

Universidade Federal de Minas Gerais
Instituto de Ciências Biológicas
Departamento de Microbiologia

Tese de Doutorado

**Taxonomia, diversidade, ecologia, caracterização de resistência a
antifúngicos e bioprospecção de fungos presentes na neve e gelo glacial da
Antártica**

Graciéle Cunha Alves de Menezes

Belo Horizonte, MG

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Antártica**

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

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Rapunzel - Filme Disney

Sumário

Colaboradores	3
Agradecimentos	4
Lista de Abreviaturas	10
Lista de Figuras.....	11
Lista de Tabelas	12
Resumo	13
Abstract.....	15
1 Introdução	17
1.1 Antártica	17
1.2 Fungos da Antártica.....	19
1.2.1 Diversidade e ecologia.....	19
1.2.2 Potencial biotecnológico.....	20
1.2.3 Fungos presentes na neve e gelo glacial da Antártica	21
1.3 Poluentes orgânicos no ambiente antártico: potenciais agentes de seleção de micro-organismos resistentes a drogas.....	23
1.4 Fungos resistentes aos antifúngicos.....	26
1.4.1 Relação dos agrotóxicos com a resistência cruzada aos antifúngicos	28
2 Justificativa e Relevância	31
3 Objetivos	33
3.1 Objetivo Geral	33
3.2 Objetivos Específicos	33
4 Materiais e Métodos	34
4.1 Áreas de Coletas	34
4.2 Coleta, isolamento e processamento das amostras de neve e gelo	35
4.2.1 Purificação e preservação dos fungos.....	37
4.3 Identificação dos fungos	37
4.3.1 Identificação dos fungos filamentosos	37

4.3.1.1 Extração do DNA total	38
4.3.1.2 Amplificação utilizando o iniciador (GTG) ₅	38
4.3.1.3 Amplificação da região ITS	39
4.3.1.4 Amplificação parcial do gene da β -tubulina	40
4.3.1.5 Amplificação parcial da RNA polimerase II	40
4.3.1.6 Caracterização morfofisiológica das espécies do gênero <i>Penicillium</i>	41
4.3.2 Identificação das leveduras	41
4.3.2.1 Extração do DNA total	41
4.3.2.2 Amplificação utilizando os iniciadores ITS1 e NL4	42
4.3.3 Purificação dos amplicons	42
4.3.4 Reações de sequenciamento	43
4.3.5 Análise computacional das sequências	44
4.4 Diversidade da comunidade fúngica: índices de abundância, riqueza, dominância e similaridade	44
4.5 Preparo dos extratos fúngicos	46
4.6 Ensaios Biológicos	47
4.6.1 Ensaio <i>in vitro</i> com a forma amastigota-like de <i>Leishmania</i> (<i>leishmania</i>) <i>amazonensis</i>	47
4.6.2 Ensaios <i>in vitro</i> com as formas tripomastigotas e as formas intracelulares amastigotas de <i>Trypanosoma cruzi</i>	47
4.6.3 Determinação da atividade herbicida	48
4.6.4 Concentração Inibitória Mínima (CIM) das drogas antifúngicas benomil, fluconazol e anfotericina B	49
4.6.4.1 Preparo do inóculos	51
4.6.5 Determinação do perfil de crescimento em diferentes temperaturas	51
5 Capítulos	53
5.1 Capítulo 1: “Chapter 6: Fungi in snow and glacial ice of Antarctica”	54

5.2 Capítulo 2: “Fungi in the seasonal snow of Antarctica: diversity, distribution, and resistance against agricultural and clinical antifungal drugs”	74
5.3 Capítulo 3: “ <i>Antarctomyces pellizariae</i> sp. nov., a new, endemic, blue, snow residente psychrophilic ascomycete fungus from Antarctica”	116
5.4 Capítulo 4: “Fungi in glacier ice of Antarctica: diversity, distribution, and bioprospection of bioactive compounds”	127
6 Discussão integrada	148
7 Conclusão e perspectivas	153
8 Atividades desenvolvidas no período	154
9 Referências bibliográficas	156
10 Anexos	173
Anexo 1: Comprovante submissão artigo científico para revista Fungal Diversity ..	173

Lista de Abreviaturas

% - por cento

BDA - Ágar Dextrose Batata

UFC - unidade formadora de colônia

C - Carbono

N - Nitrogênio

P - Fósforo

CTAB - Brometo de cetil trimetilamonio

DMSO - Dimetilsulfóxido

DNA - Ácido desoxirribonucléico

rDNA - Ácido desoxirribonucleico ribossomal

rRNA - Ácido ribonucleico ribossomal

dNTP - Desoxirribonucleotídeos fosfatados

EDTA - ácido etilenodiamino tetra-acético

Mm - milímetro

cm - centímetro

m - metro

$\mu\text{g.mL}^{-1}$ - miligramas por mililitro

mL - mililitro

L- litros

ng - nanogramas

g - gramas

M - molar

μm - micrômetro

mg.L^{-1} - miligramas por litro

mg.mL^{-1} - miligramas por mililitro

min - minutos

s - segundos

h - horas

r.p.m- rotação por minuto

$^{\circ}\text{C}$ - graus Celsius

pH - concentração hidrogênica

v/v - volume por volume

Lista de Figuras

Figura 1. Mapa da Antártica com suas principais porções em destaque: Antártica Oriental (Continente Antártico) e Antártica Ocidental (Península Antártica), também em destaque o arquipélago da Shetland do Sul (DUTRA; JASPER, 2015).....	18
Figura 2. Esquema das etapas envolvidas no acúmulo de produtos químicos orgânicos em regiões remotas/polar (WANIA, 2003 com modificações).	24
Figura 3. Mapa da Antártica (A) e Península Antártica (B) com os pontos de coleta de neve (1 ao 6 em laranja) e gelo glacial (1 ao 9 em verde). Fonte: GOOGLE EARTH-MAPS, 2017.	34
Figura 4. Supplementary Figure 1 Dendrogram showing the comparison of (a) Sorensen (b) Bray-Curtis similarities measures for the fungal assemblages isolated from seasonal snow of different regions of Antarctica.....	111
Figura 5. Supplementary Figure 2 Dendrograms showing the comparison of Sorensen and Bray-Curtis similarities measures for the fungal assemblages isolated from each segment per region of seasonal snow of Antarctica.Segment 1 (top layer), segment 2 (middle layer), and segment 3 (base layer) Fungal assemblages of (a) Deception Island, (b) King George Island, (c) Trinity Peninsula, (d) Snow Island, (e) and Robert Island.	114
Figura 6. Supplementary Figure 3 Similarity among the seasonal snow fungal assemblages of Antarctica using the Venn diagrams.	115
Figura 7. Supplementary Figure 1. Dendrogram showing the comparison of (a) Sorensen and (b) Bray-Curtis similarities measures for the fungal assemblages isolated from fragment glacial ice of different sites of Antarctic Peninsula. KGA = King George Island, Ajax/Stain House Glaciar; APR = Arctowski Peninsula, Rozier/Woodbury; GIT = Greenwich Island, Troub; GIF = Greenwich Island, Fuerza Aerea; LIC = Livingston Island, Charity; LIH = Livingston Island, Huron; APS= Antarctic Peninsula, Sikorsky; APL= Antarctic Peninsula, Leonardo/Blanchard; and APB= Antarctic Peninsula, Blérnot.....	147

Lista de Tabelas

Tabela 1. Pontos de coleta de neve e gelo glacial da Antártica.	35
Tabela 2. Table 1 Fungi obtained from Antarctic snow segments and identified by sequence comparison with the BLASTn match with the NCBI GenBank database.....	101
Tabela 3. Table 2 Physicochemical parameters of melted snow sampled in different sites of Antarctic Peninsula and diversity indices.....	108
Tabela 4. Table 3 Minimal inhibitory concentration of benomyl, fluconazole, and amphotericin B against fungi isolated from seasonal snow of Antarctica	109
Tabela 5. Supplementary Table 1 Growth profile of fungi isolated from winter seasonal snow of Antarctica that displayed antifungal resistance	110
Tabela 6. Table 1. Fungi obtained from Antarctic glacial ice fragments and identified by sequence comparison with the BLASTn match with the NCBI GenBank database	140
Tabela 7. Table 2 Physico-chemical parameters of melted ice and diversity indices of fungal assemblages at the different sites.....	143
Tabela 8. Table 3 Biological activities of extracts obtained from cultures of <i>Penicillium</i> species isolated from glacial ice from Antarctica.....	144
Tabela 9. Supplementary Table 1 Sites where the fragment of glacial ice were sampled in Antarctica.....	146

Resumo

A Antártica possui 13.8 milhões de km² e a maior parte de sua área é coberta permanentemente por neve e gelo, substratos estes que, apesar de pouco provável, constituem um habitat natural para diferentes grupos microbianos. Apesar de representarem a paisagem dominante na Antártica, poucos estudos foram realizados até o momento com o objetivo de conhecer a comunidade de fungos que habitam a neve e o gelo glacial da Antártica, bem como o potencial desses fungos como produtores de substâncias de interesse em processos biotecnológicos. Neste contexto, esta tese teve como objetivos caracterizar a comunidade fúngica presente na neve sazonal e no gelo glacial da Antártica, determinar o perfil de resistência dos fungos presentes na neve aos antifúngicos clínicos fluconazol e anfotericina e o ambiental benomil, bem como, avaliar o potencial dos fungos obtidos do gelo glacial como produtores de metabólitos biotivos. Ao final do processo de isolamento, foram obtidos 234 isolados fúngicos (99 filamentosos e 135 leveduras) da neve sazonal da Antártica, os quais foram identificados por técnicas de biologia molecular e morfofisiológicas em 51 táxons, pertencentes a 26 gêneros. Os índices ecológicos mostraram que a comunidade de fungos da neve é rica e diversificada e apresenta espécies que ainda não haviam sido descritas na literatura. Entre os fungos presentes na neve, 11 espécies de leveduras foram dominantes, pois apresentaram as maiores densidades encontradas (≤ 300 UFC L⁻¹). Entre elas, *Phenoliferia glacialis* mostrou ampla distribuição e foi isolada em todos os locais amostrados. *Debaryomyces hansenii*, *Rhodotorula mucilaginosa*, *Penicillium chrysogenum*, *Penicillium* sp. 3 e *Penicillium* sp. 4 demonstraram resistência aos antifúngicos azólicos benomil e fluconazol. Além da resistência ao fluconazol, os isolados de *R. mucilaginosa* foram capazes de crescer a 37 °C, porém foram suscetíveis a anfotericina B. Em relação as amostras de gelo glacial, 66 isolados fúngicos (45 filamentosos e 21 leveduras) foram obtidos e caracterizados em 27 táxons pertencentes a 14 gêneros. *Penicillium palitans*, *Penicillium* sp. 1, *Thelebolus balaustiformis*, *Glaciozyma antarctica*, *Penicillium* sp. 7, *Rhodotorula mucilaginosa* e *Rhodotorula dairenensis* foram os táxons dominantes com as maiores densidades encontradas ($\leq 40,66$ UFC L⁻¹). A diversidade e riqueza da comunidade fúngica que compõem o gelo glacial foram altas e a dominância moderada. Espécies de *Penicillium* ocorreram no gelo glacial de todos os locais amostrados e *Penicillium chrysogenum* foi o que apresentou a distribuição mais ampla. *Penicillium chrysogenum*, *P. palitans* e *Penicillium* spp. foram capazes de produzir substâncias com seletivas atividades tripanosomicidas, leishmanicidas e herbicidas. A espécie *P. chrysogenum* apresentou as bioatividades mais elevadas. A partir dos resultados obtidos, o presente estudo demonstrou que a neve sazonal acumulada na Península

Antártica abriga uma comunidade fúngica diversa, dominada por espécies basidiomicéticas cosmopolitas, anteriormente encontradas em ecossistemas tropicais, temperados e polares. As altas densidades desses fungos cosmopolitas na neve sugerem que eles podem estar presentes no ar que chega à Península Antártica, por meio de massas de ar oriundas de fora do Continente Antártico. Adicionalmente, detectamos na neve isolados fúngicos ambientais resistentes a antifúngicos agrícolas e clínicos e alguns deles capazes de crescer a 37 °C, o que merece novos estudos para determinar a patogenicidade destes fungos e seu risco para animais e humanos. Apesar de representar um ambiente mais extremo que a neve, o gelo glacial antártico também se mostrou favorável como habitat para uma comunidade fúngica rica e diversificada. Essa comunidade fúngica inclui espécies não relatadas antes para o gelo glacial do Ártico e da Antártica e dominadas pelo gênero *Penicillium*, os quais podem estar presentes na forma de esporos e fragmentos de hifas nas bolhas de gás presas no interior das amostras de gelo. Os representantes do gênero *Penicillium* obtidos do gelo glacial foram capazes de produzir metabólitos bioativos com diferentes atividades biológicas e podem representar linhagens antigas e selvagens com vias genéticas e bioquímicas ainda não conhecidas para a produção novos metabólitos bioativos; esses metabólitos podem servir como moléculas protótipos para o desenvolvimento de novos fármacos ou pesticidas menos tóxicos para o meio ambiente.

Palavras – chave: Antártica, fungos, neve, gelo glacial, resistência antifúngica, metabólitos secundários.

Abstract

Antarctica has approximately 13.8 million km² and most of its area is covered permanently by snow and ice, substrates that although unlikely, represent a natural habitat for different microbial groups. Although snow and glacial ice dominate the Antarctic landscape, few studies have been done so far with the objective of knowing the community of fungi that inhabit snow and glacial ice and the potential of these fungi as producers of substances of interest in biotechnological processes. In this context, this work aimed to characterize the fungal community present in the seasonal snow and glacial ice of Antarctica, characterize the resistance profile for clinical and environmental antifungal fungi present in the snow and evaluate the potential of fungi obtained from glacial ice as producers of bioactive metabolites. A total of 234 fungal isolates (99 filaments and 135 yeasts) were obtained from Antarctica seasonal snow and could be assigned to 51 taxa of 26 genera. Ecological indices showed that the snow fungal community is rich and diverse and has species that have not yet been described in the literature. Eleven yeast species displayed the highest densities (≥ 300 UFC L⁻¹). Among them, *Phenoliferia glacialis* showed a broad distribution and was detected at all sites that were sampled. *Debaryomyces hansenii*, *Rhodotorula mucilaginosa*, *Penicillium chrysogenum*, *Penicillium* sp. 3, and *Penicillium* sp. 4 displayed resistance against the antifungals benomyl and fluconazole azoles. Despite showing resistance to fluconazole, *R. mucilaginosa* isolates were able to grow at 37 °C, but were susceptible to amphotericin B. The glacial ice collections yielded 66 fungal isolates (45 filaments and 21 yeasts) identified in 27 taxa of 14 genera. *Penicillium palitans*, *Penicillium* sp. 1, *Thelebolus balaustiformis*, *Glaciozyma antarctica*, *Penicillium* sp. 7, *Rhodotorula mucilaginosa*, and *Rhodotorula dairenensis* were the dominant taxa with the highest densities. The diversity and richness of the fungal community of glacial ice were high and moderate dominance. *Penicillium* species occurred in samples of all sites and *Penicillium chrysogenum* displayed the broadest distribution. Among them, *P. chrysogenum*, *P. palitans*, and *Penicillium* spp. produced trypanocidal, leishmanicidal, and herbicidal compounds. *P. chrysogenum* showed the highest herbicidal, trypanocidal, and leishmanicidal activities. Our results show that the winter seasonal snow of the Antarctic Peninsula contains a diverse fungal community dominated by cosmopolitan ubiquitous basidiomycetes species previously found in tropical, temperate, and polar ecosystems. The high densities of these cosmopolitan fungi suggest that they could be present in the air that arrives to the Antarctic Peninsula by air masses from outside Antarctica. Additionally, we detected environmental fungal isolates that were resistant to agricultural and clinical antifungals and able to grow at 37 °C. Our study shows that glacial ice shelters a rich

and diverse fungal community, including species unreported before to Arctic and Antarctica, dominated by the airborne genus *Penicillium*, which might be present in the gas bubbles trapped of the ice samples. These *Penicillium* taxa were able to produce bioactive compounds and may represent old and wild strains with genetic and biochemical pathways to produce new secondary bioactive metabolites.

Key words: Antarctica; snow, glacial ice, fungi, antifungal resistance, secondary metabolites.

1 Introdução

1.1 Antártica

5 A Antártica surgiu da separação do supercontinente Gondwana, a partir do resultado da movimentação das placas tectônicas no período do Cretáceo, a cerca de 120 milhões de anos atrás (BRUNATI et al., 2009) e foi isolada geograficamente dos demais continentes após a separação da Península Antártica da América do Sul, a aproximadamente 30 milhões de anos (BARGAGLI, 2008). A posteriori, a abertura e o aprofundamento da Passagem de Drake
10 (encontro dos oceanos Pacífico e Atlântico), entre a extremidade austral da América do Sul e no início da Península Antártica, permitiram o estabelecimento da Corrente Circumpolar Antártica (CCA) e do Vórtice Ciclônico Circumpolar (VCC). Todos estes fenômenos contribuíram para o isolamento geográfico da Antártica e suas comunidades de organismos (BARGAGLI, 2008) em relação às demais regiões do planeta.

15 O continente Antártico é comumente dividido em Antártica Oriental e Ocidental, regiões estas bem delimitadas pelas Montanhas Transantárticas (HERZFELD, 2004) (**Figura 1**). A Península Antártica é a única parte do continente que se estende de maneira significativa para o norte até a latitude 63°S. É uma região montanhosa estreita com uma largura média de 70 km e uma altura média de 1.500 m (TURNER et al., 2009). O arquipélago Shetland do Sul
20 compõe um grande grupo de ilhas que se estendem a 540 km do nordeste ao sudeste e consiste em quatro agrupamentos distintos: Ilhas Clarence e Elefante estão no Nordeste, Ilhas Rei George e Nelson estão a nordeste das Ilhas Robert, Greenwich, Livingston, Snow e Deception; as Ilhas Smith e Low estão no extremo sudoeste da cadeia de ilhas (ISLAND, 2001). A mais distinta das ilhas do arquipélago é Deception, que apresenta atividade
25 vulcânica, onde fumarolas subaéreas e locais de desgaseificação subaquática são comuns em várias áreas dentro e fora da costa da ilha (SERRANO; LÓPEZ-MARTÍNEZ, 2000).

A maior parte da camada de gelo que cobre o planeta Terra (atualmente cerca de 10%) está na Antártica, concentrando-se nas regiões leste e oeste da região continental (DE MENEZES et al., 2019). Cerca de 99% do continente é coberto permanentemente por neve e
30 gelo (TURNER et al., 2009), com espessura média de 2 km e máxima com mais de 4 km (BARGAGLI, 2008). Essas características tornam a Antártica uma das regiões mais inóspitas do mundo com o clima mais frio, dinâmico (ventos mais fortes do planeta) e médias anuais de precipitação entre 30 e 70 mm (OCHYRA et al., 2008). Devido a estas características, a Antártica é considerada um deserto branco. A temperatura mínima registrada, em um cume
35 elevado no leste do platô antártico, foi de -93 °C em julho de 2013 (NASA, 2013).



Figura 1. Mapa da Antártica com suas principais porções em destaque: Antártica Oriental (Continente Antártico) e Antártica Ocidental (Península Antártica) e Arquipélago da Shetland do Sul (DUTRA; JASPER, 2015).

10

A Península Antártica, localizada no Hemisfério Ocidental, possui clima mais brando em relação a porção continental, por apresentar maior umidade (média de precipitação anual de 500-800 mm) (BAÑON; VASALLO, 2015), temperaturas mais amenas (média mensal $> 0^{\circ}\text{C}$ durante o verão e no inverno $< -10^{\circ}\text{C}$) e ventos moderados, o que possibilitou o desenvolvimento e presença de algumas espécies vegetais e animais (OCHYRA et al., 2008). As ilhas deste conjunto são aproximadamente 80% glaciadas e cobrem 3.688 Km^2 (ISLAND, 2001). Entretanto, a Península Antártica e as ilhas adjacentes possuem menos de 1% do volume de gelo da Antártica. Devido ao pequeno tamanho dessas massas de gelo e também por estarem mais próximas do ponto de fusão sob pressão, respondem mais rapidamente às

5 mudanças climáticas e, por isso, representa a região do planeta que sofre os maiores impactos das mudanças climáticas globais (DE MENEZES et al., 2019).

Apesar das condições ambientais extremas da Antártica, algumas formas de vida estão presentes tais como: algas, bactérias, fungos, líquens, plantas, invertebrados, aves e mamíferos. A flora antártica inclui quase que exclusivamente briófitas, além de apenas duas
10 plantas vasculares nativas, *Deschampsia antarctica* Desv. e *Colobanthus quitensis* (Kunth) Bartl. (SMITH, 2003; OCHYRA et al., 2008; ROSA et al., 2009). A fauna é composta principalmente por mamíferos aquáticos, aves marinhas e migratórias eo abundante crustáceo *Krill* é uma das principais fontes da cadeia alimentar para baleias, lulas, focas e pinguins (FERREIRA, 2009; BÜDEL; COLESIE, 2014).

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1.2 Fungos da Antártica

1.2.1 Diversidade e ecologia

As formas de vida mais abundantes presentes nos ecossistemas extremos do Continente Antártico são as microbianas (ROSA et al., 2019). Dentre os micro-organismos
20 antárticos, destacam-se os fungos que possuem diversos mecanismos de tolerância a condições extremas. A maioria dos fungos registrados no Continente Antártico está na forma anamórfica (RUISI et al., 2007), a qual tem como vantagem menos gasto de energia quando comparada com a forma sexuada (de HOOG et al., 2005).

Os fungos na Antártica se distribuem nos diferentes substratos presentes na região, tais
25 como solos (FELL et al., 2006; COX et al., 2016; GOMES et al., 2018), rochas (SELBMANN et al., 2005; GONÇALVES et al., 2017a; ALVES et al., 2019), vegetação (BRADNER et al., 2000; ZHANG et al., 2013; YU et al., 2018), macroalgas (GODINHO et al., 2013; FURBINO et al., 2018), água do mar (GONÇALVES et al., 2017b), sedimento marinho (GONÇALVES et al., 2013; WENTZEL et al., 2019), lagos (GONÇALVES et al., 2012b; TSUJI et al., 2013),
30 invertebrados marinhos (GODINHO et al., 2019), *permafrost*¹ (KOCHKINA et al., 2012; GOORDIAL et al., 2016), neve (ANTONY et al., 2016; de MENEZES et al., 2017) e gelo (KNOWLTON et al., 2013; SANYAL et al., 2018). Mais de 1.000 espécies de fungos não

¹**Permafrost ou pergelissolo** (em português) é o solo permanentemente congelado. Esse tipo de solo encontrado na região do Ártico e da Antártica.

5 liquenizados² já foram descritos para a Antártica e região Subantártica, os quais pertencem aos filos *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota*, *Chytridiomycota* e *Glomeromycota* (BRIDGE et al., 2008), porém este número vem crescendo gradativamente, conforme os estudos na região vem avançando. Entre os fungos antárticos não liquenizados, além das espécies endêmicas³, também são encontradas espécies nativas⁴, as quais são
10 capazes de apresentar crescimento ativo e se reproduzir nas condições extremas da Antártica (RUISI et al., 2007). Dentre os fungos endêmicos para o Continente Antártico, já foram reportadas até o presente momento, as espécies *Thelebolus globosus*, *Thelebolus ellipsoideus* (de HOOG et al., 2005) e o gênero *Antarctomyces*, representado por apenas duas espécies, *Antarctomyces psychrotrophicus* (STCHIGEL et al., 2001) e *Antarctomyces pellizariae* (de
15 MENEZES et al., 2016). Entretanto, táxons mesofílicos, psicrofílicos e mesofílicos tolerantes ao frio também ocorrem na Antártica e alguns deles em alta frequência (RUISI et al., 2007). De acordo com Godinho et al. (2013) a maioria dos fungos filamentosos isolados de amostras antárticas é psicrotolerante.

Algumas espécies de fungos parecem estar bem estabelecidas em nichos ecológicos
20 específicos nos ecossistemas antárticos (BRIDGE; SPOONER, 2012) e alguns podem desempenhar um papel chave na biodegradação de matéria orgânica e ciclagem de nutrientes em baixas temperaturas nestes ecossistemas (SHIVAJI; PRASAD, 2009; ANTONY et al., 2016). Os fungos antárticos também representam importantes indicadores da complexidade trófica dentro da comunidade microbiana da Antártica (RAO et al., 2012).

25

1.2.2 Potencial biotecnológico

Os fungos têm a capacidade de produzir diferentes metabólitos como resposta fisiológica aos múltiplos estressores bióticos e abióticos (MACHELEIDT et al., 2016). Dentre as substâncias produzidas pelos fungos, os metabólitos secundários bioativos tem seu
30 destaque, os quais são caracterizados por apresentam baixo peso molecular e serem produzidos durante as fases estacionárias e de declínio (morte) do ciclo celular

2 **Fungos liquenizados ou líquens** refere-se à estrutura resultante de um processo biológico (simbiose) entre fungos (micobionte) e uma alga ou cianobactéria (fotobionte). Portanto, fungos não liquenizados refere-se a um único fungo sem nenhuma associação com outro organismo.

3 **Espécie endêmica** é aquela que ocorre especificamente em um determinado ecossistema, área ou região geográfica.

4 **Espécie nativa ou indígena** é aquela originária de um determinado ecossistema, área ou região geográfica, mas que também pode ocorrer em outras áreas e regiões geográficas.

(BRAKHAGE, 2013). Os fungos antárticos apresentam-se como promissoras fontes de terpenóides, policetídeos, alcalóides e poliacetilenos com diferentes atividades biológicas (ROSA et al., 2019). Essas moléculas bioativas produzidas pelos fungos representam um rico reservatório químico para a descoberta de novas substâncias como antioxidantes, antibióticos, anticancerígenos, imunomoduladores e antiparasitários (SANTIAGO et al., 2012).

Fungos presentes em diferentes substratos da Antártica foram descritos como produtores de metabólitos com diferentes atividades biológicas, tais como antimicrobiana (BRUNATI et al., 2009; GODINHO et al., 2013; FURBINO et al., 2014; ALBORÉS et al., 2018; PURÍĆ et al., 2018), citotóxica (GONÇALVES et al., 2015; DING et al., 2016), antiprotozoária (SANTIAGO et al., 2012; GODINHO et al., 2013; GOMES et al., 2018), antiviral (FURBINO et al., 2014; GODINHO et al., 2015; GOMES et al., 2018), antioxidante (HENRÍQUEZ et al., 2014; MELO et al., 2014; KOSTADINOVA et al., 2017), inseticida (GODINHO et al., 2015a) e herbicida (GODINHO et al., 2015; GOMES et al., 2018). Além disso, novas moléculas bioativas vem sendo isoladas e caracterizadas a partir de fungos de diferentes ambientes antárticos com atividade antimicrobiana (IVANOVA et al., 2007), antifúngica (LI et al., 2008), antibacteriana (LI et al., 2008, 2018), citotóxica (LI et al., 2012) e antiviral (WANG et al., 2016). Dentre os fungos, o maior número de substâncias e espécies conhecidas pertencem ao gênero *Penicillium* (WU et al., 2012, 2013; LIN et al., 2014; ZHOU et al., 2018), o qual é tradicionalmente estudado por ser considerado um excelente produtor de substâncias bioativas (NICOLETTI; TRINCONE, 2016).

1.2.3 Fungos presentes na neve e gelo glacial da Antártica

A neve precipitada ao longo dos anos é compactada gradualmente até a formação completa do gelo glacial. Durante este processo de compactação, aprisiona amostras de ar em formato de bolhas que ficam retidas no gelo glacial, os quais representam microhabitats únicos para obtenção de amostras de ar de centenas de anos atrás, bem como os micro-organismos ali presentes aprisionados no tempo e espaço (ABYZOV, 1993; MA et al., 1999). Desta forma, o gelo glacial pode ser considerado uma excelente matriz para a preservação a longo prazo de micro-organismos e permite, como tal, o estudo da diversidade microbiana contemporânea e antiga (GUNDE-CIMERMAN et al., 2003). Em ambiente remotos, como a Antártica, a origem da vida microbiana pode ser devido às correntes de ar (ABYZOV, 1993; PEARCE et al., 2009), as quais sob o efeito da precipitação da neve, representa um processo capaz de decantar micro-organismos suspensos na atmosfera, vindos de diferentes partes do planeta (BARGAGLI, 2008).

De acordo com Hodson et al. (2008), são reconhecidos 2 (dois) ecossistemas glaciais capazes de abrigar diferentes formas de vida:

(1) **Ecossistema supraglacial** – representado pela superfície da geleira. É caracterizado pela presença de um diverso consórcio de organismos (bactérias, diatomáceas, fitoflagelados, fungos, vírus, rotíferos ocasionais e tardígrados), os quais estão no interior da camada de neve, córregos supraglaciais, piscinas de derretimento e orifícios denominados *cryoconite* [orifícios verticais cilíndricos que se formam na superfície do gelo com uma fina camada de partículas, detritos e micro-organismos depositados no fundo e são preenchidos com água (ANESIO et al., 2010)];

(2) **Ecossistema subglacial** – representa o interior da camada de gelo e é caracterizado pela prevalência de bactérias aeróbias/anaeróbias e vírus em misturas basais do gelo até os lagos subglaciares.

Os micro-organismos, incluindo os fungos, que habitam esses ecossistemas de neve e gelo são considerados extremófilos por se encontrarem expostos à alta incidência de radiação UV, baixa disponibilidade de nutrientes e água no estado líquido, bem como temperaturas negativas (MITEVA, 2008). Para sobreviver a tais condições extremas, os fungos possuem estratégias fisiológicas para se proteger do congelamento (DUMAN; OLSEN, 1993), como a produção de proteínas anticongelantes (AFPs) e/ou glicoproteínas anticongelantes (AFGPs) (EWART; LIN; HEW, 1999; GILBERT et al., 2004; XIAO et al., 2010a, 2010b), exopolissacarídeos (EPS) (TIBBETT; SANDERS; CAIRNEY, 1998; POLEZHAEVA et al., 2014), enzimas frio-ativas (FELLER; GERDAY, 1997; ROBINSON, 2001) e ácidos graxos insaturados (SINGH et al., 2014). Considerando que alguns fungos possuem adaptações para sobreviver à baixa atividade de água, altas concentrações de sal e baixas temperaturas, eles podem manter uma colonização contínua na neve e no gelo (GUNDE-CIMERMAN et al., 2003). Neste contexto, já foi demonstrado a capacidade de linhagens pertencentes aos gêneros *Cryptococcus* e *Rhodotorula*, isoladas do gelo glacial antártico, conseguirem permanecer metabolicamente ativos sob condições de congelamento (AMATO; DOYLE; CHRISTNER, 2009). D'elia et al. (2009) detectaram a presença de fungos dos filos *Ascomycota* e *Basidiomycota* psicotolerantes em amostras de gelo antártico com cerca de 5 mil anos de idade. Knowlton et al. (2013) encontraram fungos dos gêneros *Alternaria*, *Davidiella* e *Rhodotorula* em camadas de gelo glacial da região continental da Antártica com datação de 500 a 175 mil anos de idade. A partir desses resultados, os autores indicaram que a viabilidade dos micro-organismos encontrados em seu estudo era dependentes do frio no

5 momento da sua deposição na neve e que as baixas concentrações de poeira atmosférica e CO₂ também estavam relacionadas ao aumento da viabilidade.

As comunidades microbianas, incluindo os fungos, presentes em habitats glaciares parecem ser ativas nos diferentes ciclos biogeoquímicos, pois podem desempenhar funções metabólicas envolvidas nos ciclos de C, N e P (HODSON et al., 2007; TELLING et al., 2011; 10 GRZESIAK et al., 2015) e, por isso, considerados promissores em processos biotecnológicos. Alguns isolados de *Vishniacozyma victoriae* isolados da neve antártica foram capazes de produzir diferentes enzimas (amilase, lipase, protease, celulase, lignina e β -galactosidase) em baixas temperaturas (ANTONY et al., 2011). Sanyal et al. (2018) verificaram que isolados de *Leucosporidium* sp. e *Curvibasidium* sp., obtidos dos orifícios de *cryoconite* antárticos, 15 demonstraram alta atividade e diversidade enzimática extracelular, com a produção das enzimas celulase, ligninase, amilase, lipase, protease e β -galactosidase em baixas temperaturas. Esses resultados dão suporte a hipótese de que comunidades microbianas residentes na neve e gelo podem modificar a química da neve por meio de suas atividades metabólicas (ANTONY et al., 2011).

20 Embora a neve e o gelo glacial sejam substratos predominantes na Antártica, estudos sobre a diversidade e ecologia das comunidades fúngicas presentes nesses substratos ainda são escassos e poucos táxons foram relatados até o momento. Apesar da pouca informação sobre essas comunidades, a detecção de fungos cosmopolitas e endêmicos já listados demonstram que a neve e o gelo da Antártica abrigam comunidades fúngicas crípticas, 25 capazes de sobreviver no limite da vida (de MENEZES, 2019).

1.3 Poluentes orgânicos no ambiente antártico: potenciais agentes de seleção de micro-organismos resistentes a drogas

Os poluentes orgânicos persistentes (POPs) são substâncias tóxicas, bioacumulativas e 30 contaminantes presentes no meio ambiente e por meio do seu fracionamento global, podem ser transportadas e se acumulam nas regiões polares e de alta latitude do planeta (WANIA; MACKAY, 1996; WANIA, 2003). Wania; Mackay (1995) confirmaram, por meio de modelos matemáticos, que a condensação a baixas temperaturas pode resultar em concentrações elevadas de contaminantes nas regiões polares. A localização e meio de 35 liberação no meio ambiente, juntamente com diversos parâmetros ambientais também influenciam significativamente o destino global dos POPs (WANIA; MACKAY, 1995, 1996).

5 Além disso, diferentes etapas estão envolvidas no acúmulo de POPs, de acordo com Wania (2003), para as regiões (**Figura 2**).

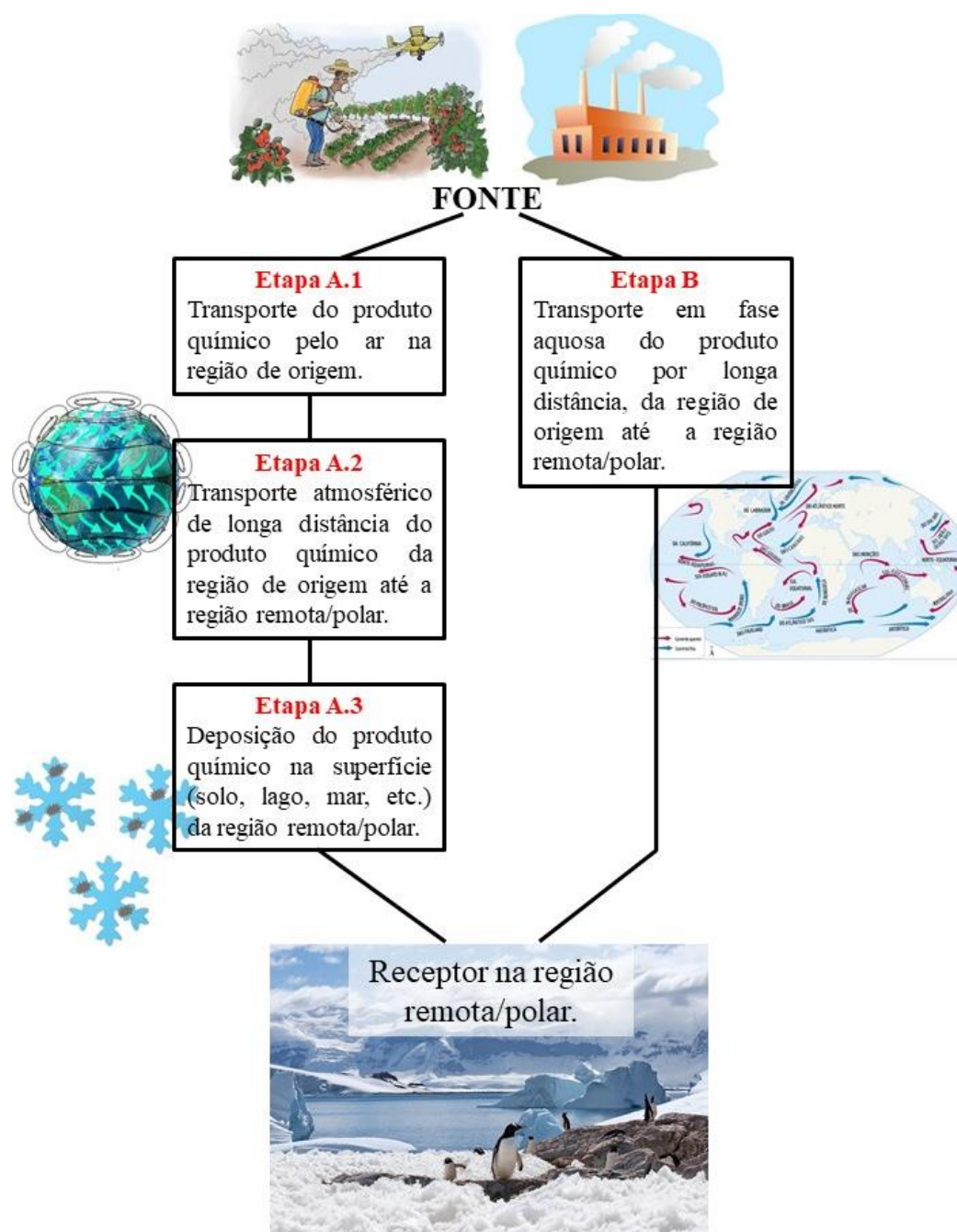


Figura 2. Esquema das etapas envolvidas no acúmulo de produtos químicos orgânicos em regiões remotas/polar (WANIA, 2003 com modificações).

Apesar do isolamento geográfico e da pouca ou ausente influência antrópica da Antártica, seus ecossistemas são afetados pela entrada de POPs, relacionados às atividades humanas atuais e do passado (CIPRO; TANIGUCHI; MONTONE, 2017). O transporte por

5 longas distâncias e ciclos de evaporação e deposição são fatores que permitem que esses contaminantes atinjam áreas remotas como a Antártica (WANIA, 2003; GOUIN et al., 2004). Estudos de monitoramento ambiental investigaram os níveis de POPs, como pesticidas organoclorados (POCs) na Antártica e detectaram a presença destes contaminantes na atmosfera em diferentes quantidades (DICKHUT et al., 2005; KHAIRY et al., 2016), água do
10 mar (GALBÁN-MALAGÓN et al., 2013), gelo marinho (BIGOT et al., 2017), solo (KLÁNOVÁ et al., 2008), aves, fitoplâncton (CIPRO et al., 2013; GALBÁN-MALAGÓN et al., 2013) e neve (WANIA, 2003; VECCHIATO et al., 2015; KHAIRY et al., 2016; CIPRO; TANIGUCHI; MONTONE, 2017).

A neve parece ser um dos principais meios de deposição dos POPs que chegam pela
15 atmosfera aos polos (DALY; WANIA, 2004). Os flocos de neve apresentam grande porosidade e podem ser mais eficazes que a chuva na captura e eliminação de partículas presentes na atmosfera (FRANZ; EISENREICH, 1998; DALY; WANIA, 2004), incluindo POPs. A camada de neve é um reservatório para substâncias orgânicas semivoláteis, em particular para os POPs, tornando-a uma importante matriz ambiental para a análise de
20 contaminantes, devido à sua grande área de superfície e suas propriedades de absorção e adsorção, especialmente em ambientes frios (DOMINE et al., 2007).

Alguns estudos avaliaram a presença e concentração de diversos contaminantes orgânicos que possuem registro de grande produção, utilização e descarte incorreto em termos mundiais como o dibenzo-p-dioxinas, dibenzofuranos policlorados, bifenilos policlorados, éteres difenílicos polibromados e hidrocarbonetos aromáticos policíclicos na neve das porções
25 continental e peninsular da Antártica (KANG et al., 2012; VECCHIATO et al., 2015; CIPRO; TANIGUCHI; MONTONE, 2017). A presença desses contaminantes foram frequentemente detectadas em baixas concentrações, quando comparados a regiões com forte influência antrópica (KANG et al., 2012; VECCHIATO et al., 2015; CIPRO; TANIGUCHI; MONTONE, 2017). Entretanto, a degradação de POPs na Antártica é lenta, devido às baixas
30 temperaturas e a pouca luminosidade no período do inverno (CORSOLINI, 2009). Os POPs que estão aprisionados na neve e no gelo podem ser liberados para o meio ambiente durante o período de descongelamento. Dessa forma, esses poluentes acabam se bioacumulando não somente no ambiente, como também nos organismos antárticos (GEISZ et al., 2008). A neve
35 antártica também pode ser considerada um micro-habitat para micro-organismos que está diretamente interligada com a precipitação atmosférica de poeira, células microbianas e outros materiais biológicos que estão suspensos no ar (ANTONY et al., 2011; MARGESIN; MITEVA, 2011).

5 1.4 Fungos resistentes aos antifúngicos

Desde o início dos anos 1980, quando iniciaram os primeiros estudos sobre o processo de resistência às drogas antifúngicas, um conjunto de informações sobre os aspectos clínicos, bioquímicos e genéticos dos fungos começaram a ser elucidados (WHITE; MARR; BOWDEN, 1998). Entretanto, o desenvolvimento da resistência antifúngica é complexo e depende de múltiplos fatores relacionados aos hospedeiros e patógenos (WHITE; MARR; BOWDEN, 1998). A partir da década de 1990, várias condições contribuíram para o aumento na incidência de infecções fúngicas sistêmicas, relacionadas a pacientes imunossuprimidos portadores de doenças como a síndrome da imunodeficiência adquirida (AIDS), tuberculose e infecções causadas por bactérias (WHITE; MARR; BOWDEN, 1998; VANDEPUTTE; FERRARI; COSTE, 2012). Desta forma, houve progressiva utilização de antifúngicos em tratamentos clínicos e, consecutivamente, aumento na resistência à antifúngicos em certas espécies de fungos (VANDEPUTTE; FERRARI; COSTE, 2012; FISHER et al., 2018).

A resistência às drogas antifúngicas pode ser definida de duas maneiras distintas: (1) resistência a partir do âmbito clínico e (2) laboratorial (SHAPIRO; ROBBINS; COWEN, 2011). A partir da perspectiva clínica, a resistência antifúngica é a persistência ou progressão de uma infecção mesmo após o emprego da terapia medicamentosa apropriada (WHITE; MARR; BOWDEN, 1998; SHAPIRO; ROBBINS; COWEN, 2011). Do ponto de vista laboratorial, refere-se a não susceptibilidade de um fungo a um agente antifúngico em testes de susceptibilidade *in vitro*, nos quais a determinação da concentração inibitória mínima (CIM) da droga excede o ponto de quebra de susceptibilidade (KANAFANI; PERFECT, 2008). O ponto de quebra é a concentração escolhida do antifúngico a ser testado para definir se o fungo em estudo é suscetível ou resistente ao antifúngico. Se a CIM for menor ou igual ao ponto de quebra de susceptibilidade, o fungo é considerado suscetível ao antifúngico. Apesar dos ensaios de CIM fornecerem uma medida de forma como o patógeno responderá ao tratamento com determinada droga, estes ensaios nem sempre são preditores precisos da resposta *in vivo*, uma vez que os níveis de CIM podem não ser a medida mais ideal de resistência (COWEN, 2001; KANAFANI; PERFECT, 2008; SHAPIRO; ROBBINS; COWEN, 2011). A resistência antifúngica pode ser primária (intrínseca) ou secundária (adquirida), onde a resistência primária é encontrada naturalmente entre determinados fungos, sem que esses fungos tenham sido expostos previamente a droga, demonstrando a importância da identificação e diferenciação de espécies ou linhagens ambientais de clínicos (KANAFANI; PERFECT, 2008). A resistência secundária ocorre em fungos previamente expostos a droga e que expressam resistência em resposta a esta exposição (PEREA;

PATTERSON, 2002). Outro tipo de resistência que pode estar ligada a mudança em um fator genético é a resistência cruzada, a qual ocorre quando um mecanismo fúngico de resistência a uma droga resulta na resistência a outras drogas pertencentes a grupos químicos distintos (FARIA-RAMOS et al., 2014; RIBAS E RIBAS et al., 2016). Frequentemente, é relatada a recorrente resistência às drogas antifúngicas pertencentes aos grupos triazóis, imidazóis e 5-flucitosina, enquanto uma menor frequência de resistência é relatada às drogas dos grupos polienos, alilaminas e equinocandinas (SHAPIRO; ROBBINS; COWEN, 2011; VANDEPUTTE; FERRARI; COSTE, 2012; ZAVREL; WHITE, 2015).

Inúmeras drogas antifúngicas foram desenvolvidas nas últimas décadas (ZAVREL; WHITE, 2015). Entre elas a anfotericina B, que pertencente a classe dos polienos e permanece sendo a substância fungicida empregada no tratamento de diversas infecções fúngicas, incluindo as sistêmicas, devido ao seu amplo espectro de ação (FILIPPIN; SOUZA, 2006). Os polienos agem se ligando ao ergosterol presente no interior da membrana plasmática fúngica, levando a formação de poros que vão alterar a permeabilidade desta membrana, acarretando no escape de pequenos íons e metabólitos, principalmente íons potássio, resultando na morte celular fúngica (FILIPPIN; SOUZA, 2006). Também há evidências de que a anfotericina B pode atuar como um agente pró-inflamatório, que estimularia a imunidade inata do hospedeiro (THOMPSON; CADENA; PATTERSON, 2009). Entretanto, a anfotericina B possui toxicidade intrínseca, com efeitos nefrotóxicos e hepatotóxicos, o que dificulta e limita seu uso clínico (FILIPPIN; SOUZA, 2006; THOMPSON; CADENA; PATTERSON, 2009). Segundo Thompson et al. (2009), a resistência aos antifúngicos poliênicos poderia estar associada a duas possibilidades: (1) ao aumento ou diminuição dos esteróides da membrana, especialmente ergosterol e seus precursores, ou (2) a ocorrência de outras modificações fenotípicas, com alterações na síntese de proteínas e enzimas.

As drogas antifúngicas mais comumente utilizadas na prática clínica são os azólicos, que atuam inibindo a via biosintética do ergosterol, componente fundamental da membrana plasmática das células fúngicas (ZAVREL; WHITE, 2015; RIBAS E RIBAS et al., 2016). A inibição da formação de ergosterol resulta na desorganização da parede celular fúngica, impedindo o crescimento do fungo e assim, o modo de ação dos azólicos é fungistático e não fungicida (HOF, 2001). Dentre o grupo dos antifúngicos azólicos, o mais propenso ao desenvolvimento de resistência é o fluconazol devido a dois fatores: (1) sua função fungistática, que pode deixar grandes populações de células sobreviventes que são expostas a forte seleção direcional para resistência e, (2) diferentes mecanismos de resistência

5 (ANDERSON, 2005). Vários mecanismos isolados ou combinados estão envolvidos na
resistência aos azólicos, entre eles, os mecanismos moleculares que promovem mutações no
gene ERG11, que é o gene alvo da droga que codifica a enzima chave 14- α -demetilase
(COWEN et al., 2015). Essas mutações no gene ERG11 podem inibir a ligação do fármaco no
alvo ou induzir a superexpressão do fármaco na síntese alvo nas células (VANDEPUTTE;
10 FERRARI; COSTE, 2012; COWEN et al., 2015). Outro mecanismo envolvido na resistência,
é a superexpressão das bombas de efluxo associadas à membrana, que aumentam o efluxo das
drogas do meio intracelular para o meio extracelular, reduzindo o efeito das drogas (COWEN
et al., 2015). As duas principais famílias de proteínas de efluxo correspondem as *Binding*
Cassette ATP (ABC) e *Major Facilitator Superfamily* (MFS) (HOF, 2001; CANNON et al.,
15 2009; CAVALHEIRO et al., 2018). As proteínas ABC são transportadoras primárias, que
possuem em geral uma especificidade mais ampla e utilizam a hidrólise de ATP, enquanto os
transportadores MFS são secundários e tem uma especificidade mais limitada de substratos,
utilizando os prótons como força motriz (CANNON et al., 2009; CAVALHEIRO et al.,
2018). De acordo com Cannon et al. (2009) os dois tipos de transportadores contêm domínios
20 proteicos distintos, os domínios de ligação a nucleotídeos (NBDs), em bombas ABC e os
domínios transmembrana (TMDs) em bombas ABC e MFS, o que confere especificidade ao
substrato. Além disso, o azólico fluconazol possui menor toxicidade e excelente
biodisponibilidade nos tecidos, em relação a anfotericina B (SANTOS et al., 2012).

Os fungos possuem genomas altamente plásticos, capacidade para se reproduzirem
25 rapidamente e a combinação destes fatores gera variantes selecionados para resistência
(FISHER et al., 2018). O uso em terapias a longo prazo esta associada à resistência ao
fluconazol (WHITE; MARR; BOWDEN, 1998). Entretanto, estudos sugerem que essa
resistência pode também estar relacionada à exposição prévia destes isolados fúngicos aos
fungicidas azólicos utilizados em agrossistemas (VERWEIJ et al., 2009; RIBAS E RIBAS et
30 al., 2016; ZHANG et al., 2017).

1.4.1 Relação dos agrotóxicos com a resistência cruzada aos antifúngicos

O uso de agrotóxicos⁵ é uma das estratégias mais utilizadas para evitar perdas no
agronegócio, o qual cresce progressivamente a cada ano (BASTOS et al., 2017). Infecções

5 **Agrotóxico** é o termo legal definido na Lei Brasileira 7802/89 (Lei de Agrotóxicos). Os termos *pesticida*, *praguicida* e *defensivos agrícolas* também são utilizados. “*Praguicida*” é a denominação utilizada pelos países de língua espanhola e “*pesticida*” utilizado na língua inglesa. Os agrotóxicos possuem várias classes

5 fúngicas em plantas causam perda considerável no rendimento de culturas no mundo inteiro e
podem prejudicar seriamente a produção de alimentos (HOF, 2001). Desta forma, os
fungicidas são utilizados na agricultura para controlar o crescimento de fungos em plantas e
frutos (HOF, 2001; EUROPEAN CENTRE FOR DISEASE CONTROL, 2013). Em
consequência, o mercado global de agrotóxicos teve uma rápida expansão na última década,
10 com aumento em torno de 93%, o que levou os agrotóxicos a constituírem um importante
problema de saúde pública (RIGOTTO; VASCONCELOS; ROCHA, 2014).

Os fungicidas azólicos são amplamente utilizados na agricultura anualmente para
proteção, prevenção e preservação de cultivos contra fungos em diversos países (HOF, 2001;
EUROPEAN CENTRE FOR DISEASE CONTROL, 2013; AVENOT et al., 2016). O
15 surgimento de isolados fúngicos resistentes aos azólicos clínicos utilizados em patógenos
humanos tem sido relacionado à prévia exposição destes fungicidas utilizados no agronegócio
(FARIA-RAMOS et al., 2014; RIBAS E RIBAS et al., 2016; ZHANG et al., 2017). Além
disso, foi proposto que a resistência aos azólicos clínicos evoluiu no ambiente e poderia ser
impulsionada pela pressão seletiva dos fungicidas azólicos, mas apesar das evidências
20 apoiarem esta hipótese, mais estudos devem ser realizados para esta comprovação
(EUROPEAN CENTRE FOR DISEASE CONTROL, 2013). Neste contexto, a eficácia dos
antifúngicos azólicos pode ser afetada devido à resistência cruzada ou quando um isolado
fúngico desenvolve resistência a todos os fungicidas em um grupo químico similar
(SNELDERS et al., 2012).

25 Com base na hipótese de que a resistência é estabelecida por meio da evolução de
micro-organismos em resposta a estresses ambientais, Rocha et al. (2016) investigaram a
influência do tetraconazol (antifúngico ambiental), na resistência *in vitro* aos azólicos clínicos
itraconazol, voriconazol e fluconazol em isolados de *Candida parapsilosis*. Os resultados
mostraram que a exposição ao tetraconazol diminuiu a suscetibilidade aos azólicos clínicos,
30 principalmente ao fluconazol e as linhagens tratadas com ambos azólicos se comportaram de
maneira semelhante. Portanto, os autores sugerem que a resistência aos azólicos de uso clínico
pode ser uma consequência do uso de azólicos nas atividades agrícolas. Bastos et al. (2017)
mostraram que a exposição de diversas linhagens de *Cryptococcus neoformans* e
Cryptococcus gattii ao antifúngico ambiental tebuconazol causou resistência cruzada, *in vitro*,
35 entre o agrotóxico e os azólicos clínicos fluconazol, itraconazol e ravuconazol.

agronômicas: inseticidas (controlar insetos), acaricidas (ácaros), nematicidas (nematóides), fungicidas (fungos),
herbicidas (plantas daninhas), entre outras (Informações retiradas do portal ANVISA).

5 O antifúngico benomil é um fungicida sistêmico de amplo espectro, que pertence à
classe dos benzimidazólicos, sendo um dos antifúngicos ambientais mais utilizados na
agricultura (MÜLLER; ACKERMANN; MARGOT, 2011). O benomil suprime o crescimento
fúngico pela inibição da divisão celular e liga-se a proteína tubulina, bloqueando sua
polimerização e a formação de microtúbulos (LONDE et al., 2000; MÜLLER;
10 ACKERMANN; MARGOT, 2011). Embora seja um dos mais importantes antifúngicos
ambientais, o benomil é propenso ao desenvolvimento de resistência, o que levou a um
declínio no seu uso para muitas culturas (MÜLLER; ACKERMANN; MARGOT, 2011).
Carneiro et al. (2019) avaliaram por meio de testes *in vitro* a influência de antifúngicos
ambientais, dentre eles o benomil, na susceptibilidade de linhagens de *C. gattii* aos
15 antifúngicos de uso clínico e observaram eventos de resistência cruzada entre o fungicida e o
antifúngico fluconazol. A resistência cruzada entre benomil e fluconazol também foi
verificada em testes *in vitro* e em modelo murinho; os resultados deste estudo mostraram que
os isolados de *C. gattii* expostos a pequenas concentrações de benomil apresentaram
alterações morfológicas e aumento da taxa de crescimento *in vitro*, demonstrando perfil de
20 susceptibilidade fúngica para os antifúngicos clínicos (CARNEIRO, 2019).

Os antifúngicos azólicos são a única classe de substâncias utilizadas tanto na
agricultura como na prática clínica, embora possuam diferenças estruturais (RIBAS E RIBAS
et al., 2016). Dessa maneira, fungos oportunistas naturais oriundos de ecossistemas
ambientais contaminados por agrotóxicos, podem ter resistência cruzada à azólicos clínicos
25 utilizados na medicina (RIBAS E RIBAS et al., 2016; FISHER et al., 2018).

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5 2 Justificativa e Relevância

Embora na última década tenha aumentado o número de estudos sobre as comunidades microbianas presentes no gelo e na neve de ambientes polares, poucas informações sobre a diversidade de comunidades fúngicas foram publicados, principalmente sobre aqueles presentes na neve e gelo glacial da Antártica. Partículas de poeira e material orgânico, contendo diversos micro-organismos originários de diferentes regiões ao redor do globo, podem ser transportados pelas correntes aéreas e depositados na superfície da neve e, posteriormente, podem ser encontrados também no gelo glacial formado ao longo dos anos a partir da compactação da neve depositada. Este material eólico varia em diversidade e quantidade, dependendo do clima, e origina diferentes sistemas ecológicos locais na neve e no gelo, os quais são caracterizados como reservatórios dinâmicos de nutrientes e, consequentemente, de diferentes comunidades microbianas (JONES, 1999). Por outro lado, o gelo glacial representa um substrato único capaz de abrigar e preservar a vida microbiana por centenas de milhares de anos (MA et al., 1999).

Apesar do isolamento geográfico e climático, bem como da pouca ou nenhuma influência antrópica na Antártica, seus ecossistemas são afetados pela entrada de poluentes orgânicos persistentes (POPs) relacionados às atividades humanas atuais e do passado (CIPRO; TANIGUCHI; MONTONE, 2017). A neve é um dos principais meios de deposição de POPs, os quais chegam pela atmosfera aos polos do planeta (DALY; WANIA, 2004), transformando a camada de neve em um reservatório para substâncias orgânicas semivoláteis, em particular os POPs (DOMINE et al., 2007). Diferentes estudos já demonstraram a presença de comunidades fúngicas constituída por espécies cosmopolitas e endêmicas na Antártica (ROSA et al., 2019). Esses fungos estão em contato direto com diferentes substâncias que podem ter sido transportadas pelo ar e depositados nessa neve, incluindo os poluentes com estruturas químicas semelhantes às drogas antifúngicas utilizadas nos agrossistemas e na medicina clínica. Dessa forma, os poluentes podem funcionar como agentes antimicrobianos seletivos para os fungos que estão na neve antártica.

Nos ecossistemas glaciais, a formação intracelular de cristais de gelo, água líquida disponível e temperatura exercem grande influência na diversidade e na determinação da comunidade microbiana, incluindo os fungos, capazes de sobreviver e/ou se desenvolver nas condições extremas da criosfera (GUNDE-CIMERMAN et al., 2003; COWAN; TOW, 2004). Neste contexto, devido às condições severas que os fungos antárticos são expostos, estes possuem alta plasticidade genética e fisiológica para conseguir sobreviver a partir da produção de diferentes macro- e micromoléculas pelos seus sistemas metabólicos

5 eucarióticos. Assim, esses micro-organismos vêm sendo considerados uma fonte promissora de metabólitos bioativos com diferentes atividades biológicas (ROSA et al., 2019). Além disso, espécies de fungos aprisionadas no antigo gelo glacial da Antártica podem incluir também linhagens antigas e selvagens produtoras de substâncias bioativas ainda desconhecidas.

10 A partir do exposto acima, a presente tese tem como objetivos caracterizar as comunidades de fungos presentes na neve sazonal e do gelo glacial da Antártica, caracterizar o perfil de resistência desses fungos aos antifúngicos clínicos e ambientais e avaliar o potencial desses fungos como produtores de metabólitos biotivos. Para isso, este estudo visa responder as seguintes questões:

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- A neve e o gelo glacial, considerados substratos ultra-oligotróficos, representam boas fontes para isolamento de fungos extremófilos?
 - Qual a riqueza, diversidade, distribuição e similaridade das comunidades de fungos presentes nos segmentos de neve e gelo glacial da Antártica?
 - Os fungos isolados da neve sazonal da Antártica possuem resistência intrínseca e/ou
- 20
- resistência cruzada entre os antifúngicos clínicos e ambientais?
 - Os fungos isolados do gelo glacial da Antártica produzem substâncias bioativas?

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5 3 Objetivos

3.1 Objetivo Geral

Caracterizar as comunidades de fungos presentes na neve sazonal e gelo da Antártica, detectar espécies/linhagens resistentes a antifúngicos agrícolas e clínicos e avaliar seu potencial como produtores de substâncias bioativas de interesse na medicina e agricultura tropical.

10

3.2 Objetivos Específicos

- Isolar e identificar todos os fungos obtidos da neve sazonal e gelo glacial utilizando técnicas taxonômicas morfofisiológicas e de biologia molecular;
- Caracterizar a diversidade das comunidades de fungos presentes na neve sazonal e gelo glacial da Antártica;
- Identificar e descrever novos táxons;
- Contribuir para preservação *ex situ* dos fungos antárticos, por meio da inclusão de todos os isolados obtidos na coleção temática de fungos extremófilos da Coleção de Micro-organismos e Células da Universidade Federal de Minas Gerais;
- Determinar as concentrações inibitórias mínimas (CIM) dos fungos isolados da neve frente aos antifúngicos clínico fluconazol e o ambiental benomil;
- Definir o perfil de crescimento em diferentes temperaturas dos fungos isolados da neve que apresentarem um perfil de resistência aos antifúngicos fluconazol e benomil;
- Determinar as concentrações inibitórias mínimas (CIM) para o antifúngico clínico anfotericina B dos isolados da neve que exibirem resistência ao fluconazol e/ou ao benomil e apresentarem crescimento à 37 °C;
- Preparar extratos brutos utilizando técnicas de fermentação no estado sólido a partir das culturas dos fungos provenientes do gelo antártico para estudos de bioprospecção;
- Determinar a atividade biológica dos extratos fúngicos obtidos para formas amastigotas de *Leishmania amazonensis* e *Trypanosoma cruzi*, bem como sementes de mono e dicotiledôneas como modelos de plantas para detecção de potencial herbicida.

5 4 Materiais e Métodos

4.1 Áreas de Coletas

As coletas das amostras de neve e gelo foram realizadas durante as Operações Antárticas XXXIV e XXXV, nos verões austrais (2015/2016), em diferentes regiões da Península Antártica (**Figura 3**), de acordo com a logística do Navio Polar Oceanográfico (NPO) Comandante Almirante Maximiano (H41). Os pontos de coleta de neve e gelo estão descritos na **Tabela 1**.

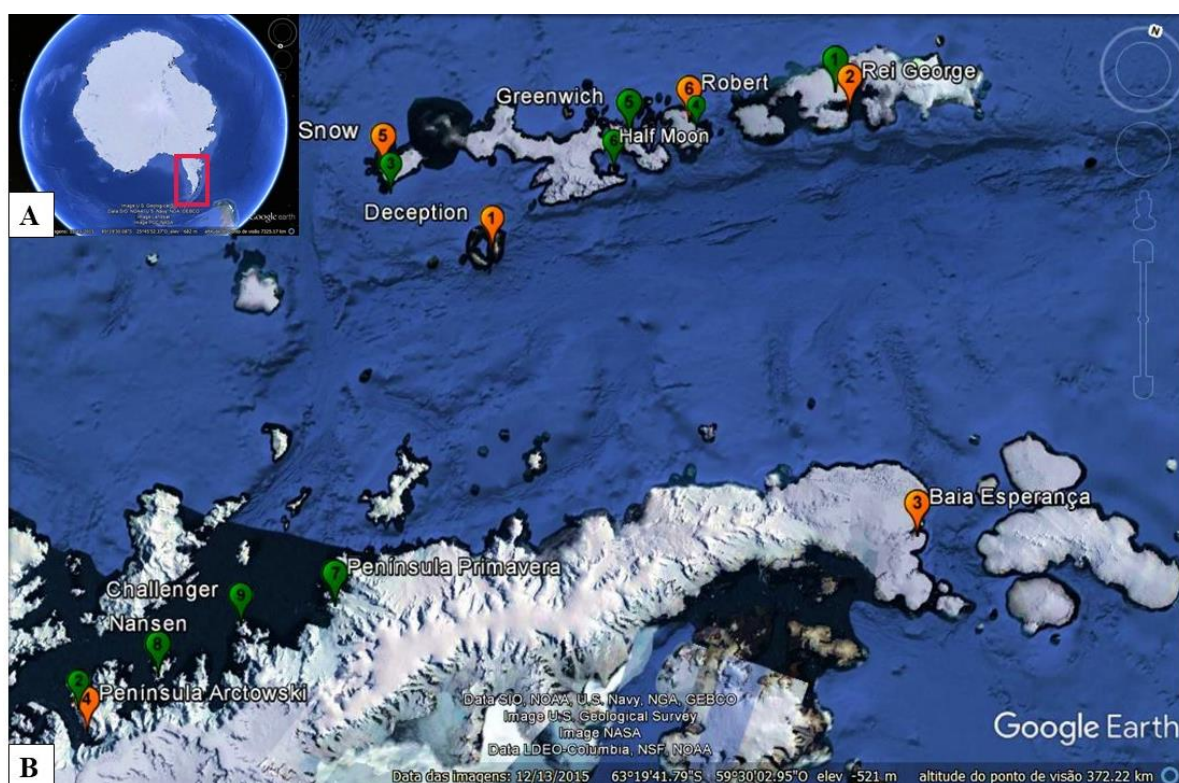


Figura 3. Mapa da Antártica (A) e Península Antártica (B) com os pontos de coleta de neve (1 ao 6 em laranja) e gelo glacial (1 ao 9 em verde). Fonte: GOOGLE EARTH-MAPS, 2017.

5 **Tabela 1.** Pontos de coleta de neve e gelo glacial da Antártica.

Pontos de coleta	Material Coletado	Coordenadas geográficas
Península Antártica - Geleira Sikorsky	Gelo	64°12'S 60°53'W
Península Antártica - Geleira Leonardo/Blanchard	Gelo	64°42'S, 61°58'W
Península Antártica - Geleira Blérnot	Gelo	64°21'7S, 61°34'W
Península Arctowski - Geleira Rozier/Woodbury	Gelo	64°45'S 62°13'W
Ilha Greenwich - Geleira Troub	Gelo	62°37'S, 059°70'W
Ilha Greenwich -Geleria Fuerza Aerea	Gelo	62°30'S 59°38'W
Ilha Rei George -Geleira Ajax/ Stain House	Gelo	62°06'S, 058°27W
Ilha Livingston - Geleira Charity	Gelo	62°44'S 60°20'W
Ilha Livingston - Gleira Huron	Gelo	62°37'50"S 60°06'50"W
Ilha Deception	Neve	62°58'55.6"S, 60°33'11.5"W
Ilha Rei George	Neve	62°06'211"S, 058°27.627"W
Península Trinity	Neve	63°24'45.6"S, 057°00'164"W
Península Arctowski	Neve	64 45'0"S, 62 25'0"W
Ilha Snow	Neve	62°46'300"S, 06°15'240'W
Ilha Robert	Neve	62°37'941"S, 059°70'400"W

4.2 Coleta, isolamento e processamento das amostras de neve e gelo

10 Para obtenção das amostras de neve, foram escavadas trincheiras de até 2 m de profundidade. A neve foi coletada em três segmentos (topo, meio e base) distintos em torno de 30-50 cm cada ou até o encontro do solo e/ou neve compactada (*firm*), os quais foram denominados de segmentos 1, 2 e 3, respectivamente. Um total de 10 L de neve foram coletados, acondicionados separadamente (por segmento) em sacos plásticos de 20 L previamente esterilizados e então transportados para o Laboratório de Microbiologia do NPo Almirante Maximiano. Para escavar as trincheiras foram utilizadas pás próprias para neve, as

5 quais foram desinfestadas com álcool 70% no momento da coleta dos diferentes segmentos de neve. A neve foi descongelada em temperatura de aproximadamente 20 °C e 1,5 L de neve filtrados através de membranas de 0,45 µm (Millipore).

Fragmentos de gelo glacial foram coletados manualmente ao longo das ilhas antárticas (**Tabela 1**), acondicionados em caixas plásticas (tipo marfinite) e transportadas para o
10 Laboratório de Microbiologia do navio NPo Almirante Maximiano. Os fragmentos de gelo de cada região amostrada foram fragmentados em pedaços menores e submetidos a um processo de desinfestação superficial, de acordo com o protocolo proposto por Rogers et al. (2004), seguindo a seguinte ordem:

- 15 1. Lavagem em hipoclorito de sódio 5% por 10 s;
 2. Lavagem em água destilada esterilizada por 10 s;
 3. Exposição a luz ultravioleta germicida por 10 min.

Após a desinfestação, os fragmentos de gelo foram acondicionados em baldes de
20 polipropileno previamente esterilizados e vedados para o derretimento a temperatura de aproximadamente 20 °C. Após o derretimento completo, até 3 L de gelo foram filtrados através de membranas de 0,45 µm (Millipore).

Para cada segmento de neve e dos fragmentos de gelo foram obtidas quatro membranas para cada substrato, das quais:

- 25 • Duas foram inoculadas em placas de Petri contendo o meio de cultura ágar Sabouraud (Himedia, India);
 • Duas foram inoculadas em placas de Petri contendo o meio de cultura Meio Mínimo (MM) (6,98 K₂HPO₄; 5,44 KH₂PO₄; 1,0 (NH₄)₂SO₄; 1,1 MgSO₄ 7H₂O, 0,25 peptone, 20 agar em g.L⁻¹, pH 6,8 ± 0.2) (GONÇALVES et al., 2012a modificado);

30

Em ambos os meios de cultura foram acrescidos 200 mg.L⁻¹ de cloranfenicol (Sigma, EUA), para evitar o crescimento de bactérias. Os meios foram selecionados a fim de oferecer uma fonte rica de nutrientes, comumente utilizados em trabalhos de micologia e uma fonte oligotrófica, para tentar selecionar fungos que possuam características de crescimento
35 específicas, como as encontradas na neve. As placas de Petri contendo as membranas e meios de cultura foram incubadas a 10 °C por até 60 dias, para obtenção das colônias dos fungos. A temperatura de 10 °C foi escolhida visando isolar organismos psicrófilos, os quais possuem capacidade de crescimento a 0 °C, com temperatura ótima de crescimento igual ou inferior a

5 15 °C e temperatura máxima de crescimento igual ou abaixo de 20 °C (MORITA, 1975; ROBINSON, 2001).

4.2.1 Purificação e preservação dos fungos

Transcorrido o período de incubação, a densidade das colônias fúngicas obtidas foram quantificadas em UFC L⁻¹ e purificadas em novas placas de Petri, contendo o meio de cultura
10 o qual os fungos foram isolados e mantidos a 10 °C. Discos de 3 mm de diâmetro de ágar contendo micélio ativo das culturas puras dos fungos filamentosos foram armazenadas em duplicatas, em água destilada esterilizada em temperatura ambiente (CASTELLANI, 1967) e em criotubos esterilizados contendo água destilada esterilizada e glicerol 15%, e depositados no ultrafreezer a -80 °C. As culturas puras de leveduras foram inoculadas em meio GYMP
15 (glicose 2%, extrato de levedura 0,5%, extrato de malte 1% e fosfato de sódio 0,2%). Após o período de 48-72 horas, foi retirada uma alíquota de 1 mL deste caldo com a levedura e colocada em criotubos esterilizados e acrescido 20% de glicerol e depositadas no ultrafreezer a -80 °C. Todos os fungos obtidos foram depositados na Coleção de Microorganismos e Células do Departamento de Microbiologia do Instituto de Ciências Biológicas da UFMG,
20 com o código UFMGCB.

4.3 Identificação dos fungos

4.3.1 Identificação dos fungos filamentosos

As culturas dos isolados de fungos obtidas foram fotografadas (frente e verso) e agrupadas de acordo com as seguintes características macromorfológicas: cor da colônia
25 (frente e verso), textura da superfície (frente e verso) e aspecto da borda. Posteriormente os grupos formados de acordo com as características macromorfológicas, foram submetidos à análise de perfis moleculares pela técnica de PCR microsatélite (PCR-MST), também chamada “DNA *fingerprint*”, utilizando o oligonucleotídeo sintético (GTG)₅. Os isolados com padrões de banda de DNA idênticos foram confirmados como pertencentes ao mesmo grupo.
30 Então, um isolado de cada grupo molecular foi selecionado para o sequenciamento da região espaçadora transcrita interna ITS-5.8S, da região gênica do rRNA. As identificações dos grupos foram confirmadas por meio do sequenciamento parcial do gene da β-tubulina e/ou da RNA Polimerase II. A escolha da região a ser sequenciada foi determinada de acordo com os gêneros encontrados, por meio do sequenciamento da região ITS, pois para alguns táxons este
35 iniciador apenas identifica a nível de gênero.

5 4.3.1.1 Extração do DNA total

A extração do DNA total foi realizada de acordo com metodologia descrita por Rosa et al. (2009), com modificações. Os fungos filamentosos foram crescidos por no máximo 20 dias em meio Sabouraud a 10 °C e fragmentos de micélio foram retirados e colocados em tubos de 1,5 mL acrescidos de 400 µL de tampão de lise (Tris-HCl – trishidroximetilaminometano 0,05 M, EDTA – ácido etilenodiamino tetra-acético 0,005 M, NaCl 0,1 M e SDS – sódio dodecil sulfato 1%) e mantido a – 20 °C, por no mínimo 30 min. Em cada tubo foram adicionadas três esferas de aço inox (3,175 mm de diâmetro) e posteriormente submetidos à trituração no equipamento Bullet Blender TM 24 (Uniscience). Em seguida, foram adicionados 162 µL de CTAB (Tris 2M, NaCl 8,2%, EDTA 2M e CTAB 0,2%), seguido de homogeneização em vórtex e incubação por 40 min – 1h a 65 °C. Sequencialmente, foram acrescentados 570 µL da mistura clorofórmio/álcool isoamílico (24:1). Após homogeneização, o tubo foi incubado por 30 min em banho de gelo. Em seguida, o conteúdo foi centrifugado a 14.000 r.p.m por 10 min e o líquido sobrenadante foi transferido para um novo tubo de 1,5 mL e acrescentado 10% do volume de uma solução de acetato de sódio 3M (pH 5,2). Após homogeneização por inversão, os tubos foram incubados por 30 min em banho de gelo e centrifugados a 14.800 r.p.m por 10 min. O sobrenadante foi transferido para um novo tubo e, em seguida, adicionado 50% do volume de isopropanol (Merck), sendo a suspensão gentilmente homogeneizada e incubada a temperatura ambiente por 40 min. Em seguida, foram adicionados 200 µL de etanol 70% (Merck) gelado, sendo os tubos homogeneizados e submetidos à centrifugação a 14.800 r.p.m por 5 min. O sobrenadante foi descartado novamente por inversão. Foi realizada mais uma lavagem com 200 µL de etanol 70% (Merck) gelado e, por último, mais uma centrifugação a 14.800 r.p.m por 5 min, seguido de descarte do sobrenadante. A amostra foi então seca, por aproximadamente 60 min a 37 °C e/ou deixada overnight em temperatura ambiente e então ressuspensa em 50 µL de Tris-EDTA (Tris-HCl 30 0,01 M e EDTA 0,001 M) e incubada a 65 °C por 60 min, para hidratação do DNA e armazenadas a –20 °C até sua utilização. Todos os produtos obtidos foram quantificados em espectrofotômetro a 260/280 nm (NanoDrop ND 1000 Thechnologies).

4.3.1.2 Amplificação utilizando o iniciador (GTG)₅

O oligonucleotídeo (GTG)₅ foi utilizado para amplificação de regiões microssatélites e para confirmar os agrupamentos morfológicos, conforme descrito por Lieckfeldt et al. (1993). A técnica de DNA *fingerprint* baseia-se na detecção de sequências repetitivas hipervariáveis (mini e microssatélites de DNAs) que são favorecidas para mostrar a variabilidade genética

dos fungos (LIECKFELDT; MEYER; BÖRNER, 1993). A Reação em Cadeia da Polimerase (PCR) foi realizada para um volume final de 25 μL , contendo 1,0 μL de DNA (de acordo com a concentração do DNA, a qual deve estar entre 50 - 400 $\text{ng} \cdot \mu\text{L}^{-1}$), 2,0 μL do iniciador (GTG)₅ 10 μmol^{-1} (MWG Biotech), 2,5 μL de tampão de PCR 5X (Fermentas), 1,5 μL de MgCl_2 25mM, 1,0 μL de dNTP 10 mM, 0,2 μL de *Taq* DNA polimerase 5U (Fermentas) e o volume final completado com água de injeção esterilizada. As reações de PCR foram realizadas utilizando o termociclador Mastercycler (Eppendorf). O programa consistiu de uma desnaturação inicial a 94 °C por dois min, seguido por 40 ciclos de 45 s de desnaturação a 94 °C, um minuto de anelamento a 50 °C, um minuto de extensão a 72 °C e uma extensão final por seis minutos a 72 °C. Os produtos de PCR foram analisados por eletroforese em gel de agarose 1,5%, em tampão TBE 0,5 X (54 g de tris base, 27,5 g de ácido bórico, 20 mL de EDTA 0,5M, pH 8,0), eluídos durante aproximadamente 1 hora e 30 min a 80 V. As bandas foram coradas com solução de GelRed (Biotium). Os géis foram visualizados sob luz ultravioleta e fotografados pelo sistema de foto-documentação de gel (Vilber Lourmat, France).

4.3.1.3 Amplificação da região ITS

Os iniciadores ITS1 (TCCGTAGGTGAACCTGCGG) e ITS4 (TCCTCCGCTTATTGATATGC) foram utilizados para amplificação da região transcrita interna ITS-5.8S da região do gene do rRNA, conforme descrito por WHITE et al. (1990). A PCR foi realizada em um volume final de 50 μL , contendo de 1,0 μL de DNA, 1,0 μL de cada iniciador ITS1 e ITS4 a 10 μmol^{-1} (MWG Biotech), 5,0 μL de tampão de PCR 5X (Fermentas), 2,0 μL de MgCl_2 25mM, 2,0 μL de dNTP 10 mM, 0,2 μL de *Taq* DNA polimerase 5U (Fermentas) e o volume final completado com água de injeção esterilizada. As reações de PCR foram realizadas utilizando o termociclador Mastercycler (Eppendorf). O programa consistiu de uma desnaturação 30 ciclos iniciais a 94 °C por cinco min, seguido por 35 ciclos de um minuto de desnaturação a 94 °C, um minuto de anelamento a 55 °C, um minuto de extensão a 72 °C e uma extensão final por cinco minutos a 72 °C. Os produtos de PCR foram analisados por eletroforese em gel de agarose 1%, em tampão TBE 0,5X (54 g de tris base, 27,5 g de ácido bórico, 20 mL de EDTA 0,5M, pH 8,0), eluídos durante aproximadamente 30 minutos a 110 V. As bandas foram coradas com solução de GelRed (Biotium), e os géis visualizados sob luz ultravioleta e fotografados pelo sistema de foto-documentação de gel (Vilber Lourmat, França).

4.3.1.4 Amplificação parcial do gene da β -tubulina

Os iniciadores BT2a (GGTAACCAAATCGGTGCTGCTTTC) e BT2b (ACCCTCAGTGTAGTGACCCTTGGC) foram utilizados para amplificação parcial do gene da β -tubulina, conforme descrito por Glass e Donaldson (1995). O sequenciamento parcial do gene da β -tubulina possui elevado índice de polimorfismo entre os fungos do filo Ascomycota (DONALDSON, 1995), por isso foi uma região escolhida para identificação de determinados táxons. A PCR foi realizada em um volume final de 50 μ L contendo de 1,0 μ L de DNA, 1,0 μ L de cada iniciador BT2a e BT2b a 10 μ mol⁻¹ (MWG Biotech), 5,0 μ L de tampão de PCR 5X (Fermentas), 2,0 μ L de MgCl₂ 25 mM, 2,0 μ L de dNTP 10 mM, 0,2 μ L de *Taq* DNA polimerase 5U (Fermentas) e o volume final completado com água de injeção esterilizada. As reações de PCR foram realizadas utilizando o termociclador Mastercycler (Eppendorf). O programa consistiu de uma desnaturação inicial a 94 °C por cinco minutos, seguido por 35 ciclos de um minuto de desnaturação a 94 °C, um minuto de anelamento a 59 °C e 90 s de extensão a 72 °C e uma extensão final por sete minutos a 72 °C. Os produtos da PCR foram analisados da mesma maneira descrita no tópico 4.3.1.3.

4.3.1.5 Amplificação parcial da RNA polimerase II

Os iniciadores RPB2 5F (GATGACCGTGGACCACTTCGG) e RPB2 7R (CCCATGGCTTGTTTGCCCAT) foram utilizados para amplificação parcial do gene da RNA polimerase II, conforme descrito por Malkus et al. (2006). O sequenciamento do gene RNA polimerase II produz uma cópia única em fungos, sendo uma região bem conservada (MALKUS et al., 2006), por este motivo foi empregada. A PCR foi realizada em um volume final de 50 μ L contendo de 1,0 μ L de DNA, 1,0 μ L de cada iniciador RPB2 5F e RPB2 7F a 10 μ mol⁻¹ (MWG Biotech), 5,0 μ L de tampão de PCR 5 X (Fermentas), 2,0 μ L de MgCl₂ 25mM, 2,0 μ L de dNTP 10 mM, 0,2 μ L de *Taq* DNA polimerase 5U (Fermentas) e o volume final completado com água para injeção esterilizada. As reações de PCR foram realizadas utilizando o termociclador Mastercycler (Eppendorf). O programa consistiu de uma desnaturação inicial a 94 °C por três minutos, seguido por 35 ciclos de 20 s de desnaturação a 94 °C, 30 s de anelamento a 55 °C e 60 segundos de extensão a 72 °C e uma extensão final por 10 min a 72 °C. Os produtos da PCR foram analisados da mesma maneira descrita no tópico

4.3.1.3.

4.3.1.6 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, foram submetidas à caracterização dos parâmetros macroscópicos. Dentre os parâmetros morfofisiológicos foram analisados: cor da colônia, textura, cor reversa, tipo de borda, esporulação, 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 agar em g L^{-1}) e CYA (1,0 K_2HPO_4 ; 10 mL concentrado de Czapek; 5 extrato de levedura; 30 sacarose 15 agar 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).

4.3.2 Identificação das 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 de acordo com as características citadas acima, foram submetidas à análise de perfis moleculares, por meio da técnica de PCR microsatélite (PCR-MST), utilizando o iniciador (GTG)₅. Os isolados que apresentaram padrões de bandas idênticos foram confirmados como pertencentes ao mesmo grupo. Então, um isolado de cada grupo molecular foi selecionado para sequenciamento dos domínios D1/D2 da subunidade maior do DNA ribossomal utilizando os iniciadores ITS1/NL4.

4.3.2.1 Extração do DNA total

A extração do DNA genômico dos isolados de leveduras seguiu Sambrook et al. (1986), com modificações. Os isolados foram crescidos em meio sólido Sabouraud, por um período máximo 72 h, a 10 °C. Após crescimento, uma alçada das colônias foi ressuspensa em 100 μL de tampão de lise (Tris-HCl – trishidroximetilaminometano 0,05 M, EDTA – ácido etilenodiamino tetra-acético 0,005 M, NaCl 0,1 M e SDS – sódio dodecil sulfato 1%) em tubos de 0,6 μL , homogeneizadas em vórtex e incubadas em banho-maria a 65 °C, por 30 min. Posteriormente, foram adicionados 200 μL da solução clorofórmio/álcool isoamílico (24:1) e homogeneizados por inversão. Em seguida, centrifugou-se a 14.000 r.p.m por 15 min. Transferiu-se a fase superior do sobrenadante para novos tubos de 0,6 μL e adicionou-se 100 μL de isopropanol (Merck) (v/v), para a precipitação do DNA, por um período de 15 min em temperatura ambiente. Após as amostras foram centrifugadas a 14.000 r.p.m por 10 min. Na

5 sequência, foram adicionados 200 µL de etanol 70% (Merck) gelado. Os tubos foram
homogeneizados e centrifugados a 14.000 r.p.m, por 10 min e o sobrenadante descartado. Foi
repetida a etapa do etanol 70%, seguido do descarte do sobrenadante. As amostras foram
secas por aproximadamente 60 min a 37 °C e/ou deixadas overnight em temperatura ambiente
e ressuspensas em 50 µl de TE (Tris 10 mM; EDTA 1 mM). As amostras foram então
10 incubadas em banho-maria a 37 °C por 30 min para hidratação do DNA e armazenadas a - 20
°C, até sua utilização. Todos os produtos obtidos foram quantificados em espectrofotômetro a
260/280 nm (NanoDrop ND 1000 Thecnologies).

4.3.2.2 Amplificação utilizando os iniciadores ITS1 e NL4

15 As leveduras foram submetidas ao sequenciamento dos espaçadores transcritos
internos (ITS1-5.8S-ITS2) do gene do rRNA. Assim, a PCR foi realizada utilizando-se os
iniciadores ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') e NL4 (5'-
CGTCCGTGTTTCAAGACGG-3') (WHITE et al., 1990; O'DONNELL, 1993), a fim de se
amplificar toda a região de interesse em uma única reação.

20 A reação de PCR foi realizada em um volume final de 50 µL contendo: 2,0 µL de
DNA; 1,0 µL de cada iniciador ITS1 e NL4 a 10 pmol µL⁻¹ (Invitrogen); 5,0 µL de tampão de
PCR 10X (Fermentas); 2,0 µL de MgCl₂ 25mM (Fermentas); 2,0 µL de dNTP 10 mM; 0,2 µL
de Taq DNA polimerase 5U µL⁻¹ (Fermentas) e o volume final completado com água
deionizada esterilizada. As amplificações foram realizadas sob as seguintes condições:
25 desnaturação inicial a 94 °C por 3 min, seguida por 30 ciclos de: desnaturação a 94 °C por 30
s, anelamento a 57 °C por 30 s e extensão a 72 °C por 1 min e 30 s, seguida por extensão final
a 72 °C por 10 min. Os amplicons, foram analisados da mesma maneira descrita no tópico
4.3.1.3.

4.3.3 Purificação dos amplicons

30 Os amplicons gerados pelas reações de PCR foram purificados utilizando etanol
absoluto e EDTA. Ao produto de PCR (47 µL) foram adicionados 11,75 µL de EDTA 125
mM e 141 µL de etanol absoluto (Merck). Os tubos foram homogeneizados por inversão e
deixados a temperatura ambiente, por 15 min. Após os tubos foram centrifugados a 14.000
r.p.m, durante 25 min. O sobrenadante foi descartado e o precipitado foi lavado com a adição
35 de 120 µL de etanol 70% gelado, sendo posteriormente homogeneizado por inversão. Após
centrifugação a 14.000 r.p.m por 10 min, o sobrenadante foi descartado novamente e os tubos
foram deixados abertos à temperatura ambiente para evaporação de todo o excesso de etanol.

5 Após, foi adicionado 10 µL de água de injeção esterilizada e os tubos incubados em banho-maria a 37 °C, por 30 min. Os produtos obtidos foram dosados em NanoDrop ND 1000 (NanoDrop Technologies, EUA) e armazenados a -20 °C, até o momento da reação de sequenciamento.

10 **4.3.4 Reações de sequenciamento**

As reações de sequenciamento dos produtos obtidos após a purificação dos amplicons foram realizadas no Laboratório de Parasitologia Celular e Molecular (LPCM) da FIOCRUZ/Instituto René Rachou, utilizando o kit Big Dye versão 3.1 5 (Applied Biosystems, EUA) em combinação com a plataforma de sequenciamento automatizado ABI 3730. A
15 reação de sequenciamento foi realizada em microplacas de 96 poços (Applied 5 Biosystems, EUA) preparada para um volume final de 10 µL, em que foram adicionados: 1 µL do iniciador (5 µmol⁻¹), 1 µL de tampão (presente no kit de sequenciamento), 1 µL de Big Dye, 1 µL de DNA (concentração do DNA deve estar entre 15 e 20 ng) e o restante de água de injeção esterilizada para completar o volume. A placa foi selada com filme adesivo
20 transparente. O programa de ciclagem utilizado consistiu em 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 °C e 4 min de extensão final a 60 °C. 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
25 vórtex e incubada por 15 min à temperatura ambiente. Após período de incubação, a placa foi centrifugada por 45 min a 4.000 r.p.m a 4 °C. 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 r.p.m, a 4 °C e o sobrenadante descartado por inversão. Para remoção do excesso de etanol, foi retirado o selante e a placa foi invertida
30 sobre um papel absorvente e submetida a um spin (pulso até a centrífuga atingir 900 r.p.m). 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 °C, protegida da luz, até injeção das amostras
35 no sistema automatizado ABI 3730 (Applied Biosystems, EUA).

4.3.5 Análise computacional das sequências

5 As sequências de DNA obtidas dos fungos filamentosos e leveduras foram comparadas com as sequências de espécies tipo ou referência depositadas no *GenBank* e pertencentes a coleções de culturas internacionais, utilizando o programa BLASTn (*Basic Local Alignment Search 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*.

10 Os fungos que apresentaram sequências com valor de $E = 0$, cobertura e identidade $\geq 99\%$, bem como, proximidade com as sequências fúngicas depositadas no *GenBank*, quando analisadas filogeneticamente utilizando o programa MEGA 6 (*Molecular Evolutionary Genetics Analysis*) (TAMURA et al., 2013), foram considerados como pertencentes à mesma espécie. Para fungos com sequências com valor de $E \neq 0$ e cobertura e identidade $\leq 98\%$

15 foram identificados em nível de gênero ou níveis hierárquicos mais altos após a análise filogenética. Além disso, 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 ≥ 350 pares de bases. As

20 árvores filogenéticas foram construídas utilizando o algoritmo de *Neighbor-joining*. O modelo *Maximum composite likelihood* foi usado para estimar a distância evolucionária. Uma análise de *bootstrap* foi feita com 1,000 repetições, utilizando os programas incluídos no MEGA 6. Informações sobre os níveis hierárquicos utilizados na taxonomia dos fungos foram obtidos no MycoBank (<http://www.mycobank.org/>) e Index Fungorum

25 (<http://www.Índexfungorum.org/>).

4.4 Diversidade da comunidade fúngica: índices de abundância, riqueza, dominância e similaridade

A abundância de cada táxon foi calculada de acordo com a seguinte fórmula: porcentagem de abundância do táxon A = número de isolados do táxon A x 100/soma de isolados de todos os táxons. Estes dados foram utilizados para determinar a prevalência de

30 cada táxon em comparação com o total de táxons presente nas comunidades fúngicas na área de coleta.

Para avaliar a diversidade de espécies no local de coleta, foram utilizados os seguintes índices: (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

5 ocorrem de forma aleatória onde, comumente, algumas espécies são tão raras que sua chance de inclusão é pequena (FISHER et al., 1943). Este índice é calculado pela 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 é uma medida utilizada em ecologia para estimar a riqueza de espécies de uma comunidade, com base na distribuição numérica dos indivíduos das diferentes espécies em função do número total de indivíduos existentes na amostra analisada. 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 local amostrado. O índice de Simpson é muitas vezes utilizado para quantificar a biodiversidade de um ecossistema. Ele leva em conta o número de espécies presentes no local, bem como a abundância de cada espécie. Trata-se de um índice de dominância que mede a probabilidade de dois indivíduos, selecionados ao acaso na amostra, pertencer à mesma espécie. O cálculo da Dominância de Simpson é dado pela fórmula:

$$D = \sum (n / N)^2$$

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 neve e gelo, 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 local (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 (Sorensen) entre os mesmos locais amostrados.

5 Uma curva de rarefação 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 resultados foram obtidos com 95% de confiança e os valores de *bootstrap* calculados a partir de 1.000 repetições. Todos os
10 índices foram calculados utilizando o programa computacional PAST 1.90.

Para ilustrar a comparação entre as comunidades fúngicas da neve e do gelo da Antártica foi utilizado o diagrama de *Venn*, de acordo com Bardou et al. (2014). O diagrama de *Venn* é um gráfico de visualização comum, que permite identificar espécies compartilhadas e não compartilhadas, fornecendo uma visão sobre similaridades dos locais amostrados. No
15 diagrama de *Venn*, cada lista é apresentada por uma forma transparente. As sobreposições das formas contêm os elementos compartilhados entre os locais ou, mais frequentemente, as contagens correspondentes (BARDOU et al., 2014).

4.5 Preparo dos extratos fúngicos

Os fungos filamentosos obtidos do gelo glacial antártico que foram identificados como pertencentes ao gênero *Penicillium* foram selecionados para a produção dos extratos e
20 posterior ensaios biológicos. Este gênero foi escolhido por ser tradicionalmente muito estudado e por ser considerado um excelente produtor de substâncias bioativas (NICOLETTI; TRINCONI, 2016).

Os fungos foram crescidos em cinco placas de Petri de 90 x 15 mm contendo meio BDA a 25 °C com umidade de 70-80%. Após 15 dias de crescimento, o meio de cultura com o
25 crescimento micelial foi transferido para frascos de vidro (tipo Erlenmeyers) de 125 mL, congelados a -80 °C por 24 horas e, posteriormente, submetidos ao processo de liofilização (Liofilizador LioTop – K105) por 96 h. Após a desidratação completa do meio contendo micélio foram acrescentados 80 mL de diclorometano P.A. (Synth). Os frascos foram incubados a temperatura ambiente e após 1 semana o sobrenadante (fase diclorometânica) de
30 cada frasco foi filtrado com auxílio de papel filtro (50 x 50 – 80g), sendo posteriormente transferidos para frascos de cintilação, onde os extratos foram secos e posteriormente pesados (ROSA et al., 2012).

5 4.6 Ensaios Biológicos

4.6.1 Ensaio *in vitro* com a forma amastigota-like de *Leishmania (leishmania) amazonensis*

A atividade leishmanicida seguiu os protocolos estabelecidos por Callahan et al. (1997) e Campos et al. (2008). A manutenção das células axênicas de *L. (L.) amazonensis* (linhagem IFLA/BR/196/PH-8) e o ensaio com amastigotas-like foram realizados por técnicos especializados pertencentes ao Grupo de Pesquisa em Química de Produtos Naturais Bioativos/Fiocruz MG. 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. As formas amastigotas foram semeadas a $1,0 \times 10^8$ parasitos/mL⁻¹ e mantidas em meio apropriado, na presença ou não dos extratos e drogas controle à temperatura de 32 °C. Após 72 horas, a atividade dos extratos testados sobre as formas amastigotas foi medida pelo método colorimétrico do MTT (metil tiazolil tetrazólio) (Sigma/EUA). Os resultados foram calculados a partir das absorbâncias medidas, onde o percentual de morte parasitária foi comparado com as absorbâncias dos controles com e sem droga de referência (anfotericina B/controlado positivo). Todos os ensaios foram realizados em triplicata. Os extratos capazes de inibir acima de 70% a proliferação dos parasitas foram considerados ativos.

25 4.6.2 Ensaios *in vitro* com as formas tripomastigotas e as formas intracelulares amastigotas de *Trypanosoma cruzi*

Os ensaios *in vitro* com as formas tripomastigotas e amastigotas de *T. cruzi* foram realizados de acordo com protocolos estabelecidos por Buckner et al. (1996) com algumas modificações com base em Romanha et al. (2010). A manutenção dos parasitas e os ensaios foram realizados por técnicos especializados do Grupo Genômica Funcional e Proteômica de *Leishmania* spp. e *Trypanosoma cruzi* (GFP) do Instituto René Rachou/Fiocruz, MG. Os extratos foram testados por meio de ensaio colorimétrico, sendo utilizada a linhagem de *Trypanosoma cruzi* (Tulahuen) e extratos fúngicos diluídos na concentração de 20 mg.mL⁻¹.

A linhagem de *T. cruzi* é transformada para expressar a enzima β -galactosidase, que possui capacidade de catalisar uma reação colorimétrica quando o vermelho de clorofenol β -D- galactopiranosídeo (CPRG) é utilizado como substrato. Para a triagem dos extratos, $4,0 \times 10^3$ células L929 (fibroblasto de camundongo) em meio RPMI (pH 7,2 - 7,4) foram semeadas por poço em placas de 96 poços, no volume de 80 μ L, seguido de incubação para a adesão das

5 células à superfície. Após incubação, a infecção foi realizada com $4,0 \times 10^4$ formas tripomastigotas por poço, no volume de 20 μL , durante 2 h. Decorrido este período, o meio contendo as formas tripomastigotas que não penetraram nas células foi substituído por 200 μL de meio fresco e a placa novamente incubada. Após 48 h, o meio foi descartado e substituído por 180 μL de meio fresco e 20 μL dos extratos a serem testados. Cada extrato foi testado em
10 duplicata. Transcorridos 7 dias após o período de incubação, o CPRG na concentração final de 100 μM e o Nonidet P-40 na concentração final de 0,1 % foram adicionadas as placas, seguidos de incubação overnight a 37 °C e a absorbância foi medida a 570 nm em leitor automático de microplaca. Como controles, foram utilizadas células não infectadas, células infectadas não tratadas (controle negativo), benzonidazol a 1 mg.mL^{-1} (3,81 μM) (controle
15 positivo) e DMSO diluído em meio na concentração final de 1 % (controle do solvente). Os resultados foram expressos como a porcentagem de redução da absorbância dos poços experimentais em comparação com a absorbância dos poços com células infectadas não tratadas. Os extratos que apresentaram atividade $\geq 50\%$ foram considerados ativos.

4.6.3 Determinação da atividade herbicida

20 A determinação da atividade herbicida foi realizada de acordo com Ferreira et al. (2017). A atividade herbicida foi determinada utilizando 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 2,5% (7 min
25 e 30 s). Posteriormente, as sementes foram lavadas em água deionizada esterilizada por 6 vezes e secas sobre papel filtro, em capela de fluxo laminar, por até 2 h. 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 – 80g) esterilizado foi colocado em cada poço. Para o controle negativo, foram utilizados 400 μL de água deionizada esterilizada. Para
30 avaliação da toxicidade do solvente utilizado na solubilização dos extratos, foram adicionados ao poço 360 μL de água e 40 μL de acetona P.A. Para controle positivo de inibição da germinação, foi utilizado o herbicida sistêmico Glifosato (Sigma/USA). Para tanto, foram adicionados ao poço 400 μL da solução aquosa de glifosato, na concentração de 3 mg.mL^{-1} . Aos poços com a solução teste, foram adicionados 360 μL de água e 40 μL da diluição do
35 extrato na concentração de 1 mg.mL^{-1} . Posteriormente, 5 sementes de cada vegetal teste foram adicionadas em cada poço. As placas foram tampadas e seladas com parafilme, sendo incubadas em estufa de fotoperíodo (BOD), sob condições de luz contínua ($120,1 \text{ umol s}^{-1}\text{m}^{-2}$)

5 a 25 °C, por 10 dias para *L. sativa* e 14 dias para *A. schoenoprasum*. O período de crescimento para cada vegetal, seguiu os padrões de germinação. Toda a preparação e montagem da placa para o bioensaio foi realizada em capela de fluxo laminar, para diminuir possível contaminação. Transcorrido o período de germinação de cada semente, foi realizada uma
10 estimativa qualitativa de fitotoxicidade, atribuindo-se uma classificação que vai de 0 a 5; onde, 0 (zero) representa que não ocorreu nenhum efeito de inibição da germinação (todas as sementes germinaram), 2 para menos de 50% de inibição da germinação, 3 para cerca de 50% de inibição da germinação, 4 para mais de 50% de inibição de germinação e 5 para não germinação das sementes. Durante o processo da leitura do bioensaio, sempre foi utilizado como padrão comparativo os controles utilizados. Todos os extratos foram avaliados em
15 duplicata.

4.6.4 Concentração Inibitória Mínima (CIM) das drogas antifúngicas benomil, fluconazol e anfotericina B

Os fungos isolados da neve antártica que apresentavam relatos na literatura como
20 patógenos oportunistas para humanos foram selecionados e submetidos à determinação da CIM dos antifúngicos benomil (Sigma, EUA) e fluconazol (Sigma, EUA) a 25 °C. Somente os isolados que apresentaram $CIM \geq 64 \mu\text{g.mL}^{-1}$ para fluconazol e que foram capazes de crescer a 37 °C foram submetidos à determinação da CIM do antifúngico anfotericina B (Sigma, EUA). O ensaio de CIM foi determinado de acordo com os documentos M27-A3,
25 referente às leveduras e M38-A2 para fungos filamentosos, ambos obtidos a partir do *Clinical and Laboratory Standards Institute* (CLSI, 2008).

Os testes de susceptibilidade foram realizados em placas de microdiluição de 96 poços, com fundo chato, em duplicata. As concentrações testadas para benomil variaram de 0,078125 a 40 $\mu\text{g.mL}^{-1}$ (TRUONG et al., 2018), fluconazol de 0,062 a 64 $\mu\text{g.mL}^{-1}$ e
30 anfotericina B de 0,007 a 8 $\mu\text{g.mL}^{-1}$ (CLSI, 2008). Uma solução estoque de cada antifúngico foi formulada de acordo com a solubilidade da droga. Benomil e anfotericina B foram dissolvidos em DMSO 100% (dimetil sulfóxido) (Sigma/EUA), enquanto fluconazol foi dissolvido em água destilada estéril. As soluções estoques foram preparadas em uma concentração inicial de 2000 $\mu\text{g.ml}^{-1}$ para o fungicida ambiental benomil e 1000 $\mu\text{g.ml}^{-1}$ para
35 fluconazol e anfotericina B. Para os testes, a solução estoque foi diluída 5 vezes em RPMI - 1640 para chegar na concentração de 2 vezes a necessária, e em seguida foram feitas as diluições.

5 A coluna 1 foi o controle de esterilidade, na qual foi inoculado somente o meio de cultura RPMI - 1640. Nas colunas 2 a 11 foram inoculados 100 µL das diluições das drogas antifúngicas 2 vezes concentradas. A coluna 2 continha a maior concentração da droga antifúngica e a fileira 11 a menor concentração. Nas fileiras, a partir da segunda coluna, foram inoculados 100 µL da suspensão dos inóculos duas vezes concentrados. O volume final de
10 cada poço foi de 200 µL, de forma que tanto as concentrações da droga quanto as concentrações dos inóculos diminuíram de 2 vezes para 1 vez, atingindo assim os valores desejados. Os poços de controle positivo de crescimento (coluna 12) continham 100 µL de meio sem droga, inoculados com 100 µL das suspensões 2 vezes concentradas dos inóculos. Após a montagem, as microplacas foram incubadas a 25 °C para fluconazol e benomil e a 37
15 °C para anfotericina B, todas por 48 horas.

A leitura do ensaio foi realizada visualmente, onde foi observada a presença ou ausência de crescimento visível nos poços, comparando ao controle de crescimento. As concentrações inibitórias mínimas foram determinadas a partir das menores concentrações da droga que levaram a uma redução de 100% do crescimento dos fungos para anfotericina B e
20 para os azólicos. Os valores de referência para os testes de sensibilidade *in vitro* (µg.mL⁻¹) para leveduras são os sugeridos para *Candida* spp., de acordo com o protocolo do CLSI (M27-A3, 2008b), já que não existem testes padronizados para as espécies de leveduras ambientais pertencentes a outros gêneros. Os valores de CIM para anfotericina B ainda não estão bem estabelecidos, mas quando se obtém um valor acima de 1 µg.mL⁻¹ para um isolado
25 de *Candida* spp. é provável que o isolado seja resistente à anfotericina B (CLSI, M27-A3, 2008b). Da mesma maneira determinada para as leveduras, ainda não existem dados suficientes para classificar os fungos filamentosos em resistentes a determinado antifúngico, mas o CLSI (M38-A2, 2008a) sugere alguns valores de referência para os testes de sensibilidade com fungos filamentosos. De maneira geral, se a CIM for ≥ 4 , o fungo pode ser
30 resistente a anfotericina B.

O antifúngico benomil foi escolhido para o teste por se tratar de um fungicida sistêmico de amplo espectro que pertence à classe dos benzimidazólicos, sendo um dos antifúngicos ambientais mais utilizados na agricultura (MÜLLER; ACKERMANN; MARGOT, 2011), além de sua estrutura química ser similar ao azólico clínico fluconazol.
35 Embora seja um dos mais importantes fungicidas ambientais, benomil é propenso ao desenvolvimento de resistência (MÜLLER; ACKERMANN; MARGOT, 2011).

5 4.6.4.1 Preparo do inóculos

Para o preparo dos inóculos das leveduras, colônias jovens dos isolados, com no máximo 72 h de crescimento, foram ressuspensas em 5 mL de solução salina esterilizada 0,145 mol L⁻¹ (8,5 g.L⁻¹ NaCl; salina a 0,85%). As suspensões resultantes dos inóculos foram homogeneizadas em vórtex por aproximadamente 15 s e as densidades celulares foram
10 ajustadas com espectrofotômetro a 530 nm, acrescentando-se solução salina suficiente para obter a transmitância de 74-76 % para *Rhodotorula muscilaginosaa*, 85-87% para *Debaromyces hansenii*. Esse procedimento fornece as suspensões-padrão das leveduras contendo 1 x 10⁶ a 5 x 10⁶ células por mL. Diluições 1:50 seguida de diluições de 1:20 das suspensões com inóculo inicial em RPMI-1640 foram realizadas para se obter as densidades
15 dos inóculos necessários para o teste duas vezes concentradas (de 1 x 10³ a 5 x 10³ células mL⁻¹).

Para o preparo dos inóculos dos fungos filamentosos, colônias com 7 dias de crescimento, foram cobertas com 1 mL de solução salina (0,85% de NaCl) esterilizada. A solução salina contendo os esporos ou fragmentos de hifas foi transferido para um tubo de
20 ensaio esterilizado contendo 5 mL de salina esterilizada e após sedimentação, por decantação em repouso de 1 a 3 min a temperatura ambiente, o sobrenadante foi transferido para outro tubo de ensaio com salina esterilizada, que foi homogeneizado em vórtex por aproximadamente 15 s. As densidades das suspensões dos esporos foram mensuradas e ajustadas para uma transmitância de acordo com o tamanho dos esporos de cada espécie
25 fúngica a 620 nm. Para isolados do gênero *Penicillium*, a transmitância ficou entre 85-87%. Estas suspensões foram diluídas a 1:50 com o meio RPMI-1640, correspondendo a 2 vezes a densidade necessária de 0,4 x 10⁴ a 5 x 10⁴ células mL⁻¹, aproximadamente.

4.6.5 Determinação do perfil de crescimento em diferentes temperaturas

30 Os fungos da neve já relatados como patógenos oportunistas em humanos foram cultivados em ágar Sabouraud a 10, 15, 20, 25, 30, 35 e 37 °C em placas de Petri (90 x 15 cm) para determinar seu perfil de crescimento em diferentes temperaturas. Os fungos filamentosos foram inoculados por transferência de plugs de ágar (4 mm²) de pré-culturas com 10 dias de crescimento a 10 °C. As leveduras foram cultivadas em YM por 72 h a 10 °C. Em seguida, 10
35 µl de células com turbidez de 0,5 na escala MacFarland (1–5 × 10⁶ células mL⁻¹ a 70% de transmitância) foram inoculadas no centro das placas de Petri contendo meio Sabouraud.

- 5 Todas as placas foram incubadas em triplicata, durante 72 h para leveduras e 14 dias para os fungos filamentosos para detectar a presença ou ausência de crescimento.

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5 5 Capítulos

Os resultados desta tese serão apresentados em forma de capítulos, dos quais dois já foram publicados, um na forma de capítulo de livro e outro na forma de artigo científico, ambos em livro e jornal indexados. Os demais capítulos foram/serão submetidos em jornais indexados.

10

5.1 Capítulo 1: “Chapter 6: Fungi in snow and glacial ice of Antarctica”

5.2 Capítulo 2: “Fungi in the seasonal snow of Antarctica: diversity, distribution, and resistance against agricultural and clinical antifungal drugs”

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5.3 Capítulo 3: “*Antarctomyces pellizariae* sp. nov., a new, endemic, blue, snow residente psychrophilic ascomycete fungus from Antarctica”

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5.4 Capítulo 4: “Fungi in glacier ice of Antarctica: diversity, distribution, and bioprospection of bioactive compounds”

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Chapter 6

Fungi in Snow and Glacial Ice of Antarctica



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6.1 The Antarctic Cryosphere

According to glaciologists and palaeoclimatologists, we live in an interglacial environment in the middle of an Ice Age, where the ice mass currently covers 10% of the planet (at the height of the last glacial age, about 18.000–20.000 years ago, 30% of the planet was covered in ice). However, from the environmental viewpoint, 90% of the volume is concentrated only in Antarctica.

Most of this ice is found in the Antarctic ice sheet, covering approximately 12.3 million km² with an average thickness of 2.020 m, encompassing 24.7 million km³ of ice. The maximum thickness of ice found here is 4.776 m, and the oldest existing ice is probably more than 1.5 million years old. The mantle is composed of several domes (the highest reaches 4.093 m in altitude), where the ice drains slowly to its shores, thousands of miles away. The surface of the Antarctic ice sheet is typically semi-parabolic, more than 2.000 km long, with a flat surface at the centre, and a slope that increases towards the coast (Fig. 6.1).

The Antarctic ice sheet can be divided into two major parts, East and West (although the nomenclature, east and west, may sound illogical for a continent centred on the geographical South Pole). The East Antarctic ice sheet is spread over a subglacial topography that is above the mean sea level, covering an area of 10.1 million km² (21.7 million km³ of ice), with a mean thickness of 2.220 m. The region around its main dome (Dome A) is the driest region (it precipitates only 1–2 cm of water, in the form of snow, per year) and the coldest place on Earth (surface tempera-

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127



Fig. 6.1 Landscape near the West Antarctic ice sheet ice divide. (Photograph of a Brazilian glaciological camp site taken in 2008. Photo Credits: Simões JC)

ture in winter may fall below -90°C). The West Antarctic ice sheet covers an area of 2.3 million km^2 (3.0 million km^3 of ice), has a mean thickness of 1.300 m and is largely spread on a continental surface positioned below the mean sea level, even covering a trench of depth 2.496 m. The removal of ice from this part of Antarctica would result in the formation of an archipelago.

Ice shelves are the floating parts of the mantle with a thickness varying between 200 and 1.600 m, generally occurring in regions where there is a large embayment of the Antarctic coast and are fixed to the coast. They gain mass by the ice flow from the mantle or by in situ ice accumulation, ending in cliffs that may be 50 m above sea level or 100–350 m below it. They lose mass by the release of icebergs or by melting caused by seawater at their bottom (which plays an important role in forming the bottom water of oceans). Ice shelves cover approximately 44% of the Antarctic coast—the largest ones, Filchner-Ronne and Ross, cover 439.920 km^2 and 510.680 km^2 , respectively. In total, it is an additional 1.5 million km^2 of ice, with an average thickness of about 700 m.

The Antarctic Peninsula and adjacent islands have less than 1% of the Antarctic ice volume (mainly comprising small ice masses, including glaciers a few 100 m long, ice caps and the ice covering the Peninsular plateau, which is approximately 120.000 km^2 in area), limited by the topography that controls its shape and flow conditions. However, owing to their small size of these ice masses and because they are closer to the melting point under pressure, they respond more quickly to changes in climatic variables. Figure 6.2 presents a perspective view of the Antarctic cryosphere, where two main components are observed: the ice sheet covering the continent and the sea ice belt. In addition to the mantle, the central plateau of the Antarctic Peninsula can also be observed, covered with ice caps, flowing to the coast in the form of steep outlet glaciers.

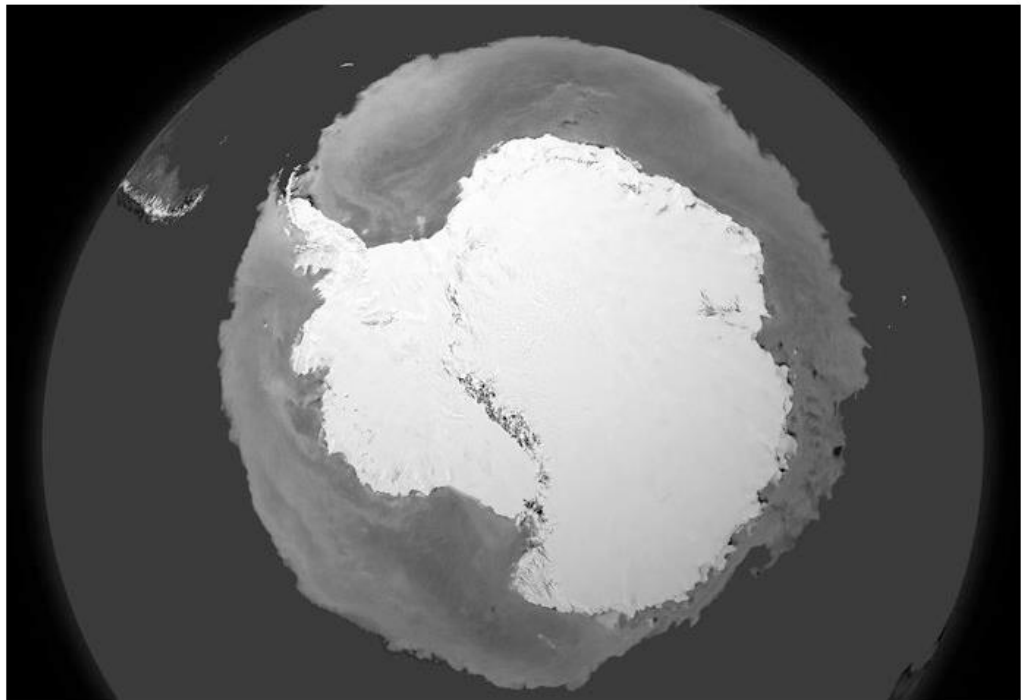


Fig. 6.2 Perspective view of the Antarctic cryosphere. The huge ice sheet (white) covers 99.7% of the continent (across 13.8 million km²) and the Antarctic Peninsula. The dark spots represent the few mountainous regions with apparent rocks. Around the continent, grey represents sea ice at its maximum annual extent (in September), which can cover up to 20 million km² of the Southern Ocean in winters. (Courtesy: NASA/USA)

Around the continent, the sea ice belt can be seen at its maximum extent, typically during the late polar winter (in September), when it covers up to 20 million km² of the Southern Ocean. Although it is only a thin layer (averaging not more than 1 m in thickness), it plays an important climatic role in isolating the underlying ocean waters from the atmosphere. Sea ice is formed by the freezing of seawater (-1.83°C), and its seasonal cycle of formation and melting is known as the environmental phenomenon with the highest annual variation (the area covered by sea ice varies, on average, from 3.0 to 18 million km² between summer and winter).

The melting and disintegration of the packed ice is rapid, and the ice coverage may get considerably reduced to only 1.6–3.0 million km² from October till the end of February. On average, the Antarctic sea ice does not survive for more than a year, except in some regions such as in the Weddell Sea to the east of the Antarctic Peninsula, the Amundsen Sea and part of the Ross Sea, where it can survive up to 3 years. Importantly, sea ice undergoes drifting by the action of the wind, moving up to 20 km per day.

6.2 Snow and Glacial Ice as Microbial Habitats

Snow is a massive component of the cryosphere that permanently or seasonally spans up to 35% of the Earth's surface (Margesin and Miteva 2011) and represents the source for the formation of glaciers (Pearce et al. 2009). In Antarctica, snow plays an important role in the formation of glacial ice by compression and gradual burial over hundreds and thousands of years (Cowan and Tow 2004). Most of the Antarctic continent is covered by snow (Fig. 6.3), which plays an important role in balancing ice masses and glaciers (Goodison et al. 1999).

As a habitat, snow is directly related to the atmosphere because of constant wind flows of dust, microbial cells and other deposited biological materials present in suspension (Margesin and Miteva 2011). In remote environments, the origin of most of the microbial life seems to be caused by air currents (Abyzov 1993; Pearce et al. 2009), which under the effect of precipitation (in the form of snow) is a capable process of decanting microorganisms suspended in the atmosphere, originating from different parts of the world, towards the terrestrial ecosystems, including Antarctica (Bargagli 2008). Microorganisms inhabiting these snow ecosystems are considered extremophiles and are generally exposed to high incidences of light, UV radiation and seasonal temperature fluctuations (Miteva 2008).

Over the years, the precipitated snow undergoes a process of compaction to become firm ice, which can be considered as a natural means of air sampling from thousands of years ago, where different microorganisms in space and time become trapped in the ice (Abyzov 1993; Ma et al. 1999). Thus, ice can be considered as an excellent matrix for the long-term preservation of microorganisms, allowing the study of contemporary and ancient microbial diversity (Gunde-Cimerman et al. 2003). Ice and snow are important and dominant substrates that are interconnected in the Antarctic environment (Price 2000; Bargagli 2008), with characteristics that restrict the development of life, and are considered as real extreme ecosystems (Maccario et al. 2015).

The glaciers on the planet represent large, thick ice masses that move slowly downwards relative to gravity because of a combination of deformations (Anesio and Laybourn-Parry 2012; Castiella 2014). Glaciers and ice sheets occupy 10% of the Earth's surface (approximately covering 15 million km²) (Anesio and Laybourn-Parry 2012), and Antarctica holds most of the planet's ice volume (Goodison et al. 1999), where approximately 99.7% of the continent is permanently covered by snow and ice (Convey et al. 2009) with an average thickness of 2 km and a maximum thickness of 4 km (Bargagli 2008) (Fig. 6.4). The active microbial growth in these environments is influenced by ice formation and consequently, the little liquid water available (Gunde-Cimerman et al. 2003). Liquid water is vital in these environments because their distribution reflects the heat balance at certain points inside the ice and the hydraulics forming the drainage system that conveys water through the ice by forming small water channels (Hodson et al. 2008), where microorganisms are able to grow and reproduce.



Fig. 6.3 Summer seasonal snow collection for fungal isolation in Livingston Island and South Shetland Islands in Antarctica. (Photos Credits: LH Rosa)



Fig. 6.4 Glaciers formed at King George Island, South Shetland Islands in Antarctica. (Photos Credits: LH Rosa)

Among physical factors, temperature has considerable influence in determining the type of microorganism that can survive and/or grow in cold ecosystems. Liquid water available in the micro-channels of ice along with the few organic molecules present are the prime conditions for the development of a resident microbial community (Poindexter 2009). Hence, most microorganisms isolated from cold environments are psychrotolerant¹ (also called psychrotrophs) or psychrophilic² and are well adapted to cold conditions (Cavicchioli et al. 2002). Therefore, the water activity in snow and glacial ice is an important factor that influences the microbial diversity and activity resident to this extreme habitat. In glacial ecosystems, there are holes called cryoconites,³ functioning as microhabitats for active microbial communities on the surface of glaciers (Bagshaw et al. 2013). Grzesiak et al. (2015) showed that the gradual exposure of ice as the surface layer of snow melts is an important factor in the spatial variation, chemical composition, abundance of cryoconite holes and the microbial diversity present on the surface of glacial ice. The melting of the superficial snow provides an important supply of inocula, nutrients and water that will cascade into the glacial ecosystems, whereas the accumulation of superficial snow exerts a critical control on the development of the subglacial drainage and determines, to some extent, the degree of interconnectivity in the integrated glacial ecosystem (Fountain et al. 2006; Hodson et al. 2008).

6.3 Antarctic Sea Ice

In the Antarctic cryosphere, beyond snow and ice, the sea ice is also considered a unique habitat that harbours different microorganisms (Fig. 6.5). The sea ice formed in different regions of the Antarctic sea is largely seasonal, with an average range that varies from minimum in February to maximum in September (Zwally et al. 2002; Comiso et al. 2011). A small fraction of the Antarctic sea ice that persists in the austral summer is mainly found in the Weddell Sea; however, in some stricter Antarctic winters, it is possible to find fragments of sea ice in other regions around the coast of the Antarctic continent (Wadhams and Comiso 1992; Vaughan et al. 2013).

Sea ice is a semi-solid heterogeneous matrix containing tiny brine channels and pockets of highly saline water between solid ice crystals (Eicken 2003). Sea ice represents a dynamic ecosystem that changes its physical structure simultaneously with the seasonality of solar radiation at high latitudes and daily fluctuations in air temperature (Hassett and Gradinger 2016). Despite these limiting characteristics,

¹ Psycho-tolerant microorganisms not only grow well at temperatures close to the freezing point of water but also show high growth rates above 20 °C.

² Psychrophilic microorganisms show optimal growth at temperatures equal to or less than 15 °C, but cannot grow above 20 °C.

³ Vertical cylindrical holes that form on the ice surface with a thin layer of particles, debris and microorganisms deposited on the bottom and filled with water.



Fig. 6.5 Sea ice sheet formed at Deception Island, Antarctica. (Photo Credits: LH Rosa)

the sea ice presents itself as a unique habitat for diverse microbial communities in polar environments (Mock and Thomas 2005). The microbial communities in sea ice are critical components of polar marine ecosystems (Robinson et al. 1997) and contribute to primary production in the local food chain (Legendre et al. 1992). It is believed that extreme conditions, together with halotolerance, are a driving force in the evolution of marine microorganisms (Powell et al. 2015), suggesting that the sea ice environment may be an important reservoir of species diversity, including for those of the fungi kingdom (Hassett and Gradinger 2016). However, it is important to note that studies on microbial communities in Antarctic sea ice have concentrated almost exclusively on algae and bacteria, and studies on fungi are inadequate.

6.4 Methods Used to Study Microbial Communities in Snow and Ice

Studies involving microbial diversity in snow, glacial ice and Antarctic sea ice are challenging for a variety of reasons; the remote location of these habitats and the difficult logistics involved in obtaining and transporting these samples are regarded as the main challenges. According to Miteva (2008), the logistic difficulty in obtaining glacial ice is still greater, as the process by which its perforation is performed is slow and requires specialist equipment to obtain the glacial ice core in the best conditions of sterility available for microbiological studies.

To conduct reliable microbiological studies with samples of snow, glacial ice and sea ice, it is important that external contamination of samples during the processing

is avoided to demonstrate that the microbial communities observed in the sample are truly native to that specific habitat, and not any external contamination associated with the methodology used in obtaining the samples. Several processing and decontamination techniques have been proposed, tested and used over the years (Rogers et al. 2004).

To reduce contaminations in snow samples, generally, about 20 cm-thick portions are removed from both the upper layer (to avoid surface contamination from particles carried by the wind) (Antony et al. 2016) and lower layer (to avoid contaminations from the soil layer in contact with the snow) before collecting the target sample. For glacial and sea ice samples, decontamination is usually performed by washing the samples with sodium hypochlorite followed by exposure to UV radiation (Rogers et al. 2004; Miteva et al. 2009), removing the outer layer of the samples (Abyzov 1993; Ma et al. 2000; D'Elia et al. 2009) and using materials previously sterilised by moist heat and/or chemicals such as 70% ethanol for sample collection (Turchetti et al. 2008; de Menezes et al. 2017). The elimination of all external contaminants is fundamental in the authentication of the species found in the samples, and effective decontamination depends on the necessary care taken during the collection, transportation and processing of samples for isolation of the microorganisms or extraction of the genetic material present (Rogers et al. 2004).

The extreme conditions in cryo-environments (minus temperatures, continuous incidence of UV radiation and low availability of water, nutrients and minerals) pose the biggest challenges to isolate and cultivate microorganisms by the direct inoculation of samples obtained from these environments, as these conditions (and the cryo-environment) provide inadequate information regarding the complex nutritional and biomass requirements of the microorganisms present. In addition, the low number of microbial cells and plants in these environments may be damaged, or simply inactive, and may not be able to grow or develop in the different culture media used for their isolation. Thus, physico-chemical studies and previous samples of the target environment that can be analysed can form the basis for preparing specific culture media and growth conditions (Miteva 2008).

Abyzov et al. (1998) utilised melted water from ice core fragments for direct inoculation into different culture media and showed that lower amounts of viable fungi are obtained by direct inoculation of water from deeper and older ice layers. Studies using the same technique of direct inoculation in various culture media were able to obtain strains of viable fungi from ancient glacial ice samples (Ma et al. 1999, 2000; D'Elia et al. 2008, 2009).

In filtration-culture techniques, snow or ice samples are melted and filtered through membranes having pores of specific size, typically 0.45 µm in diameter, for the retention of fungal particles (spores or hyphal fragments), which are subsequently seeded in different culture media compositions (Table 6.1). This technique was effective in getting viable, cultivable microorganisms from Antarctic ice and snow (Turchetti et al. 2008; de Menezes et al. 2017). Elster et al. (2007) used three different pre-concentration methods (filtration, centrifugation or lyophilisation) before inoculating the samples in different solid and liquid culture media. Their study demonstrated that these pre-concentration methods optimised cultivation of

Table 6.1 Culture media used for the isolation of fungi from snow, glacial ice and sea ice

Media	Composition per litre	References
Sabouraud agar	10 g enzymatic digest of casein, 40 g dextrose (glucose), 20 g agar, pH 5.6 ± 0.2	Jacobs et al. (1964), Ma et al. (2000), D'Elia et al. (2008, 2009), Knowlton et al. (2013), de Menezes et al. (2017)
Yeast-malt extract agar (YMA)	5 g peptic digest of animal tissue, 3 g yeast extract, 3 g malt extract, 10 g dextrose; 20 g agar, pH 6.2 ± 0.2	Ma et al. (2000)
Nutrient agar (NA)	3 g beef extract, 5 g peptone, 15 g agar, pH 6.8 ± 0.2	Ma et al. (2000), D'Elia et al. (2008, 2009), Knowlton et al. (2013), Sanyal et al. (2018)
Malt extract agar (MEA)	12.8 g maltose, 2.7 g dextrin, 2.4 g glycerol, 0.8 g peptone, 15 g agar, pH 4.7 ± 0.2	Ma et al. (2000), D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Potato dextrose agar (PDA)	40 g potato starch; 20 g dextrose, 15 g agar, pH 5.6 ± 0.2	Ma et al. (2000), D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Mycobiotic agar (MA)	15 g agar, 10 g soybean hydrolysate, 10 g dextrose, 0.5 g cycloheximide, 0.05 g chloramphenicol, pH 6.5 ± 0.2	Ma et al. (2000)
Oatmeal agar (OA)	60 g oat flakes, 12.5 g agar, pH 6.0 ± 0.2	Ma et al. (2000) and Knowlton et al. (2013)
Yeast Extract Peptone agar (YEP)	5 g yeast extract; 5 g bacteriological peptone, 3 g KH_2PO_4 ; 3 g $(\text{NH}_4)_2\text{SO}_4$, 2 g glucose, 15 g agar, pH 7 ± 0.2	Thomas-Hall and Watson (2002)
Supplemented PDA	4 g potato starch, 20 g dextrose, 15 g agar, 0.5 g yeast autolysate, pH 5.6 ± 0.2	Abyzov et al. (2004)
Winogradsky medium	10 g sucrose or glucose, trace CaCO_3 , 20 g agar, pH 7.2 ± 0.2	Abyzov et al. (2004)
Z medium	0.467 g NaNO_3 , 0.059 g $\text{Ca}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$, 0.003 g K_2HPO_4 , 0.025 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.021 g Na_2CO_3 , 10 mL Fe-EDTA, pH 6.6, for solid media add 15 g of agar	Elster et al. (2007)
Bold's Basal Medium (BBM)	10 mL NaNO_3 (25 g), 10 mL $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (2.5 g), 10 mL $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (7.5 g), 10 mL K_2HPO_4 (7.5 g), 10 mL KH_2PO_4 (17.5 g), 10 mL NaCl (2.5 g), 1 mL EDTA solution, 1 mL Fe solution (0.498 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 0.1 mL H_2SO_4 (96%)), 1 mL H_3BO_3 (11.42 g), 1 mL Trace metals solution (8.82 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.44 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.71 g MoO_3 , 1.57 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.49 g $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), pH 6.6 ± 0.2 , for solid media add 15 g of agar	Elster et al. (2007)

(continued)

Table 6.1 (continued)

Media	Composition per litre	References
BG-11	1.5 g NaNO ₃ , 0.04 g K ₂ HPO ₄ , 0.075 g MgSO ₄ ·7H ₂ O, 0.036 g CaCl ₂ ·2H ₂ O, 0.006 g citric acid; 0.006 g ferric ammonium citrate, 0.001 g EDTA (disodium salt), 0.02 g Na ₂ CO ₃ , 1 mL Trace metal mix A5 (2.86 g H ₃ BO ₃ , 1.81 g MnCl ₂ ·4H ₂ O, 0.22 g ZnSO ₄ ·7H ₂ O, 0.39 g NaMoO ₄ ·2H ₂ O, 0.079 g CuSO ₄ ·5H ₂ O, 0.49 g CO(NO ₃) ₂ ·6H ₂ O), pH 7.1 ± 0.2, for solid media add 10 g of agar	Elster et al. (2007)
Rose Bengal agar (RB)	5 g soytone, 10 g dextrose, 1 g KH ₂ PO ₄ , 0.05 g rose Bengal, 15 g agar, pH 7.2 ± 0.2	D'Elia et al. (2008, 2009), Turchetti et al. (2008)
Acidic yeast extract agar	30 g yeast extract, 30 g malt extract, 5 g peptone, 10 g dextrose, 20 g agar, pH 4.5 ± 0.2	D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Meat-liver agar	20 g meat-liver base, 0.75 g D (+)-glucose, 0.75 g starch, 1.2 g sodium sulphite, 0.5 g ammonium ferric citrate, 11 g agar, pH 7.6 ± 0.2	D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Blood agar	15 g pancreatic digest of casein, 5 g papaic digest of soybean meal, 5 g NaCl, 50 g sheep's blood, 15 g agar, pH 7.3. ± 0.2	D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Water agar	Sterile distilled water with 20 g agar	D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Yeast extract agar	30 g yeast extract, 30 g malt extract, 5 g peptone, 1 g dextrose, 20 g agar, pH 6.2 ± 0.2	D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Dichloran 18% glycerol agar (DG18)	5 g casein enzymatic digest, 10 g D-glucose, 1 g potassium dihydrogen phosphate, 0.5 g magnesium sulphate, 0.002 g dichloran, 0.1 g chloramphenicol, 15 g agar, pH 5.6 ± 0.2	Turchetti et al. (2008)
Dichloran rose Bengal agar (DRB)	5 g peptic digest of animal tissue, 10 g dextrose, 1 g Monopotassium phosphate 0.5 g magnesium sulphate, 0.025 g rose Bengal 0.1 g chloramphenicol, 0.002 g dichloran, 15 g agar, pH 5.6 ± 0.2	Turchetti et al. (2008)
Tryptone soy broth (TSB)	17 g tryptone, 3 g Soytone—enzymatic digest of soybean meal, 5 g NaCl, 2.5 g glucose, 2.5 g K ₂ HPO ₄ , pH 7.3 ± 0.2	Antony et al. (2016)
Zobell marine broth	5 g peptone, 1 g yeast extract, 0.1 g C ₆ H ₅ FeO ₇ , 19.45 g NaCl, 8.8 g MgCl ₂ , 3.24 g Na ₂ SO ₄ , 1.8 g CaCl ₂ , 0.55 g KCl, 0.16 g NaHCO ₃ , 0.8 g KBr, 0.034 g SrCl ₂ , 0.022 g H ₃ BO ₃ , 0.004 g Na ₂ SiO ₃ , 0.0016 g (NH ₄)(NO ₃), 0.008 g Na ₂ HPO ₄ , 0.0024 g NaF, pH 7.6 ± 0.2	Antony et al. (2016)
Tryptone soy agar (TSA)	15 g tryptone, 5 g soytone - enzymatic digest of soybean meal, 5 g NaCl, 15 g agar, pH 7.3 ± 0.2	Sanyal et al. (2018)

viable microorganisms, and that liquid media provided the best conditions for the isolation of microorganisms in Antarctic ice and snow samples.

Although widely used, some cultivation methods can arbitrarily favour certain taxa in the microbial community to the detriment of others, by either enabling the faster growth rate of some species (based on the type of culture medium and temperature chosen) or owing to the fact that several microorganisms are simply not cultivable. Individually or collectively, these factors can interfere with the accurate interpretation of the microbial diversity in samples, including that for fungi. About 1% of the local microbial community of most ecosystems is able to grow in culture media (Amann et al. 1995). Based on this understanding, independent methods of cultivation have gained interest in recent years, with emphasis on the use of metagenomic techniques.

Metagenomics is based on the direct extraction of DNA from microorganisms in different environmental samples, without requiring their cultivation, which can be used to evaluate microbial diversity (Escobar-Zepeda et al. 2015) by sequencing their amplicons (Antony et al. 2016). In permanently frozen environments, the constant low temperatures provide the ideal conditions for long-term preservation of nucleic acids due to the reduction of the molecular degradation rate (Willerslev et al. 2004). Molecular methods based on polymerase chain reaction (PCR) have the advantage of detecting live, dead, dormant or damaged cell DNA, providing a better representation of the local microbial diversity (Miteva 2008). However, obtaining the low biomass in addition to the difficulty of lysing the obtained cells and obtaining sufficient DNA may hinder the amplification process and the subsequent cloning and analysis of the sequences (Miteva et al. 2009). The popularisation of independent cultivation techniques in terms of cost and analytical tools, as well as the integration of “omics”⁴ technologies into all biological approaches will still challenge scientific standards on the ecology of extremophiles (Cowan et al. 2015).

Scanning electron microscopy and fluorescence-based techniques are used in studies involving the characterisation of microbial communities in Antarctic ice and snow to determine cell viability, enumeration and size of cells (Abyzov 1993; Abyzov et al. 2004; D’Elia et al. 2008; Knowlton et al. 2013). Abyzov et al. (2004) have reported the advantage of using epifluorescence microscopy to detect cells and possibly estimate their physiological state in samples selected for study.

Interestingly, Antony et al. (2016) reported notable differences in both the quantity and variety of fungal taxa determined using both culture-dependent and independent techniques in their work focused on the microbial communities in Antarctic snow. Thus, when reviewing studies on microbial diversity, we must bear in mind that only a single methodological approach cannot provide data regarding the real diversity of the microbial communities inhabiting a particular environment. The application of polyphase methodologies (culture-dependent and independent techniques and microscopy) in the evaluation of microbial diversity can increase the accuracy of the results (Elster et al. 2007; Miteva 2008). Although the use of culture-dependent methods may not be able to determine the actual microbial diversity in particular samples/ecosystems, one of the main advantages of these methods is the production of pure microbial cultures (Abyzov et al. 2004). Accordingly, we can

consider the possibility of using these pure microbial cultures in future biotechnology studies for valuable applications.

6.5 Diversity and Ecology of Fungal Community in Antarctic Snow and Ice

Taxonomic studies on fungi in Antarctic snow and ice samples are scarce, and few taxa have been published thus far. Table 6.2 shows the known taxa identified from the samples collected from different substrates in Antarctica. Jacobs et al. (1964) conducted pioneering studies in the central part of the Antarctic continent and isolated the yeast *Candida parapsilosis* and filamentous fungi of the genera *Penicillium* and *Trichoderma* from the snow and glacial ice samples obtained from this region. They concluded that these isolates were probably transported to the region by their own Antarctic expedition or by other previous expeditions and represented a source of human contamination.

According to Abyzov et al. (2004), different methods were used to detect microorganisms in old layers of a glacier in Antarctica at depths of 1.500–2.400 m. Fungi of the genera *Cryptococcus albidus*, *Cystofilobasidium*, *Rhodotorula*, *Pseudozyma*, *Aureobasidium*, *Aspergillus*, and *Penicillium* were isolated from older sections of the Antarctic ice core, dating back 1.000–5.000 years on Lake Vostok in continental Antarctica, and some isolates were found to be heterotrophic psychrotolerant (D’Elia et al. 2009). Furthermore, the number of total and viable yeast cells from the samples of Lake Vostok was higher for the ice accretion obtained from a depth of 3.582 m (D’Elia et al. 2008, 2009). These data indicate that the ecological conditions in these regions of deep ice are sufficient to support heterotrophic metabolism and a high diversity of microorganisms, including fungi (D’Elia et al. 2009). Sanyal et al. (2018) isolated *Leucosporidium* and *Curvibasidium* from glacial cryoconite holes in three distinct regions of East Antarctica. Their study suggested that fungal communities from cryoconite hole environments can provide a rich source of microorganisms, carbon and nutrients to the surrounding glacial ecosystems and participate in the cycling processes of these ecosystems.

Ice core accretions of Vostok 5G evaluated by sequence analysis using metagenomic/metatranscriptomic techniques showed that only 6% of the obtained sequences belonged to the Eukarya domain, especially fungi of the phyla Ascomycota and Basidiomycota (Shtarkman et al. 2013). Another study on ice sections dating back 500.000–175.000 years obtained from two regions on the mainland of Antarctica employed cultivation, microscopy, metagenomics and metatranscriptomic techniques and isolated species belonging to the genera *Alternaria*, *Davidiella* and *Rhodotorula*, and predominantly *Rhodotorula* species (Knowlton et al. 2013). These results indicate that the viability of these microorganisms was “independent of the cold” or “not dependent on cold” conditions at the time of their deposition in the snow, and that low concentrations of atmospheric dust and CO₂ were also related to increased microbial viability.

Table 6.2 Fungi isolated from snow and glacial ice from different regions of Antarctica

Substrate	Location	Taxon	References
Snow	Lichen Valley, Vestvold Hills	<i>Holtermanniella nyarrowii</i>	Thomas-Hall and Watson (2002)
	Lichen Valley, South Pole	<i>Mrakia frigida</i>	Thomas-Hall et al. (2010)
	Victoria Land	<i>Vishniacozyma victoriae</i>	Antony et al. (2016)
	Coppermine Peninsula, Robert Island	<i>Antarctomyces pellizariae</i>	de Menezes et al. (2017)
Glacial ice	Ross Island, South Pole	<i>Trichoderma viride</i>	Jacobs et al. (1964)
		<i>Penicillium lanosum</i>	
		<i>Penicillium commune</i>	
		<i>Penicillium chrysogenum</i>	
		<i>Candida parapsilosis</i>	
	Lake Vostok, South Pole	<i>Cryptococcus albidus</i>	Abyzov et al. (2004)
		<i>Rhodotorula</i> spp.	
	Lake Vostok, South Pole	<i>Aureobasidium pullulans</i>	D'Elia et al. (2009)
		<i>Cystofilobasidium informominiatum</i>	
		<i>Rhodotorula mucilaginosa</i>	
		<i>Penicillium chrysogenum</i>	
		<i>Rhodotorula</i> sp.	
		<i>Pseudozyma</i> sp.	
		<i>Cryptococcus</i> sp.	
		<i>Penicillium</i> sp.	
		<i>Aspergillus</i> sp.	
	Lake Vostok 5G region, South Pole	<i>Alternaria tenuissimum</i>	Knowlton et al. (2013)
		<i>Davidiella tassiana</i>	
		<i>Rhodotorula mucilaginosa</i>	
	Byrd region, South Pole	<i>Alternaria alternata</i>	Knowlton et al. (2013)
		<i>Alternaria</i> sp.	
	Dronning Maud Land, East Antarctica	<i>Leucosporidium</i> spp.	Sanyal et al. (2018)
	Larsemann Hills, East Antarctica	<i>Leucosporidium</i> spp.	Sanyal et al. (2018)
		<i>Curvibasidium</i> sp.	

Most organisms are capable of supporting only transient periods of cold; micro-organisms that inhabit permanently cold environments have cellular processes adapted for growth at low temperatures (Cavicchioli et al. 2002). The formation of ice in the intracellular environment is lethal to living organisms. Fungi have developed various physiological strategies to protect themselves from freezing at low temperatures (Duman and Olsen 1993), which include production of antifreeze proteins

(AFPs) and/or antifreeze glycoproteins (AFGPs) (Ewart et al. 1999; Gilbert et al. 2004; Xiao et al. 2010a, b), exopolysaccharides (EPS) (Tibbett et al. 1998; Polezhaeva et al. 2014), cold-active enzymes (Feller and Gerday 1997; Robinson 2001) and unsaturated fatty acids (Singh et al. 2014).

Furthermore, some fungal species synthesise trehalose in their cells/spores and hyphae, which functions as an important storage substance, protects the cytosol against general stress and stabilises the cell membranes during dehydration (Robinson 2001). Some fungi may increase the concentration and production of glycerol and mannitol to maintain turgor pressure (Robinson 2001). Mannitol appears to play an important role in protecting against water stress and can act as a cryoprotectant (Weinstein et al. 2000).

Different strains of yeast species (*Cryptococcus* and *Rhodotorula*) isolated from Antarctic ice were able to grow and reproduce when subjected to negative temperatures. The incorporation rate of leucine was evaluated to compare macromolecular synthesis under liquid and frozen conditions from -5 to -15 °C in these yeasts. All yeast strains were able to incorporate exogenous leucine in their cellular material. These results demonstrate that these strains of *Cryptococcus* and *Rhodotorula* remain metabolically active under freezing conditions (Amato et al. 2009). Some fungi are adapted to conditions of low water availability, high salt concentrations and low temperatures and can sustain a continuous colonisation on snow and ice (Gunde-Cimerman et al. 2003). Cold-tolerant yeast possibly use a combination of various physiological mechanisms for their survival in the cryosphere (Robinson 2001).

6.6 Biotechnological Applications of Fungi Isolated from Snow and Ice

Microbial communities, including fungi in glacial habitats, appear to be active in various biogeochemical cycles and can perform metabolic functions involved in the cycles of C, N and P (Hodson et al. 2007; Telling et al. 2011; Grzesiak et al. 2015). Yeasts such as *Vishniacozyma victoriae* (synonymous to *Cryptococcus victoriae*) isolated from Antarctic snow can produce different enzymes (amylase, lipase, protease, cellulase, ligninase and β -galactosidase) at low temperatures. These studies suggest that microbial communities growing in the snow could potentially modify the snow chemistry through their metabolic activities (Antony et al. 2016). All isolates of the yeast *Mrakia frigida* obtained from snow samples from different regions of Antarctica produced high amounts of intracellular lipids and exhibited active lipid unsaturation in the fatty acyl chain; these characteristics demonstrate the potential industrial application of *M. frigida* in biofuel synthesis at low temperatures (Thomas-Hall et al. 2010).

Sanyal et al. (2018) evaluated the degradation potential of organic carbon dissolved in Antarctic cryoconite holes and found that *Leucosporidium* sp. and *Curvibasidium* sp. isolates demonstrated high enzymatic activity and extracellular

enzymatic diversity at 4 °C with the production of cellulase, ligninase, amylase, lipase, protease, and β -galactosidase. These results demonstrate that these psychrophilic fungi have significant potential in biotechnological applications because of their diverse and high enzymatic activity at low temperatures and, furthermore, offer economic benefits, such as energy savings in large-scale commercial bioprocesses, which would not require the expensive use of reactors (Feller and Gerday 2003).

6.7 Conclusions and Perspectives

Fungi are broadly distributed in the different substrates and ecosystems of Antarctica. Despite the limited studies on fungal communities in snow and ice published thus far, the detection of cold-tolerant cosmopolitan and endemic taxa suggests that both cold substrates, snow and ice, harbour interesting fungal species living at the edge of life in terms of temperature, low nutrient availability and high exposure to UV radiation. Collecting core ice samples from Antarctic glaciers usually involves expensive and difficult logistics to drill and obtain the old glacial ice fragments. However, with the melting and fragmentation of several glaciers in Antarctica, especially in the Antarctic Peninsula (Fig. 6.6), many small fragments have become available in the sea and beaches. These fragmented ice samples can



Fig. 6.6 Fragments of ice released from glaciers in the Antarctica Peninsula. (Photo Credits: LH Rosa)

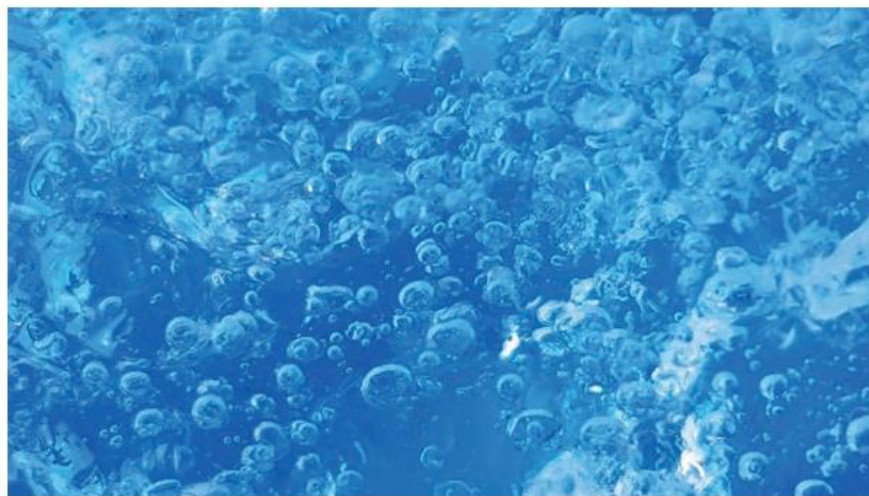


Fig. 6.7 Details of bubbles trapped in fragments of ice collected in Antarctica from glaciers containing atmospheric air of ancient Earth. (Photo Credits: LH Rosa)

be studied as a source of ancient fungi trapped for thousands of years in Antarctica, and after the ice melts, they become available to be dispersed across and out of Antarctica.

In addition, glaciologists use samples of old snow and ice to understand the physico-chemical composition of the atmosphere of the past and associate it to current global climatic changes. Some old ice samples can reveal past atmospheric composition and temperatures of the Earth from the gases trapped in the bubbles of old ice. Consequently, the fungal spores or hyphal fragments trapped in these bubbles may be very old as well (Fig. 6.7).

These fungi inhabiting Antarctic snow and ice may represent taxa that lived in the Earth's atmosphere thousands of years ago, which might provide information regarding the microbial compositions of the planet in the past. In addition, fungi adapted to the extreme conditions of Antarctic snow and ice may exhibit genetic and biochemical pathways to produce antifreeze compounds, tolerance to low nutrient availability and photoprotective activity, which can be of significant value in biotechnological applications.

References

- Abyzov SS (1993) Microorganisms in the Antarctic ice. In: Friedmann EI (ed) *Antarctic microbiology*. Wiley, New York, pp 265–295
- Abyzov SS, Mitskevich IN, Poglazova MN, Barkov NI, Lipenkov VY, Bobin NE, Koudryashov BB, Pashkevich VM (1998) Long-term conservation of viable microorganisms in the ice sheet of Central Antarctica. In: Richard BH (ed) *Instruments, methods, and missions for astrobiology* international society for optics and photonics, San Diego, pp 75–85

- Abyzov SS, Hoover RB, Imura S, Mitskevich IN, Naganuma T, Poglazova MN, Ivanov MV (2004) Use of different methods for discovery of ice-entrapped microorganisms in ancient layers of the Antarctic glacier. *Adv Space Res* 33:1222–1230
- Amann RI, Ludwig W, Schleifer KH, Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* 59:143–169
- Amato P, Doyle S, Christner BC (2009) Macromolecular synthesis by yeasts under frozen conditions. *Environ Microbiol* 11:589–596
- Anesio AM, Laybourn-Parry J (2012) Glaciers and ice sheets as a biome. *Trends Ecol Evol* 27:219–225
- Antony R, Sanyal A, Kapse N, Dhakephalkar PK, Thamban M, Nair S (2016) Microbial communities associated with Antarctic snow pack and their biogeochemical implications. *Microbiol Res* 192:192–202
- Bagshaw EA, Tranter M, Fountain AG, Welch K, Basagic HJ, Lyons WB (2013) Do cryoconite holes have the potential to be significant sources of C, N, and P to downstream depauperate ecosystems of Taylor Valley, Antarctica? *Arct Antarct Alp Res* 45:440–454
- Bargagli R (2008) Environmental contamination in Antarctic ecosystems. *Sci Total Environ* 400:212–226
- Castiella MC (2014) Caracterización dinámica del glaciar Hurd combinando observaciones de campo y simulaciones numéricas. Dissertation, Polytechnic University of Madrid
- Cavicchioli R, Siddiqui KS, Andrews D, Sowers KR (2002) Low-temperature extremophiles and their applications. *Curr Opin Biotechnol* 13:253–261
- Comiso JC, Kwok R, Martin S, Gordon AL (2011) Variability and trends in sea ice extent and ice production in the Ross Sea. *J Geophys Res Oceans* 116(C4):C04021
- Convey P, Bindenschadler R, Di Prisco G, Fahrbach E, Gutt J, Hodgson DA, Mayewski PA, Summerhayes CP, Turner J, Acce Consortium (2009) Antarctic climate change and the environment. *Antarct Sci* 21:541–563
- Cowan DA, Tow LA (2004) Endangered Antarctic environments. *Annu Rev Microbiol* 58:649–690
- Cowan DA, Ramond JB, Makhallanyane TP, De Maayer P (2015) Metagenomics of extreme environments. *Curr Opin Microbiol* 25:97–102
- D'Elia T, Veerapaneni R, Rogers SO (2008) Isolation of microbes from Lake Vostok accretion ice. *Appl Environ Microbiol* 74:4962–4965
- D'Elia T, Veerapaneni R, Theraisnathan V, Rogers SO (2009) Isolation of fungi from Lake Vostok accretion ice. *Mycologia* 101:751–763
- 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* 21:259–269
- Duman JG, Olsen TM (1993) Thermal hysteresis protein activity in bacteria, fungi, and phylogenetically diverse plants. *Cryobiology* 30:322–328
- Eicken H (2003) From the microscopic, to the macroscopic, to the regional scale: growth, microstructure and properties of sea ice. In: Thomas DN, Dieckmann GS (eds) *Sea ice—an introduction to its physics, chemistry, biology and geology*, vol 25. Blackwell, Oxford, pp 22–81
- Elster J, Delmas RJ, Petit JR, Reháková K (2007) Composition of microbial communities in aerosol, snow and ice samples from remote glaciated areas (Antarctica, Alps, Andes). *Biogeosci Discuss* 4:1779–1813
- Escobar-Zepeda A, Vera-Ponce de León A, Sanchez-Flores A (2015) The road to metagenomics: from microbiology to DNA sequencing technologies and bioinformatics. *Front Genet* 6:348
- Ewart KV, Lin Q, Hew CL (1999) Structure, function and evolution of antifreeze proteins. *Cell Mol Life Sci* 55:271–283
- Feller G, Gerday C (1997) Psychrophilic enzymes: molecular basis of cold adaptation. *Cell Mol Life Sci* 53:830–841
- Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold adaptation. *Nat Rev Microbiol* 1:200

- Fountain AG, Nylen TH, MacCLUNE KL, Dana GL (2006) Glacier mass balances (1993–2001), Taylor Valley, McMurdo Dry Valleys, Antarctica. *J Glaciol* 52:451–462
- Gilbert JA, Hill PJ, Dodd CE, Laybourn-Parry J (2004) Demonstration of antifreeze protein activity in Antarctic lake bacteria. *Microbiology* 150:171–180
- Goodison BE, Brown RD, Crane RG, Alley R, Bales R, Barber D, Barry R, Bentley C, Carrol T, Cline D, Duguay CR, Flato GM, Hall DK, Harrington R, Kargel J, Kieffer H, Munro S, Parkinson C, Raup B, Rothrock A, Sharp M (1999) Cryospheric systems. In: King M D (ed) *NASA EOS Science Plan: The State of Science in the EOS Program* pp 261–307
- Grzesiak J, Zdanowski MK, Górniak D, Świątecki A, Aleksandrak-Piekarczyk T, Sztraj K, Sasin-Kurowska J, Nieckarz M (2015) Microbial community changes along the Ecology Glacier ablation zone (King George Island, Antarctica). *Polar Biol* 38:2069–2083
- Gunde-Cimerman N, Sonjak S, Zalar P, Frisvad JC, Diderichsen B, Plemenitaš A (2003) Extremophilic fungi in arctic ice: a relationship between adaptation to low temperature and water activity. *Phys Chemis Earth Parts A/B/C* 28:1273–1278
- Hassett BT, Gradinger R (2016) Chytrids dominate arctic marine fungal communities. *Environ Microbiol* 18:2001–2009
- Hodson A, Anesio AM, Ng F, Watson R, Quirk J, Irvine-Fynn T, Dye A, Clark C, McCloy P, Kohler J, Sattler B (2007) A glacier respire: quantifying the distribution and respiration CO₂ flux of cryoconite across an entire Arctic supraglacial ecosystem. *J Geophys Res Biogeosci* 112(G4):G04S36
- Hodson A, Anesio AM, Tranter M, Fountain A, Osborn M, Prisco J, Laybourn-Parry J, Sattler B (2008) Glacial Ecosystems. *Ecol Monogr* 78:41–67
- Jacobs PH, Taylor HC, Shafer JC (1964) Studies of fungi at Amundsen-Scott IGY South Pole Base. *Arch Dermatol* 89:117–123
- Knowlton C, Veerapaneni R, D'Elia RSO (2013) Microbial analyses of ancient ice core sections from Greenland and Antarctica. *Biology* 2:206–232
- Legendre L, Ackley SF, Dieckmann GS, Gulliksen B, Horner R, Hoshiai T, Melnikov IA, Reeburgh WS, Spindler M, Sullivan CW (1992) Ecology of sea ice biota. *Polar Biol* 12:429–444
- Ma L, Catranis CM, Starmer WT, Rogers SO (1999) Revival and characterization of fungi from ancient polar ice. *Mycologist* 13:70–73
- Ma LJ, Rogers SO, Catranis CM, Starmer WT (2000) Detection and characterization of ancient fungi entrapped in glacial ice. *Mycologia* 1:286–295
- Maccario L, Sanguino L, Vogel TM, Larose C (2015) Snow and ice ecosystems: not so extreme. *Res Microbiol* 166:782–795
- Margesin R, Miteva V (2011) Diversity and ecology of psychrophilic microorganisms. *Res Microbiol* 162:346–361
- Miteva V (2008) Bacteria in snow and glacier ice. In: Margesin R (ed) *Psychrophiles: from biodiversity to biotechnology*, 2nd edn. Springer, Berlin, pp 31–50
- Miteva V, Teacher C, Sowers T, Brenchley J (2009) Comparison of the microbial diversity at different depths of the GISP2 Greenland ice core in relationship to deposition climates. *Environ Microbiol* 11:640–656
- Mock T, Thomas DN (2005) Recent advances in sea-ice microbiology. *Environ Microbiol* 7:605–619
- Pearce DA, Bridge PD, Hughes KA, Sattler B, Psenner R, Russell NJ (2009) Microorganisms in the atmosphere over Antarctica. *FEMS Microbiol Ecol* 69:143–157
- Poindexter JS (2009) Low-Nutrient Environments. In: Schaechter M (ed) *Encyclopedia of Microbiology*, 3rd Elsevier, San Diego, pp 240–257
- Polezhaeva TV, Zaitseva OO, Khudyakov AN, Laptev DS, Golovchenko VV, Gordiyenko EA, Kuleshova LG (2014) Use of pectic polysaccharides for cryopreservation of biological objects. *Arch Biol Sci* 66:1025–1033
- Powell MJ, Letcher PM, Chambers JG, Roychoudhury S. (2015) A new genus and family for the misclassified chytrid, *Rhizophlyctis harderi*. *Mycologia* 107:419–431
- Price PB (2000) A habitat for psychrophiles in deep Antarctic ice. *Proc Natl Acad Sci* 97:1247–12451

- Robinson CH (2001) Cold adaptation in Arctic and Antarctic fungi. *New Phytol* 151:341–353
- Robinson DH, Kolber Z, Sullivan CW (1997) Photophysiology and photoacclimation in surface sea ice algae from McMurdo Sound, Antarctica. *Mar Ecol Prog Ser* 147:243–256
- Rogers SO, Theraisnathan V, Ma LJ, Zhao Y, Zhang G, Shin SG, Castello JD, Starmer WT (2004) Comparisons of protocols for decontamination of environmental ice samples for biological and molecular examinations. *Appl Environ Microbiol* 70:2540–2544
- Sanyal A, Antony R, Samui G, Thamban M (2018) Microbial communities and their potential for degradation of dissolved organic carbon in cryoconite hole environments of Himalaya and Antarctica. *Microbiol Res* 208:32–42
- 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
- Singh SM, Singh PN, Singh SK, Sharma PK (2014) Pigment, fatty acid and extracellular enzyme analysis of a fungal strain *Thelebolus microsporus* from Larsemann Hills, Antarctica. *Pol Polar Rec* 50:31–36
- Telling J, Anesio AM, Tranter M, Irvine-Fynn T, Hodson A, Butler C, Wadham J (2011) Nitrogen fixation on Arctic glaciers, Svalbard. *J Geophys Res Biogeosci* 116(G3):1–8
- Thomas-Hall S, Watson K (2002) *Cryptococcus nyarrowii* sp. nov., a basidiomycetous yeast from Antarctica. *Int J Syst Evol Microbiol* 52:1033–1038
- Thomas-Hall SR, Turchetti B, Buzzini P, Branda E, Boekhout T, Theelen B, Watson K (2010) Cold-adapted yeasts from Antarctica and the Italian Alps-description of three novel species: *Mrakia robertii* sp. nov., *Mrakia blollopis* sp. nov. and *Mrakiella niccombsii* sp. nov. *Extremophiles* 14:47–59
- Tibbett M, Sanders FE, Cairney JW (1998) The effect of temperature and inorganic phosphorus supply on growth and acid phosphatase production in arctic and temperate strains of ectomycorrhizal Hebeloma spp. in axenic culture. *Mycol Res* 102(2):129–135
- Turchetti B, Buzzini P, Goretti M, Branda E, Diolaiuti G, D’Agata C, Smiraglia C, Vaughan-Martini A (2008) Psychrophilic yeasts in glacial environments of Alpine glaciers. *FEMS Microbiol Ecol* 63:73–83
- Vaughan DG, Comiso JC, Allison CJ, Kaser G, Kwok R, Mote P, Murray T, Paul F, Ren J, Rignot E (2013) Observations: cryosphere. *Clim Chang* 2103:317–382
- Wadhams P, Comiso JC (1992) The ice thickness distribution inferred using remote sensing techniques. In: Carsey FD (ed), *Geophysical Monograph Book Series*, American Geophysical Union, Washington DC, pp 375–383
- Weinstein RN, Montiel PO, Johnstone K (2000) Influence of growth temperature on lipid and soluble carbohydrate synthesis by fungi isolated from fellfield soil in the maritime Antarctic. *Mycologia* 1:222–229
- Willerslev E, Hansen AJ, Poinar HN (2004) Isolation of nucleic acids and cultures from fossil ice and permafrost. *Trends Ecol Evol* 19:141–147
- Xiao N, Inaba S, Tojo M, Degawa Y, Fujiu S, Kudoh S, Hoshino T (2010a) Antifreeze activities of various fungi and Stramenopila isolated from Antarctica. *North Am Fungi* 5:215–220
- Xiao N, Suzuki K, Nishimiya Y, Kondo H, Miura A, Tsuda S, Hoshino T (2010b) Comparison of functional properties of two fungal antifreeze proteins from *Antarctomyces psychrotrophicus* and *Typhula ishikariensis*. *FEBS J* 277:394–403
- Zwally HJ, Comiso JC, Parkinson CL, Cavalieri DJ, Gloersen P (2002) Variability of Antarctic sea ice 1979–1998. *J Geophys Res Oceans* 107:1029–1047

5 **5.2 Capítulo 2: “Fungi in the seasonal snow of Antarctica: diversity, distribution, and resistance against agricultural and clinical antifungal drugs”**

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Fungi in the seasonal snow of Antarctica: diversity, distribution, and resistance against agricultural and clinical antifungal drugs

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Running title: Fungal in snow of Antarctica

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5 Abstract

We characterized the fungal community found in the winter seasonal snow of the Antarctic Peninsula and its natural resistance to antifungal drugs used in agriculture and medicine. From the samples of snow, 234 fungal isolates were obtained and could be assigned to 51 taxa of 26 genera. Ecological indices showed that the snow fungal community is rich and diverse. Eleven yeast species displayed the highest densities; among them, *Phenoliferia glacialis* showed a broad distribution and was detected at all sites that were sampled. *Debaryomyces hansenii*, *Rhodotorula mucilaginosa*, *Penicillium chrysogenum*, *Penicillium* sp. 3, and *Penicillium* sp. 4 displayed resistance against the antifungals benomyl and fluconazole. Despite showing resistance to fluconazole, *R. mucilaginosa* isolates were able to grow at 37 °C. Our results show that the winter seasonal snow of the Antarctic Peninsula contains a diverse fungal community dominated by cosmopolitan ubiquitous fungal species previously found in tropical, temperate, and polar ecosystems. The high densities of these cosmopolitan fungi suggest that they could be present in the air that arrives to the Antarctic Peninsula by air masses from outside Antarctica. Additionally, we detected environmental fungal isolates that were resistant to agricultural and clinical antifungals and able to grow at 37 °C. Further studies will be needed to characterize the virulence potential of these fungi in humans and animals.

Keywords: Antarctica; antifungal resistance, fungi; snow

5 Introduction

Antarctica is composed of special ecosystems that allow for the study of the taxonomy and ecology of resident life forms under extreme conditions (Rosa et al. 2019). Among the eukaryote organisms living in Antarctica, fungi have received special attention due their ability to colonize and survive in different Antarctic habitats/substrates. Most studies on fungi in Antarctica have focused on fungi living in terrestrial and marine environments, such as lakes (Ellis-Evans 1985; Tsuji et al. 2013), soils (Fell et al. 2006; Gomes et al. 2018), historic woods (Arenz et al. 2006), plants (Bradner et al. 2000; Yu et al. 2018), and macroalgae (Loque et al. 2010; Furbino et al. 2018). In these Antarctic habitats, fungi are represented mostly by taxa of the phyla *Ascomycota* and its anamorphs, followed by *Basidiomycota*, *Mortierellomycota*, and *Chytridiomycota* (Bridge and Spooner 2012; Rosa et al. 2019).

Snow habitats are ecosystems characterized by permanently low temperatures, low nutrient composition, and high levels of ultraviolet radiation at high altitudes (Miteva 2008). Snow represents an important component of the cryosphere and occupies about 35% of the Earth's surface during the Northern Hemisphere winter (Margesin and Miteva 2011), covering over 99% of the 13.8 million km² of the Antarctic continent. Antarctic seasonal snow, that is the snow deposited in the last winter season, can be considered a cryptic microhabitat for microorganisms directly related to the atmosphere, the precipitation of dust, and the microbial cells, as well as other organic materials suspended in the air ((Margesin and Miteva 2011).

Industrial and agricultural activities have a great impact on these natural habitats due the dispersion of greenhouse gases, nitrogen compounds, and other synthetic chemicals, which, when released in high amounts, are dispersed in the atmosphere and transported by winds to remote areas of the planet, including the polar regions (Segawa et al. 2013). According to Kang et al. (Kang et al. 2012), falling snow contributes greatly to the deposition of airborne contaminants via the removal of atmospheric aerosols and the absorption of gas phase vapours. Previous studies have already demonstrated that several pesticides and semi-volatile

5 organic compounds circulate and accumulate in remote ecosystems across the world,
including high-elevation and high-latitude habitats (Halsall 2004; Daly and Wania 2005; Muir
et al. 2005). Kang et al. (2012) detected 22 persistent organic pollutants (POPs), identified as
organochlorine pesticides (OCPs), in snow in Antarctica; among them, α -
hexachlorocyclohexane (HCH), γ -HCH, and hexachlorobenzene (HCB) were frequently
10 found in snow at different concentration ranges. The Antarctic Peninsula is close to South
America, known for its intense agricultural and industrial activities, which employ the use of
chemicals to combat plant and animal pests and diseases. These chemicals are then
transported by winds to the Antarctic atmosphere, to be then deposited in the snow by
precipitation.

15 Although studies on the fungi found in snow in Antarctica are scarce, the presence of cold-
tolerant cosmopolitan and psychrophilic endemic fungi living at the edge of life has been
demonstrated (Thomas-Hall and Watson 2002; Thomas-Hall et al. 2010; Antony et al. 2016;
de Menezes et al. 2017; de Menezes et al. 2019), which are in contact with different
compounds transported by the air and deposited in the snow, including pollutants with
20 chemical structures similar to agricultural and clinical antifungal drugs. These pollutants may
work as selective antimicrobial agents on Antarctic fungi living in snow. In this context, the
present study aimed to characterize the fungal diversity present in the seasonal snow of the
Antarctic Peninsula islands and investigate the possible resistance of species and strains
against agricultural and clinical antifungal drugs.

25

Methods

Snow sampling, concentration, and fungal isolation

Seasonal snow samples (snow deposited during the last winter season) were collected in six
different regions of the Antarctic Peninsula and in the South Shetland Islands between

5 November and December 2015 (Suppl. Table 1; Suppl. Fig. 1). Approximately 10 kg of snow from the top, middle, and base layers (10 cm above soil) were collected in triplicate using a sterile plastic scoop and stored in sterilized 20-L plastic bags. Sterilized suits and gloves were worn during sample collection to minimize contamination. The samples were thawed in a microbiology laboratory on board the Brazilian polar ship, Admiral Maximiano, over a period of 12 h with occasional mixing. This process resulted in 10 L of water. A total of 1.5 L of melted snow was filtered through a 0.45 µm membrane with a diameter of 47 mm (Millipore, USA), in triplicate experiments. The membranes were placed on Sabouraud agar (Acumedia, India) with Minimal Medium (6.98 K₂HPO₄; 5.44 KH₂PO₄; 1.0 (NH₄)₂SO₄; 1.1 MgSO₄ 7H₂O, 0.25 peptone, 20 agar in g L⁻¹, pH 6.8 ± 0.2), both containing 200 µg mL⁻¹ of chloramphenicol (Sigma, USA), and incubated at 10 °C for 30 days. The fungi were purified in fresh Petri dishes containing Sabouraud agar and deposited in the Collection of Microorganisms and Cells of the Universidade Federal de Minas Gerais, Brazil, under the code UFMGCB in cryotubes at -80 °C and in distillate-sterilized water (Castellani 1967) at room temperature. The samples are available to 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. (Gonçalves et al. 2015). The yeasts

5 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. (Lachance et al. 1999). The fungal sequences were assembled, edited, and aligned with the program MEGA7 (Altschul et al. 1990). They were compared with type and/or annotated
10 fungal sequences deposited in the GenBank database using the Basic Local Alignment Search Tool (BLAST at <http://www.ncbi.nlm.nih.gov/>) (Kumar et al. 2016). 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
15 from the NCBI GenBank database using BLAST (Altschul et al. 1997). The information about fungal classification generally followed the databases of dictionary Kirk et al. (2008), and websites MycoBank (<http://www.mycobank.org>) and the Index Fungorum (<http://www.indexfungorum.org>).

20 *Morphological characterization*

After sequence analysis, *Penicillium* species with unknown taxonomic positions were characterized according to their macroscopic properties (colony colour, texture, reverse colour, border type, sporulation, and the production of yellow soluble pigments). The diameters of the colonies were observed on YES and CYA medium at 25 and 30 °C,
25 respectively for 14 days (Houbraken *et al.*, 2012). The resulting colours were graded according to the specifications proposed by Kornerup and Wanscher (1984).

5 *Diversity, richness, dominance, and distribution*

To quantify species diversity, richness and evenness, we used the following indices: (i) Fisher's α , (ii) Margalef's, and (iii) Simpson's, respectively. The similarities among fungal taxa from different regions were estimated using the Sorensen coefficient (QS) and Bray-Curtis (B) measures. All of the results were obtained with 95% confidence, and bootstrap values were calculated from 1.000 iterations. All diversity and similarity indices calculations were performed using the computer programme PAST, version 1.90 (Hammer et al. 2001). Venn diagram was prepared according to Bardou et al. (2014) to illustrate the comparison among fungal assemblages of the seasonal snow Antarctic.

15 *Fungal growth responses to temperature*

Fungi known to be opportunistic in humans were grown on Sabouraud agar at 10, 15, 20, 25, 30, 35, and 37 °C on Petri dishes to determine their growth profile at different temperatures. The filamentous fungi were inoculated by transferring blocks (4 mm²) from 10-day-old pre-cultures grown at 10 °C. Microtiter plates were incubated in triplicate for 7 days. Yeast was cultured on YM for 72 h at 10 °C. Then, 10 µL of cells with a turbidity of 0.5 on the MacFarland scale ($1-5 \times 10^6$ cells mL⁻¹ at 70% transmittance) were inoculated at the centre of the Petri dishes containing Sabouraud media. All plates were incubated in triplicate for 14 days to detect the presence or absence of growth.

25 *Minimum inhibitory concentration (MIC) of antifungal drugs benomyl, fluconazole, and amphotericin B*

Fungi known to be opportunistic in humans were subjected to MIC determination for the antifungal drugs benomyl and fluconazole (Sigma, USA). Isolates with a MIC ≥ 64 µg/mL for fluconazole that also exhibited growth at 37 °C were subjected to MIC determination for the

antifungal drug amphotericin B (Sigma, USA). The MIC protocol was performed using a modified version of the method used for yeast (NCCLS M27-A3, vol. 28, n°14, 2008a) and filamentous fungi (CLSI M38-A2, vol. 28, n°16, 2008b) in Roswell Park Memorial Institute (RPMI-1640) medium (INLAB, Diadema, SP, Brazil), using 96-well microtiter plates. The yeast cells and fungal spores were treated with benomyl concentrations ranging from 0.078125 to 40 $\mu\text{g mL}^{-1}$ (Truong et al., 2018), fluconazole from 0.062 to 64 $\mu\text{g mL}^{-1}$, and amphotericin B from 0.007 to 8 $\mu\text{g mL}^{-1}$. Microtiter plates containing spores and yeast cells were incubated at 25 °C for 48 h for benomyl and fluconazole and at 37 °C for 48 h for amphotericin B. All MIC assays were performed in duplicate.

Results

Snow fungal community

From the different snow samples, 234 fungal isolates were obtained and identified as 51 taxa of 26 genera (Table 1). Among the yeast species identified, *Bannozyma yamatoana*, *Cystobasidium pallidum*, *Glaciozyma antarctica*, *Leucosporidium fragarium*, *Leucosporidium golubevii*, *Phenoliferia glacialis*, *Phenoliferia psychrophenolica*, *Phaeococcomyces* sp., *Rhodotorula mucilaginosa*, *Hamamotoa singularis*, and *Vishniacozyma victoriae* displayed the highest densities (≤ 300 UFC L⁻¹). In contrast, filamentous fungi taxa showed the lowest densities values. Twenty-nine fungi (56.8%) occurred in low densities (≤ 50 UFC L⁻¹), representing singletons and considered rare taxa within the seasonal snow fungal community. Twenty-two taxa showed low molecular similarities or inconclusive taxonomic definitions when compared with the sequences of known fungi deposited in the GenBank database. Among them, *Bionectriaceae* sp., *Cystobasidium* sp., *Genolevuria* sp., *Helotiales* sp., *Galactomyces* sp., and *Hyaloscyphaceae* sp. showed the lowest query coverage and identity percentages and may represent new species.

5 *Diversity and distribution*

The seasonal snow fungal community showed high values of diversity (Fisher α), richness (Margalef's), and dominance (Simpson's) (Table 2). The highest ecological indices were found in the snow of King George, followed by Snow, Trinity, Deception, Robert islands, and the Arctowski Peninsula, respectively. In addition, the similarities between the fungal
10 assemblages of the different snow segments of each site showed that, except for those of King George Island, segments 2 and 3 were more similar than segment 1 (Suppl. Figure 2). Eleven yeast species displayed the highest densities; among them, *Phenoliferia glacialis* showed a broad distribution and was detected at all sites that were sampled (Suppl. Figure 3). We did not detect any correlation between the fungal diversity indices and the physicochemical
15 properties of seasonal Antarctic snow.

Opportunistic virulence potential

Five isolates of *R. mucilaginosa*, one of *P. chrysogenum*, *Penicillium* sp. 3, and *Penicillium* sp. 4 showed capacity for resistance to the clinical antifungal drug fluconazole at 25 °C. Two
20 isolates of *D. hansenii* were resistant to benomyl, but susceptible to fluconazole (Table 3). In addition, isolates of *R. mucilagina* displayed MIC values of 0.03 and 0.125 $\mu\text{g mL}$ against amphotericin B at 37 °C (Suppl. Table 2). However, we did not detect a clear correlation between the antifungal benomyl, used in agriculture, with cross-resistance of fungi against fluconazole and amphotericin B, used in clinical therapy.

25

Discussion

Taxonomy, diversity, and distribution

In the winter seasonal snow of the Antarctic Peninsula, we detected a rich fungal community. Within this community, the yeasts *B. yamatoana*, *C. pallidum*, *G. antarctica*, *L. fragarium*, *L.*

5 *golubevii*, *P. glacialis*, *P. psychrophenolica*, *R. mucilaginosa*, *H. singularis*, and *V. victoriae*
which are known to occur in tropical, temperate, and polar environments, were found at high
densities.

Rhodotorula includes pigmented yeast species that are found worldwide and can abundantly
grow in extreme environments (Sampaio 2004; Margesin et al. 2007). *Rhodotorula*
10 *mucilaginosa* is ubiquitous and is present in different habitats and substrates, including cold
and extreme environments (Sampaio 2011). In Antarctica, *R. mucilaginosa* are commonly
isolated from terrestrial and marine substrates (Vaz et al. 2011; Godinho et al. 2013). Despite
the extensive snow cover of the Antarctic continent, our study represents the first report on
the high density of *R. muscilagionsa* in snow samples of different sites in Antarctica.

15 Of all 51 taxa found in the Antarctic snow only *Phenoliferia glacialis* (synonym of
Rhodotorula glacialis) was isolated from all sites sampled in this work and with high
densities. *Phenoliferia glacialis* is a psychrophilic yeast that exhibits high metabolic
versatility, and together with *P. psychrophenolica* (*Rhodotorula psychrophenolica*) were
originally obtained from the Etendard Glacier in France and the Stubaier Glacier and
20 cryoconite in Austria (Margesin et al. 2007). Recently, Ferreira et al. (2019) isolated *P.*
glacialis and *P. psychrophenolica* from leaves of *Deschampsia antarctica*, mosses, and
different biofilms present in the Antarctic Peninsula. *Cystobasidium pallidum* (*Rhodotorula*
pallida) was obtained from different cold environments, such as the rhizosphere in Russia
(Bab'eva and Belianin, 1966) and seawater of the Indian Ocean (Fell 1967). In Antarctica, *C.*
25 *pallidum* was detected in snow, rocks, and the Ross Dependency in the Antarctic Peninsula
(Di Menna 1966; Rovati et al. 2013).

Vishniacozyma victoriae (synonym of *Cryptococcus victoriae*) was first described in
Antarctica (Vishniac 2006), but was subsequently detected in penguin guano, soil, sediment,
freshwater samples, the rhizosphere of *D. antarctica* (Vaz et al. 2011), macroalgae (Furbino et

5 al. 2018), lichens (Santiago et al. 2015), and as endophytes of *D. antarctica* and *C. quitensis* (Santiago et al. 2017). In addition, *V. victoriae* was detected in environments outside of Antarctica, such as soil and rhizosphere soil in Korea (Hong et al. 2002), seawater in Portugal (Gadanhó et al. 2003), the gut of the insect *Chrysoperla rufilabris* in the USA (Woolfolk 2004), an industrial malting area and indoor air in Finland (Laitila et al. 2006; Pitkäranta et al. 10 2008), in glacial ice from the Arctic (Butinar et al. 2007), in a dry meat processing factory in Norway (Asefa et al. 2009), and in the Italian Alps (Turchetti et al. 2008; Branda et al. 2010). Garcia et al. (Garcia et al. 2012) proposed that *V. victoriae* represents a generalist species with the ability to tolerate different stressful environments. Antony et al. (Antony et al. 2016) previously reported *V. victoriae* in the snow of Victoria Land, Antarctica.

15 The *Glaciozyma antarctica* species represents a re-classification of *Leucosporidium antarcticum*, originally described by Turchetti et al. (2011). In Antarctica, *G. antarctica* was previously isolated from Antarctic marine waters (Fell et al. 1969), soils around to Lake Fletcher, Lichen and Taylor Valleys, and on dead sponge (Turchetti et al. 2011), and in the seawater of the Northern Antarctic Peninsula (Gonçalves et al. 2017a).

20 *Leucosporidium fragarium* was obtained from the melt water river of the Frias Glacier of Mount Tronador (de Garcia et al. 2007; de Garcia et al. 2015). *Leucosporidium golubevii* was isolated from river water in the north of Portugal (Sampaio et al. 2003) and as endophytic fungus present in leaves of *C. quitensis* (Santiago et al. 2017). *Bannozyma yamatoana* (*Bensingtonia yamatoana*) was reported in Antarctica, present in soil and ornithogenic soil 25 (Vaz et al. 2011), and associated with thalli of lichen *Usnea antarctica* (Santiago et al. 2015). Generally, in extreme environments fungal communities are dominated by *Ascomycota* filamentous fungi (Connell et al. 2008). However, our results demonstrate that the basidiomycetous yeast assemblage was the dominant form in the winter seasonal snow of Antarctica. Similar results were obtained by de García et al. (de Garcia et al. 2007) from

5 yeasts isolated from glacial melt water rivers originating from glaciers in the Argentinean Patagonia. Butinar et al. (2007) detected viable yeasts in the ice layers of Arctic glaciers in the Svalbard Islands (Norway) and Turchetti et al. (2008) from yeasts obtained from Alpine glaciers; however, in both studies, the yeasts occurred at low densities.

10 *Resistance against antifungal drugs*

We screened fungi previously reported as potential pathogenic taxa against the antifungal compounds benomyl (used as a pesticide in agriculture), as well as fluconazole and amphotericin B (used in medicine), which have structures composed of aromatic rings, and similar to some POPs and OCPs previously found in snow (Kang et al. 2012). *Debaromyces*
15 *hansenii*, *R. mucilaginosa*, *P. chrysogenum*, and *Penicillium* sp. 3 and sp. 4 showed resistance against the antifungal drugs tested.

Debaryomyces hansenii is considered a cosmopolitan yeast species that is able to occupy and colonize different ecological niches and ecosystems. In Antarctica, *D. hansenii* was previously recovered from the rhizosphere of *D. antarctica* and soil (Vaz et al. 2011), soil and
20 thalli of lichens (Duarte et al. 2013), macroalgae (Duarte et al. 2016), freshwater, and marine water (Brandão et al. 2017). However, *D. hansenii* (also reported as *Candida famata*, its anamorph) has been previously found to cause opportunistic human infections (St-Germain and Laverdiere 1986; Wagner et al. 2005; Gupta et al. 2006). Moreover, there are reports of higher MICs values of fluconazole for *D. hansenii* than for pathogenic yeasts species (Breuer
25 and Harms 2006). Although several isolates found in the snow show susceptibility to fluconazole and are unable to grow at 37 °C, *D. hansenii* is able to synthesize or tolerate several toxins and has been reported to show resistance against several environmental pesticides, among them benomyl (Breuer and Harms 2006). Similarly, we found isolates of *D. hansenii* that were resistant to benomyl.

5 *Rhodotorula mucilaginosa* has recently emerged as an opportunistic fungal pathogen. *Rhodotorula mucilaginosa* is able to cause outbreaks of skin infections in chickens and lung infections in sheep (Aruo 1980; Monga and Garg 1980), as well as worsen the condition of immunosuppressed patients (Wirth and Goldani 2012). Here, *Rhodotorula mucilaginosa* present in snow in Antarctica were found to be resistant to fluconazole and susceptible to

10 amphotericin B. Similar results were reported by Spiliopoulou et al. (2012), who found that opportunistic *Rhodotorula* species was resistant to fluconazole, but susceptible to echinocandins and amphotericin B. Generally, opportunistic *R. mucilaginosa* has been reported to be able grow at 30 °C, but not at 37 °C. However, *R. mucilaginosa* isolates found in the snow in Antarctica were resistant to fluconazole and able to grow at 37 °C.

15 *Penicillium chrysogenum* was previously reported as an opportunistic fungus in immunocompromised patients, resulting in otomycoses, endophthalmitis, keratitis, endocarditis, cutaneous infections, and systemic infection (de Hoog et al. 2000), as well as able to cause bloodstream infection in immunocompromised patients (Swoboda et al. 2000) and cerebral disease in healthy individuals (Lyrtatzopoulos et al. 2000). *Penicillium* taxa

20 recovered from ornithogenic soil samples and rocks of from continental and Antarctic Peninsula (de Souza et al. 2017; Gonçalves et al. 2017b; Alves et al. 2019) displayed different pathogenic potential to human *in vitro*.

Kang et al. (2012) detected organochlorine pesticides (OCPs) at different concentrations in the snow of East Antarctica and suggested that the air masses that arrive at the sampling sites

25 mainly originate from the Indian and Atlantic Oceans, which travel over the Antarctic continent, suggesting that the OCPs were subjected to long-range atmospheric transport and were deposited in surface snow. In recent years, several studies have focused on fungal cross-resistance between agricultural pesticides and clinical antifungal drugs (Snelders et al. 2012; Bastos et al. 2017; Carneiro et al. 2019). Opportunistic fungi are also ubiquitous in the

5 environment and can come into contact with pesticides and antifungal agents used in crops
(Hof 2001). Since OCPs have chemical structures that are similar to those of antifungal
azoles, their presence in the snow in Antarctica may explain the detection of *D. hansenii*, *R.*
mucilacinosa, and *P. chrysogenum* resistant to agricultural and clinical antifungal drugs.

10 **Conclusion**

Our results show that the winter seasonal snow in the Antarctic Peninsula contains a diverse
fungal community dominated by cosmopolitan ubiquitous basidiomycetous yeasts species
previously found in tropical, temperate, and polar ecosystems. These species were found to
display specific adaptations for their successful survival and colonization in the Antarctic
15 snow microhabitat. The high densities of these cosmopolitan fungi suggest that they may be
present in the air masses arriving at the Antarctic Peninsula from outside Antarctica, which
are then pushed down by snow precipitation, and compacted at high concentrations in the
snow each year. Additionally, we detected environmental fungal isolates resistant to
agricultural and clinical antifungals, some of which were able to grow at 37 °C. Further
20 studies will be needed to characterize the innate virulence potential of these fungi against
humans and animals.

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5 References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403-410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389-3402. <https://doi.org/10.1093/nar/25.17.3389>
- Alves IM, Gonçalves VN, Oliveira FS, Schaefer CE, Rosa CA, Rosa LH (2019). The diversity, distribution, and pathogenic potential of cultivable fungi present in rocks from the South Shetlands archipelago, Maritime Antarctica. *Extremophiles* 23:1-10. <https://doi.org/10.1007/s00792-019-01086-8>
- Antony R, Sanyal A, Kapse N, Dhakephalkar PK, Thamban M, Nair S (2016) Microbial communities associated with Antarctic snow pack and their biogeochemical implications. *Microbiol Res* 192:192-202. <https://doi.org/10.1016/j.micres.2016.07.004>
- 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. <https://doi.org/10.1016/j.soilbio.2006.01.016>
- Aruo SK (1980) Necrotizing cutaneous rhodotorulosis in chickens in Uganda. *Avian Dis* 24: 1038-1043.
- Asefa DT, Gjerde RO, Sidhu MS, Langsrud S, Kure CF, Nesbakken T, Skaar I (2009) Moulds contaminants on Norwegian dry-cured meat products. *Int J Food Microbiol* 128:435-9. <https://doi.org/10.1016/j.ijfoodmicro.2008.09.024>
- Bab'eva IP, Belianin AI (1966) Yeast in the rhizosphere. *Mikrobiologiya* 35:7 12-720.

- 5 Bardou P, Mariette J, Escudié F, Djemiel C, Klopp C (2014). Jvenn: an interactive Venn diagram viewer. BMC Bioinformatics 15:293. <https://doi.org/10.1186/1471-2105-15-293>
- Bastos RW, Carneiro HCS, Oliveira LVN, Rocha KM, Freitas GJC, Costa MC et al (2018) Environmental triazole induces cross-resistance to clinical drugs and affects morphophysiology and virulence of *Cryptococcus gattii* and *C. neoformans*. Antimicrob Agents Chemother 62:e01179-17. <https://doi.org/10.1128/AAC.01179-17>
- 10 Bradner JR, Sidhu RK, Yee B, Skotnicki ML, Selkirk PM, Nevalainen KMH (2000) A new microfungus isolate, *Embellisia* sp., associated with the Antarctic moss *Bryum argenteum*. Polar Biol 23:730-732. <https://doi.org/10.1007/s0030000000161>
- Branda E, Turchetti B, Diolaiuti G, Pecci M, Smiraglia C, Buzzini P (2010) Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone Glacier, Apennines, Italy). FEMS Microbiol Ecol 72:354-369. <https://doi.org/10.1111/j.1574-6941.2010.00864.x>
- 15 Brandão LR, Aline BM, Vaz ABM, Lilia C, Santo LC, Pimenta RS, Morais PB, Libkind D, Rosa LH, RosanCA (2017) Diversity and biogeographical patterns of yeast communities in Antarctic, Patagonian and tropical lakes. Fungal Ecol 28:33-43. <https://dx.doi.org/10.1016/j.funeco.2017.04.003>
- 20 Breuer U, Harms H (2006) *Debaryomyces hansenii*-an extremophilic yeast with biotechnological potential. Yeast 23:415-437. <https://doi.org/10.1002/yea.1374>
- Bridge PD, Spooner BM (2012) Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem? Fungal Ecol 5:381-394. <https://doi.org/10.1016/j.funeco.2012.01.007>

- 5 Butinar L, Spencer-Martins I, Gunde-Cimerman N (2007) Yeasts in high Arctic glaciers: the discovery of a new habitat for eukaryotic microorganisms. *Antonie Van Leeuwenhoek* 91:277-289. <https://doi.org/10.1007/s10482-006-9117-3>
- Carneiro H.CS, Ribeiro NQ, Bastos RW, and Santos DA (2019). Effect of non-antifungal agrochemicals on the pathogenic fungus *Cryptococcus gattii*. *Med Mycol* 1-7.
10 <https://doi.org/10.1093/mmy/myz018>
- Castellani A (1967) Maintenance and cultivation of common pathogenic fungi in distilled water. *J Trop Med Hygien* 42:181-184
- CLSI. (2008a) Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard CLSI document M38-A2. Clinical and Laboratory
15 Standards Institute, Wayne, P.A, USA
- CLSI. (2008b) Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-third edition; CLSI document M27-A3. Clinical and Laboratory Standards Institute, Wayne, P.A, USA
- Connell LB, Redman R, Craig SD, Scorzetti G, Iszard M, Rodriguez R (2008) Diversity of
20 soil yeasts isolated from South Victoria Land, Antarctica. *Microb Ecol* 56:448-459. <https://doi.org/10.1007/s00248-008-9363-1>
- Daly GL, Wania F (2005) Organic contaminants in mountains. *Environ. Sci. Technol* 39:385-398. <https://doi.org/10.1021/es048859u>
- de Garcia V, Brizzio S, Libkind D, Buzzini P, Van Broock M (2007) Biodiversity of cold-
25 adapted yeasts from glacial meltwater rivers in Patagonia, Argentina. *FEMS Microbiol Ecol* 59:331-341. <https://doi.org/10.1111/j.1574-6941.2006.00239.x>

- 5 de Garcia V, Coelho M, Maia T et al (2015) Sex in the cold: taxonomic reorganization of psychrotolerant yeasts in the order *Leucosporidiales*. FEMS Yeasts Res. 15:pii: fov019. <https://doi.org/10.1093/femsyr/fov019>
- de Hoog GS, Queiroz-Telles F, Haase G et al. (2000) Black fungi: clinical and pathogenic approaches. Med Mycol 38:243-250
- 10 de Menezes, GCA, Porto JC, Rosa CA, Rosa LH (2019) Fungi in snow and glacial ice of Antarctica. In: Rosa LH (eds) Fungi of Antarctica, 1st edn. Springer, Switzerland AG (in press).
- de Menezes, GCA, Godinho VM, Porto BA, Gonçalves VN, Rosa LH. (2017) *Antarctomyces pellizariae* sp. nov., a new, endemic, blue, snow resident psychrophilic ascomycete fungus
- 15 from Antarctica. Extremophiles 21:259-269. <https://doi.org/10.1007/s00792-016-0895-x>
- de Sousa JRP, Gonçalves VN, de Holanda RA et al (2017) Pathogenic potential of environmental resident fungi from ornithogenic soils of Antarctica. Fungal Biol 12:991-1000. <https://doi.org/10.1016/j.funbio.2017.09.005>
- Di Menna ME (1966) Yeasts in Antarctic soils. Antonie Van Leeuwenhoek 32:29-38.
- 20 <https://doi.org/10.1007/BF02097443>
- Duarte AWF, Dayo-Owoyemi I, Nobre FS, Pagnoca FC, Chaud LCS, Pessoa A, Felipe MGA, Sette LD (2013) Taxonomic assessment and enzymes production by yeasts isolated from marine and terrestrial Antarctic samples. Extremophiles 17:1023-1035. <https://doi.org/10.1007/s00792-013-0584-y>
- 25 Duarte AWF, Passarini MRZ, Delforno TP, Pelizzari FM, Cipro CVZ, Montene RC, Petry MV, Putzke J, Rosa LH, Sette LD. (2016) Yeasts from macroalgae and lichens that inhabit

- 5 the South Shetland Islands, Antarctica. *Env Microbiol* 8:874-885.
<https://doi.org/10.1111/1758-2229.12452>
- Ellis-Evans JC (1985) Fungi from maritime Antarctic freshwater environments. *Br Antarct Surv Bull* 68:37-45
- Fell JW, Scorzetti G, Connell L, Craig S (2006) Biodiversity of micro-eukaryotes in Antarctic
 10 Dry Valley soils with <5% soil moisture. *Soil Biol. Biochem.* **38**:3107-3119.
<https://doi.org/10.1016/j.soilbio.2006.01.014>
- Fell JW (1967) Distribution of yeasts in the Indian Ocean. *Bull Mar Sci* 17:454-470.
- Fell JW, Statzell AC, Hunter IL, Phaff L (1969) *Leucosporidium* gen. n., the
 heterobasidiomycetous stage of several yeasts of the genus *Candida*. *Antonie Van*
 15 *Leeuwenhoek* 35:433-462. <https://doi.org/10.1007/BF02219163>
- Ferreira EMS, de Sousa FMP, Rosa LH, Pimenta RS (2019) Taxonomy and richness of yeasts
 associated with angiosperms, bryophytes, and meltwater biofilms collected in the Antarctic
 Peninsula. *Extremophiles* 23:151-159. <https://doi.org/10.1007/s00792-018-1069-9>
- Furbino LE, Pellizzari FM, Neto PC, Rosa CA, Rosa LH (2018) Isolation of fungi associated
 20 with macroalgae from maritime Antarctica and their production of agarolytic and
 carrageenolytic activities. *Polar Biol* 41:527-535. <https://doi.org/10.1007/s00300-017-2213-1>
- Gadanhho M, Almeida JMF, Sampaio JP (2003) Assessment of yeast diversity in a marine
 environment in the south of Portugal by microsatellite-primed PCR. *Antonie Van*
Leeuwenhoek 84:217-227. <https://doi.org/10.1023/A:1026038213195>
- 25 Garcia V, Zalar P, Brizzio S, Gunde-Cimerman N, Van Broock M (2012) *Cryptococcus*
 species (Tremellales) from glacial biomes in the southern (Patagonia) and northern (Svalbard)

- 5 hemispheres. FEMS Microbiol Ecol 82:523-539. <https://doi.org/10.1111/j.1574-6941.2012.01465.x>
- 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 61:1323-1330
- 10 Godinho VM, Furbino LE, Santiago IF et al (2013) Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. ISME J 7:1434-1451. <https://doi.org/10.1038/ismej.2013.77>
- Gomes ECQ, Godinho VM, Silva DAS et al (2018) Cultivable fungi present in Antarctic soils: taxonomy, phylogeny, diversity, and bioprospecting of antiparasitic and herbicidal
15 metabolites. Extremophiles 22:381-393. <https://doi.org/10.1007/s00792-018-1003-1>
- Gonçalves VN, Cantrell CL, Wedge DE et al. (2015) Fungi associated with rocks of the Atacama Desert: taxonomy, distribution, diversity, ecology and bioprospection for bioactive compounds. Environ Microbiol 18:232-245. <https://doi.org/10.1111/1462-2920.13005>
- Gonçalves VN, Vitoreli GA, de Menezes GC, Mendes CR, Secchi ER, Rosa CA, Rosa LH
20 (2017) Taxonomy, phylogeny and ecology of cultivable fungi present in seawater gradients across the Northern Antarctica Peninsula. Extremophiles 21:1005-1015. <https://doi.org/10.1007/s00792-017-0959-6>
- Gonçalves VN, Oliveira FS, Carvalho CR, Schaefer CEGR, Rosa CA, Rosa LH (2017 b) Antarctic rocks from continental Antarctica as source of potential human opportunistic fungi.
25 Extremophiles 21:851-860. <https://doi.org/10.1007/s00792-017-0947-x>

- 5 Gupta A, Mi H, Wroe C, Jaques B, Talbot D. (2006) Fatal *Candida famata* peritonitis complicating sclerosing peritonitis in a peritoneal dialysis patient. *Nephrol Dial Transplant* 21:2036-2037
- Halsall CJ (2004) Investigating the occurrence of persistent organic pollutants (POPs) in the Arctic: Their atmospheric behaviour and interaction with the seasonal snow pack. *Environ Pollut* 128:163-175. <https://doi.org/10.1016/j.envpol.2003.08.026>
- 10 Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Paleontol Electron* 4:1-9
- Hof H (2001) Critical annotations to the use of azole antifungals for plant protection. *Antimicrob Agents Chemother* 45:2987-2990. [https://doi.org/10.1128/AAC.45.11.2987-](https://doi.org/10.1128/AAC.45.11.2987-2990.2001)
- 15 2990.2001
- Hong SG, Lee KH, Bae KS (2002) Diversity of yeasts associated with natural environments in Korea. *J Microbiol* 40:55-62
- Houbraken J, Frisvad JC, Seifert KA, Overy DP, Tuthill DM, Valdez JG, Samson RA (2012) New penicillin-producing *Penicillium* species and an overview of section *Chrysogena*. *Persoonia* 29:78-100. <https://doi.org/10.3767/003158512X660571>
- 20 Persoonia 29:78-100. <https://doi.org/10.3767/003158512X660571>
- Kang JH, Son MH, Hur SD, Hong S, Motoyama H, Fukui K, Chang YS (2012) Deposition of organochlorine pesticides into the surface snow of East Antarctica. *Sci Total Environ* 433:290-295. <https://doi.org/10.1016/j.scitotenv.2012.06.037>
- Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) *Dictionary of the Fungi*, CAB International, Wallingford
- 25 International, Wallingford
- Kornerup A, Wanscher JH (1984) *Methuen Handbook of Colour*. Eyre Methuen, London

- 5 Kumar S, Stecher G, Tamura K (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and Evolution* 33:1870-1874. <https://doi.org/10.1093/molbev/msw054>
- Kurtzman CP, Fell JW, Boekhout T (2011) *The Yeasts: A taxonomic study*. Elsevier, Amsterdam
- 10 Lachance MA, Bowles JM, Starmer WT, Barker JSF (1999) *Kodamaea kakaduensis* and *Candida tolerans*, two new yeast species from Australian Hibiscus flowers. *Can J Microbiol* 45:172-177. <https://doi.org/10.1139/w98-225>
- Laitila A, Wilhelmson A, Kotaviita E, Olkku J, Home S, Juvonen R (2006) Yeasts in an industrial malting ecosystem. *J Microbiol Biotechnol* 33:953-966.
- 15 <https://doi.org/10.1007/s10295-006-0150-z>
- 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. <https://doi.org/10.1007/s00300-009-0740-0>
- Lyratzopoulos G, Ellis M, Nerringer R, Denning DW (2002) Invasive Infection due to *Penicillium* species other than *P. marneffe*. *J Infection* 45:184-207. <https://doi.org/10.1053/jinf.2002.1056>
- 20 *Penicillium* species other than *P. marneffe*. *J Infection* 45:184-207. <https://doi.org/10.1053/jinf.2002.1056>
- Margesin R, Fonteyne PA, Schinner F, Sampaio JP (2007) *Rhodotorula psychrophila* sp. nov., *Rhodotorula psychrophenolica* sp. nov. and *Rhodotorula glacialis* sp. nov., novel psychrophilic basidiomycetous yeast species isolated from alpine environments. *Int J Syst Evol Microbiol* 57:2179-2184. <https://doi.org/10.1099/ijs.0.65111-0>
- 25 *Evol Microbiol* 57:2179-2184. <https://doi.org/10.1099/ijs.0.65111-0>

- 5 Margesin R, Miteva V. (2011) Diversity and ecology of psychrophilic microorganisms. *Res Microbiol* 162:346-361. <https://doi.org/10.1016/j.resmic.2010.12.004>

Miteva V. (2008) Bacteria in snow and glacier ice. In: Margesin R, Schinner F, Marx J-C, Gerday (eds). *Psychrophiles: From Biodiversity to Biotechnology*, 1st edn. Springer, Berlin, pp 31-50. https://doi.org/10.1007/978-3-540-74335-4_3
- 10 Monga DP, Garg DN (1980) Ovine pulmonary infection caused by *Rhodotorula rubra*. *Mykosen* 23:208-211. <https://doi.org/10.1111/j.1439-0507.1980.tb01787.x>

Muir DCG, Shearer RG, Van Oostdam J, Donaldson SG, Furgal C. (2005) Contaminants in Canadian Arctic biota and implications for human health: Conclusions and knowledge gaps. *Sci Total Environ* 351:539-546. <https://doi.org/10.1016/j.scitotenv.2005.08.030>
- 15 Pitkäranta M, Meklin T, Hyvärinen M, Paulin L, Auvinen P, Nevalainen A, Rintala H (2008) Analysis of fungal flora in indoor dust by ribosomal DNA sequences analysis, quantitative PCR, and culture. *Appl Environ Microbiol* 74:233-244. <https://doi.org/10.1128/AEM.00692-07>

Rosa LH, Vaz ABM, Caligorne RB, Campolina S, Rosa CA (2009) Endophytic fungi associated with the Antarctic Grass *Deschampsia antarctica* Desv. (*Poaceae*). *Polar Biol* 32:161-167. <https://doi.org/10.1007/s00300-008-0515-z>
- 20 Rosa LH, Zani CL, Cantrell CL, Duke SO, Dijck PV, Desideri A, Rosa CA (2019) Fungi in Antarctica: diversity, ecology, effects of climate change, and bioprospection for bioactive compounds. In: Rosa LH (eds) *Fungi of Antarctica*, 1st edn. Springer, Switzerland AG (in press). <https://doi.org/10.1007/978-3-030-18367-7>
- 25

- 5 Rovati JI, Pajot HF, Ruberto L, Mac Cormack W, Figueroa LI (2013) Polyphenolic substrates and dyes degradation by yeasts from 25 de Mayo/King George Island (Antarctica). Yeast 30:459-70. <https://doi.org/10.1002/yea.2982>
- 10 Sampaio JP, Gadanho M, Bauer R, Weiß M (2003) Taxonomic studies in the Microbotryomycetidae: *Leucosporidium golubevii* sp. nov., *Leucosporidiella* gen. nov. and the new orders Leucosporidiales and Sporidiobolales. *Mycol Prog* 2:53-68. <https://doi.org/10.1007/s11557-006-0044-5>
- 15 Sampaio JP (2011) *Rhodotorula* Harrison (1928). In: Kurtzman CP, Fell JW, Boekhout T (eds). *The Yeasts: A Taxonomic Study*, 5 th edn. Elsevier, The Netherlands, pp 1873-1927
- 20 Sampaio JP (2004) Diversity, phylogeny and classification of basidiomycetous yeasts. In: Agerer R, Blanz P, Piepenbring M (eds). *Frontiers in Basidiomycete Mycology* IHW-Verlag, Germany, pp 49-80
- 25 Santiago IF, Rosa CA, Rosa LH (2017) Endophytic symbiont yeasts associated with the Antarctic angiosperms *Deschampsia antarctica* and *Colobanthus quitensis*. *Polar Biol* 40:177-183. <https://doi.org/10.1007/s00300-016-1940-z>
- 30 Santiago IF, Rosa CA, Rosa LH (2015) Lichensphere: a protected natural microhabitat of the non-lichenised fungal communities living in extreme environments of Antarctica. *Extremophiles* 19:1087-1097. <https://doi.org/10.1007/s00792-015-0781-y>
- 35 Segawa T, Takeuchi N, Rivera A et al (2013) Distribution of antibiotic resistance genes in glacier environments. *Environ Microbiol Rep* 5:127-134. <https://doi.org/10.1111/1758-2229.12011>

- 5 Snelders E, Camps SMT, Karawajczyk A et al (2012) Triazole fungicides can induce cross-resistance to medical triazoles in *Aspergillus fumigatus*. PLoS ONE 7:e31801. <https://doi.org/10.1371/journal.pone.0031801>
- Spiliopoulou A, Anastassiou ED, Christofidou M. (2012) *Rhodotorula* fungemia in an intensive care unit patient and review of published cases. *Mycopathologia* 174:301-309.
- 10 <https://doi.org/10.1007/s11046-012-9552-9>
- St-Germain G, Laverdiere M (1986) *Torulopsis candida*, a new opportunistic pathogen. J. Clin. Microbiol 24:884-885.
- Swoboda-Kopec E, Wroblewska MM, Rokoz A, Luczak M (2000) Mixed bloodstream infection with *Staphylococcus aureus* and *Penicillium chrysogenum* in an
- 15 immunocompromised patient: case report and review of the literature. Clin Microbiol Infect 9:1116-1117. <https://doi.org/10.1046/j.1469-0691.2003.00718.x>
- Thomas-Hall SR, Watson K (2002) *Cryptococcus nyarrowii* sp. nov., a basidiomycetous yeast from Antarctica. Int J Syst Evol Microbiol 52:1033-1038. . <https://doi.org/10.1099/00207713-52-3-1033>
- 20 Thomas-Hall SR, Turchetti B, Buzzini P, Branda E, Boekhout T, Theelen B, Watson K (2010) Cold-adapted yeasts from Antarctica and the Italian Alps-description of three novel species: *Mrakia robertii* sp. nov., *Mrakia blollopis* sp. nov. and *Mrakiella niccombsii* sp. nov. Extremophiles 14:47-59. <https://doi.org/10.1007/s00792-009-0286-7>
- Truong M, Monahan LG, Carter DA, Charles IG (2018) Repurposing drugs to fast-track
- 25 therapeutic agents for the treatment of cryptococcosis. Peer J 6:e4761. <https://doi.org/10.7717/peerj.4761>

- 5 Tsuji M, Fuhui S, Xiao N et al. (2013) Cold adaptation of fungi obtained from soil and lake sediment in the Skarvsnes ice-free area, Antarctica. FEMS Microbiol 346:121-130. <https://doi.org/10.1111/1574-6968.12217>
- Turchetti B, Buzzini P, Goretti M et al. (2008) Psychrophilic yeasts in glacial environments of Alpine glaciers. FEMS Microbiol Ecol 6373-83. <https://doi.org/10.1111/j.1574-6941.2007.00409.x>
- 10 Turchetti B, Hall SRT, Connell LB, Branda E, Buzzini P, Theelen B, Müller WH, Boekhout T (2011) Psychrophilic yeasts from Antarctica and European glaciers: description of *Glaciozyma* gen nov, *Glaciozyma martini* sp. nov. and *Glaciozyma watsonii* sp. nov. Extremophiles 15:573-586. <https://doi.org/10.1007/s00792-011-0388-x>
- 15 Vaz ABM, Rosa LH, Vieira MLA et al. (2011) The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. Braz J Microbiol 42:937-947. <http://dx.doi.org/10.1590/S1517-83822011000300012>
- Vishniac HS (2006) Yeast biodiversity in the Antarctic. In: Rosa CA, Péter G (eds). Biodiversity and ecophysiology of yeasts, Springer, Berlin, pp 419-440. https://doi.org/10.1007/3-540-30985-3_16
- 20 Wagner D, Sander A, Bertz H, Finke J, Kern WV (2005) Breakthrough invasive infection due to *Debaryomyces hansenii* (teleomorph *Candida famata*) and *Scopulariopsis brevicaulis* in a stem cell transplant patient receiving liposomal amphotericin B and caspofungin for suspected aspergillosis. Infection 33:397-400. <https://doi.org/10.1007/s15010-005-5082-4>
- 25 White TJ, Bruns T, Lee SJWT, Taylor JL (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White

5 TJ (eds). PCR protocols: a guide to methods and applications, 1 st edn. Academic Press San Diego, pp 315-322

Wirth F, Goldani LZ (2012) Epidemiology of *Rhodotorula*: an emerging pathogen. Interdiscip Perspect Infec Dis 465717:1-7. <http://dx.doi.org/10.1155/2012/465717>

10 Woolfolk SW, Inglis G. (2004) Microorganisms associated with field-collected *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) adults with emphasis on yeast symbionts. Biol Control 29:155-168. [https://doi.org/10.1016/S1049-9644\(03\)00139-7](https://doi.org/10.1016/S1049-9644(03)00139-7)

Yu NH, Park SY, Kim JA et al. (2018) Endophytic and endolichenic fungal diversity in maritime Antarctica based on cultured material and their evolutionary position among Dikarya. Fungal Syst Evol 2:3-22. <https://doi.org/10.3114/fuse.2018.02.07>

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5 **Tabela 2. Table 1** TaFungi obtained from Antarctic snow segments and identified by sequence comparison with the BLASTn match with the NCBI GenBank database

Region	Snow segment	Density (CFU L ⁻¹)	UFMGCB ^a	Top BLAST search results (GenBank accession number)	Query cover (%)	Identity (%)	N° of bp analyzed	Proposed taxa (GenBank acc. n°)
Deception Island	1	>300	18371	<i>Leucosporidium fragarium</i> (NG058330) ^e	100	99	831	<i>Leucosporidium fragarium</i> (MN0655470) ^j
		36.66	12598	<i>Penicillium cavernicola</i> (MH862709) ^b	100	100	466	<i>Penicillium</i> sp. 1 (MK981319) ^g
		10	12377	<i>Mortierella elongatula</i> (MH859811) ^b	100	96	483	<i>Mortierella</i> sp. (MK889363) ^g
		3.3	18377	<i>Rhodotorula mucilaginosa</i> (MH636067) ^e	100	99	495	<i>Rhodotorula mucilaginosa</i> (MN0655160) ^j
	2	>300	18378	<i>Phenoliferia glacialis</i> (LC203727) ^e	100	100	815	<i>Phenoliferia glacialis</i> (MN065548) ^j
		9.9	12592	<i>Cladosporium phyllactiniicola</i> (MH863929) ^b	100	100	432	<i>Cladosporium</i> sp. 1 (MK981325) ^g
		6	12376	<i>Antarctomyces psychrotrophicus</i> (MH874317) ^b	100	100	432	<i>Antarctomyces psychrotrophicus</i> (MK889356) ^g
		4	12597	<i>Thelebolus balaustiformis</i> (NR159056) ^b	99	100	443	<i>Thelebolus balaustiformis</i> (MK889369) ^g
		2	12375	<i>Penicillium cavernicola</i> (MH862709) ^b	100	100	430	<i>Penicillium solitum</i> (MK981306) ^g
				<i>Penicillium albocoremium</i> (KU896812) ^c	99	97	405	(MN017378) ^h
				<i>Penicillium solitum</i> (KU904363) ^d	100	100	689	(MN056972) ⁱ
								<i>Leucosporidium</i> sp. (MN065549) ^j
	3	>300	18368	<i>Leucosporidium golubevii</i> (KY108283) ^e	99	98	670	
		41.3	12371	<i>Leohumicola minima</i> (NR121307) ^b	100	91	474	<i>Helotiales</i> sp. 1 (MK889360) ^g
		4.6	12428	<i>Thelebolus balaustiformis</i> (NR159056) ^b	100	100	487	<i>Thelebolus balaustiformis</i> (MK889370) ^g
		5.3	12741	<i>Penicillium cavernicola</i> (MH862709) ^b	100	99	468	<i>Penicillium</i> sp. 1 (MK981334) ^g
		4	12372	<i>Penicillium rubens</i> (NR111815) ^b	100	100	464	<i>Penicillium</i> sp. 2 (MK981307) ^g
				<i>Penicillium glycyrrhizicola</i> (KF021538) ^c	99	98	398	(MN0173379) ^h
		2	12380	<i>Penicillium tardochrysogenum</i>	100	99	470	<i>Penicillium chrysogenum</i>

King George Island	1	12373	(MH865983) ^b	100	97	377	(MK981309) ^g
			<i>Penicillium chrysogenum</i> (AY495981) ^c				(MN045349) ^h
			<i>Penicillium roqueforti</i> (MH855127) ^b	100	100	463	<i>Penicillium</i> cf.
			<i>Penicillium carneum</i> (AY674386) ^c	96	97	321	<i>psychrosexualis</i>
	1	>300	<i>Penicillium psychrosexualis</i> (KU904362) ^d	100	99	554	(MK981308) ^g
							(MN045348) ^h
			<i>Phenoliferia psychrophénolica</i> (KY108774) ^e	100	100	551	<i>Phenoliferia psychrophénolica</i> (MN065550) ⁱ
			<i>Phenoliferia glacialis</i> (NG058369) ^e	94	99	555	<i>Phenoliferia</i> cf. <i>glacialis</i> (MN065514) ^j
			<i>Phaeococcomyces mexicanus</i> (NG058078) ^e	100	98	490	<i>Phaeococcomyces</i> sp. (MN065551) ^j
			<i>Rhodotorula mucilaginosa</i> (KY218728) ^e	100	99	622	<i>Rhodotorula mucilaginosa</i> (MN065517) ^j
			<i>Penicillium tardochrysogenum</i> (MH865983) ^b	100	100	476	<i>Penicillium</i> sp. 3 (MK981320) ^g
			<i>Thelebolus globosus</i> (MH862951) ^b	100	99	499	<i>Thelebolus globosus</i> (MK889371) ^g
			<i>Alternaria multiformis</i> (MH862776) ^b	100	100	447	<i>Alternaria multiformis</i> (MK889355) ^g
			<i>Penicillium rubens</i> (NR111815) ^b	98	99	469	<i>Penicillium chrysogenum</i> (MK981310) ^g
			<i>Penicillium chrysogenum</i> (JF909937) ^d	98	99	936	<i>Neomicrosphaeropsis</i> cf. <i>italica</i> (MK889364) ^g
			<i>Neomicrosphaeropsis italica</i> (NR158247) ^b	100	98	503	
			<i>Penicillium tardochrysogenum</i> (MH865983) ^b	100	99	467	<i>Penicillium chrysogenum</i> (MK981311) ^g
			<i>Penicillium chrysogenum</i> (JF909937) ^d	100	100	920	<i>Bionectriaceae</i> sp. 1 (MK889358) ^g
			<i>Ijuhya vitelina</i> (NR154100) ^b	100	88	511	<i>Thelebolus balaustiformis</i> (MK889374) ^g
			<i>Thelebolus balaustiformis</i> (NR159056) ^b	100	100	474	
			<i>Mrakia gelida</i> (KY108585) ^e	98	99	554	<i>Mrakia</i> sp. (MN065528) ^j
	2	>300	<i>Hamamotota singularis</i> (KY107777) ^e	100	99	435	<i>Hamamotota singularis</i> (MN065527) ^j
			<i>Rhodotorula mucilaginosa</i> (KY218730) ^e	100	100	673	<i>Rhodotorula mucilaginosa</i> (MN065518) ^j

	>300	18391	<i>Hamamotoa singularis</i> (KY107777) ^e	100	97	551	<i>Hamamotoa</i> sp. (MN065526) ^j
	>300	18478	<i>Vishniacozyma victoriae</i> (LC203739) ^e	100	99	642	<i>Vishniacozyma victoriae</i> (MN065520) ^j
	88	18381	<i>Glaciozyma antarctica</i> (NG057664) ^e	100	100	553	<i>Glaciozyma antarctica</i> (MN065525) ^j
	7.3	12734	<i>Ijuhya vitelina</i> (NR154100) ^b	100	87	453	<i>Bionectriaceae</i> sp. 1 (MK889359) ^g
	6	12385	<i>Penicillium cavernicola</i> (MH862709) ^b	100	100	465	<i>Penicillium</i> sp. 1 (MK981312) ^g
			<i>Penicillium glandicola</i> (KU896814) ^c	93	82	407	(MN045350) ^h
	1.3	12594	<i>Cladosporium phyllactiniicola</i> (MH863929) ^b	100	100	465	<i>Cladosporium</i> sp. 1 (MK981333) ^g
	1	12384	<i>Neomicrosphaeropsis italica</i> (NR158247) ^b	100	98	482	<i>Neomicrosphaeropsis</i> cf. <i>italica</i> (MK889365) ^g
							<i>Phenoliferia</i>
	3	>300	<i>Phenoliferia psychrophenolica</i> (KY108774) ^e	100	100	565	<i>psychrophenolica</i> (MN065553) ^j
	>300	18393	<i>Rhodotorula mucilaginosa</i> (MF927654) ^e	100	99	546	<i>Rhodotorula mucilaginosa</i> (MN065519) ^j
	>300	18403	<i>Glaciozyma antarctica</i> (LC202043) ^e	100	100	501	<i>Glaciozyma antarctica</i> (MN065524) ^j
	>300	18493	<i>Phenoliferia glacialis</i> (LC203727) ^e	100	100	590	<i>Phenoliferia glacialis</i> (MN065540) ^j
	8	12386	<i>Penicillium cavernicola</i> (MH862709) ^b	100	100	425	<i>Penicillium</i> sp. 1 (MK981313) ^g
			<i>Penicillium albocoremium</i> (KU896812) ^c	95	97	370	(MN045351) ^h
	2.6	12735	<i>Penicillium caseifulvum</i> (MH862722) ^b	100	100	373	<i>Penicillium</i> sp. 4 (MK981321) ^g
	4	12387	<i>Penicillium commune</i> (NR111143) ^b	100	100	466	<i>Penicillium palitans</i> (MK981314) ^g
			<i>Penicillium palitans</i> (KU904360) ^d	100	100	577	
	4	12582	<i>Cladosporium asperulatum</i> (MH863916) ^b	100	100	475	<i>Cladosporium</i> sp. 2 (MK981326) ^g
	4	12740	<i>Leohumicola minima</i> (NR121307) ^b	100	91	482	<i>Helotiales</i> sp. 1 (MK889361) ^g
Trinity Peninsula	1	>300	<i>Phenoliferia glacialis</i> (LC203723) ^e	100	99	678	<i>Phenoliferia glacialis</i> (MN065531) ^j
	>300	18417	<i>Bannozya yamatoana</i> (AF189896) ^e	100	99	563	<i>Bannozya yamatoana</i> (MN065523) ^j

	>300	18489	<i>Vishniacozyma victoriae</i> _(LC203739) ^e	100	100	559	<i>Vishniacozyma victoriae</i> (MN065555) ^j
	>300	18480	<i>Cystobasidium pallidum</i> (NG059006) ^e	100	93	529	<i>Cystobasidium</i> sp. (MN065556) ^j
	110	18410	<i>Phenoliferia psychrophenolica</i> (KY108774) ^e	100	99	543	<i>Phenoliferia</i> <i>psychrophenolica</i> (MN065530) ^j
	46	12405	<i>Penicillium caseifulvum</i> (MH862722) ^b	100	100	495	<i>Penicillium</i> sp. 4 (MK981316) ^g
			<i>Penicillium longisporum</i> (KJ834467) ^c	88	98	336	(MN075199) ^h
	31.3	18408	<i>Dioszegia hungarica</i> (KY107643) ^e	100	99	488	<i>Dioszegia hungarica</i> (MN065557) ^j
	34	18423	<i>Dioszegia crocea</i> (KX096670) ^e	100	100	448	<i>Dioszegia crocea</i> (MN065520) ^j
	12.6	18411	<i>Genolevuria bromeliarum</i> (NG058291) ^e	98	94	542	<i>Genolevuria</i> sp. (MN065564) ^j
			<i>Genolevuria bromeliarum</i> (NG058291) ^b	61	95	860	(MN065510) ^g
	6	12429	<i>Penicillium oregonense</i> (MH865652) ^b	100	96	419	<i>Penicillium</i> sp. 5 (MK981315) ^g
			<i>Penicillium glandicola</i> (KU896814) ^c	93	82	407	(MN045352) ^h
	2	12404	<i>Thelebolus balaustiformis</i> (NR159056) ^b	99	100	488	<i>Thelebolus balaustiformis</i> (MK889373) ^g
2	>300	18413	<i>Rhodotorula mucilaginosa</i> (KP223715) ^e	100	100	596	<i>Rhodotorula mucilaginosa</i> (MN065512) ^j
	>300	18425	<i>Phenoliferia psychrophenolica</i> (KY108774) ^e	100	100	508	<i>Phenoliferia</i> <i>psychrophenolica</i> (MN065535) ^j
	106	12427	<i>Penicillium tardochrysogenum</i> (NR1383081) ^b	100	99	504	
			<i>Penicillium chrysogenum</i> (JF909937) ^c	100	100	651	<i>Penicillium chrysogenum</i>
			<i>Penicillium chrysogenum</i> (AY495981) ^d	100	100	342	(MK981317) ^g (MN075200) ^h
	8.6	18426	<i>Debaryomyces hansenii</i> (KX981201) ^e	100	100	712	<i>Debaryomyces hansenii</i> (MN065522) ^j
	2.6	18416	<i>Leucosporidium muscorum</i> (KY108280) ^e	100	99	599	
			<i>Leucosporidium creatinivorum</i> (KC455908) ^b	99	100	654	<i>Leucosporidium</i> sp. (MN121386) ^g (MN121387) ^j
	1.3	18422	<i>Candida sake</i> (KY102375) ^e	100	100	656	<i>Candida sake</i> (MN065558) ^j
3	>300	18414	<i>Phenoliferia glacialis</i> (LC203723) ^e	100	99	653	<i>Phenoliferia</i> cf <i>glacialis</i>

							(MN0655320) ^j	
Arctowski Peninsula		>300	18415	<i>Phenoliferia psychrophenumolica</i> (KY108774) ^e	100	99	499	<i>Phenoliferia psychrophenumolica</i> (MN065533) ^j
		5.3	12402	<i>Penicillium tardochrysogenum</i> (MH865983) ^b	100	99	419	<i>Penicillium</i> sp. 3 (MK981335) ^g
		2.6	12732	<i>Cladosporium phyllactiniicola</i> (MH863929) ^b	100	100	428	<i>Cladosporium</i> sp. 1 (MK981327) ^g
	1	>300	18430	<i>Phenoliferia psychrophenumolica</i> (KY108774) ^e	100	100	533	<i>Phenoliferia psychrophenumolica</i> (MN065534) ^j
		120	18428	<i>Genolevuria amylolytica</i> (NG057728) ^e	100	99	455	<i>Genolevuria amylolytica</i> (MN065559) ^j
		3.65	12432	<i>Antarctomyces psychrotrophicus</i> (MH874317) ^b	100	100	435	<i>Antarctomyces psychrotrophicus</i> (MK889357) ^g
		2	12433	<i>Cladosporium phaenocomae</i> (MH865096) ^b	100	100	435	<i>Cladosporium</i> sp. 3
				<i>Cladosporium longisporum</i> (KJ834467) ^c	94	97	355	(MK981324) ^g (MN075202) ^h
		2	18436	<i>Debaryomyces hansenii</i> (LC219506) ^e	100	100	546	<i>Debaryomyces hansenii</i> (MN065521) ^j
	2	>300	18431	<i>Phenoliferia psychrophenumolica</i> (KY108774) ^e	100	100	571	<i>Phenoliferia psychrophenumolica</i> (MN065536) ^j
		>300	18477	<i>Phenoliferia glacialis</i> (LC203727) ^e	100	100	510	<i>Phenoliferia glacialis</i> (MN065537) ^j
		15.33	12431	<i>Cladosporium phyllactiniicola</i> (MH863929) ^b	100	100	462	<i>Cladosporium</i> sp. 1 (MK981330) ^g
		7.3	12408	<i>Cladosporium variabile</i> (MH863132) ^b	100	100	481	<i>Cladosporium</i> sp. 3 (MK981331) ^g
		1.3	12587	<i>Penicillium caseifulvum</i> (MH862722) ^b	100	100	488	<i>Penicillium</i> sp. 4 (MK981323) ^g
		1	12407	<i>Thelebolus balaustiformis</i> (NR159056) ^b	98	100	439	<i>Thelebolus</i> cf. <i>balaustiformis</i> (MK889366) ^g
Snow Island	1	>300	18455	<i>Phenoliferia psychrophenumolica</i> (KY108774) ^e	100	100	527	<i>Phenoliferia psychrophenumolica</i> (MN065538) ^j
		157.3	18439	<i>Mrakia gelida</i> (LC203698) ^e	100	100	618	<i>Mrakia gélida</i> (MN065529) ^j

	19.95	18441	<i>Phenoliferia glacialis</i> (LC203727) ^e	100	100	631	<i>Phenoliferia glacialis</i> (MN065539) ^j
	28	18475	<i>Holtermanniella wattica</i> (LC203693) ^e	100	100	458	<i>Holtermanniella wattica</i> (MN065560) ^j
	6.3	18443	<i>Rhodotorula mucilaginosa</i> (KY218730) ^e	100	100	543	<i>Rhodotorula mucilaginosa</i> (MN065513) ^j
	3.3	18491	<i>Genolevuria bromeliarum</i> (NG058291) ^e	96	98	542	<i>Genolevuria</i> sp. (MN065563) ^j
	1.3	18440	<i>Kabatiella bupleuri</i> (JN886792) ^e	98	98	420	<i>Kabatiella</i> cf. <i>bupleuri</i> (MN065565) ^j
			<i>Kabatiella bupleuri</i> (JN886792) ^b	100	98	577	(MN655511) ^g
	2	12590	<i>Clathrosphaerina zalewskii</i> (MH856474) ^b	100	91	423	<i>Hyaloscyphaceae</i> sp. (MK889362) ^g
2	>300	18445	<i>Phenoliferia psychrophenolica</i> (KY108774) ^e	100	100	553	<i>Phenoliferia psychrophenolica</i> (MN065540) ^j
	>300	18484	<i>Phenoliferia glacialis</i> (LC203727) ^e	100	100	571	<i>Phenoliferia glacialis</i> (MN065561) ^j
	0.6	18448	<i>Vishniacozyma victoriae</i> (LC203739) ^e	100	99	599	<i>Vishniacozyma victoriae</i> (MN065562) ^j
	2	12434	<i>Cladosporium tenuissimum</i> (MH864840) ^b	100	99	471	<i>Cladosporium</i> sp. 4 (MK981332) ^g
	2	12411	<i>Penicillium tardochrysogenum</i> (NR1383081) ^b	100	100	523	<i>Penicillium chrysogenum</i> (MK981318) ^g (MN075201) ^h
			<i>Penicillium chrysogenum</i> (AY4959811) ^c	100	99	416	
3	>300	18437	<i>Phenoliferia glacialis</i> (LC203727) ^e	100	100	571	<i>Phenoliferia glacialis</i> (MN065541) ^j
	>300	18456	<i>Phenoliferia psychrophenolica</i> (FN550378) ^e	100	99	514	<i>Phenoliferia psychrophenolica</i> (MN065542) ^j
	6.65	12739	<i>Pseudogymnoascus destructans</i> (EU88492) ^b	100	99	453	<i>Pseudogymnoascus destructans</i> (MK889375) ^g
	2.66	12412	<i>Thelebolus globosus</i> (KX576510) ^b	100	99	410	<i>Thelebolus globosus</i> (MK889367) ^g
	2.6	12589	<i>Pseudogymnoascus verrucosus</i> (KJ755525) ^b	100	100	400	<i>Pseudogymnoascus verrucosus</i> (MK889376) ^g
	4	12588	<i>Pseudogymnoascus pannorum</i> (MH861038) ^b	99	98	414	<i>Pseudogymnoascus</i> sp. (MK889378) ^g

Robert Island	1	>300	18470	<i>Phenoliferia psychrophenolica</i> (FN550378) ^e	100	100	525	<i>Phenoliferia psychrophenolica</i> (MN065543) ⁱ
		>300	18461	<i>Phenoliferia glacialis</i> (KX773532) ^e	100	100	506	<i>Phenoliferia glacialis</i> (MN065544) ⁱ
		3.3	12420	<i>Cladosporium phyllactiniicola</i> (MH863929) ^b	98	100	430	<i>Cladosporium</i> sp. 1 (MK981328) ^g
		4	12419	<i>Thelebolus balaustiformis</i> (NR159056) ^b	100	99	511	<i>Thelebolus</i> cf. <i>balaustiformis</i> (MK889368) ^g
		1.3	12423	<i>Pseudocamarosporium africanum</i> (NR154294) ^b	99	100	528	<i>Pseudocamarosporium africanum</i> (MK889379) ^g
				<i>Pseudocamarosporium africanum</i> (JX496368) ^c				
	2	>300	18464	<i>Phenoliferia psychrophenolica</i> (KY108774) ^e	100	99	538	<i>Phenoliferia psychrophenolica</i> (MN065545) ⁱ
		2.66	12414	<i>Thelebolus balaustiformis</i> (NR159056) ^b	100	100	425	<i>Thelebolus balaustiformis</i> (MK889372) ^g
		1.33	12413	<i>Cladosporium phyllactiniicola</i> (MH863929) ^b	100	100	469	<i>Cladosporium</i> sp. 1 (MK981329) ^g
		1.3	12733	<i>Penicillium rubens</i> (NR111815) ^b	98	100	434	<i>Penicillium</i> sp. 2 (MK981322) ^g
	3	>300	18472	<i>Phenoliferia psychrophenolica</i> (KY108774) ^e	100	100	528	<i>Phenoliferia psychrophenolica</i> (MN065546) ⁱ
		>300	18490	<i>Rhodotorula mucilaginosa</i> (KY218730) ^e	100	100	605	<i>Rhodotorula mucilaginosa</i> (MN065515) ⁱ
		14	12416	<i>Antarctomyces pellizariae</i> (KX576510) ^b (KX790790) ^c (KY100007) ^d	100	100	545	<i>Antarctomyces pellizariae</i> (KX576510) ^g (KX790790) ^h (KY100007) ⁱ
		1	12438	<i>Pseudogymnoascus verrucosus</i> (KJ755525) ^b	98	99	488	<i>Pseudogymnoascus</i> cf. <i>verrucosus</i> (MK889377) ^g

^aUFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. Taxa subjected to BLAST analysis based on the ^bITS, ^c β -tubulin, ^dPolymerase II, and ^eD1/D2 regions for elucidation of taxonomic positions. ^fTaxonomic position suggested. Sequences of ^gITS, ^h β -tubulin and/or ⁱPolymerase II and ^jD1/D2 sequences deposited in GenBank database.

5 **Tabela 3. Table 2** Physicochemical parameters of melted snow sampled in different sites of Antarctic Peninsula and diversity indices

Parameters/diversity indices/density	Sites sampled					
	Deception	King George	Trinity	Arctowski	Snow	Robert
	Island	Island	Peninsula	Peninsula	Island	Island
Conductivity ($\mu\text{S cm}^{-1}$)	50	173	20	28	52	53
Resistivity ($\text{M}\Omega \text{ cm}^{-1}$)	0.024	0.006	0.047	0.023	0,026	0.039
Total dissolved solids (ppm)	24	86	11	14	14.6	27
Oxidation Reduction Potential (mV)	190.9	310	256.8	255.9	361.2	230.2
pH	6.76	6.3	6.74	6.24	6.1	6.5
Salinity (ppt)	0.02	0.08	0.01	0.01	0.02	0.02
Number of taxa	16	31	21	11	20	14
Fisher α	2.69	4.63	3.09	1.71	3.17	2.06
Margalef's	2.16	3.65	2.52	1.44	2.55	1.73
Simpson's	0.74	0.92	0.90	0.74	0.84	0.84

- 5 **Tabela 4. Table 3** Minimal inhibitory concentration of benomyl, fluconazole, and amphotericin B against fungi isolated from seasonal snow of Antarctica

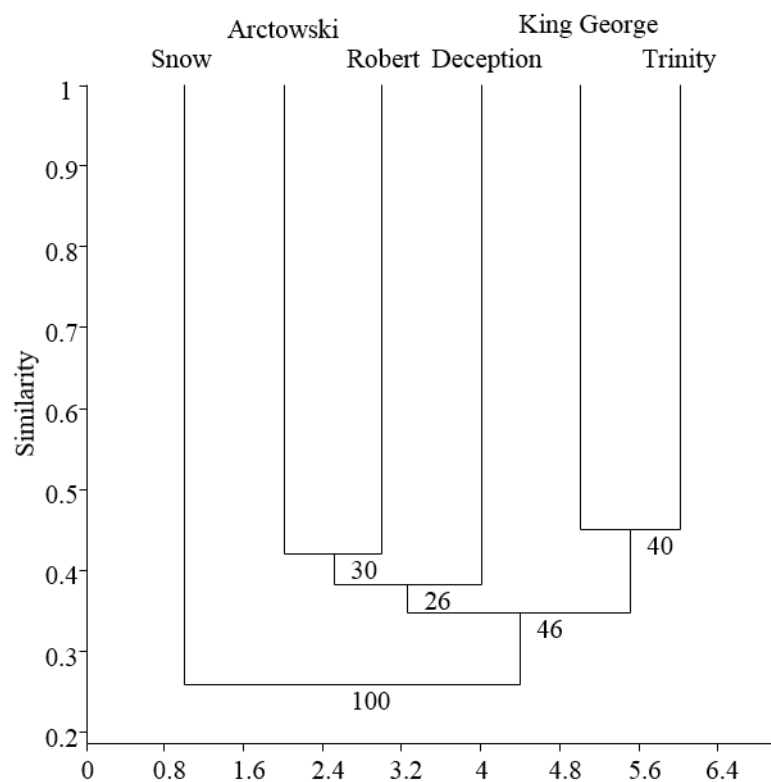
Fungi	UFMGCB ^a	MIC ^b at 25 °C			MIC ^b at 37 °C ^d
		Benomyl	Fluconazole	Amphoterecin B	
<i>Debaryomyces hansenii</i>	18426	40	8	-	
<i>D. hansenii</i>	18436	40	4	-	
<i>Rhodotorula mucilaginosa</i>	18377	0.3125	64	0.03	
<i>R. mucilaginosa</i>	18400	0.3125	64	0.03	
<i>R. mucilaginosa</i>	18393	0.3125	64	0.03	
<i>R. mucilaginosa</i>	18413	0.3125	64	0.03	
<i>R. mucilaginosa</i>	18443	0.3125	64	0.125	
<i>Penicillium chrysogenum</i>	12380	0	32	-	
<i>P. chrysogenum</i>	12427	0	64	-	
<i>Penicillium</i> sp. 3	12430	2.5	64	-	
<i>Penicillium</i> sp. 2	12733	2.5	1	-	
<i>Penicillium</i> sp. 4	12405	2.5	64	-	

^aUFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. ^bMIC = Minimal inhibitory concentration at µg/mL. ^dFor selected fungi with MIC ≥ 64 µg/mL and able to growth at 37 °C. 0 = absence of growth. - = fungi incapable to grow at 37°C.

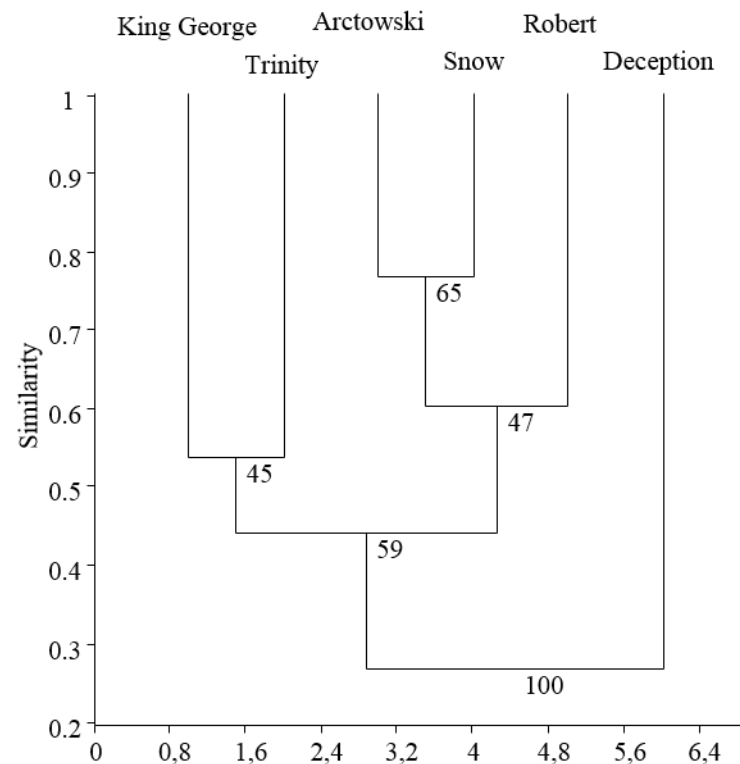
- 5 **Tabela 5. Supplementary Table 1** Growth profile of fungi isolated from winter seasonal snow of Antarctica that displayed antifungal resistance

Taxa	UFMGCB ^a	Temperature (°C)						
		10	15	20	25	30	35	37
<i>Debaryomyces hansenii</i>	18426	+	+	+	+	+	+	-
<i>D. hansenii</i>	18436	+	+	+	+	+	+	-
<i>R. mucilaginosa</i>	18377	+	+	+	+	+	+	+
<i>R. mucilaginosa</i>	18400	+	+	+	+	+	+	+
<i>R. mucilaginosa</i>	18393	+	+	+	+	+	+	+
<i>R. mucilaginosa</i>	18413	+	+	+	+	+	+	+
<i>R. mucilaginosa</i>	18443	+	+	+	+	+	+	+
<i>Penicillium chrysogenum</i>	12380	+	+	+	+	+	+	-
<i>P. chrysogenum</i>	12427	+	+	+	+	+	+	-
<i>Penicillium</i> sp. 3	12430	+	+	+	+	+	+	-
<i>Penicillium</i> sp. 2	12733	+	+	+	+	+	+	-
<i>Penicillium</i> sp. 4	12405	+	+	+	+	+	+	-

^aUFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. + = presence of growth; - = absence of growth. Yeasts growth for 72 h and filamentous fungi 14 days.



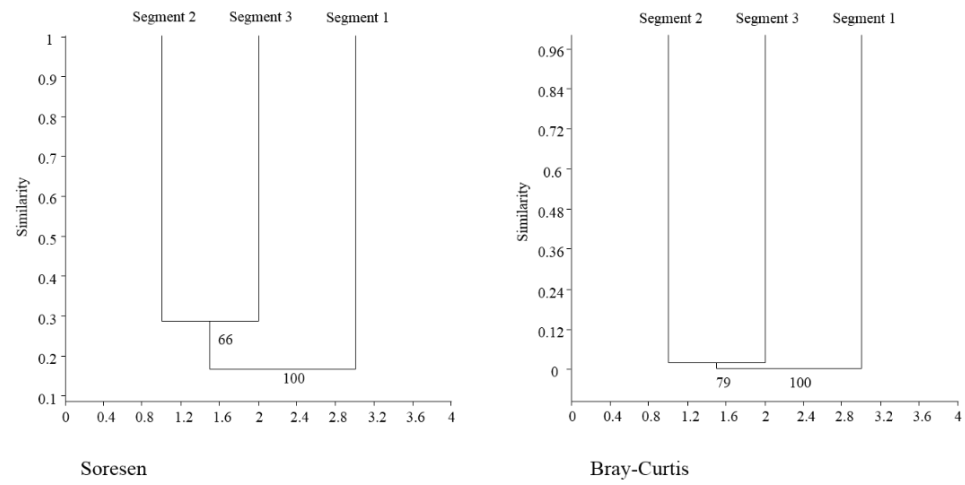
(a)



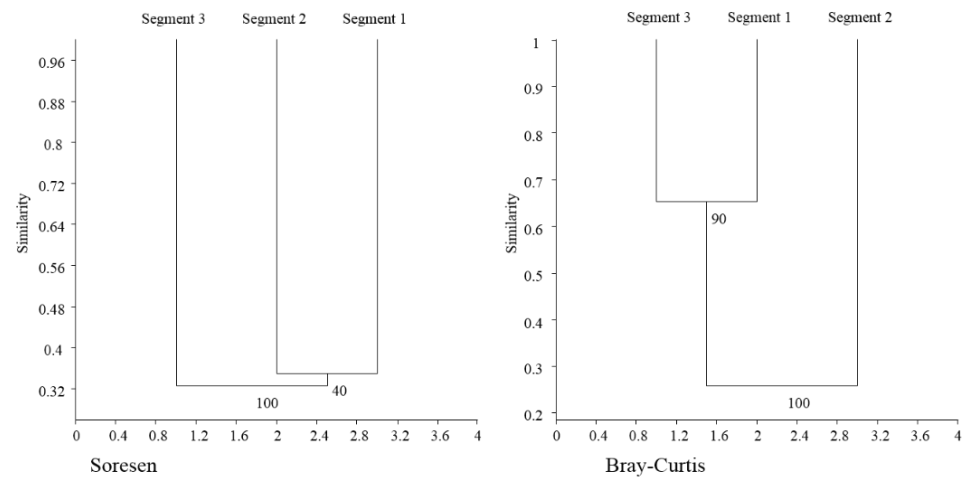
(b)

Figure 4. Supplementary Figure 1 Dendrogram showing the comparison of (a) Sorensen (b) Bray-Curtis similarities measures for the fungal assemblages isolated from seasonal snow of different regions of Antarctica.

Deception Island



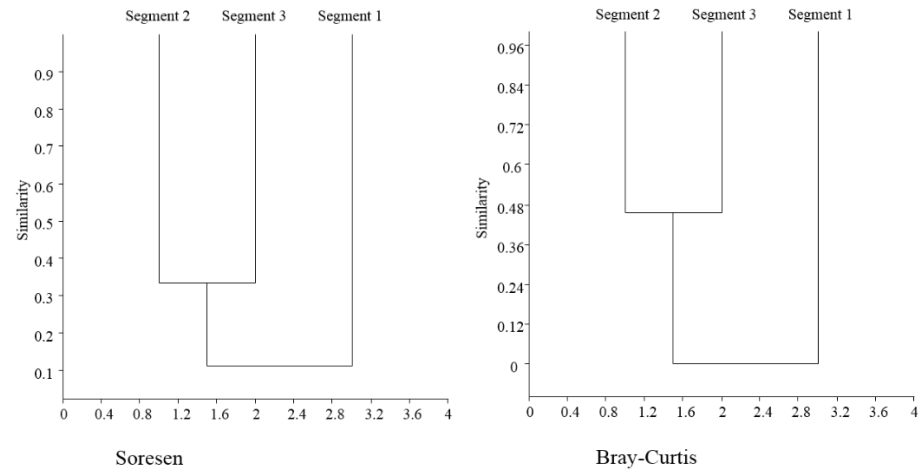
King George Island



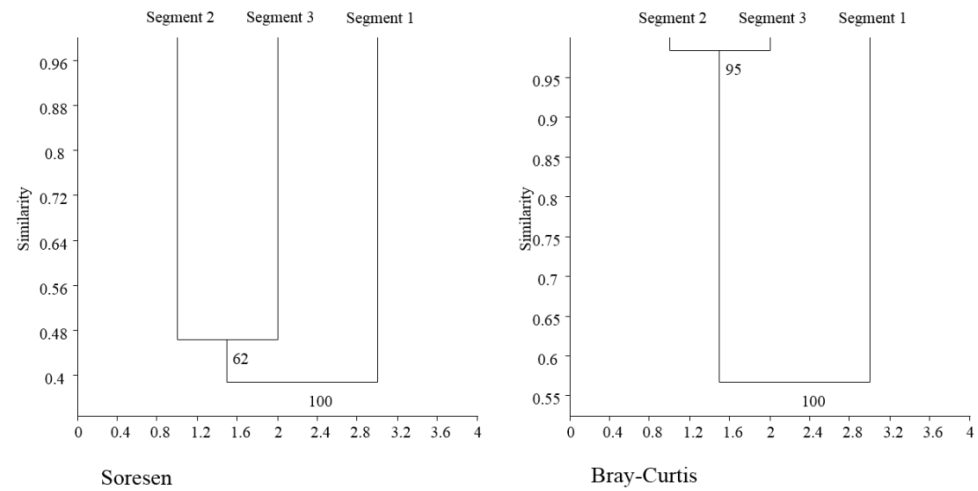
(a)

(b)

Trinity Peninsula

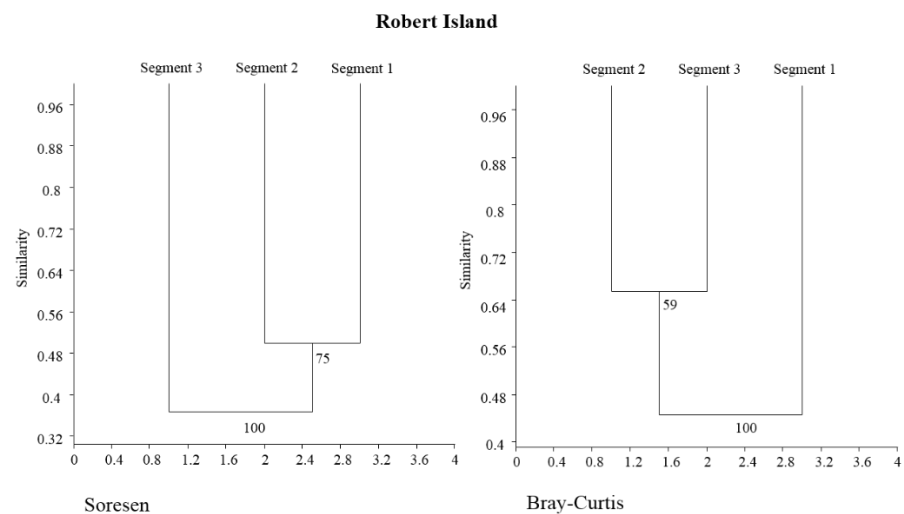


Snow Island



(c)

(d)



(e)

Figura 5. Supplementary Figure 2 Dendrograms showing the comparison of Sorensen and Bray-Curtis similarities measures for the fungal assemblages isolated from each segment per region of seasonal snow of Antarctica. Segment 1 (top layer), segment 2 (middle layer), and segment 3 (base layer) Fungal assemblages of (a) Deception Island, (b) King George Island, (c) Trinity Peninsula, (d) Snow Island, (e) and Robert Island.

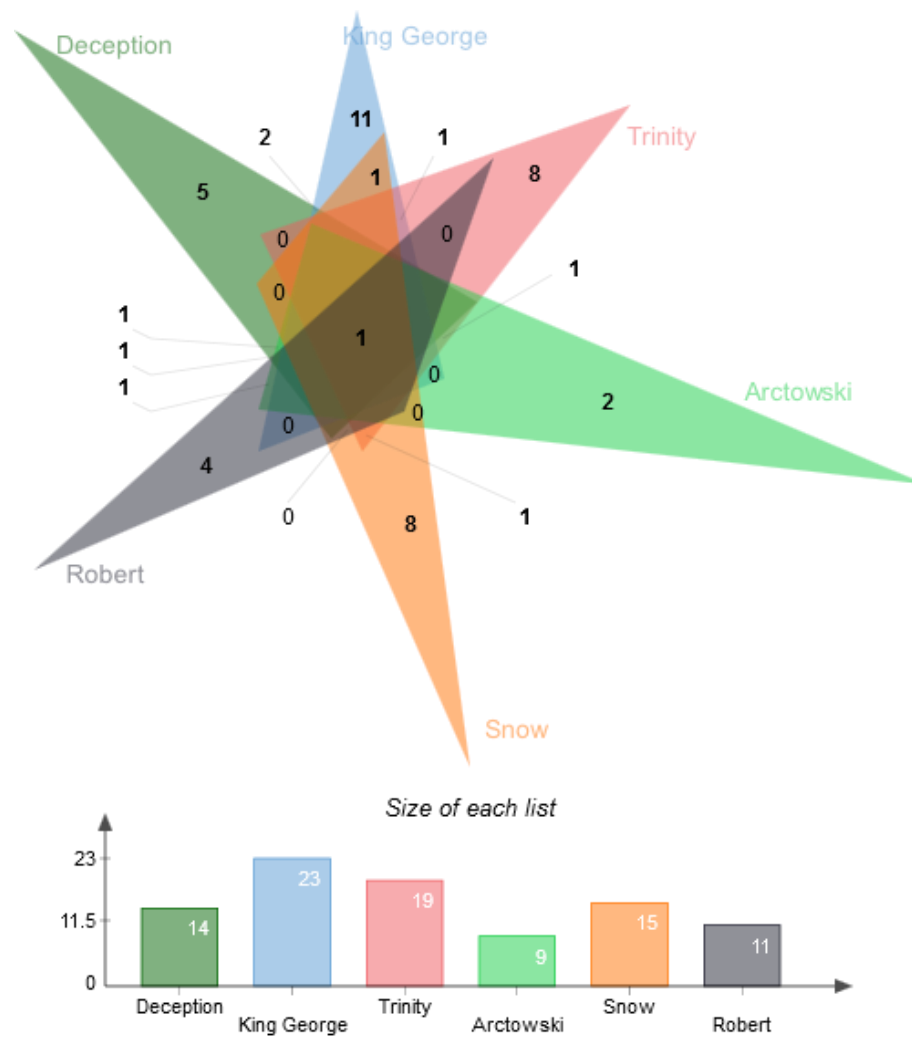


Figura 6. Supplementary Figure 3 Similarity among the seasonal snow fungal assemblages of Antarctica using the Venn diagrams.

5.3 Capítulo 3: “*Antarctomyces pellizariae* sp. nov., a new, endemic, blue, snow residente psychrophilic ascomycete fungus from Antarctica”

Extremophiles
DOI 10.1007/s00792-016-0895-x



ORIGINAL PAPER

Antarctomyces pellizariae sp. nov., a new, endemic, blue, snow resident psychrophilic ascomycete fungus from Antarctica

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Abstract In the present study, we have identified and characterised a new snow resident ascomycete blue stain fungus from Antarctica named *Antarctomyces pellizariae* sp. nov. Menezes, Godinho, Porto, Gonçalves and Rosa, using polyphasic taxonomy techniques. This fungal species was recovered from the seasonal snow of the Antarctic Peninsula. *Antarctomyces pellizariae* displayed different macro- and micromorphology when compared with *A. psychrotrophicus* Stehlig and Guarro, the only other *Antarctomyces* species reported until date. *Antarctomyces pellizariae* showed psychrophilic behavior and very low growth rate at 22–25 °C, quite different from *A. psychrotrophicus* that has a higher growth rate at mesophilic temperatures. In addition, micromorphological characteristics and the analysis of the nuclear rDNA internal transcribed spacer, β -tubulin, and RNA polymerase II regions revealed that *A. pellizariae* is a new species that is related to *A. psychrotrophicus* and *Thelebolus* species. Since the Antarctic Peninsula is reported to be one of the main regions of the earth experiencing the effects of global change in climate, species, such as *A. pellizariae*, might provide information about these effects on the endemic Antarctic biota. In addition, *A. pellizariae* displayed psychrophilic behavior and

might be a source of interesting anti-freeze compounds that might prove useful in biotechnological processes.

Keywords Antarctica · *Antarctomyces* · Fungi · New species · Snow

Introduction

Antarctica is the largest continent on the planet, with an extreme climate. This makes it a unique field laboratory for taxonomy, diversity, ecology, evolution, and biotechnological studies on polar fungi. Fungi represent an important part of the microbiota of Antarctica. Important representatives of the polar fungi community are the filamentous fungi consisting of species in the phyla Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota, and Basidiomycota (Ruisi et al. 2007). In Antarctica, fungi have been reported in different substrates, such as soil, rocks, woody components, freshwater, macroalgae, and plants (Ellis-Evans 1985; Tosi et al. 2002; Arenz et al. 2006; Rosa et al. 2009; Loque et al. 2010; Bridge and Spooner 2012).

Among the different substrates that shelter microbial communities in Antarctica, snow represents an interesting and unknown habitat for bacteria, archaea, and eukarya. Approximately 35% of the land surface is covered by snow (Miteva 2008; Margesin and Miteva 2010). These resident microbial communities might have an active influence on the nutrient dynamics and hydrochemistry of snow (Larose et al. 2013). According to Margesin and Miteva (2010), the snow habitat represents the constant aeolian fluxes of dust, microorganisms, and other biological material deposited with precipitation. According to Miteva (2008), snow habitats are characterised by permanent low temperatures, low nutrient availability, and in high altitudes and polar regions,

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and high levels of UV radiation. Few studies have characterised the microbial communities in the snow of Antarctica, with most of them characterising bacteria and microalgae (González-Toril et al. 2008; Fujii et al. 2010; Lopatina et al. 2013); only Fujii et al. (2010) described the yeasts found in the red snow in Antarctica.

In the Antarctic summer of 2015, we collected snow samples from different Antarctic islands to isolate its fungal communities. Among these fungal communities, we discovered a filamentous fungus with deep blue-coloured colonies on Sabouraud agar, first identified as *Antarctomyces* sp. UFMGCB 12416 by preliminary molecular taxonomy. The detailed polyphasic analysis showed strong evidence that *Antarctomyces* UFMGCB 12416 represented a new ascomycete species.

Methods

Snow sampling, concentration, and fungus isolation

The snow sample was collected in the Coppermine Peninsula (62°37'941"S; 59°70'400"W), Robert Island, in the South Shetland Islands, Antarctica in the austral summer season in the December of 2015. Approximately 10 kg of snow from the uppermost 1.5 m layer was collected using a sterile plastic scoop and stored in sterilised plastic bags of 30 L capacity. Sterilised suits and gloves were worn during sample collection to minimise contamination during sampling. The snow was melted in the microbiology laboratory on board the Brazilian polar ship admiral Maximiano over a period of about 12 h with occasional mixing. This process resulted in 10 L of water. A total of 1.5 L of melted snow was filtered through a 0.45 µm membrane with 47 mm diameter (Millipore, USA) in triplicate. The membranes were placed on Sabouraud agar (Acumedia, India) containing 200 µg mL⁻¹ of chloramphenicol (Sigma, USA) and incubated at 10 °C for 30 days. The fungus was purified in new Petri dishes containing Sabouraud agar and deposited in the Collection of Microorganisms and Cells of the Universidade Federal de Minas Gerais, Brazil, under the code UFMGCB 12416 in cryotubes at -80 °C and in distillate-sterilised water (Castellani 1967) at room temperature, which are available to be accessed by other scientists.

Macro- and micromorphological characterization

Fungal macroscopic parameters (colony colour, texture, reverse colour, border type, and radial growth rate) and colony diameters were observed on different media, as described in the following. Colours follow the specification proposed by Kornerup and Wanscher (1984). The *Q* values were obtained by length/width of individual ascospores,

conidia, and chlamydospores. *Antarctomyces* sp. UFMGCB 12416 was inoculated into three point cultures on the following media: potato carrot agar [PCA; potatoes 20 g, carrot 20 g, agar agar 20 g (Kasvi, Brazil), and sterilised distilled water 1 L], oatmeal agar [OA; oat flakes 30 g, agar agar 15 g (Kasvi, Brazil), and sterilised distilled water 1 L], potato dextrose agar (PDA; Merck, Germany), malt extract agar [MEA; glucose 20 g (Synth, Brazil), malt extract 15 g (Merck, Germany), peptone 1 g (Kasvi, Brazil), and agar 20 g (Kasvi, Brazil)], and Sabouraud agar. All media were incubated for 7 and 14 days at 4, 10, 15, and 25 ± 2 °C. Media under the same conditions were used to determine the microscopic parameters (hyphae, conidiophores, and conidia) using slide cultures mounted in lactophenol and viewed under a microscope (Leica DM750, Germany).

For scanning electron microscopy, the fungus *Antarctomyces* sp. UFMGCB 12416 was inoculated on PCA, PDA, and Sabouraud agar media and incubated at 10 and 25 °C for 14 days. The mycelia were fixed in 2% glutaraldehyde in 0.1 M NaPO₄ buffer and washed in buffered 1% OsO₄ for 2 h. The material was dehydrated using a graded ethanol series (10, 25, 40, 60, 75, 85, 95, and 100%) for 15 min per concentration. The material was dried in a critical point drying apparatus, sputter-coated with gold, and viewed with an FEI Quanta 200 SEM, USA.

Fungal growth responses to different temperatures

Plugs of 4 mm² from 7-day-old pre-cultures of *Antarctomyces* sp. UFMGCB 12416, grown at 10 °C on PDA, were inoculated onto Petri dishes containing PDA medium and incubated at 5, 10, 15, 20, and 25 ± 2 °C. Plates were incubated in triplicate, and after 7 and 14 days, the colony diameters were measured in mm.

Molecular identification

DNA was extracted as per the protocol described previously in Rosa et al. (2009). The internal transcribed spacer (ITS) region was amplified with 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 RNA polymerase II genes (RPB2) (Houbaken et al. 2012), which are commonly utilised to identify fungal species of the same genera with low intraspecific variation was done with the Bt2a/Bt2b and RPB2-5F-Pc/RPB2-7CR-Pc 7CR primers, respectively. Representative consensus sequences of *A. pellizariae* were deposited into GenBank (KX576510, KX790790, and KY100007), TreeBASE (19625), and Mycobank (MB 817786). To achieve species-rank identification based on ITS, β-tubulin, and RNA polymerase II, the consensus

sequence was aligned with sequences from related species retrieved from the NCBI GenBank database using BLAST (Altschul et al. Altschul et al. 1997). Sequences of *A. pellizariae* were compared with different sequences of type species as well as the ITS sequence of the ex-type species *Antarctomyces psychrotrophicus* (AJ133431), both deposited in the GenBank database with estimations conducted using MEGA Version 6.0 (Tamura et al. 2013). The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1000 replicate runs. The information about fungal classification generally followed the dictionary of Kirk et al. (2008), MycoBank (<http://www.mycobank.org>), and the Index Fungorum (<http://www.indexfungorum.org>).

Results

Preliminary taxonomic analysis

Melting snow from Antarctica revealed a fungus that was labelled UFMGCB 12416. It displayed an unusual deep blue colour on Sabouraud agar at 10 °C (Suppl. Fig. 1), which drew our attention, as it could possibly be a new taxon. Preliminary BLAST-based comparison of the ITS sequences of the UFMGCB 12416 taxon with the GenBank sequences showed that the three closest species were fungal endophyte (HQ335307) recovered from the moss *Polypodium strictum* at Barton Peninsula, Antarctica (query coverage = 99%, identity = 100%); uncultured fungus (KC965762) isolated from the soil of Happy Valley, Alaska, USA (query coverage = 100%, identity = 99%); and *Antarctomyces psychrotrophicus* (GU004189) obtained from the soil from an undescribed region in Antarctica (query coverage = 98%, identity = 100%).

According to Stchigel et al. (2001), *Antarctomyces* possess peculiar morphological features that make the genus easy to identify. These characteristics include the following: mainly submerged mycelium, composed of septate, branched and unbranched, anastomosing, hyaline hyphae; ascomata composed of naked asci, without excipulum; asci that are ellipsoidal to subglobose, unitunicate, non-catenate, and eight-spored; absent paraphyses; ascospores that are ellipsoidal to fusiform, hyaline, spinulose, without germ pores, and one-celled.

Macro- and micromorphological analyses

After the detection of the preliminary genetic and morphological characteristics, we identified the fungus UFMGCB 12416 as an *Antarctomyces* species. However, the fungus UFMGCB 12416 when cultured on PDA at 22–25 °C displayed slower growth, different from that of

A. psychrotrophicus. The mycelial characteristic suggested that the fungus UFMGCB 12416 could represent a new species, and its detailed molecular and morphological characteristics were determined. The macro- and micromorphological characteristics of *A. pellizariae* on five different media (Figs. 1, 2) are described below.

PCA after 14 days

At 11–12 °C

The colonies were 26–27 mm in diameter, and plane, thin, zonate, vegetative mycelium was mainly submerged and uncoloured, reverse uncoloured. Ascomatal initials began to develop from the coiling of two side branches, occasionally disposed in tandem. Ascomata composed of naked asci were seen, singly or in groups of six, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were scarce, 17–23 × 8–17 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thickwalled, non-catenate, eight-spored, and developed form croziers. Ascospores were 6.5–9 × 4.5–6 µm in size ($Q = 1.4–1.5$), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines c. 0.3–0.5 µm long. Anamorph stage: conidia and chlamydospores were absent.

At 22–25 °C

The colonies were 16.5–18 mm in diameter, plane, thin, white with yellowish centre, with irregular margins, anastomosing, reverse uncoloured, with septate hyphae; hyphae 1.5–2 µm broad, thin to thick-walled. Ascomatal initials were scarce and began developing from the coiling of the two side branches, occasionally disposed in tandem. Ascomata were scarce in number, composed of naked asci, single, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were 8.5–20 × 6–13 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were 5–8.5 × 3–6 µm in size ($Q = 1.7–1.4$), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, one-celled; spines c. 0.3–0.5 µm long. Anamorph stage: conidia were absent. Chlamydospores were abundant, 3–5 × 5–10 µm in size ($Q = 0.6–0.5$), irregular, single or forming long chains, and one or two-celled.

PDA after 14 days

At 11–12 °C

The colonies were 15–16.5 mm in diameter at 11–12 °C, rough, with long radial furrows, irregular margins,

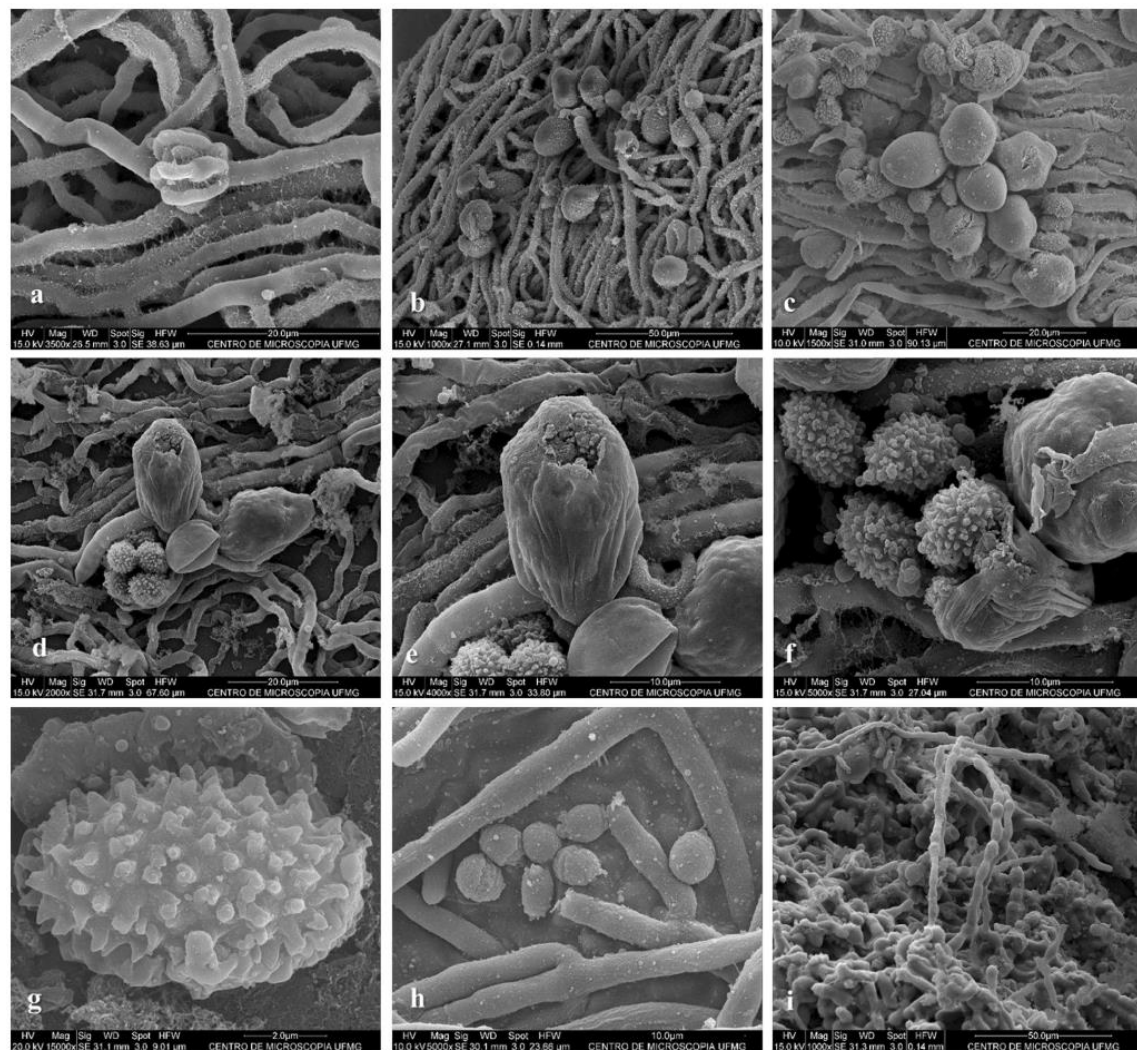


Fig. 1 Optical micrographs of *Antartomyces pellizariae* after 14 days of growth. **a** Asci on PCA at 10–12 °C. **b** Free ascospores with spinulose surface and the thick wall on PCA at 10–12 °C. **c** Cluster of conidia on PDA at 22–25 °C. **d** Anastomosis and chlamydospores on PCA at 22–25 °C. **e** Ascomata initials on PCA at 22–25 °C. **f**

Ascospores on OA at 10–12 °C. **g** Asci with ascospores on OA at 10–12 °C. **h** View of septate hypha and ascomata initial on MEA at 4–6 °C. **i** Asci and ascospores on MEA at 10–12 °C. **j** Asci and ascospores on Sb at 10–12 °C. *PCA* potato carrot agar, *PDA* potato dextrose agar, *OA* oat agar, *MEA* malt extract agar, *Sb* Sabouraud agar

vegetative mycelium mainly submerged, dull blue (M 23D4), light blue funicles in the central area, composed of sterile hyphae, anastomosing, reverse with the same colour. Abundant ascumatal initials began to develop from the coiling of two side branches. Ascumata composed of naked asci, single or in groups of two, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were 6–11 × 10–19 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate,

thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were 3–5 × 4–7 µm in size ($Q = 0.8–0.7$), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines c. 0.3–0.5 µm long. Anamorph stage: conidia were 6.5–7.5 × 4–5 µm in size ($Q = 1.6–1.5$), subglobose to irregularly cylindrical, hyaline, smooth, thick-walled, aggregated in slimy masses, and one-celled. Chlamydospores were 9–11 × 6–7 µm in size

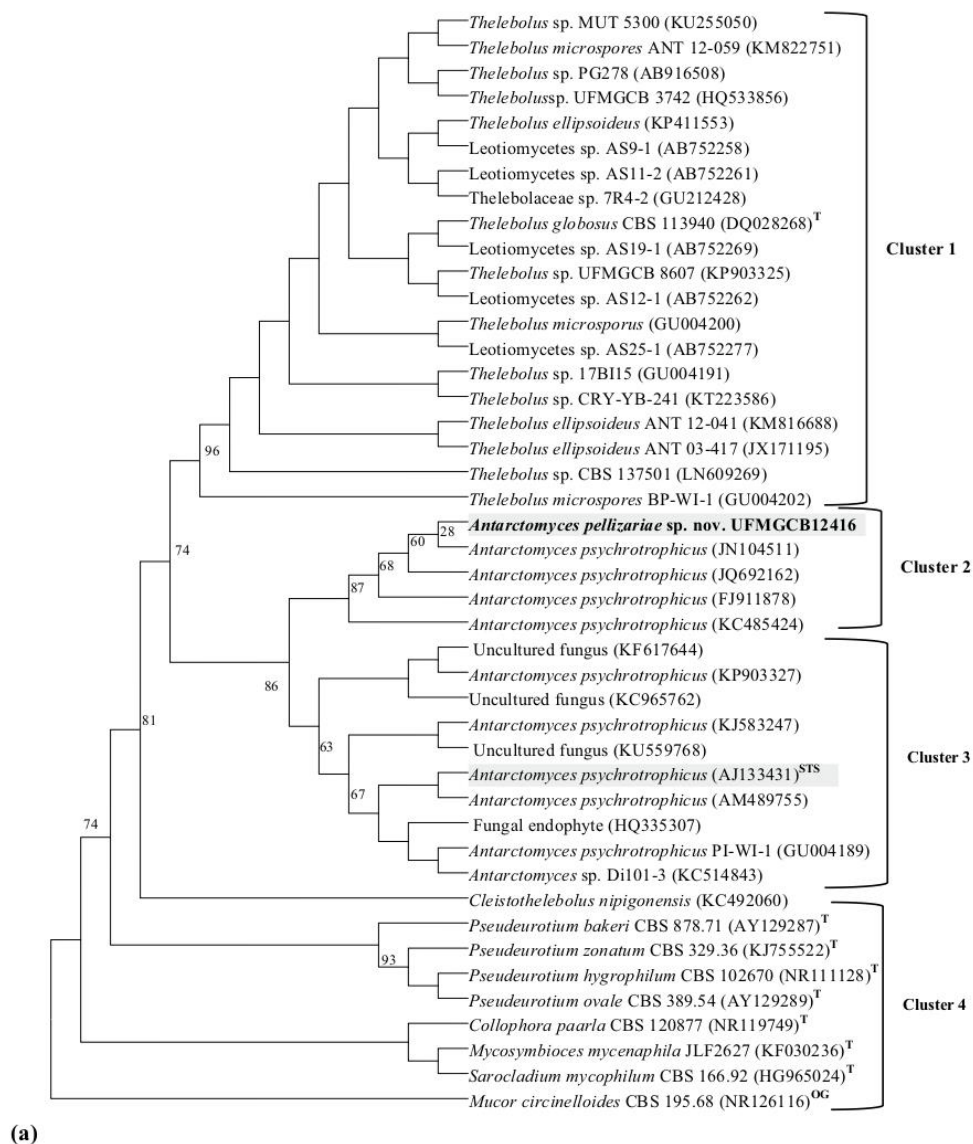


Fig. 2 Scanning electron micrographs of *Antarctomyces pellizariae* on potato carrot agar (PCA) at 10–12 °C after 14 days. **a** Ascomata initials. **b** Young asci. **c** Cluster of young asci. **d** Mature asci and

release ascospores. **e** Mature asci. **f** Ascospores releasing of the asci. **g** Free ascospores with spinulose surface and the thick wall. **h** Different conidia. **i** Chlamydospores

($Q = 1.5$ – 1.6), irregular, single or forming long chains, and one or two-celled.

At 22–25 °C

The colonies were 6–7 mm in diameter after 14 days, rough, margins irregular, vegetative mycelium mainly submerged,

yellowish, reverse yellowish. Asci were absent. Anamorph stage: conidia were 4.5 – 9×4 – $5 \mu\text{m}$ in size ($Q = 1.1$ – 1.8), subglobose to irregularly cylindrical, hyaline, smooth, thick-walled, aggregated in slimy masses, and one-celled. Chlamydospores were abundant, 7 – 14×6 – $11 \mu\text{m}$ in size ($Q = 1.2$ – 1.3), irregular, single or forming long chains, and one or two-celled.

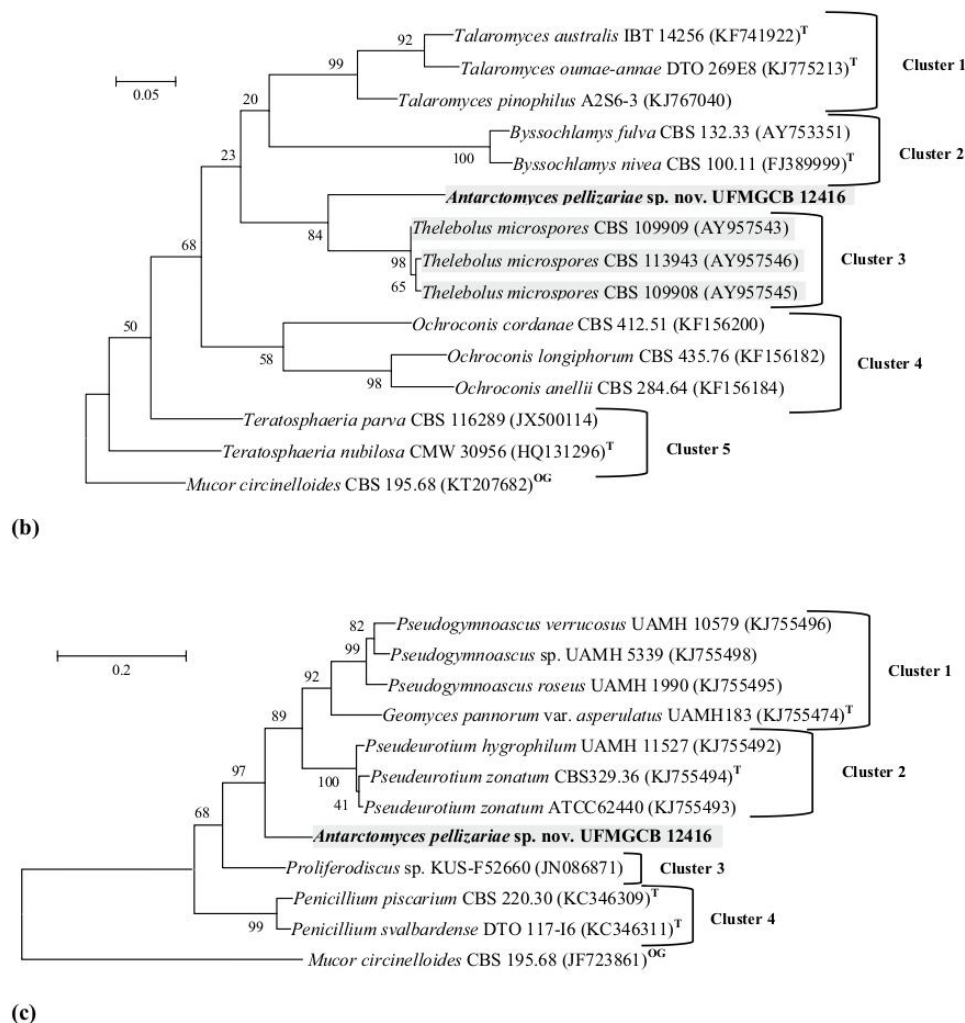


Fig. 2 continued

OA after 14 days*At 11–12 °C*

The colonies were 20–21 mm in diameter at 11–12 °C, plane and thin; vegetative mycelium mainly submerged, uncoloured, reverse uncoloured. Ascomata were composed of naked asci, single or in groups of 1–4, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci measured 10–23 × 10–16.5 µm in size, were subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were 7–8 × 3–4 µm in size

($Q = 2.3$ –2), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines with 0.3–0.5 µm long. In the anamorph stage, conidia were absent. Chlamydospores were scarce and measured 3–6 × 3–5 µm in size ($Q = 1$ –1.2), were irregular, single, or forming long chains, and were one or two-celled.

At 22–25 °C

The colonies were 10–21 mm in diameter, plane, thin, fimbriate margins, vegetative mycelium mainly submerged, uncoloured, reverse uncoloured, anastomosing. Ascomatal initials, scarce in number, began to develop from

the coiling of two side branches, occasionally disposed in tandem. Asci were absent. Anamorph stage: conidia were $5\text{--}9 \times 3\text{--}4 \mu\text{m}$ in size ($Q = 1.7\text{--}2.6$), subglobose to irregularly cylindrical, hyaline, smooth, thick-walled, aggregated in slimy masses, and one-celled. Chlamydospores were very abundant in long chains, $6\text{--}11 \times 5\text{--}7 \mu\text{m}$ in size ($Q = 1\text{--}1.6$), irregular, single or forming long chains, and one or two-celled.

MEA after 14 days

At 10–12 °C

The colonies were 55–59 mm in diameter at 10–12 °C and 30–32.5 mm in diameter at 4–6 °C, plane, with the vegetative mycelium mainly submerged, yellowish, reverse yellowish, anastomosing. Moniliform mycelium and asci were present. Abundant ascomatal initials began to develop from the coiling of two side branches. Ascomata were composed of naked asci, single or in groups of 2–4, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci measured $16\text{--}22 \times 9\text{--}14 \mu\text{m}$ in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were $5\text{--}7 \times 3\text{--}4 \mu\text{m}$ in size ($Q = 1.7\text{--}1.8$), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines with $0.3\text{--}0.5 \mu\text{m}$ long. Anamorph stage: conidia absent. Chlamydospores were $5\text{--}11 \times 5\text{--}7 \mu\text{m}$ in size ($Q = 1\text{--}1.6$), irregular, single or forming long chains, and one or two-celled.

At 22–25 °C

The colonies were 8 mm in diameter, with the same culture characteristics as in OA. Moniliform mycelium was present. Asci, chlamydospores, and conidia were absent.

Sabouraud agar after 14 days

At 10–12 °C

The colonies were 26–27 mm in diameter, cerebriform, front and reverse white to soft blue. Ascomatal initials began to develop from the coiling of two side branches, occasionally disposed in tandem. Ascomata were composed of naked asci, single or in groups of 2–5, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were $18.5\text{--}20 \times 13\text{--}15 \mu\text{m}$ in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were $13\text{--}18.5 \times 15\text{--}20 \mu\text{m}$ in

size ($Q = 0.85\text{--}0.9$), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines c. $0.3\text{--}0.5 \mu\text{m}$ long. Anamorph stage: conidia and chlamydospores were absent.

At 22–25 °C

The colonies were 26–27 mm in diameter, irregular, vegetative mycelium mainly submerged, front reverse yellowish, mycelium mainly submerged, composed of hyaline, branched and unbranched, anastomosing, with septate hyphae; hyphae $2\text{--}4 \mu\text{m}$ broad, thin to thick-walled. Ascomatal initials began to develop from the coiling of two side branches, occasionally disposed in tandem. Ascomata were composed of naked asci, single, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were $11.5\text{--}14 \times 6.5\text{--}7 \mu\text{m}$ in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were $4.5\text{--}8.5 \times 3\text{--}6 \mu\text{m}$ in size ($Q = 1.5\text{--}1.4$), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines c. $0.3\text{--}0.5 \mu\text{m}$ long. Anamorph stage: conidia were $7\text{--}9 \times 4 \mu\text{m}$ in size ($Q = 1.75\text{--}2$), subglobose to irregularly cylindrical, hyaline, smooth, thick-walled, aggregated in slimy masses, and one-celled. Chlamydospores were abundant, $7\text{--}11 \times 4.5\text{--}7 \mu\text{m}$ in size ($Q = 1.5\text{--}1.6$), irregular, single or forming long chains, and one or two-celled. Sporothrix-like anamorph was present.

In summary, the morphology of *A. pellizariae* is different when compared with that of *A. psychrotrophicus* (Table 1). The colonies growing on all the media (mainly on MEA) at 22–25 °C were 8 mm in diameter in the case of *A. pellizariae* where as the same for *A. psychrotrophicus* was 54–58 mm. The two species also differed with respect to the number of asci groups, asci, ascospores, chlamydospores measurements, and absence of conidia. The colour of the colonies of *A. pellizariae* on PDA, OA, and MEA are different compared with that of *A. psychrotrophicus*. In addition, *A. pellizariae* showed abundant asci on MEA at 10–12 °C in contrast with *A. psychrotrophicus*, which, according to Stchigel et al. (2001), does not produce any asci.

We also evaluated the mycelial growth rate of *A. pellizariae* at different temperatures for 7 and 14 days (Suppl. Fig. 3). At 14 day, *A. pellizariae* displayed the most growth at 15 and 20 ± 2 °C (colonies diameters of 43.5 ± 4 and 57 ± 5.5 mm, respectively), followed by 10 and 4 ± 2 °C (33.5 ± 4 and 26 ± 5.5 mm diameter, respectively). In addition, *A. pellizariae* showed the slowest growth at 25 ± 2 °C (11.5 ± 0.5 mm diameter), indicating its psychrophilic behavior.

Table 1 Main macro- and micromorphological differences between *Antarctomyces pellizariae* and *Antarctomyces psychrotrophicus*

	Colour and measurement (in mm) of the colonies on different media at 22–25 °C					Micromorphology characteristics (measurement in µm) on PCA at 22–25 °C				
	PCA	PDA	OA	MEA	Sb	Asci number	Asci	Ascospores	Chlamydospores	Conidia
<i>Antarctomyces pellizariae</i>	Yellowish 16.5–18	Yellowish 6–7	Uncoloured 10–21	Uncoloured 8	Yellowish 26–27	Single	8.5–20 × 6–13	5–8 × 3–6 (<i>Q</i> = 1.7–1.3)	3–5 × 5–10 (<i>Q</i> = 0.6–0.5)	Absent
<i>Antarctomyces psychrotrophicus</i>	White 33–47	Uncoloured 65–71	Uncoloured 50–55	Uncoloured 54–58	nd	Single or groups of 2–7	15–19 × 12–13	7–10 × 4–5.5 (<i>Q</i> = 1.7–1.8)	10–15 × 5–8 (<i>Q</i> = 2–1.9)	3–20 × 2–5 (<i>Q</i> = 1.5–4)

Characteristics of the *Antarctomyces psychrotrophicus* are according to Stchigel et al. (2001)

PCA potato carrot agar, PDA potato dextrose agar, OA oat agar, MEA malt extract agar, Sb Sabouraud agar, nd not determined

Phylogenetic analysis

To prove our hypothesis and to clarify the taxonomic position of the new *Antarctomyces* species, we performed a phylogenetic study based on the sequences of the ITS, β -tubulin, and RNA polymerase II regions (Fig. 3). Detailed phylogenetic analysis of the ITS region of the sequences of *A. pellizariae* with the nearest taxa obtained from GenBank (Fig. 3a) showed that *A. pellizariae* forms a distinct cluster (cluster 2) similar to four *Antarctomyces* taxa (JN104511, JQ692162, FJ911878, and KC485424), which might also be *A. pellizariae* taxa, but different from cluster 3, which includes the type species *A. psychrotrophicus* (AJ133431). In addition, the *A. pellizariae* cluster showed phylogenetic differences when compared with *Thelebolus* (a sister species) and Leotiomycetes and *Pseudeurotium*, *Collophora*, *Mycosymbiodes*, and *Sarocladium* taxa. In addition, the ITS sequence of *A. pellizariae* displayed 11 different nucleotides when compared with the sequence of the type species *A. psychrotrophicus* (AJ133431). No sequence of β -tubulin or RNA polymerase II of *Antarctomyces* was found in the GenBank. However, we obtained these sequences from *A. pellizariae*, which were then compared with the sequences of the nearest taxa. The β -tubulin sequence analyses placed *A. pellizariae* between the *Thelebolus* (sister genus) (cluster 3) and *Byssoschlamys* (cluster 2) groups (Fig. 3b). The RNA polymerase II sequence of *A. pellizariae* (Fig. 3c) formed a separate cluster, but is closed to clusters composed of *Pseudogymnoascus*, *Pseudeurotium*, *Proliferodiscus*, and *Penicillium*.

Antarctomyces pellizariae Menezes, Godinho, Porto, Gonçalves & Rosa, sp. nov.-MB 817786; Figs. 1, 2; Suppl. Fig. 2.

At 22–25 °C, the colonies were 16.5–18 mm in diameter, plane, thin, white with yellowish centre, with irregular margins, anastomosing, reverse uncoloured, with septate hyphae; hyphae 1.5–2 µm broad, thin to thick-walled. Ascomatal initials were scarce and began developing from the coiling of the two side branches, occasionally disposed in tandem. Ascomata were scarce in number, composed of naked asci, single, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were 8.5–20 × 6–13 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were 5–8.5 × 3–6 µm in size (*Q* = 1.7–1.4), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, one-celled; spines *c.* 0.3–0.5 µm long. Anamorph stage: conidia were absent. Chlamydospores were abundant, 3–5 × 5–10 µm in size (*Q* = 0.6–0.5), irregular, single or forming long chains, and one or two-celled.

Typus: Antarctica: South Shetland Islands, Robert Island, Coppemine Peninsula, ex snow, 9 Dec

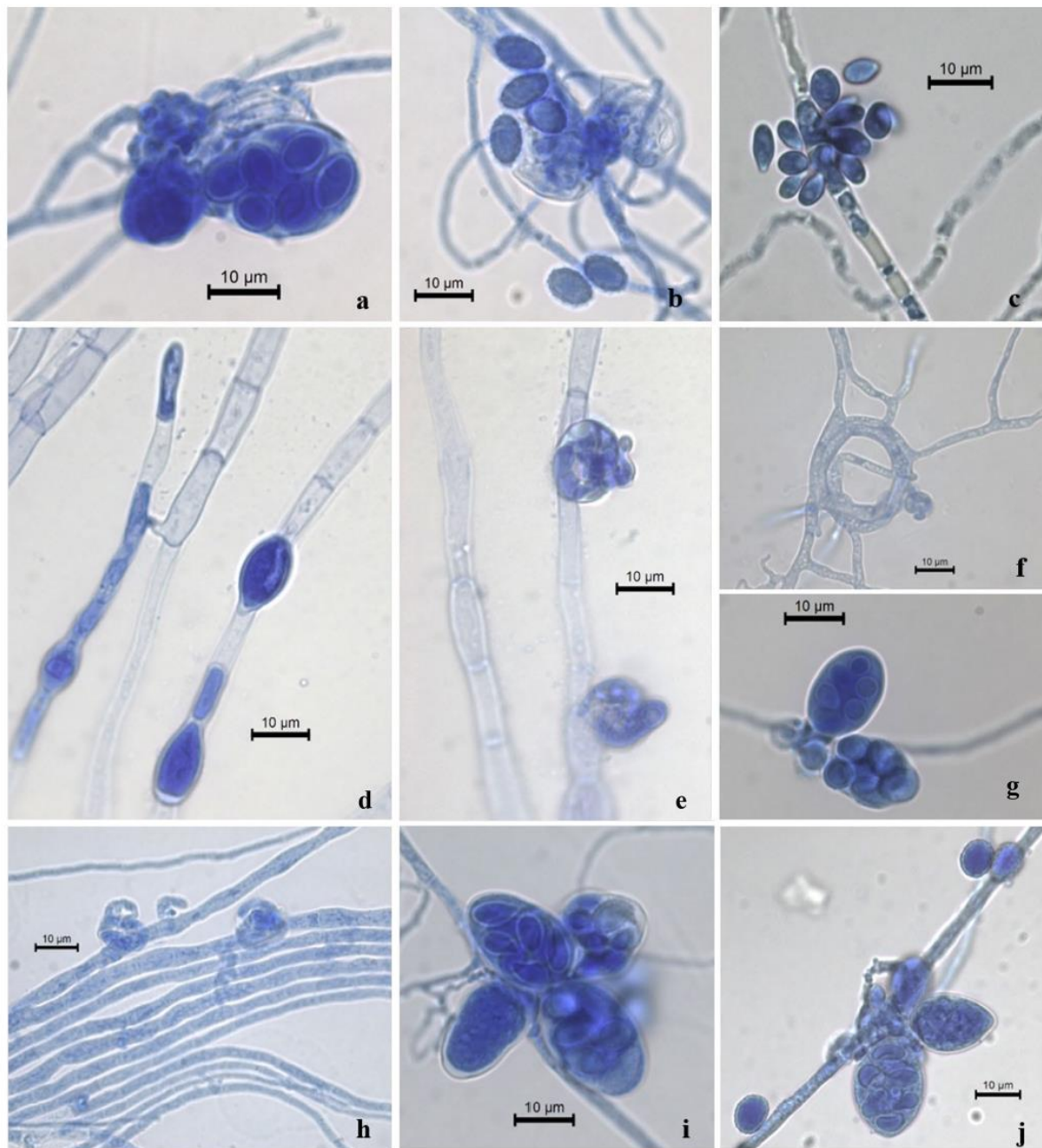


Fig. 3 Phylogenetic analysis of the sequence of *Antarctomyces pellizariae* UFMGCB 12416 compared with sequences of the closest species following BLAST analysis, deposited in the GenBank database. The trees were constructed on the basis of the ITS (a), beta-

tubulin (b), and polymerase II (c) regions sequences using the maximum composite likelihood method. T = sequences of type species. STS = sequence of type species. Sequence of *Mucor circinelloides* was used as outgroup (OG)

2015, G. C. A. Menezes, V. N. Gonçalves, V. M. Godinho and L. H. Rosa (UFMGCB 12416—holotype, CBS 142036—ex-type).

Etymology: *Antarctomyces pellizariae* [pe.lli'za.riæ. N.L. fem. gen. n. *pellizariae*, of Pellizari, referring to Dr. Vivian Helena Pellizari, a Full Professor of Oceanographic

Institute of University of São Paulo (USP), Brazil, in recognition of her important contributions to microbiology researches in the Brazilian Antarctic Program (PROANTAR), which inspire new Polar Brazilian Microbiologist].

Antarctomyces pellizariae holotypus has been deposited in the Collection of Microorganisms and Cells of Federal University of Minas Gerais (Coleção de Micro-organismos e Células da Universidade Federal de Minas Gerais, UFMG), Belo Horizonte, Minas Gerais, Brazil, as strain UFMGCB 12416, and is permanently preserved in a metabolically inactive state at in cryotubes at -80°C and in distillate-sterilised water (Castellani 1967) at room temperature. Ex-type culture has been deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 142036. The Mycobank number is MB 817786.

Discussion

In the present study, we have described a novel species *A. pellizariae* (Thelebolaceae, Ascomycota) that is endemic to the Antarctic region, after careful consideration of its biological, morphological, physiological, and phylogenetic aspects. The ITS, beta-tubulin, and RNA polymerase II phylogenies showed that *A. pellizariae* is genetically distinct from *A. psychrotrophicus* and other taxa of Thelebolaceae.

The genus *Antarctomyces* Stchigel & Guarro, represented only by the species *A. psychrotrophicus* Stchigel and Guarro, was originally isolated from the soil of South Shetland Islands, King George Island, Antarctica (Stchigel et al. 2001). However, *A. psychrotrophicus* has been recovered from different substrates of Antarctica as a symbiont endophyte of the Antarctic grass *Deschampsia antarctica* (Rosa et al. 2009), in association with macroalgae (Loque et al. 2010; Godinho et al. 2013; Furbino et al. 2014), in fresh water lakes (Gonçalves et al. 2012) and thalli of lichens (Santiago et al. 2015). In addition, as *A. psychrotrophicus* has been isolated only from substrates of Antarctic environments, it is considered an endemic psychrophilic species of Thelebolales (Santiago et al. 2015).

The cold biosphere environment characterised by habitats with prolonged cold and freezing temperatures work as a selective ecological filter for all immigrant and resident organisms. These habitats are dominated by cold-adapted (psychrophilic) and cold-tolerant (psychrotolerant) microorganisms (Harding et al. 2011). In contrast with *A. psychrotrophicus*, which has the best growth rate at 25°C , *A. pellizariae* displayed the best growth at 15 and $20 \pm 2^{\circ}\text{C}$ and the worst growth at $25 \pm 2^{\circ}\text{C}$, indicating that it is indeed a psychrophilic fungus. Usually, psychrophilic microbes possess lipids, enzymes, anti-freezing proteins, and/or

carbohydrate-based extracellular polymeric substances, which serve as cryo- and osmo-protectants (Krembs et al. 2011; Stibal et al. 2012). These anti-freezing biological strategies help the microorganisms to support cellular membrane homeostasis as well as the biochemical catalysis (Feller and Gerday 2003). Xiao et al. (2010) characterised *A. psychrotrophicus* as a producer of anti-freeze proteins (Xiao et al. 2010). We have similarly detected that *A. pellizariae* the second described species of the genus, has psychrophilic behavior suggesting that it might serve as a source of anti-freeze compounds. However, further studies are necessary to characterise the possible anti-freeze capabilities of these endemic *Antarctomyces* species.

Conclusion

Antarctic environments present promising habitats to isolate and describe new fungal species. Applying a polyphasic taxonomical approach, we identified a new, endemic, and unique ascomycete named *A. pellizariae*, which represents the second species in the endemic *Antarctomyces*. Since the Antarctic Peninsula has been reported to be one of the main regions of the earth experiencing the effects of global change in climate, species endemic to the Antarctic Peninsula, such as those from the *Antarctomyces* taxa, may provide information about these effects on the resident Antarctic biota. The presence of this unique and endemic fungus, *A. pellizariae* in the Coppermine Peninsula, a specially protected area (SPA) in Antarctica, reinforces the importance of an SPA as a source of new species as well as a biological sanctuary in Antarctica. Finally, *A. pellizariae* displayed psychrophilic behavior and might be a source of interesting anti-freeze compounds that might prove useful in biotechnological processes.

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References

- Altschul SF, Madden TL, Schaffer AA, Zhang JH, 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
- 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. doi:10.1016/j.soilbio.2006.01.016
- Bridge PD, Spooner BM (2012) Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem? *Fungal Ecol* 5:381–394. doi:10.1016/j.funeco.2012.01.007

- Castellani A (1967) Maintenance and cultivation of common pathogenic fungi in distilled water. *J Trop Med Hygien* 42:181–184
- Ellis-Evans JC (1985) Fungi from maritime Antarctic freshwater environments. *Br Antarct Surv Bull* 68:37–45
- Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold adaptation. *Nat Rev Microbiol* 1:200–208. doi:10.1038/nrmicro773
- Fujii M, Takano Y, Kojima H, Hoshino T, Tanaka R, Fukui M (2010) Microbial community structure, pigment composition and nitrogen source of red snow in Antarctica. *Microb Ecol* 59:466–475. doi:10.1007/s00248-009-9594-9
- Furbino LE, Godinho VM, Santiago IF et al (2014) Diversity patterns, ecology and biological activities of fungal communities associated with the endemic macroalgae across the Antarctic Peninsula. *Microb Ecol* 67:775–787. doi:10.1007/s00248-014-0374-9
- 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* 61:1323–1330
- Godinho VM, Furbino LE, Santiago IF, Pellizzari FM, Yokoya N et al (2013) Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. *ISME J* 7:1434–1451. doi:10.1038/ismej.2013.77
- 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. doi:10.1111/j.1574-6941.2012.01424.x
- González-Toril E, Amils R, Delmas RJ, Petit JR, Komárek J, Elster J (2008) Diversity of bacteria producing pigmented colonies in aerosol, snow and soil samples from remote glacial areas (Antarctica, Alps and Andes). *Biogeosci Discuss* 5:1607–1630. doi:10.5194/bgd-5-1607-2008
- Harding T, Jungblut AD, Lovejoy C, Vincent WF (2011) Microbes in high arctic snow and implications for the cold biosphere. *Appl Environ Microbiol* 77:3234–3243. doi:10.1128/AEM.02611-10
- Houbraken J, Frisvad JC, Seifert KA, Overby DP, Tuthill DM, Valdez JG et al (2012) New penicillin-producing *Penicillium* species and an overview of section *Chrysogena*. *Persoonia* 29:78–100. doi:10.3767/003158512X660571
- Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the fungi, 10th edn. CAB International, Wallingford
- Kornerup A, Wanscher JH (1984) Methuen handbook of colour, 3rd edn. Eyre Methuen, London
- Krembs C, Eicken H, Deming JW (2011) Exopolymer alteration of physical properties of sea ice and implications for the ice habitability and biogeochemistry in a warmer Arctic. *Proc Natl Acad Sci USA* 108:3653–3658. doi:10.1073/pnas.1100701108
- Larose C, Prestat E, Cecillon S, S Berger, Malandain C et al (2013) Interactions between snow chemistry, mercury inputs and microbial population dynamics in an arctic snowpack. *PLoS One* 8:e79972. doi:10.1371/journal.pone.0079972
- Lopatina A, Krylenkov V, Severinov K (2013) Activity and bacterial diversity of snow around Russian Antarctic stations. *Res Microbiol* 164:949–958. doi:10.1016/j.resmic.2013.08.005
- 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. doi:10.1007/s00300-009-0740-0
- Margesin R, Miteva V (2010) Diversity and ecology of psychrophilic microorganisms. *Res Microbiol* 162:346–361. doi:10.1016/j.resmic.2010.12.004
- Miteva V (2008) Bacteria in snow and glacier ice. In: Margesin R, Schinner F, Marx J-C, Gerday (eds), *Psychrophiles: from biodiversity to biotechnology*. Springer, Berlin, pp 31–50. doi:10.1007/978-3-540-74335-4
- Rosa LH, Vaz ABM, Caligiore RB, Campolina S, Rosa CA (2009) Endophytic fungi associated with the Antarctic Grass *Deschampsia antarctica* Desv. (Poaceae). *Polar Biol* 32:161–167. doi:10.1007/s00300-008-0515-z
- Ruissi S, Barreca D, Selbmann L, Zucconi L, Onofri S (2007) Fungi in Antarctica. *Rev Environ Sci Biotechnol* 6:127–141. doi:10.1007/s11157-006-9107-y
- Santiago IF, Soares MA, Rosa CA, Rosa LH (2015) Lichensphere: a protected natural microhabitat of the non-lichenised fungal communities living in extreme environments of Antarctica. *Extremophiles* 19:1087–1097. doi:10.1007/s00792-015-0781-y
- Stehlig AM, Cano J, MacCormack CW (2001) *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. *Mycol Res* 105:377–382. doi:10.1017/S0953756201003379
- Stibal M, Hasan F, Wadham JL, Sharp MJ, Anesio AM (2012) Prokaryotic diversity in sediments beneath two polar glaciers with contrasting organic carbon substrates. *Extremophiles* 16:255–265. doi:10.1007/s00792-011-0426-8
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729. doi:10.1093/molbev/mst197
- Tosi S, Casado B, Gerdol R, Caretta G (2002) Fungi isolated from antarctic mosses. *Polar Biol* 25:262–268. doi:10.1007/s00300-001-0337-8
- White TJ, Bruns TD, Lee SB (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis NA, Gelfand J, Sninsky J et al (eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp 315–322
- Xiao N, Suzuki K, Nishimiya Y, Kondo H, Miura A, Tsuda S, Hoshino T (2010) Comparison of functional properties of two fungal antifreeze proteins from *Antarctomyces psychrotrophicus* and *Typhula ishikariensis*. *FEBS J* 277:394–403. doi:10.1111/j.1742-4658.2009.07490.x

5 **5.4 Capítulo 4: “Fungi in glacier ice of Antarctica: diversity, distribution, and
bioprospection of bioactive compounds”**

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Running title: Fungi in glacier ice of Antarctica

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Abstract

25 We identified the resident fungi present in samples of glacier ice of Antarctica. The
collections yielded 66 isolates identified by molecular biology methods into 27 taxa of 14
genera. *Penicillium palitans*, *Penicillium* sp. 1, *Thelebolus balaustiformis*, *Glaciozyma*
antarctica, *Penicillium* sp. 7, *Rhodotorula mucilaginosa*, and *Rhodotorula dairenensis* were
the dominant taxa with the highest densities. The diversity and richness of ice fungal
30 community were high and dominance moderate. *Penicillium* species occurred in ice of all
sites samples and *Penicillium chrysogenum* displayed the broadest distribution. Among them,
P. chrysogenum, *P. palitans*, and *Penicillium* spp. produced trypanocidal, leishmanicidal, and
herbicidal compounds. *Penicillium chrysogenum* showed the highest herbicidal, trypanocidal,
and leishmanicidal activities. We discover a rich and diverse fungal community, including
35 species unreported before to glacial ice of Arctic and Antarctica. We found in the glacial ice
of Antarctica a fungal community dominated by the airborne genus *Penicillium*, which might

5 be present in the gas bubbles trapped of the ice samples. These *Penicillium* taxa were able to produce bioactive compounds and may represent old and wild strains with genetic and biochemical pathways to produce new secondary bioactive metabolites.

Key words: Antarctica, ancient ice, fungi, secondary metabolites

10 **Introduction**

Antarctica is one of most pristine regions where occurs different extreme conditions of low, acid and alkaline pH, ultra-oligotrophic conditions, freeze-thaw cycles, difference of salinity, strong winds, and high UV radiation; these characteristics offer unique opportunities to study the biology of extremophile organisms (Rosa et al. 2019). Antarctica presents the largest amount of glacial ice of the planet, which represents one unknown ecosystem where resident organisms to survive have to show biochemistry mechanisms against low temperatures, water activity, nutrients, and high level of solar radiation (de Menezes et al. 2019a).

The life in icy environments seems to be dominated by microorganisms; however, the majority of the studies characterized in glacial ice Archaea and bacteria, and rarely fungi (Gunde-Cimerman et al. 2003; Sonjak et al. 2006). In Antarctica scarce studies were made to recover and identify fungi in glacial ice and only few species of the genera *Alternaria*, *Aspergillus*, *Aureobasidium*, *Candida*, *Curvibasidium*, *Cystofilobasidium*, *Davidiella*, *Penicillium*, *Cryptococcus*, *Leucosporodinium*, *Pseudozyma*, *Trichoderma*, and *Rhodotorula* were characterized until now (Jacobs and Shafer 1964; Abyzov et al. 2004; D'Elia et al. 2009; Knowlton et al. 2013; Sanyal et al. 2018) and the resident ice fungal community remains practically unknown.

As glacial ice has its origin on the snow precipitation, followed by compaction over the several years, its represent an interesting and unique habitat for special microorganisms, including spores and mycelia fragments of fungi, present in the air from thousand years ago and trapped in ice matrix (Abyzov 1993; Gunde-Cimerman et al. 2003). In addition, according to Sonjak et al. (2006) viable fungi cells were already isolated from Arctic and Antarctic ice, ranging in age from 10.000 and up to 140.000 years and considered as randomly entrapped fungal Aeolian propagules originating from close and distant locations.

For the reasons described above, in the present study, we recover and identified the cultivable fungi present in fragments of glacial ice of different sites of Antarctica and evaluated their potential as producers of bioactive secondary metabolites useful against neglected tropical diseases and as herbicides in agriculture.

5 **Methods**

Ice sampling

The samples of glacier fragment ice were collected in the South Shetland Islands, Antarctica in the austral summer season in the December of 2015 and 2016 (Suppl. Table 1). Approximately 10 kg of each ice fragment was collected using sterilized suits and gloves to
10 minimize contamination. The ice samples were disinfested with sodium hypochlorite at 5%, (10 s), sterilized distilled water (10 s), and expose under ultraviolet radiation (10 min) (Rogers et al. 2004). After that, the ice fragments were melted in sterilized bucket in the Microbiology Laboratory on board the Brazilian polar ship admiral Maximiano over a period of about 48 h. This process resulted in 5 L of water. The physico-chemical parameters (temperature,
15 conductivity, salinity, pH, chlorophyll a) of the melted ice of each site were obtained using the Hanna HI 9829 multi-parameter display system.

Fungal isolation

A total of 3 L of melted ice from each sample was filtered through a 0.45 µm membrane with
20 47 mm diameter (Millipore, USA) in triplicate. The membranes were placed on Sabouraud agar (Acumedia, India) and Minimal Medium (6.98 K₂HPO₄; 5.44 KH₂PO₄; 1.0 (NH₄)₂SO₄; 1.1 MgSO₄ 7H₂O, 0.25 peptone, 20 agar in g L⁻¹, pH 6.8 ± 0.2), both containing 200 µg mL⁻¹ of chloramphenicol (Sigma, USA) and incubated at 10 °C for 60 d. The fungi were purified in new Petri dishes containing Sabouraud agar and deposited in the Collection of
25 Microorganisms and Cells of the Universidade Federal de Minas Gerais, Brazil, under the code UFMGCB in cryotubes at -80 °C and in distillate-sterilized water (Castellani 1967) at room temperature, which are available to be accessed by other scientists.

Fungal identification

30 The molecular biology identification of fungi was made using different strategy for filamentous and unicellular (yeast) fungi. The protocol for DNA extraction was described previously in Rosa et al. (2009). Briefly, the dried DNA was re-suspended in 100 µL TE-buVer (10 mM Tris, 10 mM Na-EDTA, pH 8.0) with 2.5 µL RNase-solution, which was prepared by adding 10 mg of pancreatic RNase 20 U mg⁻¹ to 1 mL 0.01 M Na-acetate,
35 heating to 100 °C for 15 min, cooling slowly to room temperature, then adjusting the pH to 7.4 with 100 µL Tris-HCl. The sample was incubated for 30 min at 37 °C and then stored at -20 °C until use. 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). 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). Yeast isolates with query coverage and identity $\geq 99\%$ 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 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 identity $\leq 98\%$ or an inconclusive taxonomic position were subjected to phylogenetic ITS analysis for comparison with sequences of ex type species deposited in the GenBank database, with estimations conducted using MEGA Version 6.0 (Tamura et al. 2013). The maximum composite likelihood method, which represents the best choice to evaluate the evolutionary distances among fungi from different genus and families, was employed to estimate evolutionary distances with bootstrap values calculated from 1.000 replicate runs. The information about fungal classification generally followed the dictionary Kirk et al. (2008), MycoBank (<http://www.mycobank.org>) and the Index Fungorum (<http://www.indexfungorum.org>).

Morphological characterization

After sequence analysis, *Penicillium* species with unknown taxonomic positions were characterized according to their macroscopic properties (colony colour, texture, reverse colour, border type, sporulation, and the production of yellow soluble pigments). The diameters of the colonies were observed on YES and CYA medium at 25 and 30 °C, respectively for 14 days (Houbraken et al. 2012). The resulting colours were graded according to the specifications proposed by Kornerup and Wanscher (1984).

Diversity, richness, dominance and distribution

To quantify species diversity, richness and evenness, we used the following indices: (i) Fisher's α , (ii) Margalef's and (iii) Simpson's, respectively. The similarities among fungal taxa from different areas were estimated using the Sorensen coefficient (QS) and Bray-Curtis (B) measures. All of the results were obtained with 95% confidence, and bootstrap values were calculated from 1.000 iterations. All diversity and similarity indices calculations were performed using the computer programme PAST, version 1.90 (Hammer et al. 2001).

5 *Fungal cultivation and extract production for biological assays*

All fungal isolates were cultivated according to protocols established by Rosa et al. (2013). Briefly, 5 mm diameter plug of each fungus was placed on 20 mL of PDA medium at the center of Petri dishes (90 mm diameter) and cultured for 15 days at 25 ± 2 °C (time enough to fungi produce secondary metabolites). These fungal cultures were lyophilized (Lyophilizer
10 LioTop-K105) for 96 h, cut in small pieces and transferred to 50 mL glass centrifuge tubes followed by the addition of 50 mL of dichloromethane (DCM, Fisher, USA). After 96 h 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/USA) to prepare a 100 mg mL⁻¹ stock solution, which was stored at -20 °C. Sterile PDA medium
15 was extracted using the same procedure. The sterile PDA medium extract was used as the control in the screening procedure.

Assay against Trypanosoma cruzi

The *in vitro* test of trypanocidal activity was performed as previously described by Romanha
20 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 μ L of supplemented medium are added to each well of a 96-well microtitre plate. After
25 an overnight incubation, 40,000 trypomastigotes in 20 μ L 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 μ L 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 μ L) and the plate is incubated for 96 h at 37°C. After this period, 50 μ L of
30 500 μ M 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 μ M (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
35 infected cells and untreated cells. Duplicates are run in two different plates.

5 *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 $\mu\text{g mL}^{-1}$. The results are expressed as percent inhibition in relation to controls without drugs.

10 Amphotericin B at 0.2 $\mu\text{g mL}^{-1}$ (Fungison® Bristol-Myers Squibb B, Brazil) was used as a positive drug control. All assays were performed in triplicate.

Herbicide assay

The herbicidal activities of fungal extracts at 1 mg mL^{-1} against *Lactuca sativa* (lettuce) and

15 *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

20 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

25 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.

30 **Results**

Fungal identification

The fungal isolates were identified by molecular sequencing of the ITS, β -tubulin, ribosomal polymerase B2, and D1-D2 domains representing 27 taxa of 14 distinct genera (Table 1). The taxa with the greatest densities were *Penicillium palitans*, *Penicillium* sp. 1, *Thelebolus*

35 *balaustiformis*, *Glaciozyma antarctica*, *Penicillium* sp. 7, *Rhodotorula mucilaginosa*, and *Rhodotorula dairenensis*. In contrast, the majority of the fungal taxa occurred as rare components within the fungal assemblages with low densities and $\text{UFC L}^{-1} \leq 3.33$.

5 *Ecological diversity indices and distribution*

In general, the diversity (Fisher α 11.02) and richness (Margalef 5.46) of ice fungal community were high and dominance moderate (Simpson 0.83). However, the indices ranged among the different sites (Table 2). The highest diversity indices were obtained from fungal assemblages present in ice samples of Livingston Island and the lowest value were obtained in
10 Arctowski Peninsula, Rozier/Woodbury and Greenwich Island, Troub. The physic-chemical measures of the all ice samples seem to be homogeneous, except for those of Livingston Island, Charity and Antarctic Peninsula, Sikorsky, which displayed values of conductivity and total dissolved solids above of others samples. The values of temperature of the ice melted, pH, and salinity were similar among the samples. No one correlation was observed among the
15 physic-chemical parameters of the different ice samples with the diversity indices of fungal assemblages.

The fungal composition was variable among the ice at different sites, which was demonstrated by the values of the Sorensen and Bray-Curtis similarity indices (Suppl. Fig. 1). The Sorensen index showed that the most similar fungal assemblages were found in Greenwich Island,
20 Troub and King George Island. However, the Bray-Curtis similarity between the fungal assemblages of ice collected in Livingston Island, Huron and Antarctic Peninsula, Blérnot indicated the highest similarity. Additionally, *Penicillium* species occurred in glacial ice of all sites and *Penicillium chrysogenum* displayed the broadest distribution.

25 *Bioprospection of bioactive secondary metabolites*

Among the 15 fungal isolates screened, 13 were able to produce compounds with biological activities against the different targets (Table 3). All of them are taxa of the *Penicillium*, including *P. chrysogenum*, *P. palitans*, and *Penicillium* spp. Extract of different of *P. chrysogenum* isolates present strong biological activities, in special the isolated UFMGCB
30 12439 showed the highest herbicidal and trypanocidal activities. *Penicillium palitans*, *Penicillium* sp. 2, *Penicillium* sp. 6, *Penicillium* sp. 7 showed selective herbicidal and trypanocidal activities.

Discussion

35 Despite to represent an extreme and ultra-oligotrophic environment, the glacial ice of Antarctica seems to shelter a rich biodiversity of fungi. According to Gunde-Cimerman et al. (2003), the diversity of the fungal community's present in cold ecosystems are little known, in special those in glacial ice where low temperatures induce the formation of ice crystals that

5 kill normal cells and also due the low water activity (aw). In our study, few species occurred in high densities, mainly those of *Penicillium*, *Thelebolus*, *Glaciozyma*, and *Rhodotorula*. However, a considerable number at low densities of taxa were detected, which represented rare taxa into the fungal community.

Thelebolus comprises species that occur in different habitats of the world (Crous et al. 2004
10 [www.mycobank.or]) and are often been isolated from Arctic and Antarctic regions (Kobayasi et al. 1967; Montemartini et al. 1993). de Hoog et al. (2005) reported *Thelebolus* was an abundant fungus present in lakes and in association with skuas, petrels and other birds in Antarctica. In addition, *Thelebolus* was the dominant genus found by Brunati et al. (2009) and Gonçalves et al. (2012) in Antarctic lakes. *Thelebolus balaustiformis* represent a new species
15 described recently and isolated from the sponge *Dysidea fragilis* in Gurraig Sound, Atlantic Ocean (Bovio et al. 2018). However, *T. balaustiformis* represent a new report to Antarctica.

The *Glaciozyma antarctica* (re-classification of *Leucosporidium antarcticum*) was firstly isolated from Antarctic marine waters (Fell et al. 1969), soils around to Lake Fletcher, Lichen and Taylor Valleys, and on dead sponge (Turchetti et al. 2011), in the seawater of the
20 Northern Antarctic Peninsula (Gonçalves et al. 2017), and snow of different sites of Antarctic Peninsula (de Menezes et al. 2019b).

Rhodotorula species occur in different ecosystems and abundantly grow in extreme environments (Sampaio 2004; Margesin et al. 2007). *Rhodotorula mucilaginosa* is ubiquitous in cold and extreme environments (Sampaio 2011). In Antarctica, *R. mucilaginosa* are
25 commonly isolated from terrestrial such soils, lake and marine sediments (Vaz et al. 2011) and associated with macroalgae (Godinho et al. 2013). *Rhodotorula mucilaginosa* was already reported in ancient sections of glacial ice (age between 500.000 -175.000 years) of Lake Vostok in Antarctica (Knowlton et al. 2013).

Penicillium species has broad worldwide distribution and different studies reported it in
30 several living and non-living substrates in Antarctica. Among them two species call attention, *P. palitans* was obtained at high densities from one site and *P. chrysogenum* obtained from glacial ice of different sites, but in low densities. *Penicillium* species are common habitant of glacial ice and different taxa already were reported in glacial ice of Arctic (Gunde-Cimerman et al., 2003; Sonjak et al. 2006) and Antarctica (D'Elia et al. 2009). *Penicillium palitans*
35 represent a cold resistant species already found in glacial ice and was obtained at moderate density in the large Arctic fjords found on the western coast of Spitsbergen (Sonjak et al. 2006). Recently, Kozlovsky et al. (2019) reported *P. palitans* from undisturbed Antarctic permafrost with age of 30.000 thousand years that was able to produce the secondary

5 metabolites festuclavine, fumigaclavines A and B. *Penicillium chrysogenum* was isolated from macroalgae thalli (Godinho et al. 2013; Furbino et al. 2014), seawater across the Antarctic Peninsula (Gonçalves et al. 2017) and continental glacial ice (D'Elia et al. 2009). *Penicillium* includes species known due to their capabilities to produce different bioactive compounds. Antipova et al. (2018) reported *Penicillium* strains isolated from Antarctic soils
10 as prolific producers of secondary bioactive metabolites. *Penicillium* from the Antarctic call attention because they can be considered as live factories producing unknown bioactive secondary metabolites, but few species and strains were investigated chemically until now (Rosa et al. 2019). According to Houbraken et al. (2012), *P. chrysogenum* is one of the species recognized producers of penicillin. Strains of *P. chrysogenum* from Antarctica to be
15 producers of rugulosin and skyrin (bioactive bis-anthraquinones) which are able to demonstrate selective antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecium*, and *Escherichia coli* (Brunati et al. 2009). Extract of *P. chrysogenum* recovered from the endemic Antarctic macroalgae *Palmaria decipiens* displayed high and selective antifungal and/or trypanocidal activities (Godinho et al. 2013). In addition, strains of *P. chrysogenum*
20 recovered from ultra-oligotrophic soils of continental Antarctica displayed trypanocidal and herbicidal activities (Godinho et al. 2015).

Despite the extreme conditions of glacial ice like low temperature, very low nutrient available (ultra-oligotrophic conditions), and low water activity, we discover a rich and diverse fungal community, including species unreported before to glacial ice of Arctic and Antarctica.
25 According de Menezes et al. (2019), one of the most important studies of glaciologists is the use of samples of glacial ice to understand the physico-chemical composition of the ancient atmosphere to understand the current global climatic changes. Old ice samples can reveal past the atmospheric composition and temperatures of the Earth from the gases trapped in the bubbles of old ice. However, in these gas bubbles trapped in old glacial ice may have fungal
30 spores or hyphal fragments, which might represent taxa taxa and/or strains that lived in the Earth's atmosphere thousands of years ago and a portion of microbial community of the planet in the past. We found in the glacial ice of Antarctica a fungal community dominated by the airborne genus *Penicillium*, which might be present in the gas bubbles trapped of the ice samples. In addition, these *Penicillium* taxa were able to produce bioactive compounds and
35 may represent old and wild strains with genetic and biochemical pathways to produce new secondary bioactive metabolites. However, further studies of whole genome and chemical dereplication will be necessary to determine the age of this species and identify their bioactive compounds, respectively.

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10 References

- Abyzov SS (1993) Microorganisms in the Antarctic ice. In: Friedmann EI (ed) Antarctic microbiology. Wiley, New York, pp 265-295
- Abyzov SS, Hoover RB, Imura S, Mitskevich IN, Naganuma T, Poglazova MN, Ivanov MV (2004) Use of different methods for discovery of ice-entrapped microorganisms in ancient layers of the Antarctic glacier. *Adv Space Res* 33:1222-1230
- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389-3402
- Antipova T V, Zhelifonova VP, Baskunov BP, Kochkina G A, Ozerskaya SM, Kozlovskii A G (2018) Exometabolites the *Penicillium* Fungi Isolated from Various High-Latitude Ecosystems. *Microbiology* 87:642-651
- Bovio E, Garzoli L, Poli A, Prigione V, Firsova D, McCormack GP, Varese GC (2018) The culturable mycobiota associated with three Atlantic sponges, including two new species: *Thelebolus balaustiformis* and *T. spongiae*. *Fungal Syst Evol* 1:141-167
- Branda E, Turchetti B, Diolaiuti G, Pecci M, Smiraglia C, Buzzini P (2010) Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone Glacier, Apennines, Italy). *FEMS Microbiol Ecol* 72:354-369
- Brunati M, Rojas JL, 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
- Callahan HL, Portal AC, Devereaux R, Grogl M (1997) An axenic amastigote system for drug screening. *Antimicrob Agents Chemother* 41:818-822
- Castellani A (1967) Maintenance and cultivation of common pathogenic fungi in distilled water. *J Trop Med Hygien* 42:181-184
- Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. *Stud Mycol* 50: 19-22
- D'Elia T, Veerapaneni R, Theraisnathan V, Rogers SO (2009) Isolation of fungi from Lake Vostok accretion ice. *Mycologia* 101:751-763
- Dayan FE, Romagni JG, Duke SO (2000) Investigating the mode of action of natural phytotoxins. *J Chem Ecol* 26: 2079-2094

- 5 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. *Stud Mycol* 51:33-76
- de Menezes GCA, Porto BA, Simões JC, Rosa CA, Rosa LH (2019) Fungi in snow and glacial ice of Antarctica. In: Rosa LH (eds) *Fungi of Antarctica*, 1st edn. Springer, Switzerland AG, pp 127-146
- 10 Fell JW, Statzell AC, Hunter IL, Phaff L (1969) *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. *Antonie Van Leeuwenhoek* 35:433-462
- Furbino LE, Godinho VM, Santiago IF, Pellizari FM et al (2014) Diversity Patterns, Ecology and Biological Activities of Fungal Communities Associated with the Endemic Macroalgae Across the Antarctic Peninsula. *Microb Ecol* 67: 775-787
- 15 Godinho VM, Furbino LE, Santiago IF et al (2013) Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. *ISME J* 7:1434-1451
- 20 Godinho VM, Gonçalves VN, Santiago IF et al (2015) Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica. *Extremophiles* 19: 585-596
- 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
- 25 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 DB, Teixeira JA, Bazzolli DMS, de Queiroz MV, de Araújo EF (2012). Use of response surface methodology to optimize production of pectinases by recombinant *Penicillium griseoroseum* T20. *Biocatal Agric Biotechnol* 1:140-146
- 30 Gunde-Cimerman N, Sonjak S, Zalar P, Frisvad JC, Diderichsen B, Plemenitaš A (2003). Extremophilic fungi in arctic ice: a relationship between adaptation to low temperature and water activity. *Phys Chem Earth, Parts A/B/C*, 28:1273-1278
- Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Paleontol Electron* 4:1-9
- 35 Houbraken J, Frisvad JC, Seifert KA, Overby DP, Tuthill DM, Valdez JG, Samson RA (2012) New penicillin-producing *Penicillium* species and an overview of section *Chrysogena*. *Persoonia* 29:78
- Jacobs PH, Taylor HC, Shafer JC (1964) Studies of fungi at Amundsen-Scott IGY South Pole
- Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) *Dictionary of the Fungi*, CAB International, Wallingford
- 40 Knowlton C, Veerapaneni R, D'Elia RSO (2013) Microbial analyses of ancient ice core sections from Greenland and Antarctica. *Biology*, 2:206-232

- 5 Kobayasi Y, Hiratsuka N, Korf RP, Tubaki K, Aoshima K, Soneda M, et al. (1967) Mycological studies of the Alaskan Arctic. Res Commun Inst Ferment Osaka 3:1-138.
- Kornerup A, Wanscher JH (1984) Methuen Handbook of Colour. Eyre Methuen, London
- Kozlovsky AG, Kochkina GA, Zhelifonova VP, Antipova TV, Ivanushkina NE, Ozerskaya SM (2019) Secondary metabolites of the genus *Penicillium* from undisturbed and anthropogenically altered Antarctic habitats. Folia Microbiol 1-8.
- 10 Kurtzman CP, Fell JW, Boekhout T (2011) The Yeasts: A taxonomic study. Elsevier, Amsterdam
- Lachance MA, Bowles JM, Starmer WT, Barker JSF (1999) *Kodamaea kakaduensis* and *Candida tolerans*, two new yeast species from Australian Hibiscus flowers. Can J Microbiol 45:172-177
- 15 Margesin R, Fonteyne PA, Schinner F, Sampaio JP (2007) *Rhodotorula psychrophila* sp. nov., *Rhodotorula psychrophenolica* sp. nov. and *Rhodotorula glacialis* sp. nov., novel psychrophilic basidiomycetous yeast species isolated from alpine environments. Int J Syst Evol Microbiol 57:2179-2184
- 20 Montemartini A, Caretta G, Del Frate G (1993) Notes on *Thelebolus microsporus* isolated in Antarctica. Mycotaxon 48:343-358
- Rogers SO, Theraisnathan V, Ma LJ et al (2004) Comparisons of Protocols for Decontamination of Environmental Ice Samples for Biological and Molecular Examinations. Appl and Environ Microbiol 70: 2540-2544.
- 25 Romanha AJ, de Castro SL, Soeiro MN. 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 LH, Zani CL, Cantrell CL, Duke SO, Dijck PV, Desideri A, Rosa CA (2019) Fungi in Antarctica: diversity, ecology, effects of climate change, and bioprospection for bioactive compounds. In: Rosa LH (eds) Fungi of Antarctica, 1st edn. Springer, Switzerland AG, 1-17
- 30 Rosa LH, Queiroz SCN, Moraes RM, et al (2013) *Coniochaeta ligniaria*: antifungal activity of the cryptic endophytic fungus associated with autotrophic tissue cultures of the medicinal plant *Smallanthus sonchifolius* (Asteraceae). Symbiosis 60: 133-142
- 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
- 35 Sampaio JP (2004) Diversity, phylogeny and classification of basidiomycetous yeasts. In: Agerer R, Blanz P, Piepenbring M (eds). Frontiers in Basidiomycete Mycology IHW-Verlag, Germany, pp 49-80
- 40 Sanyal A, Antony R, Samui G, Thamban M (2018) Microbial communities and their potential for degradation of dissolved organic carbon in cryoconite hole environments of Himalaya and Antarctica. Microbiol Res 208:32-42

- 5 Sonjak S, Frisvad JC, Gunde-Cimerman N (2006) *Penicillium* mycobiota in Arctic subglacial ice. *Microb Ecol* 50:207-216
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30: 2725-2729

Tabela 6. Table 1. Fungi obtained from Antarctic glacial ice fragments and identified by sequence comparison with the BLASTn match with the NCBI GenBank database

Region/Island/Glacier	Density (CFU L ⁻¹)	UFMGCB ^a	Top BLAST search results (GenBank accession number)	Query cover (%)	Identity (%)	N° of bp analyzed	Proposed taxa (GenBank acc. n°) ^e
King George Island, Ajax/Stain House	20.66	12440	<i>Penicillium rubens</i> (NR111815) ^b	100	100	468	<i>Penicillium</i> sp. 1
			<i>Penicillium tardochrysogenum</i> (JX996898) ^c	97	99	359	(MN105129) ^g
	5.33	12439	<i>Penicillium tardochrysogenum</i> (MH865983) ^b	100	100	495	<i>Penicillium</i>
			<i>Penicillium chrysogenum</i> (AY495981) ^c	100	99	352	<i>chrysogenum</i>
			<i>Penicillium chrysogenum</i> (JX996668) ^d	100	100	879	(MN105128) ^g
	11.33	12441	<i>Thelebolus balaustiformis</i> (NR159056) ^b	100	100	494	<i>Thelebolus</i> <i>balaustiformis</i> (MN105143) ^g
	1.6	13080	<i>Chaetomium globosum</i> (MH858130) ^b	99	99	452	<i>Chaetomium</i> cf. <i>globosum</i> (MN105150) ^g
Antarctic Sikorsky Peninsula,	40.66	12446	<i>Penicillium caseifulvum</i> (MH862722) ^b	100	100	498	<i>Penicillium palitans</i>
			<i>Penicillium palitans</i> (KU904360) ^d	99	99	429	(MN105130) ^g
	0.66	12448	<i>Preussia flanaganii</i> (MH860647) ^b	100	99	422	<i>Preussia flanaganii</i> (MN105146) ^g
	1.9	13070	<i>Penicillium tardochrysogenum</i> (MH865983) ^b	100	100	406	<i>Penicillium</i> sp. 2 (MN105141) ^g
	0.3	13069	<i>Antarctomyces psychrotrophicus</i> (MH874317) ^b	100	99	500	<i>Antarctomyces</i> <i>psychrotrophicus</i> (MN105151) ^g
	1.6	18498	<i>Meyerozyma guilliermondii</i> (KY1085411) ^e	100	100	563	<i>Meyerozyma</i> <i>guilliermondii</i>
	4.9	18501	<i>Rhodotorula mucilaginosa</i> (KY2187281) ^e	100	100	565	<i>Rhodotorula</i> <i>mucilaginosa</i>
Arctowski Peninsula, Rozier/Woodbury	0.3	13065	<i>Penicillium rubens</i> (NR1118151) ^b	100	99	467	<i>Penicillium</i> sp. 3 (MN105142) ^g
Livingston Island,	0.66	12600	<i>Neomicrosphaeropsis italica</i> (NR158247) ^b	100	98	478	<i>Neomicrosphaeropsis</i> cf.

Charity							<i>italica</i> (MN105148) ^g
			<i>Penicillium commune</i> (NR111143) ^b	100	100	486	<i>Penicillium</i> cf.
3.33	12577		<i>Penicillium caseifulvum</i> (MH862722) ^d	100	100	379	<i>caseifulvum</i>
							(MN105131) ^g
1.33	12579		<i>Penicillium cavernicola</i> (MH862709) ^b	100	100	474	<i>Penicillium</i> sp. 4
							(MN105140) ^g
1.33	12575		<i>Penicillium resticulosum</i> (MH862492) ^b	100	99	511	<i>Penicillium</i> sp. 5
							(MN105132) ^g
							<i>Thelebolus</i>
0.66	12580		<i>Thelebolus balaustiformis</i> (NR159056) ^b	100	100	487	<i>balaustiformis</i>
							(MN105144) ^g
Greenwich Island, Troub			<i>Penicillium rubens</i> (NR111815) ^b				
0.66	12447		<i>Penicillium tardochrysogenum</i>	100	100	476	<i>Penicillium</i>
			(JX996898) ^c	100	99	270	<i>chrysogenum</i>
			<i>Penicillium chrysogenum</i> (JX9966681) ^d	100	99	478	(MN105133) ^g
Greenwich Island, Fuerza Aérea			<i>Penicillium rubens</i> (NR111815) ^b				
2.00	12444		<i>Penicillium tardochrysogenum</i>	100	100	465	<i>Penicillium</i>
			(JX996898) ^c	100	100	315	<i>chrysogenum</i>
			<i>Penicillium chrysogenum</i> (JX996668) ^d	100	99	614	(MN105134) ^g
0.65	13071		<i>Penicillium tardochrysogenum</i>	100	100	430	<i>Penicillium</i> sp. 2
5	18497		(MH865983) ^b	100	100	574	(MN105137) ^g
			<i>Rhodotorula dairenensis</i> (KY108996) ^e	100	100	574	<i>Rhodotorula dairenensis</i>
5.6	18510		<i>Glaciozyma antarctica</i> (AY040665) ^e	100	99	677	
			<i>Glaciozyma antarctica</i> (AY040665) ^b	83	100	505	<i>Glaciozyma antarctica</i>
2.6	18514		<i>Glaciozyma litoralis</i> (MG736026) ^e	100	100	545	<i>Glaciozyma litoralis</i>
Livingston Island, Huron			<i>Penicillium rubens</i> (NR111815) ^b				
0.66	12445		<i>Penicillium tardochrysogenum</i>	100	100	476	<i>Penicillium</i>
			(JX996898) ^c	100	99	415	<i>chrysogenum</i>
			<i>Penicillium chrysogenum</i> (JN121487) ^d	97	99	938	(MN105135) ^g
							<i>Thelebolus globosus</i>
0.6	13067		<i>Thelebolus globosus</i> (MH862951) ^b	100	100	484	(MN105145) ^g
							<i>Graphium rubrum</i>
0.6	13075		<i>Graphium rubrum</i> (MH855487) ^b	97	100	522	(MN105149) ^g

	1.6	18496	<i>Papiliotrema laurentii</i> (NG056281) ^e	100	98	549	<i>Papiliotrema</i> sp.
	2.3	18499	<i>Rhodotorula mucilaginosa</i> (KY109056) ^e	100	100	583	<i>Rhodotorula mucilaginosa</i>
	3.3	18500	<i>Metschnikowia australis</i> (KY108453) ^e	77	100	605	<i>Metschnikowia australis</i>
	1	18513	<i>Sakaguchia dacryoidea</i> (KY1095291) ^e	98	99	579	<i>Sakaguchia dacryoidea</i>
	1	18515	<i>Glaciozyma litoralis</i> (HF9340091) ^e	96	100	593	<i>Glaciozyma litoralis</i>
Antarctic Peninsula, Leonardo/Blanchard	3	13062	<i>Penicillium chrysogenum</i> (MK178856) ^b	100	100	464	<i>Penicillium</i> sp. 6 (MN105138) ^g
	5.6	13081	<i>Penicillium kongii</i> (NR138336) ^b	100	99	465	<i>Penicillium</i> sp. 7 (MN105139) ^g
	13	18503	<i>Rhodotorula mucilaginosa</i> (KP2237141) ^e	100	100	556	<i>Rhodotorula mucilaginosa</i>
Antarctic Blérnot			<i>Penicillium tardochrysogenum</i> (HQ0267451) ^b	100	99	472	<i>Penicillium chrysogenum</i> (MN105136) ^g
	0.3	13066	<i>Penicillium chrysogenum</i> (JX996668) ^d	100	100	880	<i>Antarctomyces psychrotrophicus</i> (MN105152) ^g
	0.3	13068	<i>Antarctomyces psychrotrophicus</i> (MH874317) ^b	100	99	408	<i>Antarctomyces psychrotrophicus</i> (MN105152) ^g
	0.3	13082	<i>Petriella asymmetrica</i> var. <i>cypria</i> (MH855955) ^b	95	99	496	<i>Petriella</i> sp. (MN105147) ^g
	1.6	18506	<i>Rhodotorula mucilaginosa</i> (KY109056) ^e	100	99	441	<i>Rhodotorula mucilaginosa</i>

^aUFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. Taxa subjected to BLAST analysis based on the ^bITS, ^c β -tubulin,

^dPolymerase II, and D1/D2^e regions for elucidation of taxonomic positions. ^fTaxonomic position suggested. Sequences of ^gITS, ^h β -tubulin, ⁱPolymerase II and/or

^jD1/D2.

Tabela 7. Table 2 Physico-chemical parameters of melted ice and diversity indices of fungal assemblages at the different sites

Parameters/diversity indices/density	Sites sampled								
	KGA	APR	LIC	LIH	GIT	GIF	APS	APL	APB
Temperature melted ice (°C)	15.6	16.1	17.5	10.1	9.6	16	15.6	14.5	4.6
Conductivity ($\mu\text{S cm}^{-1}$)	25	23	59	18	21	23.5	50	6	9
Resistivity ($\text{M}\Omega \text{ cm}^{-1}$)	0.04	0.06	0.01	0.10	0.04	0.06	0.08	0.16	0.11
Total dissolved solids (ppm)	12	11.5	30	9	11	11.5	25.5	3	4
Oxidation Reduction Potential (mV)	520.1	540.7	765.4	494.7	750.4	540.7	509.5	188.8	211.5
pH	6.51	6.65	6.62	6.9	6.7	6.7	7.4	6.5	6.6
Salinity (ppt)	0.01	0.01	0.03	0	0.02	0.01	0.02	0	0
Number of taxa	4	1	5	8	1	5	6	3	4
Fisher α	1.12	-	6.97	12.98	-	2.52	1.78	0.95	0
Margalef	0.82	-	2.48	3.36	-	1.51	1.28	0.65	0
Simpson	0.61	-	0.71	0.82	-	0.73	0.33	0.55	0.55

*The numbers in parenthesis represent the lower and upper diversity values, respectively, with 95% of confidence and bootstrap values calculated from 1.000 iterations. KGA = King George Island, Ajax/Stain House Glaciär; APR = Arctowski Peninsula, Rozier/Woodbury; GIT = Greenwich Island, Troub; GIF = Greenwich Island, Fuerza Aerea; LIC = Livingston Island, Charity; LIH = Livingston Island, Huron; APS= Antarctic Peninsula, Sikorsky; APL= Antarctic Peninsula, Leonardo/Blanchard; and APB= Antarctic Peninsula, Blérnot. - = no diversity indices.

Tabela 8. Table 3 Biological activities of extracts obtained from cultures of *Penicillium* species isolated from glacial ice from Antarctica

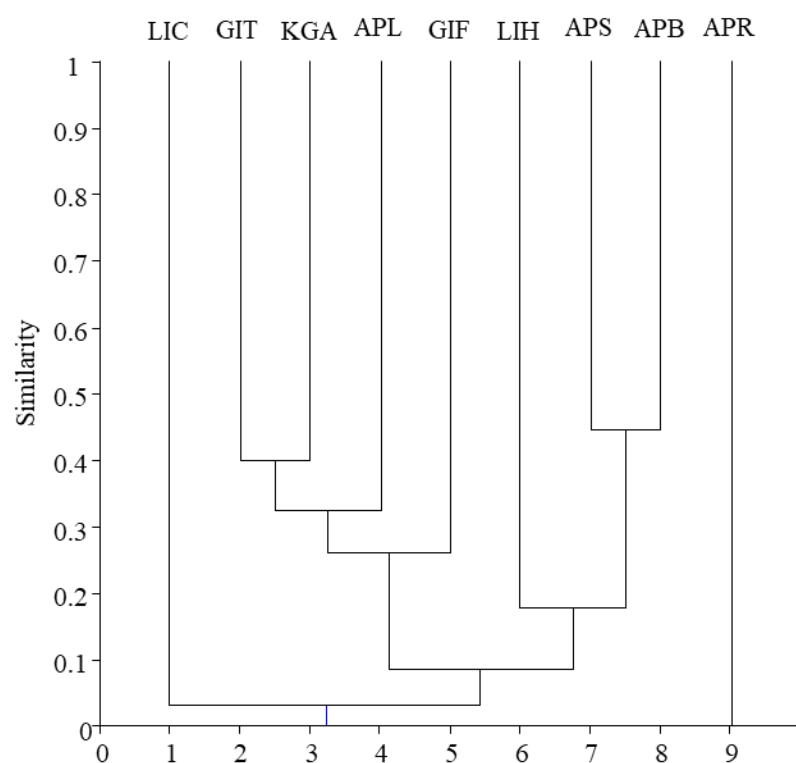
Fungal species	UFMGCB ^a	Biological activities			
		Herbicidal ^b		Antiparasitic ^c	
		<i>L. sativa</i>	<i>A. schoenoprasum</i>	<i>T. cruzi</i>	<i>L. amazonensis</i>
<i>Penicillium chrysogenum</i>	12439	5 ± 0	3.5 ± 2	100 ± 3	55 ± 5
<i>P. chrysogenum</i>	12447	4.5 ± 0.7	1.5 ± 0.7	72 ± 6	40 ± 0
<i>P. chrysogenum</i>	12444	4 ± 0	2.5 ± 2	72 ± 5	44 ± 1
<i>P. chrysogenum</i>	12445	5 ± 0	3.5 ± 0.7	82 ± 1.5	45 ± 0
<i>P. chrysogenum</i>	13066	5 ± 0	1 ± 1.4	67 ± 2	44 ± 1
<i>Penicillium palitans</i>	12446	5 ± 0	1.5 ± 0.7	100 ± 2	37 ± 1
<i>Penicillium</i> sp. 1	12440	2.5 ± 2	2 ± 0	66.8 ± 4	53 ± 5
<i>Penicillium</i> sp. 2	13070	5 ± 0	1 ± 1.4	99 ± 0.6	43 ± 1
<i>Penicillium</i> sp. 2	13071	2.5 ± 0.7	2.5 ± 0.7	84.5 ± 0.8	59 ± 1
<i>Penicillium</i> sp. 3	13065	5 ± 0	1 ± 1.4	93 ± 7	43 ± 0
<i>Penicillium</i> sp. 5	12575	4.5 ± 0.7	2.5 ± 0.7	87 ± 7	41 ± 0
<i>Penicillium</i> sp. 6	13062	4 ± 1.4	1.5 ± 0.7	100 ± 2	47 ± 0
<i>Penicillium</i> sp. 7	13081	5 ± 0	5 ± 0	94 ± 10	35 ± 1
Controls	Extract of PDA	2 ± 0	1 ± 0	0	0
	Glyphosate ^d	4.5 ± 0.7	5 ± 0	-	-
	Benzonidazole ^d	-	-	71 ± 3	-

Amphotecin B ^d	-	-	-	86 ± 6
DMSO ^e 1%	-	-	0 ± 0	10 ± 4
Acetone ^e	1 ± 0	1 ± 1.4	-	-
Sterilised Water ^f	0 ± 0	0 ± 0	-	-

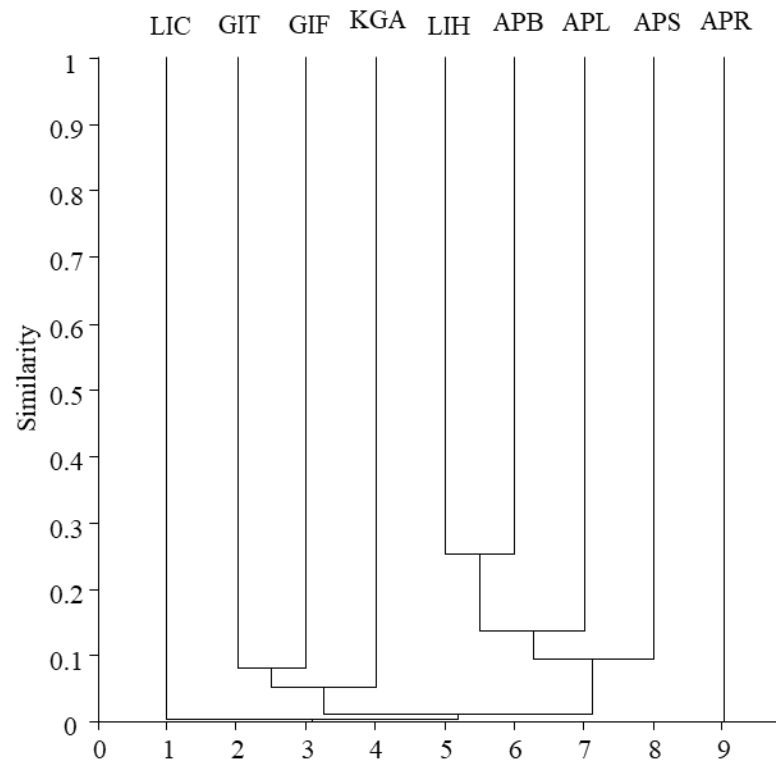
^aUFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. ^bThe qualitative estimate of phytotoxicity was evaluated by using a rating scale of 0-5, where 0 = no effect and 5 = no growth or no germination of the seeds. Plant targets: *L. sativa* = *Lactuca sativa* (lettuce), *A. schoenoprasum* = *Allium schoenoprasum* (chive). ^cResults of effective extract with inhibition of $\geq 50\%$ of *Trypanosoma cruzi* and $\geq 70\%$ of *Leishmania amazonensis* ^dPositive control, ^esolvent control and ^fnegative control. PDA = Potato dextrose agar.

Tabela 9. Supplementary Table 1 Sites were the fragment of glacial ice were sampled in Antarctica

Sites	Glacier	Coordinates
Antarctic Peninsula	Sikorsky	64°12'S 60°53'W
Antarctic Peninsula	Leonardo/Blanchard	64°42'S, 61°58'W
Antarctic Peninsula	Blérnot	64°21'7S, 61°34'W
Arctowski Peninsula	Rozier/Woodbury	64°45'S 62°13'W
Greenwich Island	Troub	62°37'S, 059°70'W
Greenwich Island	Fuerza Aerea	62°30'S 59°38'W
King George Island	Ajax/Stain House	62°06'S, 058°27W
Livingston Island	Charity	62°44'S 60°20'W
Livingston Island	Huron	62°37'50"S 60°06'50"W



(a)



(b)

Figure 7. Supplementary Figure 1. Dendrogram showing the comparison of **(a)** Sorensen and **(b)** Bray-Curtis similarities measures for the fungal assemblages isolated from fragment glacial ice of different sites of Antarctic Peninsula. KGA = King George Island, Ajax/Stain House Glaciér; APR = Arctowski Peninsula, Rozier/Woodbury; GIT = Greenwich Island, Troub; GIF = Greenwich Island, Fuerza Aerea; LIC = Livingston Island, Charity; LIH = Livingston Island, Huron; APS= Antarctic Peninsula, Sikorsky; APL= Antarctic Peninsula, Leonardo/Blanchard; and APB= Antarctic Peninsula, Blérnot.

5 6 Discussão integrada

O gelo e a neve da Antártica representam um ambiente extremo e ultra-oligotrófico e de acordo com Gunde-Cimerman et al. (2003) a diversidade microbiana em ambientes extremamente frios está sob influência da formação de gelo intracelular e, conseqüentemente, de pouca água líquida disponível. Porém, nós detectamos uma rica e diversa comunidade fúngica na neve sazonal de inverno da Península Antártica, composta em sua maioria por espécies de leveduras basidiomicéticas em altas densidades (≤ 300 UFC L⁻¹), que ocorrem também em ambientes tropicais, temperados e polares. Resultados semelhantes foram obtidos por de García et al. (2007), que isolaram leveduras de rios formados por água de degelo de geleiras da Patagônia argentina. Estas leveduras basidiomicéticas também foram obtidas de camadas de geleiras do Ártico, nas Ilhas Svalbard, Noruega (BUTINAR; SPENCER-MARTINS; GUNDE-CIMERMAN, 2007) e em geleiras dos Alpes da Itália (TURCHETTI et al., 2008). Contudo, em ambos os estudos, as leveduras ocorreram em baixas densidades. Geralmente, em ambientes extremos, as comunidades de fungos são dominadas por fungos filamentosos pertencentes ao filo *Ascomycota* (CONNELL et al., 2008). Porém, a alta densidade de leveduras do filo *Basidiomycota* na neve antártica pode ser explicada pela grande diversidade do grupo e por representarem fungos dimórficos capazes de ocupar variados habitats, incluindo ambientes ultra-oligotróficos de ambientes extremos (SAMPAIO, 2004).

Dos 51 táxons encontrados na neve sazonal da Antártica, somente *Phenoliferia glacialis* (sinônimo de *Rhodotorula glacialis*) foi isolada de todos os locais amostrados e em altas densidades. *Phenoliferia glacialis* é uma levedura psicrófila que apresenta alta versatilidade metabólica e já foi isolada de geleiras na França e Áustria (MARGESIN et al., 2007). Recentemente, *P. glacialis* foi isolada de folhas de *Deschampsia antarctica*, musgos e de diferentes biofilmes presentes na Península Antártica (FERREIRA et al., 2019). Dentre as leveduras com alta densidade encontradas na neve está *R. mucilaginosa*, levedura pigmentada, ubíqua, presente em diferentes habitats e substratos, incluindo ambientes frios e extremos (SAMPAIO, 2004; 2011). *Rhodotorula mucilaginosa* é comumente isolada de substratos terrestres e marinhos da Antártica (VAZ et al., 2011; GODINHO et al., 2013). Apesar da extensa cobertura de neve do continente antártico, este estudo representa o primeiro relato sobre a alta densidade de *R. mucilaginosa* em amostras de neve de diferentes locais da Península Antártica.

Embora a comunidade fúngica da neve antártica seja composta em sua maior parte por leveduras, também encontramos espécies de fungos filamentos cosmopolitas e endêmicos. Após considerarmos aspectos morfológicos, fisiológicos e filogenéticos, caracterizamos e descrevemos uma nova espécie de fungo filamentoso ascomiceto, o *Antarctomyces pellizariae* (*Thelebolaceae*, *Ascomycota*). *Antarctomyces pellizariae* foi isolado da neve sazonal na Ilha Robert, Península Coppermine e é uma espécie endêmica da Antártica. As análises filogenéticas das regiões ITS, β -tubulina e RNA polimerase II mostraram que *A. pellizariae* é geneticamente distinto da outra espécie do gênero *A. psychrotrophicus* e outros táxons de *Thelebolaceae*, como demonstrado no artigo do **Capítulo 3. *Antarctomyces psychrotrophicus***, por ter sido isolado apenas em ambientes antárticos, é considerado uma espécie psicrófila endêmica de *Thelebolales* (SANTIAGO et al., 2015) e o gênero *Antarctomyces* era representado apenas pela espécie *A. psychrotrophicus*, a qual foi originalmente isolada a partir de solo da Ilha Rei George (STCHIGEL et al., 2001). Desta forma, *A. pellizariae* é a segunda espécie descrita para o gênero.

Os táxons isolados da neve antártica, previamente relatados na literatura como patógenos oportunistas humanos, foram avaliados quanto ao seu perfil de resistência ao fungicida ambiental benomil e aos antifúngicos clínicos fluconazol e anfotericina B. O azólicos testados possuem estruturas compostas por anéis aromáticos, similares a alguns POPs encontrados anteriormente na neve antártica (KANG et al., 2012). *Debaromyces hansenii*, *R. mucilaginosa*, *P. chrysogenum*, *Penicillium* sp. 3 e *Penicillium* sp. 4 demonstraram resistência frente os antifúngicos azólicos testados. *Debaryomyces hansenii* é considerada uma levedura cosmopolita, capaz de ocupar e colonizar diferentes nichos ecológicos e ecossistemas. *Debaryomyces hansenii* também já foi descrita como agente causador de infecções oportunista em humanos (WAGNER et al., 2005; GUPTA et al., 2006). No continente antártico, *D. hansenii* já foi isolada de diversos substratos, como solo e rizosfera (VAZ et al., 2011), talos de líquens (DUARTE et al., 2018), macroalgas (DUARTE et al., 2016) e de águas continental e marinha (BRANDÃO et al., 2017). No entanto, já foi relatado que isolados ambientais de *D. hansenii* apresentam elevados valores de CIMs para fluconazol, em relação as espécies de leveduras patogênicas (BREUER; HARMS, 2006). Embora os isolados de *D. hansenii* encontrados na neve antártica tenham apresentado suscetibilidade ao fluconazol, eles não cresceram a 37 °C. Contudo, da mesma forma que os resultados encontrados neste estudo, *D. hansenii* possui capacidade de sintetizar e/ou tolerar várias toxinas e tem sido relatada como resistente à vários pesticidas ambientais, dentre eles o benomil (BREUER; HARMS, 2006).

5 *Rhodotorula mucilaginosa* é considerada um agente patogênico fúngico oportunista emergente, principalmente em pacientes imunossuprimidos (WIRTH; GOLDANI, 2012). Isolados de *R. mucilaginosa* presentes na neve da Antártica foram resistentes ao fluconazol e suscetíveis a anfotericina B e ao benomil. Resultados semelhantes foram encontrados por Spiliopoulou et al. (2012), que verificaram que espécies oportunistas humanas de
10 *Rhodotorula* foram resistentes ao antifúngico fluconazol, mas suscetíveis a equinocandinas e a anfotericina B. Em geral, espécies oportunistas de *R. mucilaginosa* tem sido referidas como capazes de crescer a 30 °C. Contudo, os isolados de *R. mucilaginosa* encontrados na neve antártica foram resistentes ao fluconazol e capazes de crescer a 37 °C. Ainda, encontramos isolados de *Penicillium* na neve antártica que mostraram resistência ao fluconazol, porém
15 estes foram suscetíveis ao benomil. Espécies de *Penicillium* isoladas de amostras de solo ornitogênico e rochas da região peninsular e continental da Antártica mostraram potencial patogênico para humanos *in vitro* (DE SOUSA et al., 2017; GONÇALVES et al., 2017a; ALVES et al., 2019). *Penicillium chrysogenum*, também obtido da neve sazonal da Antártica, é reportado como um fungo oportunista em pacientes imunocomprometidos (DE HOOG et
20 al., 2000; SWOBODA-KOPEC et al., 2003), mas também já foi relatado causando doença cerebral em pacientes imunocompetentes (LYRATZOPOULOS et al., 2002)

Kang et al. (2012) detectaram pesticidas organoclorados (POCs) em diferentes concentrações na neve do leste da Antártica e sugeriram que as massas de ar que chegam aos locais amostrados são originárias principalmente dos oceanos Índico e Atlântico que viajam
25 pelo continente antártico, sugerindo que os POCs foram submetidos a transporte atmosférico de longo alcance e depositados na superfície da neve antártica. Nos últimos anos, alguns estudos têm focado na resistência cruzada de fungos frente a antifúngicos agrícolas e clínicos (SNELDERS et al., 2012; BASTOS et al., 2017; CARNEIRO et al., 2019), pois alguns fungos oportunistas também são cosmopolitas no meio ambiente e podem entrar em contato
30 com agrotóxicos e agentes antifúngicos utilizados na agricultura (HOF, 2001). Neste contexto, como os POCs possuem estruturas químicas semelhantes às dos antifúngicos azólicos, sua presença na neve na Antártica pode explicar a detecção de resistência intrínseca a antifúngicos agrícolas e clínicos em *D. hansenii*, *R. mucilaginosa*, *P. chrysogenum*, *Penicillium* sp. 3 e sp. 4.

35 Em contraste com a neve, o gelo glacial da Antártica apresentou uma comunidade fúngica dominada por *Ascomycota* e caracterizada por um número considerável de táxons em baixas densidades ($\text{UFC L}^{-1} \leq 3,33$), representando táxons raros dentro da comunidade. Poucas espécies ocorreram em altas densidades no gelo glacial antártico, dentre elas, estão os

5 representantes dos gêneros *Penicillium*, *Thelebolus*, *Glaciozyma* e *Rhodotorula*. Foram obtidos 66 isolados fúngicos do gelo glacial antártico, onde apenas espécies do gênero *Penicillium* ocorreram em todos os locais amostrados e *P. chrysogenum* apresentou ampla distribuição. *Glaciozyma antarctica* (reclassificação de *Leucosporidium antarcticum*), obtida da neve sazonal da Antártica, foi primeiramente isolada de águas marinhas da Antártica (FELL et al., 1969), posteriormente em solos da região continental (TOMAS-HALL, 2004) e na água do mar do Norte da Península Antártica (GONÇALVES et al., 2017). As espécies do gênero *Rhodotorula* ocorrem em diferentes ecossistemas e crescem abundantemente em ambientes extremos (SAMPAIO, 2004; MARGESIN et al., 2007). Assim como na neve antártica, *R. mucilaginosa* ocorreu em altas densidades no gelo glacial antártico em mais de uma região amostrada. *Rhodotorula mucilaginosa* já foi encontrada em seções antigas de gelo glacial (idade entre 500 mil – 175 mil anos) do lago de Vostok, na porção continental da Antártica (KNOWLTON et al., 2013).

O gênero *Thelebolus* compreende espécies que ocorrem em diferentes habitats do mundo (CROUS et al., 2004 [<http://www.mycobank.org/>]) e são frequentemente isolados das regiões do Ártico e Antártica (KOBAYASI et al., 1967; MONTEMARTINI et al., 1993). Na Antártica, espécies de *Thelebolus* ocorrem em abundância em lagos e em associação com skuas, petréis e outras aves (DE HOOG et al., 2005; GONÇALVES et al., 2012b). A espécie *Thelebolus balaustiformis*, obtida do gelo glacial antártico, foi isolada originalmente da esponja *Dysidea fragilis* no Oceano Atlântico e descrita recentemente por Bovio et al. (2018). No entanto, *T. balaustiformis* representa um novo relato para a Antártica. O gênero *Penicillium* possui ampla distribuição mundial e diferentes estudos citam espécies do gênero em variados substratos na Antártica (MCRAE; SEPPELT; HOCKING, 1999; GODINHO et al., 2013; ALVES et al., 2019). Entre eles, duas espécies chamam a atenção, *P. palitans* que foi isolado em altas densidades do gelo glacial apenas da Península Antártica, geleira Sikorsky e *P. chrysogenum*, isolado a partir do gelo glacial de cinco dos nove locais amostrados, mas em baixas densidades. Espécies de *Penicillium* são habitantes comuns do gelo glacial e diferentes táxons já foram relatados no gelo glacial do Ártico (GUNDE-CIMERMAN et al., 2003; SONJAK; FRISVAD; GUNDE-CIMERMAN, 2006) e da Antártica (D'ELIA et al., 2009). *Penicillium palitans* representa uma espécie resistente ao frio já encontrada no gelo glacial e foi obtida em densidade moderada nos grandes fiordes árticos (SONJAK; FRISVAD; GUNDE-CIMERMAN, 2006).

A partir de isolados de *Penicillium* do gelo glacial antártico, foram produzidos e testados 15 extratos fúngicos, dos quais 13 apresentaram atividades biológicas contra

5 diferentes alvos. Antipova et al. (2018) relataram espécies de *Penicillium* isoladas de solos antárticos como produtores de diferentes metabólitos secundários bioativos. *Penicillium* spp. isolados da Antártica chamam a atenção por serem considerados “fábricas vivas”, produtoras de metabólitos secundários bioativos que podem ser ainda desconhecidos, porém poucas espécies e linhagens foram investigadas quimicamente até o momento (ROSA et al., 2019).

10 Nossos resultados mostram que extratos de diferentes isolados de *P. chrysogenum* exibiram forte atividade biológica, em especial o isolado UFMGCB 12439, que apresentou as maiores atividades herbicidas e tripanosomicidas. Resultados semelhantes foram obtidos por Godinho et al. (2015), que isolaram linhagens de *P. chrysogenum* de solos ultra-oligotróficos da Antártica continental, os quais apresentaram atividades tripanosomicidas e herbicidas.

15 Além disso, o extrato de *P. chrysogenum*, isolado da macroalga antártica *Palmaria decipiens*, apresentou elevadas atividades antifúngica e tripanocida seletivas (GODINHO et al., 2013). Brunati et al. (2009) encontraram linhagens de *P. chrysogenum* da Antártica produtoras de rugulosina e skirin (bis-antraquinonas bioativas), substâncias que possuem atividade antibacteriana seletiva contra *Staphylococcus aureus*, *Enterococcus faecium* e *Escherichia*

20 *coli*. Recentemente, Kozlovsky et al. (2019) relataram *P. palitans* isolado de permafrost antártico antigo (datado com 30 mil anos) que foi capaz de produzir os metabólitos secundários festuclavina e fumigaclavinas A e B. O extrato produzido a partir de *P. palitans* isolado do gelo glacial antártico apresentou seletiva atividade herbicida e tripanocida.

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7 Conclusão e perspectivas

Nossos resultados mostram que a neve sazonal e o gelo glacial da Antártica, mesmo como substratos ultra-oligotróficos, demonstram ser uma rica fonte para o isolamento de fungos em todas as regiões amostradas, bem como substratos promissores para a caracterização de novas espécies. A neve sazonal de inverno da Península Antártica possui uma comunidade fúngica diversa, dominada por espécies de leveduras basidiomicéticas cosmopolitas já reportadas, para ecossistemas tropicais, temperados e polares. Entretanto, também apresenta espécies fúngicas que ainda não haviam sido descritas na literatura e relatadas pela primeira vez para a Antártica. As altas densidades encontradas de fungos cosmopolitas sugerem que eles podem estar presentes nas massas de ar que vem de outros locais do globo e chegam à Península Antártica, sendo então empurradas pela precipitação de neve, depositadas e compactadas em altas concentrações na neve a cada ano. Também detectamos isolados fúngicos ambientais resistentes a antifúngicos agrícolas e clínicos, dos quais alguns foram capazes de crescer a 37 °C. No entanto, não foi detectada uma correlação clara entre o fungicida benomil, que é utilizado na agricultura, com resistência cruzada frente aos antifúngicos clínicos fluconazol e anfotericina B. Mais estudos serão necessários para caracterizar o potencial de virulência intrínseca desses fungos contra humanos e animais, além de análises prévias da presença e quantidade de POPs na neve antártica.

Apesar das condições extremas do gelo glacial, como baixa temperatura, baixa disponibilidade de nutrientes, baixa atividade de água e congelamento, descobrimos uma comunidade fúngica rica e diversificada, incluindo espécies não relatadas antes para o gelo glacial do Ártico e da Antártica. Além disso, esta comunidade foi dominada por táxons do gênero *Penicillium*, que podem estar presentes nas bolhas de gás presas no interior do gelo glacial. Estes *Penicillium* foram capazes de produzir substâncias bioativas e podem representar linhagens antigas e selvagens, com vias genéticas e bioquímicas inéditas, capazes de gerar novos metabólitos secundários bioativos. No entanto, mais estudos utilizando técnicas de relógio molecular, genoma completo e desreplicação química serão necessários para determinar a idade, o número de diferenças moleculares dessas espécies, identificar e caracterizar seus compostos bioativos.

8 Atividades desenvolvidas no período

- 5 • **Co-orientação** de Trabalho de Conclusão de Curso: PORTO, B. A. Identificação de fungos isolados de segmentos de gelo glacial da Antártica por técnicas de taxonômica molecular, 2017. (Bacharelado em Ciências Biológicas) - Universidade Federal de Minas Gerais.
- 10 • **Avaliadora** da 6ª FEBRAT - Feira Brasileira de Colégios de Aplicação e Escolas Técnicas. Universidade Federal de Minas Gerais. 2018.
- 15 • **Organização de eventos:**
V SYMPOSIUM APECS-BRASIL Achievements and challenges in a decade of changes in national and international scene. 2018.
MUSEU ITINERANTE PONTO UFMG na 69ª Reunião Anual da SBPC (Sociedade Brasileira para o Progresso da Ciência) Jovem. 2017.
- 20 • **Publicações de artigos:**
DE MENEZES, G. C. A. et al. *Antarctomyces pellizariae* sp. nov., a new, endemic, blue, snow resident psychrophilic ascomycete fungus from Antarctica. **Extremophiles**, v. 21, n. 2, p. 1–11, 2017.
GONÇALVES, V. N. et al. Taxonomy, phylogeny and ecology of cultivable fungi present in seawater gradients across the Northern Antarctica Peninsula. **Extremophiles**, v. 21, n. 6, p. 1005–1015, 30 nov. 2017.
25 DE MENEZES, G. C. A. et al. Study of physiological and enzymatic properties and characterization of pathogenic activity of a fungus isolated from moss *Sanionia uncinata* (Hedw.) Loeske in Antarctica. **Polar Biology**, v. 42, p. 2473, 2019.
- 30 • **Capítulos de livros:**
GONÇALVES, V. N. et al. Biologia e Biotecnologia de Fungos. In: **Microbiologia Industrial – Bioprocessos**. 2017. p. 137-179.
DE MENEZES, G. C. A. et al. Fungi in Snow and Glacial Ice of Antarctica. In: **Fungi of Antarctica**. Cham: Springer International Publishing, 2019. p. 127–146.
35 DUARTE, A. W. F. Antarctic Fungi as Producers of Pigments. In: **Fungi of Antarctica**. Cham: Springer International Publishing, 2019. p. 127–146.
- 40 • **Resumos** apresentados no IX Congreso Latinoamericano de Ciencia Antártica 2017:
DE MENEZES, G. C. A. et al. Aislamiento e Identificación de los Hongos Presente em el Hielo Glacial de la Antártida.
ROSA, L. H., et al. Estudio Taxonómico de Hongos Presente Anillo Cocéntricos de Musgos Enfermeros que Viven en la Pensínsula Antártida.
GONÇALVES, V. N. Diversidad, Distribución, Filogenia y Ecología de las
45 Comunidades de Hongos Presente em el Gradiente de Agua de Mar a Través de la Antártida del Norte.
- 50 • **Resumo** apresentado no V SYMPOSIUM APECS-BRASIL Achievements and challenges in a decade of changes in national and international scene, 2018:
DE MENEZES, G. C. A. et al. Taxonomy and diversity of fungi present in snow and ice of the Antarctica.

- 5 • **Resumo** apresentado no POLAR 2018 - Where the Poles come together, 2018: de Menezes, G. C. A. et al. Diversity and Antifreeze Activity of Fungi in Snow of Antarctic Peninsula.
- 10 • **Participação em três Operações Antárticas Brasileiras** (OP XXXIV, OP XXXV e OPXXXVII nos anos de 2015, 2016 e 2018), pelo Programa Antártico Brasileiro (PROANTAR) a bordo do navio Polar Brasileiro Almirante Maximiano.
- 15 • **Participação em banca de avaliação** de Thaís Felix Miranda. Fungos endofíticos em espécies agrícolas de importância econômica. 2019. Monografia (Aperfeiçoamento/Especialização em Programa de Pós-Graduação em Microbiologia) - Universidade Federal de Minas Gerais.
- 20 • **Textos em revistas de divulgação científica:**
 DE MENEZES, G. C. A.; ROSA, LUIZ H. Fungo antártico azul, raro, novo e endêmico. Informativo APECS-Brasil, p. 20 - 20, 01 jul. 2017.
 SOUZA, J. S.; DE MENEZES, G. C. A. V Simpósio da APECS-Brasil Discutindo ciência polar e celebrando os 10 anos do nosso comitê nacional. Informativo APECS-BRASIL, p. 11 - 12, 01 jan. 2018.
 DE MENEZES, G. C. A.; SOUZA, J. S. Divulgando a Ciência Antártica Brasileira: apresentações orais e mini vídeos durante o V Simpósio da APECS-Brasil. Informativo APECS-BRASIL, p. 12 - 12, 01 jan. 2018.
 SOUSA, J. B. et al. Relatos sobre a XXI Semana Polar Internacional na Escola Estadual Menino Jesus de Praga sob a visão de pesquisadores polares e professores. Informativo APECS-Brasil, Brasil, p. 8 - 9, 31 dez. 2018.
- 30 DE MENEZES, G. C. A. et al. Como o conhecimento da Biodiversidade de Fungos da Antártica pode ajudar a melhorar o mundo? InformativoAPECS-Brasil, p. 23 - 24, 02 set. 2017.
- 35 • **Premiação** como melhor apresentação oral no V SYMPOSIUM APECS-BRASIL Achievements and challenges in a decade of changes in national and international scene, 2018, com o trabalho DE MENEZES, G. C. A. et al. Taxonomy and diversity of fungi present in snow and ice of the Antarctica.

9 Referências bibliográficas

- 5 ABYZOV, S. S. Microorganisms in the Antarctic ice. **Antarctic Microbiology**, v. 1, n. 1, p. 265-296, 1993.
- ALBORÉS, S. et al. Biodiversity and antimicrobial activity of antarctic fungi from the fildes peninsula, king george island. **Sydowia**, v. 70, n. August, p. 185–192, 2018. Disponível em: <<https://www.scopus.com/inward/record.uri?partnerID=HzOxMe3b&scp=85061613718&origin=inward>>.
- 10 ALVES, I. M. S. et al. The diversity, distribution, and pathogenic potential of cultivable fungi present in rocks from the South Shetlands archipelago, Maritime Antarctica. **Extremophiles**, v. 23, n. 3, p. 327–336, 9 maio 2019. Disponível em: <<https://doi.org/10.1007/s00792-019-01086-8>>.
- 15 AMATO, P.; DOYLE, S.; CHRISTNER, B. C. Macromolecular synthesis by yeasts under frozen conditions. **Environmental Microbiology**, v. 11, n. 3, p. 589–596, mar. 2009. Disponível em: <<http://doi.wiley.com/10.1111/j.1462-2920.2008.01829.x>>.
- ANDERSON, J. B. Evolution of antifungal-drug resistance: mechanisms and pathogen fitness. **Nature Reviews Microbiology**, v. 3, n. 7, p. 547–556, 10 jul. 2005. Disponível em: <<http://www.ncbi.nlm.nih.gov/pubmed/15953931>>.
- 20 ANESIO, A. M. et al. Carbon fluxes through bacterial communities on glacier surfaces. **Annals of Glaciology**, v. 51, n. 56, p. 32–40, 1 dez. 2010. Disponível em: <<http://openurl.ingenta.com/content/xref?genre=article&issn=0260-3055&volume=51&issue=56&spage=32>>.
- 25 ANTIPOVA, T. V. et al. Exometabolites the Penicillium Fungi Isolated from Various High-Latitude Ecosystems. **Microbiology**, v. 87, n. 5, p. 642–651, 3 set. 2018. Disponível em: <<http://link.springer.com/10.1134/S002626171805003X>>.
- ANTONY, R. et al. Organic Carbon in Antarctic Snow : Spatial Trends and Possible Sources. p. 9944–9950, 2011.
- 30 ANTONY, R. et al. Microbial communities associated with Antarctic snow pack and their biogeochemical implications. **Microbiological Research**, v. 192, p. 192–202, nov. 2016. Disponível em: <<http://dx.doi.org/10.1016/j.micres.2016.07.004>>.
- ANVISA- Agência Nacional de Vigilância Sanitária. **Agrotóxico, Herbicida e Pesticida**. Disponível em: <<http://www.portal.anvisa.gov.br/agrotoxico>>. Acesso em 19 de maio de 2019.
- 35 AVENOT, H. F. et al. Sensitivity and cross-resistance patterns to demethylation-inhibiting fungicides in California populations of *Alternaria alternata* pathogenic on pistachio. **Crop Protection**, v. 88, p. 72–78, out. 2016. Disponível em: <<http://dx.doi.org/10.1016/j.cropro.2016.05.012>>.
- 40 BARDOU, P. et al. jvenn: an interactive Venn diagram viewer. **BMC Bioinformatics**, v. 15, n. 1, p. 293, 2014. Disponível em: <<http://www.biomedcentral.com/1471-2105/15/293>>.
- BARGAGLI, R. Environmental contamination in Antarctic ecosystems. **Science of The Total**

- 5 **Environment**, v. 400, n. 1–3, p. 212–226, ago. 2008. Disponível em: <<http://dx.doi.org/10.1016/j.scitotenv.2008.06.062>>.
- BASTOS, R. W. et al. Environmental Triazole Induces Cross-Resistance to Clinical Drugs and Affects Morphophysiology and Virulence of *Cryptococcus gattii* and *C. neoformans*. **Antimicrobial Agents and Chemotherapy**, v. 62, n. 1, p. e01179–17, 6 nov. 2017.
- 10 Disponível em: <<https://aac.asm.org/lookup/doi/10.1128/AAC.01179-17>>.
- BIGOT, M. et al. Spring Melt and the Redistribution of Organochlorine Pesticides in the Sea-Ice Environment: A Comparative Study between Arctic and Antarctic Regions. **Environmental Science & Technology**, v. 51, n. 16, p. 8944–8952, 15 ago. 2017. Disponível em: <<http://pubs.acs.org/doi/10.1021/acs.est.7b02481>>.
- 15 BOVIO, E. et al. The culturable mycobiota associated with three Atlantic sponges, including two new species: *Thelebolus balaustiformis* and *T. spongiae*. **Fungal Systematics and Evolution**, v. 1, n. 1, p. 141–167, 1 jun. 2018. Disponível em: <<http://www.ingentaconnect.com/content/10.3114/fuse.2018.01.07>>.
- BRADNER, J. R. et al. A new microfungus isolate, *Embellisia* sp., associated with the Antarctic moss *Bryum argenteum*. **Polar Biology**, v. 23, n. 10, p. 730–732, 22 set. 2000.
- 20 Disponível em: <<http://link.springer.com/10.1007/s003000000161>>.
- BRAKHAGE, A. A. Regulation of fungal secondary metabolism. **Nature Reviews Microbiology**, v. 11, n. 1, p. 21–32, 26 jan. 2013. Disponível em: <<http://dx.doi.org/10.1038/nrmicro2916>>.
- 25 BRAY, J. R., CURTIS, J. T. An ordination of upland forest communities of southern Wisconsin. **Ecological Monographs**, v. 27, p. 325–349, 1957.
- BRANDÃO, L. R. et al. Diversity and biogeographical patterns of yeast communities in Antarctic, Patagonian and tropical lakes. **Fungal Ecology**, v. 28, p. 33–43, ago. 2017. Disponível em: <<https://linkinghub.elsevier.com/retrieve/pii/S1754504817300387>>.
- 30 BREUER, U.; HARMS, H. *Debaryomyces hansenii* — an extremophilic yeast with biotechnological potential. **Yeast**, v. 23, n. 6, p. 415–437, 30 abr. 2006. Disponível em: <<http://doi.wiley.com/10.1002/yea.1374>>.
- BRIDGE, P. D.; SPOONER, B. M. Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem? **Fungal Ecology**, v. 5, n. 4, p. 381–394, ago. 2012.
- 35 Disponível em: <<http://dx.doi.org/10.1016/j.funeco.2012.01.007>>.
- BRIDGE, P. D.; SPOONER, B. M.; ROBERTS, P. J. Non-lichenized fungi from the Antarctic region. **Mycotaxon**, v. 106, p. 485–490, 2008.
- BRUNATI, M. et al. Diversity and pharmaceutical screening of fungi from benthic mats of Antarctic lakes. **Marine Genomics**, v. 2, n. 1, p. 43–50, 2009. Disponível em: <<http://dx.doi.org/10.1016/j.margen.2009.04.002>>.
- 40 BUCKNER, F. S.; VERLINDE, C. L. M. J.; FLAMME, A. C. L. A. Efficient technique for screening drugs for activity against *Trypanosoma cruzi* using parasites expressing beta-galactosidase. **Antimicrobial Agents and Chemotherapy**, v. 40, n. 11, p. 2592–2597, 1996.

- 5 BÜDEL, B.; COLESIE, C. **Antarctic Terrestrial Microbiology**. Berlin, Heidelberg: Springer Berlin Heidelberg, 2014.
- BUTINAR, L.; SPENCER-MARTINS, I.; GUNDE-CIMERMAN, N. Yeasts in high Arctic glaciers: the discovery of a new habitat for eukaryotic microorganisms. **Antonie van Leeuwenhoek**, v. 91, n. 3, p. 277–289, 14 mar. 2007. Disponível em:
10 <<http://link.springer.com/10.1007/s10482-006-9117-3>>.
- CALLAHAN HL, PORTAL AC, DEVEREAUX R, G. M. An axenic amastigote system for drug screening. **Antimicrobial Agents and Chemotherapy**, v. 41, n. 4, p. 818–822, 1997. Disponível em:
15 <<http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L27150538>>.
- CAMPOS, F. F. et al. Leishmanicidal Metabolites from *Cochliobolus* sp., an Endophytic Fungus Isolated from *Piptadenia adiantoides* (Fabaceae). **PLoS Neglected Tropical Diseases**, v. 2, n. 12, p. e348, 16 dez. 2008. Disponível em:
<<http://dx.plos.org/10.1371/journal.pntd.0000348>>.
- 20 CANNON, R. D. et al. Efflux-Mediated Antifungal Drug Resistance. **Clinical Microbiology Reviews**, v. 22, n. 2, p. 291–321, 1 abr. 2009. Disponível em:
<<http://cmr.asm.org/cgi/doi/10.1128/CMR.00051-08>>.
- CARNEIRO, H. C. S. **Influência do agroquímico Benomyl na Morfologia-fisiologia, virulência e susceptibilidade de *Cryptococcus gattii* aos antifúngicos clínicos in vitro e em modelo murino**. 2019. Universidade Federal de Minas Gerais, 2019.
- 25 CARNEIRO, H. C. S. et al. Effect of non-antifungal agrochemicals on the pathogenic fungus *Cryptococcus gattii*. **Medical Mycology**, p. 1–7, 19 mar. 2019. Disponível em:
<<https://academic.oup.com/mmy/advance-article/doi/10.1093/mmy/myz018/5393278>>.
- CASTELLANI, A. Maintenance and cultivation of common pathogenic fungi in distilled 35 water. **The Journal of Tropical Medicine and Hygiene**, v. 42, p. 181-184, 1967.
- CAVALHEIRO, M. et al. Host-Pathogen Interactions Mediated by MDR Transporters in Fungi: As Pleiotropic as it Gets! **Genes**, v. 9, n. 7, p. 332, 2 jul. 2018. Disponível em:
<<http://www.mdpi.com/2073-4425/9/7/332>>.
- CIPRO, C. V. Z. et al. Persistent organic pollutants in bird, fish and invertebrate samples from King George Island, Antarctica. **Antarctic Science**, v. 25, n. 4, p. 545–552, 11 ago. 2013. Disponível em:
35 <https://www.cambridge.org/core/product/identifier/S0954102012001149/type/journal_article>.
- CIPRO, C. V. Z.; TANIGUCHI, S.; MONTONE, R. C. Organic Pollutants in Snow and Seasonal Melting Water from King George Island, Antarctica. **Water, Air, & Soil Pollution**, v. 228, n. 4, p. 149, 20 abr. 2017. Disponível em: <<http://link.springer.com/10.1007/s11270-017-3325-5>>.
- 40 C.L.S.I. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard CLSI document M38-A2. **Clinical and Laboratory Standards Institute**, Wayne, 2008a.
- 45

- 5 C.L.S.I. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-third edition; CLSI document M27-A3. **Clinical and Laboratory Standards Institute**, Wayne, 2008b.
- CONNELL, L. et al. Diversity of Soil Yeasts Isolated from South Victoria Land, Antarctica. **Microbial Ecology**, v. 56, n. 3, p. 448–459, 6 out. 2008. Disponível em: <<http://link.springer.com/10.1007/s00248-008-9363-1>>.
- 10 CORSOLINI, S. Industrial contaminants in Antarctic biota. **Journal of Chromatography A**, v. 1216, n. 3, p. 598–612, jan. 2009. Disponível em: <<https://linkinghub.elsevier.com/retrieve/pii/S0021967308013411>>.
- 15 COWAN, D. A.; TOW, L. A. Endangered Antarctic Environments. **Annual Review of Microbiology**, v. 58, n. 1, p. 649–690, out. 2004. Disponível em: <<http://www.annualreviews.org/doi/10.1146/annurev.micro.57.030502.090811>>.
- COWEN, L. Predicting the emergence of resistance to antifungal drugs. **FEMS Microbiology Letters**, v. 204, n. 1, p. 1–7, 16 out. 2001. Disponível em: <[http://doi.wiley.com/10.1016/S0378-1097\(01\)00362-7](http://doi.wiley.com/10.1016/S0378-1097(01)00362-7)>.
- 20 COWEN, L. E. et al. Mechanisms of Antifungal Drug Resistance. **Cold Spring Harbor Perspectives in Medicine**, v. 5, n. 7, p. a019752, jul. 2015. Disponível em: <www.perspectivesinmedicine.org>.
- COX, F. et al. Not poles apart: Antarctic soil fungal communities show similarities to those of the distant Arctic. **Ecology Letters**, v. 19, n. 5, p. 528–536, 2016.
- 25 CROUS, P. W. et al. MycoBank: An online initiative to launch mycology into the 21st century. **Studies in Mycology**, v. 50, n. 1, p. 19–22, 2004.
- D'ELIA, T. et al. Isolation of fungi from Lake Vostok accretion ice. **Mycologia**, v. 101, n. 6, p. 751–763, 20 nov. 2009. Disponível em: <<http://aem.asm.org/cgi/doi/10.1128/AEM.02501-07>>.
- 30 DALY, G. L.; WANIA, F. Simulating the Influence of Snow on the Fate of Organic Compounds. **Environmental Science & Technology**, v. 38, n. 15, p. 4176–4186, ago. 2004. Disponível em: <<https://pubs.acs.org/doi/10.1021/es035105r>>.
- DE GARCÍA, V. et al. Biodiversity of cold-adapted yeasts from glacial meltwater rivers in Patagonia, Argentina. **FEMS Microbiology Ecology**, v. 59, n. 2, p. 331–341, 2007.
- 35 DE HOOG, G. S. et al. Black fungi: Clinical and pathogenic approaches. **Medical Mycology**, v. 38, n. SUPPL. 1, p. 243–250, 2000. Disponível em: <<http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L32094072%0Ahttp://bj7rx7bn7b.search.serialssolutions.com?sid=EMBASE&issn=13693786&id=doi:&atitle=Black+fungi%3A+Clinical+and+pathogenic+approaches&stitle=Med.+Mycol.&title=Medical+Myc>>.
- 40 DE HOOG, G. S. et al. Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. **Studies in Mycology**, v. 51, n. 2003, p. 33–76, 2005.
- DE MENEZES, G. C. A. et al. *Antarctomyces pellizariae* sp. nov., a new, endemic, blue,

- 5 snow resident psychrophilic ascomycete fungus from Antarctica. **Extremophiles**, v. 21, n. 2, p. 259–269, 30 mar. 2017. Disponível em: <<http://link.springer.com/10.1007/s00792-016-0895-x>>.
- DE MENEZES, G. C. A. et al. Fungi in Snow and Glacial Ice of Antarctica. In: **Fungi of Antarctica**. Cham: Springer International Publishing, 2019. p. 127–146.
- 10 DE SOUSA, J. R. P. et al. Pathogenic potential of environmental resident fungi from ornithogenic soils of Antarctica. **Fungal Biology**, v. 121, n. 12, p. 991–1000, dez. 2017. Disponível em: <<https://linkinghub.elsevier.com/retrieve/pii/S1878614617301289>>.
- DICKHUT, R. M. et al. Atmospheric Concentrations and Air–Water Flux of Organochlorine Pesticides along the Western Antarctic Peninsula. **Environmental Science & Technology**, v. 39, n. 2, p. 465–470, jan. 2005. Disponível em: <<https://pubs.acs.org/doi/10.1021/es048648p>>.
- 15 DING, Z. et al. Richness and bioactivity of culturable soil fungi from the Fildes Peninsula, Antarctica. **Extremophiles**, v. 20, n. 4, p. 425–435, 3 jul. 2016. Disponível em: <<http://link.springer.com/10.1007/s00792-016-0833-y>>.
- 20 DOMINE, F. et al. Adsorption of Phenanthrene on Natural Snow. **Environmental Science & Technology**, v. 41, n. 17, p. 6033–6038, set. 2007. Disponível em: <<https://pubs.acs.org/doi/10.1021/es0706798>>.
- DONALDSON, N. L. G. G. C. Development of Primer Sets Designed for Use with the PCR To Amplify Conserved Genes from Filamentous Ascomycetes. **APPLIED AND ENVIRONMENTAL MICROBIOLOGY**, v. 61, n. 4, p. 1323–1330, 1995. Disponível em: <<http://doi.wiley.com/10.1002/jobm.3620330609>>.
- 25 DUARTE, A. W. F. et al. Yeasts from macroalgae and lichens that inhabit the South Shetland Islands, Antarctica. **Environmental Microbiology Reports**, v. 8, n. 5, p. 874–885, out. 2016. Disponível em: <<http://ci.nii.ac.jp/naid/10011292528/%5Cnpapers2://publication/uuid/2C7AFE13-7675-420B-99F2-FE9B9D8B2A3D>>.
- 30 DUARTE, A. W. F. et al. Cold-adapted enzymes produced by fungi from terrestrial and marine Antarctic environments. **Critical Reviews in Biotechnology**, v. 38, n. 4, p. 600–619, 2018. Disponível em: <<https://doi.org/10.1080/07388551.2017.1379468>>.
- 35 DUMAN, J. G.; OLSEN, T. M. Thermal Hysteresis Protein Activity in Bacteria, Fungi, and Phylogenetically Diverse Plants. **Cryobiology**, v. 30, n. 3, p. 322–328, jun. 1993. Disponível em: <<http://linkinghub.elsevier.com/retrieve/pii/S001122408371031X>>.
- DUTRA, T. L.; JASPER, A. Fósseis da Antártica. In: **Paleontologia, Conceitos e Métodos**. 3. ed. [s.l.: s.n.].p. 597–631.
- 40 EUROPEAN CENTRE FOR DISEASE CONTROL. Risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in *Aspergillus* species. **Technical report**, 2013.
- EWART, K. V.; LIN, Q.; HEW, C. L. Structure, function and evolution of antifreeze proteins. **Cellular and Molecular Life Sciences CMLS**, v. 55, n. 2, p. 271–283, fev. 1999. Disponível

- 5 em: <<http://link.springer.com/10.1007/s000180050289>>.
- FARIA-RAMOS, I. et al. Development of cross-resistance by *Aspergillus fumigatus* to clinical azoles following exposure to prochloraz, an agricultural azole. **BMC Microbiology**, v. 14, n. 1, p. 155, 2014. Disponível em: <<http://bmcmicrobiol.biomedcentral.com/articles/10.1186/1471-2180-14-155>>.
- 10 FELL, J. W. et al. *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. **Antonie van Leeuwenhoek**, v. 35, n. 1, p. 433–462, dez. 1969. Disponível em: <<http://link.springer.com/10.1007/BF02219163>>.
- FELL, J. W. et al. Biodiversity of micro-eukaryotes in Antarctic Dry Valley soils with <5% soil moisture. **Soil Biology and Biochemistry**, v. 38, n. 10, p. 3107–3119, out. 2006.
- 15 Disponível em: <<https://linkinghub.elsevier.com/retrieve/pii/S0038071706000939>>.
- FELLER, G.; GERDAY, C. **Psychrophilic enzymes: Molecular basis of cold adaptation**, 1997. . (Nota t?cnica).
- FERREIRA, E. M. S. et al. Taxonomy and richness of yeasts associated with angiosperms, bryophytes, and meltwater biofilms collected in the Antarctic Peninsula. **Extremophiles**, v. 23, n. 1, p. 151–159, 29 jan. 2019. Disponível em: <<https://doi.org/10.1007/s00792-018-1069-9>>.
- 20 FERREIRA, F. R. G. **O Sistema do Tratado da Antártica: evolução do regime e seu impacto na política externa brasileira**. Brasília: Fundação Alexandre de Gusmão, 2009.
- FERREIRA, M. et al. New Pesticidal Diterpenoids from *Vellozia gigantea* (Velloziaceae), an Endemic Neotropical Plant Living in the Endangered Brazilian Biome Rupestrian Grasslands. **Molecules**, v. 22, n. 1, p. 175, 21 jan. 2017. Disponível em: <<http://www.mdpi.com/1420-3049/22/1/175>>.
- 25 FILIPPIN, F. B.; SOUZA, L. C. Eficiência terapêutica das formulações lipídicas de anfotericina B. **Revista Brasileira de Ciências Farmacêuticas**, v. 42, n. 2, p. 167–194, jun. 2006. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-93322006000200003&lng=pt&nrm=iso&tlng=pt>.
- 30 FISHER, M. C. et al. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. **Science**, v. 360, n. 6390, p. 739–742, 18 maio 2018. Disponível em: <http://tclocal.org/2011/01/health_and_food_security.html>.
- 35 FISHER, R. A., CORBET, A. S., WILLIAMS, C. B. The relation between the number of species and the number of individuals in a random sample of an animal population. **Journal of Animal Ecology**, v. 12, p. 42–58, 1943. Disponível em: <<http://www.math.mcgill.ca/dstephens/556-2008/Papers/Fisher1943.pdf>>.
- 40 FRANZ, T. P.; EISENREICH, S. J. Snow Scavenging of Polychlorinated Biphenyls and Polycyclic Aromatic Hydrocarbons in Minnesota. **Environmental Science & Technology**, v. 32, n. 12, p. 1771–1778, jun. 1998. Disponível em: <<https://pubs.acs.org/doi/10.1021/es970601z>>.
- FURBINO, L. E. et al. Diversity Patterns, Ecology and Biological Activities of Fungal Communities Associated with the Endemic Macroalgae Across the Antarctic Peninsula.

- 5 **Microbial Ecology**, v. 67, n. 4, p. 775–787, 2014.
- FURBINO, L. E. et al. Isolation of fungi associated with macroalgae from maritime Antarctica and their production of agarolytic and carrageenolytic activities. **Polar Biology**, v. 41, n. 3, p. 527–535, 31 mar. 2018. Disponível em: <<http://link.springer.com/10.1007/s00300-017-2213-1>>.
- 10 GALBÁN-MALAGÓN, C. J. et al. Polychlorinated Biphenyls, Hexachlorocyclohexanes and Hexachlorobenzene in Seawater and Phytoplankton from the Southern Ocean (Weddell, South Scotia, and Bellingshausen Seas). **Environmental Science & Technology**, v. 47, n. 11, p. 5578–5587, 4 jun. 2013. Disponível em: <<http://pubs.acs.org/doi/10.1021/es400030q>>.
- 15 GEISZ, H. N. et al. Melting Glaciers: A Probable Source of DDT to the Antarctic Marine Ecosystem. **Environmental Science & Technology**, v. 42, n. 11, p. 3958–3962, jun. 2008. Disponível em: <<https://pubs.acs.org/doi/10.1021/es702919n>>.
- GILBERT, J. A. et al. Demonstration of antifreeze protein activity in Antarctic lake bacteria. **Microbiology**, v. 150, n. 1, p. 171–180, 2004.
- 20 GODINHO, V. M. et al. Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. **The ISME journal**, v. 7, n. 7, p. 1434–51, 2013. Disponível em: <<http://www.ncbi.nlm.nih.gov/pubmed/23702515>>.
- GODINHO, V. M. et al. Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica. **Extremophiles**, v. 19, n. 3, p. 585–596, 2015a. Disponível em: <<http://dx.doi.org/10.1007/s00792-015-0741-6>>.
- 25 GODINHO, V. M. et al. Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica. **Extremophiles**, v. 19, n. 3, p. 585–596, 26 maio 2015b. Disponível em: <<http://dx.doi.org/10.1007/s00792-015-0741-6>>.
- 30 GODINHO, V. M. et al. Diversity and distribution of hidden cultivable fungi associated with marine animals of Antarctica. **Fungal Biology**, maio 2019. Disponível em: <<https://linkinghub.elsevier.com/retrieve/pii/S1878614618304057>>.
- GOMES, E. C. Q. et al. Cultivable fungi present in Antarctic soils: taxonomy, phylogeny, diversity, and bioprospecting of antiparasitic and herbicidal metabolites. **Extremophiles**, v. 22, n. 3, p. 381–393, 13 maio 2018. Disponível em: <<https://doi.org/10.1007/s00792-018-1003-1>>.
- 35 GONÇALVES, D. B. et al. Use of response surface methodology to optimize production of pectinases by recombinant *Penicillium griseoroseum* T20. **Biocatalysis and Agricultural Biotechnology**, v. 1, n. 2, p. 140–146, abr. 2012a. Disponível em: <<http://linkinghub.elsevier.com/retrieve/pii/S1878818112000187>>.
- 40 GONÇALVES, V. N. et al. Diversity and distribution of fungal communities in lakes of Antarctica. **FEMS Microbiology Ecology**, v. 82, n. 2, p. 459–471, 2012b.
- GONÇALVES, V. N. et al. *Penicillium solitum*: a mesophilic, psychrotolerant fungus present in marine sediments from Antarctica. **Polar Biology**, v. 36, n. 12, p. 1823–1831, 7 dez. 2013. Disponível em: <<http://link.springer.com/10.1007/s00300-013-1403-8>>.

- 5 GONÇALVES, V. N. et al. Antibacterial, antifungal and antiprotozoal activities of fungal communities present in different substrates from Antarctica. **Polar Biology**, v. 38, n. 8, p. 1143–1152, 14 ago. 2015. Disponível em: <<http://link.springer.com/10.1007/s00300-015-1672-5>>.
- 10 GONÇALVES, V. N. et al. Antarctic rocks from continental Antarctica as source of potential human opportunistic fungi. **Extremophiles**, v. 21, n. 5, p. 851–860, 28 set. 2017a. Disponível em: <<http://link.springer.com/10.1007/s00792-017-0947-x>>.
- 15 GONÇALVES, V. N. et al. Taxonomy, phylogeny and ecology of cultivable fungi present in seawater gradients across the Northern Antarctica Peninsula. **Extremophiles**, v. 21, n. 6, p. 1005–1015, 30 nov. 2017b. Disponível em: <<http://link.springer.com/10.1007/s00792-017-0959-6>>.
- GOOGLE™ EARTH SOFTWARE versão 7.1.5. 1557. **Mapa da Península Antártica e das ilhas que compõem o arquipélago Shetland do Sul**. Disponível em: <<http://www.google.com/earth/>>. Acesso em 19 de julho de 2017.
- 20 GOORDIAL, J. et al. Nearing the cold-arid limits of microbial life in permafrost of an upper dry valley, Antarctica. **The ISME Journal**, v. 10, n. 7, p. 1613–1624, 19 jul. 2016. Disponível em: <<http://dx.doi.org/10.1038/ismej.2015.239>>.
- 25 GOUIN, T. et al. Evidence for the “grasshopper” effect and fractionation during long-range atmospheric transport of organic contaminants. **Environmental Pollution**, v. 128, n. 1–2, p. 139–148, mar. 2004. Disponível em: <<https://linkinghub.elsevier.com/retrieve/pii/S0269749103003385>>.
- GRZESIAK, J. et al. Microbial community changes along the Ecology Glacier ablation zone (King George Island, Antarctica). **Polar Biology**, v. 38, n. 12, p. 2069–2083, 27 dez. 2015. Disponível em: <<http://link.springer.com/10.1007/s00300-015-1767-z>>.
- 30 GUNDE-CIMERMAN, N. et al. Extremophilic fungi in arctic ice: A relationship between adaptation to low temperature and water activity. **Physics and Chemistry of the Earth**, v. 28, n. 28–32, p. 1273–1278, 2003.
- 35 GUPTA, A. et al. Fatal *Candida famata* peritonitis complicating sclerosing peritonitis in a peritoneal dialysis patient. **Nephrology Dialysis Transplantation**, v. 21, n. 7, p. 2036–2037, 1 jul. 2006. Disponível em: <<http://academic.oup.com/ndt/article/21/7/2035/1821801/Antioxidant-treatment-in-dialysis-patients>>.
- 40 HENRÍQUEZ, M. et al. Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial, antitumoral and antioxidant potential. **World Journal of Microbiology and Biotechnology**, v. 30, n. 1, p. 65–76, 4 jan. 2014. Disponível em: <<http://link.springer.com/10.1007/s11274-013-1418-x>>.
- HERZFELD, U. C. **Atlas of Antarctica**. Berlin, Heidelberg: Springer Berlin Heidelberg, 2004.
- 45 HODSON, A. et al. A glacier respire: Quantifying the distribution and respiration CO₂ flux of cryoconite across an entire Arctic supraglacial ecosystem. **Journal of Geophysical Research: Biogeosciences**, v. 112, n. G4, p. n/a–n/a, dez. 2007. Disponível em:

- 5 <<http://doi.wiley.com/10.1029/2007JG000452>>.
- HODSON, A. et al. GLACIAL ECOSYSTEMS. **Ecological Monographs**, v. 78, n. 1, p. 41–67, fev. 2008. Disponível em: <<http://doi.wiley.com/10.1890/07-0187.1>>.
- HOF, H. Critical Annotations to the Use of Azole Antifungals for Plant Protection. **Antimicrobial Agents and Chemotherapy**, v. 45, n. 11, p. 2987–2990, 1 nov. 2001.
10 Disponível em: <<http://aac.asm.org/cgi/doi/10.1128/AAC.45.11.2987-2990.2001>>.
- HOUBRAKEN, J. et al. New penicillin-producing *Penicillium* species and an overview of section *Chrysogena*. **Persoonia - Molecular Phylogeny and Evolution of Fungi**, v. 29, n. 1, p. 78–100, 31 dez. 2012. Disponível em: <<http://openurl.ingenta.com/content/xref?genre=article&issn=0031-5850&volume=29&issue=1&page=78>>.
15
- ISLAND, G. South Shetland Islands. **Antarctic Science**, v. 7, p. 99–113, 2001. Disponível em: <http://media.lonelyplanet.com/shop/pdfs/2548-Antarctica_-_South_Shetland_Islands__Chapter_.pdf>.
- IVANOVA, V. et al. Diphenylether and Macrotriolides Occurring in a Fungal Isolate from the Antarctic Lichen *Neuropogon*. **Preparative Biochemistry and Biotechnology**, v. 37, n. 1, p. 39–45, 18 jan. 2007. Disponível em: <<http://www.tandfonline.com/doi/abs/10.1080/10826060601039436>>.
20
- JONES, H. G. The ecology of snow-covered systems: a brief overview of nutrient cycling and life in the cold. **Hydrological Processes**, v. 13, n. 1415, p. 2135–2147, out. 1999.
25 Disponível em: <<http://doi.wiley.com/10.1002/%28SICI%291099-1085%28199910%2913%3A14%2F15%3C2135%3A%3AAID-HYP862%3E3.3.CO%3B2-P>>.
- KANAFANI, Z. A.; PERFECT, J. R. Resistance to Antifungal Agents: Mechanisms and Clinical Impact. **Clinical Infectious Diseases**, v. 46, n. 1, p. 120–128, 1 jan. 2008. Disponível em: <<http://academic.oup.com/cid/article-lookup/doi/10.1086/524071>>.
30
- KANG, J. H. et al. Deposition of organochlorine pesticides into the surface snow of East Antarctica. **Science of the Total Environment**, v. 433, p. 290–295, 2012. Disponível em: <<http://dx.doi.org/10.1016/j.scitotenv.2012.06.037>>.
- KHAIRY, M. A. et al. Levels, sources and chemical fate of persistent organic pollutants in the atmosphere and snow along the western Antarctic Peninsula. **Environmental Pollution**, v. 216, p. 304–313, set. 2016. Disponível em: <<http://dx.doi.org/10.1016/j.envpol.2016.05.092>>.
35
- KLÁNOVÁ, J. et al. Persistent organic pollutants in soils and sediments from James Ross Island, Antarctica. **Environmental Pollution**, v. 152, n. 2, p. 416–423, mar. 2008. Disponível em: <<https://linkinghub.elsevier.com/retrieve/pii/S0269749107003077>>.
- KNOWLTON, C. et al. Microbial Analyses of Ancient Ice Core Sections from Greenland and Antarctica. **Biology**, v. 2, n. 1, p. 206–232, 25 jan. 2013. Disponível em: <www.mdpi.com/journal/biology>.
40
- KOBAYASI, Y. et al. Mycological studies of the Alaskan Arctic. **Res Commun Inst Ferment Osaka**, v. 3, p.1-138, 1967.

- 5 KOCHKINA, G. et al. Ancient fungi in Antarctic permafrost environments. **FEMS Microbiology Ecology**, v. 82, n. 2, p. 501–509, 2012.
- KORNERUP, A., WANSCHER, J.H. (eds). **Methuen Handbook of Colour**, 3rd (edn.) Eyre Methuen, London, 1984.
- 10 KOSTADINOVA, N. et al. Comparison of the oxidative stress response of two Antarctic fungi to different growth temperatures. **Polish Polar Research**, v. 38, n. 3, p. 393–408, 1 set. 2017. Disponível em: <<http://content.sciendo.com/view/journals/popore/38/3/article-p393.xml>>.
- 15 KOZLOVSKY, A. G. et al. Secondary metabolites of the genus *Penicillium* from undisturbed and anthropogenically altered Antarctic habitats. **Folia Microbiologica**, 13 abr. 2019. Disponível em: <<http://link.springer.com/10.1007/s12223-019-00708-0>>.
- LACHANCE, M.-A. et al. *Kodamaea kakaduensis* and *Candida tolerans*, two new ascomycetous yeast species from Australian *Hibiscus* flowers. **Canadian Journal of Microbiology**, v. 45, n. 2, p. 172–177, 1999. Disponível em: <http://www.nrc.ca/cgi-bin/cisti/journals/rp/rp2_abst_e?cjm_w98-225_45_ns_nf_cjm45-99>.
- 20 LI, L. et al. Cytotoxic Metabolites from the Antarctic Psychrophilic Fungus *Oidiodendron truncatum*. **Journal of Natural Products**, v. 75, n. 5, p. 920–927, 25 maio 2012. Disponível em: <<http://pubs.acs.org/doi/10.1021/np3000443>>.
- 25 LI, W. et al. Antibacterial constituents from Antarctic fungus, *Aspergillus sydowii* SP-1. **Natural Product Research**, v. 32, n. 6, p. 662–667, 19 mar. 2018. Disponível em: <<https://doi.org/10.1080/14786419.2017.1335730>>.
- LI, Y. et al. Bioactive Asterric Acid Derivatives from the Antarctic Ascomycete Fungus *Geomyces* sp. **Journal of Natural Products**, v. 71, n. 9, p. 1643–1646, set. 2008. Disponível em: <<http://pubs.acs.org/doi/10.1021/np8003003>>.
- 30 LIECKFELDT, E.; MEYER, W.; BÖRNER, T. Rapid identification and differentiation of yeasts by DNA and PCR fingerprinting. **Journal of Basic Microbiology**, v. 33, n. 6, p. 413–425, 1993. Disponível em: <<http://doi.wiley.com/10.1002/jobm.3620330609>>.
- LIN, A. et al. New eremophilane-type sesquiterpenes from an Antarctic deep-sea derived fungus, *Penicillium* sp. PR19 N-1. **Archives of Pharmacal Research**, v. 37, n. 7, p. 839–844, 28 jul. 2014. Disponível em: <<http://link.springer.com/10.1007/s12272-013-0246-8>>.
- 35 LONDE, L. N. et al. Efeito do benomyl e identificação de fitopatógenos em meio MS para controle da contaminação na micropropagação de *Anacardium humile* (Anacardiaceae). **Bioscience Journal**, v. 23, n. 3, p. 94–100, 2000.
- 40 LYRATZOPOULOS, G. et al. Invasive Infection due to *Penicillium* Species other than *P. marneffei*. **Journal of Infection**, v. 45, n. 3, p. 184–195, out. 2002. Disponível em: <<http://linkinghub.elsevier.com/retrieve/pii/S016344530291056X>>.
- MA, L. et al. Revival and characterization of fungi from ancient polar ice. **Mycologist**, v. 13, n. 2, p. 70–73, 1999. Disponível em: <<http://www.sciencedirect.com/science/article/pii/S0269915X99800123>>.

- 5 MACHELEIDT, J. et al. Regulation and Role of Fungal Secondary Metabolites. **Annual Review of Genetics**, v. 50, n. 1, p. 371–392, 23 nov. 2016. Disponível em: <<http://www.annualreviews.org/doi/10.1146/annurev-genet-120215-035203>>.
- MALKUS, A. et al. RNA polymerase II gene (RPB2) encoding the second largest protein subunit in *Phaeosphaeria nodorum* and *P. avenaria*. **Mycological Research**, v. 110, n. 10, p. 1152–1164, 2006.
- 10 MARGESIN, R. et al. *Rhodotorula psychrophila* sp. nov., *Rhodotorula psychrophenolica* sp. nov. and *Rhodotorula glacialis* sp. nov., novel psychrophilic basidiomycetous yeast species isolated from alpine environments. **International Journal of Systematic and Evolutionary Microbiology**, v. 57, n. 9, p. 2179–2184, 1 set. 2007. Disponível em: <<http://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/ijms.0.65111-0>>.
- 15 MARGESIN, R.; MITEVA, V. Diversity and ecology of psychrophilic microorganisms. **Research in Microbiology**, v. 162, n. 3, p. 346–361, abr. 2011. Disponível em: <<https://linkinghub.elsevier.com/retrieve/pii/S0923250810002810>>.
- 20 MCRAE, C. F.; SEPPELT, R. D.; HOCKING, A. D. *Penicillium* species from terrestrial habitats in the Windmill Islands, East Antarctica, including a new species, *Penicillium antarcticum*. **Polar Biology**, v. 21, n. 2, p. 97–111, 1999.
- MELO, I. S. et al. Isolation and biological activities of an endophytic *Mortierella alpina* strain from the Antarctic moss *Schistidium antarctici*. **Extremophiles**, v. 18, n. 1, p. 15–23, 15 jan. 2014. Disponível em: <<http://link.springer.com/10.1007/s00792-013-0588-7>>.
- 25 MITEVA, V. Bacteria in Snow and Glacier Ice. In: **Psychrophiles: from Biodiversity to Biotechnology**. Berlin, Heidelberg: Springer Berlin Heidelberg, 2008. p. 31–50.
- MONTEMARTINI, A., CARETTA, G., DEL FRATE, G. Notes on *Thelebolus microsporus* isolated in Antarctica. **Mycotaxon**, v. 48, p. 343–358, 1993.
- MORITA, R. Y. Psychrophilic bacteria. **Bacteriol Reviews**, v. 39, n. 2, p. 144, 1975.
- 30 MÜLLER, F.; ACKERMANN, P.; MARGOT, P. Fungicides, Agricultural, 2. Individual Fungicides. In: **Ullmann's Encyclopedia of Industrial Chemistry**. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, 2011.
- NICOLETTI, R.; TRINCONE, A. Bioactive Compounds Produced by Strains of *Penicillium* and *Talaromyces* of Marine Origin. **Marine Drugs**, v. 14, n. 2, p. 37, 18 fev. 2016.
- 35 Disponível em: <<http://www.mdpi.com/1660-3397/14/2/37>>.
- OCHYRA, R.; I., L.-S. R.; B. OCHYRA, H. **The Illustrated Moss Flora of Antarctica**. First ed. New York: Cambridge University Press, 2008.
- O'DONNELL, K. *Fusarium* and its near relatives. In: Reynolds, D. R.; Taylor, J. W. (Eds.). *The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematic*.
- 40 **Wallingford: CAB International**, p. 225-233, 1993
- PEARCE, D. A. et al. Microorganisms in the atmosphere over Antarctica. **FEMS Microbiology Ecology**, v. 69, n. 2, p. 143–157, ago. 2009. Disponível em: <<https://academic.oup.com/femsec/article-lookup/doi/10.1111/j.1574-6941.2009.00706.x>>.

- 5 PEREA, S.; PATTERSON, T. F. Antifungal Resistance in Pathogenic Fungi. **Clinical Infectious Diseases**, v. 35, n. 9, p. 1073–1080, nov. 2002. Disponível em: <<https://academic.oup.com/cid/article-lookup/doi/10.1086/344058>>.
- POLEZHAEVA, T. V. et al. Use of pectic polysaccharides for cryopreservation of biological objects. **Archives of Biological Sciences**, v. 66, n. 3, p. 1025–1033, 2014.
- 10 PURÍĆ, J. et al. Activity of Antarctic fungi extracts against phytopathogenic bacteria. **Letters in Applied Microbiology**, v. 66, n. 6, p. 530–536, jun. 2018. Disponível em: <<http://doi.wiley.com/10.1111/lam.12875>>.
- RAO, S. et al. Low-diversity fungal assemblage in an Antarctic Dry Valleys soil. **Polar Biology**, v. 35, n. 4, p. 567–574, 2012.
- 15 RIBAS E RIBAS, A. D. et al. Is the emergence of fungal resistance to medical triazoles related to their use in the agroecosystems? A mini review. **Brazilian Journal of Microbiology**, v. 47, n. 4, p. 793–799, out. 2016. Disponível em: <<http://dx.doi.org/10.1016/j.bjm.2016.06.006>>.
- 20 RIGOTTO, R. M.; VASCONCELOS, D. P. e; ROCHA, M. M. Pesticide use in Brazil and problems for public health. **Cadernos de Saúde Pública**, v. 30, n. 7, p. 1360–1362, 2014. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-311X2014000701360&lng=en&tlng=en>.
- ROBINSON, C. H. Cold adaptation in Arctic and antartic fungi.pdf. **New Phytologist**, v. 151, n. 151, p. 341–353, 2001. Disponível em: <www.newphytologist.com>.
- 25 ROCHA, M. F. G. et al. Cross-resistance to fluconazole induced by exposure to the agricultural azole tetraconazole: an environmental resistance school? **Mycoses**, v. 59, n. 5, p. 281–290, maio 2016. Disponível em: <<http://doi.wiley.com/10.1111/myc.12457>>.
- 30 ROGERS, S. O. et al. Comparisons of Protocols for Decontamination of Environmental Ice Samples for Biological and Molecular Examinations. **Applied and Environmental Microbiology**, v. 70, n. 4, p. 2540–2544, 2004.
- ROMANHA, A. J. et al. In vitro and in vivo experimental models for drug screening and development for Chagas disease. **Memorias do Instituto Oswaldo Cruz**, v. 105, n. March, p. 233–238, 2010.
- 35 ROSA, L. H. et al. Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv. (Poaceae). **Polar Biology**, v. 32, n. 2, p. 161–167, fev. 2009. Disponível em: <<http://link.springer.com/10.1007/s00300-008-0515-z>>.
- 40 ROSA, L. H. et al. Antifungal activity of extracts from endophytic fungi associated with *Smallanthus* maintained in vitro as autotrophic cultures and as pot plants in the greenhouse. **Canadian Journal of Microbiology**, v. 58, n. 10, p. 1202–1211, out. 2012. Disponível em: <<http://www.nrcresearchpress.com/doi/10.1139/w2012-088>>.
- ROSA, L. H. et al. Fungi in Antarctica: Diversity, Ecology, Effects of Climate Change, and Bioprospection for Bioactive Compounds. In: **Fungi of Antarctica**. Cham: Springer International Publishing, 2019. p. 1–17.

- 5 RUISE, S. et al. Fungi in Antarctica. **Reviews in Environmental Science and Bio/Technology**, v. 6, n. 1–3, p. 127–141, 12 jan. 2007. Disponível em: <<http://link.springer.com/10.1007/s11157-006-9107-y>>.
- SAMBROOK, J., FRITSCH, E. F., MANIATIS, T. **Molecular cloning: a laboratory manual**. Cold spring harbor laboratory press, 1989.
- 10 SAMPAIO, J.P. *Rhodotorula* Harrison (1928). In: KURTZMAN, C. P., FELL, J. W., BOEKHOUT, T. (eds). **The Yeasts: A Taxonomic Study**, 5 th (edn.) Elsevier, The Netherlands, p 1873–1927, 2011.
- SAMPAIO, J. P. Diversity, phylogeny, and classification of basidiomycetous yeasts. In: AGERER R, BLANZ P, P. M. (Ed.). **Frontiers in Basidiomycete Mycology**, v. 1 p. 49–80, 15 2004.
- SANTIAGO, I. F. et al. Leishmanicidal and antitumoral activities of endophytic fungi associated with the Antarctic angiosperms *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. **Extremophiles**, v. 16, n. 1, p. 95–103, 2012.
- 20 SANTIAGO, I. F. et al. Lichensphere: a protected natural microhabitat of the non-lichenised fungal communities living in extreme environments of Antarctica. **Extremophiles**, v. 19, n. 6, p. 1087–1097, 23 nov. 2015. Disponível em: <<http://link.springer.com/10.1007/s00792-015-0781-y>>.
- SANTOS, J. R. A. et al. Dynamic Interaction between Fluconazole and Amphotericin B against *Cryptococcus gattii*. **Antimicrobial Agents and Chemotherapy**, v. 56, n. 5, p. 2553–2558, maio 2012. Disponível em: <<http://aac.asm.org/lookup/doi/10.1128/AAC.06098-11>>.
- 25 SANYAL, A. et al. Microbial communities and their potential for degradation of dissolved organic carbon in cryoconite hole environments of Himalaya and Antarctica. **Microbiological Research**, v. 208, p. 32–42, 2018. Disponível em: <<http://dx.doi.org/10.1016/j.micres.2018.01.004>>.
- 30 SELBMANN, L. et al. Fungi at the edge of life: Cryptoendolithic black fungi from Antarctic desert. **Studies in Mycology**, v. 51, n. Friedmann 1982, p. 1–32, 2005.
- SERRANO, E.; LÓPEZ-MARTÍNEZ, J. Rock glaciers in the South Shetland Islands, Western Antarctica. **Geomorphology**, v. 35, n. 1–2, p. 145–162, out. 2000. Disponível em: <<http://linkinghub.elsevier.com/retrieve/pii/S0169555X00000349>>.
- 35 SHAPIRO, R. S.; ROBBINS, N.; COWEN, L. E. Regulatory Circuitry Governing Fungal Development, Drug Resistance, and Disease. **Microbiology and Molecular Biology Reviews**, v. 75, n. 2, p. 213–267, 1 jun. 2011. Disponível em: <<http://www.ncbi.nlm.nih.gov/pubmed/21646428>> <<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3122626>>.
- 40 SHIVAJI, S.; PRASAD, G. S. Antarctic Yeasts: Biodiversity and Potential Applications. In: **Yeast Biotechnology: Diversity and Applications**. Dordrecht: Springer Netherlands, 2009. p. 3–18.
- SINGH, S. M. et al. Pigment, fatty acid and extracellular enzyme analysis of a fungal strain *Thelebolus microsporus* from Larsemann Hills, Antarctica. **Polar Record**, v. 50, n. 01, p. 31–

- 5 36, 7 jan. 2014. Disponível em:
<<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4009855&tool=pmcentrez&rendertype=abstract>>.
- SNELDERS, E. et al. Triazole fungicides can induce cross-resistance to medical triazoles in *Aspergillus fumigatus*. **PLoS ONE**, v. 7, n. 3, p. e31801, 1 mar. 2012. Disponível em:
10 <<http://dx.plos.org/10.1371/journal.pone.0031801>>.
- SONJAK, S.; FRISVAD, J. C.; GUNDE-CIMERMAN, N. *Penicillium* Mycobiota in Arctic Subglacial Ice. **Microbial Ecology**, v. 52, n. 2, p. 207–216, 8 ago. 2006. Disponível em:
<<http://link.springer.com/10.1007/s00248-006-9086-0>>.
- SPILIOPOULOU, A.; ANASTASSIOU, E. D.; CHRISTOFIDOU, M. *Rhodotorula* Fungemia of an Intensive Care Unit Patient and Review of Published Cases. **Mycopathologia**, v. 174, n. 4, p. 301–309, 11 out. 2012. Disponível em: <<http://link.springer.com/10.1007/s11046-012-9552-9>>.
- STCHIGEL, A. M. et al. *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. **Mycological Research**, v. 105, n. 3, p. 377–382, mar. 2001. Disponível em:
20 <<http://linkinghub.elsevier.com/retrieve/pii/S0953756208618572>>.
- SWOBODA-KOPEC, E. et al. Mixed bloodstream infection with *Staphylococcus aureus* and *Penicillium chrysogenum* in an immunocompromised patient: case report and review of the literature. **Clinical Microbiology and Infection**, v. 9, n. 11, p. 1116–1117, nov. 2003. Disponível em: <<https://linkinghub.elsevier.com/retrieve/pii/S1198743X14638209>>.
- 25 TAMURA, K. et al. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. **Molecular Biology and Evolution**, v. 30, n. 12, p. 2725–2729, dez. 2013. Disponível em: <<https://academic.oup.com/mbe/article-lookup/doi/10.1093/molbev/mst197>>.
- TELLING, J. et al. Nitrogen fixation on Arctic glaciers, Svalbard. **Journal of Geophysical Research**, v. 116, n. G3, p. G03039, 28 set. 2011. Disponível em:
30 <<http://doi.wiley.com/10.1029/2010JG001632>>.
- THOMPSON, G. R.; CADENA, J.; PATTERSON, T. F. Overview of Antifungal Agents. **Clinics in Chest Medicine**, v. 30, n. 2, p. 203–215, jun. 2009. Disponível em: <<http://dx.doi.org/10.1016/j.ccm.2009.02.001>>.
- TIBBETT, M.; SANDERS, F. E.; CAIRNEY, J. W. G. The effect of temperature and inorganic phosphorus supply on growth and acid phosphatase production in arctic and temperate strains of ectomycorrhizal *Hebeloma* spp. in axenic culture. **Mycological Research**, v. 102, n. 2, p. 129–135, fev. 1998. Disponível em:
35 <<http://linkinghub.elsevier.com/retrieve/pii/S0953756208608448>>.
- TRUONG, M. et al. Repurposing drugs to fast-track therapeutic agents for the treatment of cryptococcosis. **PeerJ**, v. 6, p. e4761, 4 maio 2018. Disponível em:
40 <<https://peerj.com/articles/4761>>.
- TSUJI, M. et al. Cold adaptation of fungi obtained from soil and lake sediment in the Skarvsnes ice-free area, Antarctica. **FEMS Microbiology Letters**, v. 346, n. 2, p. 121–130, set. 2013. Disponível em: <<https://academic.oup.com/femsle/article-lookup/doi/10.1111/1574-6968.12217>>.
- 45

- 5 TURCHETTI, B. et al. Psychrophilic yeasts in glacial environments of Alpine glaciers. **FEMS Microbiology Ecology**, v. 63, n. 1, p. 73–83, jan. 2008. Disponível em: <<https://academic.oup.com/femsec/article-lookup/doi/10.1111/j.1574-6941.2007.00409.x>>.
- TURNER, J. et al. **Antarctic Climate Change and the Environment**. [s.l.: s.n.]
- 10 VANDEPUTTE, P.; FERRARI, S.; COSTE, A. T. Antifungal Resistance and New Strategies to Control Fungal Infections. **International Journal of Microbiology**, v. 2012, p. 1–26, 2012. Disponível em: <<http://www.hindawi.com/journals/ijmicro/2012/713687/>>.
- 15 VAZ, A. B. M. et al. The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. **Brazilian Journal of Microbiology**, v. 42, n. 3, p. 937–947, set. 2011. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-83822011000300012&lng=en&nrm=iso&tlng=en>.
- VECCHIATO, M. et al. Persistent Organic Pollutants (POPs) in Antarctica: Occurrence in continental and coastal surface snow. **Microchemical Journal**, v. 119, p. 75–82, mar. 2015. Disponível em: <<http://dx.doi.org/10.1016/j.microc.2014.10.010>>.
- 20 VERWEIJ, P. E. et al. Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? **The Lancet Infectious Diseases**, v. 9, n. 12, p. 789–795, dez. 2009. Disponível em: <[http://dx.doi.org/10.1016/S1473-3099\(09\)70265-8](http://dx.doi.org/10.1016/S1473-3099(09)70265-8)>.
- 25 VINET, L.; ZHEDANOV, A. A ‘missing’ family of classical orthogonal polynomials. **Journal of Physics A: Mathematical and Theoretical**, v. 44, n. 8, p. 085201, 25 fev. 2011. Disponível em: <<https://academic.oup.com/femsl/article-lookup/doi/10.1111/1574-6968.12217>>.
- 30 WAGNER, D. et al. Breakthrough Invasive Infection Due to *Debaryomyces hansenii* (teleomorph *Candida famata*) and *Scopulariopsis brevicaulis* in a Stem Cell Transplant Patient Receiving Liposomal Amphotericin B and Caspofungin for Suspected Aspergillosis. **Infection**, v. 33, n. 5–6, p. 397–400, out. 2005. Disponível em: <<http://link.springer.com/10.1007/s15010-005-5082-4>>.
- WANG, J. et al. Antiviral Meroterpenoids Produced by the Antarctic Fungus *Aspergillus ochraceopetaliformis* SCSIO 05702. **Journal of Natural Products**, v. 79, n. 1, p. 59–65, 22 jan. 2016. Disponível em: <<http://pubs.acs.org/doi/10.1021/acs.jnatprod.5b00650>>.
- 35 WANIA, F. Assessing the Potential of Persistent Organic Chemicals for Long-Range Transport and Accumulation in Polar Regions. **Environmental Science & Technology**, v. 37, n. 7, p. 1344–1351, abr. 2003. Disponível em: <<https://pubs.acs.org/doi/10.1021/es026019e>>.
- 40 WANIA, F.; MACKAY, D. Science of the Total Environment 1995 Wania....pdf. v. 161, p. 211–232, 1995.
- WANIA, F.; MACKAY, D. Peer Reviewed: Tracking the Distribution of Persistent Organic Pollutants. **Environmental Science & Technology**, v. 30, n. 9, p. 390A–396A, ago. 1996. Disponível em: <<https://pubs.acs.org/doi/10.1021/es962399q>>.
- WENTZEL, L. C. P. et al. Fungi from Admiralty Bay (King George Island, Antarctica) Soils

- 5 and Marine Sediments. **Microbial Ecology**, v. 77, n. 1, p. 12–24, 19 jan. 2019. Disponível em: <<http://link.springer.com/10.1007/s00248-018-1217-x>>.
- WHITE, T. J. et al. PCR Protocols: A guide methods and applications. In: (Ed.). **Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics**, p. 315-322, 1990.
- 10 WHITE, T. C.; MARR, K. A.; BOWDEN, R. A. Clinical, Cellular, and Molecular Factors That Contribute to Antifungal Drug Resistance. **Clinical Microbiology Reviews**, v. 11, n. 2, p. 382–402, 1 abr. 1998. Disponível em: <<http://cmr.asm.org/lookup/doi/10.1128/CMR.11.2.382>>.
- 15 WIRTH, F.; GOLDANI, L. Z. **Epidemiology of rhodotorula: An emerging pathogen** **Interdisciplinary Perspectives on Infectious Diseases**, 2012. . Disponível em: <<http://www.hindawi.com/journals/ipid/2012/465717/>>.
- WU, G. et al. Penilactones A and B, two novel polyketides from Antarctic deep-sea derived fungus *Penicillium crustosum* PRB-2. **Tetrahedron**, v. 68, n. 47, p. 9745–9749, nov. 2012. Disponível em: <<http://dx.doi.org/10.1016/j.tet.2012.09.038>>.
- 20 WU, G. et al. Four New Chloro-Eremophilane Sesquiterpenes from an Antarctic Deep-Sea Derived Fungus, *Penicillium* sp. PR19N-1. **Marine Drugs**, v. 11, n. 4, p. 1399–1408, 23 abr. 2013. Disponível em: <<http://www.mdpi.com/1660-3397/11/4/1399>>.
- XIAO, N. et al. Comparison of functional properties of two fungal antifreeze proteins from *Antarctomyces psychrotrophicus* and *Typhula ishikariensis*. **FEBS Journal**, v. 277, n. 2, p. 394–403, 2010a.
- 25 XIAO, N. . B. et al. Antifreeze activities of various fungi and stramenopila isolated from Antarctica. **North American Fungi**, v. 5, n. 5, p. 215–220, 2010b. Disponível em: <<http://www.scopus.com/inward/record.url?eid=2-s2.0-78650542345&partnerID=40&md5=da0a8c12210a71ec52c9439ac39eaa5a>>.
- 30 YU, N. H. et al. Endophytic and endolichenic fungal diversity in maritime Antarctica based on cultured material and their evolutionary position among Dikarya. **Fungal Systematics and Evolution**, v. 1, n. June, p. 13–22, 2018. Disponível em: <<http://www.ingentaconnect.com/content/10.3114/fuse.2018.02.07>>.
- 35 ZAVREL, M.; WHITE, T. C. Medically important fungi respond to azole drugs: an update. **Future Microbiology**, v. 10, n. 8, p. 1355–1373, ago. 2015. Disponível em: <<https://www.futuremedicine.com/doi/10.2217/FMB.15.47>>.
- ZHANG, J. et al. Evolution of cross-resistance to medical triazoles in *Aspergillus fumigatus* through selection pressure of environmental fungicides. **Proceedings of the Royal Society B: Biological Sciences**, v. 284, n. 1863, p. 20170635, 27 set. 2017. Disponível em: <<http://rspb.royalsocietypublishing.org/lookup/doi/10.1098/rspb.2017.0635>>.
- 40 ZHANG, T. et al. Molecular analysis of fungal diversity associated with three bryophyte species in the Fildes Region, King George Island, maritime Antarctica. **Extremophiles**, v. 17, n. 5, p. 757–765, 2013.
- ZHOU, Y. et al. Furanone derivative and sesquiterpene from Antarctic marine-derived fungus

- 5 *Penicillium* sp. S-1-18. **Journal of Asian Natural Products Research**, v. 20, n. 12, p. 1108–1115, 2 dez. 2018. Disponível em: <<http://doi.org/10.1080/10286020.2017.1385604>>.

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10 Anexos

Anexo 1: Comprovante submissão artigo científico para revista Fungal Diversity

21/06/2019

Gmail - FUDI-D-19-00057 - Submission Notification to co-author



Graciéle Alves de Menezes <graciele.cunhaalves@gmail.com>

FUDI-D-19-00057 - Submission Notification to co-author

FUDI <em@editorialmanager.com>

20 de junho de 2019 08:46

Responder a: FUDI <saranya.sekar@springernature.com>

Para: Graciéle de Menezes <graciele.cunhaalves@gmail.com>

FUDI-D-19-00057

Re: "Fungi in the seasonal snow of Antarctica: diversity, distribution, and resistance against agricultural and clinical antifungal drugs"

Full author list: Graciéle de Menezes; Soraya Amorim, Dr; Vívian Gonçalves, Dr; Valéria Godinho, Dr; Jefferson Simões, Dr; Carlos Rosa; Luiz Rosa, Ph.D.

Dear Ms Graciéle de Menezes,

We have received the submission entitled: "Fungi in the seasonal snow of Antarctica: diversity, distribution, and resistance against agricultural and clinical antifungal drugs" for possible publication in Fungal Diversity, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. Dr. Luiz Rosa who will be able to track the status of the paper through his/her login.

If you have any objections, please contact the editorial office as soon as possible. If we do not hear back from you, we will assume you agree with your co-authorship.

Thank you very much.

With kind regards,

Springer Journals Editorial Office
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