



Diversity, distribution, and xerophilic tolerance of cultivable fungi associated with the Antarctic angiosperms

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Abstract

We characterized the diversity, distribution, systematic colonization, and xerophilic capabilities of fungi associated with the Antarctic angiosperms *Colobanthus quitensis* and *Deschampsia antarctica* collected at different sites of the South Shetlands Islands, Antarctic Peninsula. A total of 684 fungal isolates were obtained and identified into 67 taxa from 32 genera. The highest fungal diversity and richness were obtained from the rhizosphere, roots, and leaves, in order, and only 11 taxa shared between both plants. *Penicillium* and *Pseudogymnoascus* were the dominant fungal genera. However, the rarefaction curves for plant fungal assemblages did not reach a plateau, suggesting that these Antarctic plants may host more fungi in their tissues and rhizospheres. A total of 460 isolates grew at water activity (a_w) = 0.95, 200 at 0.90, 110 at 0.81, and 47 at 0.66. *Antarctomyces*, *Cladosporium*, *Mortierella*, *Leptosphaeria*, *Penicillium*, *Pseudogymnoascus*, and *Thelebolus* taxa grew at a_w = 0.81 and 0.66 and considered highly xerophilic. In addition, specific isolates of *Penicillium* and *Thelebolus* exhibited the highest mycelial growth at a_w = 0.66. Our results show that the internal tissues and rhizosphere of Antarctic angiosperms host rich and diverse fungal communities dominated by cold-adapted and endemic taxa, which seem to coexist as symbionts and decomposer fungi. In addition, specific fungi are able to colonize different parts of the plant, suggesting a high ecological relationship with their hosts. Finally, different fungi living in the rhizosphere displayed remarkable xerophilic tolerance, representing promising candidates for further biotechnological studies, including identification of genes for applications in industry and agriculture.

Keywords Antarctica · Bioprospecting · Ecology · Endophytes · Xerophiles

Introduction

Antarctic shelter environments can support different life forms adapted to extreme physical and chemical conditions such as cold temperature, water availability, high UV radiation, strong winds, different pH, and salinity ranges (Rosa et al. 2019). The dynamic spatial distribution of living species in Antarctica ranges according to the availability of organic matter. Biological diversity decreases from the

Peninsular to continental regions as environmental effects become more extreme.

The major part of Antarctica is permanently covered by ice or snow, and only about 0.3% of its area is available for colonization by plants, mainly along the Antarctic Peninsula, its archipelagos, and the coastal region of the Antarctic continent (Convey et al. 2009). Antarctic angiosperms and bryophytes have been demonstrated to represent a microhabitat hotspot for different life forms such as viruses, bacteria, fungi, and invertebrates (Möller and Dreyfuss 1996; Pearce and Wilson 2003; Ebach et al. 2008; Teixeira et al. 2013). The two native Antarctic angiosperms capable of surviving the extreme conditions of the Antarctic Peninsula during the winter season are the dicot Antarctic pearlwort *Colobanthus quitensis* (Kunth) Bartl. and the monocot Antarctic hairgrass *Deschampsia antarctica* E. Desv. (Convey et al. 2014).

Microorganisms display genetic and biochemical characteristics promoting survival and colonization in different habitats and ecosystems of Antarctica. Among the Antarctic

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microbial life identified, fungi of the phyla Ascomycota, Basidiomycota, Mortierellomycota, and Mucoromycota have been discovered in macroalgae thalli, sediment, seawater, invertebrates in marine environment, soil, rocks, permafrost, snow, ice, and plants in terrestrial ecosystems (Rosa et al. 2019). However, despite the increase in fungal studies in recent years, fungal diversity in Antarctica remains poorly catalogued.

Within the microbiome of Antarctic plants, fungi can occur as symbiotic endophytes inside leaf and root tissues, as epiphytes on the surface, and can also live in the rhizosphere (Carvalho et al. 2019). The major studies on fungi associated with *C. quitensis* and *D. antarctica* involve endophytes (Möller and Dreyfuss 1996; Rosa et al. 2009, 2010; Upson et al. 2009; Santiago et al. 2012, 2017). However, few studies have analysed the systematic colonization of endophytes or fungi living in the rhizosphere across different regions of the Antarctic Peninsula. For these reasons, here, we examined the diversity, distribution, systematic colonization across plant parts, and xerophilic capacity of endophytic and rhizosphere fungi associated with the Antarctic angiosperms *C. quitensis* and *D. antarctica* collected at different sites of the South Shetlands Islands and Antarctic Peninsula.

Materials and methods

Plant samples and isolation of associated fungi

Three samples from each plant species were collected from different sites of the South Shetlands Islands in the Antarctic Peninsula (Fig. 1). Five fragments of healthy leaves were subjected to surface disinfestation and inoculated onto Potato Dextrose Agar (PDA) (Difco, USA) containing $100 \mu\text{g mL}^{-1}$ chloramphenicol (Sigma, USA) for isolation of endophytic fungi according to the protocol established by Carvalho et al. (2012).

For endophytes from roots, five fragments of healthy roots were subjected to surface disinfestation according to Upson et al. (2009) and inoculated onto PDA (Difco, USA), Dichloran-Glycerol (DG-18, Acumedia, USA), Hagem agar [KH_2PO_4 0.5 g, NH_4Cl 0.5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, FeCl_3 (1%) 0.5 mL, glucose 5 g, malt extract 5 g, thiamine HCl 50 μg , aureomycin 35 mg, agar 15 g, 1000 mL distilled water, pH adjusted to 4.5–5], Melin-Norkrans [MMN, KH_2PO_4 0.5 g, NH_4Cl 0.25 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.15 g, FeCl_3 (1%) 0.5 mL, CaCl_2 0.05 g, NaCl 0.025 g, glucose 10 g, thiamine HCl 100 μg , aureomycin 35 mg, agar 15 g, 1000 mL distilled water, pH adjusted to 5.7–6.2], and PGK (glucose 10 g, peptone 3.33 g, yeast extract 0.67 g, NH_4NO_3 1 g, KH_2PO_4 0.264 g, K_2HPO_4 0.628 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.33 g,

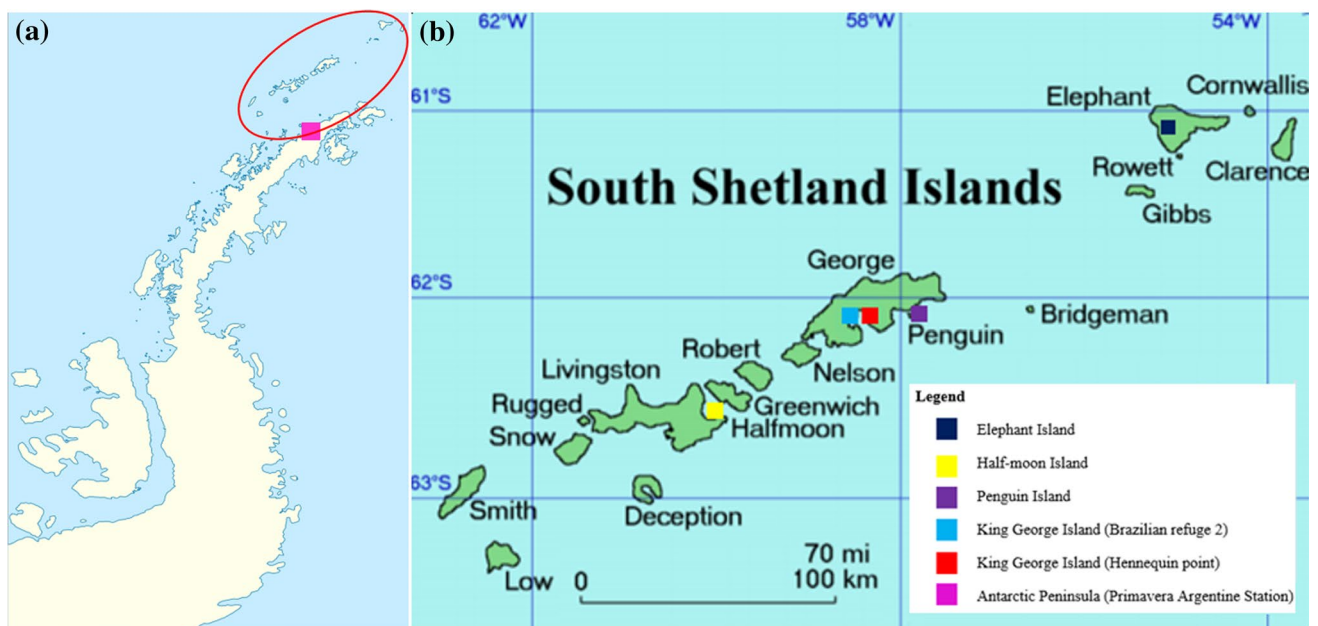


Fig. 1 Map with the sites where the Antarctic angiosperms were sampled. **a** Antarctic Peninsula and **b** South Shetland with Elephant Island [close to the Brazilian Emílio Goeldi refuge (S 61°13′18.2″; W 55°21′54.3″)], King George Island [close to the Brazilian refuge 2 (S 62°04′89.4″; W 58°23′65.8″) and Hennequin point (S 62°07′22.9″;

W 58°23′ 46.1″)], Penguin Island (S 62°06′04.7″; W 57°55′13.0″) Half Moon Island (S 62°35′43.8″; W 59°55′05.9″), and Antarctic Peninsula [close to the Primavera Argentine station (S 64°09′18.6″; W 60°57′20.1″)

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.0021 g, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.0006 g, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.0005 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.0004 g, 1000 mL of distilled water, pH adjusted to 5.8), both media containing 100 $\mu\text{g mL}^{-1}$ chloramphenicol.

For isolation of rhizosphere fungi, 1 g of each sample root was added to 9 mL of a 0.85% NaCl solution, sonicated, and 100 μL of a 1000-fold dilution was inoculated onto Petri dishes containing PDA, DG-18, Hagem agar, Melin-Norkrans agar, or PGK. All plates were incubated at 15 °C for 60 days. All fungi obtained were deposited in the Collection of Microorganisms and Cells of the Federal University of Minas Gerais, Brazil, under the code UFMGCB.

Fungal identification

The protocol for DNA extraction followed Rosa et al. (2009). Filamentous fungal isolates were grouped into different morphospecies according to their characteristics of the culture: colony color and texture, border type, and radial growth rate on PDA agar (Fröhlich et al. 2000). Isolates with identical morphological characteristics were grouped together and subjected to PCR fingerprinting using the microsatellite-primed PCR technique (GTG)₅ by Lieckfeldt et al. (1993). Based on the electrophoretic profile of PCR-amplified products with primer (GTG)₅, an isolated among those who showed the same pattern of bands was selected for sequencing of the nuclear ribosomal RNA gene. 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), which are commonly utilized to fungal taxa with low intraspecific variation, was completed with the Bt2a/Bt2b primers, according to protocols established by Gonçalves et al. (2015). The yeasts were grouped and identified according to protocols established by Kurtzman et al. (2011). Yeast molecular identities were confirmed by sequencing the D1–D2 variable domains of the large-subunit rRNA gene using the primers NL1 and NL4 as described by Lachance et al. (1999). Fungal 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 (Online Resource 1). To achieve species-rank identification based on ITS and β -tubulin data, 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 $\leq 98\%$ identity or an inconclusive taxonomic position were subjected to phylogenetic ITS and β -tubulin based analysis for comparison with sequences of ex type species deposited in the GenBank database. 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>).

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, considering the total specimens number of each plant species. All of the results were obtained with 95% confidence, and bootstrap values were calculated from 1000 iterations. The rarefaction curve was calculated using the Mao Tao index. All diversity and similarity indices calculations were performed using PAST, version 1.90 (Hammer et al. 2001). Venn diagrams were prepared according to Bardou et al. (2014) to illustrate the comparison of fungal assemblages associated with the plants with high sampling.

Fungal xerophilic tolerance to different water activities

To determine the xerophilic capacity, all fungi obtained from the rhizosphere were grown progressively on DG18 [18% glycerol; water activity (a_w) = 0.95], DG36 (36% glycerol; a_w = 0.90), DG54 (54% glycerol; a_w = 0.81), and DG72 (72% glycerol; a_w = 0.66) media in 48-well plates. Plates were incubated at 15 °C for 9–27 days. Mycelial growth rate of all fungi that grew on DG72 (a_w = 0.66) was determined on DG18, DG36, DG54, and DG72. A 3 mm plug of mycelia was inoculated on 90 × 15 mm rectangular Petri dishes containing 20 mL of DG72 medium, incubated for 27 days at 15 °C, and the colony diameter was measured in millimeters every 9 days. All assays were conducted in triplicate and analyzed using the Tukey's honest significance test. Water activity (a_w) was calculated using RAOULT' Law according to formula $a_w = \text{number of moles of H}_2\text{O} / \text{number of moles of H}_2\text{O} + \text{number of moles of glycerol}$.

Results

Fungal taxonomy

From leaves, roots, and the rhizosphere of *C. quitensis* and *D. antarctica*, 684 fungi that were identified represented 67 taxa of 32 genera (Online Resource 1). For both plants, the highest fungal diversity and richness were obtained from the rhizosphere, followed by the roots and leaves. *Penicillium* and *Pseudogymnoascus* were the dominant genera and *Antarctomyces pellizariae*, *Penicillium* sp. 3, *Penicillium* sp. 4, *Pseudogymnoascus destructans*, *Vishniacozyma tephrensis*, and *Volucrispora graminea* represented the dominant taxa.

Table 1 Diversity indices of fungal assemblages associated with Antarctic plants *Deschampsia antarctica* and *Colobanthis quitensis* sampled in South Shetlands, Antarctic Peninsula

Diversity indices Plant species/site	<i>Deschampsia antarctica</i>						<i>Colobanthis quitensis</i>				
	Elephant Island		King George R2		Penguin Island		King George R2		Penguin Island		Total
	HP	King George Island	HP	King George Island	Half Moon Island	Antarctic Peninsula	King George R2	Half Moon Island	Antarctic Peninsula	Total*	
N° of taxa	16	16	11	13	13	20	7	20	20	58	23
Fisher α	5.11	5.15	2.75	5.07	3.99	5.81	2.57	4.28	5.81	4.49	5.58
Margalef	3.18	3.19	2.00	2.92	2.61	3.68	1.67	0.90	3.68	2.23	2.81
Simpson	0.71	0.85	0.52	0.86	0.85	0.88	0.65	8.26	0.88	0.96	0.92

King George HP = Hennequin Point at King George Island; King George R2 = Brazilian Refuge 2 at King George Island

*Diversity indices values considering the total specimens number of each plant species

Diversity, richness, dominance, and distribution

In general, we observed a high fungal diversity associated with the Antarctic angiosperms when compared with endophytic fungal communities associated with plants of polar and temperate environments (Table 1); especially fungi associated with *C. quitensis* displayed the highest diversity indices when compared to *D. antarctica*. In addition, diversity indices varied among the sampling sites. The fungal assemblage associated with *D. antarctica* from the Antarctic Peninsula site displayed the highest values of diversity, richness, and dominance, followed by samples from Hennequin Point at King George Island and Elephant Island. For fungal assemblages of *C. quitensis*, sample from the Penguin Island displayed the highest diversity values. The rarefaction curves of Mao Tao (Fig. 2) for both plant fungal assemblages did not reach a plateau, suggesting that, despite the high fungal richness detected, these Antarctic plants may host more fungi in their tissues and rhizospheres.

The fungal distribution between the plants is shown in Fig. 3a and that among their tissues and rhizosphere in Fig. 3b, c. Eleven taxa were associated with both plants. Analysis of the fungal distribution across *D. antarctica* revealed that no fungus occurred simultaneously in tissues of leaves and roots. *Mortierella parvispora* occurred in the leaves and rhizosphere, *Antarctomyces pellizariae*, *Helotiales* sp., *Penicillium* sp. 1, *Penicillium* sp. 3, *Penicillium* sp. 4, *Penicillium* sp. 6, *Mortierella fimbriocystis*, *Neosascochyta paspali*, and *Volucrispora graminea* occurred in the root tissues and rhizosphere, and only *Pseudogymnoascus destructans* was recovered from leaves, roots, and rhizosphere. For *C. quitensis*, *Alpinaria rhododendri*, *Glarea* sp., and *Penicillium* sp. 3 were retrieved from root tissues and the rhizosphere.

Fungal xerophilic tolerance to ranging water activities

A total of 463 fungal isolates obtained from the rhizosphere of *C. quitensis* and *D. antarctica* were screened for their ability to grow in different water activity conditions. From those, 460 isolates grew at 18% glycerol ($a_w = 0.95$), 200 at 36% ($a_w = 0.90$), 110 at 54% ($a_w = 0.81$), and 47 at 72% ($a_w = 0.66$). Fungi that grew at 54 and 72% glycerol were considered highly xerophilic and represented the taxa *Antarctomyces*, *Cladosporium*, *Mortierella*, *Leptosphaeria*, *Penicillium*, *Pseudogymnoascus*, and *Thelebolus*. Mycelial growth rate of the 42 fungal isolates that grew at 72% glycerol were measured at different glycerol concentrations (Table 2). Specific isolates of *Penicillium* and *Thelebolus* exhibited the highest mycelial growth on DG72 (≥ 30 mm) (Online Resource 2).

Table 2 Mycelial rate growth of fungi obtained from the Antarctic angiosperm rhizospheres at different water activities values after 27 days at 15 °C

Fungi	UFMGCB	Mycelial growth in mm			
		DG18 ($a_w = 0.95$)	DG36 ($a_w = 0.90$)	DG54 ($a_w = 0.81$)	DG72 ($a_w = 0.66$)
<i>Mortierella antarctica</i>	13135	1.50	0.92	0.37	0.23
<i>Mortierella elongulata</i>	13232	1.33	1.14	0.36	0.28
<i>Mortierella gamsii</i>	13468	1.86	0.72	0.43	0.19
<i>Mortierella</i> sp. 1	13219	1.62	1.34	0.38	0.27
<i>Penicillium</i> sp. 2	13334	1.97	0.99	0.30	0.21
<i>Penicillium</i> sp. 3	13464	0.99	0.61	0.29	0.20
<i>Penicillium</i> sp. 3	13467	1.65	0.93	0.35	0.15
<i>Penicillium</i> sp. 3	13469	1.17	0.63	0.46	0.22
<i>Penicillium</i> sp. 3	13472	0.95	0.74	0.29	0.22
<i>Penicillium</i> sp. 3	13473	0.91	0.72	0.57	0.19
<i>Penicillium</i> sp. 3	13474	1.74	0.67	0.39	0.30
<i>Penicillium</i> sp. 3	13476	1.00	0.83	0.32	0.19
<i>Penicillium</i> sp. 3	13497	0.94	0.85	0.22	0.20
<i>Penicillium</i> sp. 3	13500	2.01	0.88	0.33	0.29
<i>Penicillium</i> sp. 3	13501	1.27	0.98	0.28	0.24
<i>Penicillium</i> sp. 3	13522	1.36	0.80	0.27	0.22
<i>Penicillium</i> sp. 3	13523	1.32	0.73	0.27	0.22
<i>Penicillium</i> sp. 3	13530	1.84	1.18	0.37	0.31
<i>Penicillium</i> sp. 3	13537	1.00	0.60	0.36	0.23
<i>Penicillium</i> sp. 3	13554	1.47	0.77	0.34	0.27
<i>Penicillium</i> sp. 3	13557	1.20	1.06	0.28	0.23
<i>Penicillium</i> sp. 3	13558	0.93	0.52	0.45	0.29
<i>Penicillium</i> sp. 3	13575	2.44	1.02	0.40	0.23
<i>Penicillium</i> sp. 3	13578	1.68	1.11	0.29	0.26
<i>Penicillium</i> sp. 3	13580	0.88	0.72	0.48	0.32
<i>Penicillium</i> sp. 1	13583	1.03	0.74	0.45	0.29
<i>Penicillium</i> sp. 3	13462	1.04	0.68	0.24	0.20
<i>Penicillium</i> sp. 4	13466	1.44	0.71	0.30	0.15
<i>Penicillium</i> sp. 11	13470	0.93	0.82	0.42	0.23
<i>Penicillium</i> sp. 4	13471	0.85	0.50	0.29	0.26
<i>Penicillium</i> sp. 4	13475	1.16	0.73	0.21	0.17
<i>Penicillium</i> sp. 4	13498	0.57	0.53	0.28	0.22
<i>Penicillium</i> sp. 4	13450	1.67	0.84	0.37	0.33
<i>Penicillium</i> sp. 4	13465	1.09	0.70	0.27	0.21

Table 2 (continued)

Fungi	UFMGCB	Mycelial growth in mm			
		DG18 ($a_w = 0.95$)	DG36 ($a_w = 0.90$)	DG54 ($a_w = 0.81$)	DG72 ($a_w = 0.66$)
<i>Penicillium</i> sp. 5	13524	1.43	0.70	0.28	0.17
<i>Penicillium</i> sp. 5	13480	1.26	0.66	0.28	0.21
<i>Penicillium</i> sp. 5	13482	1.05	0.56	0.34	0.26
<i>Penicillium</i> sp. 6	13222	1.03	0.83	0.65	0.52
<i>Penicillium</i> sp. 8	13227	1.89	0.80	0.55	0.36
<i>Penicillium</i> sp. 8	13146	2.04	1.20	0.54	0.31
<i>Penicillium</i> sp. 11	13584	1.39	0.65	0.43	0.34
<i>Thelebotus balaustiformis</i>	13448	1.64	1.19	0.53	0.47

UFMGCB = code of the Fungal Culture Collection of Universidade Federal de Minas Gerais. a_w = water activity value. DG18 = dichloran medium with 18% of glycerol, DG36 = dichloran medium with 36% of glycerol, DG54 = dichloran medium with 54% of glycerol, and DG72 = dichloran medium with 72% of glycerol
 In bold the fungi with the best mycelial growth on DG72

Discussion

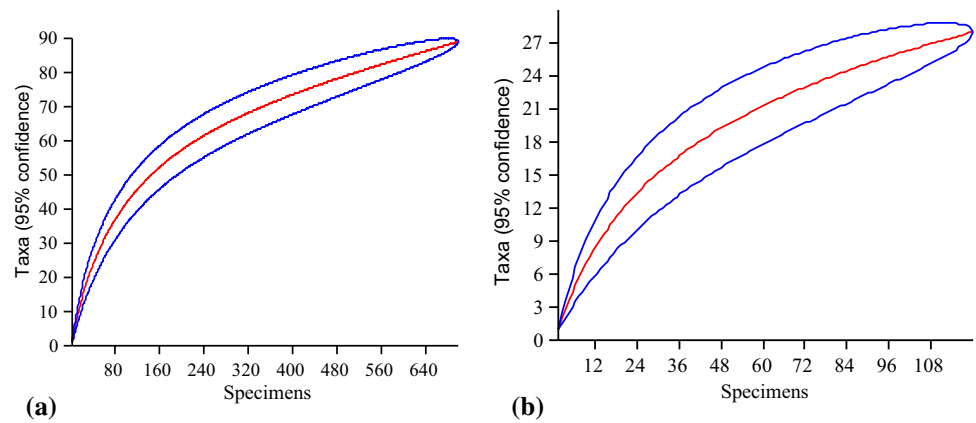
Taxonomy and fungal diversity

In the polar ecosystems of Antarctica, there are few plant species in comparison to temperate and tropical regions. However, Antarctic plants seem to play an important role in providing microhabitats, which are considered hotspots of microbial diversity (Carvalho et al. 2019). Among the Antarctic plants, the only angiosperms adapted to the Antarctic environmental conditions are *C. quitensis* and *D. antarctica*, which have been studied as hosts for fungi (Möller and Dreyfuss 1996; Rosa et al. 2009, 2010; Upson et al. 2009; Vaz et al. 2011; Santiago et al. 2012, 2017; Hereme et al. 2020). However, few of these studies focused on the systematic fungal colonization of internal tissues (leaves and roots) and the external rhizosphere in different regions of the Antarctic Peninsula. In our study, no fungi were recovered from leaves of *C. quitensis*. However, Rosa et al. (2010) obtained 188 fungal isolates from leaves of *C. quitensis* sampled in the Admiralty Bay, at King George Island and identified as endophytes species of *Aspergillus*, *Cadophora*, *Davidiella*, *Entrophospora*, *Fusarium*, *Pseudogymnoascus*, *Gyoerffyella*, *Microdochium*, *Mycocentrospora*, and *Phaeosphaeria*. As Rosa et al. (2010) sampled 180 specimens of *C. quitensis*, perhaps, in our study, the number of *C. quitensis* specimens (18) obtained were not enough to recover the endophytic fungi.

The fungal community associated with the two Antarctic angiosperms displayed high diversity, mainly due to the richness detected in the rhizosphere of both plants. In general, *Penicillium* and *Pseudogymnoascus* dominate the community. Species of the genus *Penicillium* represent abundant cosmopolitan cold-adapted taxa that are widespread and well adapted in different environments and substrates in Antarctica (Rosa et al. 2019). *Pseudogymnoascus* are abundant in Antarctica and have been isolated from different substrates and environments, many of which have been reported at the genus level only, and thus, may include new species different from those reported from the northern hemisphere (Rosa et al. 2019). *Pseudogymnoascus destructans* occurs in both plant microbiomes and is characterized as a psychrophilic bat pathogenic fungus, the causative agent of white-nose syndrome (WNS) in temperate regions (Lorch et al. 2011). Similar results were reported by Santiago et al. (2015), who detected a high frequency of *P. destructans* in the lichensphere in Antarctica.

In addition, *Antarctomyces pellizariae*, *Vishniacozyma tephrensensis*, and *Volucrispora graminea* were detected in different parts of the Antarctic angiosperms. There are two reported species of the *Antarctomyces* genera endemic of Antarctica, *Antarctomyces psychrotrophicus* (isolated from

Fig. 2 Species accumulation rarefaction curves **a** fungal assemblages associated with the Antarctic angiosperms species *Deschampsia antarctica* and **b** *Colobanthus quitensis* based on Mao’s Tau estimator. Dotted line shows 95% confidence limits



soil) and *A. pellizariae* (isolated from snow) on the King George Island from the South Shetland Islands (Stchigel et al. 2001; de Menezes et al. 2017). *A. psychrotrophicus* has already been identified as a symbiotic endophyte of *D. antarctica* (Rosa et al. 2009). Species of *Vishniacozyma* (previously reported as *Cryptococcus*) are frequently found in different Antarctic substrates, and many of them are psychrotolerant (Vishniac 2006). *Vishniacozyma* species are commonly found in tissues (Santiago et al. 2017) and soils close to plants of temperate (Renker et al. 2004) and Antarctic environments (Vaz et al. 2011). *Volucrispora graminea* (previously reported as *Ypsilina graminea*) is recognized as an aquatic hyphomycete present in Arctic streams or on decaying sedges or grasses (Gulis et al. 2012). Möller and Dreyfuss (1996) isolated *V. graminea* from thalli of lichens

and internal tissues of *C. quitensis* and *D. antarctica*, suggesting that they are psychrotolerant. In addition, Rosa et al. (2020) reported *V. graminea* in the fairy ring of *Sanionia uncinata* on the Antarctic Peninsula.

Xerophilic tolerance

Xerophile fungi are able to grow at or below a water activity (a_w) of 0.85 (Pettersson and Leong 2001). Some compounds with protection activity against drying produced by xerophilic organisms may be useful in industry and agriculture biotechnology. Among these organisms, xerophilic fungi are efficient producers of different hydrolytic enzymes such as amylases, cellulases, lipases, and proteases, which are useful in biotechnological process (Chamekh et al. 2019). Fungi of

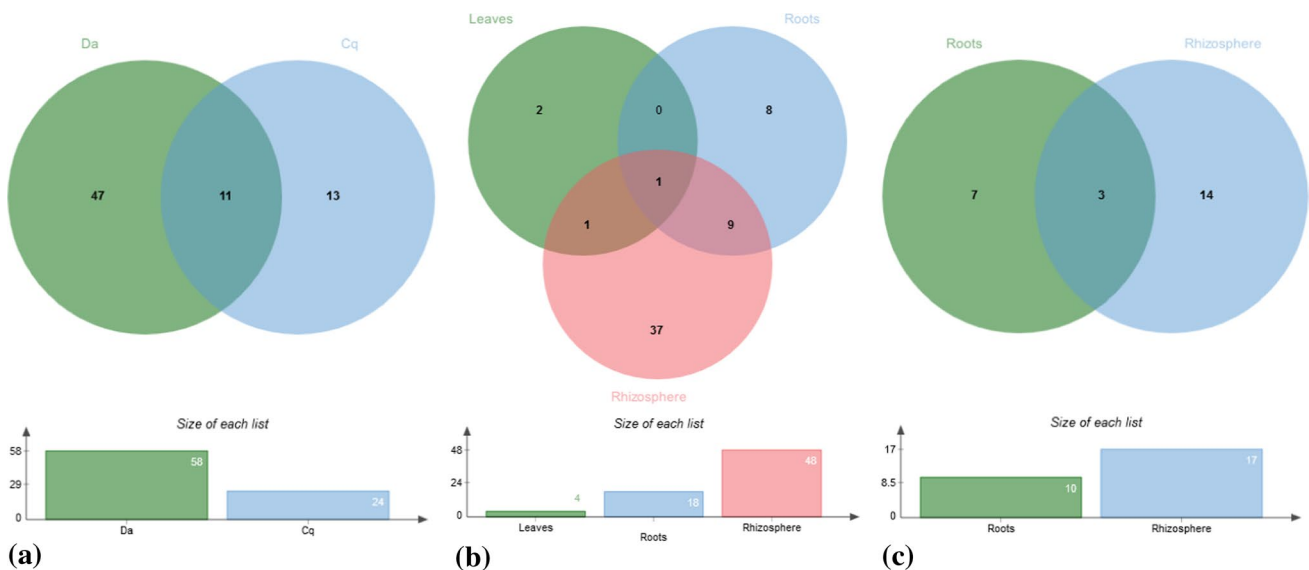


Fig. 3 Similarity of fungal assemblages associated with the Antarctic angiosperms *Deschampsia antarctica* and *Colobanthus quitensis* sampled in South Shetlands Archipelago in the Antarctic Peninsula represented by Venn diagrams. **a** represents the total fungal distribu-

tion between $Da = D. antarctica$ and $Cq = C. quitensis$, **b** shows the fungal distribution in the different parts sampled of *D. antarctica*, and **c** shows the fungal distribution in the different parts sampled of *C. quitensis*

seven genera obtained from the rhizosphere tolerated low water activity conditions, among which, *Mortierella* and *Penicillium* occurred frequently. According to Pettersson and Leong (2001), xerophilic microorganisms display different biochemical pathways to survive and colonize environments where little water is available, including membrane osmosensors involved in glycerol regulation within the cell. *Mortierella* species are commonly found in Antarctica in different substrates such as mosses (Tosi et al. 2020), lichens (Santiago et al. 2015), soil (Bridge and Newsham 2009; Gomes et al. 2018), freshwater lakes (Gonçalves et al. 2012), thalli of macroalgae (Godinho et al. 2013), and in the rhizosphere of *D. antarctica* (Gonçalves et al. 2015). *Mortierella* species produce polyunsaturated fatty acids (Eroshin and Dedyukhina 2002; Melo et al. 2014), promoting tolerance to low water activity conditions.

Penicillium taxa isolated from *C. quitensis* and *D. antarctica* rhizosphere exhibit range about their capabilities at different values of *aw*. *Penicillium* species, which may represent the most adapted fungal group in Antarctica (Rosa et al. 2019), can survive in substrates with low water availability, such as plant surfaces (Fletcher et al. 1985), oligotrophic soils (McRae et al. 1999; Gomes et al. 2018), ultraoligotrophic soils of continental Antarctica (Godinho et al. 2015), permafrost (Zuconi et al. 2012), and rocks (Gonçalves et al. 2017). According to Corry (1987), xerophily is a common physiological property of many *Penicillium* species. Among the *Penicillium* taxa obtained, isolates of *Penicillium* sp. 6 and *Penicillium* sp. 8 demonstrated high xerophilic tolerance, deserving further polyphasic taxonomic identification at the species level.

Thelebolus balaustiformis isolated from the rhizosphere of *C. quitensis* grew on DG72. *Thelebolus* includes species living in different habitats of the world (Crous et al. 2004; Vanderwolf et al. 2018; Bovio et al. 2018), often recovered from polar environments of Arctic and Antarctica (Kobayasi et al. 1967; Montemartini et al. 1993; Sazanova et al. 2019; Alves et al. 2019). *Thelebolus balaustiformis* represents a new species isolated from the sponge *Dysidea fragilis* in the Atlantic Ocean (Bovio et al. 2018) and in fragments of ice in Antarctica (de Menezes et al. 2020).

Conclusion

Our results show that the Antarctic angiosperms *C. quitensis* and *D. antarctica* host in their internal tissues and rhizospheres rich and diverse fungal communities dominated by cold-adapted and endemic taxa, which seem to coexist with their hosts as symbionts and decomposer fungi. In addition, some of these fungi are able to colonize systematically different parts of the plant, suggesting a high ecological

relationship with their hosts. Different fungi living in the rhizosphere of the Antarctic angiosperms displayed xerophilic tolerance. Despite the fact that xerophiles cause considerable economic losses in storage food products, they might represent promising candidates for further biotechnological studies to detect byproducts (such as carbohydrate-active enzymes, proteins, and polysaccharides) and/or genes for applications in industry and agriculture.

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Author contributions LCC and LHR conceived the study. LCC and CRC performed fungi isolation. LCC, CRC, LHR and CAR identified the fungi. LCC performed the xerophilic experiments. LCC, CRC, CAR and LHR analyzed the results and wrote the manuscript. All authors read and approved of the final manuscript.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

References

- Altschul SF, Madden TL, Schäffer AA et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402
- Alves IM, Gonçalves VN, Oliveira FS et al (2019) The diversity, distribution, and pathogenic potential of cultivable fungi present in rocks from the South Shetlands archipelago, Maritime Antarctica. *Extremophiles* 23:327–336
- Bardou P, Mariette J, Escudé F, Djemiel C, Klopp C (2014) Jvarkit: an interactive Venn diagram viewer. *BMC Bioinform* 15:293
- Bovio E, Garzoli L, Poli A et al (2018) The culturable mycobiota associated with three Atlantic sponges, including two new species: *Thelebolus balaustiformis* and *T. spongiae*. *Fungal Syst Evol* 1:141–167
- Bridge PD, Newsham KK (2009) Soil fungal community composition at Mars Oasis, a southern maritime Antarctic site, assessed by PCR amplification and cloning. *Fungal Ecol* 2:66–74
- Carvalho CR, Gonçalves VN, Pereira CB et al (2012) The diversity, antimicrobial and anticancer activity of endophytic fungi associated with the medicinal plant *Stryphnodendron adstringens* (Mart.) Coville (Fabaceae) from the Brazilian savannah. *Symbiosis* 57:95–107
- Carvalho CR, Santiago IF, Coelho LC et al (2019) Fungi associated with plants and lichens of Antarctica. In: Rosa LH (ed) *Fungi of Antarctica: diversity ecology and biotechnological applications*. Springer, Cham, Switzerland, pp 165–200
- Chamekh R, Deniel F, Donot C et al (2019) Isolation, identification and enzymatic activity of halotolerant and halophilic fungi from the Great Sebkhia of Oran in Northwestern of Algeria. *Mycobiology* 47:230–241

- Convey P, Bindschadler R, Di Prisco G et al (2009) Antarctic climate change and the environment. *Sustain Sci* 3:9–22
- Convey P, Chown SL, Clarke A et al (2014) The spatial structure of Antarctic biodiversity. *Ecol Monogr* 84:203–244
- Corry JEL (1987) Relationships of water activity to fungal growth. In: Beuchat LR (ed) *Food and beverage mycology*. Van Nostrand Reinhold, New York, pp 51–99
- Crous PW, Gams W, Stalpers JA et al (2004) MycoBank: an online initiative to launch mycology into the 21st century. *Stud Mycol* 50:19–22
- 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 from Antarctica. *Extremophiles* 21:259–269
- de Menezes GCA et al (2020) Fungi in glacial ice of Antarctica: diversity, distribution and bioprospecting of bioactive compounds. *Extremophiles* 24:367–376
- Ebach M, Pugh PJA, Convey P (2008) Surviving out in the cold: Antarctic endemic invertebrates and their refugia. *J Biogeogr* 35:2176–2186
- Eroshin V, Dedyukhina E (2002) Effect of lipids from *Mortierella hygrophila* on plant resistance to phytopathogens. *World J Microbiol Biotechnol* 18:165–167
- Fletcher LD, Kerry EJ, Weste GM (1985) Microfungi of Mac. Robertson and Enderby Lands, Antarctica. *Polar Biol* 4:81–88
- Fröhlich J, Hyde KD, Petrini O (2000) Endophytic fungi associated with palms. *Mycol Res* 104:1202–1212
- 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 et al (2013) Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. *ISME J* 7:1434–1451
- 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
- Gomes EC, Godinho VM, Silva DA et al (2018) Cultivable fungi present in Antarctic soils: taxonomy, phylogeny, diversity, and bioprospecting of antiparasitic and herbicidal metabolites. *Extremophiles* 22:381–393
- Gonçalves VN, Vaz ABM, Rosa CA, Rosa LH (2012) Diversity and distribution of fungal communities in lakes of Antarctica. *FEMS Microbiol Ecol* 82:459–471
- Gonçalves VN, 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
- Gonçalves VN, Oliveira FS, Carvalho C et al (2017) Antarctic rocks from continental Antarctica as source of potential human opportunistic fungi. *Extremophiles* 21:851–860
- Gulis V, Baschien C, Marvanova L (2012) Two new *Tricladium* species from streams in Alaska. *Mycologia* 104:1510–1516
- Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontol Electron* 4:9
- Hereme R, Morales-Navarro S, Ballesteros G et al (2020) Fungal endophytes exert positive effects on *Colobanthus quitensis* under water stress but neutral under a projected climate change scenario in Antarctica. *Front Microbiol* 11:264
- Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) *Dictionary of the fungi*, 10th edn. CAB International, Wallingford
- Kobayasi Y, Hiratsuka N, Korf RP et al (1967) Mycological studies of the Alaskan Arctic. *Annu Rep Inst Ferment Osaka* 3:1–138
- Kurtzman CP, Fell JW, Boekhout T, Robert V (2011) Methods for isolation, phenotypic characterization and maintenance of yeasts. In: Kurtzman CP, Fell JW, Boekhout T (eds) *The yeasts, a taxonomic study*. Elsevier, Amsterdam, pp 87–110
- 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
- Lieckfeldt E, Meyer W, Borner T (1993) Rapid identification and differentiation of yeast by DNA and PCR fingerprinting. *J Basic Microbiol* 33:413–426
- Lorch JM, Meteyer CU, Behr JM et al (2011) Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* 480:376–378
- McRae CF, Hocking AD, Seppelt RD (1999) *Penicillium* species from terrestrial habitats in the Windmill Islands, East Antarctica, including a new species, *Penicillium antarcticum*. *Polar Biol* 21:97–111
- Melo IS, Santos SN, Rosa LH et al (2014) Isolation and biological activities of an endophytic *Mortierella alpina* strain from the Antarctic moss *Schistidium antarctici*. *Extremophiles* 18:15–23
- Möller C, Dreyfuss MM (1996) Microfungi from Antarctic lichens, mosses and vascular plants. *Mycologia* 88:922–933
- Montemartini A, Caretta G, Del Frate G (1993) Notes on *Thelebolus microsporus* isolated in Antarctica. *Mycotaxon* 48:343–358
- Pearce DA, Wilson WH (2003) Viruses in Antarctic ecosystems. *Antarct Sci* 15:319–331
- Pettersson OV, Leong SL (2001) Fungal xerophiles (Osmophiles). In: eLS (ed) <https://doi.org/10.1002/9780470015902.a0000376.pub2>
- Renker C, Zobel M, Öpik M et al (2004) Structure, dynamics, and restoration of plant communities: do arbuscular mycorrhizae matter? In: Temperton VM, Hobbs RJ, Nuttle T, Halle S (eds) *Assembly rules and restoration ecology—bridging the gap between theory and practice*. Island Press, Washington, pp 189–229
- 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
- Rosa LH, Vieira MLA, Santiago IF et al (2010) Endophytic fungi community associated with the dicotyledonous plant *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) in Antarctica. *FEMS Microbiol Ecol* 73:178–189
- Rosa LH, Zani CL, Cantrell CL et al (2019) Fungi in Antarctica: diversity, ecology, effects of climate change, and bioprospection for bioactive compounds. In: Rosa LH (ed) *Fungi of Antarctica: diversity ecology and biotechnological applications*. Springer, Cham, pp 1–18
- Rosa LH, Sousa JRP, de Menezes GCA et al (2020) Opportunistic fungi present in fairy rings are present on different moss species in the Antarctic Peninsula. *Polar Biol* 43:587–596
- Santiago IF, Alves TM, Rabello A et al (2012) Leishmanicidal and antitumoral activities of endophytic fungi associated with the Antarctic angiosperms *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. *Extremophiles* 16:95–103
- Santiago IF, Soares MA, Rosa CA, Rosa LH (2015) Lichensphere: a protected natural microhabitat of the non-lichenised fungal communities living in extreme environments of Antarctica. *Extremophiles* 19:1087–1097
- 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
- Sazanova KV, Senik SV, Kirtsideli IY, Shavarda AL (2019) Metabolomic profiling and lipid composition of Arctic and Antarctic strains of *Micromycetes Geomyces pannorum* and *Thelebolus microsporus* grown at different temperatures. *Microbiology* 88:282–291

- Stehigel AM, Cano J, MacCormack CW (2001) *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. *Mycol Res* 105:377–382
- Teixeira LCRS, Yergeau E, Balieiro FC et al (2013) Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. *PLoS One* 8:e66109
- Tosi S, Casado B, Gerdol R (2020) Fungi isolated from Antarctic mosses. *Polar Biol* 25:262–268
- Upton R, Newsham KK, Bridge PD et al (2009) Taxonomic affinities of dark septate root endophytes of *Colobanthus quitensis* and *Deschampsia antarctica*, the two native Antarctic vascular plant species. *Fungal Ecol* 2:184–196
- Vanderwolf KJ, Malloch D, McAlpine DF (2018) Psychrotolerant microfungi associated with deer mice (*Peromyscus maniculatus*) in a White-nose Syndrome positive bat hibernaculum in eastern Canada. *Can Field Nat* 131:238–245
- Vaz ABM, Rosa LH, Vieira MLA (2011) The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. *Braz J Microbiol* 42:937–947
- Vishniac HS (2006) Yeast biodiversity in the Antarctic. In: Rosa CA, Péter G (eds) *Biodiversity and ecophysiology of yeasts*. Springer, Berlin, pp 419–440
- White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp 315–322
- Zucconi L, Selbmann L, Buzzini P et al (2012) Searching for eukaryotic life preserved in Antarctic permafrost. *Polar Biol* 35:749–757

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