25 % de DMSO; d) células infectadas e tratadas com 300 $UI.mL^{-1}$ e 600 $UI.mL^{-1}$ de interferon alfa 2b (INREC). As placas foram reincubadas em estufa a 37°C com 5% de CO_2 por 72 h. A atividade antiviral foi avaliada pelo sistema de classificação do efeito citopático peculiar (CPE) causado por DENV-2 observado por microscopia óptica seguido de ensaio colorimétrico utilizando 3-(4,5-Dimetil-2-tiazolil)-2,5-difenil-2H-tetrazólio (MTT) (Sigma Aldrich - USA) (MOSMANN, 1983) Para isso, o sobrenadante das placas foi removido e em seguida foram adicionados 30 μL em cada poço de

uma solução de MTT a 2 mg mL^{-1} dissolvido em PBS. As placas foram incubadas novamente por 90 min a 37°C. Em seguida, foram adicionados 130 µL de DMSO puro em cada poço e as placas forammantidas em agitação por 5 min em vórtex de placas na velocidade de 200 rpm para dissolver os cristais de formazan. A densidade óptica da reação foi determinada em leitura feita a 540 nm em leitor de microplacas (SpectraMaxM5 -Molecular Devices). Os resultados são expressos como a porcentagem de inibição viral em relação aos controles virais sem extratos. Todos os ensaios antivirais foram repetidos pelo menos três vezes.

4.7.6 Determinação de atividade herbicida

A determinação da atividade herbicida foi realizada de acordo com Ferreira *et al*. (2017) com modificações. A atividade herbicida é determinada utilizando-se sementes de alface crespa (*Lactuca sativa*) como modelo para dicotiledôneas e sementes de cebolinha (*Allium schoenoprasum*) como modelo para monocotiledôneas. Para a realização dos ensaios, todas as sementes foram desinfestadas superficialmente em álcool 70% (1 minuto), hipoclorito de sódio a 2-2,5% (7 min e meio). Posteriormente lavadas em água deionizada autoclavada por quatro vezes e secas ao ar em um ambiente esterilizado. Os bioensaios foram realizados em duplicata em placas de poliestireno de 24 poços esterilizadas (TPP 92024). Um disco de papel de filtro qualitativo (50 x 50, 80 g), foi colocado em cada poço a ser utilizado. Aos poços controle foram adicionados 400 µL de água deionizada autoclavada. Para avaliação de toxicidade do solvente utilizado na solubilização dos extratos foi realizado o controle de solvente. Para tanto, foi adicionado ao poço 360 µL de água e 40 µL de acetona P.A. Aos poços testes foram adicionados 360 µL de água e 40 µL da diluição da amostra na concentraçao de mg mL^{-1}. Os ensaios foram feitos em fluxo laminar para evitar possíveis contaminações. Cinco sementes de cada espécie foram adicionadas a cada poço. As placas foram seladas incubadas a 25 °C sob condições de luz contínua (120,1 µmol $m^{-2}s^{-1}$) por 14 dias. Após o período de incubação, uma estimativa qualitativa de fitotoxicidade foi feita atribuindo-se uma classificação de 0 para onde não ocorreu nenhum efeito de inibição da germinação (as plantas dos poços teste encontraram-se idênticas às plantas controle), 2 para menos de 50% de inibição da germinação, 3 para cerca de 50 % de inibição de germinação, 4 para mais de 50% de inibição de germinação e 5 para não germinação das sementes. Como controle postivo de inibição da germinação usou-se o herbicida glifosato a 3 mg mL^{-1} diluído em água deionizada. A Figura 5 mostra a disposição dos controles e extratos nas placas de 24 poços.

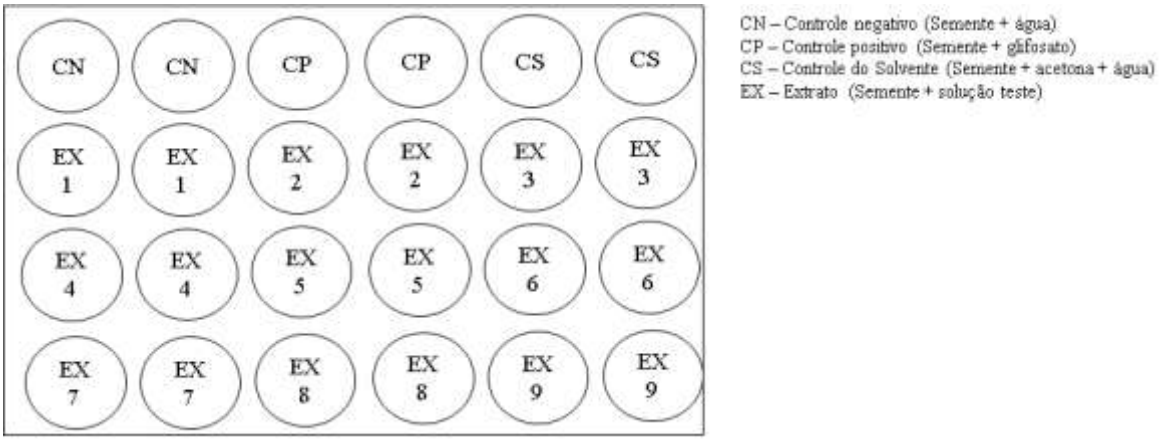


Figura 6. Modelo da disposição dos extratos e controles na placa de 24 poços utilizada nos ensaios herbicidas.

5

4.7.7 Determinação da atividade nematicida

Os ensaios nematicidas foram realizados pela aluna de doutorado Roberta Oliveira Viana sob a orientação da professora Dra. Viviane de Souza Alves do Laboratório de Biologia Celular de Microrganismos - UFMG. Para os ensaios foi utilizada a linhagem de vermes *Caenorhabditis elegans* tipo selvagem N2 Bristol gentilmente cedida pelo do CGC (*Caenorhabdtitis* Genetics Stock Center).

O cultivo dos vermes se deu em meio NGM - *Nematode Growth Media* sólido (NaCl, peptona, KPO_4, $MgSO_4$, $CaCl_2$) a 16 °C, contendo *Esherichia coli* OP50 para alimentação normal (MUHAMMED *et al*. 2012). Após o período de crescimento, os vermes foram estocados em solução de congelamento (NaCl, KH_2PO_4, Glicerol) a -80°C. Para os ensaios, os nematódeos foram mantidos no estágio larval (L4) por meio de sincronização, utilizando o método descrito por Porta de La Riva *et al*. (2012), no qual verme adultos *C. elegans* grávidos e/ou ovos são coletados em tampão M9, lavados em M9, e lisados com solução v/v de de NaOH e hipoclorito de sódio; sob estas condições apenas os ovos são mantidos íntegros. Os ovos foram lavados, incubados por 18h em tampão M9 sob agitação e em seguida foram adicionados a placas contendo NGM e *E. coli* OP50, e incubados até chegarem no estágio larval L4 (adulto jovem) a 16 ou 20 °C.

Para os testes de toxicidade de *C. elegans* aos extratos fúngicos, os vermes foram expostos a diferentes concentrações de extratos (400, 160, 80, 40 e 20 $\mu g\ mL^{-1}$). O ensaio foi realizado em placas de 96 poços de fundo chato, onde houve exposição crônica de larvas L4 (jovem adulto) em tampão M9 com os extratos, até a morte de todos os vermes (contagem realizada a cada 48 h). Todas as exposições foram realizadas a 25 °C, na presença de alimentação normal (*E. coli* OP50), utilizando DMSO como controle negativo. Para o ensaio de exposição crônica, a FUdR, um inibidor de timidilato sintase, foi adicionada ao meio para impedir a produção de descendência de *C. elegans* (MITCHELL *et al*., 1979).

5. CAPÍTULOS

Os resultados obtidos foram apresentados em forma de capítulos, dos quais os capítulos 1 e 2 já foram publicados no livro "*Fungi of Antarctica: Diversity, Ecology and Biotechnological Applications*", editora *Springer*. Os demais capítulos foram ou serão submetidos em jornais indexados.

- Capítulo 1. Capitulo de livro: "Diversity and ecology of fungal assemblages present in lakes of Antarctica."
- Capítulo 2. Capitulo de livro: "Sub-antarctic and Antarctic marine ecosystems: An unexplored ecosystem of fungal diversity."
- Capítulo 3. Artigo: "Cultivable fungi present in deep-sea sediments of Antarctica: Taxonomy, diversity, and bioprospection of bioactive compounds." Submetido a revista Envinromental Microbiology.
- Capítulo 4. Artigo: "Diversity, ecology, and bioprospection of fungi in lakes under anthropogenic effects in Antarctic Peninsula." Será submetido para a revista FEMS Microbiology Ecology.
- Capítulo 5. Artigo: "Diversity and bioprospection of cultivable fungal community in sediments of lakes at Antarctic Peninsula." Será submetido para a revista FEMS Microbiology Ecology.

5.1 Capítulo 1: "Diversity and ecology of fungal assemblages present in lakes of Antarctica."

Chapter 4
Diversity and Ecology of Fungal Assemblages Present in Lakes of Antarctica

Mayara Baptistucci Ogaki, Rosemary Vieira, Juan Manuel Lírio, Carlos Augusto Rosa, and Luiz Henrique Rosa

4.1 Introduction

The aquatic ecosystems of Antarctica comprise a range of habitats covered by temporary or perennial water bodies, such as saline and freshwater lakes, swamps, streams, rivers, estuaries, melting ice areas, sea, and ocean. Usually, the freshwater aquatic zones of Antarctica are in direct contact with rocks, soil, and mud and are bordered by vegetation like *Deschampsia antarctica*, *Colobanthus quitensis*, mosses, macroalgae, and lichens. In addition, the Antarctic lakes have an interface with air, named as extra-aquatic zone; consequently, these lakes represent important sites for the study of microbial diversity and ecology.

Almost the entire land surface of Antarctica is covered by a vast ice cap (more than 70% of the world's freshwater). Nevertheless, water bodies that contain water in a liquid state (unfrozen water) for at least part of a year are a common feature of the Antarctic landscape. Most of subaerial Antarctic lakes are formed when ice recedes and because of exposed depressions in the terrain, formed either by glacial erosion or by the deposition of terminal moraines, or because of folds and depressions in the underlying geological topography (Hodgson 2012).

Seasonal ice-free areas occur in the marginal ice zones, some containing lakes, ponds, streams, and wetlands. The majority of lakes are found in the islands and in the coastal zones of McMurdo Dry Valleys, Vestfold Hills, Larsemann Hills, Bunger Hills, and Schirmacher in continental Antarctica (Vicent et al. 2008; Sokratova

M. B. Ogaki (✉) · C. A. Rosa · L. H. Rosa
Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

R. Vieira
Departamento de Geografia, Universidade Federal Fluminense, Rio de Janeiro, Brazil

J. M. Lírio
Instituto Antártico Argentino, Buenos Aires, Argentina

L. H. Rosa (ed.), *Fungi of Antarctica*,
https://doi.org/10.1007/978-3-030-18367-7_4

2011; Phartiyal et al. 2011). However, maritime and subantarctic islands in Antarctica, such as Signy and King George Islands, also have ice-free lakes (Pienitz et al. 2008) and are the most intensively studied areas.

All major fungal phyla identified within freshwater systems, including water and lake sediments, are *Ascomycota*, *Basidiomycota*, traditional *Zygomycota*, and *Chytridiomycota* (Shearer et al. 2007; Monchy et al. 2011; Wurzbacher et al. 2011). Additionally, some *Oomycetes* taxa (Stramenopila) have been described (Shearer et al. 2007; Gonçalves et al. 2012). Moreover, some fungi are largely spread in different Antarctic freshwater environments and are represented by decomposer and parasitic taxa that actively participate in the cycling of organic matter and nutrients (Knox and Peterson 1973; McInnes 2003; Hao et al. 2005). As decomposers, the major functional role of fungi in freshwater ecosystems is breakdown and mineralisation of allochthonous and autochthonous organic matter (Kuehn 2015).

The mycota living in the Antarctic lakes is under the influence of various adverse factors, such as extreme low temperatures, frequent freeze-thaw cycles, high salinity, alkaline and acidic pH values, high UV radiation, and low nutrient availability (Gonçalves et al. 2012). Researches conducted on fungi in Antarctic lakes include studies of different microhabitats, such as water bodies, biofilms composed of plants and microalgae, and sediments (Baublis et al. 1991; de Hoog et al. 2005; Tsuji et al. 2013; Conell et al. 2018), which, despite the extreme conditions, shelter rich and diverse fungal assemblages.

4.2 Lakes of Maritime Antarctica

The maritime Antarctic region includes the west coast of the Antarctic Peninsula and the associated islands of the Scotia Arc and extends from the South Sandwich Islands through South Orkney and South Shetland Islands and down the western side of the Antarctic Peninsula to approximately 72°S (Camacho 2006). In Antarctica, the highest increases in temperature were observed in the Antarctic Peninsula, especially on its west coast. Between 2000 and 2009, the intensity of the Antarctic Peninsula melt accounted for more than 50% of the total intensity of Antarctic surface melt, a finding supported by regional climate modelling of surface meltwater production over a period of 1979–2010 (Abram et al. 2013).

Maritime Antarctica presents lakes and ponds formed in the rocky substratum, as well as coastal lagoons influenced by marine water intrusions (Camacho et al. 2012). The lacustrine system is located in areas, climatically less extreme than the interior of the continent. Therefore, although temperatures less than 0 °C predominate, in summer, the events of fusion and liquid precipitation are common, owing to a more active hydrological cycle with sediment and nutrient circulation throughout the lacustrine basin and in the glaciomarine environment (Quayle et al. 2002; Mckay et al. 2009; Camacho et al. 2012). These climatic conditions favour a large number of freshwater ecosystems that melt out and become ice-free in summer (Toro et al. 2006).

Fig. 4.1 Aerial photo of Kroner Lake, Deception Island (maritime Antarctica). The photo shows the connection between the sea (on the left) and the lake (on the right). (Photo credits, LH Rosa)

Most of the Antarctic lakes have arisen because of glacial retreat, and a few have been formed by tectonic activity; in addition, some lakes may have been formed by volcanic activity (Priddle and Heywood 1980). Volcanoes are still active in some areas of Antarctica such as the South Shetland Islands. Kroner Lake (Fig. 4.1) on Deception Island (located in the South Shetland Islands), occupies a shallow, circular depression in a lava plain; the fumarole activity supplies heat to the lake, which makes it a unique geothermally heated lake in Antarctica (Priddle and Heywood 1980).

Kroner Lake is an example of a lake with marine water intrusion, which has a vast environmental and biological heterogeneity because of its connection with the sea on the margins of the lagoon (Izagirre et al. 2006). Besides, some types of lakes are formed in volcano areas or volcanic depressions, which contain deposits of pyroclastic material on South Shetlands Islands, such as the Crater Lake at Deception Island (Fig. 4.2b) and a smaller Crater Lake at the Penguin Island (Fig. 4.2a).

Two of the largest ice-free areas of Southern Shetland Islands are found on the Byers Peninsula, on the Livingston Island (Oliva et al. 2017), and Fildes Peninsula, King George Island (Peter et al. 2008). In these areas, permafrost dynamics control the interaction between lacustrine and terrestrial ecosystems (Izaguirre et al. 2012). Limnological processes are influenced by a complex set of feedback mechanisms, driven by climatic conditions (e.g. biological productivity or lake ice cover) and other factors directly controlled by permafrost (e.g. hydrological, geomorphological, and sedimentological processes). Therefore, the permafrost controls geomorphic processes, which in turn influence the limnological processes and patterns of lake sedimentation (Oliva et al. 2017). The permafrost may also contain considerable stocks of ancient organic matter that is released during melting (Quesada et al. 2006).

Fig. 4.2 Lakes formed in volcano depressions (called Crater lakes) on South Shetlands Islands. (**a**) depicts a small Crater lake at Penguin Island (area affected by the last volcanic eruption in 1905) and (**b**) Crater lake on Deception Island (area affected by the last eruption in 1969). (Photo credits, LH Rosa)

The Byers Peninsula on Livingston Island holds a high number of lakes, ponds, and flooded or wet areas. Lakes are found both in the central part of the peninsula, in a plateau of 100 m high, and in coastal areas, the latter being accessible to marine animals. The shallow lagoons located in coastal areas are found on low relief lands, and their surrounding areas may vary from sandy and dry terrains to those that are largely covered by moss cushions and plants (Toro et al. 2006; Camacho et al. 2012).

Regarding King George Island, about 60 freshwater lakes of different types and origins are located on the Fildes Peninsula. Most of the lakes are small and shallow endorheic ponds located in depressions formed by deglaciation. However, large and deep lakes also exist. During summer, the lakes are mostly ice-free, and snowmelt water is transported by a local streamflow network (Shevnina and Kourzeneva 2017). The peninsula's vast ice-free area experiences different conditions, transitioning from paraglacial to periglacial, as one moves away from the Collins Glacier. Periglacial landforms occupy approximately 70% of the Fildes Peninsula, and the remaining 30% are composed of structural landforms and rock outcrops shaped by glacial erosion (Michel et al. 2014).

The basins of most lakes are over-deepened glacial basins, and the valleys of the largest streams are glacial troughs (Michel et al. 2014). Solids and particulate matter from catchment areas are carried by water and deposited in lake sediments, contributing to the lake biogeochemical processes (Alfonso et al. 2015); consequently, the microbial community may be affected by such constant changes in the solute composition of lake habitats.

4.3 Lakes of Continental Antarctica

Continental aquatic habitats differ from each other; the ice-free areas have larger lakes. Most of them have a typical concentration of ions and solutes, which make them saline or hypersaline. The differences in salinity and ionic composition of the lakes are related, in part, to how the lakes have responded to temperature changes. These lakes acquire a significant fraction of salt content when glacial meltwaters come in contact with soils and sources that surround them. Furthermore the concentrations of all the ions increase with the distance that the meltwater travels to these lakes before finding slots for percolation (Green and Canfiel 1984; Priscu and Foreman 2009).

The Antarctic oases are ice-free regions with distinct weather patterns and are located in relief depressions or old marine lagoons (Laybourn-Parry and Wadham 2014; Shevnina and Kourzeneva 2017). The lakes and ponds found in these regions are shallow and relatively warm, but there are also some large, deep, and cold-water lakes, which occur in tectonic faults or in proximity to glaciers (Sokratova 2011; Shevnina and Kourzeneva 2017). Till date, many of these lakes show high-density stratification with hypersaline bottom water, thought to have formed during glacial

lowstands. Water levels in this hyperarid environment are controlled by the balance between surface inflow and sublimation of the perennial ice cover (Hall et al. 2010).

The presence of unfrozen water (in the form of a system of seasonal streams and non-freezing lakes) makes the Antarctic oases unique landscapes (Sokratova 2011). The perennial ice can have a profound impact on the lake dynamics. The thick perennial ice cover can minimise wind-generated currents, limit lake circulation, restrict light penetration, increase dissolved gas concentrations, and cause heterogeneous sedimentation on the lake bottom (Doran et al. 2000; Phartiyal et al. 2011).

Many lakes and ponds having various salt contents are scattered in the depressions of valleys and are generally covered with ice, 3–5 m thick, throughout the year (McKay et al. 1985; Matsumoto 1993; Doran et al. 1996). The salt composition of lake waters of the Antarctic oases is formed because of the transport of marine aerosols by precipitation, salt freezing, and ion inflow from the upper layers of the soils of lake basins (Sokratova 2011).

The distribution of total salts and chloride ion concentrations corresponds to freshwater and saline lakes, reflecting the presence and absence of outflows, respectively. The local arid condition enables subsurface waters to contain salts, possibly resulting from the weathering of rocks and evaporation of waters. Nutrients are concentrated in the anoxic bottom waters because of the lack of circulation, producing an oligotrophic status of the lakes and ponds (Bishop et al. 1996, 2014). However, these extreme habitats host active biosystems: microbial mat communities that can flourish in the lake bottom sediments, owing to the absence of a significant foraging fauna (Bird et al. 1991; Bishop et al. 1996, 2014).

The McMurdo Dry Valleys consist of three major valleys, namely, Victoria, Wright, and Taylor, and these comprise the largest ice-free area (2500 km^2) in Antarctica (Vocke and Hanson 1981). The Vestfold Hills on Princess Elizabeth Land are at the margin of the East Antarctic ice sheet, and they have more than 150 hypersaline and low-salinity lakes, because of the retreat of the ice from ice sheets (Bird et al. 1991). In contrast, in the East Antarctic Shield, about 100 km southwest of the Vestfold Hills, are the Larsemann Hills that consist of several ice-free peninsulas and islands along the coast, with more than 100 freshwater lakes, ranging from small ponds to deep lakes (Stüwe et al. 1989; Shevnina and Kourzeneva 2017). Furthermore, the Bunger Hills are one of the largest ice-free areas on the Antarctic continent, located near the coast of East Antarctica at about 100″ longitude, covering an area of about 300 km^2 and consisting of low rocky hills and glacially deepened valleys (Sheraton et al. 1993). In this area, there are a few perennially ice-covered lakes at the oasis edge, and these lakes are largely in contact with glacier ice (Doran et al. 2000). Most of the lakes are in the centre of the oasis, and thus become ice-free in the summer months, while the lakes at the edge of the oasis, in contact with glacier ice, mostly retain their ice covers around the whole year (Doran et al. 1996).

In general, continental Antarctica presents epiglacial lakes found on the surface of the ice sheets, glaciers, and ice shelves. Since 1970s, subglacial lakes, rivers, and

wetlands were discovered using airborne radio echo, deep beneath the Antarctic ice cap (Robin et al. 1977). According to Wright and Siegert (2012), satellite observations and radar measurements revealed that there are active and partially interconnected subglacial hydrological systems connecting more than 379 lakes, existing extensively beneath the Antarctic ice sheet.

All these lakes are subject to high pressure (approximately 350 atmosphere), low temperatures (about −3 °C), and permanent darkness. However, dissolved oxygen is available on the surface of the lake because of an equilibrium with the air hydrates released by melting basal layers of glacier ice (Siegert et al. 2001). They lie up to 4200 m under the Antarctic ice sheet and range in sizes from 1 to 241 km long (Hodgson et al. 2004). These lakes have been isolated from the outside world for thousands of years, and combined with these characteristics are some of the most extreme environments on Earth. Lake Vostok, for example, was continually buried under glacial ice for 15 million years (Rogers et al. 2013); therefore Lake Vostok and others subglacial lakes may be an ecosystem still awaiting to be explored.

Lake Vostok is the best-known subglacial lake in Antarctica; it is the largest and deepest lake in East Antarctica (240 km long, 50 km wide) and lies between 3750 m (at the south of the lake) and 4150 m (at the north) beneath the central-east Antarctic ice sheet (Siegert et al. 2001). The freshwater in Lake Vostok is kept in a liquid state by the pressure of the ice overburden (equivalent to −350 atmosphere) and, perhaps, by geothermal heating (Karl et al. 1999). The water residence time of this lake is estimated to be around 50,000 years, and there is speculation that the lake may contain microbes, which have remained isolated from the rest of the biological world for thousands of years (Hodgson et al. 2004). In spite of its isolation from the ice surface, Lake Vostok and similar lakes may contain previously undescribed relic populations of microorganisms that are adapted to life in these presumably oligotrophic (low-nutrient, low-biomass, and low-energy flux) habitats (Karl et al. 1999).

4.4 Diversity of Fungi in Antarctica Lakes

There are few detailed reports of fungal communities in freshwater lakes in the Antarctic continent, most of them in maritime Antarctica as compared to those in continental Antarctica (Ellis-Evans 1996; Brunatti et al. 2009; Gonçalves et al. 2012). Regarding the diversity of lake microbiota, there is variation in the communities of microorganisms according to the temperature gradient that occurs latitudinally in the continent. A summary of the main research involving the mycobiota of lakes of the Antarctica with respect to the regions of study is given in Table 4.1.

Table 4.1 List of fungal species isolated from different lakes of Antarctica

Region	Lake	Substrate	Fungal taxa	Reference
Maritime Antarctica				
Deception Island	Kroner, Relict, and two unnamed lakes	Surface and depth water	*Rhodotorula*, *Candida* and one unknown filamentous fungus	Stanley and Rose (1967)
	Lake in Port Foster	Sediment	*Cystobasidium laryngis*	Vaz et al. (2011)
	Crater lake	Water	*Pseudogymnoascus pannorum*, *Mortierella* sp., *Cladosporium* cf. *cladosporioides*, *Penicillium* sp., *Davidiella tassiana*, *Cladosporium* sp., and *Trichoderma longibrachiatum*	Gonçalves et al. (2012)
King George Island	Lake in Agat point	Water	*P. pannorum*, *Phaeosphaeria* sp., *Cadophora malorum*, *D. tassiana*, *Helotiales* sp., *Gibberella moniliformis*, *Penicillium paneum*, and *Penicillium* cf. *verrucosum*	Gonçalves et al. (2012)
	Lake next to the Brazilian Refuge II	Water	*Antarctomyces psychrotrophicus*, *Cladosporium cladosporioides*, *D. tassiana*, *Helgardia* sp., *P. pannorum*, *Microdochium* sp., *Microdochium nivale*, *Mortierella* sp., *Pleosporales* sp., *Saprolegniaceae* sp., and *Thelebolus* sp.	Gonçalves et al. (2012)
	Lake next to the Brazilian Station	Sediment	*Helotiales* sp. and *Schizophyllum commune*	Gonçalves et al. (2015)
	Lake next to Copacabana USA Refuge	Freshwater and sediment	*Candida glaebosa*, *Nadsonia commutate* (freshwater); *Issatchenkia* (*Pichia*) *orientalis*, *Kodamaea ohmeri*, *Meyerozyma guilliermondii*, *Rhodotorula mucilaginosa*, and *Vishniacozyma victoriae* (sediment)	Vaz et al. (2011)
	Lake in Jardew point	Sediment	*Annulohypoxylon* sp. and *Cosmospora* sp.	Gonçalves et al. (2015)

	Two lakes next to Machu Picchu Station	Freshwater	*Aureobasidium pullulans*, *Exophiala xenobiotica*, *Leucosporidium creatinivorum*, *Microglossum* sp., *Rhodotorula mucilaginosa*, *Sporidiobolus salmonicolor*, *V. victoriae*	Vaz et al. (2011)
	One lake next to Machu Picchu Station	Freshwater	*Cladosporium* sp., *C. malorum*, *Fontanospora* sp., *P. pannorum*, *Helgardia* sp., *Mortierella* cf. *alpina*, *Phoma* cf. *paspali*, *Phaeosphaeria* sp., and *Thelebolus* sp.	Gonçalves et al. (2012)
	Three lakes next to Machu Picchu Station	Sediment	*Aspergillus* sp., *C. malorum*, *Pseudogymnoascus* sp., *Penicillium* sp., *Pleosporaceae* sp., and *Sordariomycetidae* sp.	Gonçalves et al. (2015)
	Lake next to Stain House glacier	Water	*A. psychrotrophicus*, *C. cladosporioides*, *Cosmospora* cf. *vilior*, *Cadophora* cf. *luteo-olivacea*,*P. pannorum*, *Helotiales* sp., *Heydenia* sp., *Microdochium* sp., *Mortierella* cf. *alpina*, *Phoma herbarum*, *Phoma fimeti*, and *Thelebolus microsporus*	Gonçalves et al. (2012)
	Lake next to Wanda glacier	Water	*Cladosporium* sp., *P. pannorum*, *Mortierella* sp., *Pseudeurotium* sp., *Phoma herbarum*, *Penicillium paneum*, and *Thelebolus* sp.	Gonçalves et al. (2012)
Signy Island (South Orkney Islands)	Signy lakes	Freshwater	*Chytriomyces* sp., *Chytriomyces willoughbyi*, and aquatic "phycomycetes" *Aphanomyces* sp.	Willoughby (1971)
		Freshwater	*Lagenidium giganteum*, *Hyphochytrium catenoides*, *Aphanomyces* sp. (*Saprolegniaceae*), 6 basidiomycetous forms including *Rhodotorula* sp., and *Leucosporidium* sp.	Ellis-Evans (1985)

(continued)

Table 4.1 (continued)

Region	Lake	Substrate	Fungal taxa	Reference
		Benthic cyanobacterial mat and sediments	*Lecophagus antarcticus*	McInnes (2003)
Ice-free areas (continental Antarctica)				
McMurdo Dry Valleys (Southern Victoria Land)	McMurdo oasis next to McMurdo Station	Freshwater, soil, and algae	*Scherffelliomyces appendiculatus*, *Chytridium versatile*, *Rhizophlyctis rosea*, *Rhizophydium proliferum*, *Phlyctochytrium recurvastomum*, *Catenophlyctis variabilis*, *Aphanomyces* (*Saprolegniales*), *Pythium tenue*, and *Pythium* sp.	Knox and Paterson (1973)
	Bonney Lake	Soil in lake side and inflow stream	*Candida australis*	Goto et al. (1969)
		Bottom water	*Dendryphiella* sp. and *Diheterospora catenulata*	Waguri et al. (1975), Waguri (1976)
	Basins Lake located in the Taylor and Miers Valleys (including two samples from Bonney Lake)	Water	*Cryptomycota* sp., *Chytridiomycota* sp., *Ascomycota* sp., *Zygomycota* sp., *Blastocladiomycota* sp., *Glaciozyma* sp., and *Mrakia* sp.	Rojas-Jimenez et al. (2017)
	Fryxell Lake	Algae in lake side	*Candida scottii*	Goto et al. (1969)
		Bottom water	*Aureobasidium foliicolum*	Waguri et al. (1975), Waguri (1976)
		Biomats	*Thelebolus ellipsoideus* and *Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Embellisia* sp., *Onychophora* sp., *Leucosporidium antarcticum* (*Glaciozyma antarctica*), and *Mrakia frigida*	Brunati et al. (2009)

		Water	*Acremonium* sp., *Aureobasidium pullulans*, *Cladosporium cladosporoides*, *Clavispora lusitaniae*, *Debaryomyces hansenii*, *Pseudogymnoascus* sp., *Heydenia alpina*, *Penicillium commune*, *Penicillium dipodomyicola*, *Thelebolus ellipsoideus*, *Thelebolus globosus*, *Toxicocladosporium strelitziae*, *Filobasidium magnus*, *Glaciozyma antarctica*, *Glaciozyma watsonii*, *Holtermanniella yarrowii*, *Mrakiella aquatica*, *Naganishia albidosimilis*, *Naganishia globosa*, *Rhodotorula mucilaginosa*, and *Vishniacozyma victoriae*	Conell et al. (2018)
	Hoare Lake	Meltwater foam, microbial mat from benthos and from meltwater	*Candida ciferrii*, *Cephalosporium acremonium*, *Aureohasidium pullulans*, *Chrysosporium pannorum*, *Geotrichum candidum*, and *Penicillium notatum*	Baublis et al. (1991)
		Biomats	*Thelebolus ellipsoideus*	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Leucosporidium antarcticum*, and *Rhodotorula mucilaginosa*	Brunati et al. (2009)
	Miers Lake	Water and an outlet stream	*Candida diffluens*, *R. texensis* (in water), and *Rhodotorula rubra* var. *miersensis* (in an outlet stream)	Goto et al. (1969)
	Vanda Lake	Water and sediment	*Cryptococcus albidus*, *Candida diffluens*, *Candida humicola*, *Trichosporon cutaneum* var. *antarcticum*, *Rhodotorula glutinis* var. *rufusa*, and *Rhodotorula texensis* (water); *Sporobolomyces antarcticus*, *C. diffluens*, *C. scottii*, and *Rhodotorula rubra* (sediment)	Goto et al. (1969)

(continued)

Table 4.1 (continued)

Region	Lake	Substrate	Fungal taxa	Reference
		Deep water (68 m)	*Aspergillus*, *Penicillium*, *Stachybotrys*, and *Trichoderma*	Kriss et al. (1976)
		Water from several depths (5–69 m)	*Candida* sp.	Nagashima et al. (1990)
Vestfold Hills	Ace Lakes	Biomats	*Thelebolus microsporus*	de Hoog et al. 2005
		Biomats	*Thelebolus* sp., *Penicillium* sp., *Cladosporium* sp., *Pseudogymnoascus* sp., unidentified filamentous fungus; *Cryptococcus albidus*, *Cryptococcus infirmo-miniatus*, *Cryptococcus laurentii*, *Leucosporidium antarcticum*, *Leucosporidium scottii*, *Mrakia frigida*, and *Rhodotorula mucilaginosa*	Brunati et al. (2009)
	Druzby Lake	Biomats	*Thelebolus ellipsoideus*, *Thelebolus globosus*, and *Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Phoma* sp., and *Leucosporidium scottii*	Brunati et al. (2009)
	Highway Lakes	Biomats	*Thelebolus microsporus*	de Hoog et al. 2005
		Biomats	*Thelebolus* sp., *Penicillium* sp., and unidentified filamentous fungus	Brunati et al. (2009)
	Organic Lake	Biomats	*Thelebolus microsporus*	de Hoog et al. 2005
		Biomats	*Thelebolus* sp. and *Penicillium* sp.	Brunati et al. (2009)

	Pendant Lake	Biomats	*Acremonium* sp., *Alternaria* sp., *Arthrinium* sp., *Aspergillus* sp., *Beauveria* sp., *Botrytis* sp., *Penicillium* sp., *Cladosporium* sp., *Curvularia* sp., *Pseudogymnoascus* sp., and unidentified filamentous fungus	Brunati et al. (2009)
	Watts Lake	Biomats	*Thelebolus globosus*	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Penicillium* sp., *Beauveria* sp., *Phialophora* sp., and unidentified filamentous fungus	Brunati et al. (2009)
Larsemann Hills (Princess Elizabeth)	Manning Lake	Biomats	*Thelebolus ellipsoideus*, *Thelebolus microsporus*, and *Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Phoma* sp., *Cladosporium* sp., *Curvularia* sp., and *Rhodotorula minuta*	Brunati et al. (2009)
	Reid Lake	Biomats	*Thelebolus microsporus*	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Phoma* sp., *Cladosporium* sp., unidentified filamentous fungus, *Candida lipolytica*, *Cryptococcus albidus*, *Debaryomyces hansenii* var. *hansenii*, *Leucosporidium scottii*, and *Rhodotorula mucilaginosa*	Brunati et al. (2009)
	Sarah Tarn Lake	Biomats	*Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Aspergillus* sp.	Brunati et al. (2009)

(continued)

Table 4.1 (continued)

Region	Lake	Substrate	Fungal taxa	Reference
Skarvsnes (Lutzow-Holm Bay, East Antarctica)	Abi-ike, Ageha-ike, Bosatsu-ike, Ebi-numa, Hyoutan-ike, Jizou-ike, Kuwai-ike, Kumogata-ike, Magoike, Naga-ike, Nisehyoutan-ike, Nyorai-ike, Ohgi-ike, Oyako-ike, Shimai-ike, and Tokkuri-ike Lakes	Surface soil around lakes and sediments	*Embellisia* sp. and *Phoma* sp., *Pseudogymnoascus* sp., *Tetracladium* sp., *Thelebolus* sp., *Mrakia* sp., *Cryptococcus* sp., *Dioszegia* sp., *Rhodotorula gracialis*, and *Leucosporidium antarcticum*	Tsuji et al. (2013)
Subglacial lakes				
Vostok Station (under surface of the central East Antarctic ice sheet)	Vostok Lake	Accretion ice	*Cystofilobasidium* sp., *Cryptococcus* sp., *Pseudozyma* sp., *Penicillium* sp., *Aeurobasidium* sp., and *Aspergillus* sp.	D'Elia et al. (2009)
		Accretion ice and surface of the southern main basin	*Ascomycota* sp., *Basidiomycota* sp., *Zygomycota* sp. (*Mucorales*), and unknown uncultured fungi	Rogers et al. (2013)

4.5 Fungi from Lakes in Maritime Antarctica

The islands of maritime Antarctica are formed by different archipelagos (located at South Shetland and South Orkney Islands) or active volcanoes (at Deception, Ross, and South Sandwich Islands) (Hodgson 2012). The maritime areas of the Antarctic Peninsula have higher temperatures around the year compared to the rest of the continental Antarctica and, consequently, influence the microbial communities of the water bodies present in the region.

The taxa of fungi found in maritime Antarctica, where there are warmer temperatures and more variations of humidity and water availability, are already described. The main fungal phyla are *Ascomycota*, *Basidiomycota*, traditional *Zygomycot*a, and *Chytridiomycota*, as well as allied species from *Oomycota*.

Different studies have described the presence of freshwater fungi, mainly on Signy Island (Willoughby 1971; Ellis-Evans 1985; McInnes 2003; Rogers et al. 2013) in the South Orkney Islands, located on the east of the South Shetland Islands (northern extremity of maritime Antarctica). The most detailed studies about the mycobiota of the South Orkney Islands were carried out in the last decades by Ellis-Evans [Ellis-Evans (1981, 1985, 1996) and McInnes (2003)], which showed that aquatic fungi of the classes *Hyphomycetes* and *Chytridiomycetes* (fungi) as well as *Oomycetes* (Stramenopila) were observed to be either associated with cyanobacterial mats or to have a predation relationship with algae and aquatic invertebrates.

Ellis-Evans (1985) described six psychrotolerant basidiomycetous taxa, including those of the genera *Rhodotorula* and *Leucosporidium*. According to Ellis-Evans (1981), yeasts can compete with bacteria in the water columns and, therefore, are considered as transient propagules, whereas in the sediments, yeasts are present in comparatively low numbers but may play some roles in nutrient cycling. Nevertheless, the ecological role of yeasts in these lakes remains unclear. The *Oomycetes* represented by *Saprolegniaceae* sp., *Aphanomyces* sp., and six species of *Chytridiomycetes* were described by Ellis-Evans (1985). In addition, an account of freshwater fungi at the Signy Island was given by Willoughby (1971), including two species, *Chytriomyces* and *Chytriomyces willoughbyi*. Isolates of *Saprolegniaceae* was also found in the Keller Peninsula, South Shetlands Islands (Gonçalves et al. 2012), and the genus *Aphanomyces* in McMurdo oasis in Ross Island (Knox and Peterson 1973), thus indicating that the order *Saprolegniales* is representative of the Antarctica lakes. In an evaluation of shallow lake margins on Signy Island, McInnes (2003) described a new species, *Lecophagus antarcticus*, a predaceous fungus of rotifers and tardigrades, collected from benthic cyanobacterial mats and sediments. However, there is little information about aquatic *Hyphomycetes* in Antarctica lakes, which mainly possess such type of an ecological relationship.

A large number of freshwater lakes with varying complexity occur in the South Shetland Islands. Stanley and Rose (1967) reported yeasts and a filamentous fungus from five lakes located on the Deception Island (Kroner Relict, and two unnamed Lakes). They obtained only one unknown filamentous fungus and four yeasts, but because of the rudimentary identification techniques available at that period of time,

only two yeasts were categorised as belonging to the genera *Rhodotorula* and *Candida*. Their growth profiles at different temperatures indicated that most of the yeasts grew optimally below 20 °C. Years later, by means of molecular identification techniques, more detailed studies about fungi in the South Shetland freshwater were published.

Vaz et al. (2011) isolated fungi from different substrates of Antarctica, including water and lake sediment. The samples were collected in areas of anthropic influence near research stations (Machu Picchu and the Copacabana United States Refuge, in King George Island) and the sealing history area – the first exploratory industry in the Antarctic (Port Foster on Deception Island). The authors reported the presence of species *Candida glaebosa*, *Nadsonia commutate*, and *Cystobasidium laryngis* in samples of the lake sediment and nine genera from the lake freshwater, of which *Exophiala xenobiotica* and *Microglossum* sp. were the most isolated taxa. All fungal taxa were evaluated for biotechnological applicability and *E. xenobiotica*, *C. laryngis*, *R. mucilaginosa*, and *Microglossum* sp. were observed to produce mycosporines and/or carotenoids, UV-protective compounds, and other pigments. In addition, most of the yeast isolates had extracellular enzymatic activities at different temperatures, indicating that the fungi would have been metabolically active in the substrate.

In the King George and Deception Islands, Gonçalves et al. (2012) isolated 128 fungi from freshwater, and the most common taxon isolated was the genus *Psedudogymnoascus*, which has been reported in other cold ecosystems, such as arctic, temperate and alpine regions (Kobayashi et al. 1967; Semenova et al. 2015; Wang et al. 2015b; Zukal et al. 2016). The endemic genera *Antarctomyces*, as well as *Mortierella* (*Zygomycota*), and one zoosporic fungus (*Oomycota*) were also identified by Gonçalves et al. (2012). The other taxa identified were only at the order or family levels, such as *Helotiales* sp. and *Pleosporales* sp., as they did not have matches with any of the species deposited in GenBank.

Gonçalves et al. (2015) isolated fungi from different substrates, including sediment from lakes situated next to the Ferraz (Brazilian) and Machu Picchu (Peru) stations, as well as Jardew point, both located in King George Island. In the freshwater of these lakes, the authors identified species such as *Schizophyllum commune* and the genera *Pseudogymnoascus*, *Penicillium*, *Cosmospora*, *Annulohypoxylon*, and *Aspergillus*. In addition, another taxon *Incertae sedis* belonging to the order *Helotiales* was obtained by Gonçalves et al. (2015), suggesting that Antarctic lakes provide a unique environment to study the origin and activities of aquatic fungal communities and trophic webs, the importance of which remains unknown. Wang et al. (2015a) described some records about psychrotolerant species in *Helotiales* (principally *Letiomicetes* class), which are fungi associated with glacier soil from China and Antarctic soil from the Great Wall (Chinese) Station in King George Island, including the new species described by the authors as *Psychrophila antarctica*.

According to Bridge et al. (2008), there are 25 fungal taxa belonging to the order *Helotiales* cited in different regions of Antarctica, including Bird Island, Signy Island, and King George Island in maritime Antarctica. From this list of non-lichenised fungi from Antarctica, most of the fungi were associated with substrates such as lichens, different species of bryophyte, plants such as *D. antarctica* and *C.*

quitensis, and soil; however, the association with the lake substrates for the order *Helotiales* is not cited in their study.

4.6 Fungi from Ice-Free Lakes of Continental Antarctica

Among the most extensively studied Antarctic lakes are those that occur in the ice-free oases on the edge of the continent region, such as the Vestfold Hills, Larsemann Hills, and McMurdo Dry Valleys (Hodgson 2012). Most of the mycobiota studies of these types of lakes are concentrated in these areas of Antarctica. In addition, research on fungi of saline lakes, situated on Victoria Land, such as Lake Bonney and Lake Vanda, has been reported.

Antarctic saline lakes have many factors that limit the species diversity and growth rates of organisms inhabiting this ecosystem. According to Wright and Burton (1981), high salinity produces a rise in osmotic strength and opacity of ice, decline in the freezing point of water, and a reduction in the ice cover. In addition, in summer, these lakes have a high exposure to light. Therefore, as a lake freezes, salts eliminated from the ice increase the salinity of the water, which remains in the water layers at the bottom, making the environment even more hostile. Most of these factors also affect organisms inhabiting the alpine lakes and Antarctic oceans. Consequently, microbial communities that constitute saline lakes in Antarctica may vary from those present in cold freshwater ecosystems.

The Taylor Valley is the southernmost valley of the three large Dry Valleys in the Transantarctic Mountains, in Victoria Land, where the three major lakes – Lake Bonney, Lake Fryxell, and Lake Hoare – are located. The permanently ice-covered lakes in the valleys vary dramatically in their geochemistry. Lake Bonney contains hypersaline deep waters where sodium chloride and magnesium chloride are predominantly present, while sulphides and methane are absent. Lake Fryxell has weakly saline deep waters and sediments that are both highly sulphidic and methanogenic, whereas Lake Hoare is essentially a freshwater system (Green and Lyons 2009; Tregoning et al. 2015). Detailed reports of the fungi obtained from these Taylor Valley saline lakes and other saline lakes from ice-free areas are given below.

Lake Vanda is a meromictic and hypersaline lake which lies about 30 km northwest of Lake Bonney in the Wright Valley (Tregoning et al. 2015). In the Lake Vanda, Goto et al. (1969) reported the presence of genera *Candida*, *Rhodotorula*, and *Cryptococcus* from the water and sediments. They also identified a new variety of *Trichosporon cutaneum* var. *antarcticum* from freshwater and a new species *Sporobolomyces antarcticus* (synonym *Pseudozyma antarctica*) from the sediment; the species was subsequently transferred to the genus *Moesziomyces* (teleomorphic genera) by Wang et al. (2015b).

Microbiological studies conducted by Sugiyama (1970) also revealed fungal species from other genera in Lake Vanda such as *Eurotium*, *Geotrichum*, *Stachybotrys*, and *Trichoderma.* Kriss et al. (1976) reported the genera *Stachybotrys* and *Trichoderma* in the same lake and other cosmopolitan fungi, such as *Aspergillus* and *Penicillium*. Other psychrophilic and non-halotolerant yeasts, *Candida* species,

were isolated at a depth of 5 m in Lake Vanda by Nagashima et al. (1990). None or very few yeast species were discovered in samples of lake water or sediment collected from Lakes Bonney and Fryxell by Goto et al. (1969). However, *Dendryphiella* sp. and *Diheterospora catenulata* were obtained from the water at the bottom of Lake Bonney, and *Aerobasidium foliicolum* and an unidentified species were recovered from Lake Fryxell (Waguri et al. 1975; Waguri 1976).

Baublis et al. (1991) demonstrated the richness of diversity of fungi from several selected microhabitats in the McMurdo Dry Valleys, including samples from Lake Hoare and Victoria Land. The highest number of fungi was found during the fall season compared to that in spring, and *Candida ciferrii*, *Cephalosporium acremonium*, *Aureohasidium pullulans*, and *Pseudogymnoascus pannorum* were identified on the meltwater foam. *P. pannorum* and *Fusarium fusavioides* were obtained from a microbial mat from the benthos, while a microbial mat from meltwater contained species, such as *Geotrichum candidum* and *Penicillium notatum*. All these species indicate that Taylor Valley contains a diverse mycobiota and occasionally high population levels, despite the severe climate and limited substrates.

A more detailed study by Brunati et al. (2009), involving fungal diversity and bioprospecting, which also covered a larger area, was carried out in the Victoria Land region. They isolated fungi associated with biomats from lakes in the Larsemann Hills (Manning and Rei Lakes), Vestfold Hills (Ace and Druzby Lakes), and McMurdo Dry Valleys (Fryxell and Hoare Lakes). Genera of psychrophilic fungi such as *Thelebolus* and *Pseudogymnoascus* were identified, as well as the cold-adapted cosmopolitan species, namely, *Aspergillus*, *Penicillium*, *Cladosporium*, and *Acremonium*. In addition, the *Beauveria* and *Curvularia* genera were also identified. *Basidiomycetes* yeasts were recurrently isolated from the biomats; however, only a single genus of ascomycetous yeasts, *Debaromyces hansenii*, was identified. Furthermore, de Hoog et al. (2005) identified some *Thelebolus* species (*T. ellipsoideus*, *T. microsporus*, and a new species, *T. globosus*) also associated with biomats in the same lakes situated in the McMurdo Dry Valleys and Vestfold Hills. According to the authors, the high frequency of members of *Thelebolus* obtained from Antarctic biomats in the diverse types of lakes was unexpected, but more recent articles have shown that the genera is widely spread in samples obtained from such lakes.

Conell et al. (2018) explored the biodiversity of culturable microfungi from the water column of Lake Fryxell; the most abundant taxa isolated were *Glaciozyma watsonii* (59%) and *Penicillium* spp. (10%). In this study, the authors reported a sharp decline in the fungal abundance in the water at 9 m below the lake with a concurrent shift in diversity; the fungi *Toxicocladosporium strelitziae*, *Clavispora lusitaniae*, and *Holtermanniella nyarrowii* were found at this depth. The genera *Aureobasidium*, *Cladosporium*, *Pseudogymnoascus* sp., *Heydenia*, *Thelebolus*, *Filobasidium*, *Mrakiella*, *Naganishia*, and *Rhodotorula* were obtained both on the surface and in the deep water. In contrast, the cosmopolitan filamentous genera *Acremonium*, *Pseudogymnoascus*, and *Penicillium* and the yeasts *Debaryomyces*, *Vishniacozyma*, and *Glaciozyma* were found only on the surface.

Rojas-Jimenez et al. (2017) using a culture-independent method of analysis explored the diversity and community composition of fungi in five permanently cov-

ered lake basins located in the Taylor and Miers Valleys (including two samples from Lake Bonney). Unculturable microorganisms represent a significant part of biodiversity of the Earth; therefore, culture-independent method of analysis makes it possible to unravel fundamental characteristics of the microbiology and ecology of microorganisms in a community (Ravin et al. 2015), including those present in lakes.

The fungal taxa found by the authors represented between 0.93% and 60.32% of the eukaryotic sequences. *Cryptomycota* and *Chytridiomycota* dominated in the fungal communities of all the lakes, and *Cryptomycota* was particularly dominant in Lakes Miers, Bonney West, and Fryxell, while *Chytridiomycota* was the dominant species in Bonney East and Hoare. In addition, members of *Ascomycota*, *Zygomycota*, *Blastocladiomycota*, and *Basidiomycota* were also present. *Basidiomycota* was dominant in the upper layer of Lake Hoare, and the main genera identified were *Glaciozyma* and *Mrakia*. Furthermore, Rojas-Jimenez et al. (2017) carried out a comparative study of the fungal composition (derived from RNA or DNA) obtained from Lake Bonney.

On the east of Antarctica, in Skarvness ice-free area, Tsuji et al. (2013) isolated fungi from lake sediments and identified fungi of the genera *Pseudogymnoascus*, *Thelebolus*, *Mrakia*, *Dioszegia*, and *Leucosporidium* (*Glaciozyma*). Some fungi species are able to secrete extracellular fatty acids and polysaccharides, to avoid getting frozen when exposed to extremely cold climatic conditions (Robinson 2001). These authors also evaluated the antifreeze activity of fungal isolates, wherein the species *G. antarctica* and *Rhodotorula glacialis* displayed antifreeze activities. Tsuji (2016) evaluated the ability of *Mrakia blollopis* isolates, obtained in a previous study, regarding their responses to cold stress. The author reported that one of the isolates was capable of accumulating high levels of tricarboxylic acid cycle metabolites, lactic acid, aromatic amino acids and polyamines, probably as an adaptation to resist cold shock. These data demonstrated the mechanisms by which fungi, isolated from frozen lakes, could remain active during periods of freezing, besides growing and decomposing organic compounds under subzero temperatures.

4.7 Fungi from Subglacial Lakes in Continental Antarctica

In 1998, Russian and French scientists completed drilling of Lake Vostok at a termination depth of 3623 m (Fig. 4.3), which represented the largest drilling ever made. Moreover, Abyzov et al. (1998) inferred that Lake Vostok could contain viable microorganisms. They analysed a portion of the ice core obtained in 1998 and samples extracted from core depths of 1500–2750 m (with corresponding ages ranging from 110,000 to 240,000 years) and demonstrated the existence of prokaryotic and eukaryotic microorganisms. Subsequently, analysis of these frozen ice cores led to inferences about the chemistry of lake water and revealed the existence of a small number of microbes (Karl et al. 1999; Siegert et al. 2001).

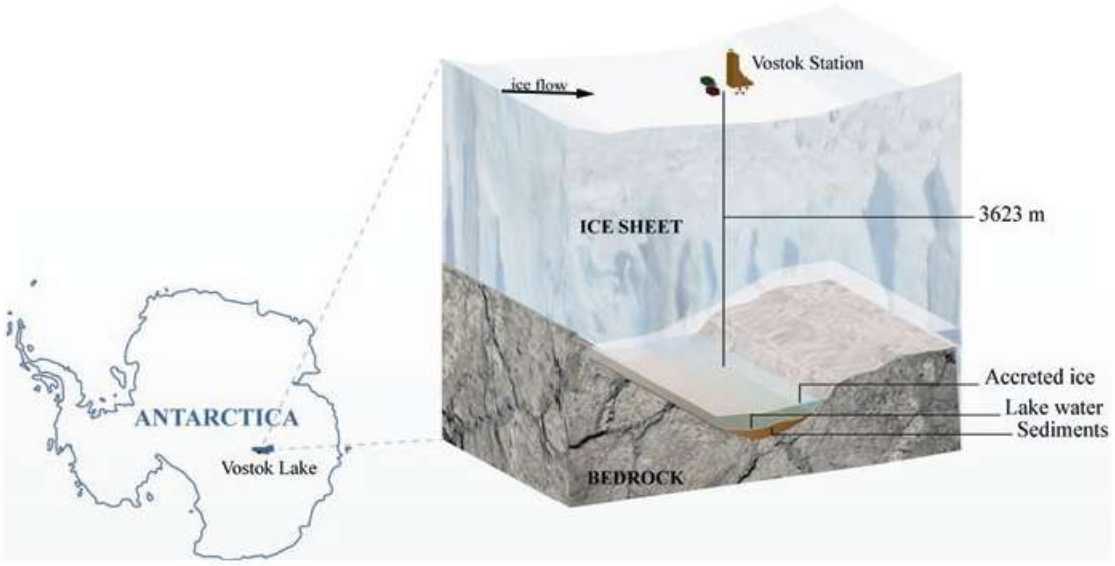


Fig. 4.3 Location of Lake Vostok in continental Antarctica and a scheme of the distance of the ice sheet drilling. Accumulation of accretion ice is depicted above the unfrozen lake water, and shown below is the accumulation of sediments. (Picture credits, M.B. Ogaki)

The most of the analyses of microbiota associated with Lake Vostok were performed with the ice found above the lake; this layer of accretion ice (frozen lake water collected above lake liquid surface) can retain linear and temporal contents of the upper surface of the lake. Karl et al. (1999) analysed a core section that contained frozen water derived from Lake Vostok (a body of liquid water located beneath glacial ice at about 4 km). They revealed viable bacterial cells (predominance of gram-negative bacteria) and low concentrations of potential growth nutrients. Other evaluations of the accretion ice revealed phylotypes closely related to extant members of the α-, β-, γ-, δ-, and *Proteobacteria* and the actinomycetes (Priscu et al. 1999; Christner et al. 2001, 2006); however, all these papers did not report the presence of fungi in the accretion ice.

D'Ellia et al. (2009) also isolated fungi from ten accretion ice sections (3300–5100 years old) from Lake Vostok. A total of 38 fungal cultures were analysed by ribosomal ITS region; the fungi belonged to four genera of *Basidiomycetes* and five of *Ascomycetes*. The species *Rhodotorula mucilaginosa* is the most frequently found, and other genera were also reported, including *Cystofilobasidium*, *Cryptococcus*, *Pseudozyma*, *Penicillium*, *Aeurobasidium*, and *Aspergillus*. The remaining taxa were classified as unknown. All the isolates identified by D'Elia et al. (2009) were related to polar taxa isolated from a variety of permanently cold environments. The presence of these types of fungi indicates that the ecological conditions within the regions of Lake Vostok are sufficient to support heterotrophic metabolism and a high diversity of microorganisms.

Shtarkman et al. (2013) evaluated two core sections that accreted in the vicinity of an embayment (at depths of 3563 and 3585 m) and two ice core sections that accreted over the southern main lake basin (at 3606 and 3621 m) using metagenomic/metatranscriptomic sequence analyses of mRNA. They obtained 3507 unique gene sequences. Among these, 1623 could be taxonomically classified from both

the core sections. Approximately 94% of the sequences obtained were from bacteria, and 6% were from eukaryotes, represented by Amoebozoa, Archaeplastida, Animalia, Chromalveolata, and Excavata. Fungi (represented by *Ascomycota*, *Basidiomycota*, *Zygomycota* taxa) represented 23% of the sequences obtained from the samples.

In the meltwater ice from Lake Vostok, Rogers et al. (2013), using metagenomic/metatranscriptomic analyses, noted the presence of thousands of species of organisms (94% *Bacteria*, 6% Eukaryota, and 2% Archaea). Two samples were analysed, one from the accretion ice over a shallow embayment and the other from the surface of the southern main basin. Only about 6% of the unique sequences were closest to eukaryotes. Of these, the phyla *Ascomycota*, *Basidiomycota*, and *Zygomycota* (*Mucorales*) and some sequences showing no matches with GenBank database (uncultured fungi) were identified. The data were very similar to those obtained by Shtarkman et al. (2013). These kinds of analyses allowed to gather information about the ecology, in a broader way, about organisms in Lake Vostok, since it was based on RNA sequences present in the samples collected, but precise identification of which types of fungi would be present in these lakes has not been evaluated.

Until now, the probability of subglacial lakes harbouring life remains poorly characterised (Kuhn et al. 2017), mainly because of potential contamination during sample collection, which are difficult to obtain. From the few samples obtained by penetration of subglacial lakes, acquiring pure samples to evaluate the taxa present would be hampered by contamination, because of the mixing of the lake water with well-drilling fluid (Lukin and Vasiliev 2014). This would result in an ambiguous analysis of the communities present. Because of these challenges in obtaining samples, practically no information is known about the fungi that inhabit the subglacial lakes of Antarctica.

According to Bulat (2016), only two studies of penetrations of Lake Vostok were performed with the viewpoint of a possible sampling of clean water. The results of these samples yielded only one new unknown bacterial phylotype, which indicates that a hitherto unrecorded microbial life could exist within water body of Lake Vostok. The search for lake inhabitants should aim to sample the cleanest lake water in order to ensure that the results are more robust. In addition, the use of clean laboratory facilities and the establishment of a library of contaminants are considered prerequisites for the research of Lake Vostok microorganisms (Bulat 2016). Therefore, further drilling is expected to be done in the future in order to analyse, more accurately, the microbial community in subglacial lakes.

4.8 Factors that Influence Fungal Communities in Antarctic Lakes

In Antarctica, lakes usually show low nutrient availability, constant low temperatures, short ice-free seasons, and different ranges of pH in comparison with other regions of the Earth; these factors influence biotic diversity in the lacustrine systems (Ellis-Evans 1996). In an evolutionary sequence report outlined, the

development of an Antarctic lake is initially seen as a function of catchment complexity and natural eutrophication (Priddle and Heywood 1980), wherein the biotic development is initiated with the colonisation of the lake bottom by algae, bacteria, and macroinvertebrates, followed by a rise in the dominance of benthic cyanobacterial mats and aquatic mosses, and finally switching to phytoplankton dominance. When nutrient inputs increase, the water column turbidity reduces light penetration (Ellis-Evans 1996).

It is known that the biological communities of the Antarctica lakes usually contain simplified and truncated food webs (Laybourn-Parry et al. 1997), absence of fish, low zooplankton biomass, and low floristic diversity at the lake borders. For these reasons, there are no plants of higher orders in the Antarctic lakes. Only benthic cyanobacterial mats are extremely common in these lakes, and annual algae and aquatic mosses can occur in some systems (Ellis-Evans 1996; Laybourn-Parry and Pearce 2007). The biota of the Antarctic lakes are primarily constituted by microorganisms (Vincent 2000) adapted to disperse in the water column or adhere to other lacustrine substrates that display supersaturated levels of dissolved oxygen, extremely low light conditions on an annual basis, and thick ice covers, which reduce or eliminate wind-induced internal water circulation (Simmons et al. 1992). All these characteristics limit colonisation and growth of many microbial species in these environments. The microbial communities in these types of extreme lakes have to survive hostile habitats and deal with high ultraviolet radiation loads, freeze-thaw cycles, and low organic resources and are dependent on long-term primary products produced by photosynthetic organisms (Tranter et al. 2004). Ice and snow covers are responsible for low levels of annual photosynthetically available radiation in the lakes; however, when the ice melts in summer, the high transparency of the water column can transmit a high intensity of light and can have an inhibitory effect on photosynthesis (Hodgson 2012). Therefore, these light-darkness and freeze-thaw cycles are decisive in establishing a trophic web in these lakes.

In the Antarctic lakes, nutrient cycling occurs because of the activity of benthic communities of cyanobacteria, bacteria, and fungi (Ellis-Evans 1996; Gonçalves et al. 2012). The low diversity of organisms limits the availability of nutrients, since most fungi in Antarctica are associated with the decomposition of organic matter (Arenz et al. 2006), as endophytes (Rosa et al. 2009) and parasites (McInnes 2003). According to Laybourn-Parry et al. (1997), the lakes of Antarctica are ultra-oligotrophic, unless they are enriched by bird droppings and plant debris. In addition, the continents and islands located in the ice-free areas support the well-known fauna of seals, penguins, and marine birds and a variety of plants and lichens (Hodgson 2012). These organisms can carry or harbour vectors and microbial propagules (Ellis-Evans and Walton 1990). In the Antarctica lakes, fungi were reported to be associated with different lacustrine substrates or were present in water columns. Among the Antarctic lacustrine substrates, fungi have been identified in the marginal microbial mats (Brunati et al. 2009), marginal soil and sediment (Vaz et al. 2011; Tsuji et al. 2013; Gonçalves et al. 2015), and water (de Hoog et al. 2005; Gonçalves et al. 2012; Rojas-Jimenez et al. 2017; Connell et al. 2018) associated with invertebrates (McInnes 2003) and macroalgae (Knox and Peterson 1973).

The colonisation of water bodies by fungi occurs mainly because of the dispersion of their propagules. Most of the terrestrial fungi disperse their spores carried by air and by precipitation or by floods, which are subsequently deposited in the water (Dix and Webster 1995). In addition, freshwater fungi produce spores adapted to dispersion in aquatic conditions. Fungi taxa reported from aquatic habitats range from those that are adapted to complete their life cycles in aquatic habitats and are not found outside the aquatic environment (indigenous or residents) to those present in different ecosystems and substrates (cosmopolitan) (Shearer et al. 2007). Most of residents are able to sporulate in water and keep their biomass at a constant level corresponding to the substrates and nutrients available (Dix and Webster 1995). In contrast, those fungi that inhabit the water randomly by being washed, leached, blown in, or dispersed by wind are present transiently. Some transient propagules enter the aquatic environment and can reach maturation but are unable to sporulate and colonise new substrates (Dix and Webster 1995; Shearer et al. 2007). In addition, transitional fungi can be immigrant or migratory. Among these, immigrants usually have an extra-aquatic habitat and their propagules, by dispersion, are deposited in the water. As for the migratory species, they alternate periodically between aquatic and extra-aquatic habitats (Dix and Webster 1995).

Antarctic lakes exhibit different physicochemical conditions and can be either freshwater or hypersaline (Laybourn-Parry et al. 1997). Chemically, these lakes range from some of the freshest lakes in the world to the most hypersaline lakes, with concentrations of salt exceeding eight times that of seawater, which prevent them from freezing over, even during winter (Hodgson 2012). Additionally, many saline lakes exhibit seasonal or permanent stratification of water columns because of temperature and salinity gradients (Hodgson 2012). The water composition depends on the material leached from the catchment areas and other intrinsic factors, such as a rocky composition and marine influence around the lakes in maritime Antarctica.

During the trajectory of the water in the formation of water bodies, there are marked changes in hydrologic, physical, chemical, and biological conditions (Townsend and Hildrew 1994). These changes of nutrients, which occur by leaching, contribute to a water composition that provides solutes essential to microbial community. In addition, oxygen availability, water temperature, and biological challenges, such as competition with other organisms, influence the occurrence and adaptations of fungi. Most of the Antarctic lakes exhibit unusual thermal profiles, and most are covered, to some degree, with perennial ice or annual ice cover (Simmons et al. 1992; Laybourn-Parry and Pearce 2007). Consequently, abiotic and biotic forces exert a high selective pressure on fungi. However, the constant cold temperature, freezing periods, and high UV radiation compel them to have adaptive mechanisms that include changes in the composition of the cell membrane, the production of intra- and extracellular antifreeze substances (trehalose, cryoprotectant sugars, proteins, polyols), and the ability to produce cold-active enzymes and protective UV compounds, such as mycosporines and pigments (Robinson et al. 2000; Vaz et al. 2011). Many of these characteristics allow mainly the psychrophilic and psychrotolerant fungi to survive. The production of such substances, as well as

other secondary metabolites, may have different applications in industrial and medical fields as well as be of great scientific interest.

4.9 Conclusions

Fungi are widely diffused by the diverse ecosystems that the Antarctic environment offers, including lake ecosystems and their range of associated substrates (biomats, invertebrates, sediment, and water columns). In this article, several studies regarding the fungal diversity among the taxa that characterise these lacustrine environments and variations between endemic species, such as *Antarctomyces psychrotrophicus*, *Thelebolus globosus*, and *Thelebolus ellipsoideus*, cosmopolitan species such as the genus *Penicillium*, and psychrotolerant fungi such as *Pseudogymnoascus* of Antarctica lakes were addressed. These fungi tend to be well adapted, since the Antarctic continent presents several adverse factors for fungal colonisation and growth, such as freezing and thawing cycles with low annual precipitation, temperature, water availability, strong winds, and high incidence of UV radiation. Therefore, the fungal communities of the lakes could have a great ecological importance in the Antarctica by playing a role in primary decomposition of organic materials, actively participating in nutrient cycling important for the balance of the micro- and macronutrients in lake systems, contributing to demonstrate the effects of the climatic global changes, and last but not the least, by their ability to produce bioproducts for further biotechnological applications.

References

Abram NJ, Mulvaney R, Wolff EW, Triest J, Kipfstuhl S, Trusel LD, Vimeux F, Fleet L, Arrowsmith C (2013) Acceleration of snowmelt in an Antarctic Peninsula ice core during the twentieth century. Nat Geosci 6:404–411

Abyzov SS, Mitskevich IN, Poglazova MN (1998) Microflora of the deep glacier horizons of central Antarctica. Microbiology 67:451

Alfonso JA, Vasquez Y, Hernandez AC, Mora A, Handt H, Sira E (2015) Geochemistry of recent lacustrine sediments from Fildes Peninsula, King George Island, maritime Antarctica. Antarct Sci 27:462–471

Arenz BE, Held BW, Jurgens JA, Farrell RL, Blanchette RA (2006) Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. Soil Biol Biochem 38:3057–3064

Baublis JA, Wharton RA, Volz PA (1991) Diversity of micro-fungi in an Antarctic dry valley. J Basic Microbiol 31:3–12

Bird MI, Chivas AR, Radnell CJ, Burton HR (1991) Sedimentological and stable-isotope evolution of lakes in the Vestfold Hills Antarctica. Palaeogeogr Palaeoclimatol Palaeoecol 84:109–130

Bishop JL, Koeberl C, Kralik C, Froeschl H, Englert PAJ, Andersen DW, Pieters CM, Wharton RA (1996) Reflectance spectroscopy and geochemical analyses of Lake Hoare sediments, Antarctica. Geochim Cosmochim Acta 60:765–785

Bishop JL, Englert PAJ, Patel S, Tirsch D, Roy AJ, Koeberl C, Böttger U, Hanke F, Jaumann R (2014) Mineralogical analyses of surface sediments in the Antarctic Dry Valleys: coordinated

analyses of Raman spectra, reflectance spectra and elemental abundances. Philos Trans R Soc, Ser A 372:20140198
Bridge PD, Spooner BM, Roberts PJ (2008) Non-lichenized fungi from the Antarctic region. Mycotaxon 106:485–490
Brunati M, Rojas JL, Sponga F, Ciciliato I, Losi D, Göttlich de Hoog S, Genilloud OE, Marinelli F (2009) Diversity and pharmaceutical screening of fungi from benthic mats of Antarctic lakes. Mar Genomics 2:43–50
Bulat SA (2016) Microbiology of the subglacial Lake Vostok: first results of borehole-frozen lake water analysis and prospects for searching for lake inhabitants. Philos Trans R Soc, Ser A 374:20140292
Camacho A (2006) Planktonic microbial assemblages and the potential effects of metazooplankton predation on the food web of lakes from the Maritime Antarctica and Sub-antarctic Islands. Rev Environ Sci Biotechnol 5:167–185
Camacho A, Rochera C, Villaescusa JA, Velázquez D, Toro M, Rico E, Fernandez-Valiente E, Justel A, Bañon M, Quesada A (2012) Maritime Antarctic lakes as sentinels of climate change. Int J Des Nat Ecodyn 7:239–250
Christner BC, Mosley-Thompson E, Thompson LG, Reeve JN (2001) Isolation of bacteria and 16S rDNAs from Lake Vostok accretion ice. Environ Microbiol 3:570–577
Christner BC, Royston-Bishop G, Foreman CM, Arnold BR, Tranter M, Welch KA, Lyons WB, Tsapin AI, Studinger M, Priscu JC (2006) Limnological conditions in subglacial Lake Vostok, Antarctica. Limnol Oceanogr 51:2485–2501
Connell L, Segee B, Redman R, Rodriguez RJ, Staudige H (2018) Biodiversity and abundance of cultured microfungi from the permanently ice-covered Lake Fryxell, Antarctica. Life 8:1–10
D'Ellia T, Veerapaneni R, Theraisnathan V, Rogers SO (2009) Isolation of fungi from Lake Vostok accretion ice. Mycologia 101:751–763
Dix NJ, Webster J (1995) Aquatic Fungi. In: Dix NJ, Webster J (eds) Fungal Ecology. Chapman & Hall, London, pp 225–283
Doran PT, McKay CP, Meyeri MA, Andersen DT, Wharton RA Jr, Hastings JT (1996) Climatology and implications for perennial lake ice occurrence at Bunger Hills Oasis, East Antarctica. Antarct Sci 8:289–296
Doran PT, Wharton RA Jr, Lyons WB, Des Marais DJ, Andersen DT (2000) Sedimentology and geochemistry of a perennially ice-covered epishelf lake in Bunger Hills Oasis, East Antarctica. Antarct Sci 12:131–140
Ellis-Evans JC (1981) Freshwater microbiology at Signy Island, South Orkney Islands Antarctica PhD dissertation, CNAA, 283pp
Ellis-Evans JC (1985) Fungi from maritime Antarctic freshwater environmentsBr. Brit Antartic Surv B 68:37–45
Ellis-Evans JC (1996) Microbial diversity and function in Antarctic freshwater ecosystems. Biodivers Conserv 5:1395–1431
Ellis-Evans J, Walton D (1990) The process of colonization in Antarctic terrestrial and freshwater ecosystems. Polar Biol 3:151–163
Gonçalves VN, Vaz AB, Rosa CA, Rosa LH (2012) Diversity and distribution of fungal communities in lakes of Antarctica. FEMS Microbiol Ecol 82:459–471
Gonçalves VN, Carvalho CR, Johann S, Mendes G, Alves TM, Zani CL, Junior PAS, Murta SMF, Romanha AJ, Cantrell CL, Rosa CA, Rosa LH (2015) Antibacterial, antifungal and antiprotozoal activities of fungal communities present in different substrates from Antarctica. Polar Biol 38:1143–1152
Goto S, Sugiyama J, Iizuka H (1969) A taxonomic study of Antarctic yeast. Mycologia 61:748–774
Green WJ, Canfield DE (1984) Geochemistry of the Onyx River (Wright Valley, Antarctica) and its role in the chemical evolution of Lake Vanda. Geochim Cosmochim Acta 48:2457–2467
Green WJ, Lyons WB (2009) The saline lakes of the McMurdo Dry Valleys Antarctica. Aquatic Geochem 15:321–348

Hall BL, Denton GH, Fountain AG, Hendy CH, Henderson GM (2010) Antarctic lakes suggest millennial reorganizations of Southern Hemisphere atmospheric and oceanic circulation. Proc Natl Acad Sci 107:21355–21359

Hao Y, Mo M, Su H, Zhang K (2005) Ecology of aquatic nematode-trapping *Hyphomycetes* in southwestern China. Aquat Microb Ecol 40:175–181

Hodgson DA (2012) Antarctic Lakes. In: Bengtsson L, Herschy RW, Fairbridge RW (eds) Encyclopedia of lakes and reservoirs. Springer, Dordrecht/London, pp 26–30

Hodgson DA, Doran PT, Roberts D, McMinn A (2004) Paleolimnological studies from the Antarctic and Subantarctic Islands. In: Pienitz R, Douglas MSV, Smol JP (eds) Long-term environmental change in Arctic and Antarctic lakes. Springer, Dordrecht - London, pp 419–474

de Hoog GS, Göttlich E, Platas G, Genilloudm O, Leotta G, van Brummelen J (2005) Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. Stud Mycol 51:33–76

Izaguirre I, Allende L, Tell G (2006) Algal communities of a geothermally heated lagoon on Deception Island (South Shetland Islands). Polar Biol 29:364–371

Izaguirre I, Pizarro H, Allende L, Unrein F, Rodríguez P, Marinone MC, Tell G (2012) Responses of a Maritime Antarctic lake to a catastrophic draining event under a climate change scenario. Polar Biol 35:231–239

Karl DM, Bird DF, Björkman K, Houlihan T, Shackelford R, Tupas L (1999) Microorganisms in the accreted ice of Lake Vostok, Antarctica. Science 286:2144–2147

Knox JS, Peterson RA (1973) The occurrence and distribution of some aquatic *Phycomycetes* on Ross Island and the Dry Valleys of Victoria Land, Antarctica. Mycologia 65:373–387

Kobayashi Y, Hiratsuka N, Korf RP, Tubaki K, Aoshima K, Soneda M, Sugiyama J (1967) Mycological studies of the Alaskan Arctic. Annu Rep Inst Ferment, Osaka 3:1–138

Kriss AE, Mitskevich IN, Rozanova EP, Osnitskaya LK (1976) Microbiological investigations of Lake Vanda (Antarctica). Microbiology 45:917–922

Kuehn KA (2015) Lentic and lotic habitats as templets for fungal communities: traits, adaptations, and their significance to litter decomposition within freshwater ecosystems. Fungal Ecol 19:135–154

Kuhn G, Hillenbrand CD, Kasten S, Smith JA, Nitsche FO, Frederichs T, Wiers S, Ehrmann W, Klages JP, Mogollón JM (2017) Evidence for a palaeo-subglacial lake on the Antarctic continental shelf. Nat Commun 8:15591

Laybourn-Parry J, Pearce DA (2007) The biodiversity and ecology of Antarctic lakes: models for evolution. Philos Trans R Soc Lond, Ser B 362:2273–2289

Laybourn-Parry J, Wadham JL (2014) Antarctic Lakes. Oxford University Press, Oxford, pp 1–40

Laybourn-Parry J, James MR, McKnight DM, Priscu J, Spaulding SA, Shiel R (1997) The microbial plankton of Lake Fryxell, Southern Victoria Land, Antarctica during the summers of 1992 and 1994. Polar Biol 17:54–61

Lukin V, Vasiliev N (2014) Technological aspects of the final phase of drilling borehole 5G and unsealing Vostok Subglacial Lake East Antarctica. Ann Glaciol 55:83–89

Matsumoto GL (1993) Geochemical features of the McMurdo Dry Valley lakes, Antarctica. In: Green WJ, Friedmann EI (eds) Physical and biogeochemical processes in Antarctica lakes, Antarctic research series, vol 59. American Geophysical Union, Washington, pp 95–118

McInnes SJ (2003) A predatory fungus (*Hyphomycetes: Lecophagus*) attacking *Rotifera* and *Tardigrada* in maritime Antarctic lakes. Polar Biol 26:79–82

McKay CP, Clow GD, Wharton RA Jr, Squyres SW (1985) Thickness of ice in perennially frozen lakes. Nature 313:561–562

McKay RM, Browne G, Carter L, Cowan E, Dunbar G, Krissek L, Naish T, Powell R, Reed J, Talarico F, Wilch T (2009) The stratigraphic signature of the late Cenozoic Antarctic Ice Sheets in the Ross Embayment. Geol Soc Am Bull 121:1537–1561

Michel RFM, Schaefer CEGR, Martínez JL, Simas FNB, Haus NW, Serrano E, Bockheim JG (2014) Soils and landforms from Fildes Peninsula and Ardley Island, Maritime Antarctica. Geomorphology 225:76–86

Monchy S, Sanciu G, Jobard M, Rasconi S, Gerphagnon M, Chabé M, Cian A, Meloni D, Niquil N, Christaki U, Viscogliosi E, Sime-Ngando T (2011) Exploring and quantifying fungal diver-

sity in freshwater lake ecosystems using rDNA cloning/sequencing and SSU tag pyrosequencing. Environ Microbiol 13:1433–1453

Nagashima H, Nishikawa J, Matsumoto GI, Izuka H (1990) Characterization and habitats of bacteria and yeasts isolated from Lake Vanda in Antarctica. Proc NIPR Symp Polar Biol 3:190–200

Oliva M, Hrbacek F, Ruiz-Fernández J, Pablo MA, Vieira G, Ramos M, Antoniades D (2017) Active layer dynamics in three topographically distinct lake catchments in Byers Peninsula (Livingston Island, Antarctica). Catena 149:548–559

Peter HU, Buesser C, Mustafa O, Pfeiffer S (2008) Risk assessment for the Fildes Peninsula and Ardley Island, and development of management plans for their designation as specially protected or specially managed areas. German Environ Agency Res Rep 203:124. 508p

Phartiyal B, Sharma A, Bera SK (2011) Glacial lakes and geomorphological evolution of Schirmacher Oasis, East Antarctica during Late Quaternary. Quat Int 235:128–136

Pienitz R, Doran PT, Lamoureux SF (2008) Origin and geomorphology of lakes in the polar regions. In: Vicent WF, Laybourn-Parry J (eds) Polar Lakes and Rivers. Oxford University Press, Oxford, pp 25–41

Priddle J, Heywood RB (1980) Evolution of Antarctic lake ecosystems. Biol J Linn Soc 14(5):1–66

Priscu JC, Foreman CM (2009) Lakes of Antarctic. In: Likens GE (ed) Encyclopedia of inland waters, vol 2. Elsevier Press, Oxford, pp 555–566

Priscu JC, Adams EE, Lyons WB, Voytek MA, Mogk DW, Brown RL, McKay CP, Takacs CD, Welch KA, Wolf CF (1999) Geomicrobiology of subglacial ice above Lake Vostok, Antarctica. Science 286:2141–2144

Quayle WC, Peck LS, Peat H, Ellis-Evans JC, Harrigan PR (2002) Extreme responses to climate change in Antarctic lakes. Science 295:645

Quesada A, Vincent WF, Kaupp E, Hobbie JE, Laurion I, Pienitz R, López-Martínez J, Durán JJ (2006) Landscape control of high latitude lakes in changing climate. In: Bergström DM, Convey P, Huiskes AHL (eds) Trends in Antarctic terrestrial and limnetic ecosystems. Antarctica as a global indicator. Springer, Dordrecht, pp 221–252

Ravin NV, Mardanov AV, Skryabin KG (2015) Metagenomics as a tool for the investigation of uncultured microorganisms. Russ J Genet 51:431

Robin Gde Q, Drewry DJ, Meldrum T (1977) International studies of ice sheet and bedrock. Philos Trans R Soc Ser A 279:185–196

Robinson CH (2001) Cold adaptation in Arctic and Antarctic fungi. New Phytol 151:341–353

Robinson CT, Gessner MO, Callies Ka Jolidon C, Ward JV (2000) Larch needle breakdown in contrasting streams of an alpine glacial floodplain. J N Am Benthol Soc 19:250–262

Rogers SO, Shtarkman YM, Koçer ZA, Edgar R, Veerapaneni R, D'Elia T (2013) Ecology of subglacial Lake Vostok (Antarctica), based on metagenomic/metatranscriptomic analyses of accretion ice. Biology (Basel) 2:629–650

Rojas-Jimenez K, Wurzbacher C, Bourne E, Chiuchiolo A, Priscu JC, Grossart HP (2017) Early diverging lineages within *Cryptomycota* and *Chytridiomycota* dominate the fungal communities in ice-covered lakes of the McMurdo Dry Valleys, Antarctica. Sci Rep 7:15348

Rosa LH, Vaz ABM, Caligiorne RB, Campolina S, Rosa CA (2009) Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv (*Poaceae*). Polar Biol 32:161–167

Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Geml J (2015) Long-term experimental warming alters community composition of ascomycetes in Alaskan moist and dry arctic tundra. Mol Ecol 24:424–437

Shearer CA, Descals E, Volkmann-Kohlmeyer B, Kohlmeyer J, Marvanova L, Padgett D, Porter D, Raja HA, Schmit JP, Thorton HA, Voglymayr H (2007) Fungal biodiversity in aquatic habitats. Biodivers Conserv 16:49–67

Sheraton JW, Tingeyi RJ, Black LP, Oliverz RL (1993) Geology of the Bunger Hills area, Antarctica: implications for Gondwana correlations. Antarct Sci 5:85–102

Shevnina E, Kourzeneva E (2017) Thermal regime and components of water balance of lakes in Antarctica at the Fildes peninsula and the Larsemann Hills. Tellus A 69:1317202

Shtarkman YM, Koçer ZA, Edgar R, Veerapaneni RS, D'Elia T, Morris PF, Rogers SO (2013) Subglacial Lake Vostok (Antarctica) accretion ice contains a diverse set of sequences from aquatic, marine and sediment-inhabiting Bacteria and Eukarya. PLoS One 8:e67221

Siegert JM, Ellis-Evans JC, Tranter M, Mayer C, Petit J, Salamatink A, Priscu JC (2001) Physical, chemical and biological processes in Lake Vostok and other Antarctic subglacial lakes. Nature 414:603–609

Simmons GM, Vestal JR, Wharton RA (1992) Environmental regulators of microbial activity in continental antarctic lakes. In: Green WJ, Friedmann EI (eds) Physical and biogeochemical processes in Antarctica Lakes, Antarctic Research Series, vol 59. American Geophysical Union, Washington, pp 165–195

Sokratova IN (2011) Hydrological investigations in the Antarctic oases. Russ Meteorol Hydrol 36:207–215

Stanley SO, Rose AH (1967) Bacteria and yeasts from lakes on Deception Island. Philos Trans R Soc, Ser A 252:199–207

Stüwe K, Braun H, Peer H (1989) Geology and structure of the Larsemann Hills area, Prydz Bay, East Antarctica. Aust J Earth Sci 36:219–241

Sugiyama J (1970) World's last frontier 111: polar mycology in Antarctica. Polar News 6:17–24

Toro M, Camacho A, Rochera C, Rico E, Bañón M, Fernández-Valiente E, Quesada A (2006) Limnological characteristics of the freshwater ecosystems of Byers Peninsula, Livingston Island, in maritime, Antarctica. Polar Biol 30:635–649

Townsend CR, Hildrew AG (1994) Species traits in relation to a habitat templet for river systems. Freshwat Biol 31:265–275

Tranter M, Fountain AG, Fritsen CH, Berry Lyons W, Priscu JC, Statham PJ, Welch KA (2004) Extreme hydrochemical conditions in natural microcosms entombed within Antarctic ice. Hydrol Proc 18:379–387

Tregoning GS, Kempher ML, Jung DO, Samarkin VA, Joye SB, Madigan MT (2015) A halophilic bacterium inhabiting the warm CaCl2-rich brine of the perennially ice-covered Lake Vanda, McMurdo Dry Valleys Antarctica. Appl Environ Microbiol 81:1988–1995

Tsuji M (2016) Cold-stress responses in the Antarctic basidiomycetous yeast *Mrakia blollopis*. Royal Soc Open Sci 31:60106

Tsuji M, Fujiu S, Xiao N, Hanada Y, Kudoh S, Kondo H, Tsuda S, Hoshino T (2013) Cold adaptation of fungi obtained from soil and lake sediment in the Skarvsnes ice-free area, Antarctica. FEMS Microbiol Lett 346:121–130

Vaz AB, Rosa LH, Vieira ML, Garcia VD, Brandão LR, Teixeira LC, Rosa CA (2011) The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. Brazilian J Microbiol 42:937–947

Vicent WF, Hobbie JE, Layboum-Parry J (2008) Introduction to the limnology of high-latitude lake and river ecosystems. In: Vicent WF, Laybourn-Parry J (eds) Polar lakes and rivers. Oxford University Press, Oxford, pp 1–24

Vincent WF (2000) Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. Antarct Sci 12:374–385

Vocke RD, Hanson GN (1981) U-Pb zircon ages and petrogenetic implications for two basement units from Victoria Valley, Antarctica. In: Mcginnis LD (ed) Dry Valley drilling project vol 33. American Geophysical Union, Washington DC, pp 248–255

Waguri O (1976) Isolation of microorganisms from salt lakes in the Dry Valley, Antarctica, and their living environment. Antarct Rec 57:80–96

Waguri O, Kawamura Y, Tubaki K (1975) Isolation of microorganisms. In: Mudrey MG Jr, McGinnis LD (eds) Dry Valley drilling project, bulletin no 5. Northern Illinois University, De Kalb, pp 111–117

Wang M, Jiang X, Wu W, Hao Y, Su Y, Cai L, Xiang M, Liu X (2015a) Psychrophilic fungi from the world's roof. Persoonia 34:100–112

Wang QM, Begerow D, Groenewald M, Liu XZ, Theelen B, Bai FY, Boekhout T (2015b) Multigene phylogeny and taxonomic revision of yeasts and related fungi in the *Ustilaginomycotina*. Stud Mycol 81:55–83

Willoughby LG (1971) Aquatic fungi from an Antarctic Island and a Tropical lake. Nova Hedwigia 22:469–488

Wright W, Burton HR (1981) The biology of Antarctic saline lakes. Hydrobiologia 82:319–338

Wright A, Siegert MA (2012) A fourth inventory of Antarctic subglacial lakes. Antarct Sci 24:659–664

Wurzbacher C, Kerr J, Grossart H (2011) Aquatic Fungi. In: Grillo O, Venora G (eds) The dynamical processes of biodiversity. Intech Open, London, pp 229–261

Zukal J, Bandouchova H, Brichta J, Cmokova A, Jaron KS, Kolarik M, Kovacova V, Kubátová A, Nováková A, Orlov O, Pikula J, Presetnik P, Šuba J, Zahradníková A Jr, Martínková N (2016) White nose syndrome without borders: *Pseudogymnoascus destructans* infection tolerated in Europe and Palearctic Asia but not in North America. Sci Rep 6(19829):1–12

5.2 Capítulo 2: "Sub-antarctic and Antarctic marine ecosystems: An unexplored ecosystem of fungal diversity."

Chapter 10
Sub-Antarctic and Antarctic Marine Ecosystems: An Unexplored Ecosystem of Fungal Diversity

Luiz Henrique Rosa, Franciane Maria Pellizzari, Mayara Baptistucci Ogaki, Maria Thereza Rafaela de Paula, Andrés Mansilla, Johanna Marambio, Pio Colepicolo, Arthur Ayres Neto, Rosemary Vieira, and Carlos Augusto Rosa

10.1 Introduction

There are various marine ecosystems in the world which are practically unexplored in terms of their microbial diversity, ecological role, and biotechnological potential. There are few published studies on the Antarctic marine microbiology, as compared with the rest of the world. The biological dynamics of marine ecosystems seem to be related to the presence of the microbiota living there, which dominate the living biomass of the oceans.

Various microorganisms, including bacteria, archaea, viruses, fungi, and protists, can be found in different ocean substrates (Glöckner et al. 2012). Microorganisms play an important role in the formation of the marine trophic pyramid by acting as photosynthetic primary producers (Richmond 2004), herbivores (Ramanan et al.

L. H. Rosa (✉) · M. B. Ogaki · M. T. R. de Paula · C. A. Rosa
Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
e-mail: lhrosa@icb.ufmg.br

F. M. Pellizzari
Laboratório de Ficologia e Qualidade de Água Marinha, Universidade Estadual do Paraná, Paranaguá, Paraná, Brazil

A. Mansilla · J. Marambio
Departamento de Ciencias y Recursos Naturales, Universidad de Magallanes, Punta Arenas, Chile

P. Colepicolo
Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil

A. A. Neto · R. Vieira
Instituto de Geociências, Universidade Federal Fluminense, Rio de Janeiro, Brazil

L. H. Rosa (ed.), *Fungi of Antarctica*,
https://doi.org/10.1007/978-3-030-18367-7_10

2016), consumers (Rivkin et al. 1996), parasites and decomposers responsible for cycling organic matter and oxygen to sustain life in the oceans (Glöckner et al. 2012), and by restoring the biogeochemical cycling of carbon, nitrogen, silica, iron, and other trace elements (Nelson et al. 1996; Moore et al. 2002; Morel and Price 2003; Voss et al. 2013).

Marine fungi are categorized into two major ecological groups by Kohlmeyer and Kohlmeyer (1979): (i) obligate marine fungi, which are described as 'those that grow and sporulate exclusively in a marine or estuarine habitat', and (ii) facultative marine fungi which are described as 'those from freshwater and terrestrial milieus able to grow and possibly also sporulate in the marine environment'. According to a taxonomic review by Jones et al. (2015), more than 700 species of obligate marine fungi and nearly 550 species of facultative and marine-derived fungi have been described. The *Halosphaeriaceae* is the largest family of marine fungi with 141 species and 59 genera; the most common genera in terms of number of species were *Aspergillus*, *Penicillium*, and *Candida* (Jones et al. 2015). However, the richness and diversity of marine fungi in the world seem to be underestimated and more than 10,000 species might exist, based on unidentified species that can be present in habitats and substrates not completely explored, such as those in deep sediments and seawater as well as abyssal organisms (Jones et al. 2009, 2015; Jones and Pang 2012).

Within the ocean ecosystem, marine fungi are important decomposers, which are able to, virtually, colonize all marine substrates, including wood and algae to sediments, muds, soils, sand, corals, calcareous tubes of molluscs, decaying leaves of mangroves, intertidal grasses, living animals, and the guts of crustaceans (Hyde and Goh 1998). Some fungi have the ability to disperse and survive in unusual or hostile environments (Sridhar 2017; Dighton and White 2017), such as those in Antarctica.

10.2 Antarctic Marine Ecosystem

Venter et al. (2004) and Sunagawa et al. (2015) studied the worldwide marine microbial diversity and showed that a large portion is represented by unknown taxa. Among the global oceans, the Southern Ocean, also called Austral Ocean or Antarctic Ocean, starts from 60 °S encircling the border of continental Antarctica (Fig. 10.1). The Antarctic Ocean represents a unique region whose microbiome is practically unexplored, including the microbial genes (Dickinson et al. 2016). Different substrates of the Southern Ocean represent promising microhabitats able to shelter microorganisms like virus, archaea, bacteria, and fungi. Among these substrates, those with availability of organic matter can be considered potentially rich in terms of microorganisms. However, virtually all substrates of Southern Ocean such as sediments, rocks, sea ice, seawater, seaweeds, invertebrates, and vertebrates might shelter microbial life.

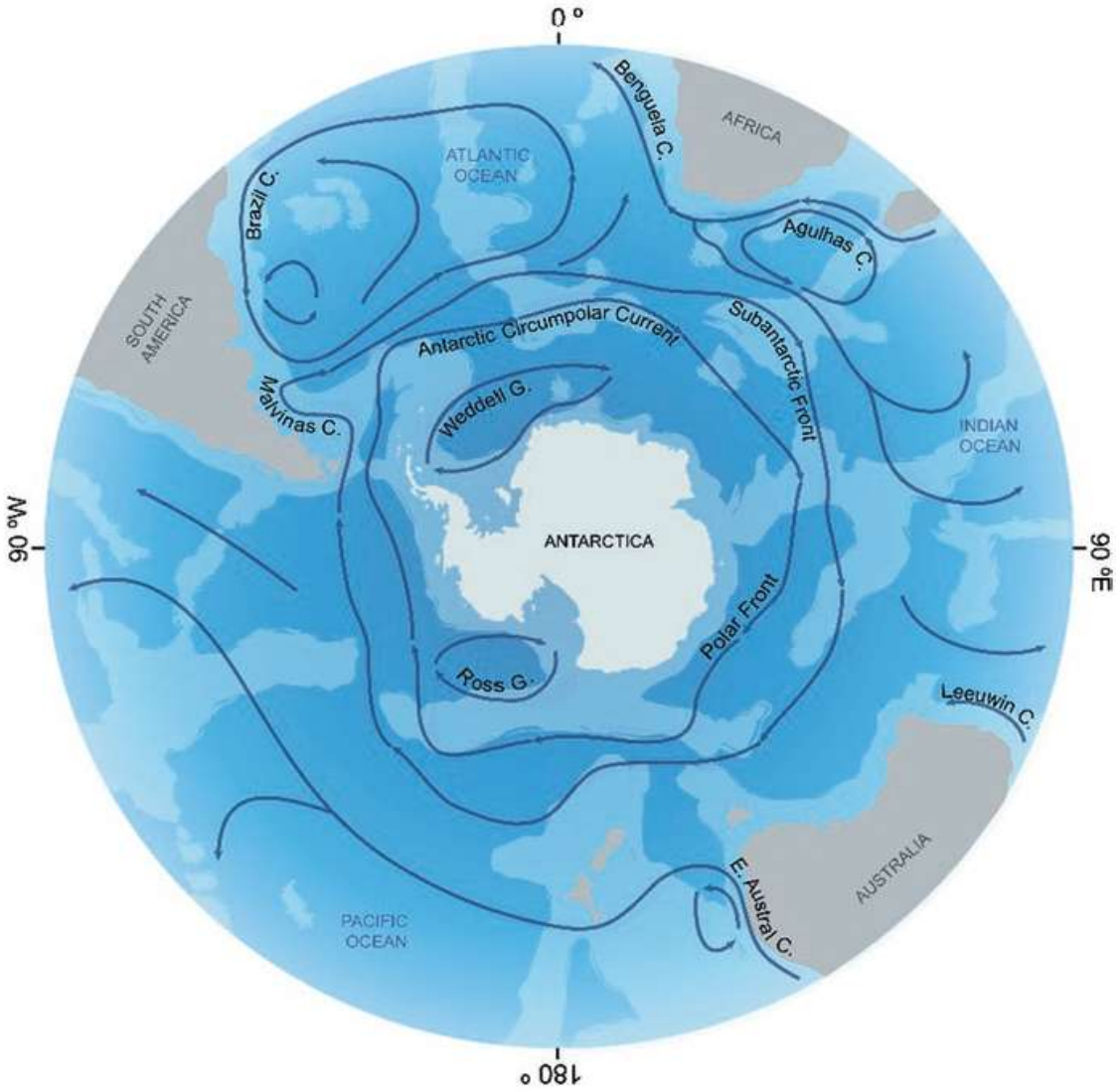


Fig. 10.1 Delimitation of the Antarctic Circumpolar Current and the nearest maritime current

10.3 Fungi in Antarctic Marine Sediments

The seafloor corresponds to two-thirds of the earth's surface and harbours a diversity of microorganisms adapted to live under conditions imposed by the sea depth (Nagano et al. 2010; Rédou et al. 2015). The sea bottom is recognized as an extreme environment, being mostly stable, characterized by the absence of sunlight, low temperatures, high hydrostatic pressure, and low availability of nutrients (Nagano et al. 2010; Raghukumar et al. 2010). Due to its uniqueness (hydrothermal, anoxic, and low temperature), marine environments represent a great potential for the study of the evolution of fungi, mainly the hypothesis that fungal divergence might be initiated in the marine habitats (Zhang et al. 2015).

On the seafloor, fungi act as decomposers of organic matters, parasites, pathogens, and mutualists associated with other marine organisms. Fungi are also

involved in denitrification processes (Cathrine and Raghukumar 2009; Jebaraj et al. 2010), because the major organic matter that is highly resistant to microbial decay is stored in marine sediments (Clarke et al. 2017). Some seagrasses, marsh plants, and mangrove detritus are rich in lignocelluloses; this structural polysaccharide is extremely resistant to decomposition (Clarke et al. 2017). These and other resistant compounds are deposited in sediments, making this substrate a site difficult for colonization by most organisms. However, fungi, present in marine sediments, have some adaptations in their structure and metabolism that demonstrate mesophilic, psychrotolerant, and/or halotolerant profiles (Raghukumar et al. 2010), which make them capable of tolerating the impositions of the deep sea. Among the fungal communities living in the marine sediments, there are some taxa that can produce oxidative and hydrolytic enzymes and can be a source of bioactive metabolites (Vaz et al. 2011; Gonçalves et al. 2013, 2015; Wentzel et al. 2019).

Some reports have demonstrated a significant fungal diversity in marine sediments of the Pacific Ocean (Lai et al. 2007; Nagano et al. 2010; Zhang et al. 2013; Rédou et al. 2015; Ahumada-Rudolph et al. 2016), Indian Ocean (Raghukumar et al. 2004; Cathrine and Raghukumar 2009; Singh et al. 2010; Zhang et al. 2014), Atlantic Ocean (Mouton et al. 2012; Orsi et al. 2013; Nagano et al. 2017), Arctic Ocean (Zhang et al. 2015), and Antarctic Ocean (López-garcía et al. 2001; Gonçalves et al. 2013, 2015) (Fig. 10.2). However, there is not much information on the fungal community in marine sediments of the Polar Regions. The genera *Cladosporium*,

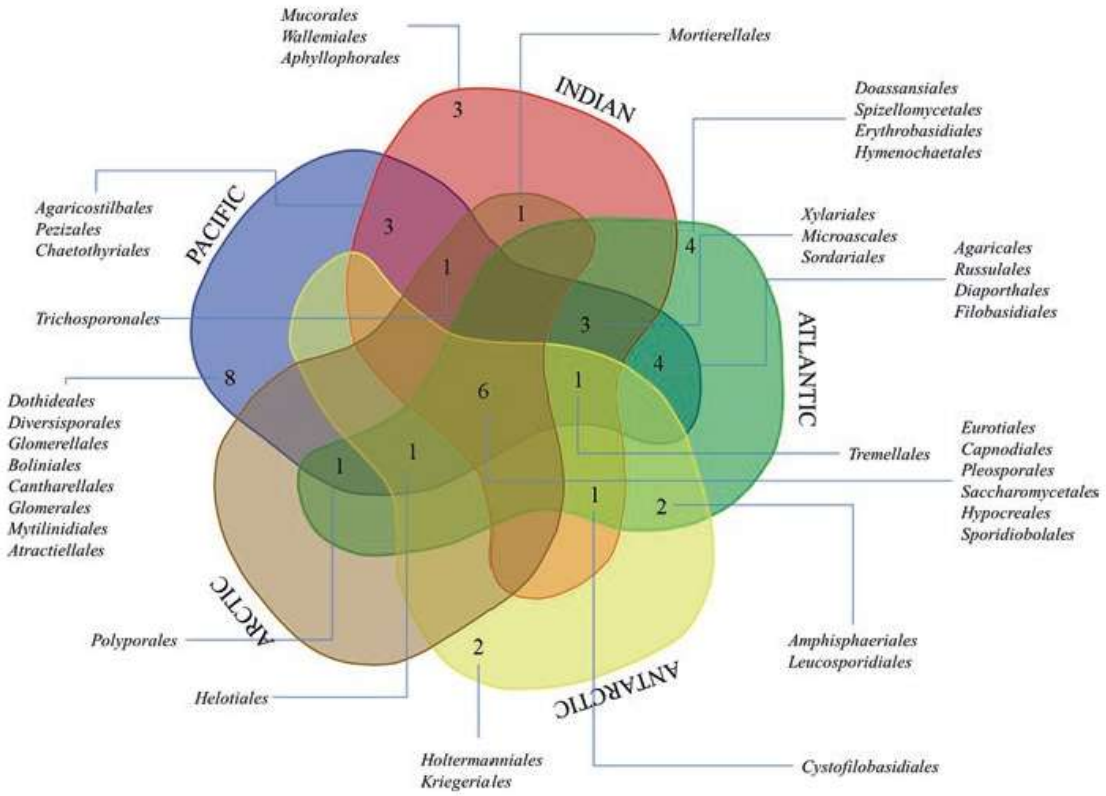


Fig. 10.2 Venn diagram showing fungal orders of isolates obtained from sediment samples of various oceans according to previous reports

Penicillium, and *Rhodotorula* are commonly identified in marine sediments of all oceans. The majority of taxa found in Antarctic marine sediments are shared with Atlantic marine sediments and the marine sediments from other oceans.

Few studies have isolated culturable fungi within the marine sediments of Antarctica, indicating the presence of several known taxa of fungi. Cosmopolitan species of *Aspergillus*, *Cladosporium*, *Penicillium*, *Fusarium*, *Candida*, and *Rhodothorula* seem to be adapted to extreme conditions of the Antarctic seawater (Raghukumar et al. 2004; Cathrine and Raghukumar 2009; Singh et al. 2010, 2012; Zhang et al. 2013). The occurrence of the orders *Kriegeriales* (*Glaciozyma* sp. and *Phenoliferia* sp.) and *Holtermanniales* (*Holtermanniella* sp.) reported by Wentzel et al. (2019) may be more frequent in the Antarctic marine sediments in comparison to other areas, since their occurrence was unique in the reports that were analysed.

To isolate microorganisms from extreme marine environments, like marine Antarctic environments, many specific isolation conditions have to be considered, such as hydrostatic pressure, salinity, nutrient profile, incubation temperature, and oxygen level, in order to simulate the limitations of the environment as much as possible. Due to this challenge, there is a major constraint in the identification of marine Antarctic fungi, and it is estimated that less than 20% of the species present have been isolated and grown in pure culture (Bridge 2007). This may explain the lack of data in the literature regarding obligate marine fungi in the region.

Due to the difficulties in isolation, molecular studies involving uncultured analyses have reported a large number of fungal taxa in marine environments, suggesting that there may be more fungi still unknown in the Antarctic environment, although there is a gap in the knowledge about their ecological role in this habitat, because Polar Regions are complex ecosystems. Some studies investigated fungi associated with marine sediments using molecular methods (clone libraries and pyrosequencing), which allow the identification of uncultivable species (Lai et al. 2007; Nagano et al. 2010; Singh et al. 2011; Xu et al. 2014; Zhang et al. 2014, 2015). Some studies have used cloning libraries to analyse samples from the deep-sea water column of the Drake Passage (Antarctic polar front) (López-garcía et al. 2001; Bass et al. 2007); however, from marine sediments, apparently, only cultivation techniques have been used to identify fungal taxa (Vaz et al. 2011; Laich et al. 2013; Gonçalves et al. 2013; Wentzel et al. 2019), which indicates that much of the fungal assemblages in this substrate remain unknown, mainly the species that are difficult to culture.

10.4 Macroalgae from Antarctic and Sub-Antarctic Areas as Hosts of Marine Fungi

In the Southern Pacific Ocean, the Magellanic biogeographic province (41 °S to 56 °S) is featured by a large extension of channels and fjords with diverse coastal environments arising from the glacial influence by direct exposure to the Pacific Ocean

(Camus 2001; Spalding et al. 2007). Two biogeographic districts have been categorized for this biogeographic province: Austral and Sub-Antarctic. The latter extends from about 52–53 °S to 56 °S (Camus 2001) or from the Magellanic Strait to Cape Horn. This territory is characterized by different environmental conditions and thus is further divided into sub-areas according to its geomorphology, orography, geology, soils, and climates (Pisano 1977).

The Magellanic province presents several ecological singularities that highlight the differences in composition, richness, and structure of macroalgae communities compared with temperate coasts of America (Ojeda 2013; Mansilla et al. 2014). The coastal habitats present high environmental heterogeneity, influencing the Sub-Antarctic macroalgae biodiversity, ruled by several factors such as geomorphology [type of substrate (Ojeda et al. 2014)], oceanographic patterns [salinity variation (Silva and Calvete 2002)], climate [seasonal variation in solar radiation, photoperiod, and temperature (Ojeda et al. 2014; Marambio et al. 2017)], and biological features [diversity of biotopes associated with macroalgae (Soto et al. 2012)]. Figure 10.3 shows macroalgae from the Sub-Antarctic ecosystem potential host of marine fungi.

The concept of macroalgal richness originated from the expeditions of Charles Darwin and Captain Robert Fitz Roy (1834) (Mansilla 2013) and current compilations have shown that the area of Magallanes and Tierra del Fuego afford about 234 macroalgae taxa, not considering the 444 registered for the entire Chilean coast (Ramírez 2010). Extensive Kelp forests cover the Magellanic coast, and *Macrocystis pyrifera* (*Phaeophyceae*) dominate the fjords and channels of the region (Mansilla and Ávila 2011). Its morphological features make this species an important engineering organism in the Magallanes region, as it provides habitat and refuge for

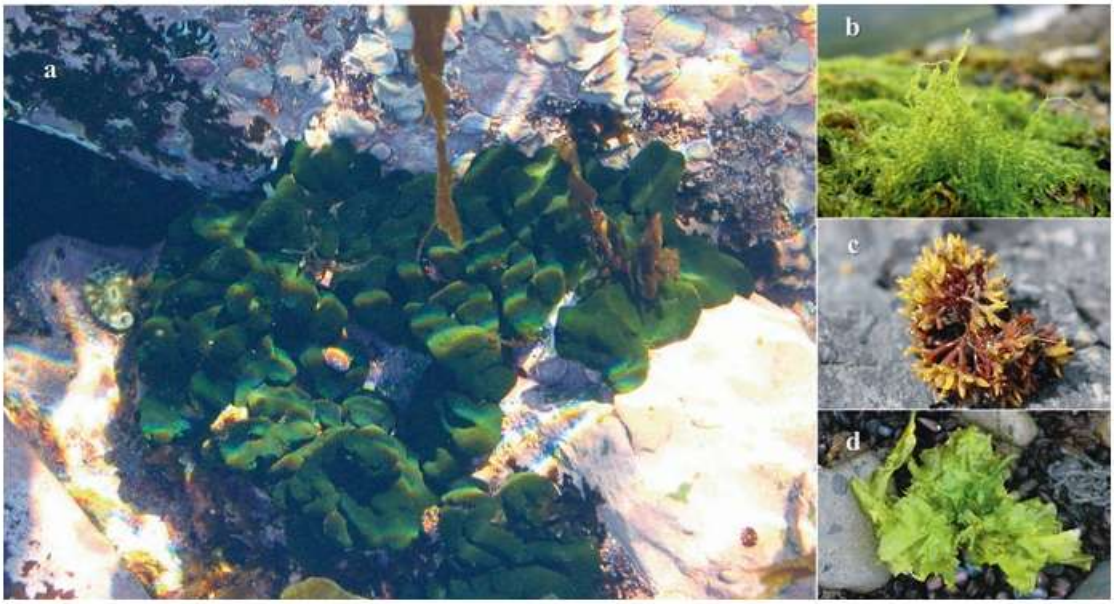


Fig. 10.3 Sub-Antarctic macroalgae (**a**) *Codium dimorphum*, (**b**) *Ulva intestinalis*, (**c**) *Nothogenia fastigiata,* and (**d**) *Ulva lactuca*. (Photos Credits: A Mansilla)

feeding and reproduction to many species (Vanella et al. 2007). The Sub-Antarctic intertidal environment is generally characterized by highly exposed shores situated in the path of the Antarctic Circumpolar Current (ACC).

Mansilla et al. (2016) reported that the Chilean Sub-Antarctic ecoregion of Magallanes hosts a distinct coastal phycobenthic community compared to other temperate continental rocky shores of South America and this could be the result of several factors such as (i) geomorphology generated by the glacial erosion during the advance and retreat of ice in the Quaternary, (ii) oceanographic gradients combining unique current flows, salinity, and thermal gradients, (iii) photoperiod and irradiance regimes, (iv) presence of glaciers and west-to-east winds and rainfall, (v) freshwater coastal discharge, and (vi) distinct substrate types, resulting in unique physical and biogeochemical seawater conditions that generate a peculiar macroalgal structure.

These unique abiotic factors at higher southern latitudes also affect the efficiency of light capture during photosynthesis. Seaweed photosynthesis demands efficient strategies for light utilization and pigment production to maintain algal performance. The environmental heterogeneity of the Sub-Antarctic and Antarctic ecosystems has shaped the evolutionary history and physiological adaptations of the local phycoflora and their associated organisms. Phylogenetic, morphological, and ecophysiological studies using the genus *Desmarestia*, conducted in the Chilean Sub-Antarctic areas, suggested that this species originated from an Antarctic ancestor, then radiated north, eventually reaching the Northern Hemisphere through long-distance natural dispersal (Peters et al. 1997). The representative *Desmarestia* species in Antarctica and the endemic *Himantothallus grandifolius* comprise a large proportion of the macroalgal biomass along the Antarctic Peninsula (Quartino et al. 2005; Pellizzari et al. 2017), both great substrates for marine fungi and yeast.

The differences in diversity, high degree of endemism, and affinities or molecular divergences between macroalgae species from the Antarctic and the Sub-Antarctic ecoregion of Magallanes are evidenced by the complex evolutionary process common in the Southern Ocean and probably due to a high degree of divergence registered between the Antarctic and South American populations of marine organisms (Poulin et al. 2014). Griffiths and Waller (2016) pointed out that the Sub-Antarctic intertidal environment is often characterized by highly exposed shores. The Sub-Antarctic Islands are located in the path of the ACC and are subjected to the force of the Southern Westerly winds. Ice-free coastlines are often dominated by dense beds of kelp *Durvillaea antarctica* (*Phaeophyceae*) and other large macrophytes. Unlike shores further south, there is often a distinct pattern of zonation from extreme high water to low water spring tides. Finally, the high environmental heterogeneity present in the Magallanes Biogeographic Province and the high richness of macroalgae species make the Magallanes region a natural laboratory for further studies.

The Antarctic intertidal environment is also the less well-sampled biome compared to the surrounding deep sea or adjacent terrestrial habitats (Griffiths and Waller 2016). This is in contrast to anywhere else in the world, since being characterized by intense seasonal scouring by ice, winter ice encasement, high UV

radiation, and seasonally large variations in temperature and salinity, it deserves continuous monitoring studies (Pellizzari et al. 2017).

Macroalgae are primary producers that act as a biogenic habitat for several other marine organisms and aid in maintaining the ocean's homeostasis through pH regulation. In addition, seaweeds possess bioactive chemical components of a high nutritional value, which can be applied in several industries, like nutraceutical and pharmaco-cosmetic industries. Recently, compounds such as terpenes, phenols, quinones, macrolides, alkaloids, lipids, chromones, and other related metabolites and extracts from Antarctic macroalgae have been reviewed as potential drugs against antiprotozoal and other neglected diseases (Torres et al. 2014; Falkenberg et al. 2018). Additionally, Antarctic macroalgae also can be a source of anticancer and antimicrobial compounds (Martins et al. 2018; Pacheco et al. 2018). Indeed, as Antarctic macroalgae synthesize large amounts of omega 3 and 6 fatty acids, they can be a source of polyunsaturated fatty acids (PUFAs) and steroids for other animals that consume them (Pereira et al. 2017; Santos et al. 2017). The Antarctic and Sub-Antarctic zones are distinct biogeographical regions, with patterns driven by a small number of widely distributed species. According to Griffiths and Waller (2016) and Sanches et al. (2016), the wide distribution of macroalgae that dominate the biogeographical formation of the intertidal zone along the Southern Ocean is most likely the result of rafting in the ACC.

Antarctic benthic biomes are characterized by winter ice encasement; spring/summer scouring by ice, intense and continuous UV radiation; and seasonal broad variations in temperature and salinity. However, despite the extreme Antarctic intertidal conditions, there are a wide and peculiar diversity of seaweeds and associated fungi/yeast growing in these substrates. In comparison, the Sub-Antarctic intertidal environment is often characterized by highly exposed shores situated in the ACC path. As reported by Astorga-España and Mansilla (2014), the Region of Magallanes, total area of 132,033 km^2, is the largest world representative of Sub-Antarctic environments (Mansilla et al. 2012), where 391 species of macroalgae have been reported (75 *Chlorophyta*, 86 *Phaeophyceae*, and 230 *Rhodophyta*). Many of these are economically crucial species for their alginates, carrageenans, or agarans, besides other compounds that are used as dietary supplements, or directly as food for human consumption (Mansilla et al. 2012).

South Georgia is an isolated island in the Southern Ocean and is the second largest hotspot of macroalgae diversity in the circum-Antarctic zone. The island presents 127 listed seaweed species (Wells et al. 2011), and some sporadic additional records include both endemic and cosmopolitan species. Until now, the origin of these species was unknown and there is lack of knowledge regarding whether they have been present in South Georgia for long periods of time. Moreover, low sampling efforts and low resolution in taxonomic inventories have either masked their presence or deterred the determination of whether they are recent additions to the seaweed flora. It may be speculated that many were not recorded due to the isolation of the area and the inaccessibility of the coastline. However, given the increased tourism and human activity, it is equally plausible that many species have been introduced in recent years through these means.

Studies focusing on seaweeds and their associated microbiota along remote polar areas are limited by logistical and safety challenges. All previous investigators relied on collections from the shore or occasionally by dredging and bottom grab, missing much of the subtidal organisms. The use of remotely operated underwater vehicle (ROV) and scuba diving, besides molecular methods, has improved the taxonomical endeavours, jointly with culturing techniques. Regarding the Maritime Antarctic Peninsula, recently Pellizzari et al. (2017) reported a total of 104 species of benthic marine algae (28 *Phaeophyceae*, 24 *Chlorophyta*, and 52 *Rhodophyta*), representing approximately 82% of all the seaweed taxa present in entire Antarctica, along eight islands of South Shetland Archipelago. The authors also listed six new species records (previously recorded only at lower latitudes), four putative taxa that were confirmed by their biogeographical distribution and two new species that were identified by their morphological and molecular features. Sanches et al. (2016) and Pellizzari et al. (2017) also suggest, using seaweed assemblages as proxy for diversity changes, that the Antarctic intertidal environment is undergoing dramatic changes, and that these sensitive communities are more connected with Sub-Antarctic and South American population than previously reported. Indeed, continuous investigations in these remote places are imperative to enable collection of accurate baseline data to monitor future changes in species composition and distributional shifts particularly due to climate change.

The Antarctic Peninsula is a transitional area and clearly a macroalgal hotspot (Pellizzari et al. 2017); this is followed by the West Antarctic Peninsula (WAP) and then, the East Antarctic Peninsula (EAP). The level of endemism was previously considered high (34% by Wiencke et al. 2014) and mainly involved *Phaeophyceae* and *Rhodophyta*. Figure 10.4 shows some macroalgal species frequently found in the Antarctic Peninsula already studied as hosts of marine fungi. However, a reduction in the endemism level (to ca. of 20%) has been reported since the study conducted

Fig. 10.4 Thalli of macroalgae from Antarctica (**a**) Bed of *Palmaria decipiens*, (**b**) *Desmarestia menziesii*, (**c**) *Iridaea cordata*, (**d**) *Pyropia endiviifolia*, (**e**) *Phaeurus antarcticus*, and (**f**) *Adenocystis utricularis*. (Photos Credits: FM Pellizari)

by Pellizzari et al. (2017), as suggested by several reports of the endemic species from Antarctica, in Patagonian samples (Argentinean and Chilean) and many Sub-Antarctic island samples (Pellizzari et al. 2017). Sanches et al. (2016) have used previously published data to establish a database for monitoring future biogeographical changes in the phycoflora of the Antarctic and Sub-Antarctic areas. The patterns seem to be altered either due to natural dispersion associated with global meteorological and oceanographic changes or due to biological invasions related to anthropogenic activities. The authors also indicate a period of changes in Antarctic diversity, suggesting that Antarctica may not be as isolated as was once thought.

10.5 Ecology and Diversity of Marine Fungi from Antarctica

Fungi occur in marine ecosystems as spores, hypha fragments, or mycelium in active or resistant forms even in unlikely locations like a hot deep-sea volcano (Connell et al. 2009), in deep sea (Damare et al. 2006; Redou et al. 2015), and anoxic hypersaline sediments (Bernhard et al. 2015) to freezing seawater substrates of Antarctica (Godinho et al. 2013; Gonçalves et al. 2015). Marine fungi can occur in harmonic and disharmonic symbiosis, as a commensal, mutualist, or parasite in the oceans (Raghukumar 2017). As in the land ecosystems, in the oceans, fungi play the main ecological role in the decomposition of organic matter including mangrove wood (Raghukumar 2017), seaweeds (Raghukumar 2006), corals, invertebrates, and vertebrates (Jones and Pang 2012). In addition, different chemical and physical environmental factors affect the diversity of marine fungi including seawater temperature, pressure, pH, and salinity (Jones 2000). However, according to Jones and Pang (2012), latitude and seawater temperature represent the limiting factor for fungal species composition in several habitats.

According to their biogeographical distribution, marine fungi are classified as tropical, temperate, polar (Arctic and Antarctic), and cosmopolitan (Jones 1993). When compared with tropical and temperate ecosystems, there are few reports of marine fungi in polar environments, mainly surrounding Antarctica. Marine Antarctic fungi have been recovered from marine animals (Henríquez et al. 2014), driftwood (Pugh and Jones 1986), in coastal waters (Grasso et al. 1997), deep sea (Lopez-Garcia et al. 2001), from macroalgae (Loque et al. 2010; Godinho et al. 2013; Furbino et al. 2014, 2018; Duarte et al. 2016), deep sediments (Gonçalves et al. 2015), and seawater (Gonçalves et al. 2017). However, these reported substrates represent a short portion habitats among those that exist in the Antarctic Ocean. Table 10.1 shows the checklist of Antarctic fungi reported in different substrates in Antarctica.

Some lignicolous marine fungi present in Antarctic seawater were studied using wood baits. In the Sub-Antarctic South Georgia and South Orkney Islands as well as in Antarctic Signy Island, Pugh and Jones (1986) found, as predominant lignicolous fungi, *Monodictys pelagica*, *Ceriosporosis tubulifera*, *Ceriosporosis halina*, and *Remispora maritima*. Grasso et al. (1997) used baits of beech and poplar wood

Table 10.1 List of taxa of marine fungi present in different substrata in Antarctica

Fungal taxa	Substrate	References
Acremonium sp.	*Palmaria decipiens* and seawater	Godinho et al. (2013), Gonçalves et al. (2017), Poveda et al. (2018)
Antarctomyces pellizariae	*Ascoseira mirabilis*	Furbino et al. (2018)
A. psychrotrophicus	*Adenocystis utricularis*, *Ulva intestinalis*, and *Pyropia endiviifolia*	Loque et al. (2010), Godinho et al. (2013), Furbino et al. (2014)
Aspergillus pseudoglaucus	Seawater	Gonçalves et al. (2017)
A. conicus	*Adenocystis* sp.	Godinho et al. (2013)
A. protuberus	*P. endiviifolia*	Furbino et al. (2014)
A. tabacinus	*M. hariotii*	Furbino et al. (2014)
A. terreus	*Phaeurus antarcticus*	Godinho et al. (2013)
Aspergillus sp.	*P. decipiens*, *P. endiviifoli*, and *Monostroma hariotii*	Godinho et al. (2013), Furbino et al. (2014)
Aureobasidium pullulans	*Desmarestia anceps*	Loque et al. (2010)
Beauveria bassiana	*Ascoseira mirabilis*	Furbino et al. (2018)
Cadophora malorum	*P. endiviifolia*	Furbino et al. (2014)
Cadophora sp.	Shallow marine sediment	Wentzel et al. (2019)
Candida sake	*Acrosiphonia arcta* and *Desmarestia menziesii*	Godinho et al. (2013), Duarte et al. (2016)
C. spencermartinsiae	Seawater	Vaz et al. (2011)
C. zeylanoides	Seawater	Vaz et al. (2011)
Ceriosporopsis halima	Wooden panels of balsa	Pugh and Jones (1986)
C. tubulifera	Wooden panels of balsa	Pugh and Jones (1986)
Chaetomium sp.	*P. decipiens*	Godinho et al. (2013)
Cladosporium lignicola	*P. endiviifolia*	Furbino et al. (2014)
C. tenuissimum	*A. arcta*	Godinho et al. (2013)
C. sphaerospermum	Seawater	Gonçalves et al. (2017)
Cladosporium sp.	*A.* mirabili, *A. arcta*, *Georgiella confluens*, *M. hariotii*, *P. endiviifolia,* and Shallow marine sediment	Godinho et al. (2013), Furbino et al. (2014), Furbino et al. (2018), Wentzel et al. (2019)
Comospora sp.	Deep marine sediment	Gonçalves et al. (2015)
Coprinellus radians	*Georgiella confluens*	Furbino et al. (2018)
Cordyciptaceae sp.	*M. hariotii*	Godinho et al. (2013)
Cryptococcus adeliensis	*M. hariotii*	Furbino et al. (2014)
C. albidosimilis	*M. hariotii*	Furbino et al. (2014)
Cryptococcus carnescens	*P. decipiens* and *Himantothallus grandifolius*	Loque et al. (2010), Duarte et al. (2016)

(continued)

Table 10.1 (continued)

Fungal taxa	Substrate	References
C. cf. *laurentii*	*M. hariotii*	Godinho et al. (2013)
C. magnus	*P. decipiens*	Duarte et al. (2016)
Vishniacozyma victoriae	*M. hariotii*, *H. grandifolius*, *U. intestinalis*, and shallow sediments	Furbino et al. (2014), Duarte et al. (2016), Godinho et al. (2013), Vaz et al. (2011)
Cryptococcus sp.	*H. grandifolius* and shallow sediment	Duarte et al. (2016), Wentzel et al. (2019)
Cystobasidium slooffiae	Seawater	Gonçalves et al. (2017)
Cystobasidium sp.	Shallow marine sediment	Wentzel et al. (2019)
Cystofilobasidium infirmominiatum	*M. hariotii*	Furbino et al. (2014)
Debaryomyces hansenii	*A. arcta* and *A. utricularis*	Godinho et al. (2013)
Dioszegia athyri	*P. decipiens*	Duarte et al. (2016)
D. xingshanensis	*H. grandifolius*	Duarte et al. (2016)
Dipodascus australiensis	*P. endiviifolia*	Furbino et al. (2014)
Doratomyces sp.	*Iridaea cordata*	Furbino et al. (2018)
Engyodontium sp.	*U. intestinalis*	Godinho et al. (2013)
Eurotium herbariorum	*P. antarcticus*	Godinho et al. (2013)
E. repens	*P. antarcticus*	Godinho et al. (2013)
Exophiala xenobiotica	Seawater	Gonçalves et al. (2017)
Fusarium sp.	*P. decipiens*	Godinho et al. (2013)
Glaciozyma litorale	*Gigartina skottsbergii*, *A. utricularis*, *Desmarestia menziesii*, and *I. Cordata*	Duarte et al. (2016)
G. martinii	*G. skottsbergii*	Duarte et al. (2016)
G. antarctica	Seawater	Gonçalves et al. (2017)
Glaciozyma sp.	Shallow marine sediment	Wentzel et al. (2019)
Graphium rubrum	Seawater	Gonçalves et al. (2017)
Guehomyces pullulans	*M. hariotii*	Furbino et al. (2014)
Helotiales sp.	*M. harioti*, *U. intestinalis*, and deep sediment	Godinho et al. (2013), Gonçalves et al. (2015)
Holtermanniella nyarrowii	*H. grandifolius*	Duarte et al. (2016)
H. festucosa	*H. grandifolius*	Duarte et al. (2016)
Holtermanniella sp.	Shallow marine sediment	Wentzel et al. (2019)
Hyaloscyphaceae sp.	*M. hariotii*	Godinho et al. (2013)
Lecanicillium sp.	*P. endiviifolia*	Furbino et al. (2014)
L. attenuatum	Seawater	Gonçalves et al. (2017)
Leucosporidiella creatinivora	*Tedania* sp. and seawater	Vaca et al. (2013), Vaz et al. (2011)

(continued)

Table 10.1 (continued)

Fungal taxa	Substrate	References
L. fragaria	*A. mirabilis* and *H. grandifolius*	Duarte et al. (2016), Furbino et al. (2018)
L. muscorum	*A. mirabilis, H. grandifolius*, *G. skottsbergii,* and shallow sediment	Furbino et al. (2018), Duarte et al. (2016), Vaz et al. (2011)
Leucosporidium scottii	Shallow marine sediment and seawater	Vaz et al. (2011)
Metschnikowia australis	*A. utriculari, D. anceps*, *P. decipien, M. hariotii*, *A. mirabilis*, *A. arcta*, *D. menziesii*, *P. endiviifolia*, *Cystosphaera jacquinotii*, *H. grandifolius*, *I. cordata*, *Curdiea racovitzae*, *G. skottsbergii*, *Curdiea racovitzae*, *Georgiella confluens*, *Dendrilla* sp., *Tedani* sp., *Hymeniacidon* sp., shallow sediment, and seawater	Loque et al. (2010), Vaz et al. (2011), Godinho et al. (2013), Furbino et al. (2014), Duarte et al. (2016), Furbino et al. (2018)
Metschnikowia sp.	Shallow marine sediment	Wentzel et al. (2019)
Meyerozyma caribbica	*A. utricularis*	Godinho et al. (2013)
M. guilliermondii	*M. hariotii* and *P. endiviifolia*	Furbino et al. (2014), Godinho et al. (2013)
Meyerozyma sp.	Shallow marine sediment	Wentzel et al. (2019)
Monodictys pelagica	wooden panels of vessel	Pugh and Jones (1986)
Mortierella antarctica	*P. endiviifolia*	Furbino et al. (2014)
Mortierella sp.	*A. arcta*	Godinho et al. (2013)
Mrakia sp.	*C. jacquinotii*, *G. skottsbergii*, *H. grandifolius*, *M. hariotii*, *D. menziesii*, and shallow sediment	Duarte et al. (2016), Wentzel et al. (2019)
Mycoarthris cf. *corallines*	*U. intestinalis*	Godinho et al. (2013)
Oidiodendron sp.	*A. utricularis*	Loque et al. (2010)
O. truncatum	*P. endiviifolia*	Furbino et al. (2014)
Paraconiothyrium sp.	Shallow marine sediment	Wentzel et al. (2019)
Penicillium chrysogenum	Seawater and *A. mirabilis*	Gonçalves et al. (2017), Furbino et al. (2018)
P. citreosulfuratum	Seawater	Gonçalves et al. (2017)
P. citrinum	*Adenocystis utricularis* and *M. hariotii*	Godinho et al. (2013), Furbino et al. (2014)
P. crustosum	*M. hariotii*	Furbino et al. (2014)
P. discolor	*U. intestinalis*	Godinho et al. (2013)
P. solitum	Shallow to deep marine sediment	Gonçalves et al. (2013)
P. spinulosum	*P. decipiens*	Godinho et al. (2013)
P. steckii	*M. hariotii* and *P. antarcticus*	Furbino et al. (2014), Godinho et al. (2013)
*P.*cf. *rubens*	Seawater	Gonçalves et al. (2017)

(continued)

Table 10.1 (continued)

Fungal taxa	Substrate	References
Penicillium sp.	*A. utricularis*, *P. decipiens*, *A. arcta, D. menziesii*, *P. endiviifolia*, *M. hariotii*, *Adenocystis* sp., *I. cordata*, *U. intestinalis*, *P. decipiens*, *P. antarcticus*, *G. skottsbergii*, *G. confluens*, seawater, and shallow sediment	Loque et al. (2010), Godinho et al. (2013), Furbino et al. (2014), Duarte et al. (2016), Furbino et al. (2018), Gonçalves et al. (2017), Wentzel et al. (2019)
Pestalotiopsis sp.	Shallow marine sediment	Wentzel et al. (2019)
Phaeosphaeria herpotrichoides	*A. utricularis*	Loque et al. (2010)
Phenoliferia sp.	Shallow marine sediment	Wentzel et al. (2019)
Phoma sp.	*A. arcta*	Godinho et al. (2013)
Pleosporaceae sp.	Deep marine sediment	Gonçalves et al. (2015)
Pseudocercosporella sp.	Shallow marine sediment	Wentzel et al. (2019)
Pseudogymnoascus destructans	*M. hariotii*	Godinho et al. (2013)
P. luteus	*U. intestinalis*	Godinho et al. (2013)
P. pannorum	*A. utricularis* and *D. anceps*	Loque et al. (2010)
Pseudogymnoascus sp.	*M. hariotii*, *P. endiviifolia*, *I. cordata*, *A. arcta*, *Adenocystis* sp., *P. decipiens*, *P. Antarcticus,* and shallow sediment	Furbino et al. (2014), Godinho et al. (2013), Gonçalves et al. (2015)
Pseudozyma tsukubaensis	*A. utricularis*	Duarte et al. (2016)
Purpureocillium lilacinum	Seawater	Gonçalves et al. (2017)
Phenoliferia glacialis	*G. skottsbergii*, *H. grandifolius*, and shallow sediment	Duarte et al. (2016), Vaz et al. (2011)
R. laryngis	*M. hariotii* and seawater	Furbino et al. (2014), Vaz et al. (2011)
R. marina	*P. decipiens*	Duarte et al. (2016)
R. minuta	*M. hariotii*	Furbino et al. (2014)
R. mucilaginosa	*A. utricularis*, *P. decipiens*, *M. hariotii,* and *G. confluens*	Loque et al. (2010), Furbino et al. (2014, 2018)
R. pinicola	*Hymeniacidon* sp.	Vaca et al. (2013)
Rhodotorula sp.	Shallow sediment	Wentzel et al. (2019)
Schizophyllum commune	Deep marine sediment	Gonçalves et al. (2015)
Simplicillium lamellicola	Deep marine sediment	Gonçalves et al. (2015)
S. aogashimaense	Seawater	Gonçalves et al. (2017)
Sporidiobolus pararoseus	*A. utricularis* and *Curdiea racovitzae*	Duarte et al. (2016)

(continued)

Table 10.1 (continued)

Fungal taxa	Substrate	References
Thelebolus globosus	*A. arcta* and *U. intestinalis*	Godinho et al. (2013), Furbino et al. (2014)
Tilletiopsis washingtonensis	*P. decipiens*	Duarte et al. (2016)
Toxicocladosporium sp.	Shallow marine sediment	Wentzel et al. (2019)
Ustilaginaceae	*A. utricularis*, *D. anceps*, and *P. decipiens*	Duarte et al. (2016)
Verticillum sp.	*P. endiviifolia*	Furbino et al. (2014)
Yamadazyma mexicana	*P. decipiens*	Godinho et al. (2013)

at a depth of 50 m, and after 1 year, they processed the baits and detected the presence of 14 *Ascomycota* species, with *Phoma* sp., *Trichocladium achrasporum*, *Trichocladium constrictum*, and *Trichocladium lignincola* being the dominant species/lineages.

Antarctic macroalgae represent the most studied marine substrate/host of marine fungi. The first study was conducted by Loque et al. (2010), who selected the macroalgae *Adenocystis utricularis*, *Desmarestia anceps*, and *Palmaria decipiens* to recover associated fungi. The authors recovered 75 fungal isolates, represented by 27 filamentous fungi and 48 yeasts of the genera *Pseudogymnoascus*, *Antarctomyces*, *Oidiodendron*, *Penicillium*, *Phaeosphaeria*, *Aureobasidium*, *Cryptococcus*, *Leucosporidium*, *Metschnikowia*, and *Rhodotorula*. After this pioneer study, Godinho et al. (2013), Furbino et al. (2014), Furbino et al. (2018), and Duarte et al. (2016) confirmed that Antarctic macroalgae shelter a rich and diverse fungal community, which include taxa with different ecological roles and possibly those which contribute to cycling organic matter in the Southern Ocean.

Fungi have already been detected in deep-sea sediments of the Atlantic, Pacific, and China Oceans. However, studies of the presence of fungi in deep sediments of the Southern Ocean surrounding Antarctica are in initial stages. Vaz et al. (2011) identified few yeast species (*Candida glaebosa*, *Vishniacozyma victoriae*, *Leucosporidiella muscorum*, *Metschnikowia australis*, *Nadsonia commutate*, and *Phenoliferia glacialis*) from the shallow marine sediments of Antarctica. Wentzel et al. (2019) processed 5 cm deep-sea sediment samples from the Admiralty Bay of the King George Island and obtained 226 fungal isolates containing species from 17 genera.

Apparently, Gonçalves et al. (2013) represented the first study focusing on cultivable fungi present in deep-sea sediments of Antarctica. They explored marine sediments from 100, 500, 700, and 1100 m depths and obtained 52 fungal isolates using the USNEL-type box corer, one of the most common devices utilized to sample deep marine sediments (Fig. 10.5). In this study, all fungal isolates were identified by polyphasic taxonomy as *Penicillium solitum*. The authors showed that conidial germination of *P. solitum* occurred at low temperatures, high salinities, and extracellular amylasic and esterasic activities, demonstrating its adaptability to extreme conditions of Southern Ocean.

Fig. 10.5 Details of the USNEL-type box corer utilized to sample deep marine sediments (**a**) General view of the process of sampling marine sediments in Antarctica using the box corer, (**b**) box corer with Antarctic marine sediments, (**c**) preparation of marine sediment corers, and (**d**) corer of Antarctic marine sediment. (Photos Credits: LH Rosa)

This pioneer study performed by Gonçalves et al. (2013) suggested that Antarctic marine sediments, mainly those from deep sea, might represent an interesting microhabitat to recover and study the biology of barophilic/psychrophilic fungi. However, to recover and better characterize these fungal communities, new studies using different culture media, pressure conditions, and metagenomic techniques are necessary to understand the complexity, ecological role, and biotechnological potential of these extremophilic fungi. An interesting result was that the sequences of *P. solitum* (Fig. 10.6) isolated from the Southern Ocean showed high query coverage and similarities with sequences of *Penicillium* taxa detected in different parts of the world, mainly those obtained from marine ecosystems like sediments of the Yellow Sea and the tropical Pacific Ocean, seawater of the East Pacific Ocean (both in China), macroalgae from the Mediterranean Sea along the coast of Spain, and marine air in Germany. These results might suggest that fungi with high genetic and physiological plasticity (like *P. solitum*) may use the marine current to disperse from Antarctica to other parts of the world and vice versa.

Lopez-Garcia et al. (2001) suggested that fungi from deep-sea environment are some of the few eukaryotes in the aphotic zone between 250 and 3000 m below the Southern Ocean. In a preliminary study on seawater samples of the Antarctic Peninsula, Vaz et al. (2011) detected the yeast species *Candida spencermartinsiae*, *Candida zeylanoides*, *Leucosporidiella creatinivora*, *Leucosporidium scottii*, *Metschnikowia australis,* and *Rhodotorula laryngis*. Further, from seawater at different depths and sites across the Gerlache and Bransfield Straits of the northern Antarctic Peninsula, using a combined Sea-Bird CTD equipped with 24 5L Niskin Sampling Bottles (Fig. 10.7), Gonçalves et al. (2017) recovered species of *Acremonium*, *Aspergillus*, *Cladosporium*, *Cystobasidium*, *Exophiala*, *Glaciozyma*, *Graphium*, *Lecanicillium*, *Metschnikowia*, *Penicillium*, *Purpureocillium*, and

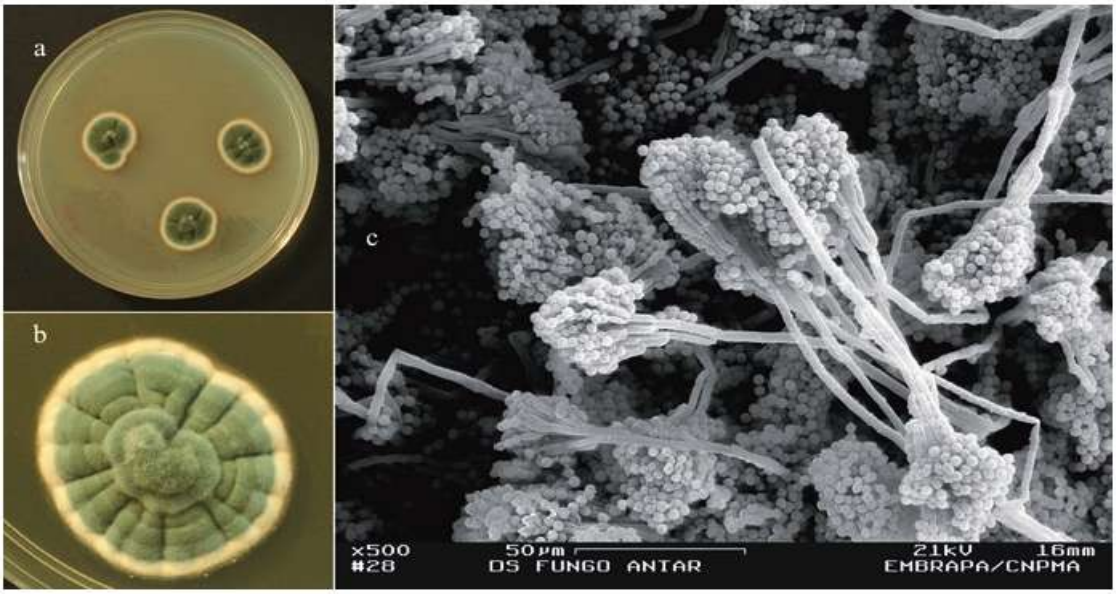


Fig. 10.6 *Penicillium solitum* isolated from marine sediments of Antarctica (**a**) Colonies on malt extract agar, (**b**) colony details, and (**c**) scanning electron microscopy details of its asexual structures. (Photos Credits: LH Rosa)

Fig. 10.7 Sequential process of seawater sampling using a combined Sea-Bird CTD equipped with 24 5L Niskin Sampling Bottles. (Photos Credits: LH Rosa)

Simplicillium. Among these, *Penicillium chrysogenum*, *Cladosporium sphaerospermum*, and *Graphium rubrum* were obtained at high densities, suggesting their ability to survive in the extreme conditions of seawater. These authors hypothesized that the marine fungal web of seawater is complex and includes species cited as barophiles, symbionts, weak and strong saprobes, parasites, pathogens, and some taxa with genetic similarities with those present in polluted environments around the world.

10.6 Conclusions and Perspectives

The different substrates and ecological niches of the Southern Ocean surrounding Antarctica represent the few unexplored frontiers of the planet that can be used to discover different extremophile fungal communities. In the Antarctic marine ecosystems, seawater, sea ice, deep-sea sediments, macroalgae, invertebrates, and vertebrates remain practically unexplored as microhabitats of Antarctic fungi. However, some abiotic factors, such as high pressure, affect access to the marine fungi for sampling purposes. Probably, there are new species and taxa able to produce bioproducts useful in biotechnological processes yet unknown in the Southern Ocean. Further interdisciplinary studies involving microbiology, phycology, oceanography, and geology have to be conducted to understand the total richness, diversity, and biotechnological potential of Antarctic marine fungi.

References

Ahumada-Rudolph R, Novoa V, Sáez K, Martínez M, Rudolph A, Torres-Diaz C, Becerra J (2016) Marine fungi isolated from Chilean fjord sediments can degrade oxytetracycline. Environ Monit Assess 188:468

Astorga-España MS, Mansilla A (2014) Sub-Antarctic macroalgae: opportunities for gastronomic tourism and local fisheries in the Region of Magallanes and Chilean Antarctic Territory. J Appl Phycol 26:973–978

Bass D, Howe A, Brown N, Barton H, Demidova M, Michelle H, Richards TA (2007) Yeast forms dominate fungal diversity in the deep oceans. Proc Biol Sci 274:3069–3077

Bernhard JM, Morrison CR, Pape E, Beaudoin DJ, Todaro MA, Pachiadaki MG, Edgcomb VP (2015) Metazoans of redoxcline sediments in Mediterranean deep-sea hypersaline anoxic basins. BMC Biol 13:105

Bridge P (2007) Fungi. In: Riffenburgh B (ed) Encyclopedia of the Antarctic. Taylor & Francis, London, pp 425–426

Camus PA (2001) Marine biogeography of continental Chile. Rev Chil Hist Nat 74:587–617

Cathrine SJ, Raghukumar C (2009) Anaerobic denitrification in fungi from the coastal marine sediments off Goa, India. Mycol Res 113:100–109

Clark GF, Stark JS, Palmer AS, Riddle MJ, Johnston EL (2017) The roles of sea-ice, light and sedimentation in structuring shallow Antarctic benthic communities. Plos One 12:e0168391

Connell L, Barrett A, Templeton A, Staudigel H (2009) Fungal diversity associated with an active deep sea volcano: Vailulu'u seamount, Samoa. Geomicrobiol J 26:597–605

Damare S, Raghukumar C, Raghukumar S (2006) Fungi in deep-sea sediments of the Central Indian Basin. Deep Sea Res Part I: Oceanogr Res Pap 53:14–27

Dickinson I, Goodall-Copestake W, Thorne MAS, Schlitt T, Ávila-Jiménez ML, Pearce DA (2016) Extremophiles in an Antarctic Marine Ecosystem. Microorganisms 4:8

Dighton J, White JF (2017) The fungal community: its organization and role in the ecosystem. CRC Press

Duarte AWF, Passarini MRZ, Delfomo TP, Pellizzari FM, Cipro CVZ, Montone RC, Petry MV, Putzke J, Rosa LH, Sette LD (2016) Yeasts from macroalgae and lichens that inhabit the South Shetland Islands, Antarctica. Environ Microbiol Rep 8:874–888

Falkenberg M, Nakano E, Zambotti-Villela L, Zatelli GA, Philippus AC, Imamura KB, Velasquez AMA, Freitas RP, Tallarico LF, Colepicolo P, Graminha MAS (2018) Bioactive compounds

against neglected diseases isolated from macroalgae: a review. J Appl Phycol 31:797. https://doi.org/10.1007/s10811-018-1572-5

Furbino LE, Godinho VM, Santiago IF, Pellizzari FM, Alves TM, Zani CL, Junior PAS, Romanha AJ, Carvalho AGO, Gil LHVG, Rosa AC, Minnis AM, Rosa LH (2014) Diversity patterns, ecology and biological activities of fungal communities associated with the endemic macroalgae across the Antarctic Peninsula. Microb Ecol 67:775–787

Furbino L, Pellizzari FM, Neto PC, Rosa CA, Rosa LH (2018) Isolation of fungi associated with macroalgae from maritime Antarctica and their production of agarolytic and carrageenolytic activities. Polar Biol 41:527–535

Glöckner FO, Stal LJ, Sandaa RA, Gasol JM, O'Gara F, Hernandez F, Labrenz M, Stoica E, Varela MM, Bordalo A, Pitta P (2012) In: Calewaert JB, McDonough N (eds) Marine Microbial Diversity and its role in Ecosystem Functioning and Environmental Change. Marine Board Position Paper 17. Marine Board-ESF, Ostend

Godinho VM, Furbino LE, Santiago IF, Pellizzari FM, Yokoya NS, Pupo D, Alves TMA, Junior PAS, Romanha AJ, Zani CL, Cantrell CL, Rosa CA, Rosa LH (2013) Diversity and bioprospecting of fungal communities associated with endemic and coldadapted macroalgae in Antarctica. ISME J 7:1434–1451

Gonçalves VN, Campos LS, Melo IS, Pellizari VH, Rosa CA, Rosa LH (2013) Penicillium solitum: a mesophilic, psychrotolerant fungus present in marine sediments from Antarctica. Polar Biol 36:1823–1831

Gonçalves VN, Carvalho CR, Johann S, Mendes G, Alves TMA, Zani CL, Junior PAS, Murta AJ, Romanha AJ, Cantrell CL, Rosa CA, Rosa LH (2015) Antibacterial, antifungal and antiprotozoal activities of fungal communities present in different substrates from Antarctica. Polar Biol 38:1143–1152

Gonçalves VN, Oliveira FS, Carvalho CR, Schaefer CEGR, Rosa CA, Rosa LH (2017) Antarctic rocks from continental Antarctica as source of potential human opportunistic fungi. Extremophiles 21:851–860

Grasso S, Bruni V, Maio G (1997) Marine fungi in Terra Nova Bay (Ross Sea, Antarctica). New Microbiol 20:371–376

Griffiths HJ, Waller CL (2016) The first comprehensive description of the biodiversity and biogeography of Antarctic and Sub-Antarctic intertidal communities. J Biogeogr 43:1143–1155

Henríquez M, Vergara K, Norambuena J, Beiza A, Maza F, Ubilla P, Araya I, Chávez R, San-Martín A, Darias J, Darias MJ, Vaca I (2014) Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial, antitumoral and antioxidant potential. World J Microbiol Biotechnol 30:65–76

Hyde KD, Goh TK (1998) Fungi on submerged wood in Lake Barrine, north Queensland, Australia. Mycol Res 102:739–749

Jebaraj CS, Raghukumar C, Behnke A, Stoeck T (2010) Fungal diversity in oxygen-depleted regions of the Arabian Sea revealed by targeted environmental sequencing combined with cultivation. FEMS Microbiol Ecol 71:399–412

Jones EBG (1993) Tropical marine fungi. In: Aspects of Tropical Mycology. Cambridge Univ Press, Cambridge pp 73–89

Jones EBG (2000) Marine fungi: some factors influencing biodiversity. Fungal Divers 4:53–73

Jones EBG, Pang KL (2012) Tropical aquatic fungi. Biodivers Conserv 21:2403–2423

Jones EBG, Sakayaroj J, Suetrong S, Somrithipol S, Pang KL (2009) Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. Fungal Divers 35:1–187

Jones EBG, Suetrong S, Sakayaroj J, Bahkali AH, Abdel-Wahab MA, Boekhout T, Pang K-L (2015) Classification of marine Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. Fungal Divers 73:1–72

Kohlmeyer J, Kohlmeyer E (1979) Marine mycology: the higher fungi. Academic Press, New York

Lai X, Cao L, Tan H, Fang S, Huang Y, Zhou S (2007) Fungal communities from methane hydrate-bearing deep-sea marine sediments in South China Sea. ISME J 1:756–762

Laich F, Vaca I, Chavez R (2013) Rhodotorula portillonensis sp. nov., a basidiomycetous yeast isolated from Antarctic shallow-water marine sediment. Int J Syst Evol Microbiol 63:3884–3891

López-García P, Rodriguez-Valera F, Pedrós-Alió C, Moreira D (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409:603–607

Loque CP, Medeiros AO, Pellizzari FM, Oliveira EC, Rosa CA, Rosa LH (2010) Fungal community associated with marine macroalgae from Antarctica. Polar Biol 33:641–648

Mansilla A. (2013) Catálogo de macroalgas y moluscos asociados a praderas naturales de Gigartina skottsbergii de la región de Magallanes. Ediciones Universidad de Magallanes, Punta Arenas, Chile, 44 pp.

Mansilla A, Ávila M (2011) Using Macrocystis pyrifera (L.) C. Agardh from southern Chile as a source of applied biological compounds. Rev Bras Farmacogn 21:262–267

Mansilla A, Ávila M, Yokoya NS (2012) Current knowledge on biotechnological interesting seaweeds from the Magellan Region, Chile. Rev Bras Farmacogn 22:760–767

Mansilla A, Rosenfeld S, Rendoll J, Murcia S, Werlinger C, Yokoya NS, Terrados J (2014) Tolerance response of Lessonia flavicans from the Sub-antarctic ecoregion of Magallanes under controlled environmental conditions. J Appl Phycol 26:1971–1977

Mansilla A, Méndez F, Murcia S, Rodríguez JP, Marambio J, Rosenfeld S, Yokoya N, Bischof K (2016) Adjustment of pigment composition in *Desmarestia* (*Desmarestiaceae*) species along a Sub-antarctic to Antarctic latitudinal gradient. Polar Res 35(1):29383

Marambio J, Mendez F, Ocaranza P, Rodriguez JP, Rosenfeld S, Ojeda J, Mansilla A (2017) Seasonal variations of the photosynthetic activity and pigment concentrations in different reproductive phases of Gigartina skottsbergii (Rhodophyta, Gigartinales) in the Magellan region, Sub-antarctic Chile. J Appl Phycol 29:721–729

Martins RM, Nedel F, Guimaraes VBS, da Silva AF, Colepicolo P, de Pereira CMP, Lund RG (2018) Macroalgae extracts from Antarctica have antimicrobial and anticancer potential. Front Microbiol 9:412

Moore JK, Doney SC, Glover DM, Fung IY (2002) Iron cycling and nutrient-limitation patterns in surface waters of the World Ocean. Deep-Sea Res II 49:463–507

Morel FMM, Price NM (2003) The biogeochemical cycles of trace metals in the oceans. Science 300:944–947

Mouton M, Postma F, Wilsenach J, Botha A (2012) Diversity and characterization of culturable fungi from marine sediment collected from St. Helena Bay, South Africa. Microb Ecol 64:311–319

Nagano Y, Nagahama T, Hatada Y, Nunoura T, Takami H, Miyazaki J, Horikoshi K (2010) Fungal diversity in deep-sea sediments–the presence of novel fungal groups. Fungal Ecol 3:316–325

Nagano Y, Miura T, Nishi S, Lima AO, Nakayama C, Pellizari VH, Fujikura K (2017) Fungal diversity in deep-sea sediments associated with asphalt seeps at the Sao Paulo Plateau. Deep Sea Res II: Top Stud Oceanogr 146:59–67

Nelson DM, DeMaster DJ, Dunbar RB, Smith WO Jr (1996) Cycling of organic carbon and biogenic silica in the Southern Ocean: Estimates of water-column and sedimentary fluxes on the Ross Sea continental shelf. J Geophys Res Oceans 101:18519–18532

Ojeda J. (2013) Dinámica estacional de macroalgas y moluscos intermareales y su relación con el conocimiento tradicional ecológico yagán, en canales subantárticos del cabo de hornos: una aproximación biocultural desde la filosofía ambiental de campo. Dissertation, Universidad de Magallanes

Ojeda J, Rosenfeld S, Marambio J, Rozzi R, Mansilla A (2014) Patrones estacionales y espaciales de la diversidad de moluscos intermareales de bahía Róbalo, canal Beagle, Reserva de la Biosfera Cabo de Hornos, Chile. Rev Biol Mar Oceanogr 49:493–509

Orsi W, Biddle JF, Edgcomb V (2013) Deep sequencing of subseafloor eukaryotic rRNA reveals active fungi across marine subsurface provinces. PLoS One 8:e56335

Pacheco BS, dos Santos MAZ, Schutze E, Martins RM, Lund RG, Seixas FK, Colepicolo P, Collares TP, FR d PCMP (2018) Cytotoxic activity of fatty acids from Antarctic macroalgae on the growth of human breast cancer cell. Front Bioeng Biotechnol 6:185

Pellizzari F, Silva MC, Silva EM, Medeiros A, Oliveira MC, Yokoya NS, Rosa LH, Colepicolo P (2017) Diversity and spatial distribution of seaweeds in the South Shetland Islands, Antarctica: an updated database for environmental monitoring under climate change scenarios. Polar Biol 40:1671–1685

Pereira CMP, Nunes CFP, Zambotti-Villela L, Streit NM, Dias D, Pinto E, Gomes CB, Colepicolo P (2017) Extraction of sterols in brown macroalgae from Antarctica and their identification by liquid chromatography coupled with tandem mass spectrometry. J Appl Phycol 29:751–757

Peters AF, van Oppen MJ, Wiencke C, Stam WT, Olsen JL (1997) Phylogenyand historical ecology of the Desmarestiaceae (Phaeophyceae) support a southern hemisphere origin 1. J Phycol 33:294–309

Pisano VE (1977) Fitogeografía de Fuego-Patagonia chilena. I. Comunidades vegetales entre las latitudes 52 y 56º S. An Inst Patagon

Poulin E, González-Wevar C, Díaz A, Gérard K, Hüne M (2014) Divergence between Antarctic and South American marine invertebrates: what molecular biology tells us about Scotia Arc geodynamics and the intensification of the Antarctic Circumpolar Current. Glob Planet Change 123:392–399

Poveda G, Gil-Durán C, Vaca I, Levicán G, Chávez R (2018) Cold-active pectinolytic activity produced by filamentous fungi associated with Antarctic marine sponges. Biol Res 51:28

Pugh GJF, Jones EBG (1986) Antarctic marine fungi: a preliminary account. The biology of marine fungi 323

Quartino ML, Zaixso HE, de Zaixso ALB (2005) Biological and environmental characterization of marine macroalgal assemblages in Potter Cove, South Shetland Islands, Antarctica. Bot Mar 48:187–197

Raghukumar C (2006) Algal-fungal interactions in the marine ecosystem: symbiosis to parasitism. Central Salt Research Institute, India

Raghukumar S (2017) The marine environment and the role of fungi. In: Fungi in coastal and oceanic marine ecosystems. Springer, Cham, pp 17–38

Raghukumar C, Muraleedharan U, Gaud VR, Mishra R (2004) Xylanases of marine fungi of potential use for biobleaching of paper pulp. J Ind Microbiol Biotechnol 31:433–441

Raghukumar C, Samir DR, Singh P (2010) A review on deep-sea fungi: occurrence, diversity and adaptations. Bot Mar 53:479–492

Ramanan R, Kim BH, Cho DH, Oh HM, Kim HS (2016) Algae–bacteria interactions: evolution, ecology and emerging applications. Biotechnol Adv 34:14–29

Ramírez ME (2010) Flora marina Bentónica de la región austral de Sudamérica y la Antártica. An Inst Patagon 38:57–71

Rédou V, Navarri M, Meslet-Cladière L, Barbier G, Burgaud G (2015) Species richness and adaptation of marine fungi from deep-subseafloor sediments. App Environ microbiol 81:3571–3583

Richmond A (2004) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd, Oxford, UK/Malden

Rivkin RB, Anderson MR, Lajzerowicz C (1996) Microbial processes in cold oceans. I. Relationship between temperature and bacterial growth rate. Aquatic Microb Ecol 10:243–254

Sanches PF, Pellizzari F, Horta PA (2016) Multivariate analyses of Antarctic and Sub-antarctic seaweed distribution patterns: an evaluation of the role of the Antarctic Circumpolar Current. J Sea Res 110:29–38

Santos MAS, Colepicolo P, Pupo D, Fujii MT, de Pereira CMP, Mesko MF (2017) Antarctic red macroalgae: a source of polyunsaturated fatty acids. J Appl Phycol 29:759–767

Silva N, Calvete C (2002) Características oceanográficas físicas y químicas de canales australes chilenos entre el golfo de Penas y el Estrecho de Magallanes (Crucero CIMAR-FIORDOS 2). Cienc Tecnol Mar 25:23–88

Singh P, Raghukumar C, Verma P, Shouche Y (2010) Phylogenetic diversity of culturable fungi from the deep-seasediments of the Central Indian Basin and their growthcharacteristics. Fungal Divers 40:89–102

Singh P, Raghukumar C, Verma P, Shouche Y (2011) Fungal community analysis in the deep-sea sediments of the Central Indian Basin by culture-independent approach. Microb Ecol 61:507–517

Singh P, Raghukumar C, Verma P, Shouche Y (2012) Assessment of fungal diversity in deep-sea sediments bymultiple primer approach. World J Microbiol Biotechnol 28:659–667

Soto E, Báez P, Ramírez EM, Letelier S, Naretto J, Rebolledo A (2012) Biotopos marinos intermareales entre Canal Trinidad y Canal Smyth, Sur de Chile. Rev Biol Mar Oceanogr 47:177–191

Spalding MD, Fox HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, Halpern S, Jorge MA, Lombana A, Lourie SA, Martin KD, McManus E, Molnar J, Recchia CA, Robertson J (2007) Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. BioScience 57:573–583

Sridhar KR (2017) Marine filamentous fungi: diversity, 5 distribution and bioprospecting. In: Satyanarayana T et al (eds) Developments in fungal biology and applied mycology, pp 59–73

Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G, Djahanschiri B, Zeller G, Mende DR, Alberti A, Cornejo-Castillo FM (2015) Structure and function of the global ocean microbiome. Science 348:1261359

Torres FAE, Passalacqua TG, Velasquez AMA, Souza RA, Colepicolo P, Graminha MAS (2014) New drugs with antiprotozoal activity from marine algae: a review. Braz J Pharmacog 24:265–276

Vaca I, Faúndez C, Maza F, Paillavil B, Hernández V, Acosta F, Levicán G, Martínez C, Chávez R (2013) Cultivable psychrotolerant yeasts associated with Antarctic marine sponges. World J Microbiol Biotechnol 29:183–189

Vanella F, Fernández D, Romero M, Calvo J (2007) Changes in the fish fauna associated with a Sub-antarctic Macrocystis pyrifera kelp forest in response to canopy removal. Polar Biol 30:449–457

Vaz AB, Rosa LH, Vieira ML, Garcia VD, Brandão LR, Teixeira LCRS, Moliné M, Libkind D, van Broock M, Rosa CA (2011) The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. Braz J Microbiol 42:937–947

Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, Wu D, Paulsen I, Nelson KE, Nelson W, Fouts DE (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304:66–74

Voss M, Bange HW, Dippner JW, Middelburg JJ, Montoya JP, Ward B (2013) The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change. Philos Trans R Soc Lon B Biol Sci 368:20130121

Wells E, Brewin P, Brickle P (2011) Intertidal and subtidal benthic seaweed diversity of South Georgia. Report for the South Georgia Heritage. Trust and Joint Nature Conservation Committee Survey:1–20

Wentzel LCP, Inforsato FJ, Montoya QV, Rossin BG, Nascimento NR, Rodrigues A, Sette LD (2019) Fungi from admiralty bay (King George Island, Antarctica) soils and marine sediments. Microb Ecol 77:12–24

Wiencke C, Amsler CD, Clayton MN (2014) Chapter 5.1. Macroalgae. In: De Broyer C, Koubbi P, Griffiths HJ, Raymond B, Udekem d'Acoz CD et al (eds) Biogeographic atlas of the Southern Ocean. Scientific Committee on Antarctic Research, Cambridge, pp 66–73

Xu W, Pang KL, Luo ZH (2014) High fungal diversity and abundance recovered in the deep-sea sediments of the Pacific Ocean. Microb Ecol 68:688–698

Zhang T, Zhang YQ, Liu HY, Wei YZ, Li HL, Su J, Yu LY (2013) Diversity and cold adaptation of culturable endophytic fungi from bryophytes in the Fildes Region, King George Island, maritime Antarctica. FEMS Microbial Lett 341:52–61

Zhang XY, Tang GL, Xu XY, Nong XH, Qi SH (2014) Insights into deep-Sea sediment fungal communities from the East Indian Ocean using targeted environmental sequencing combined with traditional cultivation. PLoS One 9:e109118

Zhang T, Wang NF, Zhang YQ, Liu HY, Li Yan YLY (2015) Diversity and distribution of fungal communities in the marine sediments of Kongsfjorden, Svalbard (High Arctic). Sci Rep 5:14524

5.3 Capítulo 3. "Cultivable fungi present in deep-sea sediments of Antarctica: Taxonomy, diversity, and bioprospection of bioactive compounds."

Artigo submetido para a revista Envinromental Microbiology (Anexo 1), no dia 14/06/2019.
Status: em análise

Cultivable fungi present in deep-sea sediments of Antarctica: taxonomy, diversity, and bioprospection of bioactive compounds

Mayara B. Ogaki[1], Lívia C. Coelho[1], Rosemary Vieira[2], Arthur Ayres Neto[2], Carlos L. Zani[3], Tânia M.A. Alves[3], Policarpo A.S. Junior[3], Silvane M.F. Murta[3], Emerson C. Barbosa[3], Jaquelline G. Oliveira[3], Isabela P. Ceravolo[3], Patrícia O. Pereira[3], Betania B. Cota[3], Roberta O. Viana[1], Viviane S. Alves[1] and Luiz H. Rosa[1]*

[1]Departamento de Microbiologia, Universidade Federal de Minas Gerais, MG, Brasil
[2]Instituto de Geociências, Universidade Federal Fluminense, RJ, Brasil
[3]Instituto René Rachou, FIOCRUZ-Minas, MG, Brasil

*Corresponding author. Departamento de Microbiologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, CEP 31270-901. Tel.: +55-31-3409 2749; Fax: +55-31-3409 2730, Brazil. E-mail: lhrosa@icb.ufmg.br

Running title: Fungi present in deep-sea sediments of Antarctica.

Abstract

We accessed the culturable mycobiota present in marine sediments at different depths in Antarctica ocean. *Acremonium fusidioides*, *Penicillium allii-sativi*, *Penicillium chrysogenum*, *Penicillium palitans*, *Penicillium solitum*, and *Pseudogymnoascus verrucosus* were identified. *Penicillium allii-sativi* was the dominant species. At least one isolate of each species was capable to present antifungal, trypanocidal, leishmanicidal, antimalarial, nematocidal, or herbicidal activities. *Penicillium* produced extracts with strong trypanocidal and antimalarial activities. Extracts of *P. solitum* and *P. chrysogenum* demonstrated strong antimalarial activities. *A. fusidioides* and *P. verrucosus* displayed strong selective herbicidal properties.

The ^{1}H NMR signals for extracts of *A. fusidioides*, *P. chrysogenum*, and *P. solitum* indicated the presence of highly functionalized secondary metabolites. In the deep marine Antarctic sediments, we detected fungal assemblages in which the *Penicillium* species were found to be dominant and demonstrated capabilities to survive and/or colonise that poly-extreme habitat. *Penicillium* being a polyextremophile Antarctic species, exhibited strong biological activities and the presence of aromatic compounds in its extracts may indicate that they are wild ancient strains with high genetic and biochemical potentials that enable them to produce bioactive compounds which can be researched in further studies and used in the chemotherapy of neglected tropical diseases as well as in agriculture.

Keywords: Antarctica; bioprospection; marine fungi; marine sediments

Introduction

The deep marine subsurface presents one of the most unknown microbial environment on the earth (Redou *et al*., 2015). Deep-sea microbial communities are mainly composed of bacteria and archaea (Whitman *et al*., 1998); however, some fungi have been reported to be present in these ecological niches in the past years (Damare *et al*., 2006; Jebaraj *et al*., 2010; Burgaud *et al*., 2009; Le Calvez *et al*., 2009; Redou *et al*., 2015). The first deep-sea fungi were isolated at a depth of 4,450 m in the Atlantic Ocean (Roth *et al*., 1964). Beyond the Atlantic, large number of fungal species were found in sediments from different regions of the world, such as the Gulf of Mexico and the Mariana Trench at a depth of 11,500 m (Takami *et al*., 1997; Thaler *et al*., 2012), the South Island of New Zealand at 1,927 m below the seafloor (Redou *et al*., 2015), and the east Indian Ocean at more than 4,500 m (Zhang *et al*., 2014). According to Giudice and Azzaro (2019), the microorganisms present in sediments of Antarctica Ocean play a major role in carbon, sulphur, nitrogen, and phosphorus cycles. Among these microorganisms, Gonçalves *et al*. (2013) isolated fungi from in the marine sediments at depths of 100, 500, 700, and 1,100 m. However, despite recent studies, the distribution and diversity of fungal communities in deep-sea environments of the Antarctic Ocean are still unknown.

Deep subseafloors present a variety of physical and chemical properties such as salinity, porosity, extreme pH, low temperature, low nutrient availability, elevated hydrostatic, and lithostatic pressure, which, either individually or in combination, limit the habitability of fungi (Redou *et al*., 2015). Majority of fungi recovered from deep sea sediments using culture-dependent and independent methods demonstrate homology with the terrestrial

species indicating the possibility of their arrival in deep-sea either via wind or terrestrial runoffs (Raghukumar *et al*., 2010). These terrestrial and freshwater fungi, which are able to grow (and possibly also sporulate) in the marine environment, are called facultative marine fungi (Kohlmeyer and Kohlmeyer, 1979). Recently Pang *et al*. (2016) proposed that a marine fungus as defined as any fungus that is recovered repeatedly from marine habitats. However, it is difficult to determine how these fungi have particularly adapted to the extreme *in situ* conditions of deep sediments.

Marine fungi are widely known to produce structurally unique secondary metabolites with promising biological and pharmacological properties (Rateb and Ebel, 2011). Majority of areas of the deep-sea bottom are stable, cold and dark, and it is assumed that most of the life forms may be present in a state of suspended animation (Raghukumar *et al*., 2010). The apparent paucity of fungal hyphae in deep-sea sediments might be because fungal propagules are present in the form of inactive spores and active fungal hyphae, but in low abundance (Damare and Raghukumar, 2008). However, Burgaud *et al*. (2015) reported that different yeasts isolated in the deep sea were active under laboratory conditions with the same pressure they experience in the deep sea. Additionally, microbial interactions between fungi, bacteria, and archaea may occur in the subsurface sediments and result in the synthesis and release of bioactive compounds useful for biotechnological applications (Redou *et al*., 2015). According to Rosa *et al*. (2019), to survive in extreme conditions of Antarctica, fungi might display unusual biochemical pathways able to generate specific or novel compounds that could be applied as prototypes to develop new drugs against different biological targets; in addition, recent studies show that fungi present in different environments of Antarctica call attention because they can be considered as live factories producing unknown bioactive secondary metabolites and few species and strains were investigated chemically until now.

For the reasons describe above, in the present study, we have characterized the cultivable fungi present in deep marine sediments of the Antarctic Ocean and evaluated their potential as producers of bioactive compounds that can be used against neglected tropical diseases and for applications as herbicides in agriculture.

Materials and methods

Study area

Four sediment samples were collected from different marine points in the South Shetlands Islands, Antarctica (Table S1) during the austral summers of 2014 and 2015. Samples of the

sediments at depths of 153, 250, 550, and 1,463 m were collected using a gravity corer and sections of 10 cm from the base of the core were sealed, placed in sterile Whirl-pack (Nasco, Ft. Atkinson, WI) bags and frozen at -20 °C for subsampling in the laboratory at the Federal University of Minas Gerais, Brazil. The core was gradually thawed at 4 °C for 24 h before carrying out the fungal isolation. Three subsamples of the central parts of each core were obtained using sterilized scoop inside of laminar flow hood under conditions of strict contamination control and processed to isolate the fungi.

Fungal isolation

Different media were used to isolate the fungal species from sediments, which are as follows: potato dextrose agar (PDA, Himedia/India), Sabouraud's agar (SAB, Himedia/ India), cornmeal agar (CMA, Himedia/India), Czapek-Dox agar (CZA, Sigma-Aldrich/USA) and malt extract agar (MEA, malt extract 2%, peptone 0.1%, agar 2%), which were diluted in a ratio of 1:5 to simulate low nutrient conditions found in the deep sediments (Damare *et al*., 2006; Redou *et al*., 2015). Additionally, the rich nutritional media used were as follows: Marine agar 2% (w/v) glucose (MAG) (Gonçalves *et al*., 2013), and Sabouraud 4% (w/v) glucose (SAG). All media contained 100 mg mL^{-1} of chloramphenicol (Sigma) to prevent bacterial growth and, with an exception of MAG, all media were prepared using a solution of water with 30 g L^{-1} sea salts (Sigma, USA).

Subsamples of the sediment were inoculated using three different methods as follows: a) Sediment dilution: 1 g of each subsample was added to 1 mL of water with sea salt at 30 g L^{-1} (Sigma, USA), and 100 µL of these 1:1 suspensions were plated; (b) Sediment smear: The muddy sediment subsamples were smeared on media plates; (c) Sediment enriched: 1 g of each subsample was added to 5 mL of liquid media (the same media cited above, but without the addition of agar) under conditions of agitation at 120 rpm at 10 °C for 7 d. After incubation, 100 µL of each suspension was plated. The plates were incubated under normal atmospheric pressure conditions at 10 °C for 60 days under aerobic and anaerobic conditions using the BD GasPak EZ Anaerobe Container Systems (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). Two replicated of the fungal colony forming units (CFUs) were counted, and subcultures of all morphologically distinct colonies obtained from each sample were made. The subcultures were grouped into different morphotypes based on their macroscopic parameters (colony colour and texture, border type, and radial growth rate) and micromorphological characteristics observed on MAG agar in order to conduct subsequent identification. All fungal isolates were deposited in the Collection of Microorganisms and

Cells of the Federal University of Minas Gerais under the code UFMGCB, the data of which can be accessed by other scientists.

Fungal identification

The protocol for DNA extraction was described previously in Rosa *et al*. (2009). For the filamentous fungi, the internal transcribed spacer (ITS) region was amplified with 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, amplification of the β-tubulin (Glass and Donaldson, 1995) and ribosomal polymerase II genes (RPB2) (Houbraken *et al*., 2012), which are commonly utilized to fungal taxa with low intraspecific variation, was completed with the Bt2a/Bt2b and RPB2-5F-Pc/RPB2-7CR-Pc 7CR primers, respectively, according to protocols established by Gonçalves *et al*. (2015). Fungi with query coverage and identity ≥ 99% were considered to represent the same taxon. Representative consensus sequences of the fungal taxa were deposited into the GenBank database (Table 1). To achieve species-rank identification based on ITS, β-tubulin data and ribosomal polymerase B2, the consensus sequence was aligned with all sequences from related species retrieved from the NCBI GenBank database using BLAST (Altschul *et al*., 1997). Taxa that displayed query coverage and ≤98% identity or an inconclusive taxonomic position were subjected to phylogenetic ITS, β-tubulin, and ribosomal polymerase B2-based analysis for comparison with sequences of ex type species deposited in the GenBank database, with estimations conducted using MEGA Version 5.0 (Tamura *et al*., 2011). The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1,000 replicate runs. The information about fungal classification generally followed the dictionary of Kirk *et al*. (2008), and databases MycoBank (http://www.mycobank.org) and the Index Fungorum (http://www.indexfungorum.org).

Morphological taxonomy

To confirm identification of the *Penicillium* species, its isolates were grown in CZA, Czapek yeast extract agar (CYA, Himedia/India) and yeast extract agar (YES, yeast extract 3%, peptone 5%, agar 2%) at 25 to 30 °C for 7 d (de Hoog *et al*., 2001). In order to determine if the isolates were the same species, their growth characteristics (mycelial colouring, border and texture of the colonies), pigment and/or exudate production, and the presence or absence of sporulation were compared with the following species: *P. alli-sativi*, *P. tardochrysogenum*,

P. chrysogenum, *P. palitans*, and *P. solitum*. The information about the description of fungal characteristics was collected from the data available on MycoBank.

Fungal cultivation and extract production for biological assays

All fungal isolates were cultivated using solid-state fermentation. Briefly, the cultures were prepared by aseptically transferring three 5-mm diameter plugs of each fungal isolate on 10 Petri dishes (90 mm diameter) containing MA medium, followed by incubation for 15 days at 10 °C. The fungal cultures were cut in small pieces and transferred to 250 mL Erlenmeyers flasks followed by the addition of 100 mL of ethyl acetate (Synth, Brazil). After 72 h of decantation at room temperature, the organic phase was filtered and the solvent removed under rotary evaporation at 40 °C. An aliquot of each dried extract was dissolved in DMSO (Merck) to prepare a 100 mg mL^{-1} stock solution, which was stored at -20 °C. Sterile MA medium was extracted using the same procedure. The sterile MA extract was used as the control in the screening procedures.

Antimicrobial activity against Cladosporium sphaerospermum

For the assay against *Cladosporium sphaerospermum* CCT 1740, this was previously grown in BDA at 25 °C for 7 to 10 days. To prepare the solution a well-filled spore loft was suspended in sterile 0.85% saline solution. The resulting suspension was homogenized on a vortex type stirrer for 15 seconds. The cell density of the suspension was standardized in a spectrophotometer (BioSpectro SP-22) for 86-88% transmittance at 620nm, corresponding to 10^6 spores mL^{-1} (NCCLS M38-A, v. 22, n ° 16). Subsequently, the obtained suspension was diluted 50-fold in RPMI1640 culture medium (INLAB Diagnostic) for use in the assay. The extracts were tested at 100mg mL^{-1}, and all tests were performed in duplicate. A total of 25 µL of the extract (dissolved in DMSO and diluted in 1 mg mL^{-1} autoclaved deionized water), 25 µL of the culture medium and 50 µL of the inoculum were inoculated into each well. As a positive control, Benomyl at 1.16 µg mL^{-1} was used. The volume of each well was 100 µL and the concentrations of 0.1% DMSO and 250 µg mL^{-1} extract. Plates were placed on a shaker (Uniscience OS-10) for 20 minutes at 200 rpm. Subsequently, they were incubated at 25 °C for 48h. The fungi growth was analysed visually and the reading of the plates was carried out on a VERSAmax microplate reader (Molecular Devices) by the program Softmax® Pro 5 (Molecular Devices), with absorbance of 620 nm. The absorbance of the test wells was compared to the absorbance of the microorganism control. Extracts with an inhibition value greater than or equal to 60% were arbitrarily considered as active.

Assay against Trypanosoma cruzi

The *in vitro* test of trypanocidal activity was performed as previously described by Romanha *et al*. (2010), using *Trypanosoma cruzi* (Tulahuen strain) expressing the *Escherichia coli* β-galactosidase gene. Infective trypomastigote forms are obtained through culture in monolayers of mouse L929 fibroblasts in RPMI-1640 medium, without phenol red, containing 10% foetal bovine serum and 2 mM glutamine. For the bioassay, 4,000 L929 cells in 80 μL of supplemented medium are added to each well of a 96-well microtitre plate. After an overnight incubation, 40,000 trypomastigotes in 20 μL are added to the cells and the cells are incubated for 2 h. Medium containing parasites that did not penetrate the cells is replaced with 200 μL of fresh medium and the plate is incubated for an additional 48 h to establish infection. The medium is then replaced with solutions of natural products at 20 mg mL^{-1} in fresh medium (200 μL) and the plate is incubated for 96 h at 37°C. After this period, 50 μL of 500 μM chlorophenol red b-D-galactopyranoside in 0.5% Nonidet P40 is added to each well and the plate is incubated for 18 h at 37 °C, after which the absorbance at 570 nm is measured. Controls with uninfected cells, untreated infected cells, infected cells treated with benznidazole at 3.8 μM (positive control) or DMSO 1% are used. The results are expressed as the percentage of *T. cruzi* growth inhibition in compound-tested cells as compared to the infected cells and untreated cells. Duplicates are run in two different plates.

Assay against Leishmania amazonensis

Promastigotes of *Leishmania* (*Leishmania*) *amazonensis* (strain IFLA/BR/196/PH-8) obtained from lesions of infected hamsters were used to detect the leishmancidal activity according to protocols established by Callahan *et al*. (1997). Each extract was tested at concentration of 20 μg mL^{-1}. The results are expressed as percent inhibition in relation to controls without drugs. Amphotericin B at 0.2 μg mL^{-1} (Fungison® Bristol-Myers Squibb B, Brazil) was used as a positive drug control. All assays were performed in triplicate.

Activity against Plasmodium falciparum *blood parasites* in vitro

Initially, 20 μg mL^{-1} of each extract sample was placed into the well of the "U" bottom 96-wells plates and their efficacies were evaluated against the parasites, and repeated at least once. The sample that inhibited the growth of the parasite with an efficacy of ≥40%, compared to the control, without addition of drugs, was considered promising. The promising samples were then titrated and the concentration at which growth inhibition of 50% of the

parasites took place was used to determine the IC_{50} value. The activity of the compounds against the bloodborne form of the *P. falciparum* parasite was tested using a chloroquine-resistant and mefloquine-sensitive W2 clone (Oduola *et al*., 1988) cultured at 37 °C, as per the method described by Trager and Jensen (1976). The activity was measured using the SYBR test with the parasite suspension (0.5% parasitemia and 2% haematocrit), as described previously (Smilkstein *et al*., 2004). After 48 h at 37 °C, the culture supernatant was removed and replaced by 100 µL of the lysis buffer solution [Tris (20 mM; pH 7.5), EDTA (5 mM), saponin (0.008%; w/v), and Triton X-100 (0.08%; v/v)] followed by the addition of 0.2 µL mL^{-1} of SYBR Safe (Sigma-Aldrich, Carlsbad, CA, USA). The contents of the plate were transferred to a flat bottom plate and incubated in the dark for 30 min at 37 °C. The plate was read in a fluorometer (Synergy H4 Hibrid Reader, Biotek) with excitation and emission at 485 and 535 nm, respectively. In all the tests, the activities of the compounds were expressed as the 50% inhibitory concentration of the parasite growth (IC_{50}) compared to the drug-free controls and estimated using the curve-fitting software Origin 8.0 (OriginLab Corporation, Northampton, MA, USA). Chloroquine was used as antimalarial reference drug (IC_{50} ~ 100 ng mL^{-1}). Compounds with IC_{50} values bellow 10 µg mL^{-1}, between 10 e 20 µg mL^{-1} and above 20 µg mL^{-1} were considered to be active, partially active and inactive, respectively.

Toxic to a mammalian cell line

The monkey kidney cell line (BGM) (ATCC, Manassas, VA, USA) was used to conduct the cytotoxicity assays, and maintained as suggested by the manufactures. The cells were cultured in bottles of 75 cm^2 dimensions with RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 40 mg L^{-1} gentamicin in a 5% CO_2 atmosphere at 37 °C. For the *in vitro* tests, a confluent cell monolayer was trypsinized, distributed in a flat-bottomed 96-well plate (2×10^5 cells mL^{-1}), and incubated for 18 h at 37 °C to ensure cell adherence. The BGM cells were incubated with 20 µL of the drugs at different concentrations (≤1 mg mL^{-1}) for 24 h in 5% CO_2 at 37 °C. The cell viability was expressed as the percentage of control absorbance of the untreated cells after subtracting the appropriate background values. The drug lethal dose of cells was determined by conducting tests at least in duplicates to calculate the dose that killed 50% of the cells (MLD_{50}), as described by do Céu de Madureira *et al*. (2002). The neutral red uptake assay was used to evaluate the lysossomal integrity and distinguishes live cells from dead by its ability to incorporate the dye (Borenfreund *et al*., 1988). Briefly, 0.2 mL medium containing 50 µg mL^{-1} was added to each well. The plate was incubated for another 3 h at 37 °C to allow the uptake of the vital dye into the lysosomes of

viable uninjured cells. After removal of the medium, 200 µL of a mixture of 0.5% formaldehyde and 1% $CaCl_2$ was added to the cells, and incubated for 5 min at 37 °C. The supernatant was removed and 100 µL of a solution of 1% acetic acid-50% ethanol was added to each well to extract the dye. After homogenization, the optical density of each well was measured at a wavelength of 540 nm using a spectrophotometer since this absorbance gives a linear relationship with the number of surviving cells. The ratio between drug cytotoxicity (MLD_{50} BGM) and activity (IC_{50} W2) was used to estimate the selective index (SI), as determined before (Bézivin *et al*., 2003), where SI ≤ 10 was indicative of toxicity.

Antiviral activity against the Dengue virus 2 and the Zika virus

For determining the antiviral activity against the Dengue virus 2 (DENV-2) and the Zika virus (ZIKV), monolayers of baby hamster kidney 21 (BHK-21) cells and Vero cells, respectively were used. Each cell was grown in flat bottom 96-well plates (Sarstedt, USA) using Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% fetal bovine serum (FBS), 100 U mL-1 penicillin, 100 µg mL^{-1} streptomycin, and 0.25 µg mL^{-1} amphotericin B (all from Gibco, Thermo Sci, USA). All fungal extracts were provided at 20 mg mL^{-1} in an aqueous 90% DMSO solution. Viral suspensions with multiplicity of infection (m.o.i.) of 2 for both viruses and the extracts were simultaneously added to the plates, in duplicates. Cells were exposed to 20 µg mL^{-1} of fungal extracts for 72 h and 96 h with DENV-2 and ZIKV, respectively. Cell controls (uninfected untreated cells, with or without DMSO), virus controls (infected untreated cells, with or without DMSO) and antiviral positive control (infected cells treated with 300 and 600 UI mL^{-1} of interferon-α 2B - INREC, Uruguay) were run in parallel during each experiment. Additionally, all extracts were tested at the same concentration in the antiviral assays to verify their cytotoxicity against both cell lines. The antiviral activity was evaluated by the grading the peculiar cytopathic effect (CPE) caused by DENV-2 or ZIKV by optic microscopy, followed by the MTT colorimetric assay using the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT) (Sigma Aldrich, USA) according to Mossman (1983). The results were expressed as the percentage of viral inhibition in relation to the virus controls. All antiviral screening assays were repeated at least twice.

Nematicidal activity using Caenorhabditis elegans

The wild-type N2 Bristol strain kindly provided by the CGC (*Caenorhabdtitis* Genetic stock Center) was maintained on plates of Nematode Growth Media (NGM) with a layer of *Escherichia coli* OP50 at 16 °C (Muhammed *et al.*, 2012). To obtain worms at the same larval

stage, gravid hermaphrodites were synchronized using the method described by Porta de La Riva *et al*. (2012), in which, only the eggs are kept intact. In order to determine the nematicidal activities of the fungal extracts, young adult worms (L4) were exposed to their different concentrations (400, 160, 80, 40, and 20 µg mL^{-1}) prepared in 200 µL of M9 buffer containing *E. coli* OP50 and supplemented with 5-fluoro-2'-deoxyuridine [FUDR (80 µg mL^{-1}), Sigma] and a thymidylate synthase inhibitor to prevent offspring production (Mitchell *et al*., 1979). The assays where performed in 96-well plates (20-30 worms/well) at 23 °C using DMSO as a negative control and the viability was examined at intervals of 48 h until the death of all the nematodes was observed. The survival curves for *C. elegans* were plotted and an estimation of the differences (log-rank and Wilcoxon tests) in survival analyzed by the Kaplan-Meier method using a GraphPad Prism software 5.01 (GraphPad Software). A p-value of 0.05 was considered to be significant and the time required to kill 50% of the worms (LT_{50}) was determined. All experiments were performed in triplicates, by at least three independent experiments.

Herbicide assay

The herbicidal activities of fungal extracts at 1 mg mL^{-1} against *Lactuca sativa* (lettuce) and *Allium schoenoprasum* (chive) were measured using methods adapted from Dayan *et al*. (2000). Briefly, seeds of both plants were disinfected with ethanol 70% for 1 min, sodium hypochlorite at 2 -2.5% for 7.5 min, washed with sterilised distilled water four times, and dried on sterilized filter paper at room temperature. Herbicidal activity was quantitatively evaluated in 24-wells microtitre plate by visually comparing the amount of germination in each well between untreated and treat samples. Samples were incubated at 26 °C in a germination incubator 710 (Thoth, Brazil) under continuous light conditions for 10 days for *L. sativa* and 14 for *A. schoenoprasum*. Test samples were dissolved in acetone to a final concentration of 10% acetone. The control wells contained 400 µL of deionized water. The control + solvent well contained 360 µL of water and 40 µL of the solvent. Glyphosate at 3 mg mL^{-1} (Sigma, USA) was used as a positive herbicide control. All sample wells contained 360 µL of water and 40 µL of the appropriate dilution of the sample. A quantitative estimate of herbicidal activity was evaluated using a rating scale of 0-5, where 0 = no effect and 5 = no growth or no germination of the seeds. All assays were performed in duplicate.

NMR spectroscopy of bioactive extracts

One-dimensional NMR experiments were acquired on a Bruker Avance DRX 400 MHz spectrometer using 0.1% tetramethylsilane as an internal standard. ^{1}H-NMR spectra of bioactive fungal extracts were recorded in $CDCl_3$ using a standard pulse sequence program.

Results

Fungal isolation and taxonomy

Thirty-one fungal isolates were recovered from Antarctic marine sediments, of which 8, 20, 2 and 1 were at depths of 1,463 m, 550 m, 250 m, and 153 m, respectively. Based on sequencing of the different regions of rDNA, the fungi from the Antarctic deep sea were identified as species of *Acremonium*, *Penicillium*, and *Pseudogymnoascus* (Table 1). Among these, *P. allii-sativi*, *P. solitum*, *P. chrysogenum*, and *P. pallitans* occurred at depths of 1,463 and 550 m; *Acremonium fusidioides* at 550 m; and *Pseudogymnoascus verrucosus* at 153 m. *P. allii-sativi* was the dominant species which displayed high densities at depths of 550 and 250 m. The difference of the fungal assemblages detected among the samples may be due the different depth and locations. Despite the variety of culture media used in the present study, there was a low frequency of isolation, with growth observed in 6 of the 7 culture media used. The most frequent species isolated was *P. chrysogenum* (39%), followed by *P. solitum* (35%), *P. allii-sativi* (13%), *P. palitans* (6%), *A. fusidioides* (3%), and *Pseudogymnoascus* sp. (3%) (Fig. S1; Table S2).

Bioprospecting of biological activities

Thirty-one fungal extracts displayed biological activity against at least one target (Table 2). Herbicidal activity was the most frequent (32%) and the different isolates of *A. fusidioides*, *P. allii-sativi*, *P. chrysogenum*, *P. palitans*, and *P. solitum* were able to produce compounds that could bring about total inhibition of germination in the seeds of *A. schoenoprasum* and *L. sativa*. Additionally, *A. fusidioides* and *P. verrucosus* were able to produce compounds with selective herbicidal activities. One extract of *P. chrysogenum* and *P. solitum* each demonstrated antifungal activity against *Cladosporium sphaerospermum*. Extracts of *P. chrysogenum, P. palitans*, and *P. solitum* isolates exhibited high trypanocidal activities. Additionally, extracts of *P. allii-sativi*, *P. chrysogenum, P. palitans,* and *P. solitum* demonstrated antiplasmodial activities; particularly, the isolate *P. chrysogenum* UFMGCB 13043 demonstrated capability to inhibit 100% of the *P. falciparum* growth at a concentration of 20 µg mL^{-1}. The extracts of *P. solitum* UFMGCB 13030 (IC_{50} of 18.4; MDL_{50} ≥200) and *P. chrysogenum* UFMGCB 13038 (IC_{50} of 18.4; MDL_{50} ≥200) were considered to be

moderately active based on the selective index (SI); however, extracts of *P. chrysogenum* UFMGCB 13035, UFMGCB 13042, and UFMGCB 13036, which presented an SI ≤ 10 μg mL^{-1}, were considered toxic. All fungal extracts demonstrated nematocidal activity against *C. elegans* and caused death of all nematodes after 10 days of incubation. The effective extracts were able to cause the death of 50% of nematodes (LT_{50} values) within 2 to 3 days of incubation at all concentrations evaluated. None of the fungal extracts displayed leishmanicidal and antiviral activities.

The most bioactive fungal extracts (*A. fusidioides* UFMGCB 13041, *P. allii-sativi* UFMGCB 13048, *P. chrysogenum* UFMGCB 13035, 13043, 13046, and 13038, *P. solitum* UFMGCB 13030 and 13056, and *P. palitans* UFMGCB 13034) were examined using ^{1}H NMR analysis to inspect for the presence of interesting secondary metabolites such as aromatic compounds and terpenoids. The extract of these fungi showed the presence of fatty acid functional groups, and the results of most extracts indicated the presence of triglycerides. However, downfield ^{1}H NMR signals for extracts of *A. fusidioides* UFMGCB 13041, *P. chrysogenum* UFMGCB 13043, and *P. solitum* UFMGCB 13030 and 13056 also indicated the presence of highly functionalized secondary metabolites due to the presence of protons in the aromatic and olefinic regions (Fig. S2) and can be potential producers of secondary bioactive compounds.

Discussion

Fungal recovery and identification

According to Nagahama and Nagano (2012), the culture media used to isolate fungi from deep-sea environments are not so different from those used for terrestrial fungi. The most commonly used media, mainly containing malt extract, peptone, yeast extract, potato starch, and glucose, are usually prepared in artificial sea water with the addition of antibiotics and are occasionally diluted to 1:5 in order to simulate the low nutrient conditions found in the deep sea (Damare *et al*., 2006; Jebaraj *et al*., 2010; Burgaud *et al*., 2009; Le Calvez *et al*., 2009; Redou *et al*., 2015). Despite this, only few species were obtained from the deep marine sediments, which may be justified by the inherent characteristics of the oligotrophic substrate itself, since there are difficulties in simulating the atmospheric temperature and pressure conditions found in the depths of the Antarctic Ocean.

Usually, presence of species only of the genera *Aspergillus*, *Penicillium*, *Candida*, *Cryptococcus*, *Leucosporidium*, *Cylindrocarpon*, *Glomerella*, *Golovinomyces*, *Hortaea*, *Lodderomyces*, *Malassezia*, *Metshnikowia*, *Nematoctonus*, *Phoma*, *Pichia*, *Rhodotorula*, *Rhizophlyctis*, *Trichoderma*, and *Trichosporon* have been reported from the marine sediment

samples around the world (Lai *et al*., 2007; Le Calvez *et al*., 2009; Ren *et al*., 2009; Nagano *et al*., 2010; Singh *et al*., 2011; Duarte *et al*., 2013; Gonçalves *et al*., 2013; Duarte *et al*., 2015). However, little information is available about the fungal communities present in deep sea environments of the Antarctic Ocean (Lopez-Garcia *et al*., 2001; Gonçalves *et al*., 2013).

We found that the fungal assemblages present in the Antarctic marine sediments were dominated by the *Penicillium* species. Among them, *P. solitum* was obtained at a depth of 1,463 m, apparently under anaerobic conditions. Gonçalves *et al*. (2013) also obtained only *P. solitum* from areas 100 to 1,100 m below the Antarctic Ocean surface, which demonstrated conidial germination at low temperatures and high salinities, and capabilities to demonstrate amylase and esterase activities. For these reasons, it is considered to be adapted to the cold and halophilic environment of the Antarctic oceans. In Antarctica, the *Penicillium* species have been identified in samples obtained from different substrates/microhabitat and environments such as soils, rocks, lakes, ice, historic wood, plants, and macroalgal (Rosa *et al*., 2019). However, our study is the first to report the presence of *A. fusidioides*, *P. allii-sativi*, *P. chrysogenum*, *P. palitans*, and *P. verrucosus* in Antarctic marine sediments.

Penicillium chrysogenum is perhaps highly adapted to the extreme conditions found in Antarctica and has been isolated from different terrestrial and marine habitats. In marine environments, *P. chrysogenum* was already reported to be associated with macroalgae (Godinho *et al*., 2013; Furbino *et al*., 2014) and was found in deep-sea waters at depths of 5 to 672 m at different sites across the Bransfield Strait and Gerlache Strait in the Antarctic Peninsula (Gonçalves *et al*., 2017). *Penicillium allii-sativi* was first isolated from garlic, soil, salterns, sorghum malt, and mixed pig feed (Houbraken *et al*., 2012). Godinho *et al*. (2015) reported the occurrence of *P. allii-sative* in the oligotrophic soils of continental Antarctica. *Penicillium palitans* was isolated from the Arctic environment (Sonjak *et al*., 2006), but until now, there are no reports on the occurrence of this species in Antarctica.

It has been described that the *Pseudogymnoascus* species are associated with mosses (Tosi *et al*., 2002), lichens (Santiago *et al*., 2015), macroalgae (Godinho *et al*., 2013; Furbino *et al*., 2014), and also present in the soils (Godinho *et al*., 2015) of Antarctica and are known to be adapted to a psychrophilic range of temperature (4 to 15 °C). The occurrence of *Pseudogymnoascus verrucosus*, found in core marine sediments at a depth of 153 m in Antarctic soils on the Coppermine Peninsula, Robert Island, was recently described (Gomes *et al*., 2018).

The genus *Acremonium* (also known as *Cephalosporium*), comprises of cosmopolitan fungi isolated commonly from decomposing plants and soils (Fincher *et al*., 1991). In the marine

environment, many species are pathogens mainly of lobsters, fishes, and oysters (Hatai *et al*., 2012). In the Antarctic regions, these genera have already been isolated from marine seawater obtained from different depths (Gonçalves *et al*., 2017) and are also associated with lichens (Nascimento *et al*., 2015).

Bioprospecting of biological activities

Penicillium species are known due to their active metabolism to produce several bioactive compounds. Recently, Antipova *et al*. (2018) studied the secondary metabolites of several *Penicillium* strains isolated from high-latitude ecosystems, including those from Antarctic soils which have been detected to be prolific producers of secondary bioactive metabolites. *Penicillium* from the Antarctic have been gaining attention because they can be considered as live factories producing unknown bioactive secondary metabolites. However, few *Penicillium* strains of the Antarctic were chemically investigated in detail. According to Houbraken *et al*., (2012), eight *Penicillium* species are recognized producers of penicillin and we detected two among these (*P*. *allii-sativi* and *P*. *chrysogenum*) in the deep Antarctic marine sediments, such that more than one strain was able to produce compounds with different biological activities.

P. *allii-sativi* produces several secondary metabolites. According to Houbraken *et al*., (2012) *P*. *allii-sativi* produces penicillin, atlantinone A, chrysogenamide, 2-(4-hydroxyphenyl)-2-oxoacetaldehydeoxim, anaptho-γ-pyrone, 2-pyruvoylaminobenzamide, roquefortine C, D, meleagrin, verrucosidin, normethylverrucosidin, deoxyverrucosidin, verrucosidinol, and others uncharacterised compounds. *P*. *chrysogenum* is best known species of *Chrysogena* that are able to produce penicillin, and for this reason, its taxonomy and search for wild strains has been receiving further attention (Houbraken *et al*., (2012). Brunati *et al*. (2009) described strains of *P*. *chrysogenum* from Antarctica to be producers of rugulosin and skyrin (bioactive bis-anthraquinones) which are able to demonstrate selective antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecium*, and *Escherichia coli*. Additionally, the extract of *P*. *chrysogenum* recovered from the endemic Antarctic macroalga *Palmaria decipiens* yielded extracts with high and selective antifungal and/or trypanocidal activities (Godinho *et al*., 2013). Additionally, distinct isolates of *P*. *allii-sativi*, *P*. *chrysogenum*, *P*. *palitans*, and *P*. *solitum* were able to present different biological activities, demonstrating intra-specific genetic/metabolic differences in their capabilities to produce bioactive compounds. The same effect was observed by Godinho *et al*. (2015) in *Penicillium* strains recovered from ultra-oligotrophic soils of continental Antarctica.

The *Pseudogymnoascus* species seem to demonstrate metabolic plasticity and tolerance to cold environments and may be the source of interesting bioactive molecules such as nitro derivatives with antibacterial and antifungal activities (Figueroa *et al*., 2015). Li *et al*. (2008) isolated the *Geomyces* sp. (*Pseudogymnoascus*) from the Antarctic soil which produced the geomycins B and C capable of demonstrating antifungal and antibacterial activities. Furbino *et al*. (2014) reported two species of *Pseudogymnoascus* associated with Antarctic macroalgae with selective antifungal activity. Gomes *et al*. (2018) detected that *Pseudogymnoascus destructans* was a producer of herbicidal and trypanocidal compounds.

According to Tian *et al*. (2017), the *Acremonium* species present promising sources of novel and bioactive secondary metabolites such as steroids, terpenoids, meroterpenoids, polyketides, alkaloids, peptides, and others, which show demonstrate antimicrobial, cytotoxic, antitumor, immunosuppressive, antioxidant, anti-inflammatory, antimalarial, phytotoxic, tremorgenic, antiviral, neuritogenic, insecticidal, and enzyme inhibiting activities. *A. fusidioides* has been described as a producer of interesting bioactive compounds. Xiao *et al*. (2016) discovered known substances and two new compounds, a steroid and a cyclopentanone derivate fusidione, produced by *A. fusidioides* RZ01.

Parasitic nematodes can cause serious damage to agriculture; furthermore, these parasites can attack and destroy a wide variety of organisms, including animals, plants, and microorganisms (Li *et al*., 2007). The life-style of the free-living worm *C. elegans* is very different from that of the parasites, but it has been used as a model in anthelmintic and nematicidal studies, mainly due to the comparative physiology and pharmacology of the Nematoda group (Holden-Dye and Walker, 2014). Majority of the fungal extracts demonstrated nematicidal activity against *C. elegans* and ^{1}H NMR analysis showed the presence of aromatic compounds. Fungi are known to be producers of nematicidal compounds such as the alkaloid paraherquamides produced by the *Penicillium* species (Ondeyka *et al*., 1990; Blanchflower *et al*., 1991, 1997).

Conclusion

Different ecosystems of Antarctica offer unique opportunities to discover fungi capable of surviving under extreme conditions, and among these, marine sediments present an unexplored environment/microhabitat with extreme conditions of temperature, availabilityle of oxygen, and high pressure. In deep marine sediments of Antarctica, we detected fungal assemblages dominated by the *Penicillium* species, which seem to demonstrate capabilities to survive and/or colonise the poly-extreme habitat. *Penicillium* is a polyextremophile of the

Antarctic species which displayed strong biological activities and the presence of aromatic compounds in its extracts, and may be present as wild ancient strains with high genetic and biochemical potential to produce bioactive compounds which can be used in further studies on chemotherapy against neglected tropical diseases as well as in agriculture as herbicidal compounds with less toxicity.

Acknowledgements

We acknowledge the financial support from CNPq PROANTAR 442258/2018-6, INCT Criosfera II, CNPq, FAPEMIG, and FNDCT. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. We are thankful to the Laboratório de Ressonância Magnética Nuclear de Alta Resolução (LAREMAR/UFMG) and IS Lula for the NMR experiments. The authors thank the Program for Technological Development of Tools for Health-PDTIS-Fiocruz for use of its facilities (Bioprospection and Chagas disease-PlaBio Tc platforms). PASJ is research fellow supported by Programa de Pós-graduação em Ciências da Saúde, Fiocruz Minas (CAPES/PNPD).

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J.H., Zhang, Z., Miller, W., *et al*. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.

Antipova, T.V., Zhelifonova, V.P., Baskunov, B.P, Kochkina, G.A., Ozerskaya, S.M., Kozlovskii, A.G. (2018) Exometabolites the *Penicillium* Fungi Isolated from Various High-Latitude Ecosystems. *Microbiology* **87**: 642–651.

Bézivin, C., Tomasi, S., Lohézic-Le Dévéhat, F., Boustie, J. (2003) Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomed* **10**:499–503

Blanchflower, S.E., Banks, R.M., Evertt, J.R., Manger, B.R., Reading, C. (1991) New paraherquamide antibiotics with anthelmintic activity. *J Antibiot* **44**: 492–97.

Blanchflower, S.E., Banks, R.M., Everett, J.R., Reading, C. (1993) Further novel metabolites of the paraherquamide family. *J Antibiot* **46**: 1355–63.

Borenfreund, E., Babich, H., Martin-Alguacil, N. (1988) Comparisons of two in vitro cytotoxicity assays: the neutral red (NR) and tetrazolium MTT tests. *Toxicol In Vitro* **2**: 1–6.

Brunati, M., Rojas, J.L., Sponga, F., Ciciliato, I., Losi, D., Göttlich, E., *et al*. (2009) Diversity and pharmaceutical screening of fungi from benthic mats of Antarctic lakes. Mar. *Genomics* **2**: 43–50.

Burgaud, G., Calvez, T.L., Arzur, D., Vandenkoornhuyse, P., Barbier, G. (2009) Diversity of culturable marine filamentous fungi from deep-sea hydrothermal vents. *Environ Microbiol* **11**: 1588–1600.

Callahan, H.L., Portal, A.C., Devereaux, R., Grogl, M. (1997) An axenic amastigote system for drug screening. *Antimicrob Agents Chemother* **41**:818–822.

Damare, S., Raghukumar, C. (2008) Fungi and macroaggregation in deep-sea sediments. *Microb Ecol* **53**:14–27.

Damare, S., Raghukumar, C. and Raghukumar, S. (2006) Fungi in deep-sea sediments of 563 the Central Indian Basin. *Deep Sea Res Part I* **53**: 14–27.

Dayan, F.E., Romagni, J.G., Duke, S.O. (2000) Investigating the mode of action of natural phytotoxins. *J Chem Ecol* **26**: 2079–2094.

de Hoog, S., Guarro, J., Gene, J., Figueras, M.J. (2001) Atlas of clinical fungi. 2th.edn. Réus: ASM Press, 1126 p.

do Céu de Madureira, M., Martins, A.P., Gomes, M., Paiva, J., Da Cunha, A.P., Do Rosário, V. (2002) Antimalarial activity of medicinal plants used in traditional medicine in S. Tomé and Príncipe islands. *J Ethnopharmacology* **81**: 23–29.

Duarte, A.W., Dayo-Owoyemi, I., Nobre, F.S., Pagnocca, F.C., Chaud, L.C., Pessoa, A., Felipe, M.G., Sette, L.D. (2013) Taxonomic assessment and enzymes production by yeasts isolated from marine and terrestrial Antarctic samples, *Extremophiles* **17**: 1023–1035.

Duarte, A.W., Lopes, A., Molino, J., Pessoa, A., Sette, L. (2015) Liquid-liquid extraction of lipase produced by psychrotrophic yeast *Leucosporidium scottii* L117 using aqueous two-phase systems. *Separ Purif Technol* 1–11.

Figueroa, L., Jiménez, C., Rodríguez, J., Areche, C., Chávez, R., Henríquez, M., *et al*. (2015) 3-Nitroasterric acid derivatives from an Antarctic sponge-derived *Pseudogymnoascus* sp. fungus. *J Nat Prod* **78**: 919–923.

Fincher, R.M., Fisher, J.F., Lovell, R.D., Newman, C.L., Espinel-Ingroff, A., Shadomy, H.J. (1991) Infection due to the fungus *Acremonium* (*Cephalosporium*). *Medicine (Baltimore)* **70**: 398–409.

Furbino, L.E., Godinho, V.M., Santiago, I.F., Pellizari, F.M., Alves, T.M.A., Zani, C.L., *et al*. (2014) Diversity patterns ecology and biological activities of fungal communities associated with the endemic macroalgae across the Antarctic Peninsula. *Microbial Ecol* **67**: 775–787.

Giudice, A.L., Azzaro, M. (2019) Diversity and ecological roles of prokaryotes in the changing antarctic marine environment. In: Castro, S. (eds) The Ecological Role of Microorganisms in the Antarctic Environment, 1st edn. Springer, Switzerland AG, 109-131.

Glass, N.L., Donaldson, G.C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* **61**: 1323–1330.

Godinho, V.M., Furbino, L.E., Santiago, I.F., Pellizzari, F.M., Yokoya, N.S., Pupo, D., *et al*. (2013) Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. *The ISME Journal* **7**: 1434–1451.

Godinho, V.M., Gonçalves, V.N., Santiago, I.F., Figueredo, H.M., Vitoreli, G.A., Schaefer, C.E., *et al*. (2015) Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica. *Extremophiles* **19**: 585–596.

Gomes, E.C.Q., Godinho, V.M., Silva, D.A.S., De Paula, M.T.R., Vitoreli, G.A., Zani, C.L., *et al*. (2018) Cultivable fungi present in Antarctic soils: taxonomy phylogeny diversity and bioprospecting of antiparasitic and herbicidal metabolites *Extremophiles* **22**: 381–393.

Gonçalves, V.N., Campos, L., Melo, I.S., Pellizari, V.H., Rosa, C.A., Rosa, L.H. (2013). *Penicillium solitum*: a mesophilic psychrotolerant fungus present in marine sediments from Antarctica. *Polar Biol* **36**: 1823–1831.

Gonçalves, V.N., Carvalho, C.R., Johann, S., Mendes, G., Alves, T.M.A., Zani, C.L., *et al*. (2015) Antibacterial antifungal and antiprotozoal activities of fungal communities present in different substrates from Antarctica. *Polar Biol* **38**: 1143–1152.

Gonçalves, V.N., Oliveira, F.S., Carvalho, C., Schaefer, C.E.G., Rosa, C.A., Rosa, L.H. (2017) Antarctic rocks from continental Antarctica as source of potential human opportunistic fungi. *Extremophiles* **21**: 851–860.

Hatai, K (2012) Diseases of fish and shellfish caused by marine fungi. In *Biology of Marine Fungi Progress in Molecular and Subcellular Biology*. Raghukumar, C. (ed.) Springer-Verlag, Berlin, pp. 15–51.

Holden-Dye, L., Walker, R. (2014) Anthelmintic drugs and nematicides: studies in *Caenorhabditis elegans*. *WormBook* **16**: 1–29.

Houbraken, J., Frisvad, J.C., Seifert, K.A., Overy, D.P., Tuthill, D.M., Valdez, J.G., *et al*. (2012) New penicillin-producing *Penicillium* species and an overview of section Chrysogena. *Persoonia* **29**: 78–100.

Jebaraj, C.S., Raghukumar, C., Behnke, A., Stoeck, T. (2010) Fungal diversity in oxygen-depleted regions of the Arabian Sea revealed by targeted environmental sequencing combined with cultivation. *FEMS Microbiol Ecol* **71**: 399–412

Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A. (2008) Dictionary of the Fungi. CAB International, Wallingford.

Kohlmeyer, J., Kohlmeyer, E. (1979) Marine mycology: the higher fungi. Academy Press, New York.

Lai, X., Cao, L., Tan, H., Fang, S., Huang, Y., Zhou, S. (2007) Fungal communities from methane hydrate-bearing deep-sea marine sediments in South China Sea. *ISME J* **1**: 756–62.

Le Calvez, T., Burgaud, G., Mahé, S., Barbier, G., Vandenkoornhuyse, P. (2009) Fungal Diversity in Deep-Sea Hydrothermal Ecosystems. *Appl Environ Microbiol* **75**: 6415–6421.

Li, Y., Sun, B., Liu, S., Jiang, L., Liu, X., Zhang, H., *et al*. (2008) Bioactive asterric acid derivatives from the Antarctic ascomycete fungus *Geomyces* sp. *J Nat Prod* **71**: 1643–1646.

López-Garcia, P., Rodriguez-Valera, F., Pedros-Allo, C., Moreira, D. (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Nature* **409**: 603–607.

Mitchell, D.H., Stiles, J.W., Santelli, J., Sanadi, D.R. (1979) Synchronous Growth and Aging of *Caenorhabditis elegans* in the Presence of Fluorodeoxyuridine. *J Gerontology* **34**: 28–36.

Mossman, T. (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* **65**: 55–63.

Muhammed, M., Fuchs, B.B., Wu, M.P., Breger, J., Coleman, J.J., Mylonakis, E. (2012) The role of mycelium production and a MAPK-mediated immune response in the *C. elegans* - *Fusarium* model system. *Med Mycol* **50**: 488–496.

Nagahama, T., Nagano, Y. (2012) Cultured and Uncultured Fungal Diversity in Deep-Sea Environments. In *Biology of Marine Fungi*. *Progress in Molecular and Subcellular Biology, vol 53*. Raghukumar, C. (eds.) Springer, Berlin, Heidelberg, pp. 173–187.

Nagano, Y., Nagahama, T., Hatada, Y., Nunoura, T., Takami, H., Miyazaki, J., *et al*. (2010) Fungal diversity in deep-sea sediments - the presence of novel fungal groups. *Fungal Ecol* **3**: 316–325.

Nascimento, T.C.E.S., Sena, A.R., Gomes, J.E.G., Santos, W.L., Montalvo, G.S.A., Medeiros, E.V., *et al*. (2015) Extracellular serine proteases by *Acremonium* sp. L1-4B isolated from Antarctica: Overproduction using cactus pear extract with response surface methodology. *Biocatalysis Agricultural Biotech* **4**: 737–744.

NCCLS (2002) Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, M38-A. *National Committee on Clinical Labora-tory Standards* **22**:16.

Oduola, A.M., Weatherly, N.F., Bowdre, J.H., Desjardins, R.E. (1988) *Plasmodium falciparum*: cloning by single-erythrocyte micromanipulation and heterogeneity in vitro. *Exp Parasitol* **66**: 86–95.

Ondeyka, J.G., Goegelman, R.T., Schaeffer, J.M., Kelemen, L., Zitano, L. (1990) Novel antinematodal and antiparasitic agents from Penicillium Charlesii. I. Fermentation, isolation and biological activity. *J Antibiot*, **38**: 1375–1379.

Porta-de-la-Riva, M., Fontrodona, L., Villanueva, A., Cerón, J. (2012) Basic *Caenorhabditis elegans* methods: synchronization and observation. *J Vis Exp* **10**: e4019.

Raghukumar, C., Damare, S.R., Singh, P. (2010) A review on deep-sea fungi occurrence, diversity and adaptations. *Bota Mar* **53**: 479–492.

Rateb, M.E., Ebel, R. (2011) Secondary metabolites of fungi from marine habitats. *Nat Prod Rep* **28**: 290-344.

Redou, V., Navarri, M., Meslet-Cladière, L., Barbier, G., Burgaud, G. (2015) Species richness and adaptation of marine fungi from deep-subseafloor sediments. *Appl Environ Microbiol* **81**: 3571–3583.

Ren, J., Yang, Y., Liu, D., Chen, W., Proksch, P., Shao, B., *et al*. (2013) Sequential determination of new peptaibols asperelines G-Z12 produced by marine-derived fungus *Trichoderma asperellum* using ultrahigh pressure liquid chromatography combined with electrospray-ionization tandem mass spectrometry. *J Chromatogr* **1309**: 90–95.

Romanha, A.J., De Castro, S.L., Soeiro, M.N.C., Lannes-Vieira, J., Ribeiro, I., Talvani, A., *et al*. (2010) In vitro and in vivo experimental models for drug screening and development for Chagas disease. *Mem Inst Oswaldo Cruz* **105**: 233–238.

Rosa, L.H., Pellizari, F.M., Ogaki, M.B., De Paula, M.T., Mansilla, A., Brito, A.A., *et al*. (2019) Sub Antarctic and Antarctic marine ecosystems: an unexplored ecosystem of fungal diversity In *Fungi of Antarctica*: *Diversity, Ecology and Biotechnological Applications*. Rosa, L.H. (ed.) Springer, Berlin, Heidelberg.

Rosa, L.H., Vaz, A.B., Caligiorne, R.B., Campolina, S., Rosa, C.A. (2009) Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv. (*Poaceae*). *Polar Biol* **32**:161–167.

Rosa, L.H., Zani, C.L., Cantrell, C.L., Duke, S.O., Dijck, P.V., Desideri, A., Rosa, C.A. (2019) Fungi in Antarctica: diversity, ecology, effects of climate change, and bioprospection

for bioactive compounds. In: Rosa, L.H. (eds) Fungi of Antarctica, 1st edn. Springer, Switzerland AG, 1-17.

Roth, F.J., Orpurt, P.A., Ahearn, D.J. (1964) Occurrence and distribution of fungi in a subtropical marine environment. *Can J Bot* **42**: 375–383.

Santiago, I.F., Soares, M.A., Rosa, C.A., Rosa, L.H. (2015) Lichensphere: a protected natural microhabitat of the non-lichenised fungal communities living in extreme environments of Antarctica. *Extremophiles* **19**: 1087–1097.

Singh, P., Raghukumar, C., Verma, P., Shouche, Y. (2012) Assessment of fungal diversity in deep-sea sediments by multiple primer approach. *World J Microbiol Biotechnol* **28**: 659–667.

Smilkstein, M., Sriwilaijaroen, N., Kelly, J.X., Wilairat, P., Riscoe, M. (2004) Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. *Antimicrob Agents Chemother* **48**: 1803–1806.

Sonjak, S., Frisvad, J.C., Gunde-Cimerman, N. (2006) *Penicillium* mycobiota in arctic subglacial ice. *Microb Ecol* **52**: 207–216.

Takami, H., Inoue, A., Fuji, F., Horikoshi, K. (1997) Microbial flora in the deepest sea mud of the Mariana Trench. *FEMS Microbiol Lett* **152**: 279–285.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**: 2731–2739.

Thaler, A.D., Dover, C.L.V., Vilgalys, R. (2012) Ascomycete phylotypes recovered from a Gulf of Mexico methane seep are identical to an uncultured deep-sea fungal clade from the Pacific. *Fungal Ecol* **5**: 270–273.

Tian, J., Lai, D., Zhou, L (2017) Secondary metabolites from *Acremonium* fungi: Diverse Structures and Bioactivities. *Med Chem* **17**: 603–632.

Tosi, S., Casado, B., Gerdol, R. (2002) Fungi isolated from Antarctic mosses. *Polar Biol* **25**: 262–268.

Trager, W., Jensen, J.B. (1976) Human malaria parasites in continuous culture. *Science* **193**: 673–675.

White, T.J., Bruns, T.D., Lee, S.B. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols*: *a guide to methods and applications*. Innis, N.A., Gelfand, J., Sninsky, J., White, T.J. (eds.) Academic Press, San Diego, pp. 315–322.

Whitman, W.B., Coleman, D.C., Wiebe, W.J. (1998) Prokaryotes: the unseen majority. *Proc Acad Natl Sci USA* **95**: 578–6583.

Xiao, A.N., Feng, B., Chen, G., Chen, S., Wang, H., Pei, Y. (2016) Isolation and identification of two new compounds from marine-derived fungus *Acremonium fusidioides* RZ01. *Chinese J Nat Medicines* **14**: 934–938.

Zhang, X., Tang, G., Xu, X., Nong, X., Qi, S. (2014) Insights into Deep-Sea Sediment Fungal Communities from the East Indian Ocean Using Targeted Environmental Sequencing Combined with Traditional Cultivation. *Plos One* **9**: e109118.

Tables and figures

Table S1. Sites where the marine sediment samples were collected in Antarctic ocean.

Site	Depth (m)	Longitude	Latitude
South Shetlands sea	550	58º 00.383'	62º 11.258'
Maxwell Bay	153	58º 57.135'	62º 14.632'
Maxwell Bay	250	58º 56.035'	62º 14.667'
Antarctic Ocean	1463	62º 45.270'	59º 00.090'

Table 1. Fungi isolated from marine sediments of Antarctica Peninsula identified by sequence comparison with the BLASTn match with the NCBI GenBank database.

UFMGCB[a]	Depth (m)	Density (CFU g^{-1})	Top BLAST search results (GenBank accession number)	No. of bp analysed	QC (%)	Identity (%)	Proposed taxa[e] (GenBank accession number)
13029	1463	80	*Penicillium solitum* (NR119494)[b]	429	100	99	*Penicillium solitum* (MK599239[f], MK984232[g], MK609958[h])
			Penicillium solitum (AY674356)[b]	255	100	99	
			Penicillium solitum (KU904363)[d]	580	100	99	
13041	550	20	*Acremonium fusidioides* (NR130687)[b]	417	100	99	*Acremonium fusidioides* (MK599240[f])
13045	550	160	*Penicillium allii-sativi* (MH865981)[b]	460	100	100	*Penicillium allii-sativi* (MK599241[f], MK984229[g], MK609959[h])
			Penicillium allii-sativi (KM656093)[c]	321	100	100	
			Penicillium allii-sativi (KM656086)[d]	639	100	100	
13035	550	20	*Penicillium chrysogenum* (MH856357)[b]	412	100	99	*Penicillium chrysogenum* (MK599242[f], MK984230[g], MK609960[h])
			Penicillium chrysogenum (JF909955)[c]	349	100	98	
			Penicillium chrysogenum (JX996668)[d]	575	100	99	
13037	550	40	*Penicillium palitans* (MK226538)[b]	461	100	100	*Penicillium palitans* (MK599243[f], MK609961[h])
			Penicillium palitans (KU904360)[d]	558	100	100	
13040	550	20	*Penicillium solitum* (NR119494)[b]	359	100	100	*Penicillium solitum* (MK599244[f], MK609962[h])
			Penicillium solitum (KU904363)[d]	301	100	99	
13053	250	260	*Penicillium allii-sativi* (MH865981)[b]	467	100	100	*Penicillium allii-sativi* (MK599245[f], MK984231[g], MK609963[h])
			Penicillium allii-sativi (KM656093)[c]	359	100	100	
			Penicillium allii-sativi (KM656086)[d]	523	100	100	
13055	153	20	*Pseudogymnoascus verrucosus* (KJ755525)[b]	469	100	99	*Pseudogymnoascus verrucosus* (MK599246[f])

[a]UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. Taxa subjected to BLAST analysis based on the [b]ITS, [c]β-tubulin, [d]Polymerase 2. [e]Taxonomic suggested. Sequences of [f]ITS, [g]β-tubulin and [h]Polymerase 2 deposited in GenBank database.

.

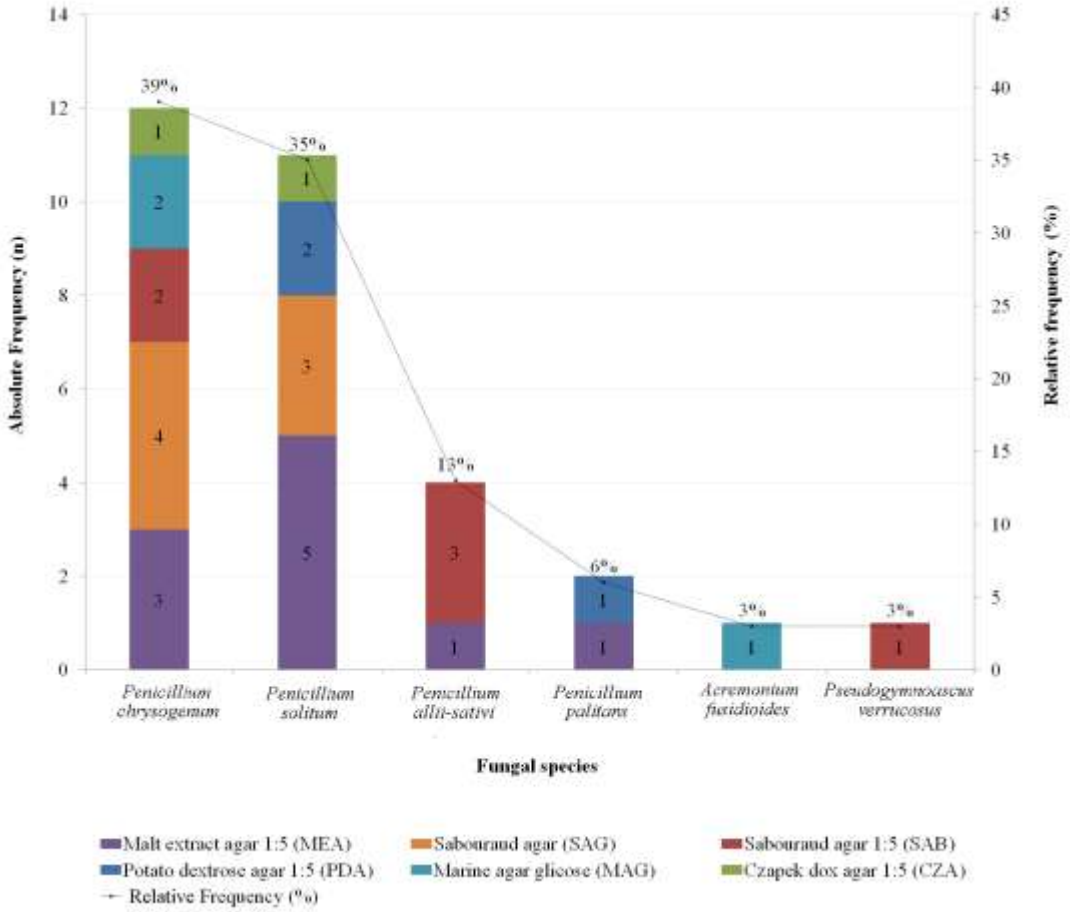


Fig. S1. Frequency of fungal isolation on different culture media.

Table S2. Density (CFU g^{-1}) of fungi isolated from marine sediments in Antarctica.

Depth (m)	Fungal species	Density (CFU g^{-1})							Number of isolates						
		PDA	SAB	CZA	MEA	MA	SAG	Total	PDA	SAB	CZA	MEA	MA	SAG	Total
1463	*Penicillium solitum*[a]	720	-	-	1440	-	2880	5040	2	-	-	3	-	3	8
550	*Acremonium fusidioides*[b]	-	-	-	-	1	-	1	-	-	-	-	1	-	1
	Penicillium allii-sativi[a]	-	720	-	-	-	-	720	-	1	-	-	-	-	1
	Penicillium allii-sativi[b]	-	3	-	-	-	-	3	-	1	-	-	-	-	1
	Penicillium chrysogenum[a]	-	1080	720	1440	-	4320	7560	-	1	1	2	-	4	8
	Penicillium chrysogenum[b]	-	5	-	2	2	-	9	-	1	-	1	2	-	4
	Penicillium palitans[a]	720	-	-	-	-	-	720	1	-	-	-	-	-	1
	Penicillium palitans[b]	-	-	-	2	-	-	2	-	-	-	1	-	-	1
	Penicillium solitum[a]	-	-	1080	2160	-	-	3240	-	-	1	2	-	-	3
250	*Penicillium allii-sativi*[c]	-	120	-	80	-	-	200	-	1	-	1	-	-	2
153	*Pseudogymnoascus verrucosus*[c]	-	40	-	-	-	-	40	-	1	-	-	-	-	1

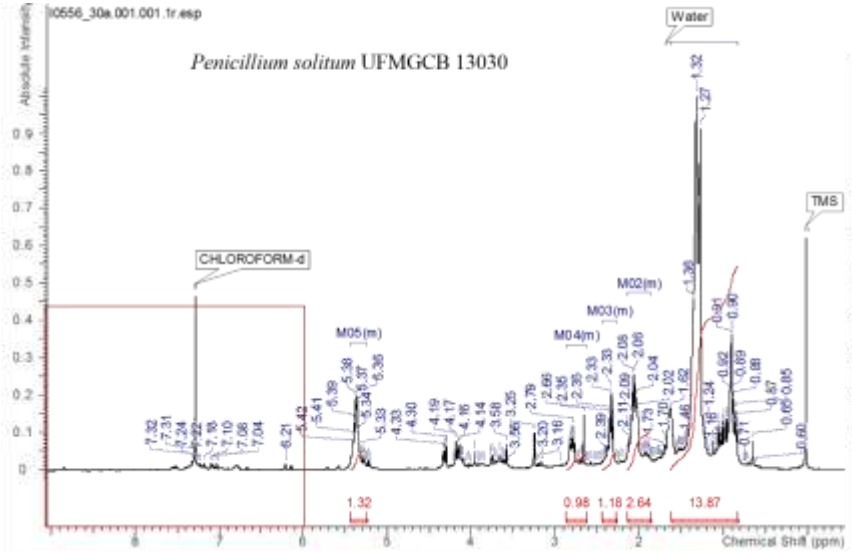
Media = PDA: Potato dextrose agar (diluted 1:5), SAB: Sabouraud agar (diluted 1:5), CZA: Czapek dox agar (diluted 1:5), MEA: malt extract agar (diluted 1:5), MA: Marine agar with 2% of glucose and SAG: Sabouraud agar. $\bar{X}$ = Arithmetic mean. N = Total of isolates recovered. Methods of isolation = a: Enrichment, b: smear, and c: dilution. (-) = absent.

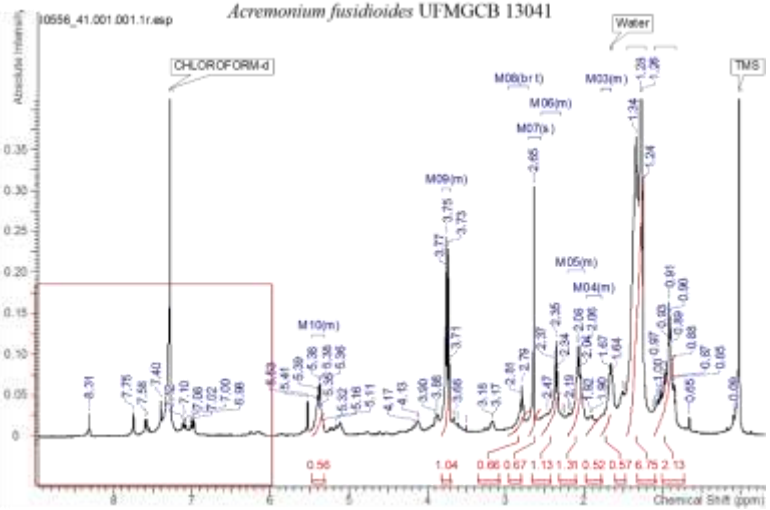
Table 2. Biological activities of extracts obtained from cultures of fungal species isolated from marine sediments from Antarctica.

	Biological activities							
		Antifungal[b]	Herbicidal[c]		Antiparasitic[d]			Nematicidal[g]
Fungal species	UFMGCB[a]	*C. sphaerospermum*	*A. schoenoprasum*	*L. sativa*	*T. cruzi*	*L. amazonensis*	*P. falciparum*	*C. elegans*
Acremonium fusidioides	13041	0 ± 0	**4 ± 0**	**5 ± 0.5**	0 ± 0	62 ± 3	0 ± 0	**2 ± 0**
Penicillium allii-sativi	13048	0 ± 0	**5 ± 0.5**	**3 ± 1**	0 ± 0	56 ± 2	**44 ± 8**	**2 ± 0**
P. allii-sativi	13045	49 ± 10	**3 ± 0.5**	1 ± 1	0 ± 0	58 ± 2	0 ± 0	**2 ± 0**
P. allii-sativi	13053	65 ± 15	2 ± 1.5	**4 ± 0**	0 ± 0	63 ± 5	0 ± 0	**2 ± 0**
P. allii-sativi	13054	0 ± 0	**3 ± 0.5**	**5 ± 0.5**	0 ± 0	53 ± 5	**41 ± 1.5**	**2 ± 0**
Penicillium chrysogenum	13035	20 ± 0	2 ± 1	**5 ± 0.5**	**93 ± 10**	64 ± 7	**75 ± 7**	**2 ± 0**
P. chrysogenum	13036	0 ± 0	2 ± 1	**4 ± 0.5**	0 ± 0	49 ± 4	0 ± 0	**3 ± 0.9**
P. chrysogenum	13039	38 ± 13	2 ± 0.5	**3 ± 1.5**	43 ± 8	46 ± 2.5	45 ± 1.4	**2 ± 0**
P. chrysogenum	13042	0 ± 0	**3 ± 1**	**5 ± 0.5**	36 ± 17	48 ± 2	**84 ± 5.5**	**2 ± 0**
P. chrysogenum	13043	0 ± 0	**3 ± 1.5**	**3 ± 0.5**	0 ± 0	50 ± 1	**100 ± 10**	**3 ± 0.9**
P. chrysogenum	13046	**73 ± 7**	**5 ± 0.5**	**4 ± 0.5**	25 ± 10	59.5 ± 3	**63.2 ± 6.7**	**2 ± 0**
P. chrysogenum	13047	50 ± 10	2 ± 0.5	2 ± 0	0 ± 0	55 ± 3	0 ± 0	**2 ± 0**
P. chrysogenum	13049	40 ± 0	**3 ± 0.5**	**4 ± 0.5**	0 ± 0	66 ± 4	0 ± 0	**2 ± 0**
P. chrysogenum	13050	1 ± 8	**4 ± 0**	2 ± 0	0 ± 0	64 ± 3	**54 ± 2**	**2 ± 0**
P. chrysogenum	13051	39 ± 0	**3 ± 0.5**	**4 ± 0.5**	0 ± 0	59 ± 2	0 ± 0	**2 ± 0**
P. chrysogenum	13052	30 ± 0	**3 ± 0**	**3 ± 0.5**	0 ± 0	60 ± 3	**53 ± 14**	**2 ± 0**
P. chrysogenum	13038	0 ± 0	2 ± 1	**5 ± 0**	**80 ± 16**	52 ± 1.5	**71.5 ± 4**	**2 ± 0**
Penicillium palitans	13034	30 ± 12	**4 ± 0**	**5 ± 0.5**	**67 ± 28**	60 ± 3	**52 ± 3**	**2 ± 0**
P. palitans	13037	0 ± 0	**4 ± 1.5**	**5 ± 0**	**71 ± 13**	62 ± 3	**50 ± 6**	**2 ± 0**
Penicillium solitum	13027	16 ± 0	**3 ± 1**	**5 ± 0**	32 ± 7.8	56 ± 2.5	0 ± 0	**2 ± 0**

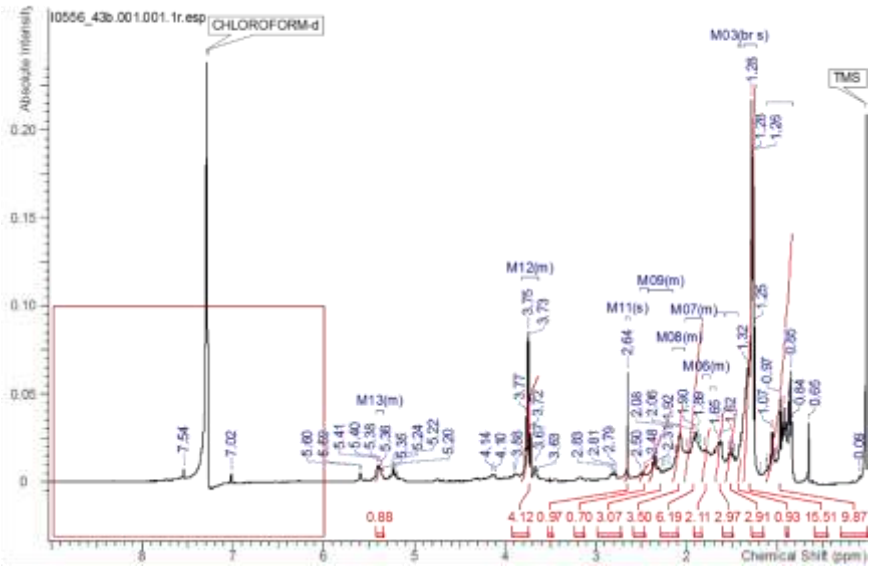
P. solitum	13028	16 ± 0	**4 ± 1**	**5 ± 0**	0 ± 0	59 ± 6	0 ± 0	**2 ± 0**
P. solitum	13029	0 ± 0	**4 ± 0.5**	**5 ± 0**	0 ± 0	60 ± 2	0 ± 0	**2 ± 0**
P. solitum	13030	0 ± 0	1 ± 0	**3 ± 0**	**69 ± 28**	53 ± 3	**45.5 ± 14**	**2 ± 0**
P. solitum	13031	**74 ± 10**	**4 ± 0.5**	**4 ± 0.5**	0 ± 0	52 ± 1	0 ± 0	**3 ± 0.9**
P. solitum	13032	23 ± 0	**5 ± 0.5**	**5 ± 0**	0 ± 0	47 ± 1	0 ± 0	**2 ± 0**
P. solitum	13033	35 ± 0	**3 ± 0**	**5 ± 0**	0 ± 0	50 ± 3	0 ± 0	**2 ± 0**
P. solitum	13040	49 ± 30	**5 ± 0**	**5 ± 0.5**	0 ± 0	0 ± 0	0 ± 0	**2 ± 0**
P. solitum	13044	60 ± 13	**3 ± 1.5**	**5 ± 0**	0 ± 0	64 ± 5	0 ± 0	**2 ± 0**
P. solitum	13056	44 ± 8	**5 ± 0.5**	**5 ± 0**	0 ± 0	45 ± 1	0 ± 0	**2 ± 0**
P. solitum	13057	0 ± 0	**5 ± 0.5**	**5 ± 0**	0 ± 0	51 ± 2	0 ± 0	**2 ± 0**
Pseudogymnoascus verrucosus	13055	42 ± 0	1 ± 1	**4 ± 0**	0 ± 0	49 ± 2	0 ± 0	**2 ± 0**
Control drugs	Benomyl	**100 ± 0**	-	-	-	-	-	-
	Glyphosate	-	**3 ± 1**	**4 ± 1**	-	-	-	-
	BenZ	-	-	-	**89 ± 14**	-	-	-
	Amph B	-	-	-	-	**80 ± 9**	-	-
	Chloroquine	-	-	-	-	-	**100 ± 5**	-

[a]UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. [b]Results of effective extract with inhibition of ≥70% of *Cladosporium sphaerospermum*. [c]The qualitative estimate of phytotoxicity was evaluated by using a rating scale of 0-5, where 0 = no effect and 5 = no growth or no germination of the seeds. Plant targets: *L sativa* = *Lactuca sativa* (lettuce), *A. schoenoprasum* = *Allium schoenoprasum* (chive). [d]Results of effective extract with inhibition of ≥40% of *Trypanossoma cruzi*, ≥70% of *Leishmania amazonensis*, ≥40% of *Plasmodium falciparum*. [g]Results of lethal time (LT_{50}) of effective extracts (evaluated in the concentration of 20µg mL^{-1}) in nematicidal assay against *Caenorhabditis elegans*. In bold extracts that presented bioactivity.





Penicillium chrysogenum UFMGCB 13043



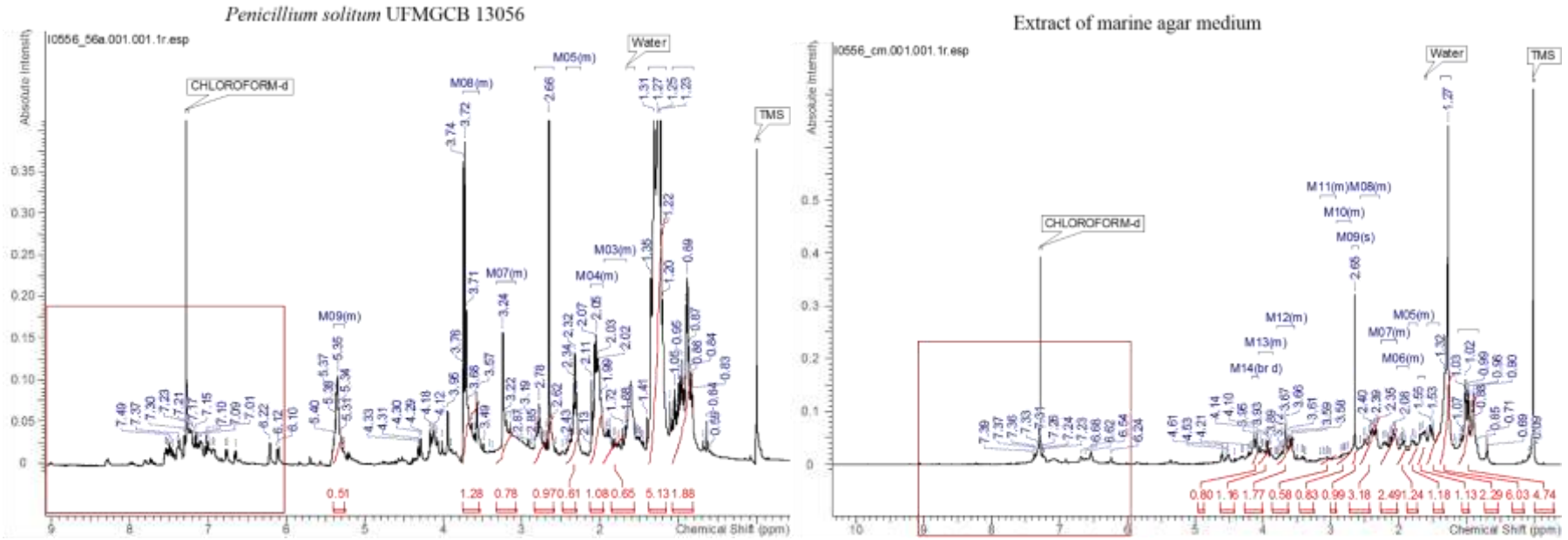


Fig. S2. ^{1}H-NMR spectrum (400 MHz, CD_3OD) of bioactive fungal extracts Regions of interest are labelled in red square above the corresponding signals of aromatic compounds produced by the fungi.

5.4 Capítulo 4. "Diversity, ecology, and bioprospection of fungi in lakes under anthropogenic effects in Antarctic Peninsula"

Artigo sera submetido à FEMS Microbiology Ecology.

Diversity, ecology, and bioprospection of fungi in lakes under anthropogenic effects in Maritime Antarctica

Mayara B. Ogaki[1], Rosemary Vieira[2], Carlos L. Zani[3], Tânia M.A. Alves[3], Policarpo A.S. Junior[3], Silvane M.F. Murta[3], Emerson C. Barbosa[3], Jaquelline G. Oliveira[3], Isabela P. Ceravolo[3], Patrícia O. Pereira[3], Carlos A. Rosa[1], and Luiz H. Rosa[1]*

[1]Departamento de Microbiologia, Universidade Federal de Minas Gerais, MG, Brasil
[2]Instituto de Geociências, Universidade Federal Fluminense, RJ, Brasil
[3]Instituto René Rachou, FIOCRUZ-Minas, MG, Brasil

*Corresponding author. Departamento de Microbiologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, CEP 31270-901. Tel.: +55-31-3409 2749; Fax: +55-31-3409 2730, Brazil. E-mail: lhrosa@icb.ufmg.br

Running title: Fungi in lakes of Antarctica

Abstract

We accessed the culturable fungal assemblages present in sediments of three lakes under anthropogenic impact in Fildes Peninsula, King George Island, Maritime Antarctica. Sixty-three different taxa were identified. *Cladosporium* sp. 2, *Pseudeurotium hygrophilum*, and *Pseudogymnoascus verrucosus* occurred in sediments of all lakes sampled. The diversity indices of the fungal assemblages and the physic-chemical conditions ranged across the lakes. In the sediment of the Central Lake that is under close influence of human activities were detected the high concentrations of metals and the lowest fungal diversity indices in comparision with the other two lakes, which are far from the Antarctic stations. Forty fungal extracts displayed at least one biological acitivities. Among them, *P. hygrophilum*, *P. verrucosus*, *Penicillium glabrum*, and *Penicillium solitum* showed strong trypanocidal,

herbicidal, and antifungal activities. Our results suggest that the increasing of the anthropogenic activities in the Fildes Peninsula, King George Island, may affect the microbial diversity and composition, and the fungal diversity in these lakes may be used as model to study the impact of anthropogenic activities in Antarctica. Additionally, we detected in the lake sediments different fungal taxa able to produce bioactive compounds, which may represent source of new drugs for use in medicine and agriculture.

Introduction

Antarctic Peninsula perhaps represents the region of the earth under the most global climate changes (Rosa *et al*., 2019). According to Flocco *et al*. (2019), different Antarctic environments have been changing due mainly to the global climate changes, but also by the impact of human activities of tourism and research over the last 50 years (Flocco *et al*., 2019). The increase of human activities in Antarctica, especially in the Antarctic Peninsula, has caused pollution problems, especially in areas where scientific stations are located, due to fuel combustion, accidental oil spills, waste incineration, sewage, and other activities linked to stations (Bargagli, 2008). Among the areas in Antarctica with intense research and tourism activities, the Fildes peninsula in the King George island shelters six permanent Antarctic stations and represents a major logistical hub in the Antarctic Peninsula, where scientific, logistic, and tourist activities are concentrated (Padeiro *et al*., 2016).

Fungi play an important role in nutrient cycling, bioconversions and energy flows in ecosystems (Peralta *et al*., 2017). According to Wong *et al*. (2008), in freshwater ecosystems, such as the lakes of Antarctica, fungi play fundamental role in decomposition of suspended particulate mater in water, like plants (degraded celluloses and lignocelluloses), and animals (exoskeletons, feathers, hairs). Abiotic factors such as UV radiations, low temperature, melting glaciers and recent snow, winds, sea spray, and sea ice extent, as well as organic influence of fauna and plants in lacustrine areas (extra-aquatic zones) also can influence the composition of the fungi communities of a lake (Ogaki *et al*., 2019a). However, there are insufficient informations on the effects of pollution and anthropogenic factors on fungal diversity and ecology in Antarctic lakes.

As anthropogenic impact pollution concentrated in Maritime Antarctica may affect the terrestrial and coastal marine natural ecosystems and influence directly the Antarctic mycobiota, in present study we characterize the diversity and ecology of cultivable fungal community present in sediment of lakes, and impacted by anthropogenic activity in the Antarctic Peninsula region. In addition, we evaluated the potential of the fungi as

producers of bioactive compounds that can be used against neglected tropical diseases and for applications as herbicides in agriculture.

Materials and methods

Study area

Three samples of lacustrine sediments were collected in three different points in Fildes Peninsula, King George Island, Antarctica (Figure S1) in austral summer of 2016 during the XXXIV Antarctic Operation. Samples were collected manually using plastic pipes (60 mm of diameter x 50 cm of height) and conditioned at - 20 °C until to be subsampling in the laboratory at the Federal University of Minas Gerais, Brazil. A gradual thawing of the core at 4 °C was performed 24 hours before the fungi isolation. Three subsamples of the central parts of each core were obtained using sterilized scoop inside of laminar flow hood under conditions of strict contamination control and processed to isolate the fungi.

Sediment analysis

For analysis of metals and trace elements present in the lake sediments, 100 g of solid sample was opened by means of a partial digestion using nitric acid, hydrochloric acid and hydrogen peroxide. The "bioavailable" metals present are oxidized and solubilized. In this process metals in structures such as silicates are not solubilized. The calculation of metal concentrations is based on the data presented by inductively coupled plasma - optical emission spectrometry (ICP-OES) equipment, considerating w/v of the ratio. Humidity data were used for calculations on dry basis or pretreatment is performed. Values of blank and replicates are checked according to the criteria registered in the CCLAS laboratory software. The concentration of the PAHs was determinated by the standard methods stabilished by US EPA 8260 (2006), which detect volatile organic compounds in solid samples by gas chromatography/mass spectrometry. The polycyclic aromatic hydrocarbon (PAHs) evaluated was acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g, h, i)perylene, benzo(k)fluoranthene, criseno, dibenzo(a, h)anthracene, phenanthrene, fluoranthene, fluorene, indene(1, 2, 3-cd)pyrene, naphthalene and pyrene.

Fungi isolation

In order to isolate fungi in the lacustrine sediment, 1 g of each subsampled sediment was resuspended in 1 mL of 0.85% saline, and 100 µL of each suspension were plated in the

media: DRBC – Dichloran Rose Bengal agar (peptone 0.5%, glicose 1%, KH_2PO_4 0.1%, $MgSO_4.7H_2O$ 0.05%, dichloran 0.0002%, rose bengal 0.0025% e agar 2%), MEA – Malt extract agar (malt extract 5%, agar 2%) and MM – Minimal media (peptone 0.025%, glucose 0.5%, K_2HPO_4 0.698%, KH_2PO_4 0.544%, $(NH4)_2SO_4$ 0.1%, $MgSO_4$. 7 H_2O 0.11%, agar 2%). All media containing 100 mg mL^{-1} of chloramphenicol (Sigma) to prevent bacterial growth and the plates were incubed at 10 °C for 60 days. Fungal colony forming units (CFUs) were counted, and subcultures were made of all of the morphologically distinct colonies from each sample. The subcultures were grouped into different morphotypes according to their macroscopic parameters (colony color and texture, border type and radial growth rate) and micromorphological characteristics on the same isolation media, except for the isolates obtained from the DRBC, whose was done in the APG – Base agar (peptone 0.5%, glucose 1%, KH_2PO_4 0.1%, $MgSO_4$.7H2O 0.05%, agar 2%). DRBC, MEA and MM culture media have distinct constitutions considered importante for the isolation of lacustrine fungi as flows: (i). MEA is a nutrient-rich media that provides a rich source of carbon, nitrogen and other nutrients of variable composition; (ii) DRBC has a basal composition and also the antifungal dichloran and rose bengal in their constitution, in order to avoid fungi of rapid radial growth which could inhibit the appearance of slower growing fungi and; (iii) MM are used to isolate fungi that could be inhibited by high sugar contents. All fungal isolates were deposited in the Collection of Microorganisms and Cells of the Federal University of Minas Gerais under code UFMGCB.

Fungal identification

The protocol for DNA extraction was described previously in Rosa *et al*. (2009). For the filamentous fungi, the internal transcribed spacer (ITS) region was amplified with 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, amplification of the β-tubulin (Glass and Donaldson, 1995) and ribosomal polymerase II genes (RPB2) (Houbraken *et al*., 2012), which are commonly utilized to fungal taxa with low intraspecific variation, was completed with the Bt2a/Bt2b and RPB2-5F-Pc/RPB2-7CR-Pc 7CR primers, respectively, according to protocols established by Gonçalves *et al*. (2015). Yeasts were grouped and identified according to protocols established by Kurtzman *et al*. (2011), and their molecular identities were confirmed by sequencing the D1–D2 variable domains of the large–subunit rRNA gene using the primers NL1 and NL4 as described by Lachance *et al*. (1999). Fungi with query coverage and identity ≥ 99% were considered to represent the same taxon.

Representative consensus sequences of the fungal taxa were deposited into the GenBank database (Table 1). To achieve species-rank identification based on ITS, β-tubulin data and ribosomal polymerase B2, the consensus sequence was aligned with all sequences from related species retrieved from the NCBI GenBank database using BLAST (Altschul *et al*., 1997). Taxa that displayed query coverage and ≤ 98% identity or an inconclusive taxonomic position were subjected to phylogenetic ITS, β-tubulin and ribosomal polymerase B2-based analysis for comparison with sequences of type species deposited in the GenBank database, with estimations conducted using MEGA Version 6.0 (Tamura *et al*., 2011). The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1,000 replicate runs. The information about fungal classification generally followed the databases of Kirk *et al*. (2008), MycoBank (http://www.mycobank.org) and the Index Fungorum (http://www.indexfungorum.org).

Diversity of the fungal community

To quantify species diversity, richness, and dominance the indices Fisher's α, Margalef's, and Simpson's were used, respectively. The matrices of OTUs (Operational taxonomic units) abundances (calculated for each OTU from the fungal density of each lake) were used to perform the following multivariate analyses to estimate the similarities among fungal taxa from different lakes points using the Sorensen and Bray-Curtis coefficient. Species accumulation curves were obtained using Mao Tao index. The principal components analyses (PCA) calculations were carried between the fungal diversity indices and the physic-chemical characteristics of the lake sediments. All results were obtained with 95% confidence, and bootstrap values were calculated from 1,000 replicates using the PAST computer program 1.90 (Hammer *et al*., 2001).

Production of the fungal extracts for biological assays

Fungal isolates were cultivated using solid-state fermentation. The cultures were prepared by aseptically transferring eight 5-mm diameter plugs of each fungal isolate on two Petri dishes (90 mm diameter) containing their same isolation media without addition of chloramphenicol, except for the fungi isolated in DRBC medium, these were plated in APG medium, very similar to the basal composition of the original medium, but without addition of antifungals. After incubation during 15 days at 15 °C, the fungal cultures were cut in small pieces and transferred to 250 mL Erlenmeyers followed by the addition of 100 mL of ethanol PA (Vetec, Brazil). After 72 h of decantation at 4 °C, the organic phase was filtered and the solvent

removed under rotary evaporation at 40 °C. The aliquot of each dried extract was dissolved in DMSO (Merck) and sterile deionized water to prepare a 100 mg mL^{-1} stock solution, which was stored at -20 °C. Sterile media were extracted using the same procedure and their extracts were used as control in the screening procedures.

Activity against Cladosporium sphaerospermum

To assess the antifungal activity of the extracts, we used a culture of *Cladosporium sphaerospermum* CCT 1740 grown in BDA at 25 °C for 7 to 10 days. To prepare the inoculum a well-filled spore loft was suspended in sterile 0.85% saline solution. The resulting suspension was homogenized on a vortex type stirrer for 15 seconds. The cell density of the suspension was standardized in a spectrophotometer (BioSpectro SP-22) for 86-88% transmittance at 620 nm, corresponding to 10^6 spores mL^{-1} (NCCLS M38-A, v. 22, n ° 16). Subsequently, the obtained spores suspension was diluted 50-fold in RPMI1640 culture medium (INLAB Diagnostic) for use in the assay. All extracts were tested at 100mg mL^{-1}, and in duplicate. A total of 25 μL of the extract (dissolved in DMSO and diluted in 1 mg mL^{-1} autoclaved deionized water), 25 μL of the culture medium and 50 μL of the inoculum were inoculated into each well. As a positive control, benomyl at 1.16 μg mL^{-1} was used. The volume of each well was 100 μL and the concentrations of 0.1% DMSO and 250 μg mL^{-1} extract. Plates were placed on a shaker (Uniscience OS-10) for 20 minutes at 200 rpm, and subsequently, incubated at 25 °C for 48h. Fungal growth was analysed visually and spectrometrically on a VERSAmax microplate reader (Molecular Devices) by the program Softmax® Pro 5 (Molecular Devices), with absorbance of 620 nm. The absorbance of the treated wells (with extracts) was compared to the absorbance of the microorganism growth control (non treated). Extracts with an inhibition value greater than or equal to 60% were arbitrarily considered as active.

Assay against Trypanosoma cruzi

The *in vitro* test of trypanocidal activity was performed as previously described by Romanha *et al*. (2010), using *Trypanosoma cruzi* (Tulahuen strain) expressing the *Escherichia coli* β-galactosidase gene. Infective trypomastigote forms are obtained through culture in monolayers of mouse L929 fibroblasts in RPMI-1640 medium, without phenol red, containing 10% foetal bovine serum and 2 mM glutamine. For the bioassay, 4,000 L929 cells in 80 μL of supplemented medium are added to each well of a 96-well microtitre plate. After an overnight incubation, 40,000 trypomastigotes in 20 μL are added to the cells and the cells

are incubated for 2 h. Medium containing parasites that did not penetrate the cells is replaced with 200 μL of fresh medium and the plate is incubated for an additional 48 h to establish infection. The medium is then replaced with solutions of natural products at 20 mg mL^{-1} in fresh medium (200 μL) and the plate is incubated for 96 h at 37°C. After this period, 50 μL of 500 μM chlorophenol red b-D-galactopyranoside in 0.5% Nonidet P40 is added to each well and the plate is incubated for 18 h at 37 °C, after which the absorbance at 570 nm is measured. Controls with uninfected cells, untreated infected cells, infected cells treated with benznidazole at 3.8 μM (positive control) or DMSO 1% are used. The results are expressed as the percentage of *T. cruzi* growth inhibition in compound-tested cells as compared to the infected cells and untreated cells. Duplicates are run in two different plates.

Assay against Leishmania amazonensis

Promastigotes of *Leishmania* (*Leishmania*) *amazonensis* (strain IFLA/BR/196/PH-8) obtained from lesions of infected hamsters were used to detect the leishmancidal activity according to protocols established by Callahan *et al*. (1997). Each extract was tested at concentration of 20 μg mL^{-1}. The results are expressed as percent inhibition in relation to controls without drugs. Amphotericin B at 0.2 μg mL^{-1} (Fungison® Bristol-Myers Squibb B, Brazil) was used as a positive drug control. All assays were performed in triplicate.

Activity against Plasmodium falciparum *blood parasites* in vitro

Initially, 20 μg mL^{-1} of each extract sample was placed into the *well* of the "U" bottom 96-wells plates and their efficacies were evaluated against the parasites, and repeated at least once. The sample that inhibited the growth of the parasite with an efficacy of ≥40%, compared to the control, without addition of drugs, was considered promising. The promising samples were then titrated and the concentration at which growth inhibition of 50% of the parasites took place was used to determine the IC_{50} value. The activity of the compounds against the blood form of the *P. falciparum* parasite was tested using a chloroquine-resistant and mefloquine-sensitive W2 clone (Oduola *et al*., 1988) cultured at 37 °C, as described by Trager and Jensen (1976). The activity was measured using the SYBR test with the parasite suspension (0.5% parasitemia and 2% haematocrit), as described previously (Smilkstein *et al*., 2004). After 48 h at 37 °C, the culture supernatant was removed and replaced by 100 μL of the lysis buffer solution [Tris (20 mM; pH 7.5), EDTA (5 mM), saponin (0.008%; w/v), and Triton X-100 (0.08%; v/v)] followed by the addition of 0.2 μL mL^{-1} of SYBR Safe (Sigma-Aldrich, Carlsbad, CA, USA). The contents of the plate were transferred to a flat bottom plate

and incubated in the dark for 30 min at 37 °C. The plate was read in a fluorometer (Synergy H4 Hibrid Reader, Biotek) with excitation and emission at 485 and 535 nm, respectively. In all the tests, the activities of the compounds were expressed as the 50% inhibitory concentration of the parasite growth (IC_{50}) compared to the drug-free controls and estimated using the curve-fitting software Origin 8.0 (OriginLab Corporation, Northampton, MA, USA). Chloroquine was used as antimalarial reference drug (IC_{50} ~ 100 ng mL^{-1}). Compounds with IC_{50} values bellow 10 μg mL^{-1}, between 10 and 20 μg mL^{-1} and above 20 μg mL^{-1} were considered to be active, partially active and inactive, respectively.

Cytotoxicity assay in BGM cells

BGM cell line, derived from African green monkey kidney (ATCC, Manassas, VA, USA) was used to conduct the cytotoxicity assays, and maintained as suggested by the manufactures. The cells were cultured in bottles of 75 cm^2 dimensions with RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 40 mg L^{-1} gentamicin in a 5% CO_2 atmosphere at 37 °C. For the *in vitro* tests, a confluent cell monolayer was trypsinized, distributed in a flat-bottomed 96-well plate (2×10^5 cells mL^{-1}), and incubated for 18 h at 37 °C to ensure cell adherence. The BGM cells were incubated with 20 μL of the drugs at different concentrations (≤1 mg mL^{-1}) for 24 h in 5% CO_2 at 37 °C. The cell viability was expressed as the percentage of control absorbance of the untreated cells after subtracting the appropriate background values. The extract lethal dose for 50% of the cells was determined by conducting tests at least in duplicates to calculate the dose that killed 50% of the cells (MLD_{50}) ou CC_{50} as described by do Céu de Madureira *et al*. (2002). The neutral red uptake assay was used to evaluate the lysossomal integrity and distinguishes live cells from dead by its ability to incorporate the dye (Borenfreund *et al*., 1988). Briefly, 0.2 mL medium containing 50 μg mL^{-1} of neutral red solution was added to each well. The plate was incubated for another 3 h at 37 °C to allow the uptake of the vital dye into the lysosomes of viable uninjured cells. After removal of the medium, 200 μL of a mixture of 0.5% formaldehyde and 1% $CaCl_2$ was added to the cells, and incubated for 5 min at 37 °C. The supernatant was removed and 100 μL of a solution of 1% acetic acid-50% ethanol was added to each well to extract the dye. The optical density of each well was measured at a wavelength of 540 nm using a spectrophotometer since this absorbance gives a linear relationship with the number of surviving cells. The ratio between extract cytotoxicity (MLD_{50} BGM) and activity (IC_{50} W2) was used to estimate the selective index (SI), as determined before (Bézivin *et al*., 2003), where SI ≤ 10 was indicative of extract cytotoxicity.

Antiviral activity against the Dengue virus 2 and the Zika virus

Fungi extracts were screened for their cytotoxic and anti-ZIKV/-DENV activities in Vero cells and BHK-21, respectively, in 96-well culture plates by measuring the reduction of the viral cytopathic effect (CPE) and by the MTT method. Briefly, lyophilized extracts were dissolved in 8 % DMSO aqueous solution and their final concentration was normalized at 20 µg mL^{-1} with fresh complete MEM supplemented with 2% FBS. Briefly, 10^4 cells per well were seeded in 96-well plate and incubated for 24 h at 37 °C and 5 % CO_2. Extract solution and virus suspension, at m.o.i.=2 for both viruses, were simultaneously added on 70 % confluent monolayers. Each extract was tested in duplicate and controls of untreated infected and untreated uninfected cells, incubated with or without DMSO. At 3-4 days pi, cell viability was observed by optic microscopy and also determined by the MTT assay, as previously described by Mosmann (1983). Briefly, 30 µL of sterile filtered (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT, Sigma) stock solution in phosphate buffered saline (PBS) pH 7.4 (5 mg ml^{-1}) were added to each well reaching a final concentration of 0.5 mg of MTT/ml. After 90 min the unreacted MTT was removed by aspiration, the insoluble formazan crystals were dissolved in 130 µL per well of dimethylsulfoxide (DMSO) (Merck, Germany) and measured spectrophotometrically in an ELISA reader Spectra Max (Molecular Devices, USA) at a wavelength of 540 nm. Antiviral activity of each extract was determined based on the percentage of cell viability of treated cultures infected with ZIKV or DENV-2 in comparison with uninfected cells and virus controls. Extracts were considered active when caused reduction of the viral CPE by at least 40%. Anti-ZIKV/DENV activity tests were run in duplicates and repeated at least three times.

Herbicide assay

The herbicidal activities of fungal extracts at 1 mg mL^{-1} against *Lactuca sativa* (lettuce) and *Allium schoenoprasum* (chive) were measured using methods adapted from Dayan *et al*. (2000). Briefly, seeds of both plants were disinfected with ethanol 70% for 1 min, sodium hypochlorite at 2 -2.5% for 7.5 min, washed with sterilised distilled water four times, and dried on sterilized filter paper at room temperature. Herbicidal activity was quantitatively evaluated in 24-wells microtitre plate by visually comparing the amount of germination in each well between untreated and treat samples. Samples were incubated at 26 °C in a germination incubator 710 (Thoth, Brazil) under continuous light conditions for 10 days for *L. sativa* and 14 for *A. schoenoprasum*. Test samples were dissolved in acetone to a final concentration of 10% acetone. The control wells contained 400 µL of deionized water. The positive control solvent well contained 360 µL of water and 40 µL of the solvent. Glyphosate

at 3 mg mL^{-1} (Sigma, USA) was used as a positive herbicide control. All sample wells contained 360 µL of water and 40 µL of the appropriate dilution of the sample. A quantitative estimate of herbicidal activity was evaluated using a rating scale of 0-5, where 0 = no effect and 5 = no growth or no germination of the seeds. All assays were performed in duplicate.

Results

Fungal isolation

Three sections of each lake sediments (top, middle, and base) were obtained, with the exception of Central Lake, where only two sections were obtained (top and base) (Table S1). A total of 260 fungi were isolated and those of *Ascomycota* were the most frequent (70.4%), followed by *Basidiomycota* (28.1%), and *Mortierellomycota* (1.1%) (Table S2). However, only the taxon of *Polypaecilum*, found in the South Lake, does not have a taxonomic position very well resolved in phylum level. In the North Lake (near the Collins glacier), a total of 122 fungi were obtained, in the Central Lake 43 isolates, and in the South Lake 95 (Table S3). All culture media used recovery fungi. The DRBC medium provided the growth of 41.5% of the fungi recovered, followed by MM 30.5% and MEA 28% media.

Fungal identification and distribution

Sixty-three OTUs (operational taxonomic units) were identified in the lakes sediment samples of Fildes Peninsula (Table 1). The hierarchical levels of the OTUs obtained are exemplified in Figure S2. Sixteen taxonomic units had a very low query cover and identity percentage when compared to the sequences of type species deposited in GenBank. Therefore, further taxonomic studies to determine if they represent new fungal species. *Cladosporium* sp. 2, *Pseudeurotium hygrophilum*, and *Pseudogymnoascus verrucosus* occurred in sediments of all lakes sampled. *Penicillium glabrum*, *Penicillium solitum*, *Pseudeurotium* sp., *Pseudogymnoascus destructans*, and *Thelebolus globosus* occurred in sediments of the North and South Lakes. The South and Central Lakes shared the endemic species *Antarctomyces pellizariae*, and no taxa were shared by Central and North Lakes. The major specific elements were present in North Lake (32 taxa), followed by South Lake (13 taxa) and Central Lake (9 taxa).

The Venn diagram (Figure S2) shows the comparison between the genera obtained in each lake. Taxa of the cosmopolitan genera *Cladosporium*, *Penicillium*, and *Pseudeurotium* occurred in sediments of the three lakes sampled. The endemic genus *Antarctomyces* was shared by South and Central Lakes, but in different sections (North middle section and South

top). The genus *Thelebolus* which presents some psychrophilic species were shared by North and South Lakes. The genus *Neobulgaria* can be represented for two possible new species in this study and it was shared between North and Central Lakes. Two other endemic species, *Glaciozyma antarctica* and *Mortierella antarctica*, were also present exclusived in the South and Central samples, respectively.

Fungal community diversity and physic-chemical sediment analysis

The diversity indices differed among the fungal assemblages of each lake evaluated (Table S3). The fungal assemblage of North Lake showed higher diversity, richness, and dominance indices in comparison with those of Central and South lakes. In addition, the same indices were compared among the fungal assemblages of the sections in the sediment cores (Figure S2a). In North Lake the fungal assemblage associated with the base layer of the sediment core (Base section) presented high diversity compared to superficial layer (top section). The Central Lake did not show significant difference between the indices values for top and middle sections, while Lake South showed a decay in the values of diversity, richness, and dominance with the increase of the depth of the core. According to Sorensen and Bray-Curtis values, the similarities among the fungal assemblages among the three lakes were low and the fungal assemblages of Central and South samples were more similar in comparison to North Lake (Figure S2b). In addition, the Mao Tao rarefaction curves reached an asymptote (Figure S4), indicating that the diversity of fungi observed reached the expected one.

The data of the physic-chemical characteristics of the lake sediments are in Table S3. In the sediment of the North Lake, which showed the highest diversity indices (Fisher-α, Margalef's, and dominance), were detected low concentration of the metals Al, Cd, Ca, Mg, and Na. In contrast, in the sediment of Central lake occurred the highest concentrations of Al, Cd, Ca, Cr, and Mg, where we detected the fungal assemblage with lowest diversity indices. The sediments of all lakes displays low values of PAHs analysed (>0.00125 mg kg^{-1}).

Biological activities

Among all 260 fungal extracts screened, 40 displayed at least one biological activity against *T. cruzi* (trypanocidal), *C. sphaerospermum* (antifungal), or weeds (herbicidal) (Table 2). Two isolates of *Pseudeurotium hygrophilum* (UFMGCB 12800 and UFMGCB 12801) showed selective trypanocidal activity. Thirty-three fungal extracts (80%) showed herbicidal activity, mainly against the dicotyledon model *L. sativa*. *Penicillium glabrum*, *Penicillium solitum*, *Pseudeurotium* sp., and *Pseudeurotium hygrophilum* displayed selective activity against the

monocotyledon *A. schoenoprasum*. The extract of *Cladosporium* sp. 2, *Cystobasidium larynges*, *Mrakia robertii*, *Patinella hyalophaea*, *Phenoliferia glacialis*, *Piskurozyma fildesensis*, *Pseudogymnoascus verrucosus*, and *Pseudogymnoascus verrucosus* strongly inhibited the germination in the seeds of *L. sativa*. Extracts of *P. glabrum*, *P. solitum*, *Pseudeurotium hygrophilum*, *Pseudeurotium* sp., and *Cladosporium* sp. 2 showed antifungal activity. No anti-ZIKV, anti-DENV-2, leishmanicidal, and antiplasmodial activities were detected.

Discussion

Fungal taxonomy and distribution

There are few published studies about fungi in samples of lakes in the Antarctic Peninsula and adjacent islands. Piooner studies published by Stanley and Rose (1967), Willoughby (1971), Ellis-Evans (1996), Vaz *et al*. (2011), and Gonçalves *et al*. (2012, 2015) identified mainly fungal taxa of the genus *Antarctomyces*, *Aphanomyces*, *Cladosporium*, *Cystobasidium*, *Leucosporidium*, *Mortierella*, *Penicillium*, *Pseudogymnoascus*, *Rhodotorula*, *Vishniacozyma*, and *Thelebolus*. In our study, the fungal assemblages present in the sediment of the Antarctic lakes under anthropogenic activities were rich and diverse and dominated (CFU >300) by taxa genus with cosmopolitan and endemic distribution.

Among the taxa identified, the species *Pseudeurotium hygrophilum* (*Pseudeurotiaceae*) and *Pseudogymnoascusus verrucosus* (*Myxotrichaceae*) occurred in the sediments of the three lakes. They were the most frequently isolated taxa in North and South lakes, with a broad distribution in all samples sections (top, middle, and base) evaluated. *Pseudeurotium hygrophilum* (old name of *Teberdinia hygrophila*) (Minnis and Lindner, 2013), was firstly isolated from montane fen soil in the Caucasus Mountains in Russia (Sogonov *et al*., 2005). Kujala *et al*. (2018) identified *P. hygrophilum* in peatlands treating mining-affected waters rich in contaminants like arsenic, sulphate, and nitrate in Northern Finland. In Antarctica the genera *Pseudeurotium* was identified in some substrates such as active layer in the ice-free oases in Continental Antarctica (Kochkina *et al*., 2014), sponges (Henríquez *et al*., 2014), and soil (Arenz and Blanchette, 2009). Kochkina *et al*. (2018) identified *P. hygrophilum* in soil with human impact in Bellingshausen Russian station (Fildes Peninsula), next to Central Lake evaluated in this study where this same taxon was identified.

Pseudogymnoascus is widespread in Polar Regions and is abundant in Antarctica, occuring in different substrates and environments (Rosa *et al*., 2019). In lakes of Antarctica, *Pseudogymnoascus* already was reported associated with biomats in Aces Lake and Pendant

Lake, Vestfold Hills (Brunatti *et al*, 2009), in sediments (Tsuji *et al*., 2013; Gonçalves *et al*., 2015), and freshwater in Fryxell Lake, Mcmurdo Dry Valleys (Conell *et al*, 2018). *Pseudogymnoascus verrucosus* was reported in different habitats such as in soil of King George and Penguin islands (Gomes *et al*., 2018), associated with marine invertebrates (Godinho *et al*., 2019), and marine sediments in Maxwell Bay in King George Island (Ogaki *et al*., 2019b).

Neobulgaria sp. also occurred in high frequence in the superficial and base sediment samples. *Neobulgaria* was identified preveously in permanently frozen soil from northeastern Siberia (Lydolph *et al*., 2005), in association with tundra in Toolik Lake region in the northern foothills of the Brooks Range, Alaska (Geml *et al*., 2005), and in permafrost thaw from moist acidic tundra near the Kuparuk River in Alaska (Coolen *et al*., 2011).

Taxa of the cosmopolitan genera *Penicillium*, *Pseudogymnoascus*, *Acremonium*, and *Cladosporium* were detected in the lake sediments of the three lakes sampled. Both genera were also identified in lacustrine samples from McMurdo Dry Valleys, Antarctica (Brunatti *et al*., 2009; Tsuji *et al*., 2013; Conell *et al*., 2018), including acretted ice layer of subglacial lakes (D'Elia *et al*, 2009). In addition, these cosmopolitan genera found in this study are widely diffused in nature, but they have also been identified in Antarctic soil in samples contaminated by vehicle tracks and oil spills (Kochkina *et al*., 2018).

In addition, psycrophilic and/or endemic species (*A. pellizariae*, *M. antarctica*, *G. antarctica*, and *T. globosus*) were detected in the lake sediments evaluated. These species already were reported in soil (Gomes *et al*., 2018), snow (de Menezes *et al*., 2017), rock (Alves *et al*., 2019), associated with macroalgae (Godinho *et al*., 2013; Furbino *et al*., 2014), and lacustrine samples (de Hoog *et al*., 2005; Brunatti *et al*., 2009; Gonçalves *et al*., 2012; Tsuji *et al*., 2013; Conell *et al*., 2018) in distinct regions of the South Shetland Islands, Maritime Antarctica.

Tetracladium, *Mrakia*, *Glaciozyma*, *Dioszegia*, and *Leucosporidium* recovered in sediments of the lakes of Fildes Peninsula alredy were reported in lakes of different region of Antarctica (Brunatti *et al*., 2009; Tsuji *et al*., 2013; Rojas-Jimenez *et al*., 2017; Conell *et al*., 2018). *Tetracladium globosum*, a cold-adapted fungus found in this study, was described firstly in Alpine glaciers on the Qinghai-Tibet Plateau (Wang *et al*., 2015) and later associated with plant in Svalbard, Arctic (Zhang and Yao, 2015). *Tetracladium* was described in lake sediments of Skarvness region in Continental Antarctica (Tsuji *et al*., 2013); however, we detected *T. globosum* at the first report in Antarctic lacustrine sediments.

The basidiomycetous yeasts *Mrakia*, *Glaciozyma*, *Dioszegia*, *Leucosporidium*, and *Vishniacozyma* include psychrophilic and psychrotolerant species that have been described for

cold habitats. *Cryptococcus* is one of the most represented yeasts in these polar habitats (Buzini *et al*., 2017). The genus *Cryptococcus* was taxonomicaly reorganised which result in some new genera (Liu *et al*., 2015). In the sediment samples studies, we identified *Piskurozyma fildesensis* (*Cryptococcus fildesensis*), *Vishniacozyma carnescens* (*Cryptococcus carnescens*), and *Vishniacozyma victoriae* (*Cryptococcus victoriae*). Among them, *V. victoriae*, commonly detected in lake sediments, has been originally described from soil at Victoria land in Antarctica (Yurkov and Pozo, 2017) and it was one of the most frequently isolated species on polar and subpolar environments (Buzini *et al*., 2017).

In contrast, some taxa like *Periconia byssoides*, *Pholiota baeosperma*, *Sarocladium dejongiae*, and *Polypaecilum* sp. occurred in only one lake sediment at a low density. These taxa may represent singlets and minor components of fungal lake assemblages. *Periconia byssoides* has already been reported in polluted freshwater enviroments, such as Lake Dianchi in Yunnan, China (Luo *et al*., 2004) and Munneru River, Telangana, India (Saikumari and Saxena, 2017). No sexual forms of *Pholiota baeosperma* (*Agaricales*) was reported in Central and South America (Coimbra, 2015), including Patagonian forests (Romano *et al*, 2016). While *Sarocladium dejongiae* was recently described in Dutch soils (Lombard, 2018).

Fungal diversity and sediment analysis

The density and diversity of the fungal assemblages living in the lakes of Fildes Peninsula under anthropogenic effects indicate that their physic-chemical characteristis may influence the composition, distribution, and ecology fungal assemblages. We did not detect PAHs in significative concentration in the samples. However, different metals were detected in high concentration. In the sediment of the Central Lake, we detected the lowest diversity and richness fungal assamblege and the highest concentration of some metals like Al, Cd, and Cr, which are considered toxic. In addition, the Central Lake perhaps represents the lake in the region under the highest anthropogenic impact, due its proximity with the Antarctic research stations and the frequent tourism in the region. In contrast, the sediment of the North Lake displayed the highest fungal diversity and richness and the lowest values of the same metals. The fact of the North Lake is far from the research stations and close to Collins glacier, which the melting ice supplies the lake, may explain the high fungal diversity detected.

Biological activities

Extracts of *Cladosporium* sp. 2, *P. glabrum*, *P. solitum*, *P. destructans*, *C. laryngis*, *M. robertii*, *P. hyalophaea*, *P. glacialis*, *P. fildesensis*, and *P. hygrophilum* were able to inhibit

the biological target screened. *Penicillium* and *Cladosporium* include taxa known as a producer of many bioactive compounds (Rosa *et al*., 2019; Khan *et al*., 2016); however, in Antarctica few species have been chemically investigated (Gonçalves *et al*., 2015). *Penicillium* and *Cladosporium* species present in Antarctic lakes already detected as producer of bioactive compounds. Brunati *et al*. (2009) found *Penicillium* and *Cladosporium* taxa associated with biomats producers of cytotoxic and antimicrobial compounds against clinic pathogens, and Gonçalves *et al*. (2015) found isolates in sediment samples able to produce antifungals against the pathogen causing human mycoses *Paracoccidiodes brasiliensis*.

Pseudogymnoascus destructans is the psychrophic pathogen of white nose syndrome (WNS) that has been deplete bat populations in temperate regions (Lorch *et al*. 2011), and in Antarctica is abundantly found on different substrates (Rosa *et al*., 2019). In this study, the isolate *P*. *destructans* UFMGCB 12886 showed selective herbicidal activity against *A. soroenoprasum*. Gomes *et al*. (2018) evaluated extracts of *P*. *destructans* obtained from Antarctic soils that showed similar results with selectivity against *A. soroenoprasum* with an high and moderate herbicidal activity. Furtheremore, the authors also demonstrate that *P*. *destructans* present trypanocidal activity (Gomes *et al*., 2018). In addition, *Pseudogymnoascus* was reported before in lake of Antarctica (Ogaki *et al*., 2019a). Some strains obtained in different substrates in Antarctic regions were recognized as producer of bioactive compounds with antifungal and antibacterial activities against clinical pathogens such as *Streptococcus pneumoniae* (Li *et al*. 2008), *Candida albicans* (Brunatti *et al*. 2009, Furbino *et al*. 2014), *Cryptococcus neoformans* (Brunatti *et al*. 2009) and other important pathogen in agriculture, *Cladosporium sphaerospermum* (Furbino *et al*. 2014).

We detected *Patinella hyalophaea*, *Cystobasidium laryngis*, *Mrakia robertii*, *Piskurozyma fildesensis*, and *Phenoliferia glacialis* able to produce compounds with selective high and moderate herbicidal activities, which can represent a high intra-specific ability to produce herbicide compounds. The increasing prevalence of herbicide resistant weeds and the misuse of pesticides have led to new pursuits for weed control strategies. Bacteria, fungi and viruses have been exploited in recent decades as potential producers of new herbicides, the benefits of this biological control are to reduce the environmental impact, increase target specificity, and reduced development costs compared to conventional herbicides (Harding and Raizada *et al*., 2015).

Pseudeurotium hygrophilum displayed expressive antifungal ans herbicide activities. *Pseudeurotium* species, obtained from sponges collected in Fildes Bay, King George Island, was already reported by by Henriquez *et al*. (2014) as producers of antimicrobial against

Staphylococcus aureus and were positive to antioxidant activity. In addition, *P. hygrophilum* was reported in Antarctic contamined soil also next to Bellingshausen station, King George Island and may be involved degradation of soil contaminants (Kochkina *et al*., 2018). The capability of *P. hygrophilum* to degradate pollunts could justify its metabolic plasticity in produce bioactive compounds.

Conclusion

Antarctic lakes represent interesting and sensible environments to study taxonomy and ecology of microbial communities under extreme conditions. Some of these lakes, such as those of Fildes Peninsula in the King George Island, despite to the extreme conditions of Antarctica, have been suffering in the last years with anthropogenic effects due the close presence and impact of several research stations and tourism. In the present study, we detected in the sediments of three lakes of the Fildes Peninsula diverse and rich fungal assemblages, which ranged their compositions among the lakes. In the sediment of the Central Lake, which is the closest to the Antarctic stations, we detected the highest metal concentration and the lowest values of the fungal diversity. In contrast, the North Lake that is far from the Antarctic stations, has the most diverse and rich fungal assemblages and also displayed the lowest metal concentrations values. Our results suggest that the increasing of anthropogenic activities in the region may affect the microbial diversity and composition. We believe that the fungal diversity in these lakes can be a useful model to study of anthropogenic activities in Antarctica**.** In addition, different fungal taxa detected in the lake sediments were able to produce antifungal, trypanocidal, and herbicidal compounds, which may represent sources of new drugs for use in medicine and agriculture.

Acknowlegments

We acknowledge the financial support from CNPq PROANTAR 442258/2018-6, INCT Criosfera II, CAPES (88887.136384/2017-00 and 88887.314457/2019-00), CNPq, FAPEMIG, and FNDCT. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The authors thank the Program for Technological Development of Tools for Health-PDTIS-Fiocruz for use of its facilities (Bioprospection and Chagas disease-PlaBio Tc platforms). PASJ is research fellow supported by Programa de Pós-graduação em Ciências da Saúde, Fiocruz Minas (CAPES/PNPD). The map indicating the Fildes Peninsula and research stations was kindly prepared by Kátia Kellem da Rosa (Polar and Climate Center - UFRGS).

References

Altschul SF, Madden TL, Schaffer AA *et al*. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl Acids Res* 1997;**25**:3389–3402.

Alves IMS, Gonçalves VN, Oliveira FS *et al*. Diversity, distribution, and pathogenic potential of fungi present in rocks of the South Shetlands Archipelago, Maritime Antarctica. *Extremophiles* 2019;**23**:327–336.

Arenz BE, Blanchette RA. Investigations of fungal diversity in wooden structures and soils at historic sites on the Antarctic Peninsula. *Can J Microbiol* 2009,**55**:46–56.

Bargagli R. Environmental contamination in Antarctic ecosystems. *Sci Total Environ* 2008;**400**:212–226.

Bézivin C, Tomasi S, Lohézic-Le DF *et al*. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomed* 2003;**10**:499–503.

Borenfreund E, Babich H, Martin-Alguacil N. Comparisons of two in vitro cytotoxicity assays: the neutral red (NR) and tetrazolium MTT tests. *Toxicol In Vitro* 1988,**2**: 1–6.

Brunati M, Rojas JL, Sponga F *et al*. Diversity and pharmaceutical screening of fungi from benthic mats of Antarctic lakes. *Mar Genomics* 2009,**2**:43–50.

Buzzini P, Turk M, Perini L *et al*. Yeasts in Polar and Subpolar Habitats. In: Buzzini P., Lachance MA, Yurkov A (eds.). Yeasts in Natural Ecosystems: Diversity. Switzerland: Springer Nature 2017, 331-365.

Callahan HL, Portal AC, Devereaux R *et al*. An axenic amastigote system for drug screening. *Antimicrob Agents Chemother* 1997;**41**:818–822.

Coimbra VRM. Checklist of Central and South American *Agaricales* (Basidiomycota) II: *Strophariaceae*. *Mycosphere* 2015;**6**(4):441–458.

Connell L, Segee B, Redman R *et al*. Biodiversity and abundance of cultured microfungi from the permanently ice-covered Lake Fryxell, Antarctica. *Life* 2018;**8**:1–10.

Coolen MJ, van de Giessen J, Zhu EY *et al*. Bioavailability of soil organic matter and microbial community dynamics upon permafrost thaw. *Environ Microbiol* 2011;**13**(8):2299-314.

D'Ellia T, Veerapaneni R, Theraisnathan V *et al*. Isolation of fungi from Lake Vostok accretion ice. *Mycologia* 2009;**101**:751–763.

Dayan FE, Romagni JG, Duke SO. Investigating the mode of action of natural phytotoxins. *J Chem Ecol* 2000;**26**:2079–2094.

de Hoog GS, Göttlich E, Platas G *et al*. Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. *Stud Mycol* 2005;**51**:33–76.

de Menezes GC, Godinho VM, Porto BA *et al*. *Antarctomyces pellizariae* sp. nov., a new, endemic, blue, snow resident psychrophilic ascomycete fungus from Antarctica. *Extremophiles* 2017;**21**:259–269.

do Céu de Madureira M, Martins AP, Gomes M *et al*. Antimalarial activity of medicinal plants used in traditional medicine in S. Tomé and Príncipe islands. *J Ethnopharmacology* 2002;**81**: 23–29.

Ellis-Evans JC. Microbial diversity and function in Antarctic freshwater ecosystems. *Biodivers Conserv* 1996;**5**:1395–1431.

Flocco CG, Mac Cormack WP, Smalla K. Chapter 7: Antarctic Soil Microbial Communities in a Changing Environment: Their Contributions to the Sustainability of Antarctic Ecosystems and the Bioremediation of Anthropogenic Pollution. In: Castro-Sowinski S. (ed.). The Ecological Role of Micro-organisms in the Antarctic Environment, Switzerland: Springer Polar Sciences, 2019, 133–161.

Furbino LE, Godinho VM, Santiago IF *et al*. Diversity patterns, ecology and biological activities of fungal communities associated with the endemic macroalgae across the Antarctic Peninsula. *Microb Ecol* 2014;**67**:775–787.

Geml J, Morgado LN, Semenova TA *et al*. Long-term warming alters richness and composition of taxonomic and functional groups of arctic fungi. *FEMS Microbiol Ecol* 2015;**91**(8):fiv095.

Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* 1995;**61**:1323–1330.

Godinho VM, Furbino LE, Santiago IF *et al*. Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. *ISME J* 2013;**7**:1434–1451.

Godinho VM, de Paula MTR, Silva DAS *et al*. Diversity and distribution of cryptic cultivable fungi associated with marine animals of Antarctica. *Fungal Biol* 2019;**123**:507-516.

Gomes EC, Godinho VM, Silva DA *et al*. Cultivable fungi present in Antarctic soils: taxonomy, phylogeny, diversity, and bioprospecting of antiparasitic and herbicidal metabolites. *Extremophiles* 2018;**22**:381–393.

Gonçalves VN, Vaz ABM, Rosa CA *et al*. Diversity and distribution of fungal communities in lakes of Antarctica. *FEMS Microbiol Ecol* 2012;**82**:459–471.

Gonçalves VN, Cantrell CL, Wedge DE *et al*. Fungi associated with rocks of the Atacama Desert: taxonomy, distribution, diversity, ecology and bioprospection for bioactive compounds. *Environ Microbiol* 2015;**18**:232–245.

Hammer Ø, Harper DAT, Ryan PD. PAST: paleon-tological statistics software package for education anddata analysis. *Palaeontol Electron* 2001;**4**:9.

Harding D P, Raizada MN. Controlling weeds with fungi, bacteria and viruses: a review. *Front Plant Sci* 2015;**6**(659):1–14.

Henríquez M, Vergara K, Norambuena J *et al*. Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial, antitumoral and antioxidant potential. *World J Microbiol Biotechnol* 2014;**30**:65–76.

Houbraken J, Frisvad JC, Seifert KA *et al*. New penicillin-producing *Penicillium* species and an overview of section Chrysogena. *Persoonia* 2012;**29**:78–100.

Khan IH, Sohrab H, Rony SR *et al*. Cytotoxic and antibacterial naphthoquinones from an endophytic fungus, *Cladosporium* sp.. *Toxicology Reports* 2016;**3**:861-865.

Kirk PM, Cannon PF, Minter DW *et al*. Dictionary of the Fungi. CAB International, Wallingford, 2008.

Kochkina GA, Ozerskaya SM, Ivanushkina NE *et al*. Fungal diversity in the Antarctic active layer. *Microbiology (Moscow)* 2014;**83**:94–101.

Kochkina GA, Ivanushkina NE, Lupachev *et al*. Diversity of mycelial fungi in natural and human‐ affected Antarctic soils. *Polar Biology* 2018;**42**:47-64.

Kujala K, Mikkonen A, Saravesi K *et al*. Microbial diversity along a gradient in peatlandstreating mining-affected Waters. *FEMS Microbiol Ecol* 2018;**94**(10):1-15.

Kurtzman CP, Fell JW, Boekhout T The yeasts: a taxonomic study. Elsevier, Amsterdam, 2011.

Lachance MA, Bowles JM, Starmer WT *et al*. Kodamaea kakaduensis and Candida tolerans, two new yeast species from Australian Hibiscus flowers. *Can J Microbiol* 1999;**45**:172–177.

Li Y, Sun B, Liu S *et al*. Bioactive asterric acid derivatives from the Antarctic ascomycete fungus *Geomyces* sp. *J Nat Prod* 2008;**71**:1643–1646.

Liu XZ, Wang QM, Göker M *et al*. Towards an integrated phylogenetic classification of the Tremellomycetes. *Stud Mycol* 2015;**81**:85-147.

Lombard L. *Sarocladium dejongiae* L. Lombard, sp. nov. *Persoonia* 2018;**41**:346-347.

Lorch JM, Meteyer CU, Behr JM *et al*. Experimental infection of bats with *Geomyces* destructans causes white-nose syndrome. *Nature* 2011;**480**:376–378.

Luo, J, Yin J, Cai L *et al*. Freshwater fungi in Lake Dianchi, a heavily polluted lake in Yunnan, China. *Fungal Diversity* 2004;**16**:93–112.

Lydolph, MC, Jacobsen, J, Arctander, P *et al*. Beringian Paleoecology Inferred from Permafrost-Preserved Fungal DNA. *Appl Environ Microbiol* 2005;**71:**1012–1017.

Margesin R, Feller G. Biotechnological applications of psychrophiles. *Environ Technol* 2010;**31**:835–844.

Martorell MM, Ruberto LAM, Fernández PM *et al*. Bioprospection of cold-adapted yeasts with biotechnological potential from Antarctica. *J Basic Microbiol* 2017;**57**(6):504–516.

Martorell MM, Ruberto LAM, de Figueroa LIC *et al*. Antarctic Yeasts as a Source of Enzymes for Biotechnological Applications. In: Rosa LH (Eds.) Fungi of Antarctica. Switzerland: Springer Nature, 2019, 285–304.

Minnis AM, Lindner DL Phylogenetic evaluation of Geomyces and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biol* 2013;**117**:638–649.

Mossman T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;**65**: 55–63.

NCCLS Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, M38-A. National Committee on Clinical Labora-tory Standards 2002;**22**:16.

Oduola AM, Weatherly NF, Bowdre JH *et al*. *Plasmodium falciparum*: cloning by single-erythrocyte micromanipulation and heterogeneity in vitro. *Exp Parasitol* 1988;**66**:86–95.

Ogaki MB, Vieria R, Lirio JM *et al*. Diversity and Ecology of Fungal Assemblages Present in Lakes of Antarctica. In: Rosa LH (Ed) Fungi of Antarctica. Switzerland: Springer Nature, 2019a, 69-97.

Ogaki MB, Coelho LC, Vieira R *et al*. Cultivable fungi present in deep-sea sediments of Antarctica: taxonomy, diversity, and bioprospection of bioactive compounds. **Unppublished**. 2019b.

Padeiro A, Amaro E, dos Santos MM *et al*. Trace Elements in the soils of Fildes Peninsula, King George Island, Antarctica. *Environ Sci Process Impacts* 2016;**18**(6):648-657.

Peralta RM, da Silva BP, Côrrea RCG *et al*. Chapter 5 - Enzymes from Basidiomycetes—Peculiar and Efficient Tools for Biotechnology. In: Brahmachari G (ed.). Biotechnology of Microbial Enzymes, San Diego: Academic Press, 2017, 119-149.

Rojas-Jimenez K, Wurzbacher C, Bourne E *et al*. Early diverging lineages within Cryptomycota and Chytridiomycota dominate the fungal communities in ice-covered lakes of the McMurdo Dry Valleys, Antarctica. *Sci Rep* 2017;**7**:15348.

Romanha AJ, de Castro SL, Soeiro MNC *et al*. In vitro and in vivo experimental models for drug screening and development for Chagas disease. *Mem Inst Oswaldo Cruz* 2010;**105**:233–238.

Romano GM, Ruiz EV, Lechner B *et al*. Track analysis of agaricoid fungi of the Patagonian forests. *Australian Systematic Botany*, 2016;**29**:440–446.

Rosa LH, Vaz ABM, Caligiorne RB *et al*. Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv (*Poaceae*). *Polar Biol* 2009;**32**:161–167.

Rosa LH, Zani CL, Cantrell CL. Fungi in Antarctica: Diversity, Ecology, Effects of Climate Change, and Bioprospection for Bioactive Compounds. In: Rosa LH (ed.) Fungi of Antarctica. Switzerland: Springer Nature, 2019,1-17.

Saikumari D, and Neeti S. Isolation and Identification of Extra-aquatic, Pathogenic Fungi from the Polluted Water of Munneru River, Telangana, India. *Int J Curr Microbiol App Sci* 2017;**6**(1): 71-77.

Smilkstein M, Sriwilaijaroen N, Kelly JX *et al*. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. *Antimicrob Agents Chemother* 2004;**48**:1803–1806.

Sogonov MV, Schroers H-J, Gams W, Dijksterhuis, Summerbell,2005. The hyphomyceteTeberdinia hygrophilagen. nov., sp.nov. and related anamorphs ofPseudeurotiumspecies. Mycologia 97: 695e709.

Stanley SO, Rose AH. Bacteria and yeasts from lakes on Deception Island. *Philos Trans R Soc, Ser A* 1967;**252**:199–207.

Tamura K, Peterson D, Peterson N *et al*. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;**28**:2731–2739.

Trager W, Jensen JB. Human malaria parasites in continuous culture. *Science* 1976;**193**:673–675.

Tsuji M, Fujiu S, Xiao N *et al*. Cold adaptation of fungi obtained from soil and lake sediment in the Skarvsnes ice-free area, Antarctica. *FEMS Microbiol Lett* 2013;**346**:121–130.

U.S. EPA. "Method 8260D (SW-846): Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)," Revision 3. Washington, DC, 2006.

Vaz AB, Rosa LH, Vieira ML *et al*. The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. *Brazilian J Microbiol* 2011;**42**:937–947.

Wang M, Jiang X, Wu W *et al*. Psychrophilic fungi from the world's roof. Persoonia 2015;**34**:100–112.

White TJ, Bruns TD, Lee SB Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis NA, Gelfand J, Sninsky J, White TJ (eds.). PCR protocols: a guide to methods and applications. San Diego: Academic Press, 1990, 315–322.

Willoughby LG Aquatic fungi from an Antarctic Island and a Tropical lake. *Nova Hedwigia* 1971;**22**:469–488.

Wong MK, Goh TK, Hodgkiss *et al*. Role of fungi in freshwater ecosystems. *Biodiv Conservation* 1998;**7**:1187.

Yurkov A. Temporal and Geographic Patterns in Yeast Distribution. In: Buzzini P., Lachance MA., Yurkov A. (eds.). Yeasts in Natural Ecosystems: Ecology. Switzerland: Springer Nature, 2017, 101-130.

Zhang T, Yao YF. Endophytic fungal communitiesassociated with vascular plants in the high Arctic zone arehighly diverse and hostplant specific. *PLoS One* 2015;**10**(6):e0130051.

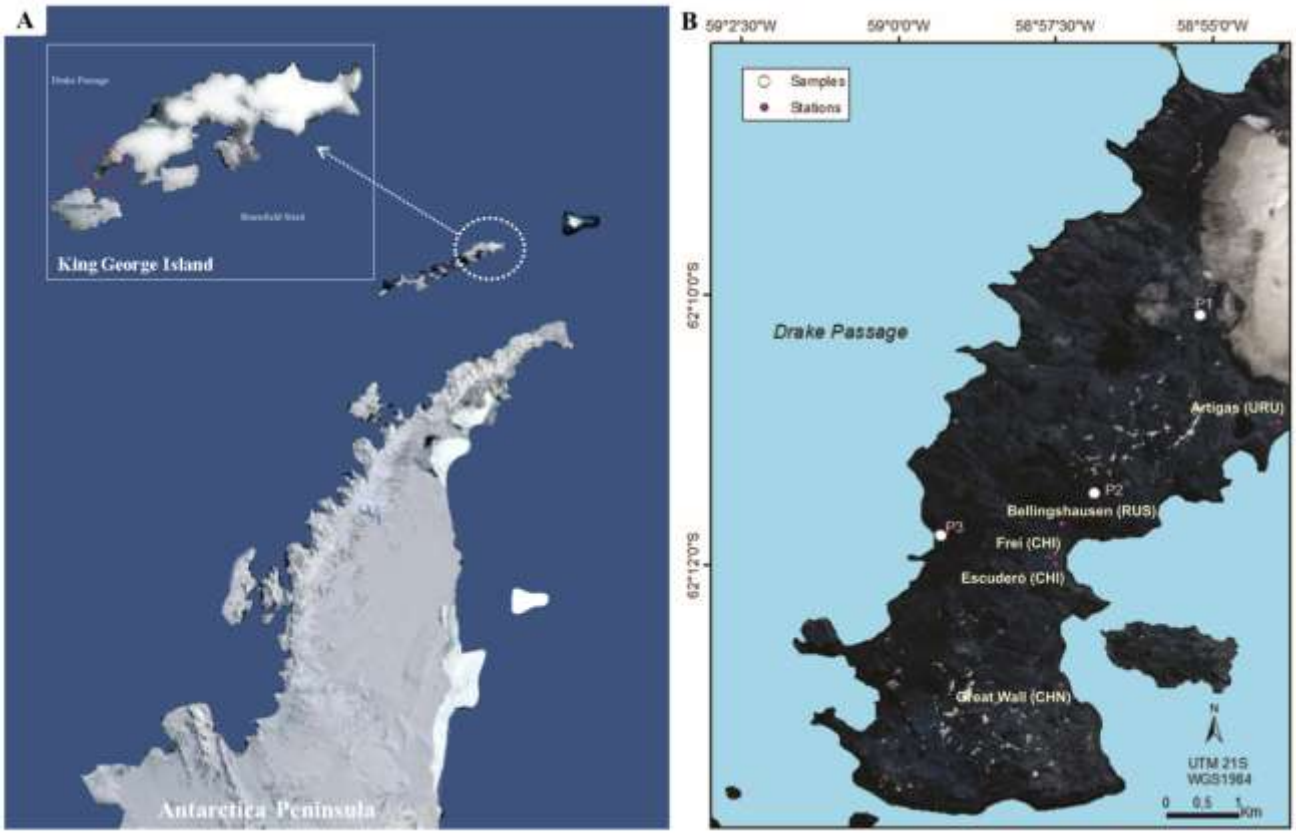


Figure S1. Maps showing the collection points of lake sediments and nearest scientific Antarctic stations. A. Antarctica Peninsula: white cicle shows King George Island and red circle shows Fildes Peninsula. B. Fildes Peninsula: P1: North Lake; P2: Central Lake; P3: South Lake (coastline of the Peninsula, further south than other points sampled).

Table S1. Collection data of lake sediment samples in Fildes Peninsula.

Lake	Core[a]	Section[b]	Longitude	Latitude
North	150	T, B	58. 5536	62. 1090
Central	200	T, M, B	58. 571296	62. 113509
South[c]	270	T, M, B	58. 5941	62. 1151

[a]size of the core in milimeters; [b]sections of the core top (T), middle (M), and base (B). [c] Lake in coastline of Peninsula, further south than other points sampled.

Table 1. Fungal taxa obtained from lakes of Peninsula Fildes and identified by sequence comparison with the BLASTn match with the NCBI GenBank database.

Collection Point	Section	UFC g^{-1}	UFMGCB[a]	Top BLAST (Number of acess on Genbank)	Query cover (%)	Identity (%)	bp analysed	Proposed taxa[f]
North Lake	Top	720	12911	*Cladosporium austrohemisphaericum* (NR152289)[b]	98	99	396	*Cladosporium* sp. 1
		40	12901	*Cladosporium tenuissimum* (MH864840)[b]	100	100	405	*Cladosporium* sp. 2
				Cladosporium fusiforme (EF101446)[d]	100	88	265	
		80	12849	*Gyoerffyella entomobryoides* (NR145302)[b]	100	98	418	*Gyoerffyella* sp.
		40	12907	*Leohumicola atra* (NR111327)[b]	99	91	369	*Helotiales* sp. 1
		200	12960	*Leohumicola minima* (NR121307)[b]	99	91	379	*Helotiales* sp. 2
		1840	12964	*Acremonium biseptum* (NR159609)[b]	97	97	362	*Hypocreales* sp.
		480	46	*Glaciozyma antarctica* (NG057664)[c]	99	98	418	*Glaciozyma* sp. 1
		40	12961	*Phenoliferia psychrophila* (NG058370)[c]	100	99	485	*Phenoliferia psychrophila*
				Phenoliferia psychrophila (NR154359)[b]	98	91	432	
		40	45	*Glaciozyma litoralis* (HF934009)[c]	100	92	694	*Microbotryomycetes* sp. 1
				Glaciozyma litoralis (NR155110)[b]	93	91	356	
		80	24	*Mrakia robertii* (AY038811)[c]	74	99	510	*Mrakia robertii*
				Mrakia robertii (AY038829)[b]	100	99	388	
		40	12844	*Neobulgaria premnophila* (MH861260)[b]	99	97	467	*Neobulgaria* sp. 1
		920	12979	*Penicillium glabrum* (MH864674)[b]	100	100	453	*Penicillium glabrum*
				Penicillium glabrum (GU981619)[d]	93	98	364	
		200	25	*Phenoliferia glacialis* (NG058369)[c]	100	100	385	*Phenoliferia glacialis*
				Phenoliferia glacialis (NR154358)[b]	98	99	364	
		120	16	*Phenoliferia psychrophenolica* (KY108774)[c]	93	100	565	*Phenoliferia psychrophenolica*
				Phenoliferia psychrophenolica (NR154359)[b]	100	99	308	
		80	12799	*Phialophora alba* (MH859216)[b]	100	99	361	*Phialophora alba*
		1100	12800	*Pseudeurotium hygrophilum* (NR111128)[b]	100	99	445	*Pseudeurotium hygrophilum*
		200	12842	*Pseudogymnoascus appendiculatus* (NR137875)[b]	100	98	445	*Pseudogymnoascus* sp. 1
		40	12910	*Pseudogymnoascus destructans* (EU884921)[b]	100	99	459	*Pseudogymnoascus destructans*
		760	12798	*Pseudogymnoascus verrucosus* (KJ755525)[b]	100	99	459	*Pseudogymnoascus verrucosus*
		160	43	*Vishniacozyma victoriae* (KY110041)[c]	100	100	442	*Vishniacozyma victoriae*
		840	12941	*Thelebolus globosus* (MH862951)[b]	100	100	413	*Thelebolus globosus*

Base	120	12855	*Cladosporium cladosporioides* (NR119839)[b]	100	99	430	*Cladosporium* sp. 3
			Cladosporium cladosporioides (MH780075)[d]	99	98	282	
			Cladosporium cladosporioides (KX288432)[e]	100	96	922	
	1080	12935	*Cladosporium psychrotolerans* (NR119607)[b]	98	99	426	*Cladosporium* sp. 4
			Cladosporium psychrotolerans (KJ596614)[d]	96	90	304	
	120	12807	*Cladosporium scabrellum* (MH863934)[b]	100	99	478	*Cladosporium* sp. 5
	40	12853	*Cladosporium halotolerans* (MH865505)[b]	100	98	297	*Cladosporium* sp. 6
			Cladosporium halotolerans (EF101424)[d]	99	87	286	
	80	12914	*Cladosporium welwitschiicola* (NR152308)[b]	100	100	435	*Cladosporium* sp. 7
	320	27	*Cystobasidium laryngis* (KY103134)[c]	100	99	532	*Cystobasidium laryngis*
	240	18	*Dioszegia fristingensis* (KY107641)[c]	100	99	550	*Dioszegia fristingensis*
	40	65	*Dioszegia hungarica* (KY107643)[c]	100	99	570	*Dioszegia hungarica*
	40	9	*Kondoa changbaiensis* (NG060244)[c]	100	99	459	*Kondoa changbaiensis*
	200	66	*Kondoa malvinella* (NG057733)[c]	100	99	538	*Kondoa malvinella*
	40	2	*Kondoa subrosea* (KY108163)[c]	100	99	318	*Kondoa subrosea*
	200	12804	*Cadophora luteo-olivacea* (GU128588)[b]	90	85	437	*Helotiales* sp. 3
	280	12967	*Acremonium biseptum* (NR159609)[b]	99	95	455	*Hypocreales* sp.
	40	7	*Leucosporidium creatinivorum* (KY108277)[c]	100	99	506	*Leucosporidium* sp. 1
	120	12805	*Penicillium glabrum* (MH854998)[b]	100	100	458	*Penicillium glabrum*
			Penicillium glabrum (GU981619)[d]	100	98	356	
	280	12854	*Penicillium solitum* (NR119494)[b]	100	99	462	*Penicillium solitum*
			Penicillium solitum (AF000934)[d]	100	98	234	
			Penicillium solitum (KU904363)[e]	100	99	693	
	40	12913	*Cosmospora butyri* (MH855967)[b]	100	100	326	*Pezizomycotina* sp.
	40	3	*Phenoliferia glacialis* (KY108773)[c]	100	100	468	*Phenoliferia glacialis*
	40	12971	*Phialophora alba* (MH859216)[b]	100	99	332	*Phialophora alba*
	320	12	*Piskurozyma fildesensis* (KC894160)[c]	100	99	508	*Piskurozyma fildesensis*
	280	12927	*Pseudeurotium desertorum* (AY129288)[b]	100	95	427	*Pseudeurotium* sp.
	240	12803	*Pseudeurotium hygrophilum* (NR111128)[b]	96	100	441	*Pseudeurotium hygrophilum*
	80	12858	*Geomyces destructans* (EU884921)[b]	100	99	409	*Pseudogymnoascus destructans*
	40	12912	*Sarocladium dejongiae* (MK069419)[b]	100	100	354	*Sarocladium dejongiae*
	80	60	*Sporidiobolus pararoseus* (KY109718)[c]	100	99	490	*Sporobolomyces pararoseus*
	200	12924	*Thelebolus globosus* (MH862951)[b]	100	100	348	*Thelebolus globosus*
	160	47	*Vishniacozyma carnescens* (KY110023)[c]	100	99	520	*Vishniacozyma carnescens*
	240	42	*Vishniacozyma victoriae* (NG057678)[c]	100	100	449	*Vishniacozyma victoriae*

Central Lake	Top	40	12975	*Cladosporium ruguloflabelliforme* (KT600458)[b]	100	100	468	*Cladosporium* sp. 8
		280	12917	*Tetracladium globosum* (JX029133)[b]	100	100	251	*Tetracladium globosum*
		440	12860	*Acremonium biseptum* (NR159609)[b]	100	94	423	*Hypocreales* sp.
		560	12812	*Neobulgaria premnophila* (MH861260)[b]	100	98	402	*Neobulgaria* sp. 2
		40	12867	*Penicillium glabrum* (GU981567)[b]	98	100	459	*Penicillium* sp. 1
				Penicillium glabrum (GU981619)[d]	96	93	246	
		40	12920	*Penicillium chrysogenum* (MH856357)[b]	100	100	462	*Penicillium* sp. 2
				Penicillium chrysogenum (AY495981)[d]	100	96	293	
		40	12976	*Pholiota baeosperma* (KY559332)[b]	100	100	488	*Pholiota baeosperma*
		80	12811	*Pseudeurotium hygrophilum* (NR111128)[b]	100	100	419	*Pseudeurotium hygrophilum*
		160	12918	*Pseudogymnoascus roseus* (MH858631)[b]	100	99	368	*Pseudogymnoascus roseus*
		280	12861	*Geomyces destructans* (EU884921)[b]	100	99	440	*Pseudogymnoascus destructans*
	Middle	160	12931	*Antarctomyces pellizariae* (KX576510)[b]	100	100	445	*Antarctomyces pellizariae*
		40	12875	*Cladosporium tenuissimum* (MH864840)[b]	100	100	359	*Cladosporium* sp. 2
		120	12872	*Tetracladium globosum* (JX029133)[b]	100	100	435	*Tetracladium globosum*
		80	12977	*Acremonium biseptum* (NR159609)[b]	99	94	501	*Hypocreales* sp.
		120	12870	*Mortierella antarctica* (JX975907)[b]	100	99	502	*Mortierella antarctica*
		200	12817	*Neobulgaria premnophila* (MH861260)[b]	100	97	469	*Neobulgaria s*p. 2
		40	12876	*Penicillium chrysogenum* (MH856357)[b]	100	100	476	*Penicillium crysogenum*
				Penicillium chrysogenum (AY495981)[d]	100	98	372	
				Penicillium chrysogenum (JX996668)[e]	100	99	395	
		80	12928	*Pseudeurotium hygrophilum* (NR111128)[b]	100	100	375	*Pseudeurotium hygrophilum*
		160	12871	*Pseudogymnoascus verrucosus* (KJ755525)[b]	100	99	385	*Pseudogymnoascus verrucosus*
South Lake	Top	40	12896	*Antarctomyces pellizariae* (KX576510)[b]	100	99	413	*Antarctomyces pellizariae*
		80	12776	*Cladosporium tenuissimum* (MH864840)[b]	100	100	425	*Cladosporium* sp. 2
				Cladosporium langeronii (EF101435)[d]	100	89	303	
		40	68	*Cystobasidium ongulense* (LC203680)[c]	100	100	744	*Cystobasidium ongulense*
		40	36	*Glaciozyma antarctica* (KY107752)[c]	100	99	510	*Glaciozyma antarctica*
		320	12952	*Glaciozyma watsonii* (NG058294)[c]	100	98	430	*Glaciozyma* sp. 2
				Glaciozyma martinii (KY103469)[b]	73	94	497	
		40	44	*Phenoliferia psychrophenolica* (KY108774)[c]	100	96	452	*Kriegeriales* sp.
		40	33	*Mrakia psychrophila* (KY108586)[c]	100	100	491	*Mrakia psychrophila*
		920	12771	*Penicillium glabrum* (GU981567)[b]	98	100	458	*Penicillium* cf *glabrum*
				Penicillium glabrum (GU981619)[d]	100	99	285	

	560	12824	*Penicillium solitum* (NR119494)[b]	100	99	464	*Penicillium solitum*
			Penicillium solitum (AY674356)[d]	95	94	211	
			Penicillium solitum (KU904363)[e]	100	100	370	
	40	12889	*Periconia byssoides* (MH859902)[b]	100	100	397	*Periconia byssoides*
	40	12775	*Polypaecilum botryoides* (MH854756)[b]	100	98	354	*Polypaecilum* sp.
	1200	12954	*Pseudeurotium desertorum* (AY129288)[b]	100	96	326	*Pseudeurotium* sp.
	1200	12769	*Pseudeurotium hygrophilum* (NR111128)[b]	100	100	375	*Pseudeurotium hygrophilum*
	280	12890	*Geomyces destructans* (EU884921)[b]	100	99	405	*Pseudogymnoascus destructans*
	3960	12770	*Pseudogymnoascus verrucosus* (KJ755525)[b]	100	99	407	*Pseudogymnoascus verrucosus*
	120	12893	*Thelebolus globosus* (MH862951)[b]	96	100	443	*Thelebolus globosus*
Middle	280	12956	*Glaciozyma watsonii* (NG058294)[c]	100	98	431	*Glaciozyma* sp. 2
			Glaciozyma martinii (KY103469)[b]	80	94	429	
	120	4	*Glaciozyma antarctica* (NG057664)[c]	100	99	500	*Glaciozyma antarctica*
	80	37	*Glaciozyma martinii* (KY107755)[c]	100	99	524	*Glaciozyma martinii*
	160	12949	*Leucosporidium intermedium* (KY104188)[b]	98	91	501	*Leucosporidium* sp. 2
	40	32	*Mrakia blollopis* (NG057710)[c]	100	99	444	*Mrakia* sp.
	80	12783	*Patinella hyalophaea* (KT876978)[b]	100	99	446	*Patinella hyalophaea*
	80	12884	*Penicillium spinuloramigenum* (MH857886)[b]	100	100	490	*Penicillium* sp. 3
	80	12781	*Pseudeurotium desertorum* (AY129288)[b]	100	95	409	*Pseudeurotium* sp.
	3520	12834	*Pseudeurotium hygrophilum* (NR111128)[b]	100	100	390	*Pseudeurotium hygrophilum*
	1520	12835	*Pseudogymnoascus destructans* (NR111838)[b]	100	99	393	*Pseudogymnoascus destructans*
	80	12789	*Pseudogymnoascus roseus* (MH858631)[b]	98	100	442	*Pseudogymnoascus roseus*
	200	12784	*Pseudogymnoascus verrucosus* (KJ755525)[b]	100	99	482	*Pseudogymnoascus verrucosus*
	40	12881	*Thelebolus globosus* (MH862951)[b]	100	100	432	*Thelebolus globosus*
Base	160	12840	*Patinella hyalophaea* (KT876978)[b]	100	100	431	*Patinella hyalophaea*
	40	12899	*Penicillium glabrum* (GU981567)[b]	99	99	453	*Penicillium* cf *glabrum*
			Penicillium glabrum (GU981619)[d]	100	99	283	
	640	12792	*Pseudeurotium hygrophilum* (NR111128)[b]	99	100	429	*Pseudeurotium hygrophilum*

[a]UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. Taxa subjected to BLAST analysis based on the [b]ITS, [c]D1-D2 domain, [d]β-tubulin, and [e]Polymerase 2. [f]Taxonomy suggested.

Table S2. Frequency of fungal isolation in lakes of the Fildes Peninsula.

Lake	Section	N° of isolates [Relative Frequency (%)]			
		Ascomycota	*Basidiomycota*	*Mortierellomycota*	*Incertae sedis*
North	Top	37 (14.2)	14 (5.4)	-	-
	Base	33 (12.7)	38 (14.6)	-	-
Central	Top	19 (7.3)	1 (0.4)	-	-
	Middle	20 (7.7)	-	3 (1.2)	-
South	Top	33 (12.7)	6 (2.3)	-	1 (0.4)
	Middle	29 (11.2)	14 (5.4)	-	-
	Base	12 (4.6)	-	-	-

(-) No fungi obtained.

Table 2. Biological activities of the extracts obtained from fungal species from lakes of Peninsula Fildes.

Taxa	UFMGCB[a]	Biological activities			
		Herbicidal[b]		Antifungic[c]	Antiparasitic[d]
		A. schoenoprasum	*L. sativa*	*C. sphaerospermum*	*T. cruzi*
Cladosporium sp. 2	12895	1 ± 0.5	**5 ± 0**	22.6 ± 9	0 ± 0
Cladosporium sp. 2	12875	0 ± 0	0 ± 0	**83 ± 25**	0 ± 0
Cladosporium sp. 6	12853	0 ± 0	**4.5 ± 0.5**	45.7 ± 2	0 ± 0
Cystobasidium laryngis	57	0 ± 0	**3.5 ± 0.5**	0 ± 0	12 ± 9.8
C. laryngis	26	0 ± 0	**4.5 ± 0.5**	0 ± 0	26.9 ± 0.7
C. laryngis	52	0 ± 0	**4 ± 0**	0 ± 0	0 ± 0
C. laryngis	58	0 ± 0	**3 ± 0**	0 ± 0	0 ± 0
Dioszegia fristingensis	18	1 ± 0.5	**3 ± 0**	0 ± 0	0 ± 0
Gyoerffyella sp.	12849	1 ± 0.5	**3 ± 1**	22.9 ± 2	0 ± 0
Hypocreales sp.	12964	0 ± 0	**3 ± 0**	0 ± 0	0 ± 0
Kondoa malvinella	22	1.5 ± 0.5	**3 ± 0**	0 ± 0	15.9 ± 1.6
K. malvinella	66	0 ± 0	**3 ± 1**	0 ± 0	12.4 ± 13
Helotiales sp. 2	12960	0 ± 0	**3 ± 1**	33.7 ± 28	0 ± 0
Mortierella antarctica	12819	**3 ± 0**	1 ± 0	0 ± 0	14.9 ± 29.1
Mrakia robertii	24	1 ± 0.5	**4 ± 1**	0 ± 0	12.9 ± 16
Patinella hyalophaea	12833	0 ± 0	**4 ± 1**	0 ± 0	2.3 ± 4.6
Penicillium glabrum	12805	0 ± 0	2 ± 0.5	**84 ± 3**	0 ± 0
P. glabrum	12820	**3 ± 0**	2.5 ± 1	0 ± 0	17.6 ± 3.6
Penicillium solitum	12824	0 ± 0	**3.5 ± 0.5**	**70 ± 30**	3.4 ± 21.1
P. solitum	12859	**2.5 ± 0.5**	1 ± 0	**70 ± 5**	0 ± 0
Phenoliferia glacialis	64	1 ± 0	**3 ± 0**	0 ± 0	2.3 ± 4.6
P. glacialis	25	1.5 ± 0.5	**4.5 ± 0.5**	0 ± 0	28.6 ± 0.1
Piskurozyma fildesensis	11	0 ± 0	**3 ± 1**	0 ± 0	24 ± 16
P. fildesensis	12	0 ± 0	**3.5 ± 0.5**	0 ± 0	10.6 ± 13.4
P. fildesensis	53	0 ± 0	**4 ± 0**	0 ± 0	0 ± 0

P. fildesensis	10	0 ± 0	**4 ± 1**	0 ± 0	0 ± 0
Pseudeurotium hygrophilum	12801	0 ± 0	0 ± 0	0 ± 0	**44 ± 40.5**
P. hygrophilum	12795	0 ± 0	**3 ± 1**	0 ± 0	23.9 ± 1
P. hygrophilum	12800	0 ± 0	2.5 ± 0.5	68.1 ± 10	**43 ± 35**
P. hygrophilum	12802	0 ± 0	2 ± 1	**74 ± 16**	0 ± 0
P. hygrophilum	12811	1.5 ± 0.5	0 ± 0	**72 ± 15**	9.8 ± 25.9
P. hygrophilum	12847	**3 ± 0**	**3 ± 0**	0 ± 0	0 ± 0
P. hygrophilum	12848	**3 ± 0**	1 ± 0.5	0 ± 0	0 ± 0
Pseudeurotium sp.	12940	1 ± 0	1 ± 0.5	**76 ± 28**	0 ± 0
Pseudogymnoascus destructans	12886	1 ± 0	**4 ± 1**	0 ± 0	24.1 ± 0
Pseudogymnoascus verrucosus	12774	0 ± 0	**3 ± 0**	20.7 ± 8	0 ± 0
P. verrucosus	12822	0 ± 0	**3 ± 0**	0 ± 0	28.3 ± 18.6
P. verrucosus	12892	3.5 ± 1	**4 ± 0**	0 ± 0	4.8 ± 0
P. verrucosus	12825	1.5 ± 0.5	**3.5 ± 0.5**	0 ± 0	11.6 ± 10.9
P. verrucosus	12827	0 ± 0	**3 ± 1**	0 ± 0	0 ± 0
Control drugs	Glyphosate	**5 ± 0**	**5 ± 0.5**	-	-
	Benomyl	-	-	**100 ± 1.9**	-
	BenZ	-	-	-	**79 ± 8**

[a]UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. [b]Results of effective extract with inhibition of ≥70% of *Cladosporium sphaerospermum*. [c]The qualitative estimate of phytotoxicity was evaluated by using a rating scale of 0-5, where 0 = no effect and 5 = no growth or no germination of the seeds. Plant targets: *L sativa* = *Lactuca sativa* (lettuce), *A. schoenoprasum* = *Allium schoenoprasum* (chive). [d]Results of effective extract with inhibition of ≥40% of *Trypanossoma cruzi*. Activity values for the bioactive isolates are in bold.

A

Lake	Phylum	Class	Order	Family	Genus
North	*Ascomycota*	*Dothideomycetes*	*Capnodiales*	*Cladosporiaceae*	*Cladosporium*
		Eurotyomycetes	*Chaetothyriales*	*Herpotrichiellaceae*	*Phialophora*
			Eurotiales	*Aspergillaceae*	*Penicillium*
		Incertae sedis	*Incertae sedis*	*Incertae sedis*	*Gyoerffyella*
		Leotiomycetes	*Helotiales*	*Helotiaceae*	*Neobulgaria*
			Incertae sedis	*Myxotrichaceae*	*Pseudogymnoascus*
				Pseudeurotiaceae	*Pseudeurotium*
			Thelebolales	*Thelebolaceae*	*Thelebolus*
		Sordariomycetes	*Hypocreales*	*Sarocladiaceae*	*Sarocladium*
	Basidiomycota	*Agaricostilbomycetes*	*Agaricostilbales*	*Kondoaceae*	*Kondoa*
		Cystobasidiomycetes	*Cystobasidiales*	*Cystobasidiaceae*	*Cystobasidium*
		Microbotryomycetes	*Kriegeriales*	*Camptobasidiaceae*	*Glaciozyma*
				Kriegeriaceae	*Phenoliferia*
			Leucosporidiales	*Incertae sedis*	*Leucosporidium*
			Sporidiobolales	*Sporidiobolaceae*	*Sporobolomyces*
		Tremellomycetes	*Cystofilobasidiales*	*Cystofilobasidiaceae*	*Mrakia*
			Filobasidiales	*Piskurozymaceae*	*Piskurozyma*
			Tremellales	*Bulleribasidiaceae*	*Vishniacozyma*
				Tremellaceae	*Dioszegia*
Central	*Ascomycota*	*Dothideomycetes*	*Capnodiales*	*Cladosporiaceae*	*Cladosporium*
		Eurotyomycetes	*Eurotiales*	*Aspergillaceae*	*Penicillium*
		Leotiomycetes	*Helotiales*	*Helotiaceae*	*Neobulgaria*
				Incertae sedis	*Tetracladium*
			Incertae sedis	*Myxotrichaceae*	*Pseudeurotium*
					Pseudogymnoascus
			Thelebolales	*Thelebolaceae*	*Antarctomyces*
	Basidiomycota	*Agaricomycetes*	*Agaricales*	*Strophariaceae*	*Pholiota*
	Mortierellomycota	*Mortierellomycetes*	*Mortierellales*	*Mortierellaceae*	*Mortierella*
South	*Ascomycota*	*Dothideomycetes*	*Capnodiales*	*Cladosporiaceae*	*Cladosporium*
			Pleosporales	*Periconiaceae*	*Periconia*
		Eurotyomycetes	*Eurotiales*	*Aspergillaceae*	*Penicillium*
		Leotiomycetes	*Helotiales*	*Dermateaceae*	*Patinella*
			Incertae sedis	*Myxotrichaceae*	*Pseudogymnoascus*
				Pseudeurotiaceae	*Pseudeurotium*
			Thelebolales	*Thelebolaceae*	*Antarctomyces*
					Thelebolus
	Basidiomycota	*Cystobasidiomycetes*	*Cystobasidiales*	*Cystobasidiaceae*	*Cystobasidium*
		Microbotryomycetes	*Kriegeriales*	*Camptobasidiaceae*	*Glaciozyma*
			Leucosporidiales	*Incertae sedis*	*Leucosporidium*
		Tremellomycetes	*Cystofilobasidiales*	*Cystofilobasidiaceae*	*Mrakia*
	Incertae sedis	*Incertae sedis*	*Incertae sedis*	*Thermoascaceae*	*Polypaecilum*

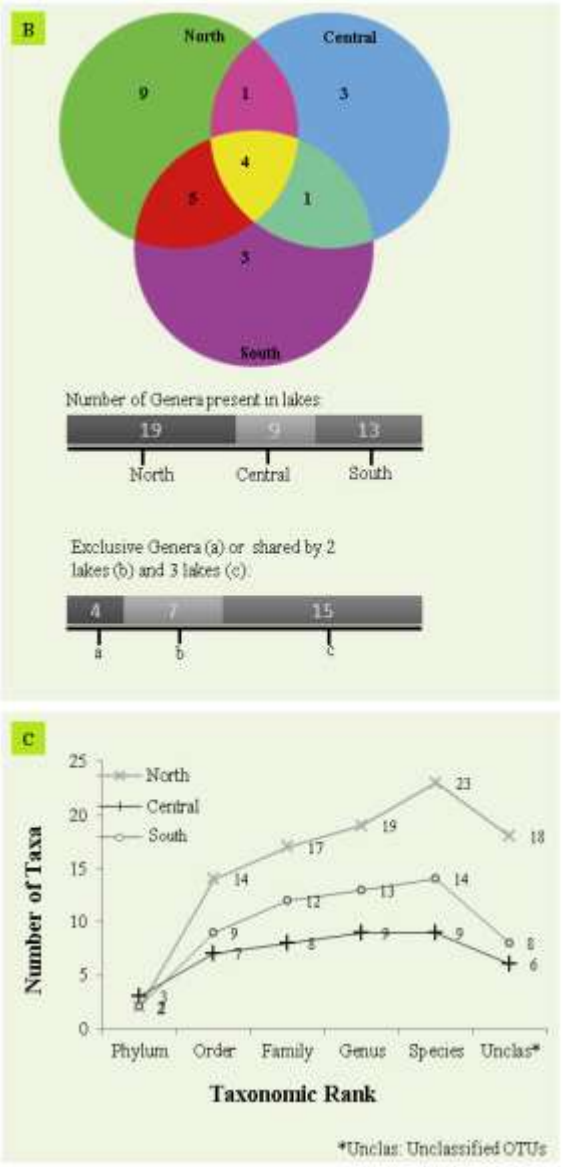


Figure S2. Taxonomy of fungi OTUs identified from lake sediments of Fildes Peninsula (a), Diagram of Venn with genera shared or exclusive identified in the North, Central and South Lakes (b), and the taxonomic rank for the OTUs identified and unclassified.

Table S3. Physico-chemical parameters of sediments and diversity indices of fungal communities associated with lake sediments of Fildes Peninsula, Kings George Island, Antarctica.

Parameters	Lakes of Fildes Peninsula		
	North	Central	South
% Humidity	20.2	18.1	19.1
Metals and solutes (mg kg^{-1})			
Aluminium	29527	54534	44864
Arsenic	4.79	0.59	1.4
Cadmium	1.57	1.89	1.84
Calcium	13068	29754	21426
Lead	2.8	1.24	2.11
Chromium	10.7	51.4	9.9
Phosphorus	524	238	377
Magnesium	9438	30087	13571
Mercury	0.11	0.11	0.09
Molybdenum	<1	<1	<1
Potassium	4494	1692	2657
Sodium	3524	5095	7536
Number of taxa	41	15	22
Number of isolates	122	43	95
Total fungal density (CFU g^{-1})	13020	2960	16040
Fisher-α (diversity)	5.25	2.06	2.51
Margalef's (richness)	4.22	1.75	2.17
Simpson's (dominance)	0.94	0.86	0.8

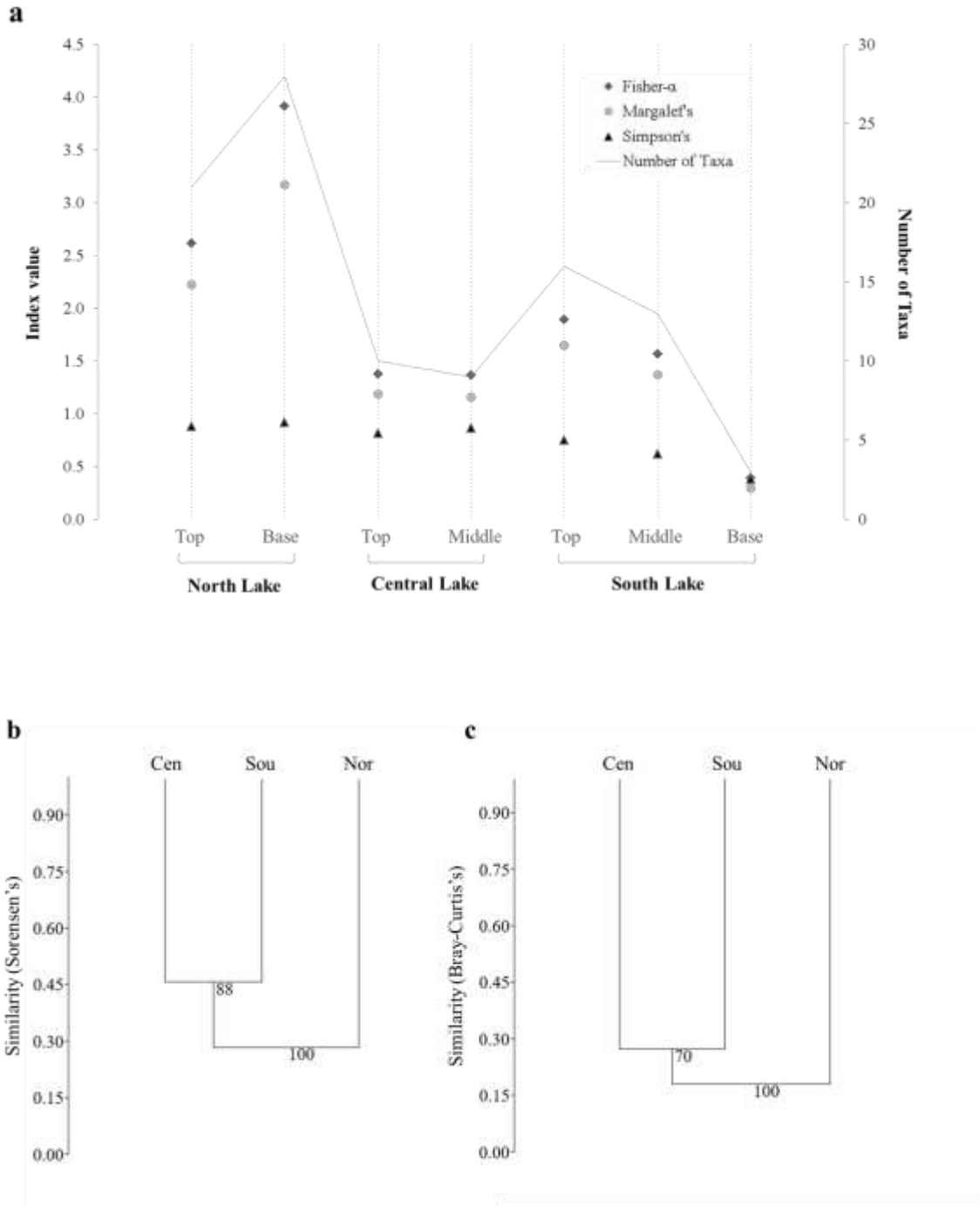


Figure S3. Diversity indices of fungal assemblages associated with lake sediment samples of Fildes Peninsula, King George Island, Antarctica. Diversity, richness and dominance indices for each section of the sediment core (a), and dendrograms showing Sorensen's (b) and Bray-Curtis's (c) similarity measures for the fungal communities associated with lake samples. The results were obtained with 95% confidence and bootstrap values calculated from 1000 iterations. Cen, Central; Sou, South and Nor, North.

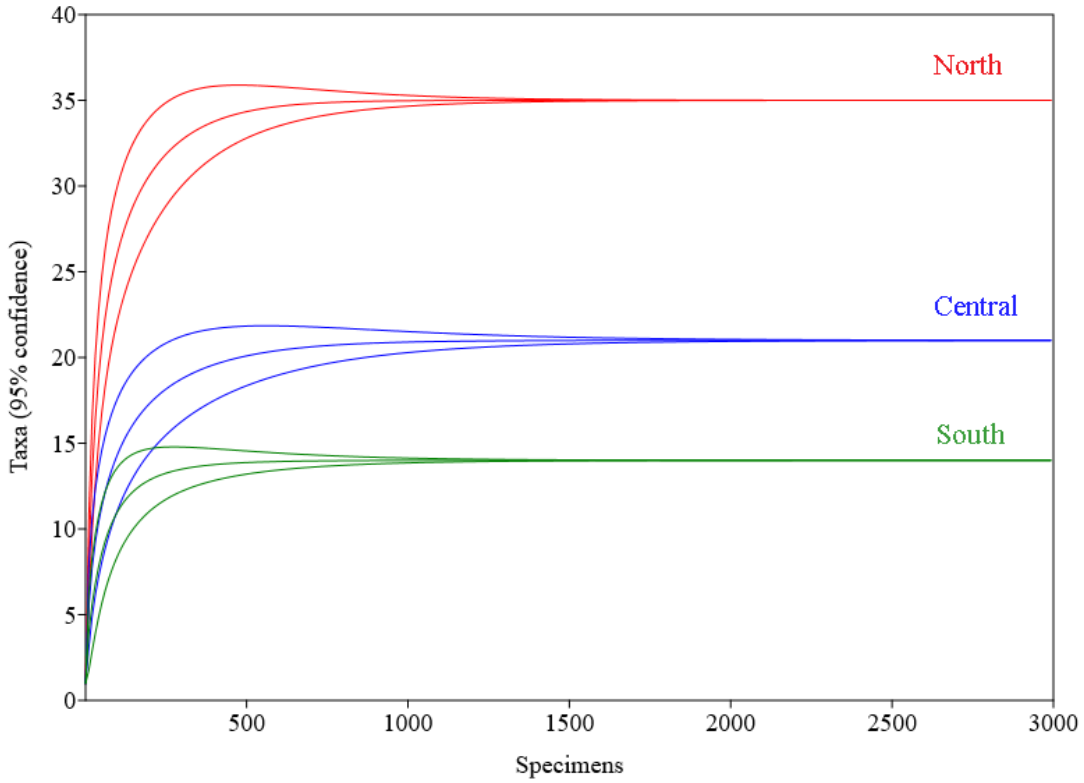


Figure S4. Species accumulation curve for the fungal assemblages associated with lake sediments of Fildes Peninsula, King George Island, Antarctica.

5.5 Capítulo 5. "Diversity and bioprospection of cultivable fungal community in sediments of lakes at Antarctic Peninsula."

Artigo formatado nas normas da revista: FEMS Microbiology Ecology.

Diversity and bioprospection of cultivable fungal community in sediments of lakes at Antarctic Peninsula

Mayara B. Ogaki[1], Daniela R. Teixeira[1], Rosemary Vieira[2], Juan M. Lírio[3], João P. Felizardo[2], Rodrigo C. Abuchacra[4], Renan P. Cardoso[5], Carlos L. Zani[6], Tânia M.A. Alves[6], Policarpo A.S. Junior[6], Silvane M.F. Murta[6], Emerson C. Barbosa[6], Jaquelline G. Oliveira[6], Isabela P. Ceravolo[6], Patrícia O. Pereira[6], Carlos A. Rosa[1], and Luiz H. Rosa[1]*

[1]Departamento de Microbiologia, Universidade Federal de Minas Gerais, MG, Brasil
[2]Instituto de Geociências, Universidade Federal Fluminense, RJ, Brasil
[3]Instituto Antártico Argentino, Buenos Aires, Argentina
[4]Departamento de Geografia, Universidade do Estado do Rio de Janeiro, RJ, Brasil
[5]Instituto de Física, Universidade Federal Fluminense, RJ, Brasil
[6]Instituto René Rachou, FIOCRUZ-Minas, MG, Brasil

*Corresponding author. Departamento de Microbiologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, CEP 31270-901. Tel.: +55-31-3409 2749; Fax: +55-31-3409 2730, Brazil. E-mail: lhrosa@icb.ufmg.br

Running title: Fungal diversity in lakes of Antarctica

Abstract

We recovered in the sediments of different lakes of Antarctic Peninsula 195 fungal isolates, which were identified as 42 taxa of of the phyla *Ascomycota*, *Basidiomycota*, and *Mortierellomycota*. *Thelebolus globosus*, *Antarctomyces psychrotrophicus*, *Pseudogymnoascus verrucosus*, *Vishniacozyma victoriae*, and *Phenoliferia* sp. were the most prevalent taxa obtained. The fungal assemblages showed high diversity and richness, but low dominance values. However, the diversity indices and fungal distribution ranged according to

the different lakes sediment. Sixty fungal extracts displayed at least one biological activity against the targets evaluated. Among them, *Pseudogymnoascus destructans* showed selective trypanocidal activity, *Cladosporium* sp. 1 and *Trichoderma polysporum* antifungal activity, and *Pseudogymnoascus appendiculatus* and *Helotiales* sp. showed the high herbicidal. We detected in the sediments of Antarctic lakes a rich and diverse fungal community composed by cold cosmopolitan and psychrophilic endemic taxa recognized as decomposer, symbiotics, pathogens, and potential new species. The dynamic and balance of this fungal community represent an interesting aquatic web model for further ecological and evolutionary studies under extreme conditions and potential climate changes in the regions. In addition, we detected fungal taxa and isolates able to produce bioactive compounds, which may represent source of prototype molecules to use in medicine and agriculture.

Keywords: Antarctica; bioprospecting; ecology; fungi; lakes; sediments; taxonomy

Introduction

Despite the extreme conditions, Antactic environments are characterized to shelter in its different habitats several life forms. Among these Antarctic environments, lagoons, streams, and lakes are prevalent in many coastal and inland locations, spanning a range of different ecosystems, from melting waters to hypersaline salines (Kirby et al. 2012). In Antarctica, there are different freshwater ecosystems such as lagoons, lakes, and saline lakes in ice-free oasis areas in maritime and continental regions (Vincent et al. 2008). Lakes and ponds are considered typical lentic habitats (regardless of climate zone) that comprise any natural aquatic environment without continuous water flow (Wong et al. 1998), where low-energy habitats can provide calm environments in which different microbial life forms can arrive, grow, and colonize (Sparrow, 1968). Sattler and Storrie-Lombardi (2009) suggested that the evolution of lake microbial communities begun in the air and that the interface air/water, named as extra-aquatic zone, represents important sites for the study of microbial diversity, which may explain the presence of a considerable number of cosmopolitan species already reported on lakes. The air transport is probably the most important dispersal route of the microorganisms, including fungi propagules, which can be transported over significant distances by wind and their distribution on the continent is constructed by low levels of airborne particles (Sattler and Storrie-Lombardi, 2009).

Fungi occur in different ecosystems in Antarctica and those communities in lakes are considered diverse, despite de extreme conditions which are expose and represented by taxa

of the phyla such as *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Murocomycota*, *Chytridiomycota*, and allied species of *Oomycot*a (Rosa et al. 2019; Ogaki et al. 2019). Fungi have been isolated from lakes sediment in Antarctica (Goto et al. 1969, McInnes, 2003, Tsuji et al. 2013, Gonçalves et al. 2015); however, the complete knowledge about the diversity, richness, and ecologigal role of fungi in sediment remains little known. In lakes of Antarctica, fungi appear to be the dominant organisms involved in the decomposition of diffferent organic matter orgined from plants, lichens, and animals (Wong et al. 1998) by the producer of different degradative enzymes (Martorell et al. 2019). In addition, fungi in lake ecosystems are able to produce bioactive secondary metabolites (Brunati et al. 2009, Gonçalves et al. 2015, Tsuji et al. 2013, Tsuji et al. 2016), which increase their capability to compete and survive in the complexe lakes environments ecosystems.

According to the panorama descrive above, studies involving fungal communities present in sediments of Antarctic lakes can contribute with knowledge of diversity and ecology of freshwater environments in Antarctica and to the discovery of species and strains potentially producer of bioactive compounds. In the present study, we characterized the diversity of culturable fungal community present in lacustrine lake sediments at different points of Antarctic Peninsula to search species able to produce bioactive compounds.

Materials and methods

Collecting samples

The lake sediments were collected in eight lakes of different islands of the Antarctic Peninsula (Table S1), during austral summer of 2016 in Brazilian Antarctic Operation XXXV. Samples were collected manually using PVC pipes (60 mm of diameter x 50 cm of height), duly disinfested to avoid contamination. Pipes were washed with 1.5% sodium hypochlorite solution for 10 min, 70% ethanol solution for 5 min and three washes with distilled water, after drying in laminar flow under UV light. The pipes were kept in sterile bags until the moment of use. The obtained cores were sectioned into 5 cm fractions (top, middle and base), depending of the total size of the core obtained (Table S1). Tree subsamples (A, B, and C) were obtained of the central parts of each core, under strict contamination control conditions, to avoid contamination, The subsamples were suitably conditioned at -20 °C until processing to isolate fungi.

Fungi Isolation

To isolate fungi in the lacustrine sediment, 1 g of each subsampled sediment was resuspended in sterilized 1 mL of 0.85% NaCl, and 100 μL of each suspension were plated in the media: DRBC - Dichloran Rose Bengal agar (peptona 0.5 %, glicose 1%, KH2PO4 0.1%, $MgSO_4.7H_2O$ 0.05%, dichloran 0.0002%, rose bengal 0.0025%, and agar 2%), MEA - Malt extract agar (malt extract 5 % and agar 2%) and MM - Minimal media (peptone 0.025%, glucose 0.5%, K_2HPO_4 0.698%, KH_2PO_4 0.544%, $(NH_4)_2SO_4$ 0.1%, $MgSO_4$ $7H_2O$ 0.11%, and agar 2%). In all media were included 100 mg mL^{-1} of chloramphenicol (Sigma, USA) to prevent bacterial growth. One hundred milliliters at the dillutin 10^{-1} dilution were inoculate the Petri dishes containing the culture media and the plates were incubed at 10 °C for 60 days. Fungal colony forming units (CFUs) were counted, and subcultures were made of all of the morphologically distinct colonies from each sample. The subcultures were grouped into different morphotypes according to their macroscopic parameters (colony color and texture, border type and radial growth rate) and micromorphological characteristics on the same isolation media, except for the isolates obtained from the DRBC, whose was done in the APG - Base agar (peptone 0.5%, glucose 1%, KH_2PO_4 0.1%, $MgSO_4.7H_2O$ 0.05%, and agar 2%). All fungal isolates were deposited in the Collection of Microorganisms and Cells of the Federal University of Minas Gerais under code UFMGCB.

Fungal identification

The protocol for DNA extraction was described previously in Rosa *et al*. (2009). For the filamentous fungi, the internal transcribed spacer (ITS) region was amplified with 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, amplification of the β-tubulin (Glass and Donaldson, 1995) and ribosomal polymerase II genes (RPB2) (Houbraken *et al*. 2012), which are commonly utilized to fungal taxa with low intraspecific variation, was completed with the Bt2a/Bt2b and RPB2-5F-Pc/RPB2-7CR-Pc 7CR primers, respectively, according to protocols established by Gonçalves *et al*. (2015). The yeasts were grouped and identified according to protocols established by Kurtzman *et al*. (2011). Yeast molecular identities were confirmed by sequencing the D1–D2 variable domains of the large–subunit rRNA gene using the primers NL1 and NL4 as described by Lachance *et al*. (1999). Fungi with query coverage and identity ≥ 99% were considered to represent the same taxon. Representative consensus sequences of the fungal taxa were deposited into the GenBank database (Table 1). To achieve species-rank identification based on ITS, β-tubulin data and

ribosomal polymerase B2, the consensus sequence was aligned with all sequences from related species retrieved from the NCBI GenBank database using BLAST (Altschul *et al*. 1997). Taxa that displayed query coverage and ≤98% identity or an inconclusive taxonomic position were subjected to phylogenetic ITS, β-tubulin, and ribosomal polymerase B2-based analysis for comparison with sequences of ex type species deposited in the GenBank database, with estimations conducted using MEGA Version 5.0 (Tamura *et al*. 2011). The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1,000 replicate runs. The information about fungal classification generally followed the dictionary of Kirk *et al*. (2008), and databases MycoBank (http://www.mycobank.org) and the Index Fungorum (http://www.indexfungorum.org).

Diversity of the fungal community

To quantify species diversity, richness, and dominance the indices Fisher's α, Margalef's, and Simpson's were used, respectively. The matrices of OTUs (Operational taxonomic units) abundances (calculated for each OTU from the fungal density of each lake) were used to perform the following multivariate analyses to estimated the similarities among fungal taxa from different lakes points using the Sorensen and Bray-Curtis coefficient. Species accumulation curves wer obtained using Mao Tao index. All results were obtained with 95% confidence, and bootstrap values were calculated from 1,000 replicates using the PAST computer program 1.90 (Hammer *et al*. 2001).

Fungal cultivation and extract production for biological assays

All fungal isolates were cultivated using solid-state fermentation. Briefly, the cultures were prepared by aseptically transferring three 5-mm diameter plugs of each fungal isolate on 10 Petri dishes (90 mm diameter) containing MA medium, followed by incubation for 15 days at 10 °C. The fungal cultures were cut in small pieces and transferred to 250 mL Erlenmeyers flasks followed by the addition of 100 mL of ethyl acetate (Synth, Brazil). After 72 h of decantation at room temperature, the organic phase was filtered and the solvent removed under rotary evaporation at 40 °C. An aliquot of each dried extract was dissolved in DMSO (Merck) to prepare a 100 mg mL^{-1} stock solution, which was stored at -20 °C. Sterile MA medium was extracted using the same procedure. The sterile MA extract was used as the control in the screening procedures.

Antimicrobial activity against Cladosporium sphaerospermum

Cladosporium sphaerospermum CCT 1740 was grown in BDA at 25 °C for 7 to 10 days. A spore loft was suspended in sterile 0.85% saline solution and homogenized on a vortex type stirrer for 15 seconds. The spore density of the suspension was adjusted to 86-88% transmittance at 620nm using a spectrophotometer (BioSpectro SP-22), corresponding to 10^6 spores mL^{-1} (NCCLS M38-A, v. 22, n ° 16). Subsequently, the suspension was diluted 50-fold in RPMI1640 culture medium (INLAB Diagnostic) for use in the assay. The extracts were diluted from a stock solution at 100 mg mL^{-1} dissolved in DMSO, and all tests were performed in duplicate. A total of 25 µL of the extract were diluted in autoclaved deionized water (at 1 mg mL^{-1}), 25 µL of the culture medium and 50 µL of the inoculum were inoculated into each well. The final volume of each well was 100 µL in the concentrations of 0.1% DMSO and 250 µg mL^{-1} extract. As a positive control, Benomyl at 1.16 µg mL^{-1} was used. Plates were placed on a shaker (Uniscience OS-10) for 20 minutes at 200 rpm. Subsequently, they were incubated at 25 °C for 48h. The fungi growth was analised by reading of the plates was carried out on a VERSAmax microplate reader (Molecular Devices) using Softmax® Pro 5 (Molecular Devices) software, by measuring the absorbance at 620 nm. The absorbance of the test wells was compared to the absorbance of the microorganism growth control without treatment. Extracts with an inhibition value greater than or equal to 60% were arbitrarily considered as active.

Assay against Trypanosoma cruzi

The *in vitro* test of trypanocidal activity was performed as previously described by Romanha *et al*. (2010), using *Trypanosoma cruzi* (Tulahuen strain) expressing the *Escherichia coli* β-galactosidase gene. Infective trypomastigote forms are obtained through culture in monolayers of mouse L929 fibroblasts in RPMI-1640 medium, without phenol red, containing 10% foetal bovine serum and 2 mM glutamine. For the bioassay, 4,000 L929 cells in 80 µL of supplemented medium are added to each well of a 96-well microtitre plate. After an overnight incubation, 40,000 trypomastigotes in 20 µL are added to the cells and the cells are incubated for 2 h. Medium containing parasites that did not penetrate the cells is replaced with 200 µL of fresh medium and the plate is incubated for an additional 48 h to establish infection. The medium is then replaced with solutions of natural products at 20 mg mL^{-1} in fresh medium (200 µL) and the plate is incubated for 96 h at 37°C. After this period, 50 µL of 500 µM chlorophenol red b-D-galactopyranoside in 0.5% Nonidet P40 is added to each well and the plate is incubated for 18 h at 37 °C, after which the absorbance at 570 nm is

measured. Controls with uninfected cells, untreated infected cells, infected cells treated with benznidazole at 3.8 μM (positive control) or DMSO 1% are used. The results are expressed as the percentage of *T. cruzi* growth inhibition in compound-tested cells as compared to the infected cells and untreated cells. Duplicates are run in two different plates.

Assay against Leishmania amazonensis

Promastigotes of *Leishmania* (*Leishmania*) *amazonensis* (strain IFLA/BR/196/PH-8) obtained from lesions of infected hamsters were used to detect the leishmancidal activity according to protocols established by Callahan *et al*. (1997). Each extract was tested at concentration of 20 μg mL^{-1}. The results are expressed as percent inhibition in relation to controls without drugs. Amphotericin B at 0.2 μg mL^{-1} (Fungison® Bristol-Myers Squibb B, Brazil) was used as a positive drug control. All assays were performed in triplicate.

Activity against Plasmodium falciparum *blood parasites* in vitro

Initially, 20 μg mL^{-1} of each extract sample was placed into the *well* of the "U" bottom 96-wells plates and their efficacies were evaluated against the parasites, and repeated at least once. The sample that inhibited the growth of the parasite with an efficacy of ≥40%, compared to the control, without addition of drugs, was considered promising. The promising samples were then titrated and *t*he concentration at which growth inhibition of 50% of the parasites took place was used to determine the IC_{50} value. The activity of the compounds against the bloodborne form of the *P. falciparum* parasite was tested using a chloroquine-resistant and mefloquine-sensitive W2 clone (Oduola *et al*. 1988) cultured at 37 °C, as per the method described by Trager and Jensen (1976). The activity was measured using the SYBR test with the parasite suspension (0.5% parasitemia and 2% haematocrit), as described previously (Smilkstein *et al*. 2004). After 48 h at 37 °C, the culture supernatant was removed and replaced by 100 μL of the lysis buffer solution [Tris (20 mM; pH 7.5), EDTA (5 mM), saponin (0.008%; w/v), and Triton X-100 (0.08%; v/v)] followed by the addition of 0.2 μL mL^{-1} of SYBR Safe (Sigma-Aldrich, Carlsbad, CA, USA). The contents of the plate were transferred to a flat bottom plate and incubated in the dark for 30 min at 37 °C. The plate was read in a fluorometer (Synergy H4 Hibrid Reader, Biotek) with excitation and emission at 485 and 535 nm, respectively. In all the tests, the activities of the compounds were expressed as the 50% inhibitory concentration of the parasite growth (IC_{50}) compared to the drug-free controls and estimated using the curve-fitting software Origin 8.0 (OriginLab Corporation, Northampton, MA, USA). Chloroquine was used as antimalarial reference drug (IC_{50} ~ 100

ng mL^{-1}). Compounds with IC_{50} values bellow 10 μg mL^{-1}, between 10 e 20 μg mL^{-1} and above 20 μg mL^{-1} were considered to be active, partially active and inactive, respectively.

Toxic to a mammalian cell line

The monkey kidney cell line (BGM) (ATCC, Manassas, VA, USA) was used to conduct the cytotoxicity assays, and maintained as suggested by the manufactures. The cells were cultured in bottles of 75 cm^2 dimensions with RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 40 mg L^{-1} gentamicin in a 5% CO_2 atmosphere at 37 °C. For the *in vitro* tests, a confluent cell monolayer was trypsinized, distributed in a flat-bottomed 96-well plate (2×10^5 cells mL^{-1}), and incubated for 18 h at 37 °C to ensure cell adherence. The BGM cells were incubated with 20 μL of the drugs at different concentrations (≤1 mg mL^{-1}) for 24 h in 5% CO_2 at 37 °C. The cell viability was expressed as the percentage of control absorbance of the untreated cells after subtracting the appropriate background values. The drug lethal dose of cells was determined by conducting tests at least in duplicates to calculate the dose that killed 50% of the cells (MLD_{50}), as described by do Céu de Madureira *et al*. (2002). The neutral red uptake assay was used to evaluate the lysossomal integrity and distinguishes live cells from dead by its ability to incorporate the dye (Borenfreund *et al*. 1988). Briefly, 0.2 mL medium containing 50 μg mL^{-1} was added to each well. The plate was incubated for another 3 h at 37 °C to allow the uptake of the vital dye into the lysosomes of viable uninjured cells. After removal of the medium, 200 μL of a mixture of 0.5% formaldehyde and 1% $CaCl_2$ was added to the cells, and incubated for 5 min at 37 °C. The supernatant was removed and 100 μL of a solution of 1% acetic acid-50% ethanol was added to each well to extract the dye. After homogenization, the optical density of each well was measured at a wavelength of 540 nm using a spectrophotometer since this absorbance gives a linear relationship with the number of surviving cells. The ratio between drug cytotoxicity (MLD_{50} BGM) and activity (IC_{50} W2) was used to estimate the selective index (SI), as determined before (Bézivin *et al*. 2003), where SI ≤ 10 was indicative of toxicity.

Antiviral activity against the Dengue virus 2 and the Zika virus

For determining the antiviral activity against the Dengue virus 2 (DENV-2) and the Zika virus (ZIKV), monolayers of baby hamster kidney 21 (BHK-21) cells and Vero cells, respectively were used. Each cell was grown in flat bottom 96-well plates (Sarstedt, USA) using Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% fetal bovine serum (FBS), 100 U mL-1 penicillin, 100 μg mL^{-1} streptomycin, and 0.25 μg mL^{-1} amphotericin B

(all from Gibco, Thermo Sci, USA). All fungal extracts were provided at 20 mg mL^{-1} in an aqueous 90% DMSO solution. Viral suspensions with multiplicity of infection (m.o.i.) of 2 for both viruses and the extracts were simultaneously added to the plates, in duplicates. Cells were exposed to 20 µg mL^{-1} of fungal extracts for 72 h and 96 h with DENV-2 and ZIKV, respectively. Cell controls (uninfected untreated cells, with or without DMSO), virus controls (infected untreated cells, with or without DMSO) and antiviral positive control (infected cells treated with 300 and 600 UI mL^{-1} of interferon-α 2B - INREC, Uruguay) were run in parallel during each experiment. Additionally, all extracts were tested at the same concentration in the antiviral assays to verify their cytotoxicity against both cell lines. The antiviral activity was evaluated by the grading the peculiar cytopathic effect (CPE) caused by DENV-2 or ZIKV by optic microscopy, followed by the MTT colorimetric assay using the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT) (Sigma Aldrich, USA) according to Mossman (1983). The results were expressed as the percentage of viral inhibition in relation to the virus controls. All antiviral screening assays were repeated at least twice.

Herbicide assay

The herbicidal activities of fungal extracts at 1 mg mL^{-1} against *Lactuca sativa* (lettuce) and *Allium schoenoprasum* (chive) were measured using methods adapted from Dayan *et al*. (2000). Briefly, seeds of both plants were disinfected with ethanol 70% for 1 min, sodium hypochlorite at 2 -2.5% for 7.5 min, washed with sterilised distilled water four times, and dried on sterilized filter paper at room temperature. Herbicidal activity was quantitatively evaluated in 24-wells microtitre plate by visually comparing the amount of germination in each well between untreated and treat samples. Samples were incubated at 26 °C in a germination incubator 710 (Thoth, Brazil) under continuous light conditions for 10 days for *L. sativa* and 14 for *A. schoenoprasum*. Test samples were dissolved in acetone to a final concentration of 10% acetone. The control wells contained 400 µL of deionized water. The control + solvent well contained 360 µL of water and 40 µL of the solvent. Glyphosate at 3 mg mL^{-1} (Sigma, USA) was used as a positive herbicide control. All sample wells contained 360 µL of water and 40 µL of the appropriate dilution of the sample. A quantitative estimate of herbicidal activity was evaluated using a rating scale of 0-5, where 0 = no effect and 5 = no growth or no germination of the seeds. All assays were performed in duplicate.

Results

Sample processing and fungal isolation

On hundred ninty-five fungal isolates were reovered on the different culture media used. The DRBC medium provided the growth of a larger number of fungi (n = 70; 35.9%), followed by the MEA (n = 68; 34.9%), and MM (n = 57; 29.2%). The sediment sampled in lake of Elephant Island supplied the highest number of isolated fungi (32.8%), followed by the lakes of Penguin (20%), Punta Hennequin (16.9%), Deception A (10.3%), Wanda A (10.3%), Wanda B (5.1%) and Kroner (4.6%). The total number of isolates and the density of colony forming units (CFU g^{-1}) per sample are represented on Table S2.

Fungi identification

The 195 fungi isolates obtained were identify in 42 OTUs (Operational taxonomic units) (Table 1). The taxonomic rank and the hierarchical order levels of the OTUs were informed in Figure S1. Taxa of the phyla *Ascomycota*, *Basidiomycota*, and *Mortierellomycota* were detected in the lacustrine sediment samples. Eighteen OTUs, including *Pleosporales* sp., *Hypocreales* sp., *Helotiales* sp., *Myxotrichaceae* sp. and *Pezizomycotina* sp., showed low query cover and identity percentage when compared to the sequences of type species deposited in GenBank and more taxonomic studies will be necessary to determine if they represent new fungal species.

The distribution of the *Ascomycota* and *Basidiomycota* assemblages obtained in the sediment of all lakes were relatively similar. *Ascomycota* represented 47.2% of the taxa, followed by *Basidiomycota* 46.1%, and *Mortierellomycota* 6.7% (Table S3). *Ascomycota* was represented by the orders *Capnodiales*, *Eurotiales*, *Helotiales*, *Hypocreales*, *Onygenales*, *Pleosporales*, *Pleosporales*, *Sordariales*, *Thelebolales*, and *Taphrinales* (Figure S1).

Within the *Ascomycota*, *Thelebolus globosus* was the most frequent (10.2%), which occurred also in lake sediments of Punta Hannequin, Deception A, and Wanda A. *Antarctomyces psychrotrophicus* (5.6%) was detected in sediments of Elephant, Kroner, and Wanda A lakes and represented the second most frequent species, followed by *Pseudogymnoascus verrucosus* (5.1%) present in sediments of Punta Hennequin and Deception A lakes.

In the sediment lakes, *Basidiomycota* was represented only by yeasts of the orders *Cystofilobasidiales*, *Filobasidiales*, *Holtermanniales*, *Kriegeriales*, *Leucosporidiales*, and *Tremellales*. The most frequent taxa obtained was *Vishniacozyma victoriae* (9.2%), followed by *Phenoliferia* sp. (7.2%), and *Microbotryomycetes* sp. 2 (5.6%). In addition,

Mortierellomycota only the order Mortierellales (*Mortierella antarctica* and *Mortierela* spp. 1) was detected.

Diversity analysis of fungal assemblages

In general, the fungal assemblages showed high diversity (Fisher's α = 4.1) and richness (Margalef = 3.5), but low dominance (Simpson = 0.88) values. However, the diversity indices ranged according to the different lakes sediment samples (Table S2). The fungal assemblage of Penguin Lake showed higher values of diversity, richness, dominance in comparison with other lakes. In contrast, the fungal assemblage of Kroner Lake displayed the lowest values of diversity, richness, and cominance.

The taxa shared among sediment samples or exclusive samples are represented by the Figure S2. Thirteen taxa were shared among the lake sediments (iFigure S2b). In the sediment of Penguin lake we detected 11 unique taxa, followed by Elephant (n = 7), Wanda B, Wanda A, Kroner, Punta Hannequin (n = 2), and Deception A (n = 1). The Sorensen and Bray-Curtis similarities of the fungal assemblages among the lakes were low (Figure S2). The sites with the most similar species assemblages were Kroner (Deception Island) and Wanda (King George Island) lakes, both saline lakes with direct connection to seawater. In addition, the Mao Tao rarefaction curves reached an asymptote (Figure S3), which indicates that the diversity observed, considering density values of fungi obtained, reached the expected one.

Biological activities

Among all fungal extracts screened, sixty (30.8%) displayed at least one biological activity, including the target *T. cruzi* (trypanocidal), *C. sphaerospermum* (antifungal), or weeds (herbicidal) (Table 2). *Pezizomycotina* sp. UFMGCB 13963 and *Pseudogymnoascus destructans* UFMGCB 13934 showed trypanocidal activity. The isolate *Trichoderma polysporum* UFMGCB 13968 present antiplasmodial activity (46.8% of inhibition). Two isolates *Pezizomycotina* sp. UFMGCB 13963 and *Mortierella* sp. 1 UFMGCB 13012 presented moderate activity against *L. amazonenesis* (61.7 and 63.2 % of inhibition, respectively). Fourteen extracts showed antifungal activity against *C. sphaerospermum* and *Cladosporium* sp. 1 UFMGB 13968 and *Trichoderma polysporum* UFMGB 13968 showed the high values of inhibition, followed by extracts of *Pseudogymnoascus appendiculatus*, *P. destructans*, *Pseudogymnoascus verrucosus*, *M. antarctica*, *Mortierella* sp. 1, *Mortierella* sp. 2, and *T. globosus*. Fourty-six fungal extracts (23.6%) showed herbicidal activity at least one target evaluted. Among them, 37 inhibited the dicotyledon *L. sativa* and 11 the monocotiledon

A. schoenoprasum. *P. appendiculatus* UFMGB 13953 and *Helotiales* sp. UFMGB 13946 showed the highest herbicidal activities. No antiviral activity were detected.

Discussion

Fungal taxonomy, diversity, and occurrence

The phyla *Ascomycota*, *Basidiomycota*, and *Mortierellomycota* are commonly identified in different samples/habitats of Antarctica (Rosa *et al*. 2019). Among the taxa present in the sediments of Antarctic lakes, *T. globosus*, *A. psychrotrophicus*, *P. verrucosus*, *V. victoriae*, and *Phenoliferia* sp. were the most prevalent. Fourteen taxa occurred as singlets in the lake sediments and represented the minor component of the fungal community. In addition, thirteen fungal taxa were shared among lakes, including the endemic species *A. pellizariae*, *A. psychrotrophicus*, *G. antarctica*, *M. antarctica*, and *T. globosus*.

Thelebolus comprises twenty-one species (www.mycobank.org) and was previously described in lakes in Maritime and Continental Antarctica (de Hoog *et al*. 2005, Brunati *et al*. 2009, Gonçalves *et al*. 2012, Tsuji *et al*. 2013, Conell *et al*. 2018). Some of the *Thelebolus* psychophilic species were reported associated with ornithogenic Antarctic soils (del Frate and Caretta *et al*. 1990) and in the digestive tract of birds in Antarctica (Leotta *et al*. 2002), indicating that the species found in the continent still active in the digestive system before being dispersed in the environment (de Hoog *et al*. 2005). *T. globosus* was described for the first time in lakes of the Eastern and Southern Antarctic, with little or no bird visitation by de Hoog *et al*. (2005). According to authors, *T. globosus* does not have an ascospore dispersion mechanism; its simple conidia and its abundance of undifferentiated hyphae indicate that it can remain submerged in the lakes during most of its life cycle, which could explain the presence of the species in the basal layers of sediment. In addition, in three lakes where *T. globosus* was detected, occur the presence of *Stercorarius pomarinus* (skua), its remnants of feathers, bones and feces.

Antarctomyces includes only two species *A. psychrotrophicus* (Stchigel *et al*. 2001) and *A. pellizariae* (de Menezes *et al*. 2017), which were identified in the lake sediments evaluated in present study. *A. psychrotrophicus* was described in soil samples where the terrain is basaltic and metamorphosed rock in King George Island (Stchigel *et al*. 2001). In addition, *A. psychrotrophicus* was detected in lake closed to Stenhouse glacier at King George Island (Gonçalves *et al*. 2012). *P. verrucosus* was first described associated with *Sphagnum* mosses in bog of Canada (Rice and Currah, 2006). In Antactica, *P. verrucosus* already detected in association with plants and others substrates in extra-aquatic zones and may play important

role in the decomposition of organic matter in lakes (Ogaki *et al*. 2019b) and lake sediments (Ogaki *et al*. 2019c).

Vishniacozyma victoriae was frequently obtained from lichens, mosses, and vascular (Vasileva-Tonkova *et al*. 2014, Santiago *et al*. 2015, Duarte *et al*. 2016, Martorell *et al*. 2017, Ferreira *et al*. 2018). In Antarctic lakes, *V. victoriae* the species was isolated from sediments and water samples in King George Island (Vaz *et al*. 2011) and Fryzell in McMurdo Dry Valleys (Conell *et al*. 2018). *P. glacialis* and *P. psychrophenolica* are typical of cold environments (Buzzini *et al*. 2017). In Antarctica, *Phenoliferia* already isolated from soil and rocks near lichens, associated with vegetal substrates such as bryophytes and *D. antarctica* (Carrasco *et al*. 2012, Duarte *et al*.2013, Martorell *et al*. 2017, Ferreira *et al*. 2018). In aquatic ecosystems, *Phenoliferia* taxa were recovered from macroalgae (Duarte *et al*.2016), biofilm samples collected in the surface of puddles formed by ice defrosting (Ferreira *et al* 2018), and in lakes (Vaz *et al*. 2011; Tsuji *et al*. 2013).

Mortierella taxa are frequently isolated from different substrates of Antarctica such as permafrost (Gilichinsk *et al*. 2007), macroalgae thalli (Godinho *et al*. 2013), floating wood in seawater (Edgington *et al*. 2014), mosses (Melo *et al*. 2014), rizhosphere of *D. antarctica* and *C. quitensis* (Gonçalves *et al*. 2015, Wentzel *et al*. 2018), soil (Newshman *et al*. 2018) and marine invertebrates (Godinho *et al*. 2019). According to Rosa *et al*. (2019), *M. antarctica* is considered endemic in Antarctica. *M. antarctica* was discovered in Antarctic soils in Victoia Land, East Antarctica for the first time in the late 1960s (Zycha *et al*. 1969). In addition, Tosi *et al*. (2002) identified *M. antarctica* associated with different species of mosses sampled in Victoria Land. In South Shetlands, *M. antarctica* was isolated in association with the macroalgae *Pyropia endiviifolia* in Elephant island (Furbino *et al*. 2014) and in soil of Nelson and Robert islands (Gomes *et al*. 2018).

Thielavia antarctica and *Glaciozyma antarctica* are considered endemic of Antarctica and already described for terrestrial and aquatic ecosystems (Arenz *et al*. 2014). The species *T. antarctica* was first isolated from a sample of the lichen *Usnea aurantio-atra* collected on King George Island (Stchigel *et al*. 2003). *G. Antarctica* was reported in water and biomats lakes samples (Brunati *et al*. 2009, Tsuji *et al*. 2013, Conell *et al*. 2018) and soil (Carrasco *et al*. 2012).

Bioprospection

Among the fungi able to produce bioactive compounds, *P. destructans, T. polysporum*, and *P. appendiculatus* displayed selective and/or high activities.

Trichoderma polysporum produce bioactive compounds against the phyopathogenic fungi *C. sphaerospermum* and *Plasmodium falciparum*. *Trichoderma* spp. are largely studied as biological control agents, being marketed as active compouds for biopesticides, biofertilizers, growth promoters and natural resistance stimulants, for example in USA, where strains *T. polysporum* are market as fungicide and in a return as a beneficial fungi (Woo *et al*. 2014). *Trichoderma* species act protecting plants, in order to increase vegetative growth and contain pathogen populations, in this sense they are considered good alternatives to chemical pesticides, in addition they can be used as inoculant in soil to improve nutrient capacity, decomposition and biodegradation (Woo *et al*. 2014).

P. destructans is recognized as a psychrophic pathogen of white nose syndrome (WNS) that affect bat populations in temperate regions (Lorch *et al*. 2011). In Antarctica *P. destructans* occur abundantly in different substrates and some strains were reported as producer of bioactive compounds (Rosa *et al*. 2019). Some strains obtained from algae, lakes, soil and other substrates in Antarctica regions were recognized as producer of bioactive compounds with antifungal and antibacterial activities against clinical pathogens such as *Streptococcus pneumoniae* (Li *et al*. 2008), *Cryptococcus neoformans* (Brunatti *et al*. 2009), *Candida albicans*, (Brunati *et al*. 2009, Furbino *et al*. 2014) and *Cladosporium sphaerospermum* (Furbino *et al*. 2014) pathogen in agriculture. Gomes *et al*. (2018) evaluated extracts of *P. destructans* obtained from Antarctica soils produce compounds with moderate herbicidal and strong trypanocidal activities.

Conclusion

In Antarctica, lakes the input of nutrient ranged to oligotrophic to ultra-oligotrophic environments. However, there are some lakes enriched by bird droppings and plant debris (Laybourn-Parry *et al*. 1997). Our study indicate that in the sediments of Antarctica lakes live a rich and diverse fungal community composed by cold cosmopolitan and psychrophilic endemic taxa dominated by *Ascomycota* and *Basidiomycota* phyla associated with extra-aquatic sources close to lakes, considering that their already were detected in different terrestrial substrates of Antarctica. Within the fungal community, we detected species recognized as strong and weak decomposer, symbiotics, pathogens, and potential new species. The dynamic and balance of this fungal community represent an interesting aquatic web model for further ecological and evolutionary studies under extreme conditions and potential climate changes in the regions. In addition, we detected fungal taxa and isolates able to

produce bioactive compounds, which may represent source of prototype molecules to use in medicine and agriculture.

Acknowledgements

We acknowledge the financial support from CNPq PROANTAR 442258/2018-6, INCT Criosfera II, CAPES (88887.136384/2017-00 and 88887.314457/2019-00), CNPq, FAPEMIG, and FNDCT. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The authors thank the Program for Technological Development of Tools for Health-PDTIS-Fiocruz for use of its facilities (Bioprospection and Chagas disease-PlaBio Tc platforms). PASJ is research fellow supported by Programa de Pós-graduação em Ciências da Saúde, Fiocruz Minas (CAPES/PNPD).

References

Altschul SF, Madden TL, Schaffer AA *et al*. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl Acids Res* 1997;**25**:3389–3402.

Arenz BE, Blanchette RA, Farrel R Fungal diversity in Antarctica soils. In: Cowan DA Antarctic Terrestrial Microbiology: Physical and Biological Properties of Antarctica soils. New York: Springer Heidelberg, 2014, 35-49.

Brunati M, Rojas JL, Sponga F *et al*. Diversity and pharmaceutical screening of fungi from benthic mats of Antarctic lakes. *Mar Genomics* 2009,**2**:43–50.

Borenfreund E, Babich H, Martin-Alguacil N. Comparisons of two in vitro cytotoxicity assays: the neutral red (NR) and tetrazolium MTT tests. *Toxicol In Vitro*, 1988;**2:**1–6.

Buzzini P, Turk M, Perini L *et al*. Yeasts in Polar and Subpolar Habitats. In: Buzzini P., Lachance MA, Yurkov A (eds.). Yeasts in Natural Ecosystems: Diversity. Switzerland: Springer Nature 2017, 331-365.

Callahan HL, Portal AC, Devereaux R *et al*. An axenic amastigote system for drug screening. *Antimicrob Agents Chemother* 1997;**41**:818–822.

Carrasco M, Rozas JM, Barahona S *et al*. Diversity and extracellular enzymatic activities of yeasts isolated from King George Island, the sub-Antarctic region. *BMC Microbiol* 2012;**12**:251.

Connell L, Segee B, Redman R *et al*. Biodiversity and abundance of cultured microfungi from the permanently ice-covered Lake Fryxell, Antarctica. *Life* 2018;**8**:1–10.

Dayan FE, Romagni JG, Duke SO. Investigating the mode of action of natural phytotoxins. *J Chem Ecol* 2000;**26**:2079–2094.

de Hoog GS, Göttlich E, Platas G *et al*. Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. *Stud Mycol* 2005;**51**:33–76.

De Menezes GC, Godinho VM, Porto BA *et al*. *Antarctomyces pellizariae* sp. nov., a new, endemic, blue, snow resident psychrophilic ascomycete fungus from Antarctica. *Extremophiles* 2017;**21**:259–269.

del Frate G, Caretta G. Fungi isolated from Antarctic material. *Polar Biol* 1990;**11**(1):1-7.

Duarte AW, Passarini MR, Delforno TP *et al*. Yeasts from macroalgae and lichens that inhabit the South Shetland Islands, Antarctica. *Environ Microbiol Rep* 2016;**8**:874–885.

Duarte AWF, Dayo-Owoyemi I, Nobre FS *et al* (2013) Taxonomic assessment and enzymes production by yeasts isolated from marine and terrestrial Antarctic samples. *Extremophiles* **17**:1023–1035.

Edgington S, Thompson E, Moore D *et al*. Investigating the insecticidal potential of *Geomyces* (*Myxotrichaceae*: *Helotiales*) and *Mortierella* (*Mortierellacea*: *Mortierellales*) isolated from Antarctica. *Springer plus* 2014;**3**:289.

Ferreira SEM, Sousa FMP, Rosa LH *et al*. Taxonomy and richness of yeasts associated with angiosperms, bryophytes, and meltwater biofilms collected in the Antarctic Peninsula. *Extremophiles* 2018;**23**:151–159.

Gilichinsky DA, Wilson GS, Friedmann EI *et al*. Microbial populations in Antarctic permafrost: biodiversity, state, age, and implication for astrobiology. *Astrobiology* 2007;**7**:275–311.

Furbino LE, Godinho VM, Santiago IF *et al*. Diversity patterns, ecology and biological activities of fungal communities associated with the endemic macroalgae across the Antarctic Peninsula. *Microb Ecol* 2014;**67**:775–787.

Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* 1995;**61**:1323–1330.

Godinho VM, Furbino LE, Santiago IF *et al*. Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. *ISME J* 2013;**7**:1434–1451.

Godinho VM, de Paula MTR, Silva DAS *et al*. Diversity and distribution of cryptic cultivable fungi associated with marine animals of Antarctica. *Fungal Biol* 2019;**123**:507-516.

Gomes EC, Godinho VM, Silva DA *et al*. Cultivable fungi present in Antarctic soils: taxonomy, phylogeny, diversity, and bioprospecting of antiparasitic and herbicidal metabolites. *Extremophiles* 2018;**22**:381–393.

Gonçalves VN, Vaz ABM, Rosa CA *et al*. Diversity and distribution of fungal communities in lakes of Antarctica. *FEMS Microbiol Ecol* 2012;**82**:459–471.

Gonçalves VN, Cantrell CL, Wedge DE *et al*. Fungi associated with rocks of the Atacama Desert: taxonomy, distribution, diversity, ecology and bioprospection for bioactive compounds. *Environ Microbiol* 2015;**18**:232–245.

Goto S, Sugiyama J, Iizuka H. A taxonomic study of Antarctic yeast. *Mycologia* 1969;**61**:748–774.

Houbraken J, Frisvad JC, Seifert KA *et al*. New penicillin-producing *Penicillium* species and an overview of section Chrysogena. *Persoonia* 2012;**29**:78–100.

Kirby BM, Easton S, Tuffin IM *et al*. Bacterial diversity in polar habitats. In: Miller RV, Whyte LG. Polar Microbiology: Life in a Deep Freeze. Washington, DC: ASM Press, 2012, 3-31.

Kirk PM, Cannon PF, Minter DW *et al*. Dictionary of the Fungi. CAB International, Wallingford, 2008.

Kurtzman CP, Fell JW, Boekhout T The yeasts: a taxonomic study. Elsevier, Amsterdam, 2011.

Lachance MA, Bowles JM, Starmer WT *et al*. Kodamaea kakaduensis and Candida tolerans, two new yeast species from Australian Hibiscus flowers. *Can J Microbiol* 1999;**45**:172–177.

Laybourn-Parry J, James MR, McKnight DM *et al*. (The microbial plankton of Lake Fryxell, Southern Victoria Land, Antarctica during the summers of 1992 and 1994. *Polar Biol* 1997;**17**:54–61.

Leotta GA, Paré JA, Sigler L *et al*. *Thelebolus microsporus* mycelial mats in the trachea of wild brown skua (*Catharacta antarctica lonnbergi*) and South Polar Skua (*C. maccormicki*) carcasses. *JWildlife Diseases*, 2002;**38**:443–447.

Li Y, Sun B, Liu S *et al*. Bioactive asterric acid derivatives from the Antarctic ascomycete fungus *Geomyces* sp. *J Nat Prod* 2008;**71**:1643–1646.

Lorch JM, Meteyer CU, Behr JM *et al*. Experimental infection of bats with *Geomyces* destructans causes white-nose syndrome. *Nature* 2011;**480**:376–378.

Martorell MM, Ruberto LAM, Fernández PM *et al*. Bioprospection of cold-adapted yeasts with biotechnological potential from Antarctica. *J Basic Microbiol* 2017;57(6):504–516.

Martorell MM, Ruberto LAM, de Figueroa LIC *et al*. Antarctic Yeasts as a Source of Enzymes for Biotechnological Applications. In: Rosa LH (Eds.) Fungi of Antarctica. Switzerland: Springer Nature, 2019, 285–304.

McInnes SJ. A predatory fungus (*Hyphomycetes*: *Lecophagus*) attacking Rotifera and Tardigrada in maritime Antarctic lakes. *Polar Biol* 2003;**26**:79–82

Melo IS, Santos SN, Rosa LH *et al*. Isolation and biological activities of an endophytic *Mortierella alpina* strain from the Antarctic moss Schistidium antarctici. *Extremophiles* 2014;**18**:15–23.

Mossman T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;**65**: 55–63.

NCCLS Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, M38-A. National Committee on Clinical Labora-tory Standards 2002;**22**:16.

Newsham KK, Garnett MH, Robinson CH *et al*. Discrete taxa of saprotrophic fungi respire different ages of carbon from Antarctic soils. *Sci Rep* 2018;**8**:7866.

Oduola AM, Weatherly NF, Bowdre JH *et al*. *Plasmodium falciparum*: cloning by single-erythrocyte micromanipulation and heterogeneity in vitro. *Exp Parasitol* 1988;**66**:86–95.

Ogaki MB, Vieria R, Lirio JM *et al*. Diversity and Ecology of Fungal Assemblages Present in Lakes of Antarctica. In: Rosa LH (Ed) Fungi of Antarctica. Switzerland: Springer Nature, 2019a, 69-97.

Ogaki MB, Coelho LC, Vieira R *et al*. Cultivable fungi present in deep-sea sediments of Antarctica: taxonomy, diversity, and bioprospection of bioactive compounds. **Unppublished**. 2019b

Ogaki MB, Vieria R, Zani CL *et al*. Diversity, ecology, and bioprospection of fungi in lakes under anthropogenic effects in Maritime Antarctica. **Unppublished**. 2019c

Rice AV, Currah RS Two new species of *Pseudogymnoascus* with *Geomyces* anamorphs and their phylogenetic relationship with *Gymnostellatospora*. *Mycologia* 2006;**98**(2):307–318.

Romanha AJ, de Castro SL, Soeiro MNC *et al*. In vitro and in vivo experimental models for drug screening and development for Chagas disease. *Mem Inst Oswaldo Cruz* 2010;**105**:233–238.

Rosa LH, Vaz ABM, Caligiorne RB *et al*. Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv (*Poaceae*). *Polar Biol* 2009;**32**:161–167.

Rosa LH, Zani CL, Cantrell CL. Fungi in Antarctica: Diversity, Ecology, Effects of Climate Change, and Bioprospection for Bioactive Compounds. In: Rosa LH (ed.) Fungi of Antarctica. Switzerland: Springer Nature, 2019,1-17.

Santiago IF, Soares MA, Rosa CA *et al*. Lichensphere: a protected natural microhabitatof the non-lichenised fungal communities living in extreme environments of Antarctica.*Extremophiles* 2015;**19**:1087–1097.

Sattler B, Storrie-Lombardi MC. Laser-induced fluorescence emission (L.I.F.E.): in situ nondestructive detection of microbial life in the ice covers of Antarctic lakes. *Astrobiology* 2009;**9**(7):659–672.

Smilkstein M, Sriwilaijaroen N, Kelly JX *et al*. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. *Antimicrob Agents Chemother* 2004;**48**:1803–1806.

Sparrow FK. Ecology of freshwater fungi. In: Ainsworth GC, Sussman A.S (eds.). The Fungi. An Advanced Treatise vol IVA. New York and London: Academic Press, 1968, 41-53.

Stchigel AM, Cano J, MacCormack CW. Antarctomyces psychrotrophicus gen. et sp. nov., a new ascomycete from Antarctica. *Mycol Res* 2001;**105**:377–382.

Stchigel AM, Guarro J, Mac Cormack W. Apiosordaria antarctica and *Thielavia antarctica*, two new ascomycetes from Antarctica. *Mycologia* 2003;**95**(6):1218-26.

Tamura K, Peterson D, Peterson N *et al*. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;**28**:2731–2739.

Tosi S, Casado B, Gerdol R *et al*. Fungi isolated from Antarctic mosses. *Polar Biol* 2002;**25**:262–268.

Trager W, Jensen JB. Human malaria parasites in continuous culture. *Science* 1976;**193**:673–675.

Tsuji M. Cold-stress responses in the Antarctic basidiomycetous yeast Mrakia blollopis. *Royal Soc Open Sci* 2016;**31**:60106.

Tsuji M, Fujiu S, Xiao N *et al*. Cold adaptation of fungi obtained from soil and lake sediment in the Skarvsnes ice-free area, Antarctica. *FEMS Microbiol Lett* 2013;**346**:121–130.

Vasileva-Tonkova E, Romanovskaya V, Gladka G *et al*. Ecophysiological properties of cultivable heterotrophic bacteria andyeasts dominating in phytocenoses of Galindez Island, maritime Antarctica. *World J MicrobiolBiotechnol* 2014;**30**:1387–1398.

Vaz AB, Rosa LH, Vieira ML *et al*. The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. *Brazilian J Microbiol* 2011;**42**:937–947.

Vincent WF, Hibbie JE, Laybourn-Parry J. Introduction of limnology of high-latitude and river ecosystems. In: Vincent WF, Laybourn-Parry J (eds.). Polar lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems. New York: Oxford University Press Inc, 2008, 1-23.

Wentzel LCP, Inforsato FJ, Montoya QV *et al*. Fungi from Admiralty Bay (King George Island, Antarctica) soils and marine sediments. *Microb Ecol* 2018;**77**:12–24.

White TJ, Bruns TD, Lee SB Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis NA, Gelfand J, Sninsky J, White TJ (eds.). PCR protocols: a guide to methods and applications. San Diego: Academic Press, 1990, 315–322.

Wong MK, Goh TK, Hodgkiss *et al*. Role of fungi in freshwater ecosystems. *Biodiv Conservation* 1998;**7**:1187.

Woo SL, Ruocco M, Vinale F *et al*. *Trichoderma*-based products and their widespread use in agriculture. *Open Mycol J* 2014;**8**:71–126.

Zycha H, Siepmann R, Linnemann G. *Mucorales*, eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe. J Cramer, 1969, 1-355.

Table S1. Collection data of lake sediment samples.

Sample identification	Lake source	Core size[a] (mm)	Section[b]	Area		
				Point	Longitude	Latitude
Punta Hennequin (Ph)	Snow melting	120	T, B	King George Island, Admiralty Bay	58. 39561	62. 12342
Deception A (Da)	Snow melting	50	T	Deception Island, Crater Lake	60. 66512	62. 98131
Deception B (Db)	Crater melting areas	70	T	Deception Island, Crater Lake	60. 65504	62. 98473
Kroner Lake (Kr)	Saline, freshwater	170	T, B	Deception Island, Whalers Bay	60. 57353	62. 98033
Wanda A (Wa)	Saline, freshwater	120	T, B	King George Island, Admiralty Bay	58. 39148	62. 08567
Wanda B (Wb)	Glacier melting	170	T, M, B	King George Island, Admiralty Bay	58. 39148	62. 08567
Elephant (El)	Snow melting	190	T, M, B	Elephant Island	55. 21543	61. 13182
Penguin (Pe)	Crater melting areas	100	T, B	Penguin Island	57. 55098	62. 06038

a – Size of the sediment core in milimeters; b – sections top (T), middle (M), and base (B).

Table 1. Fungal taxa obtained sediment of Lakes of Antarctic Peninsula and identified by sequence comparison with the BLASTn match with the NCBI GenBank database.

Collection Point	Section	UFC g^{-1}	N. of isolates	UFMGCB[a]	Top BLAST (Number of acess on Genbank)	Query coverage (%)	Identity (%)	bp analysed	Proposed taxa
Pa	Top	1000	2	13929	*Antarctomyces pellizariae* (KX576510)[b]	100	100	451	*Antarctomyces pellizariae*
		1040	4	13937	*Pseudogymnoascus verrucosus* (KJ755525)[b]	100	99	483	*Pseudogymnoascus verrucosus*
		1160	2	12987	*Mortierella antarctica* (NR111580)[b]	99	99	531	*Mortierella antarctica*
		80	1	13012	*Mortierella elongatula* (MH859811)[b]	100	96	514	*Mortierella* sp. 1
		3120	5	13008	*Mortierella parvispora* (NR077185)[b]	100	95	446	*Mortierella* sp. 2
		160	1	13970	*Myxotrichum cancellatum* (NR160089)[b]	100	99	311	*Myxotrichum cancellatum*
		3200	8	13011	*Thelebolus globosus* (NR138367)[b]	100	100	445	*Thelebolus globosus*
	Base	3000	5	13940	*Pseudogymnoascus verrucosus* (KJ755525)[b]	100	99	482	*Pseudogymnoascus verrucosus*
		2000	2	12991	*Mortierella antarctica* (JX975907)[b]	100	99	543	*Mortierella antarctica*
		1040	1	13017	*Mortierella parvispora* (NR077185)[b]	99	96	506	*Mortierella* sp. 2
		400	1	13014	*Thelebolus globosus* (NR138367)[b]	100	100	434	*Thelebolus globosus*
		40	1	72	*Goffeauzyma gilvescens* (NG058297)[c]	100	100	424	*Goffeauzyma gilvescens*
Da	Top	40	1	13969	*Mortierella antarctica* (NR111580)[b]	100	99	530	*Mortierella antarctica*
		21560	8	13943	*Thelebolus globosus* (NR138367)[b]	100	100	448	*Thelebolus globosus*
		800	1	13979	*Pseudogymnoascus verrucosus* (KJ755525)[b]	100	99	478	*Pseudogymnoascus verrucosus*
		5360	6	73	*Goffeauzyma gastrica* (KY107767)[c]	100	100	444	*Goffeauzyma gastrica*
		2160	3	77	*Leucosporidium muscorum* (KY108280)[c]	100	100	550	*Leucosporidium muscorum*
		8000	1	80	*Vishniacozyma victoriae* (KY110041)[c]	100	99	484	*Vishniacozyma victoriae*
Kr	Top	400	6	13015	*Antarctomyces psychrotrophicus* (MH874317)[b]	100	100	449	*Antarctomyces psychrotrophicus*
		400	1	13007	*Cladosporium subuliforme* (MH864124)[b] *Cladosporium cladosporioides* (KX288432)[d]	100 100	100 90	467 408	*Cladosporium* sp. 1
		120	2	13006	*Thielavia antarctica* (MH863307)[b]	100	100	408	*Thielavia antarctica*
Wa	Top	280	4	13000	*Antarctomyces psychrotrophicus* (MH874317)[b]	100	100	457	*Antarctomyces psychrotrophicus*
		120	2	13958	*Pseudeurotium hygrophilum* (NR111128)[b]	96	100	430	*Pseudeurotium hygrophilum*
		240	3	13020	*Thelebolus globosus* (MH862951)[b]	100	100	450	*Thelebolus globosus*

		40	1	13976	*Pseudoagymnoascus destructans* (EU884921)[b]	100	99	443	*Pseudoagymnoascus destructans*
		80	3	135	*Glaciozyma antarctica* (NG057664)[c]	100	100	393	*Glaciozyma antarctica*
	Base	40	1	13956	*Cladosporium rugulovarians* (KT600459)[b] *Cladosporium psychrotolerans* (KJ596614)[d]	100 96	99 93	472 302	*Cladosporium* sp. 2
		40	1	13985	*Gregarithecium curvisporum* (NR154049)[b]	100	92	434	*Pleosporales* sp.
		40	1	13932	*Antarctomyces pellizariae* (KX576510)[b]	100	100	459	*Antarctomyces pellizariae*
		40	1	13026	*Bipolaris gossypina* (MH864410)[b]	100	99	387	*Bipolaris* sp.
		60	3	127	*Glaciozyma antarctica* (KY107752)[c]	100	100	522	*Glaciozyma antarctica*
Wb	Top	120	1	13001	*Cladosporium rugulovarians* (KT600459)[b] *Cladosporium psychrotolerans* (KJ596614)[b]	100 82	100 93	478 352	*Cladosporium* sp. 2
		520	1	13946	*Cadophora luteo-olivacea* (GU128588)[b]	99	97	307	*Helotiales* sp. 1
		120	5	13971	*Coleophoma paracylindrospora* (NR154806)[b]	96	86	424	*Pezizomycotina* sp.
		120	2	14000	*Phenoliferia psychrophenolica* (EF151246)[c] *Phenoliferia psychrophenolica* (KY108774)[b]	84 100	91 98	495 316	*Kriegeriales* sp.
	Base	40	1	13002	*Epicoccum italicum* (NR158264)[b]	100	100	360	*Epicoccum italicum*
El	Top	400	1	13016	*Antarctomyces pellizariae* (KX576510)[b]	100	100	436	*Antarctomyces pellizariae*
		19960	5	14005	*Leucosporidium intermedium* (KY104188)[b]	90	96	402	*Microbotryomycetes* sp. 1
		40	1	13949	*Pseudeurotium hygrophilum* (NR111128)[b]	96	100	431	*Pseudeurotium hygrophilum*
		400	2	94	*Mrakia gelida* (KY108585)[c]	100	99	438	*Mrakia* sp.
		2720	1	96	*Mrakia aquatica* (KY108574)[c]	100	99	373	*Mrakia aquatica*
		320	2	149	*Glaciozyma antarctica* (KY107752)[c]	100	100	461	*Glaciozyma antarctica*
		40	1	162	*Piskurozyma fildesensis* (KC894160)[c]	92	100	517	*Piskurozyma fildesensis*
		144	4	144	*Goffeauzyma gilvescens* (NG058297)[c]	100	100	477	*Goffeauzyma gilvescens*
		280	1	133	*Leucosporidium fragarium* (KY108279)[c]	100	100	523	*Leucosporidium fragarium*
		7600	11	145	*Phenoliferia psychrophenolica* (KY108774)[c]	c99	100	486	*Phenoliferia* sp.
		3440	9	148	*Vishniacozyma victoriae* (NG057678)[c]	100	100	514	*Vishniacozyma victoriae*
	Middle	12360	5	13994	*Sampaiozyma vanillica* (NR137036)b *Sampaiozyma vanillica* (KY109537)c	99 100	93 93	458 552	*Microbotryomycetes* sp. 2
		160	3	168	*Phenoliferia psychrophila* (NG058370)c	100	99	437	*Phenoliferia* sp.
		240	4	123	*Vishniacozyma victoriae* (NG057678)c	100	100	477	*Vishniacozyma victoriae*
		80	1	165	*Leucosporidium fragarium* (NG058330)c	100	100	440	*Leucosporidium fragarium*

		40	1	101	*Mrakia gelida* (KY108585)c	100	99	432	*Mrakia* sp.
		40	1	136	*Glaciozyma antarctica* (NG057664)c	100	100	522	*Glaciozyma antarctica*
	Base	1800	6	14006	*Sampaiozyma vanillica* (NR137036)[b] *Sampaiozyma vanillica* (KY109537)[c]	96 100	94 93	417 559	*Microbotryomycetes* sp. 2
		800	5	13973	*Pseudeurotium hygrophilum* (NR111128)[b]	96	100	445	*Pseudeurotium hygrophilum*
Pe	Top	40	1	13022	*Antarctomyces psychrotrophicus* (MH874317)[b]	100	100	433	*Antarctomyces psychrotrophicus*
		400	1	13957	*Chrysosporium oceanitesii* (KT155793)[b]	100	100	319	*Chrysosporium oceanitesii*
		80	2	13962	*Pseudoagymnoascus destructans* (EU884921)[b]	100	99	459	*Pseudoagymnoascus destructans*
		40	3	13961	*Myxotrichum cancellatum* (NR160089)[b]	100	99	354	*Myxotrichum cancellatum*
		40	1	13991	*Oidiodendron truncatum* (NR111036)[b]	100	96	439	*Myxotrichaceae* sp.
		440	2	13023	*Pseudogymnoascus appendiculatus* (NR137875)[b]	97	100	454	*Pseudoagymnoascus appendiculatus*
		120	2	13951	*Pseudogymnoascus roseus* (MH858631)[b]	98	99	478	*Pseudoagymnoascus roseus*
	Base	80	1	13966	*Pseudoagymnoascus destructans* (EU884921)[b]	100	99	482	*Pseudoagymnoascus destructans*
		920	3	13990	*Leohumicola minima* (NR121307)[b]	100	90	444	*Helotiales* sp. 2
		680	3	13964	*Myxotrichum cancellatum* (NR160089)[b]	100	99	411	*Myxotrichum cancellatum*
		40	1	13999	*Acremonium biseptum* (NR159609)[b]	96	97	369	*Hypocreales* sp.
		120	1	13025	*Penicillium jamesonlandens* (NR119570)[b] *Penicillium jamesonlandense* (DQ309448)[d] *Penicillium jamesonlandense* (DQ309448)[e]	97 99 100	100 98 98	501 363 340	*Penicillium jamesonlandense*
		320	2	13954	*Pseudogymnoascus appendiculatus* (NR137875)[b]	97	99	487	*Pseudoagymnoascus appendiculatus*
		40	1	13968	*Trichoderma polysporum* (NR134448)[b]	98	99	341	*Trichoderma polysporum*
		80	2	103	*Goffeauzyma gastrica* (KY107768)[c]	100	99	474	*Goffeauzyma gastrica*
		1440	7	105	*Holtermanniella wattica* (NG058307)[c]	100	100	526	*Holtermanniella wattica*
		40	1	139	*Leucosporidium creatinivorum* (NR073329)[c]	100	100	381	*Leucosporidium* sp.
		2160	4	110	*Vishniacozyma victoriae* (NG057678)[c]	100	100	521	*Vishniacozyma victoriae*
		40	1	125	*Protomyces inouyei* (NG042406)[c]	100	99	499	*Protomyces inouyei*

[a]UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. Taxa subjected to BLAST analysis based on the [b]ITS, [c]D1-D2 domain, [d]β-tubulin, [e]Polymerase 2. [f]Taxonomic suggested. Sequences of [g]ITS, [h]D1-D2 domain, [i]β-tubulin and [j]Polymerase 2 deposited in GenBank database. Lakes sediment samples: Ph – Punta Hanequin, Da – Deception A, Db – Deception B, Kr – Kroner Lake, Wa – Wanda A, Wb – Wanda B, El – Elephant, Pe – Penguin.

Table S3. Frequency of fungal isolation in Lakes sediments of Antarctic Peninsula.

Lake	Section	N° of isolates [Relative Frequency (%)]		
		Ascomycota	*Basidiomycota*	*Mortierellomycota*
Ph	Top	15 (7.7)	-	8 (4.1)
	Base	6 (3.1)	1 (0.5)	3 (1.5)
Da	Top	9 (4.6)	10 (5.1)	1 (0.5)
Db	Top	-	-	-
Kr	Top	9 (4.6)	-	-
	Base	-	-	-
Wa	Top	10 (5.1)	3 (1.5)	-
	Base	4 (2.1)	3 (1.5)	-
Wb	Top	7 (3.6)	2 (1.0)	-
	Middle	-	-	-
	Base	1 (0.5)	-	-
El	Top	2 (1.0)	36 (18.5)	-
	Middle	-	15 (7.7)	-
	Base	5 (2.6)	6 (3.1)	-
Pe	Top	12 (6.2)	-	-
	Base	13 (6.7)	14 (7.2)	-
Total		92 (47.2)	90 (46.1)	13 (6.7)

(-) No fungi obtained. Lakes sediment samples: Ph – Punta Hanequin, Da – Deception A, Db – Deception B, Kr – Kroner Lake, Wa – Wanda A, Wb – Wanda B, El – Elephant, Pe – Penguin.

Table S2. Diversity indices of fungal assemblages present in sediments of lakes at Antarctic Peninsula.

Diversity indices	Lakes							
	Ph	Da	Db	Kr	Wa	Wb	El	Pe
Number of taxa	8	6	0	3	10	5	13	15
Number of isolates	33	20	0	9	20	10	64	39
Total fungal density (CFU g^{-1})	16240	37920	0	920	980	920	50904	7080
Fisher-α (diversity)	0.81	0.61	0	0.60	0.81	0.63	0.79	0.82
Margalef's (richness)	0.72	0.47	0	0.29	1.16	0.59	1.11	1.58
Simpson's (dominance)	0.78	0.54	0	0.39	1.37	0.70	1.22	1.81

[a]Sediment temperature (15 cm of depth) in the data of core collection. Lakes sediment samples: Ph – Punta Hanequin, Da – Deception A, Db – Deception B, Kr – Kroner Lake, Wa – Wanda A, Wb – Wanda B, El – Elephant, Pe – Penguin.

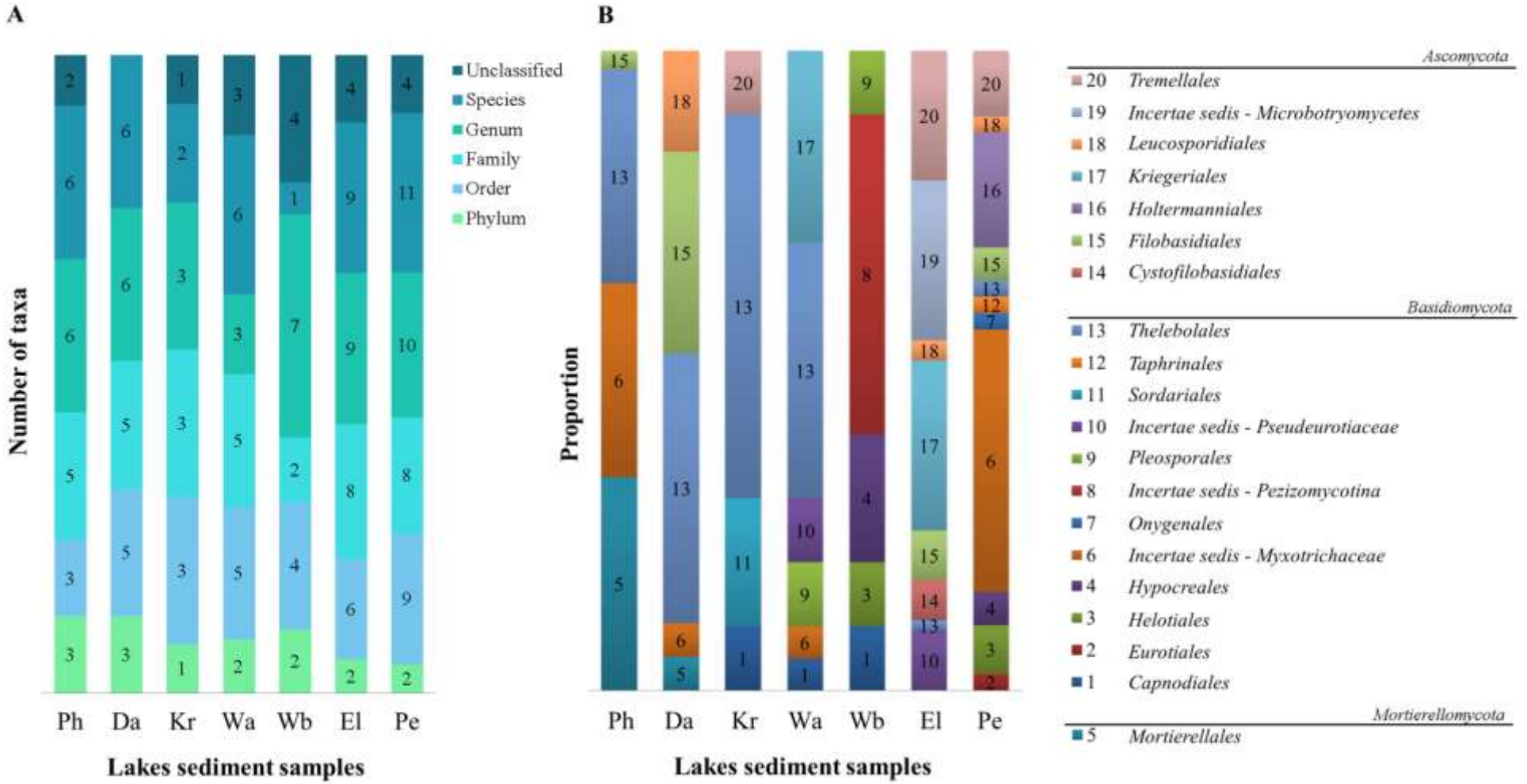


Figure S1. Taxonomic rank and classes of fungi represented across lakes sediment samples of Antarctic Peninsula. Lakes sediment samples: Ph – Punta Hanequin, Da – Deception A, Db – Deception B, Kr – Kroner Lake, Wa – Wanda A, Wb – Wanda B, El – Elephant, Pe – Penguin.

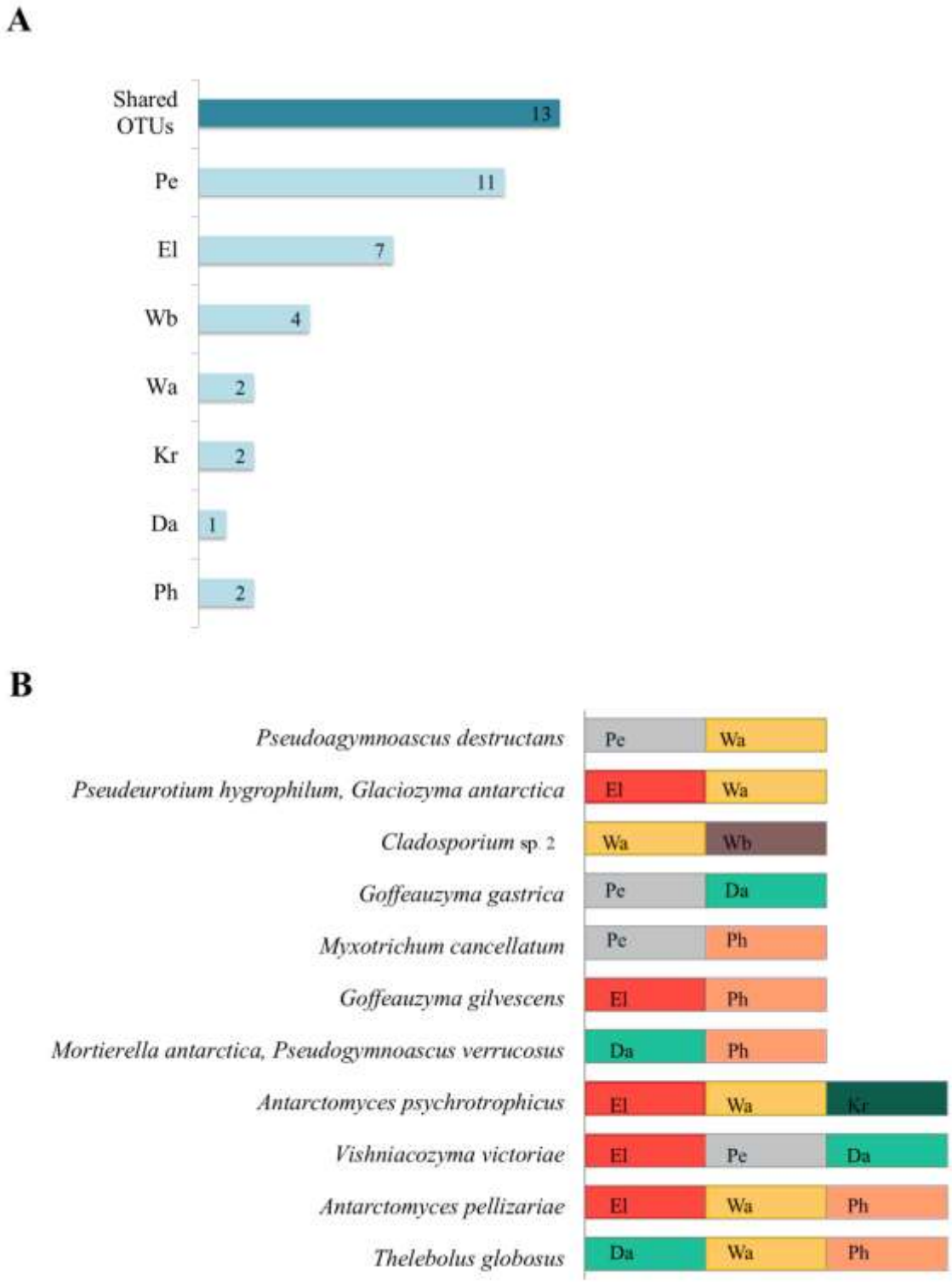


Figure S2. Exclusive and shared OTUs among lake sediment assemblages in Antarctic Peninsula. (A) Number of OTUs shared among two or more lakes and number of exclusive OTUs for each lake. (B) OTUs shared between lakes. Lakes sediment samples: Ph – Punta Hanequin, Da – Deception A, Db – Deception B, Kr – Kroner Lake, Wa – Wanda A, Wb – Wanda B, El – Elephant, Pe – Penguin.

Table 3. Biological activities of the extracts obtained from fungal species from sediment of lakes at Antarctic Peninsula.

Taxa	UFMGCB[a]	Biological activities					
		Herbicidal[c]		Antifungic[b]	Antiparasitic[d]		
		A. schoenoprasum	*L. sativa*	*C. sphaerospermum*	*T. cruzi*	*P. falciparum*	*L. amazonensis*
Antarctomyces psychrotrophicus	13000	0 ± 0	**3 ± 0**	30.7 ± 5	0.09 ± 12	4.1 ± 1	29.5 ± 1
A. psychrotrophicus	13930	1.5 ± 0.5	**4 ± 0**	0 ± 0	5.6 ± 6	0 ± 0	57.3 ± 2
Cladosporium sp. 1	13007	1.5 ± 0.5	0 ± 0	**97.7 ± 15**	4.55 ± 4	0 ± 0	49.4 ± 2
Cladosporium sp.2	13001	1.5 ± 0.5	**4.5 ± 0.5**	56.6 ± 10	0 ± 0	0 ± 0	30.2 ± 2
Glaciozyma antarctica	131	0 ± 0	**3 ± 0**	0 ± 0	9.14 ± 21	7.3 ± 1	32.2 ± 1
G. antarctica	155	1.5 ± 0.5	**3.5 ± 0.5**	0 ± 0	6.13 ± 14	12.1 ± 0	33.9 ± 1
Goffeauzyma gastrica	75	0 ± 0	**3 ± 1**	0 ± 0	13.2 ± 13	0 ± 0	29.6 ± 1
G. gastrica	79	0 ± 0	**3 ± 0**	0 ± 0	28.3 ± 28	4.6 ± 2	28.2 ± 1
G. gastrica	113	0 ± 0	**4.5 ± 0.5**	0 ± 0	11.7 ± 14	10.5 ± 2	27.7 ± 4
G. gastrica	115	0 ± 0	**4 ± 0**	0 ± 0	0 ± 0	12.9 ± 1	31.7 ± 2
Goffeauzyma gilvescens	84	0 ± 0	**3 ± 0**	0 ± 0	0 ± 0	0 ± 0	27.3 ± 1
G. gilvescens	91	0 ± 0	**4 ± 0**	0 ± 0	23 ± 25	16.5 ± 1	28 ± 1
Helotiales sp. 1	13946	**3 ± 0.5**	**3.5 ± 0.5**	0 ± 0	0 ± 0	0 ± 0	29.8 ± 1
Holtermanniella wattica	109	**3 ± 0.5**	0 ± 0	0 ± 0	2.09 ± 2	0 ± 0	31.6 ± 1
H. wattica	126	**3 ± 0.5**	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1	31.7 ± 0
Thielavia antarctica	13006	**3 ± 0.5**	0 ± 0	0 ± 0	5.9 ± 1	0 ± 0	43 ± 2
Pezizomycotina sp.	13963	2 ± 0	0 ± 0	0 ± 0	**52.3 ± 45**	0 ± 0	61.7 ± 3
Mortierella antarctica	13009	2 ± 1	0 ± 0	**75.5 ± 19**	0 ± 0	0 ± 0	41 ± 1
M. antarctica	13004	0 ± 0	1.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	29.1 ± 5
Mortierella sp. 1	13012	1 ± 0	1 ± 0	**77 ± 7**	7.79 ± 5	0 ± 0	63.2 ± 4
Mortierella sp. 2	13008	0 ± 0	2 ± 1	**93.6 ± 2**	1.65 ± 7	0 ± 0	35.5 ± 1
Mrakia sp.	94	0 ± 0	**3 ± 0**	0 ± 0	30 ± 13	7.8 ± 0	28 ± 1
Myxotrichum cancellatum	13961	**3 ± 0.5**	2.5 ± 1	0 ± 0	0 ± 0	7.9 ± 0	39.2 ± 0

M. cancellatum	13972	0 ± 0	**3 ± 0**	0 ± 0	0 ± 0	0 ± 0	7.5 ± 1
M. cancellatum	13970	0 ± 0	**3 ± 1**	0 ± 0	0 ± 0	0 ± 0	31 ± 0
Penicillium jamesonlandense	13025	0 ± 0	**3 ± 0**	0 ± 0	0 ± 0	0 ± 0	28 ± 2
Phenoliferia sp.	88	1 ± 0	**4 ± 1**	0 ± 0	0 ± 0	12.2 ± 2	32.5 ± 0
Phenoliferia sp.	89	0 ± 0	**4.5 ± 0.5**	0 ± 0	3.53 ± 4	11.6 ± 2	28.5 ± 1
Phenoliferia sp.	122	0 ± 0	**3 ± 0**	0 ± 0	0 ± 0	7.2 ± 1	29.9 ± 1
Phenoliferia sp.	152	0 ± 0	**3 ± 0**	0 ± 0	3.35 ± 5	16.5 ± 0	34 ± 3
Phenoliferia sp.	164	1 ± 0	**4.5 ± 0.5**	0 ± 0	8.54 ± 9	14 ± 1	23.5 ± 1
Phenoliferia sp.	168	1 ± 0	**3 ± 0**	0 ± 0	3.99 ± 5	0 ± 0	30 ± 2
Pleosporales sp.	13985	1.5 ± 0.5	**3 ± 0**	59.6 ± 28	0 ± 0	0 ± 0	30.7 ± 1
Protomyces inouyei	125	0 ± 0	**3 ± 1**	0 ± 0	0 ± 0	0 ± 0	28.4 ± 2
Pseudoagymnoascus appendiculatus	13023	0 ± 0	**3 ± 1**	**83.4 ± 21**	0 ± 0	0 ± 0	25.2 ± 0
P. appendiculatus	13954	**3 ± 0.5**	0 ± 0	**72.4 ± 32**	0.3 ± 1	7.3 ± 0	26.2 ± 1
P. appendiculatus	13953	**4 ± 1**	**4 ± 0**	**73.7 ± 32**	0 ± 0	0 ± 0	30.4 ± 1
Pseudoagymnoascus destructans	13934	1.5 ± 0.5	**4 ± 1**	**71.9 ± 11**	**47.3 ± 45**	0.6 ± 2	55.6 ± 3
P. destructans	13935	2 ± 0	**3 ± 0**	54.7 ± 2	6.87 ± 18	0 ± 0	55.1 ± 2
P. destructans	13974	**3 ± 0**	0 ± 0	0 ± 0	0 ± 0	0 ± 0	27.7 ± 2
Pseudogymnoascus verrucosus	13938	**3 ± 0.5**	0 ± 0	55.7 ± 3	7.05 ± 5	0 ± 0	38.6 ± 1
P. verrucosus	13951	**3 ± 0.5**	2.5 ± 1	0 ± 0	0 ± 0	0 ± 0	9.1 ± 29
P. verrucosus	13952	1 ± 0	2 ± 0	**89.3 ± 42**	0 ± 0	0 ± 0	4.6 ± 18
Pseudeurotium hygrophilum	13950	0 ± 0	**4 ± 1**	21.6 ± 21	0 ± 0	0 ± 0	21.4 ± 8
P. hygrophilum	13973	0 ± 0	**3.5 ± 0.5**	0 ± 0	5.45 ± 0	0 ± 0	35.8 ± 0
Thelebolus globosus	12994	1 ± 0	1 ± 0	**95.2 ± 13**	0 ± 0	0 ± 0	23.4 ± 0
T. globosus	13011	1 ± 0	0 ± 0	**86.9 ± 6**	11.83 ± 6	0 ± 0	49.6 ± 3
T. globosus	13020	0 ± 0	1 ± 0	**83.2 ± 8**	6.85 ± 6	0 ± 0	35.2 ± 1
T. globosus	13024	1 ± 0	1.5 ± 0.5	**73.7 ± 9**	0 ± 0	0 ± 0	30.9 ± 2
T. globosus	13944	0 ± 0	**3 ± 0**	**75.7 ± 1**	0 ± 0	0 ± 0	58.1 ± 3
Trichoderma polysporum	13968	0 ± 0	0 ± 0	**96.5 ± 1**	0 ± 0	**46.8 ± 12**	31 ± 1

Vishniacozyma victoriae	117	0 ± 0	**3 ± 0**	0 ± 0	0 ± 0	0 ± 0	23.4 ± 2
V. victoriae	134	0 ± 0	**4 ± 1**	0 ± 0	0 ± 0	12.8 ± 3	28.8 ± 0
V. victoriae	169	2 ± 0	**3.5 ± 0.5**	0 ± 0	0 ± 0	0 ± 0	55.5 ± 2
V. victoriae	170	**3 ± 0**	1 ± 0	0 ± 0	0 ± 0	0 ± 0	50.8 ± 0
V. victoriae	81	0 ± 0	**4 ± 1**	0 ± 0	7.55 ± 19	0 ± 0	28.6 ± 5
V. victoriae	118	1.5 ± 0.5	**3.5 ± 0.5**	0 ± 0	0 ± 0	0 ± 0	25.7 ± 0
V. victoriae	80	0 ± 0	**3 ± 0**	0 ± 0	39.5 ± 44	12.52 ± 1	29.6 ± 2
V. victoriae	85	1.5 ± 0.5	**4 ± 1**	0 ± 0	0 ± 0	0 ± 0	31.8 ± 2
Control drugs	Glifosate	**5 ± 0**	**4.7 ± 0.5**	-	-	-	-
	BenZ	-	-	-	**89.2 ± 14**	-	-
	Benomyl	-	-	**100 ± 1.9**	-	-	-
	Amph B	-	-	-	-	-	**84.5 ± 9**
	Chloroquine	-	-	-	-	**100 ± 5**	-

[a]UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. [b]Results of effective extract with inhibition of ≥70% of *Cladosporium sphaerospermum*. [c]The qualitative estimate of phytotoxicity was evaluated by using a rating scale of 0-5, where 0 = no effect and 5 = no growth or no germination of the seeds. Plant targets: *L sativa* = *Lactuca sativa* (lettuce), *A. schoenoprasum* = *Allium schoenoprasum* (chive). [d]Results of effective extract with inhibition of ≥40% of *Trypanossoma cruzi*.

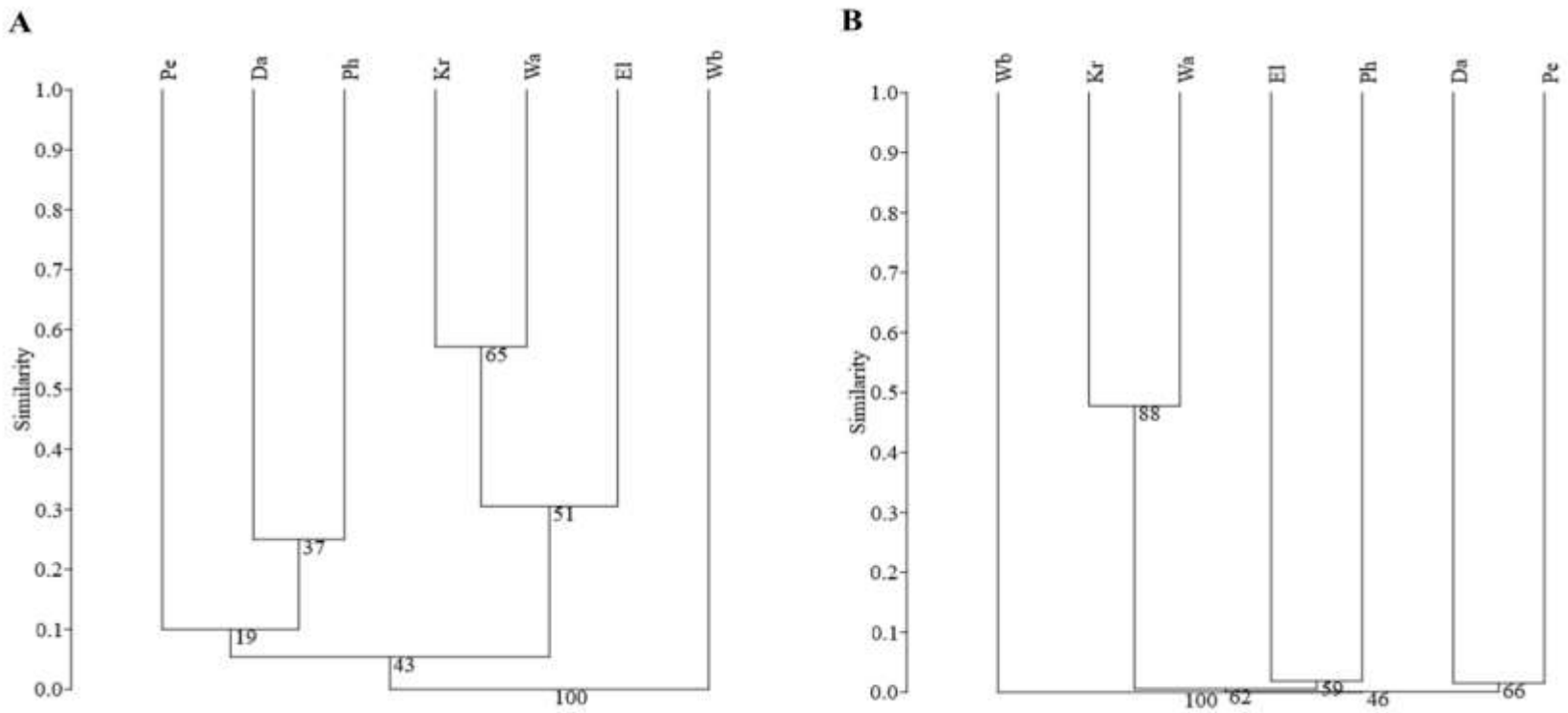


Figure S2. Dendrograms showing Sorensen's (A) and Bray-Curtis's (B) similarity measures for the fungal assemblages present in lake sediment samples. The results were obtained with 95% confidence and bootstrap values calculated from 1000 iterations. Lakes sediment samples: Ph – Punta Hennequin, Da – Deception A, Db – Deception B, Kr – Kroner Lake, Wa – Wanda A, Wb – Wanda B, El – Elephant, Pe – Penguin.

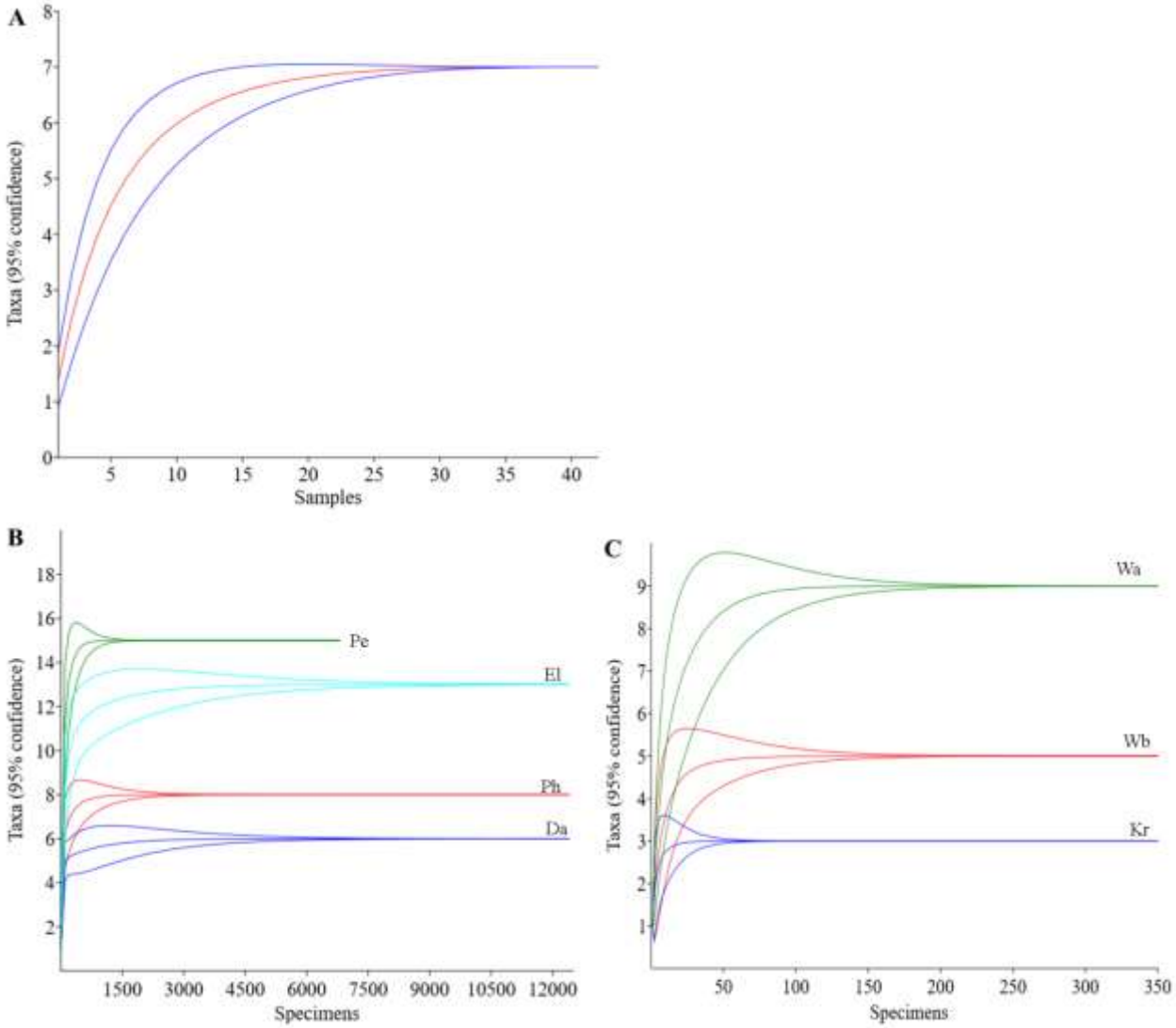


Figure S3. Species accumulation rarefaction curves based on Mao's Tau estimator, dotted line shows 95% confidence limits. A. Represents the total number of species as a function of the number of samples for the fungal assemblages of sediment lakes of Antarctic Peninsula. . B. Individual-based rarefaction curves showing the estimated richness for Penguin (Pe), Elephant (El), Punta Hennequin (Ph) and Deception A (Da) lakes. C. Individual-based rarefaction curves showing the estimated richness for Wanda A (Wa), Wanda B (Wb) and Kroner (Kr) lakes.

6. DISCUSSÃO INTEGRADA

Os sedimentos marinhos, de lagos e rios excedem em área e volume todo o solo presente na superfície da Terra (FENCHEL, 2012). O sedimento é caracterizado pela presença de matéria orgânica e partículas minerais originárias do intemperismo das rochas, precipitados químicos da água, poeira sedimentada proveniente da areia do deserto, cinzas vulcânicas e pequenas partículas atmosféricas ou cósmicas; todos esses materiais são carreados pela energia da água, vento ou geleiras e depositados abaixo da camada aquosa em lagos, lagoas, rios, alagados e mares (RABINOWITZ & VOGEL, 2009; FENCHEL 2012; WAIT *et al*., 2015). Comunidades microbianas são responsáveis pela decomposição e modificação da matéria orgânica presentes nestes sedimentos e influenciados pela temperatura e natureza da matéria orgânica depositada – proveniente das fontes de captação (solo) e fontes bióticas (algas e animais); bem como pela presença de determinados tipos de micro-organismos decompositores (JONES *et al*., 1985).

Micro-organismos decompositores, como os fungos, atuam na denitrificação da matéria orgânica altamente resistente ao decaimento microbiano (CLARKE *et al*., 2017). Em ambientes polares como os da Antártica, os processos de decomposição da matéria orgânica nos sedimentos também são realizados por fungos, os quais já foram relatadas em substratos lacustes (OGAKI *et al*., 2019) e marinhos (ROSA *et al*., 2019b). Em um dos primeiros trabalhos que relata fungos no oceano antártico usando técnicas independentes de cultivo, Bass *et al*. (2007) avaliaram amostras de água da Passagem de Drake em diferentes profundidades (250-500 m e 200-3000 m) e concluíram que os fungos são relativamente raros nestes habitats. Outros trabalhos envolvendo ensaios dependentes de cultivo já foram mencionados não somente em águas profundas (GONÇALVES *et al*., 2017) como em sedimentos profundos da Antártica (GONÇALVES *et al*., 2013; 2015).

Nas amostras de sedimento marinho avaliadas neste estudo (Capítulo 3), as comunidades microbianas foram compostas predominantemente por espécies de *Penicillium* com elevada densidade (de 200 a >300 UFC g^{-1}) que mostraram ser capazes de sobreviver e/ou colonizar esses habitats extremos em questões de temperatura, pressão hidrostática e anoxicidade. *Penicillium solitum* foi obtida na maior profundidade (463 m), aparentemente em condições anaeróbicas. Gonçalves *et al*. (2013) também obtiveram apenas *P. solitum* de 100 a 1100 m de profundidade no Oceano Antártico. Além de *Penicillium*, poucos trabalhos isolaram espécies fúngicas em sedimento marinho da Antártica como espécies cosmopolitas de *Aspergillus*, *Cladosporium*, *Fusarium*, *Candida* e *Rhodothorula* (ROSA *et al*., 2019b).

Também já foram relatas nos sedimentos marinhos superficiais leveduras basidiomicéticas das ordens *Kriegeriales* (*Glaciozyma* sp. e *Phenoliferia* sp.), *Holtermanniales* (*Holtermanniella* sp.) e *Tremellales* (*Vishniacozyma victoriae*) (VAZ *et al*., 2011; WENTZEL *et al*., 2019). Neste estudo, além de *P. solitum*, ao que tudo indica, *A. fusidioides, P. allii-sativi, P. chrysogenum, P. palitans* e *P. verrucosus,* representam os primeiros relatos em sedimentos marinhos antárticos.

Existem limitações ao isolar micro-organismos de ambientes marinhos extremos, devido às dificuldades de simulação do substrato, onde se deve considerar a pressão hidrostática, salinidade, perfil de nutrientes, temperatura de incubação e nível de oxigênio no momento do cultivo (ROSA *et al*., 2019b). Frente a este desafio, existe uma subestimação na identificação de fungos antárticos marinhos e acredita-se que menos de 20% das espécies presentes no substrato já foram isoladas e cultivadas em cultura pura (BRIDGE, 2007); portanto, apesar da utilização de diferentes meios de cultivo para isolamento dos fungos, grande parte da comunidade fúngica desse substrato ainda pode ser desconhecida, principalmente as espécies fastidiosas de difícil cultivo.

Diferentemente dos ecossistemas marinhos da Antártica, os habitats lênticos de água continental da Antártica, como os lagos, sofrem com a alta luminosidade/radiação UV, o derretimento de geleiras e da neve recente, os ventos, a atividade vulcânica, o spray proveniente do mar e a extensão de gelo marinho (OGAKI *et al*., 2019). Nos ecossistemas de água continental, os fungos também atuam na decomposição, tanto de plantas (degradação da celulose e lignocelulose) quanto de animais (exoesqueletos, penas, pelos) (WONG *et al*., 1998). Essas influências biológicas das áreas de captação juntamente aos fatores abióticos podem influenciar na composição da comunidade microbiana de um lago.

Sessenta e três UTOs foram obtidas dos lagos impactados por atividade antrópica na Peninsula Fildes, Ilha Rei George, Antartica (Capítulo 4). Os lagos Norte, Central e Sul da Península Fildes possuem características fisico-químicas que os diferenciam e que podem ter influenciado nos altos índices de diversidade apresentados pela comunidade fúngica em cada lago. O lago Norte é o mais distante das estações de pesquisa, situa-se próximo à geleira Collins. A presença da geleira gera um gradiente sul-norte de condições periglaciais para paraglaciais (MICHEL *et al*., 2014), e desta forma, no lago há influência direta da água de derretimento glacial e seus arredores recebem influência das chuvas, atividades de bacia hidrográfica, fluxos de derretimentode de neve; e também dos musgos das áreas de captação que margeiam o lago. A presença de vegetação afeta as comunidades microbianas, uma vez que podem ocorrer associações entre esses organismos, como já descrito por Tosi *et al*. (2002)

e Carvalho *et al*. (2019). O lago Central se localiza próximo à estação de pesquisa Russa Bellingshausen e recebe as águas de derretimento da neve recente e também do fluxo de escoamento das geleiras ao longo de seu percurso, tratando-se também de uma região com alta influência dos ventos. Já o Lago Sul por se encontrar às margens da ilha, sofre influência do spray marinho, da extensão do gelo marinho durante o inverno e também das colônias de elefantes marinhos próximas.

As comunidades de fungos nos sedimentos dos três lagos sob atividade antrópica apresentaram elevados índices de diversidade e riqueza e dominados por táxons com distribuição cosmopolita e endêmica. Os táxons mais frequentes foram as espécies cosmopolistas *Pseudeurotium hygrophilum* (*Pseudeurotiaceae*) e *Pseudogymnoascusus verrucosus* (*Myxotrichaceae*). A espécie *P. hygrophilum* (antigo nome de *Teberdinia hygrophila*) (MINNIS & LINDNER, 2013) já foi relatada associada a substratos vegetais em áreas alpinas no hemisfério norte (SOGONOV *et al*., 2005), e também em turfeiras ricas em contaminantes como arsênico, sulfato e nitrato no norte da Finlândia (KUJALA *et al*. 2018). Na Antártica, o gênero foi identificado na camada ativa em oásis livres de gelo da Antártica Continental (KOCHKINA *et al*., 2014), esponjas (HENRÍQUEZ *et al*., 2014), solo (ARENZ & BLANCHETTE, 2009) e a espécie *P. hygrophilum* foi idetificada em solo impactado por combustíveis da estação Russa de Bellingshausen (Península de Fildes) (KOCHKINA *et al*. 2018). No presente trabalho, *P. hygrophilum* também foi identificada no lago Central próximo à estação Russa, mas também nos lagos com menor influência antrópica como o Norte e o Sul.

Já o gênero *Pseudogymnoascus* é bem difundido nas regiões polares e abundante na Antártida, ocorrendo em diferentes substratos e ambientes (ROSA *et al*., 2019a). Em lagos da Antártida, *Pseudogymnoascus* já foi relatado associado com consórcio microbiano (BRUNATTI *et al*., 2009), em sedimentos (TSUJI *et al*., 2013; GONÇALVES *et al*., 2015) e água de lagos (CONELL *et al*., 2018). *Pseudogymnoascus verrucosus* já foi relatada em diferentes habitats, como solos (GOMES *et al*., 2018) e associados a invertebrados marinhos (GODINHO *et al*., 2019). Neste estudo, *P. verrucosus* esteve presente tanto nos sedimentos marinhos da Baía Maxwell quanto nos lagos impactados e não impactados, mostrando-se bem difundida no continente. Fungos dominantes são eurotópicos com uma faixa de distribuição muito ampla (DING *et al*.,. 2016), isso se confirmou no caso deste trabalho, pois a presença desses táxons nas amostras foi independente ao estado de impacto ambiental da região amostrada.

Nos lagos amostrados sem aparente impacto antrópico (Capítulo 5) nas ilhas Pinguim, Elefante, Deception e Rei George foram identificadas 42 UTOs. Entre os táxons presentes nos sedimentos de lagos, *Thelebolus globosus*, *Antarctomyces psychrotrophicus*, *P. verrucosus*, *V. victoriae* e *Phenoliferia* sp. foram os mais prevalentes. A espécie mais frequentemente isolada nos lagos não impactados foi *T. globosus*, seguida de *A. psychrotrophicus*.

Algumas espécies psicrofílicas de *Thelebolus* já foram descritas em solos antárticos ornitogênicos e no trato digestivo de aves na Antártida (DEL FRATE & CARETTA *et al*. 1990; LEOTTA *et al*. 2002). O gênero *Thelobolus* já foi relatado em amostras lacustres (de HOOG *et al*., 2005), água de algos (GONÇALVES *et al*., 2012), sedimento e solo marginal (TSUJI *et al*., 2013). *Thelobolus globosus* foi descrito em lagos na Antártica continental (de HOOG *et al*., 2005; CONELL *et al*., 2018), porém esta espécie é amplamente encontrada em outros substratos na Antártica (ALIAS *et al*., 2013; FURBINO *et al*., 2014; SANTIAGO *et al*., 2015; ALVES *et al*., 2019; GODINHO *et al*., 2019). Já o gênero *Antarctomyces* inclui duas espécies *A. psychrotrophicus* (STCHIGEL *et al*., 2001) e a recentemente identificada como *A. pellizariae* (MENEZES *et al*., 2017). Na Ilha Rei George a espécie *A. psychrotrophicus* já foi descrita em amostras de solo (STCHIGEL *et al*., 2001), em lago próximo à geleira Stain House (GONÇALVES *et al*., 2012), já nos lagos avaliados neste estudo esteve presente não somente na Ilha Rei George, como também em amostras de lago da Ilha Deception e Pinguim. Ademais, por também se tratar de uma espécie endêmica como *T. globosus*, já foi encontrada em diversos substratos no continente (FURBINO *et al*., 2014; SANTIAGO *et al*., 2015; ABNEUF *et al*., 2016; GODINHO *et al*., 2019).

Na Antártica, os continentes e ilhas localizados nas áreas livres de gelo abrigam uma fauna de focas, pinguins e aves marinhas e uma variedade de plantas e líquens (HODGSON, 2012) e sabe-se que os lagos são ultra-oligotróficos, exceto quando eles são enriquecidos por excrementos de pássaros e restos de plantas (LAYBOURN-PARRY *et al*., 1997), provenientes de áreas marginais. Os táxons encontrados nos lagos não impactados avaliados parecem estar associados às fontes extra-aquáticas dos lagos, considerando a presença de espécies de fungos associadas a diferentes substratos terrestres da Antártida já mencionados na literatura, como aves, musgos, gramíneas, rochas e solo (DEL FRATE & CARETTA *et al*. 1990; LEOTTA *et al*. 2002; de HOOG *et al*., 2005; TOSI *et al*., 2002; SANTIAGO *et al*., 2015; GOMES *et al*., 2018; GONÇALVES *et al*., 2019).

Além disso, de forma geral nas amostras de sedimentos lacustres da Antártica avaliadas, das 105 UTOs identificadas para os 11 lagos amostrados, os gêneros *Bipolaris*, *Epicoccum*, *Chrysosporium*, *Myxotrichum*, *Neobulgaria*, *Patinella*, *Pholiota*, *Polypaecilum*,

Protomyces, *Sarocladium* e *Thielavia* foram relatados pela primeira vez. Além disso, 34 UTOs podem se tratar de espécies novas.

Para os ensaios biológicos foram avaliados 486 extratos produzidos a partir do metabolismo dos fungos quanto à atividade herbicida, nematicida, antifúngica, antiparasitária e antiviral. Os extratos dos fungos marinhos apresentaram amplo espectro de atividades em relação aqueles presentes nos sedimentos lacustres. Espécies de *Penicillium* são amplamente conhecidas devido ao seu metabolismo ativo capaz de produzir substâncias bioativas, como as penicilinas (ZIEMONS *et al*., 2017). Entre as espécies reconhecidas produtoras de penicilina foram detectadas *P*. *allii-sativi* e *P*. *chrysogenum* nos sedimentos profundos marinhos. Entre os isolados de maior profundidade (1463 m), *P*. *solitum* UFMGCB 13029 se mostrou interessante por sua alta atividade herbicida, nematicida e moderada atividade contra *L*. *amazonensis*. Outros isolados de *Penicillium* exibiram grande atividade biológica contra alvos clínicos como *P*. *falciparum* e *T*. *cruzi*.

Pesticidas abrangem herbicidas, inseticidas e fungicidas que são aplicados em plantas e animais considerados ameaças à produtividade de alimentos ou à saúde humana (MARQUES, 2019). O Brasil é um dos maiores usuário de produtos químicos na agricultura, sendo os agrotóxicos mais empregados o glifosato (herbicida), apontado por pesquisadores como nocivo à saúde e, o acefato (inseticida); tais substâncias apresentam nocividade, constatada ou potencial, para a saúde humana e para o meio ambiente (MARQUES, 2019). Por isso, a busca por alternativas naturais aos químicos é de grande importância, a fim de reduzir os impactos ambientais e também à saúde humana.

Todos os extratos de fungos marinhos apresentaram atividade nematicida e 96.8% dos isolados apresentaram atividade herbicida contra *A*. *schoenoprasum* e/ou *L*. *sativa*, a maioria exibiu elevada atividade (inibição da germinação de 4-5 sementes). Dois isolados de *Penicillium* apresentaram atividade antifúngica contra o fitopatógeno *C*. *sphaerospermum*, os mesmos isolados também foram ativos contra ambos os alvos do ensaio herbicida. No geral, os extratos apresentaram potencial satisfatório nos ensaios contra os alvos de interesse na agricultura e novas investigações devem ser realizadas para a aplicabilidade desses fungos, e/ou das substâncias produzidas pelos mesmos, como pesticidas menos nocivos.

Quantos aos extratos produzidos a partir de fungos de sedimento lacustre, 20,4% apresentaram atividade herbicida contra pelo menos um dos alvos. Contra T. *cruzi*, destacaram-se os extratos de *P*. *hygrophilum* UFMGCB 12800 e 12801, *P*. *destructans* UFMGCB 13934 e *Pezizomycotina* sp. UFMGCB 13963. Entre eles *P*. *desctructans* também apresentou alta atividade herbicida e antifúngica. Resultados similares são mencionados por

Gomes *et al*. (2018), cujos extratos de *P*. *destructans* obtidos a partir de solos da Antártica produziram compostos com atividades herbicidas moderadas e com forte atividade tripanocida.

O extrato do isolado *Trichoderma polysporum* UFMGCB 13968 apresentou baixa atividade antiplasmodial e atividade antifúngica contra o fitopatógeno *C*. *sphaerospermum*. *Trichoderma* spp. são amplamente estudadas como agentes de controle biológico e comercializados como biopesticidas, biofertilizantes, promotores de crescimento e estimulantes de crescimento em plantas (WOO *et al*., 2014). Além de *Trichoderma*, outros táxons como *Cladosporium* sp., *Mortierella* sp. e *Thelebolus globosus* também apresentaram elevadas atividades antifúngica (>90% de inibição).

7. CONCLUSÕES

Os ambientes aquáticos abrangem uma gama de habitats de água continentais e marinhas que abrigam comunidades microbianas distintas. Os sedimentos marinhos representam um ambiente/microhabitat inexplorado com condições extremas de temperatura, disponibilidade de oxigênio e alta pressão hidrostática. Neste estudo, as comunidades de fungos associadas às amostras de sedimento marinho da Antártica mostraram-se abundantes em espécies de *Penicillium*, já conhecidas por sua expressiva produção de compostos bioativos. Todos os fungos marinhos obtidos (31 isolados, distribuídos em 6 UTOs) mostraram atividade antifúngica, antiplasmodial, tripanossomicida e/ou herbicida. Além disso, a caracterização química dos extratos mais ativos indicou a presença de moléculas aromáticas, as quais podem ser responsáveis pelas atividades biológicas apresentadas.

Os ambientes lacustres antárticos são interessantes e sensíveis para o estudo de taxonomia e ecologia de comunidades microbianas extremófilas. Lagos como os da Península Fildes (Ilha Rei George) vêm sofrendo nos últimos anos com efeitos da atividade antrópica, devido ao impacto ambiental causado pela presença de estações de pesquisa e do turismo na região. Neste estudo, os fungos obtidos de sedimento lacustre da Península Fildes (260 isolados, 63 UTOs) mostraram alta diversidade e riqueza de comunidades fúngicas, as quais variaram de composição em cada lago amostrado. O Lago Central próximo à estação de pesquisa de Bellingshausen apresentou altas concentrações de metais traço e pesados, e baixos índices de riqueza e diversidade, ao contrário do Lago Norte, distante das estações de pesquisa, em que o perfil foi oposto. Sugere-se, então, que o aumento das atividades antrópicas na região afetaram a diversidade e a composição microbiana dos lagos. Além disso, extratos produzidos pelos fungos de sedimento lacustre foram capazes de produzir compostos antifúngicos, tripanocidas e herbicidas; com destaque para o táxon *Pseudeurotium hygrophilum*, um dos mais frequentemente isolados e que também apresentou atividade contra todos os alvos biológicos testados.

Já nos lagos sem influência antrópica, os táxons isolados incluíram espécies endêmicas e psicrófilas, como *Thelebolus globosus* e *Antarctomyces psychrotrophicus*, e também espécies cosmopolitas, associadas a fontes extra-aquáticas próximas a lagos (um total de 195 isolados e 42 UTOs), considerando que os táxons encontrados já têm sido relatados em diferentes ambientes terrestres da Antártica que constituem as fontes de captação para os lagos.

Entre as 111 UTOs obtidas no geral das amostras de sedimento lacustres e marinhas, um total de 34 unidades taxonômicas podem se tratar de espécies novas, as quais precisam de maior elucidação taxonômica para a confirmação dessa hipótese. No entanto, ao que tudo indica, os lagos, onde foram obtidas todas as possíveis espécies novas, representam ambientes que podem ser considerados *hotspot* de diversidade de comunidades fúngicas. Além disso, considerando a atividade biológica apresentada por uma parte considerável dos fungos avaliados (26.4%) em ambos os habitats, fungos bioprospectados em ambientes extremos como os sedimentos, onde há acúmulo de matéria orgânica, baixa oxigenação, diferenças de osmolaridade e pressão hidrostática, representam um maquinário metabólico a ser explorado biotecnologicamente, devido às características que permitiram sua resistência e sobrevivência às intempéries impostas pelas condições abióticas da Antártica. Esses metabólitos bioativos, uma vez investigados podem servir de protótipos para novos fármacos e herbicidas-pesticidas menos tóxicos para a agricultura.

8. REFERÊNCIAS BIBLIOGRÁFICAS

ABNEUF MA, KRISHNAN A, ARAVENA MG, PANG K, CONVEY P, MOHAMAD-FAUZI N, RIZMAN-IDID M, ALIAS SA (2016) Antimicrobial activity of microfungi from maritime Antarctic soil. **Czech Polar Rep**, 6:141–154.

ALFONSO J A, VASQUEZ Y, HERNANDEZ AC, MORA A, HAND H, SIRA H (2015) Geochemistry of recent lacustrine sediments from Fildes Peninsula, King George Island, maritime Antarctica. **Antarctic Science**, 27(5):462–471.

ALIAS SA, SMYKLA J, MING CY, RIZMAN-IDID M, CONVEY P (2013) Diversity of microfungi in Ornithogenic soils from Beaufort Island, continental Antarctic. **Czech Polar Rep**, 3:144–156.

ALVES IMS, GONÇALVES VN, OLIVEIRA F S, CEGR S, ROSA CA, ROSA LH (2019). Diversity, distribution, and pathogenic potential of fungi present in rocks of the South Shetlands Archipelago, Maritime Antarctica. **Extremophiles**, 23:327–336.

ARENZ B E, BLANCHETTE R A (2009) Investigations of fungal diversity in wooden structures and soils at historic sites on the Antarctic Peninsula. **Can J Microbiol**, 55:46–56.

ARENZ BE, HELD BW, JURGENS JA, FARRELL RL, BLANCHETTE RA (2006) Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. **Soil Biol Biochem**, 38:3057-3064.

BANDARANAYAKE W M (1998) Mycosporines: are they nature's sunscreens? **Natural Product Rep**, 159–171.

BARDOU P, MARIETTE J, ESCUDIÉ F, DJEMIEL C, KLOPP C (2014) JVENN: an interactive Venn diagram viewer. **BMC Bioinformatics**, 15(40):293.

BASS D, HOWE A, BROWN N, BARTON H, DEMIDOVA M, MICHELLE H, LI L, SANDERS H, WATKINSON S, WILLCOCK S, RICHARDS TA. 2007. Yeast forms dominate fungal diversity in the deep oceans. **Proc R Soc B**, 274:3069-3077.

BÉRDY J (2005) Bioctive Microbial Metabolites**. J Antibiotics** 58(1):1-26.

BIDDLE JF, LIPP JS, LEVER MA, LLOYD KG, SØRENSEN KB. ANDERSON R. *et al*. (2006). Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. **Proc Natl Acad Sci USA**, 103:3846–3851

BOMMER E, ROUSSEAU M (1905) Champignons en resultats du voyage du SY Belgica expedition Antarctique Beige 1897 - 1899. **Rapports Scientifiques Botanique**, 1–5.

BRAY J R, CURTIS J T (1957) An ordination of upland forest communities of southern Wisconsin. **Ecological Monographs**, 27: 325-349.

BRIDGE PD, SPOONER BM (2012) Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem? **Fungal Ecol**, 5:381-394.

BRIDGE P (2007) Fungi. In: Riffenburgh B (ed) **Encyclopedia of the Antarctic**. Taylor & Francis, London, p. 425–426.

BRUNATI M, ROJAS JL, SPONGA F, CICILIATO I, LOSI D, GÖTTLICH E, DE HOOG S, GENILLOUD O, MARINELLI F (2009). Diversity and pharmaceutical screening of fungi from benthic mats of Antarctic lakes. **Mar Genomics**, 2:43-50.

BUCKNER FS, VERLINDE CL, LA-FLAME AC, VAN-VOORHIS WC (1996). Efficient technique for screening drugs for activity against *Trypanossoma cruzi* using parasites expressing beta-galactosidase. **Antimicrobial Agents Chemother**, 40:2592-2597.

BUGNI TS, IRELAND CM (2004) Marine-derived fungi: a chemically and biologically diverse group of microorganisms. **Nat Prod Rep**, 21:143-163.

BUTINAR L, SPENCER-MARTINS I, GUNDE-CIMERMAN N. 2007. Yeasts in high Arctic glaciers: the discovery of a new habitat for eukaryotic microorganisms. **A van Leeuwenhoek**, 91:277–289.

BUZZINI P, BRANDA E, GORETTI M, TURCHETTI B (2012) Psychrophilic yeasts from worldwide glacial habitats: diversity adaptation strategies and biotechnological potential**. FEMS Microbiol Ecol**, 82:217-241.

CALLAHAN HL, PORTAL AC, DEVEREAUX R, GROGL M. (1997). An axenic amastigote system for drug screening. **Antimicrob Agents Chemother**, 41:818–822.

CARRETO JI, CARIGNAN MO (2011) Mycosporine-Like Amino Acids: Relevant Secondary Metabolites. Chemical Ecological Aspects. **Mar Drugs**, 9(3): 387–446.

CARVALHO CR, SANTIAGO IF, COELHO LC, CÂMARA PEAS, SILVA MC, STECH M, ROSA CA, ROSA LH (2019) Fungi Associated with Plants and Lichens of Antarctica. . In: Rosa LH (Ed) **Fungi of Antarctica: Diversity**, **Ecology and Biotechnological Applications**.. Springer Nature Switzerland, p. 165-199.

CASTELLANI A (1967). Maintenance and cultivation of common pathogenic fungi in distilled water. **Am J Trop Med Hyg**, 42:181–184.

CHOI D, PARK YK, OH SK, YOON HJ, KIM JC, SEO WJ, CHA SH (1997) Distribution of airborne microorganisms in yellow sands of Korea. **J Microbiol**, 35:1-9.

CHOWN ST (2007) Biodiversity terrestrial. In: RIFFENBURGH B. (Ed) **Encycolpledia of the Antarctic**. New York: Routledge Taylor & Francis Group, 149-156.

CHRISTENSEN M (1989). A view of fungal ecology. **Mycologia**, 81:1-19.

CHUGH JK, WALLACE BA (2001) "Peptaibols: models for ion channels". **Biochem Soc Transactions**, 29 (4): 565–570.

CLARK GF, STARK JS, PALMER AS, RIDDLE MJ, JOHNSTON EL (2017) The roles of sea-ice, light and sedimentation in structuring shallow Antarctic benthic communities**. Plos One**, 12:e0168391

COLWELL RK, MAO CX, CHANG J (2004) Interpolating extrapolating and comparing incidence-based species accumulation curves. **Ecology**, 85(10):2717-2727.

CONNELL L, SEGEE B, REDMAN R, RODRIGUEZ RJ, STAUDIGE H (2018). Biodiversity and abundance of cultured microfungi from the permanently ice-covered Lake Fryxell, Antarctica. **Life**, 8:1–10.

CONVEY P (2007) Biogeography In: RIFFENBURGH B. (Ed) **Encycolpledia of the Antarctic**. New York: Routledge Taylor & Francis Group, 156-160.

COWAN DA, TOW LA (2004) Endangered Antartic Environments. **Annu Rev Microbiol**, 58:649-690.

COWIE GL, HEDGES JI (1994) Biochemical indicators of diagenetic alteration in natural organic matter mixtures. **Nature**, 369:304–307.

CUI X, ZHU G, LIU H, JIANG G, WANG J, ZHU W (2016). Diversity and function of the Antarctic krill microorganisms from *Euphausia superba*. **Sci Rep**, 6:36496.

D'ELLIA T, VEERAPANENI R, THERAISNATHAN V, ROGERS SO (2009) Isolation of fungi from Lake Vostok accretion ice. **Mycologia**, 101:751–763.

DE HOOG GS, GÖTTLICH E, PLATAS G, GENILLOUD O, LEOTTA G, VAN BRUMMELEN J (2005) Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. **Studies in Mycology**, 51:33–76.

DE MENEZES GC, GODINHO VM, PORTO BA, GONÇALVES VN, ROSA LH (2017) *Antarctomyces pellizariae* sp. nov., a new, endemic, blue, snow resident psychrophilic ascomycete fungus from Antarctica. **Extremophiles**, 21(2):259-269.

DEL FRATE G, CARETTA G (1990) Fungi isolated from Antarctic material. **Polar Biol**, 11(1):1-7.

DELONG EF, PACE NR (2001) Environmental diversity of bacteria and archaea. **Syst Biol**, 50:470-478.

DEMAIN AL, VAISHNAV P (2011) Natural products for cancer chemotherapy. **Microb. Biotechnol**, 4(6):687–699.

DING Z, LI L, CHE Q, LI D, GU Q, ZHU T (2016) Richness and bioactivity of culturable soil fungi from the Fildes Peninsula, Antarctica. **Extremophiles**, 20:425–435.

DIX NJ, WEBSTER J (1995) **Fungal Ecology**. 1ed. London: Chapman and Hall, 549 p.

DUARTE AW1, DAYO-OWOYEMI I, NOBRE FS, PAGNOCCA FC, CHAUD LC, PESSOA A, FELIPE MG, SETTE LD (2013) Taxonomic assessment and enzymes production by yeasts isolated from marine and terrestrial Antarctic samples, **Extremophiles**, 17(6):1023-35.

EDGCOMB VP, BEAUDOIN D, GAST R, BIDDLE JF, TESKE A (2011) Marine subsurface eukaryotes: the fungal majority. **Environ Microbiol**, 13:172-183.

ELLIS- EVANS JC (1996) Microbial diversity and function in Antarctic freshwater ecosystems**. Biodivers Conserv** 5: 1395–1431.

FELLER G, GERDAY C (2003) Psychrophilic enzymes: hot topics in cold adaptation. **Nat Rev Microbiol**, 1:200-208.

FENCHEL, T. BLACKBURN, H.; KING, G.M. (2012). Chapter 7: Aquatic Sediments. In: Fenche, T, Blackburn H, King GM. **Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling**. Elsevier, London UK, p.121-142.

FERREIRA MC, CANTRELL CL, DUKE SO, ALI A, ROSA LH (2017) New Pesticidal Diterpenoids from *Vellozia gigantea* (*Velloziaceae*) an Endemic Neotropical Plant Living in the Endangered Brazilian Biome Rupestrian Grasslands. **Molecules**, 22(1):175.

FERRER M, GOLYSHINA O, BELOQUI A, GOLYSHIN PN (2007) Mining enzymes from extreme environments. **Current Opin Microbiol**, 10:207-214.

FISHER RA, CORBET AS, WILLIAMS CB (1943) The relation between the number of species and the number of individuals in a random sample of an animal population. **J Animal Ecol**, 12:42-58.

FURBINO LE, GODINHO VM, SANTIAGO IF, PELLIZARI FM, ALVES TMA, ZANI CL, JUNIOR PAS, MURTA SMF, ROMANHA AJ, CARVALHO AGO, GILL HVG, ROSA CA, MINNIS AM ROSA LH (2014) Diversity patterns ecology and biological activities of fungal

communities associated with the endemic macroalgae across the Antarctic Peninsula. **Microbial Ecol**, 67:775-787.

FURBINO L, PELLIZZARI FC, NETO PA, ROSA C, ROSA LH (2017) Isolation of fungi associated with macroalgae from maritime Antarctica and their production of agarolytic and carrageenolytic activities. **Polar Biology**, 41:527.

GAMUNDI I.J. SPINEDI H.A. *Sclerotinia antarctica* sp. nov. the teleomorph of the first fungus described from Antarctica. **Mycotaxon** v.29 p.81-89 1987.

GARRISON VH, SHINN EA, FOREMAN WT, GRIFFI N, DW, HOLMES CW, KELLOGG CA, MAJEWSKI MS, RICHARDSON LL, RITCHIE KB, SMITH GW (2003) African and Asian dust: From desert soils to coral reefs. **Bioscience**, 53: 469–480.

GLASS NL, DONALDSON GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. **Appl Environ Microbiol**, 61(4):1323-1330.

GLÖCKNER FO, STAL LJ, SANDAA R-A, GASOL JM, O'GARA F, HERNANDEZ F, LABRENZ M, STOICA E, VARELA MM, BORDALO A, PITTA P (2012) Marine Microbial Diversity and its role in Ecosystem Functioning and Environmental Change. In: CALEWAERT JB, MCDONOUGH N (Eds). **Marine Board Position Paper 17**. Marine Board-ESF, Ostend, Belgium, p.1-84.

GODINHO VM, FURBINO LE, SANTIAGO IF, PELLIZZARI FM, YOKOYA NS, PUPO D, ALVES TMA, JUNIOR PAS, ROMANHA AJ, ZANI CL, CANTRELL CL, ROSA CA, ROSA LH (2013) Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. **The ISME Journal**, 7(7):1434-1451.

GODINHO VM, GONÇALVES VN, SANTIAGO IF, FIGUEREDO HM, VITORELI GA, SCHAEFER CEGR, BARBOSA EC, OLIVEIRA JG, ALVES TMA, ZANI CL, JUNIOR PAS, MURTA SMF, ROMANHA AJ, KROON EG, CANTRELL CL, WEDGE DE, DAVID E, DUKE SO (2015). STEPHEN O. ALI A. ROSA C.A. ROSA L.H. Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica. **Extremophiles**, 19:585-596.

GODINHO VM, DE PAULA MTR, SILVA DAS, PARESQUE K, MARTINS AP, COLEPICOLO P, ROSA CA, ROSA LH (2019) Diversity and distribution of cryptic cultivable fungi associated with marine animals of Antarctica. **Fungal Biol**, 123:507-516.

GOMES ECQ, GODINHO VM, SILVA DAS, DE PAULA MTR, VITORELI GA, ZANI CL, ALVES TMA, JUNIOR PAS, MURTA SMF, BARBOSA EC, OLIVEIRA JG, OLIVEIRA FS, CARVALHO CR, FERREIRA MC, ROSA CA, ROSA LH (2018). Cultivable fungi present in Antarctic soils: taxonomy phylogeny diversity and bioprospecting of antiparasitic and herbicidal metabolites **Extremophiles**, 22(3)381-393.

GONÇALVES VN, VAZ ABM, ROSA CA, ROSA LH (2012) Diversity and distribution of fungal communities in lakes of Antarctica. **FEMS Microbiol Ecol**, 82:459–471.

GONÇALVES VN, CAMPOS L, MELO IS, PELLIZARI VH, ROSA CA, ROSA LH (2013) *Penicillium solitum*: a mesophilic psychrotolerant fungus present in marine sediments from Antarctica. **Polar Biol**, 36:1823-1831.

GONÇALVES VN, CARVALHO CR, JOHANN S, MENDES G, ALVES TMA, ZANI CL, JUNIOR PAS, MURTA SMF, ROMANHA AJ, CANTRELL CL, ROSA CA, ROSA LH (2015) Antibacterial antifungal and antiprotozoal activities of fungal communities present in different substrates from Antarctica. **Polar Biol**, 38(8):1143-1152.

GONÇALVES VN, OLIVEIRA FS, CARVALHO C, SCHAEFER CEG, ROSA CA, ROSA LH (2017) Antarctic rocks from continental Antarctica as source of potential human opportunistic fungi. **Extremophiles** 21:851–860.

GONÇALVES VN, ALVES IMS, OLIVEIRA FS, SCHAEFER CEGR, Turbay, CVG, ROSA CA, ROSA LH (2019) Rock-Inhabiting Fungi in Antarctica: New Frontiers of the Edge of Life. In: Rosa LH (Ed) Fungi of Antarctica: **Diversity**, **Ecology and Biotechnological Applications**. Springer Nature Switzerland, p. 99-126

GROSSART HP, ROJAS-JIMENEZ K (2016). Aquatic fungi: targeting the forgotten in microbial ecology. **Curr Opin Microbiol**, 31:140-145.

GUNDE-CIMERMAN N, SONJAK S, ZALAR P, FRISVAD JC, DIDERICHSEN B, PLEMENITAS A (2003) Extremophilic fungi in Arctic ice: a relationship between adaptation to low temperature nd water activity. **Physic and Chemistry of the Earth** 28:1273-1278.

HAMMER Ø, HARPER DAT, RYAN PD (2001) PAST: paleontological sta-tistics software package for education and data analysis. **Paleont Electron**, 4:1–9

HASAN S, ANSARI MI, AHMAD A, MISHRA M (2015). Major bioactive metabolites from marine fungi: A Review. **Bioinformation** 11(4):176-18.

HEDGES JI, KEIL RG (1995) Sedimentary organic matter preservation: An assessment and speculative synthesis**. Marine Chemistry**, 49:(2-3):81-115

HENRÍQUEZ M, VERGARA K, NORAMBUENA J, BEIZA A, MAZA F, UBILLA P, ARAYA I, CHÁVEZ R, SAN-MARTÍN A, DARIAS J, DARIAS MJ, VACA I (2013) Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial antitumoral and antioxidant potential. **World J. Microbiol Biotechnol**, 30(1):65-76.

HODGSON DA (2012) Antarctic Lakes. In: Bengtsson L, Herschy RW, Fairbridge RW (eds) **Encyclopedia of lakes and reservoirs**. Springer, Dordrecht/London, p. 26–30.

HONGXIANG X, MIN W, XAOGU W, JUNYI Y, CHUNSHENG W (2008) Bacterial diversity in deep-sea sediment from northeastern Pacific Ocean. **Acta Ecol Sin**, 28(2):479-485.

HOSHINO T, MATSUMOTO N (2012) Cryophilic fungi to denote fungi in cryosphere. **Fungal Biol Rev**, 26: 102–105.

HOUBRAKEN, J., FRISVAD, J.C., SEIFERT, K.A., OVERY, D.P., TUTHILL, D.M., VALDEZ, J.G., Samson RA (2012) New penicillin-producing *Penicillium* species and an overview of section *Chrysogena*. **Persoonia 29**: 78–100.

HYDE KD, HONGSANAN S, JEEWON R, BHAT DJ, MACKENZIE EHC, JONES EBG, PHOOKAMSAK R, ARIYAWANSA HA, BOONME *et al*. (2016). Fungal diversity notes 367-491: taxonomic and phylogenetic contributions to fungal taxa. **Fungal Diversity**. 80:1-270.

HYDE KD, JONES GEB, LEAÑO E, POINTING SB, POONYTH AD, VRIJMOED LLP (1998) Role of fungi in marine ecosystems. **Biodiversity Conserv**, 7: 1147.

INAGAKI F, NUNOURA T, NAKAGAWA S, TESKE A, LEVER M, LAUER A, SUZUKI M, TAKAI K, DELWICHE M, COLWELL FS, NEALSON KH, HORIKOSHI K, D'HONDT S, JORGENSEN BB (2006) Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. **Proc Natl Acad Sci USA**, 103:2815–2820.

JONES GEB, SUETRONG S, SAKAYAROJ J, BAHKALI AH, ABDEL-WAHAB MA, BOEKHOUT T, PANG K (2015) Classification of marine *Ascomycota*, *Basidiomycota*, *Blastocladiomycota* and *Chytridiomycota*. **Fungal Diversity**, 73: 1-72.

JONES JG, BERNER RA, MEADOWS OS, DURAND B, EGLINTON G (1985) "Microbes and Microbial Processes in Sediments [and Discussion]." **Philosophical Transactions of the Royal Society of London. Series A**, **Mathematical and Physical Sciences**, 315(1531):3–17.

JOYCE SA, LANGO L, CLARKE DJ (2011). The Regulation of Secondary Metabolism and Mutualism in the Insect Pathogenic Bacterium *Photorhabdus luminescens*. **Ads Appl Microbiol**, 76:1-25.

KIRK PM, CANNON PF, MINTER DW, STALPERS JA (2008). **Dictionary of the Fungi**. 10ed. Wallingford: CABI 640 p.

KOCHKINA GA, OZERSKAYA SM, IVANUSHKINA NE, CHIGINEVA NI, VASILENKO OV, SPIRINA EV, GILICHINSKII DA (2014) Fungal diversity in the Antarctic active layer. **Microbiology (Moscow)**, 83:94–101.

KOCHKINA GA, IVANUSHKINA NE, LUPACHEV AV, STARODUMOVA IP, VASILENKO OV, OZERSKAYA SM (2018) Diversity of mycelial fungi in natural and human‐affected Antarctic soils. **Polar Biol**, 42: 47-64.

KOGEJ T, GOSTINCAR C, VOLKMANN M, GORBUSHINA AA, GUNDE-CIMERMAN N (2006) Mycosporines in Extremophilic Fungi — Novel Complementary Osmolytes? **Environ Chem**, 3:105–110.

KOHLMEYER J, KOHLMEYER E (1979) **Marine mycology: the higher fungi**. Academy Press, New York.

KOHLMEYER J, VOLKMANN-KOHLMEYER B, NEWELL SY (2004) Marine and estuarine mycelial Eumycota and Oomycota. In: MUELLER G M, BILLS G G, FOSTER M S (eds) **Biodiversity of fungi: inventory and monitoring methods**. Elsevier Academic Press, New York.

KOIVUSAARI P, TEJESVI MV, TOLKKINEN M, MARKKOLA A, MYKRÄ H, PIRTTILÄ AM (2019) Fungi Originating From Tree Leaves Contribute to Fungal Diversity of Litter in Streams. **Front Microbiol**, 10(651):1-15.

KUJALA K, MIKKONEN A, SARAVESI K, RONKANEN A, MARJA T (2018) Microbial diversity along a gradient in peatlandstreating mining-affected Waters. **FEMS Microbiol Ecol**, 94(10):1-15.

LACHANCE MA, BOWLES JM, STARMER WT, BARKER JSF (1999) *Kodamaea kakaduensis* and *Candida tolerans* two new ascomycetous yeasts species from Australian Hibiscus flowers. **Canadian J Microbiol** 45:172-177.

LAICH F, VACA I, CHAVEZ R (2013) *Rhodotorula portillonensis* sp. nov., a basidiomycetous yeast isolated from Antarctic shallow-water marine sediment. **Int J Syst Evol Microbiol**, 63:3884–3891

LAYBOURN-PARRY J, JAMES MR, MCKNIGHT DM, PRISCU J, SPAULDING SA, SHIEL R (1997) The microbial plankton of Lake Fryxell Southern Victoria Land Antarctica during the summers of 1992 and 1994. **Polar Biol**, 17:54–61.

LE CALVEZ T, BURGAUD G, MAHÉ S, BARBIER G, VANDENKOORNHUYSE P (2009) Fungal Diversity in Deep-Sea Hydrothermal **Eco Appl Environ Microbiol**, 75:6415-21.

LEE JK, PARK KS, PARK S, PARK H, SONG YH, KANG SH, KIM HJ (2010) An extracellular ice-binding glycoprotein from an Arctic psychrophilic yeast. **Cryobiology**, 60:222–238.

LEOTTA GA, PARÉ JA, SIGLER L, MONTALTI D, VIGO G, PETRUCCELLI M, REINOSO EH (2002) *Thelebolus microspores* mycelial mats in the trachea of wild brown skua (*Catharacta antarctica lonnbergi*) and South Polar Skua (*C. maccormicki*) carcasses. **J Wildlife Diseases**, 38:443–447.

LI Y, SUN B, LIU S, JIANG L, LIU X, ZHANG H, CHE Y (2008) Bioactive asterric acid derivatives from the Antarctic ascomycete fungus *Geomyces* sp. **J Nat Prod** 71:1643–1646.

LIANG WL, LE X, LI HJ, YANG XL, CHEN JX, XU J, LIU HL, WANG LY, WANG KT, HU KC, YANG DP, LAN WJ (2014) Exploring the chemodiversity and biological activities of the secondary metabolites from the marine fungus Neosartorya pseudofischeri. **Mar Drugs**, 12(11):5657-76.

LIECKFELDT E, MEYER W, FAU-BORNER T, BORNER T (1993) Rapid identification and differentiation of yeasts by DNA and PCR fingerprinting. **J Basic Microbiol**, 33(6)413-25.

LIU JT, HU B, GAO Y, ZHANG JP, JIAO BH, LU XL, LIU XY (2014) Bioactive tyrosine-derived cytochalasins from fungus *Eutypella* sp. D-1. **Chem Biodivers**, 11(5):800-6.

LÓPEZ-GARCIA P, RODRIGUEZ-VALERA F, PEDROS-ALLO C, MOREIRA D, (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. **Nature**, 409:603-607

LUNA GM, STUMM K, PUSCEDDU A, DANOVARO R (2009) Archaeal diversity in deep-sea sediments estimated by means of different terminal-restriction fragment length polymorphisms (T-RFLP) Protocols. **Curr Microbiol**, 59:356-361.

MALKUS A, CHANG PF, ZUZGA SM, CHUNG KR, SHAO J, CUNFER BM, ARSENIUK E, UENG PP (2014) RNA polymerase II gene (RPB2) encoding the second largest protein subunit in *Phaeosphaeria nodorum* and *P. avenaria*. **Chem Biodivers**, 11(5):800-6.

MANIMEGALAI K, DEVI NA, PADMAVATHY S (2013) Marine fungi as a source of secondary metabolites of antibiotics. **Int J Biotechnol Bioeng Res**, 4:275-282.

MARQUES L (2019) Atlas do envenenamento alimentar no Brasil. Disponível em: <https://www.unicamp.br/unicamp/ju/artigos/luiz-marques/atlas-do-envenenamento-alimentar-no-brasil>. Acesso: ago, 2019.

MAYER AM, RODRÍGUEZ AD, TAGLIALATELA-SCAFATI O, FUSETANI N (2013). Marine pharmacology in 2009-2011: marine compounds with antibacterial antidiabetic antifungal anti-inflammatory antiprotozoal antituberculosis and antiviral activities affecting the immune and nervous systems and other miscellaneous mechanisms of action. **Mar Drugs** 11(7):2510-73.

MCINNES SJ (2003) A predatory fungus (*Hyphomycetes: Lecophagus*) attacking Rotifera and Tardigrada in maritime Antarctic lakes. Polar Biol 26:79–82.

MIDDELBURG, J.J., 1989. A simple rate model for organic-matter decomposition in marine-sediments. **Geochim Cosmochim Acta**, 53:1577–1581.

MICHEL RFM, SCHAEFER CEGR, SIMAS FMB, FRANCELINO MR, FERNANDES-FILHO EI, LYRA GB, BOCKHEIM JG (2014). Active-layer thermal monitoring on the Fildes Peninsula, King George Island, maritime Antarctica. Solid Earth, 5:1361–1374.

MINNIS AM, LINDNER DL (2013) Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. **Fungal Biol**, 117:638–649.

MITCHELL DH, STILES JW, SANTELLI J, SANADI DR (1979) Synchronous Growth and Aging of *Caenorhabditis elegans* in the Presence of Fluorodeoxyuridine. **J Gerontology**, 34:28–36.

MOORE, JK, DONEY SC, GLOVER DM, FUNG IY (2002). Iron cycling and nutrient-limitation patterns in surface waters of the World Ocean. **Deep-Sea Research II** 49:463–507

MOREL FMM, PRICE NM (2003). The Biogeochemical Cycles of Trace Metals in the Oceans. **Science**, 300:944

MORITA R.Y. Psychrophilic bacteria. **Bacteriol. Rev.** v.39 p.144-167 1975.

MOSMANN T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. **J Immuno Methods**, 65(1-2):55-63

MUHAMMED M, FUCHS BB, WU MP, BREGER J, COLEMAN JJ, MYLONAKIS E (2012) The role of mycelium production and a MAPK-mediated immune response in the *C. elegans* - Fusarium model system. **Med Mycol**, 50:488–496.

NAGANO Y. NAGAHAMA T. HATADA Y. NUNOURA T. TAKAMI H. MIYAZAKI J. TAKAI K. HORIKOSHI K. Fungal diversity in deep-sea sediments – the presence of novel fungal groups. **Fungal Ecol.** v.3 p.316-325 2010.

NASA. Antarctica Sea Ice. Disponível em: <http://earthobservatory.nasa.gov/Features/SeaIce/page4.php>. Acesso em: 12 ago 2015.

NASA. What Is Antarctica? Disponível em: <https://www.nasa.gov/audience/forstudents/5-8/features/nasa-knows/what-is-antarctica-58.html>. Acesso em: 16 abr 2018.

NCBI (National Center for Biotechnology Information). (2019a) PubChem Database. Cephalosporin C, CID=65536, Disponível em:<https://pubchem.ncbi.nlm.nih.gov/compound/Cephalosporin-C>. Acesso em: 6 ago, 2019.

NCBI (National Center for Biotechnology Information). (2019b) PubChem Database. Geomycin. Disponível em: <https://pubchem.ncbi.nlm.nih.gov/#query=geomycin>.Acesso em: 6 ago, 2019.

NCCL – National Committee for Clinical Laboratory Standards (2002) Reference method for broth dilution antifungal susceptibility testing of filamentous fungi approved standard second ed. NCCLS Document M 38-A vol.22 No. 16 NCCLS Wayne Pa pp. 1-29.

NELSON, DM, DEMASTER, DJ; DUNBAR, RB, SMITH WOJ (1996) Cycling of organic carbon and biogenic silica in the Southern Ocean: Estimates of water-column and sedimentary fluxes on the Ross Sea continental shelf. **J Geophys Res**, 101(8):18519-18532.

NEWSHAM KK, HOPKINS DW, CARVALHAIS LC, FRETWELL PT, RUSHTON SP, O'DONNELL AG, DENNIS PG (2015) Relationship between soil fungal diversity and temperature in the maritime Antarctic. **Nature Climate Change** 6:182-186.

NIU G, TAN H (2013) Biosynthesis and regulation of secondary metabolites in microorganisms **Sci China Life Sci**, 56(7):581–583.

OGAKI MB, VIEIRA R, LÍRIO JM, ROSA CA, ROSA LH (2019) Diversity and ecology of fungal assemblages present in lakes of Antarctica. In: ROSA LH (Ed) **Fungi of Antarctica:**

Diversity, **Ecology and Biotechnological Applications**. Springer Nature, Switzerland, p.69-97.

ONI OE, SCHMIDT F, MIYATAKE T, KASTEN S, WITT M, HINRICHS KU, FRIEDRICH MW (2015) Microbial Communities and Organic Matter Composition in Surface and Subsurface Sediments of the Helgoland Mud Area North Sea. **Frontiers Microbiol**, 6:1290.

PASCALE D, DE SANTI C, FU J, LANDFALD B (2012) The microbial diversity of Polar environments is a fertile ground for bioprospecting. **Marine Genomics**, 8:15-22.

PETTIT RK (2011) Culturability and secondary metabolite diversity of extreme microbes: Expanding contribution of deep sea and deep-sea vent microbes to natural product discovery. **Mar Biotechnol**, 13:1-11.

PHADTARE S (2004) Recent developments in bacterial cold-shock response. **Curr Issues Mol Biol** 6:125-136.

PHILLPOT HR (1985) geography-climate. In: BONNER WN, WALTON DWH. (Ed.) **Key Environments-Antarctica.** Oxford: Pergamon Press, p.23-38.

PORTA-DE-LA-RIVA M, FONTRODONA L, VILLANUEVA A, CERÓN J (2012) Basic Caenorhabditis elegans methods: synchronization and observation. **J Vis Exp**, 10: e4019.

QUAYLE WC, PECK LS, PEAT H, ELLIS-EVANS JC, HARRIGAN PR (2002) Extreme responses to climate change in Antarctic lakes. **Science**, 295:645.

RABINOWITZ H, VOGEL S (2009) Chapter 11: Style and Usage in Earth and Environmental Sciences. In: RABINOWITZ H, VOGEL S (Eds) **The Manual of Scientific Style**. Elsevier, Oxford UK, p.445.

RAGHUKUMAR C (2008) Marine fungal biotechnology: an ecological perspective. **Fungal Diversity**, 31:19-35.

RAGHUKUMAR C, DAMARE SR, SINGH P (2010) A review on deep-sea fungi occurrence diversity and adaptations. **Bota Mar**, 53:479-492.

RAGHUKUMAR C, RAGHUKUMAR S, SHEELU G, GUPTA SM, NAGENDER NATH B, RAO BR (2004) Buried in time: Culturable Fungi in a Deep-sea Sediment Core from the Chagos Trench Indian Ocean. **Deep-Sea Res**, 51:1759-1768.

RAGHUKUMAR S (2017) Fungi in Coastal and Oceanic Marine Ecosystems. Marine Fungi. Springer Germany, pp. 378.

RÉDOU V, NAVARRI M, MESLET-CLADIÈRE L, BARBIER G, BURGAUD G (2015). Marine Fungi from Deep Subseafloor Sediments: Species richness and Adaptation. **Appl Environ Microbiol**, 15;81(10):3571-83.

REN J, XUE C, TIAN L, XU M, CHEN J, DENG Z, PROKSCH P, LIN W (2009) Asperelines A–F peptaibols from the marine-derived fungus *Trichoderma asperellum*. **J Nat Prod**, 72:1036–1044.

REN J, YANG Y, LIU D, CHEN W, PROKSCH P, SHAO B, LIN W (2013) Sequential determination of new peptaibols asperelines G-Z12 produced by marine-derived fungus *Trichoderma asperellum* using ultrahigh pressure liquid chromatography combined with electrospray-ionization tandem mass spectrometry. **J Chromatogr**, 1309:90–95.

ROBINSON CH (2001) Cold adaptation in Arctic and Antarctic fungi. New Phytol 151:341–353.

ROBINSON CT, Gessner MO, Callies Ka Jolidon C, Ward JV (2000) Larch needle breakdown in contrasting streams of an alpine glacial floodplain. **J N Am Benthol Soc**, 19:250–262

ROGERS SO, SHTARKMAN YMS, KOÇER ZA, EDGAR R, VEEPANENI R, D'ÉLIA T (2013) Ecology of subglacial Lake Vostok (Antarctica), based on metagenomic/metatranscriptomic analyses of accretion ice. **Biology**, 2:629–650.

ROMANHA AJ, DE CASTRO SL, SOEIRO MNC, LANNES-VIEIRA J, RIBEIRO I, TALVANI A, BOURDIN B, BLUM B, OLIVIERI B, ZANI CL, SPADAFORA C, CHIARI E, CHATELAIN E, *et al*. (2010) In vitro and in vivo experimental models for drug screening and development for Chagas disease. **Mem Inst Oswaldo Cruz**, 105:p.233–238.

ROSA LH, VAZ AB, CALIGIORNE RB, CAMPOLINA S, ROSA CA (2009) Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv. (*Poaceae*). **Polar Biol**, 32(2):161-167.

ROSA LH, Zani CL, Cantrell CL, Duke SO, Van Dijck P , Desideri A, Rosa CA (2019a) Fungi in Antarctica: Diversity, Ecology, Effects of Climate Change, and Bioprospection for Bioactive Compounds. In: Rosa LH (Ed) **Fungi of Antarctica: Diversity, Ecology and Biotechnological Applications**. Springer Nature Switzerland, p.1-17.

ROSA LH, PELLIZARI FM, OGAKI MB, DE PAULA MT, MANSILLA A, MARAMBIO J, COLEPICOLO P, NETO AA, VIEIRA R, ROSA CA (2019b) Sub Antarctic and Antarctic marine ecosystems: an unexplored ecosystem of fungal diversity In: Rosa LH (Ed) **Fungi of Antarctica: Diversity, Ecology and Biotechnological Applications**, Springer, Berlin, Heidelberg. p.221-242.

ROTH FJ, Orpurt PA, Ahearn DJ (1964) Occurrence and distribution of fungi in a subtropical marine environment. **Can J Bot**, 42:375-383.

RUISI S, BARRECA D, SELBMANN L, ZUCCONI L, ONOFRI S (2007) Fungi in Antarctica. **Rev Env Sci Biotechnol** 6:127-141.

RUSSELL NJ (1990). Cold adaptation of microorganisms. **Philos Trans Roy Soc London Ser B**, 326:595-611.

SANTIAGO IF, ALVES TMA, RABELLO A, JUNIOR PAS, ROMANHA AJ, ZANI CL, ROSA CA, ROSA LH (2012). Leishmanicidal and antitumoral activities of endophytic fungi associated with the Antarctic angiosperms *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. **Extremophiles**, 16:95-103.

SANTIAGO IF, SOARES MA, ROSA CA, ROSA LH (2015) Lichensphere: a protected natural microhabitatof the non-lichenised fungal communities living in extreme environments of Antarctica.Extremophiles, 19:1087-1097.

SASS H, PARKES RJ (2011). Sub-seafloor sediments: an extreme but globally significant prokaryotic habitat (taxonomy diversity ecology). In: HORIKOSHI K. ANTRANIKIAN G. BULL A.T. ROBB F.T. STETTER K.O. (Ed.) **Extremophiles Handbook**. Tokyo: Springer, p.1016-1036.

SATTLER B, STORRIE-LOMBARDI M, (2009) Laser-induced fluorescence emission (L.I.F.E.): in situ nondestructive detection of microbial life in the ice covers of Antarctic lakes. **Astrobiology**, 9(7):659-672.

SATYANARAYANA T, RAGHUKUMAR C, SHIVAJI S (2005) Extremophilic microbes: Diversity and perspectives. **Current Science**, 89(1) 78-90.

SHEARER CA, DESCALS H, KOHLMEYER B, KOHLMEYER J, MARVANOVÁ L, PADGETT D, PORTER D, RAJA HÁ, SCHMIT JP, THORTON HA, VOGLYMAYR H (2007) Fungal biodiversity in aquatic habitats, Biod Conservation, 16:49–67.

SHIVAJI S, PRASAD GS (2009). Antarctic yeasts: biodiversity and potential applications. In: SATYANARAYANA T, KUNZE G. **Yeast biotechnology: Diversity and applications**. Netherlands: Springer, p. 3-18.

SINGH P, RAGHUKUMAR C, MEENA RM, VERMA P, SHOUCHE Y (2012) Fungal diversity in deep-sea sediments revealed by culture-dependent and culture-independent approaches. **Fungal Ecol**, 5:543-553.

SMILKSTEIN M, SRIWILAIJAROEN N, KELLY JX, WILAIRAT P, RISCOE M. (2004) Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. **Antimicrob Agents Chemother**, 48(5):1803-6.

SMITH RIL (1991) Exotic sporomorpha as indicators of potential immigrant colonists in Antarctica, **Grana**, 30:2, 313-324.

Ambio, 26:578-582.

SOGIN M, MORRISON HG, HUBER JA, WELCH DM, HUSE SM, NEAL PR, ARRIETA JM, HERNDL GJ (2006) Microbial diversity in the deep sea and underexplored rare biosphere. **Proc Natl Acad Sci USA**, 1030(32):12115-12120.

STANLEY SO, ROSE AH (1967) Bacteria and yeasts from lakes on Deception Island. **Philos Trans R Soc**, Ser A 252:199–207.

STCHIGEL AM, CANO J, CORMACK WM, GARRO J (2001) *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. **Mycological Research**, 105(3):377–382.

STERFLINGER K, TESEI D, ZAKHAROVA K (2009). Fungi in hot and cold deserts with particular reference to microcolonial fungi. **Fungal Ecol**, 5:453-462.

TAMURA K, STECHER G, PETERSON D, FILIPSKI A, KUMAR S (2013). MEGA 6: Molecular Evolutionary Genetics Analysis Version 6.0. **Mol Biol Evol**, 30:2725-–2739.

TIWARI K (2014) The Future Products: Endophytic Fungal Metabolites. **J Biodivers Biopros Dev**, 2(1):145.

TOSI S, CASADO B, GERDOL R, CARETTA G (2002) Fungi isolated from Antarctic mosses. Polar Biol 25:262–268

TSUJI M (2016) Cold-stress responses in the Antarctic basidiomycetous yeast Mrakia blollopis. **Royal Soc Open Sci**, 31:60106.

TSUJI M, FUJIU S, XIAO N, HANADA Y, KUDOH S, KONDO H, TSUDA S, HOSHINO T (2013) Cold adaptation of fungi obtained from soil and lake sediment in the Skarvsnes ice-free area, Antarctica. **FEMS Microbiol Lett**, 346:121–130.

VAZ ABM, ROSA LH, VIEIRA MLA, GARCIA V, BRANDÃO LR, TEIXEIRA LCRS, MOLINÉ M, LIBKIND D, van BROOCK M, ROSA CA (2011) The diversity extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. **Braz J Microbiol**, 42:937-947.

VINCENT WI (2000). Evolutionary origins of Antarctic microbiota: invasion selection and endemism. **Antarctic Science**, 12(3):374-385.

VOSS M, BANGE HW, DIPPNER JW, MIDDELBURG JJ, MONTOYA JP, WARD B (2013) The marine nitrogen cycle: recent discoveries uncertainties and the potential relevance ofclimate change. **Phil Trans R Soc B**, 368:121.

WAIT D, RAMSAY C, MANEY J (2015) Chapter 4: The measurement process. In: Murphy, B. L. Morrison, RD (Ed) **Introduction to Environmental Forensics**. 3ed, Academic press, pp.65-97.

WAKEHAM SG, LEE C, HEDGES JI, HERNES PJ, PETERSON MJ (1997) Molecular indicators of diagenetic status in marine organic matter. **Geochim Cosmochim Acta**, 61:5363–5369.

Wentzel LCP, Inforsato FJ, Montoya QV, Rossin BG, Nascimento NR, Rodrigues A, Sette LD (2018) Fungi from Admiralty Bay (King George Island, Antarctica) soils and marine sediments. **Microb Ecol**, 77:12–24,

WHITE TJ, BRUNS T, LEE S, TAYLOR J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: GURR S. J. **PCR Protocols: A guide methods and applications**. London: Academic Press, p.315-322.

WHITMAN WB, COLEMAN DC, WEIBE WJ (1998). Prokaryotes: the unseen majority. **P Natl Acad Sci USA**, 95:6578-6583.

WILKINS D, YAU S, WILLIAMS TJ, ALLEN MA, BROWN MV, DEMAERE MZ, LAURO FM, CAVICCHIOLI R (2013). Key microbial drivers in Antarctic aquatic environments. **FEMS Microbiol Rev**, 37:303-335.

WONG MKM, GOH TK, HODGKISS J, HYDE KD, RANGHOO M, TSUI CKM, HO WH, WONG WSW, YUEN TK (1998) Role of fungi in freshwater ecosystems. **Biodiversity and Conservation**, 7:1187-1206.

WOO SL, RUOCCO M, VINALE F, NIGRO M, MARRA R, LOMBARDI N, PASCALE A, LANZUISE S, MANGANIELLO G, LORITO M. (2014) *Trichoderma*-based products and their widespread use in agriculture. **Open Mycol J**, 8:71–126.

WURZBACHER CM, BÄRLOCHER F, GROSSART F (2010) Fungi in lake ecosystems. **Aquat Microb Ecol**, 59:125–149.

WURZBACHER C, KERR J, GROSSART H (2011) Aquatic Fungi. In: Grillo O, Venora G (Eds) **The dynamical processes of biodiversity**. Intech Open, London, pp 229–261

WYNN-WILLIAMS DD (1990) Ecological Aspects of Antarctic Microbiology. In: MARSHALL KC (Ed) **Advances in Microbial Ecology.** 11. ed. New York: Plenum Press, p.71-146.

XIAO N, INABA S, TOJO M, DEGAWA Y, FUJIU S, KUDOH S, HOSHINO T (2010) .Antifreeze activities of various fungi and *Stramenopila* isolated from Antarctica. **N Am Fungi**, 5(5):215-220.

XIAO AN, BAO-MIN FENG, GANG CHEN, SHAO-FEI CHEN, HAI-FENG WANG, YUE-HU PEI (2016) Isolation and identification of two new compounds from marine-derived fungus *Acremonium fusidioides* RZ01, **Chinese J Nat Medicines**, 14(2): 934-938.

ZHANG C, KIM S (2012) Anti- metastasis effect of anthraquinones from marine fungus *Microsporum* sp. In: KIM S. (Ed.) **Marine Medicinal Foods: Implications and Applications: Animals and Microbes.** Oxford: Academic press, p.415-420.

ZHANG X, LI S J, LI JJ, LIANG ZZ, ZHAO CQ (2018) Novel Natural Products from Extremophilic Fungi. **Marine drugs**, 16(6): 194.

ZIEMONS S, KOUTSANTAS, K, BECKER K, DAHLMANN T, KÜCK U (2017) “Penicillin production in industrial strain Penicillium chrysogenum P2niaD18 is not dependent on the copy number of biosynthesis genes.” **BMC biotechnology**, 17(1):16.

ZONNEVELD KAF, VERSTEEGH GJM, KASTEN S, EGLINTON TI, EMEIS KC, HUGUET C, KOCH BP, DE LANGE GJ, DE LEEUW JW, MIDDELBURG JJ, MOLLENHAUER G, PRAHL FG, RETHEMEYER J, WAKEHAM SG (2010) Selective preservation of organic matter in marine environments; processes and impact on the sedimentary record. **Biogeosciences**, 7:483–511.

ZYCHA H, SIEPMANN R, LINNEMANN G (1969) Mucorales. Eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe. **Cramer Lehr**, p. 1-355.

9. PRODUÇÃO ACADÊMICA

Resumos expandidos apresentados em congressos e postados em revista de divulgação científica:

- OGAKI, M. B.; RUAS, D. T.; VIEIRA, R.; MUNIZ, M. C.; ZANI, C. L.; ALVES, T. M. A.; ROSA, L. H. Fungi isolated from lacustrine sediment in Fildes Peninsula, King George Island, Antarctica. In: V Simpósio APECS-Brasil, 2018, Belo Horizonte.

- OGAKI, M. B.; COELHO, L. C.; ROSA, L. H. Fungi present in marine sediment of Antartica. In: IX Congreso Latinoamericano de Ciencia Antártica, 2017, Punta Arenas.

- OGAKI, M. B.; SILVA, T. H.; ROSA, L. H. Fungos da Antártica: estudos de micro-organismos em condições extremas. Programa de Mentalidade Marítima - PROMAR, 2017 (Artigo Revista Informativa - Marinha do Brasil).

- RUAS, D.T.; OGAKI, M. B.; ROSA, L. H. Produção de extratos etanólicos de fungos de sedimento lacustre da Antártica. XXI Semana do conhecimento e X Semana Nacional de Ciência e Tecnologia, Belo Horizonte, 2017.

- DRUMOND, L.; OGAKI, M. B.; ROSA, L. H. Isolation of cellulolytic fungi in lake water in Deception Island, Antarctica. In: V Simpósio APECS-Brasil, 2018, Belo Horizonte.

Co-orientações:

- Trabalho de conclusão de curso:
  LÍVIA DA COSTA COELHO. Caracterização de fungos cultiváveis presentes em sedimentos marinhos da Antártica. 2016. Trabalho de Conclusão de Curso (Graduação em Abi - Ciências Biológicas) - Universidade Federal de Minas Gerais.

- Trabalho de conclusão de curso:
  LÁUREN DRUMOND. Diversidade de fungos presentes na coluna d'água de lago de cratera na Ilha Deception, Antártica. Trabalho de Conclusão de Curso (Graduação em Abi - Ciências Biológicas) - Universidade Federal de Minas Gerais.

- Trabalho de conclusão de curso:
  DANIELA TEIXEIRA RUAS. Caracterização de extratos de fungos marinhos da Antártica. Trabalho de Conclusão de Curso (Graduação em Abi - Ciências Biológicas) - Universidade Federal de Minas Gerais.

- Iniciação científica:
  DANIELA TEIXEIRA RUAS. Produção de extratos etanólicos de fungos de sedimento lacustre da Antártica. Iniciação Científica, Conselho Nacional de Desenvolvimento Científico e Tecnológico. Período: ano de 2017.

  LÁUREN DRUMOND. Diversidade de fungos presentes na coluna d'água de lago de cratera na Ilha Deception, Antártica. Iniciação Científica, Conselho Nacional de Desenvolvimento Científico e Tecnológico. Período: ano de 2018

DANIELA TEIXEIRA RUAS. Produção e caracterização de extratos de fungos de sedimento marinho da Antártica. Iniciação Científica, Conselho Nacional de Desenvolvimento Científico e Tecnológico. Período: ano de 2019

Bancas de monografia:

- OGAKI, M. B.; SILVA, T. H.; ROSA, L. H.. Participação em banca de DÉBORA AMORIM SARAIVA SILVA. Isolamento e taxonomia de fungos presentes em permafrost da Ilha Deception, Antártica. 2017. Trabalho de Conclusão de Curso (Graduação em Abi - Ciências Biológicas) - Universidade Federal de Minas Gerais.

- OGAKI, M. B.; ROSA, L. H.; MENEZES, G. C. A.. Participação em banca de BÁRBARA ALVES PORTO. Identificação de fungos isolados de segmentos do gelo glacial da Antártica. 2017. Trabalho de Conclusão de Curso (Graduação em Abi - Ciências Biológicas) - Universidade Federal de Minas Gerais.

Capítulos de livro:

- OGAKI, M.B.; DE PAULA, M. T.; RUAS, D. T. PELLIZZARI, F. M.; LAVIÑA, C. G. ROSA, L.H. (2019) Marine fungi associated with Antarctic macroalgae. In: CASTRO-SOWINSKI, S. (Eds). The Ecological Role of Micro-organisms in the Antarctic Environment. Springer Polar Sciences. p. 239-255.

- OGAKI, M.B.; VIEIRA, R.; LÍRIO, J.M.; ROSA, C.A.; ROSA, L.H. Diversity and ecology of fungal assemblages present in lakes of Antarctica. 2019. In: ROSA, L.H. (Ed). In: ROSA, L.H. Fungi of Antarctica: Diversity, Ecology and Biotechnological Applications. Springer Nature, Switzerland, p.69-97.

- ROSA, L.H.; PELLIZZARI, F.M.; OGAKI, M.B.; DE PAULA, M.T.; MANSILLA, A.; MARAMBIO, J.; COLEPICOLO, P.; NETO, A.A..N.; VIEIRA, R.; ROSA, C.A. Sub-Antartic and Antartic Marine Ecosystems: Na unexplored ecosystem of fungal diversity. 2019. In: ROSA, L.H. (Ed). In: ROSA, L.H. Fungi of Antarctica: Diversity, Ecology and Biotechnological Applications. Springer Nature, Switzerland, p.221-242.

Artigos submetidos:

- OGAKI, M.B. COELHO, L.C.; VIEIRA, R.; NETO, A.A.; ZANI, C.L.; ALVES, T.M.A.; POLICARPO Jr., A.S; MURTA, S.M.F.; BARBOSA, E.C.; OLIVEIRA, J.G.; CERAVOLO, I.P.; PEREIRA, P.O.; COTA, B.B.; VIANA, R.O.; ALVES, V.S.; ROSA, L.H. Cultivable fungi present in deep-sea sediments of Antarctica: Taxonomy, diversity, and bioprospection of bioactive compounds. (ANEXO 1).

10. ANEXOS

Environmental Microbiology - Account Created in Manuscript Central

Joan Timmis <onbehalfof@manuscriptcentral.com>
Sex, 14/06/2019 11:00
Para: mayaraogaki@hotmail.com <mayaraogaki@hotmail.com>

14-Jun-2019

Dear Ms. Ogaki:

A manuscript titled Cultivable fungi present in deep-sea sediments of Antarctica: taxonomy, diversity, and bioprospection of bioactive compounds (EMI-2019-0812) has been submitted by Ms. Mayara Ogaki to Environmental Microbiology.

You are listed as a co-author for this manuscript. The online peer-review system, Manuscript Central, automatically creates a user account for you. Your USER ID and PASSWORD for your account is as follows:

Site URL: https://mc.manuscriptcentral.com/emi
USER ID: mayaraogaki@hotmail.com
PASSWORD: https://mc.manuscriptcentral.com
/emi?URL_MASK=ced2071a44e8464885da2f2bdf6d2361

You can use the above USER ID and PASSWORD to log in to the site and check the status of papers you have authored/co-authored. This password is case-sensitive and temporary. Please log in to https://mc.manuscriptcentral.com/emi to update your account information and change your password.

Thank you for your participation.

Sincerely,

Environmental Microbiology Editorial Office

By submitting a manuscript to or reviewing for this publication, your name, email address, and affiliation, and other contact details the publication might require, will be used for the regular operations of the publication, including, when necessary, sharing with the publisher (Wiley) and partners for production and publication. The publication and the publisher recognize the importance of protecting the personal information collected from users in the operation of these services, and have practices in place to ensure that steps are taken to maintain the security, integrity, and privacy of the personal data collected and processed. You can learn more at https://authorservices.wiley.com/statements/data-protection-policy.html. In case you don't want to be contacted by this publication again, please send an email to emi.mbt@gmail.com.