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Diversity and distribution of hidden cultivable fungi associated with marine animals of Antarctica

Valéria Martins Godinho [a], Maria Theresa Rafaela de Paula [a],
Débora Amorim Saraiva Silva [a], Karla Paresque [b], Aline Paternostro Martins [c],
Pio Colepicolo [c], Carlos Augusto Rosa [a], Luiz Henrique Rosa [a, *]

[a] Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[b] Laboratório de Bentologia, Universidade Federal de Alagoas, Maceió, AL, Brazil
[c] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil

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ABSTRACT

In the present study, we surveyed the distribution and diversity of fungal assemblages associated with 10 species of marine animals from Antarctica. The collections yielded 83 taxa from 27 distinct genera, which were identified using molecular biology methods. The most abundant taxa were *Cladosporium* sp. 1, *Debaryomyces hansenii, Glaciozyma martinii*, *Metschnikowia australis*, *Pseudogymnoascus destructans*, *Thelebolus* cf. *globosus*, *Pseudogymnoascus pannorum*, *Tolypocladium tundrense*, *Metschnikowia australis*, and different *Penicillium* species. The diversity, richness, and dominance of fungal assemblages ranged among the host; however, in general, the fungal community, which was composed of endemic and cold-adapted cosmopolitan taxa distributed across the different sites of Antarctic Peninsula, displayed high diversity, richness, and dominance indices. Our results contribute to knowledge about fungal diversity in the marine environment across the Antarctic Peninsula and their phylogenetic relationships with species that occur in other cold, temperate, and tropical regions of the World. Additionally, despite their extreme habitats, marine Antarctic animals shelter cryptic and complex fungal assemblages represented by endemic and cosmopolitan cold-adapted taxa, which may represent interesting models to study different symbiotic associations between fungi and their animal hosts in the extreme conditions of Antarctica.

1. Introduction

Despite extreme conditions, Antarctica has different microhabitats in its terrestrial and marine environments, which are dominated by different microorganism groups (Ruisi et al., 2007). Fungi represent an important group of microorganisms, which according to their substrate, display complex assemblages, and can survive under extreme environmental conditions such as low temperatures (including frequent freeze-thawing cycles), high salinity, different pH values, high UV radiation, and low nutrient availability in the terrestrial and marine environments (Fell et al., 2006; Godinho et al., 2013).

The oceans shelter a great diversity of animals; of the 32 animal phyla currently recognised, 14 are exclusively marine (Brusca et al., 2016) and only one phylum (Onycophora) has no representative in the marine habitat (McGinn, 1999). Invertebrates represent about 60 % of the marine animal diversity of the oceans (Ausubel et al., 2010). In Antarctica, invertebrates are even more representative, accounting for approximately 90 % of over 7200 marine species recorded (Griffiths, 2010). Marine animals occur both in the water column (pelagic environment) and associated with the ocean floor (benthic environment). In the benthic environment, invertebrates may live among the grains of sand or in consolidated substrates, such as rocky shores and coral reefs; on hard substrates, it is common for a large diversity of animals to occur associated with other organisms, providing both food and shelter against predation and desiccation for the associated fauna, such as sponges, corals and ascidians or with macroalgae and marine grasses (Christie et al., 2009).

Apparently, the first published studies of fungi in aquatic Antarctic environments involved those present in freshwater

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* Corresponding author. Laboratório de Microbiologia Polar e Conexões Tropicais, Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, P. O. Box 486, CEP 31270-901, Brazil. Fax: +55 31 3409 2730.
E-mail address: lhrosa@icb.ufmg.br (L.H. Rosa).

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(Ellis-Evans, 1985; Montemartini-Corte, 1991). These aquatic fungi described belonged to the old phycomycetes hierarchical level, represented by fungi with non-septate hypha. Among the phycomyces taxa, Ellis-Evans (1996) reported *Apanomyces* sp., *Aphanomyces* sp., *Langenidium giganteum*, *Hyphochytrium catenoides* (Oomycete), and the chytrids *Chytridium versatile*, *Chytriomyces* sp., and *Chytriomyces willoughbyi*.

Marine fungi colonize a wide range of organic substrata in the oceans, such as sponges, corals, invertebrates, and macroalgae, and sea grasses (Jones, 2011). According to Yarden (2014), there is no consensus on the definition of marine fungi, which can be described as marine-derived, marine-associated, facultative-marine, and obligate-marine fungi. Murray (1893) present the concerns whether fungi are able or not grow in sea water, followed by Church (1893) that reported the parasitism by a marine fungus (albeit on an algae), which seem to represent the first studies of marine fungi (Yarden, 2014). According to Jone (2011a, b) over 800 marine fungal species were described. However, Blackwell (2011) proposed based on high-throughput sequencing methods that about 5.1 million fungal species exist, indicating that the diversity of marine fungi can be larger than the number proposed. Ecologically, fungi and marine animals (mainly cnidarian), might have co-evolved together and, consequently, may shelter novel lineages of fungi that are unknown to science (Amend et al., 2012).

In Antarctica, a representative of all known fungal phyla was already reported in the different substrates or hosts (Ruisi et al., 2007; Bridge and Spooner, 2012; Kochkina et al., 2012). However, most mycological studies in Antarctica focused on the fungi living in soil, ice, freshwater, and associated with plants and macroalgae. Limited attention has thus far been devoted to the fungal diversity of the Antarctic marine environments, especially from those associated with marine animals.

Until now, there is no consensus that defines and delimits marine fungi. Kohlmeyer and Kohlmeyer (1979) proposed that marine fungi are separated in 2 major ecological groups: (a) obligate: those that grow and sporulate exclusively in marine or estuarine habitats, and (b) facultative: those that are from freshwater and terrestrial milieus, and grow and sporulate in the marine environment. However, compared with the fungal diversity known in terrestrial environments, the diversity of those in marine ecosystems, especially those associated with marine invertebrates, is not well known. According to Yarden (2014) fungi have been isolated from marine organisms over the years, mainly from sponges (tropical and subtropical), and temperate waters. The first study that detected fungi associated with invertebrates of Antarctica seems to be that published by Donachie and Zdanowski (1998) who isolated the yeast *Metschnikowia australis* from the stomach of Antarctic krill *Euphausia superba*. After that, recently, Henriquez et al. (2014) is the only study to report fungi from invertebrates from Antarctica, which detected the presence of 10 genera associated with 4 species of sponges (*Pseudogymnoascus*, *Penicillium*, *Epicoccum*, *Pseudeurotium*, *Thelebolus*, *Cladosporium*, *Aspergillus*, *Aureobasidium*, *Phoma*, *Trichocladium*) besides 36 other unidentified fungi at genus level. For the reasons described above, in this study, we sought to present information on the diversity and distribution of fungal assemblages associated with marine animals of 8 different phyla living along the South Shetlands Islands and Antarctic Peninsula.

2. Materials and methods

2.1. Collection and identification of the animals

Invertebrates were collected in the Austral Summer of 2015 at an intertidal zone (Suppl. Figure 1) associated with macroalgae scraped from the rocks and sediments obtained using the box-core device. The specimens were grouped in lots by taxon, date and locality of collections (Table 1). Each lot was partitioned to keep specimens for fungal isolation and for further identification by macro and micro morphological analyses. Animal specimens for morphological studies were preserved in 98 % ethanol. Further analyses under stereo- and compound light microscopes were performed. Morphological identification was performed based on specific literature for each group and the name validity checked in the World Register of Marine Species (WoRMS Editorial Board, 2018). Voucher specimens were deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP), Brazil.

2.2. Fungal isolation

Each animal species was washed twice using sterile (autoclaved) local seawater for 2 min. After that, they were smashed in Petri dishes containing marine agar (MA, Difco, USA) supplemented with 2 % glucose and chloramphenicol (Sigma, St. Louis, MO USA) at a concentration of 200 μg mL^{-1} for inhibition of bacteria contamination. Marine Agar includes in its composition several salts in concentration similar from those present in marine environments; for this reason, this medium was choosen to isolate the fungi associated with marine animals of Antarctica. The inoculated Petri dishes were incubated for up to 60 d at 10 °C, and individual colonies of fungi were counted in CFUs mL^{-1} and purified on MA. Fungal colonies $\leq$300 CFU mL^{-1} were considered uncountable. Long-term preservation of fungi was carried out at −80 °C using cryotubes with sterile 15 % glycerol. The fungal isolates examined in this work were deposited in the Culture Collection of Microorganisms and Cells of the Universidade Federal of Minas Gerais, Brazil, under codes UFMGCB.

2.3. Fungal identification

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, 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 represent the best choice to evaluate the evolutionary distances among fungi from different genus and

Table 1
Taxa, locations, methods of collection, and number of samples of Antarctic marine animals from Antarctica.

Invertebrate taxa	Phylum	Island/site	Coordinates	Methods of collection	N° of samples
Laevilacunaria antarctica	Mollusca	Livingston, Hannah Point	−62.650596, −60.592004	Associated with macroalgae	40
	Mollusca	Antarctic Peninsula, Hope Bay	−63.398854, −56.991600	Associated with macroalgae	12
Antarctonemertes valida	Nemertea	King George, Punta Plaza	−62.090548, −58.408251	Associated with macroalgae	7
		Antarctic Peninsula, Hope Bay	−63.398854, −56.991600	Associated with macroalgae	2
		Deception, Whalers Bay	−62.987827, −60.553085	Associated with macroalgae	6
		Wiencke, Dorian Bay	−64.831690, −63.483691	Associated with macroalgae	
		Antarctic Peninsula, Orne Bay	−64.728572, −62.590356	Associated with macroalgae	2
Ascidacea sp.	Chordata	Deception, Whalers Bay	−62.985688, −60.565771	Sediment at 94 m depth	2
Halyclystus antarticus	Cnidaria	Sissini	−62.381224, −59.784774	Associated with macroalgae	4
		Greenwich	−62.446397, −59.738973	Associated with macroalgae	1
		Wiencke, Dorian Bay	−64.831690, −63.483691	Associated with macroalgae	30
		Snow	−62.775282, −61.285822	Associated with macroalgae	20
		Robert	−62.462499, −59.501382	Associated with macroalgae	30
Magelonidae sp.	Annelida	Robert	−62.382434°−59.694424°	Sediment at 94 m depth	1
Nacella concinna	Mollusca	Deception, Whalers Bay	−62.987827, −60.553085	Associated with macroalgae	10
		Greenwich	−62.446397, −59.738973	Associated with macroalgae	40
		Wiencke, Dorian Bay	−64.831690, −63.483691	Associated with macroalgae	06
Ophiuroidea sp.	Echinodermata	Deception, Whalers Bay	−62.985688, −60.565771	Sediment at 94 m depth	1
Tigriopus kingsejongensis	Arthropoda (Crustacea)	Robert	−62.462499, −59.501382	Associated with macroalgae	10
		Livingston, Hannah point	−62.650596, −60.592004	Associated with macroalgae	10
		King George, Tourret Point	−62.070562, −57.986038	Associated with macroalgae	37
		Snow	−62.775282, −61.285822	Associated with macroalgae	5
Trepaxonemata sp.	Platyhelmintes	Wiencke, Dorian Bay	−64.831690, −63.483691	Associated with macroalgae	13
		Snow	−62.775282, −61.285822	Associated with macroalgae	1

families, was employed to estimate evolutionary distances with bootstrap values calculated from 1000 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).

2.4. 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 1000 iterations. All diversity and similarity indices calculations were performed using the computer programme PAST, version 1.90 (Hammer et al., 2001). Venn diagram were prepared according to Bardou et al. (2014) to illustrate the comparison of fungal assemblages associated with the animals with high sampling.

3. Results

3.1. Animal collection and fungal identification

Ten taxa of eight phyla of marine invertebrates were obtained from different sites in the South Shetlands Islands and Antarctic Peninsula (Table 1). These animals yielded 67 fungal taxa from 26 genera of the phyla *Ascomycota*, *Basidiomycota*, and *Mortierellomycota* (Table 2; Fig. 1). The number of fungal taxa and diversity indices differed among the different animal hosts. *Cladosporium* sp. 1, *Debaryomyces hansenii, Glaciozyma martinii*, *M. australis*, *Pseudogymnoascus destructans*, *Thelebolus* cf. *globosus*, *Pseudogymnoascus pannorum*, *Tolypocladium tundrense*, *Metschnikowia australis,* and different *Penicillium* and species occurred in highest densities ($\leq$300 UFC/invertebrate). However, 52 (77.6 %) fungi displayed low densities ($\leq$100 UFC/invertebrate) representing singletons, characterizing rare taxa associated with the Antarctic animals. Thirty-seven fungal taxa showed low molecular similarities or inconclusive taxonomic definitions compared with known fungal ITS sequences deposited in the GenBank database. Among them, the taxa *Letendraea* sp., *Phoma* sp., *Cladosporium* sp., *Galactomyces* sp., and *Mortierella* sp. showed lowest query coverage and identity percentages and may represent new species.

3.2. Diversity and distribution

In general, the animals that showed the recovered fungal assemblages presented high values of diversity (Fisher's $\alpha = 11.77$), richness (Margalef's = 8.6), and dominance (Simpson's = 0.98) indices. *Nacella concinna*, *Lumbricillus* sp., and *Antarctonemertes valida* displayed the highest diversity, richness, and dominance indices (Table 3). The similarities of the fungal assemblages among the invertebrates were variable according to the Sorensen and Bray–Curtis indices (Fig. 2). In Sorensen index, the most similar fungal assemblages were found in *H. antarcticus* and Magelonidae sp. In Bray–Curtis index, the most similar fungal assemblages were between *T. kingsejongensis* and Trepaxonemata sp. The fungi *Pseudogymnoascus* cf. *destructans*, *Pseudogymnoascus verrugcosus*, *Antarctomyces psychrotrophicus*, *M. australis*, *Thelebolus* cf. *globosus*, as well as *Penicillum* and *Aspergillus* taxa occurred in association with more than 3 animals hosts. However, the genera *Metschnikowia*, *Pseudogymnoascus*, and *Penicillium* occurred in several hosts.

Additionally, *A. valida* and *T. kingsejongensis*, sampled in 5 and 4 different sites (Table 1), respectively, were used as a model to compare the similarity of their fungal assemblages (at genera level) using the Veen diagrams (Suppl. Fig. 2A, B). Associated with *A. valida*, only *Penicillium* was present in all sites sampled, followed by *Pseudogymnoascus* (3 sites), *Aspergillus*, *Metshnikowia*, and *Mortierella* (2 sites), and *Cladosporium* and *Glaciozyma* (2 sites). For *T. kingsejongensis*, *Penicillium* and *Metshnikowia* were common from two sites.

4. Discussion

Studies on the diversity of fungi in Antarctica have been increasing, especially those focussing on terrestrial environments. In contrast, the knowledge of fungal diversity of Antarctic marine ecosystems is still poor. In the present work, despite the use of

Table 2
Fungal taxa obtained from marine invertebrates of Maritime Antarctica and identified by sequence comparison with the BLASTn match with the NCBI GenBank database.

Invertebrate host	UFMGCB[a]	UFC/ invertebrate	Top BLAST search results (GenBank accession number)	N° of bp analysed	Query cover (%)	Identity (%)	Proposed taxa[c](GenBank acc. n°)
Laevilacunaria antarctica	12052	100	*Antarctomyces psychrotrophicus* (MH874317)	486	100	99	*Antarctomyces psychrotrophicus* (MK182421)[d]
	GASpPH6-1L	>300	*Metschnikowia australis* (KY108453)	392	100	99	*Metschnikowia australis* (MK123272)[e]
	12054	>300	*Pseudogymnoascus destructans* (NR111838)	479	99	99	*Pseudogymnoascus destructans* (MK182422)[d]
	12057	100	*Pseudogymnoascus verrucosus* (KJ755525)	406	100	100	*Pseudogymnoascus verrucosus* (MK182423)[d]
	GASpPH2-1L	100	*Vishniacozyma victoriae* (NG057678)	577	100	99	*Vishniacozyma victoriae* (MK123273)[e]
	12202	100	*Pseudogymnoascus destructans* (NR111838)	444	98	98	*Pseudogymnoascus* cf. *destructans* (MK182424)[d]
Antarctonemertes valida	12490[b]	100	*Cladosporium verrucocladosporioides* (MH875399)	415	100	100	*Cladosporium* sp. 1 (MK182425)[d]
	12004	100	*Didymella longicolla* (MH863382)	381	100	99	*Didymella longicolla* (MK182426)[d]
	OP34.44	100	*Glaciozyma martinii* (LC203689)	574	100	100	*Glaciozyma martinii* (MK123274)[e]
	OP34.30	200	*Metschnikowia australis* (KY108454)	783	92	100	*Metschnikowia australis* (MK123275)[e]
	12561	100	*Mollisia dextrinospora* (NR119489)	473	100	97	*Mollisia* sp. (MK182427)[d]
	12549	100	*Mortierella fimbricystis* (NR152949)	397	99	97	*Mortierella* sp. 1 (MK182428)[d]
	OP34.29	160	*Mrakia* sp. (AM922288)	667	100	100	*Mrakia* sp. (MK123276)[e]
	12547[b]	100	*Penicillium brevicompactum* (NR121299)	428	100	100	*Penicillium brevicompactum* (MK182429)[d]
	12483[b]	>300	*Penicillium cavernicola* (MH862709)	461	100	99	*Penicillium* sp. 1 (MK182430)[d]
	12472[b]	>300	*Penicillium rubens* (NR111815)	549	100	100	*Penicillium* sp. 2 (MK208317)[d]
	12005[b]	100	*Penicillium tardochrysogenum* (MH865983)	463	100	100	*Penicillium* sp. 3 (MK182431)[d]
	12002	100	*Pestalotiopsis kenyana* (NR147549)	363	100	100	*Pestalotiopsis kenyana* (MK182432)[d]
Ascidia sp.	12453	100	*Antarctomyces psychrotrophicus* (MH874317)	412	100	99	*Antarctomyces psychrotrophicus* (MK182433)[d]
Halyclystus antarticus	OP34.1	>300	*Metschnikowia australis* (MH447359)	538	92	100	*Metschnikowia australis* (MK123277)[e0]
	OP34.34	>300	*Metschnikowia australis* (MH447359)	692	92	100	*Metschnikowia australis* (MK123278)[e]
Lumbricillus sp.	12067[b]	200	*Aspergillus creber* (NR135442)	468	100	99	*Aspergillus* sp. 1 (MK121980)[d]
	12209[b]	100	*Aspergillus puulaauensis* (NR135445)	445	100	100	*Aspergillus* sp. 2 (MK182435)[d]
	12065	200	*Didymella coffeae-arabicae* (MH863293)	453	100	100	*Didymella coffeae-arabicae* (MK182436)[d]
	12069	200	*Letendraea cordylinicola* (NR154118)	538	95	99	*Letendraea* sp. (MK182437)[d]
	OLIGOLARBD1-1L	>300	*Metschnikowia australis* (MH447359)	395	100	100	*Metschnikowia australis* (MK123279)[e]
	12070	200	*Nothophoma macrospora* (NR158272)	381	100	99	*Nothophoma macrospora* (MK182438)[d]
	12208[b]	100	*Penicillium brasilianum* (MH857470)	436	100	100	*Penicillium* sp. 1 (MK182439)[d]
	12205[b]	100	*Penicillium swiecickii* (NR121254)	391	100	99	*Penicillium swiecickii* (MK182440)[d]
	12075[b]	200	*Penicillium tardochrysogenum* (MH865983)	477	100	100	*Penicillium* sp. 2 (MK182434)[d]
	12206	100	*Pestalotiopsis kenyana* (NR147549)	444	100	100	*Pestalotiopsis kenyana* (MK182442)[d]
	12210	100	*Phoma schachtii* (MH861770)	415	99	92	*Phoma* sp. (MK182443)[d]
	12063	150	*Pseudogymnoascus destructans* (NR111838)	446	98	99	*Pseudogymnoascus* cf. *destructans* (MK182444)[d]
	12020	>300	*Thelebolus globosus* (MH862951)	398	98	100	*Thelebolus* cf. *globosus* (MK208318)[d]
Magelonidae sp.	OP34.11	100	*Metschnikowia australis* (MH447359)	557	92	100	*Metschnikowia australis* (MK123281)[e]
	OP34.12	100	*Mrakia frigida* (MF193493)	733	100	99	*Mrakia frigida* (MK123282)[e]
Nacella concinna	12466	182	*Antarctomyces psychrotrophicus* (MH874317)	565	100	100	*Antarctomyces psychrotrophicus* (MK208319)[d]
	12485[b]	100	*Aspergillus sydowii* (NR131259)	485	98	100	*Aspergillus* sp. 1 (MK182445)[d]
	12027[b]	100	*Aspergillus venenatus* (NR135448)	391	100	99	*Aspergillus* sp. 2 (MK182459)[d]
	OP34.10	100	*Candida* sp. (MG004794)	623	100	100	*Candida* sp. (MK123283)[e]
	OP34.20	100	*Candida spencermartinsiae* (HQ695011)	515	100	100	*Candida spencermartinsiae* (MK123284)[e]
	OP34.16	100	*Candida zeylanoides* (KY106918)	675	100	100	*Candida zeylanoides* (MK123285)[e]
	12494[b]	100	*Cladosporium halotolerans* (NR119605)	469	98	100	*Cladosporium halotolerans* (MK182446)[d]
	12532[b]	>300	*Cladosporium verrucocladosporioides* (MH875399)	476	100	99	*Cladosporium* sp. 1 (MK182447)[d]
	OP34.8	300	*Clavispora lusitaniae* (MK026240)	207	100	100	*Clavispora lusitaniae* (MK123286)[d]
	OP34.4	100	*Debaryomyces hansenii* (MH844410)	675	100	100	*Debaryomyces hansenii* (MK123287)[e]
	12499	100	*Geomyces destructans* (EU884921)	414	100	99	*Pseudogymnoascus destructans* (MK182448)[d]
	12562	200	*Geotrichum silvicola* (NR077071)	300	94	98	*Galactomyces* sp. (MK182449)[d]
	OP34.22	>300	*Glaciozyma martinii* (LC203689)	667	100	100	*Glaciozyma martinii* (MK123288)[e]

Table 2 (*continued*)

Invertebrate host	UFMGCB[a]	UFC/ invertebrate	Top BLAST search results (GenBank accession number)	N° of bp analysed	Query cover (%)	Identity (%)	Proposed taxa[c](GenBank acc. n°)
	OP34.9	100	*Holtermanniella festucosa* (JX188167)	744	100	99	*Holtermanniella festucosa* (MK123289)[e]
	OP34.31	>300	*Metschnikowia australis* (MH447359)	598	92	100	*Metschnikowia australis* (MK161033)[e]
	OP34.19	100	*Meyerozyma guilliermondii* (MH844419)	566	100	100	*Meyerozyma guilliermondii* (MK123290)[e]
	12019	200	*Mortierella elongatula* (JX975967)	438	99	97	*Mortierella* sp. 2 (MK182460)[d]
	12531	100	*Mortierella elongatula* (NR111582)	470	99	96	*Mortierella* sp. 3 (MK182450)[d]
	12520	100	*Mortierella fimbricystis* (NR152949)	473	100	97	*Mortierella* sp. 1 (MK182451)[d]
	OP34.6	100	*Mrakia frigida* (AM922288)	720	100	99	*Mrakia frigida* (MK123291)[e]
	12508[b]	100	*Penicillium cavernicola* (MH862709)	464	100	100	*Penicillium* sp. 1 (MK182452)[d]
	12028[b]	>300	*Penicillium goetzii* (NR111820)		100	100	*Penicillium* sp. 4 (MK182461)[d]
	12026[b]	>300	*Penicillium rubens* (NR111815)	463	100	100	*Penicillium* sp. 2 (MK182462)[d]
	12459[b]	100	*Penicillium tardochrysogenum* (MH865983)	507	100	99	*Penicillium* sp. 3 (MK182454)[d]
	12559	121	*Polypaecilum botryoides* (MH854756)	431	99	100	*Polypaecilum botryoides* (MK182455)[d]
	12518	106	*Pseudogymnoascus destructans* (NR111838)	424	100	98	*Pseudogymnoascus* cf. *destructans* (MK182456)[d]
	12022	>300	*Pseudogymnoascus pannorum* (MH861038)	409	100	99	*Pseudogymnoascus pannorum* (MK208321)[d]
	12525	120	*Pseudogymnoascus verrucosus* (KJ755525)	370	100	99	*Pseudogymnoascus verrucosus* (MK182457)[d]
	OP34.38	100	*Rhodotorula mucilaginosa* (KP223715)	484	100	100	*Rhodotorula mucilaginosa* (MK123292)[e]
	12765	120	*Thelebolus balaustiformis* (MG813184)	434	100	100	*Thelebolus balaustiformis* (MK182458)[d]
	12462	300	*Thelebolus globosus* (MH862951)	400	98	100	*Thelebolus* cf. *globosus* (MK208320)[d]
	12025[b]	>300	*Tolypocladium tundrense* (MH861781)	351	100	100	*Tolypocladium tundrense* (MK182463)[d]
Ophiuroidea	12545	100	*Pseudogymnoascus destructans* (NR111838)	450	97	99	*Pseudogymnoascus* cf. *destructans* (MK208322)[d]
	12552[b]	100	*Penicillium camerunense* (KC411743)	408	100	99	*Penicillium* sp. 5 (MK121969)[d]
	OP34.32	100	*Metschnikowia australis* (MH447359)	691	92	100	*Metschnikowia australis* (MK123294)[e]
Tigriopus kingsejongensis	COPp-1L	>300	*Debaryomyces hansenii* (MH844410)	544	100	100	*Debaryomyces hansenii* (MK123295)[e]
	12030	100	*Pseudogymnoascus pannorum* (MH861038)	380	99	100	*Pseudogymnoascus pannorum* (MK121970)[d]
	12031[b]	>300	*Penicillium camerunense* (KC411743)	336	100	100	*Penicillium* sp. 5 (MK121971)[d]
	12197[b]	200	*Penicillium tardochrysogenum* (MH865983)	389	100	100	*Penicillium* sp. 3 (MK121972)[d]
	12037	100	*Pseudogymnoascus appendiculatus* (NR137875)	328	100	99	*Pseudogymnoascus appendiculatus* (MK121973)[d]
	12035	100	*Pseudogymnoascus destructans* (NR111838)	414	97	98	*Pseudogymnoascus* cf. *destructans* (MK121974)[d]
	12034	100	*Pseudogymnoascus verrucosus* (KJ755525)	339	100	99	*Pseudogymnoascus verrucosus* (MK121975)[d]
	12038	100	*Septoria chromolaenae* (MH862925)	418	100	100	*Septoria chromolaenae* (MK121976)[d]
Trepaxonemata sp.	12012[b]	100	*Cladosporium verrucocladosporioides* (MH875399)	280	100	100	*Cladosporium* sp. 1 (MK121977)[d]
	PLABD7-2L	>300	*Debaryomyces hansenii* (MH844410)	495	100	100	*Debaryomyces hansenii* (MK123296)[e]
	12013[b]	>300	*Penicillium tardochrysogenum* (MH865983)	420	100	100	*Penicillium* sp. 3 (MK121978)[d]
	12076	100	*Pseudogymnoascus destructans* (NR111838)	443	98	98	*Pseudogymnoascus* sp. (MK121979)[d]

[a]UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. Taxa subjected to phylogenetic analysis based on the [b]ITS region for elucidation of taxonomic positions. [c]Taxonomic position suggested by the phylogenetic analyses according to [d]ITS and [e]D1/D2 sequences deposited.

culturing screen to recover the associated marine fungi, we detected a diverse fungal assemblages associated with marine animals, when compared with studies of fungi present in other Antarctic marine substrates such as sponges (Henriquez et al., 2014), marine sediments (Gonçalves et al., 2015), macroalgae (Godinho et al., 2013; Furbino et al., 2014), and seawater (Gonçalves et al., 2017). In general, marine animals are poorly known as sources of fungi. This knowledge was critical when we searched for the symbiotic fungi living in association with marine invertebrates in the Antarctica Ocean. Few studies have reported fungi in association with marine animals in Antarctica. Donachie and Zdanowski (1998) isolated the yeast *M. australis* from the stomach of Antarctic krill *E. superba* and Henriquez et al. (2014) described fungal taxa associated with 4 sponge (Porifera) taxa (11 samples), and found cosmopolitan species of *Penicillium*, *Aspergillus*, *Pseudeurotium*, *Cladosporium*, *Aureobasidium*, and *Phoma*, but also possible endemic species such as *Pseudogymnoascus* and *Thelebolus*. However, in contrast to a study by Henriquez et al. (2014), our study focused on fungi associated with different marine animals, from 10 taxa (of 8 phyla) across different sites from Antarctic Islands and Peninsula. In our study, cosmopolitan genera (*Aspergillus*, *Cladosporium*, and *Penicillium*) were detected. However, we also characterized endemic or found until now only in Antarctica (*A. psychrotrophicus* and *M. australis*) and those present in

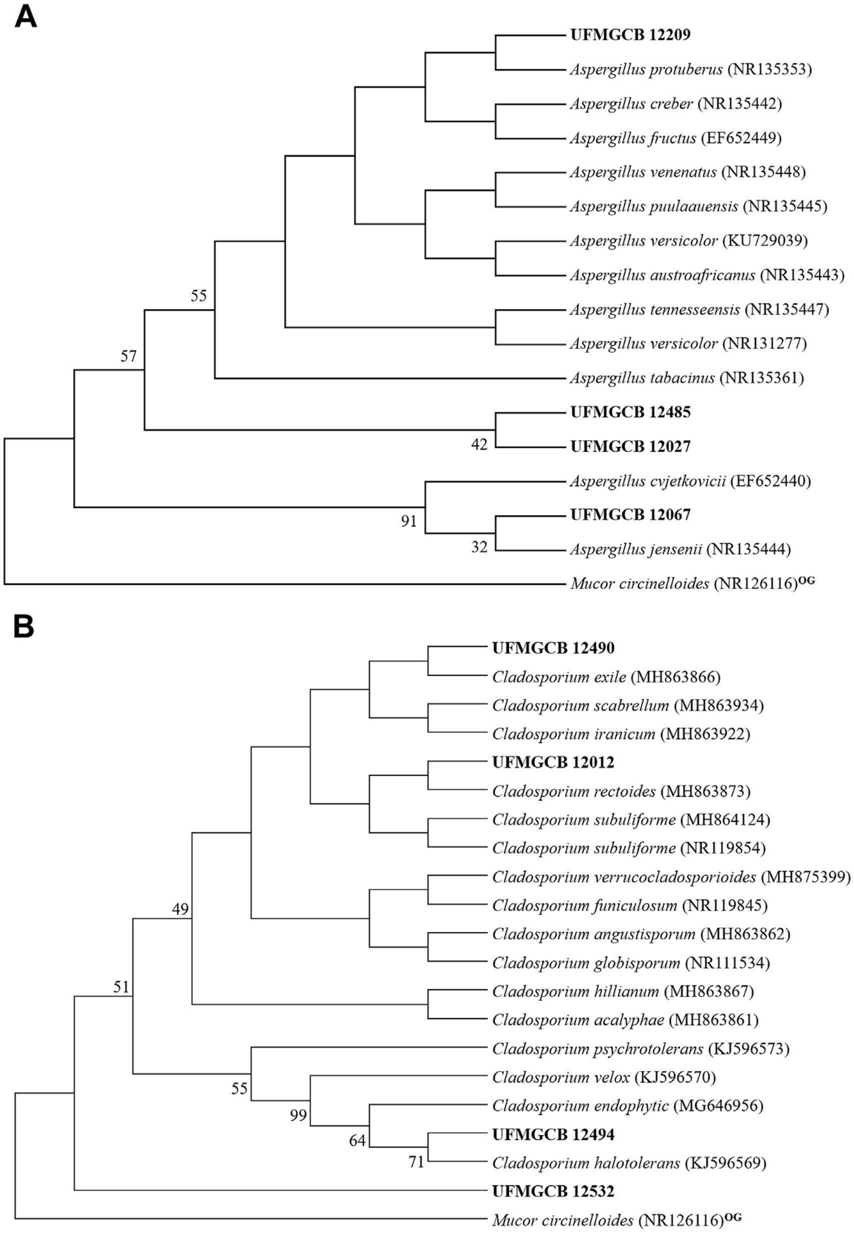

Fig. 1. Phylogenetic analysis of the sequences of fungi (in bold) associated with marine animals from Antarctica in comparison with type sequences of the nearest species, following BLAST analysis, deposited in the GenBank database. The trees were constructed based on the ITS1-5.8S-ITS2 rRNA gene sequences using the maximum composite likelihood model.

temperate environments of continent with optimal growth rate at 10–20 °C called cold adapted species (*P. destructans* and *T.* cf. *globosus*) in different animal taxa.

We detected few dominant taxa represented by *M. australis*, *P. destructans*, *T.* cf. *globosus*, *Glaciozyma martini*, *T. tundrense*, and *Penicillium* species in one or more animal hosts. These taxa include endemic, psychrophilic, and cosmopolitan mesophilic-psychrotolerant fungi, which may exhibit an interesting ecological symbiosis with their hosts.

The yeast *M. australis* is described as endemic to Antarctica and was isolated from Antarctic seawater (Fell and Hunter, 1968), the stomach of the Antarctic krill species *E. superba* (Donachie and Zdanowski, 1998), thalli of different macroalgae (Loque et al., 2010; Godinho et al., 2013; Furbino et al., 2014), marine sediment, and freshwater in Antarctica (Vaz et al., 2011). The recovery of *M. australis* from different invertebrates reinforces that this yeast occupies abundantly different substrates in the Antarctic Ocean.

Pseudogymnoascus includes species with worldwide distribution (Lorch et al., 2013; Minnis and Lindner, 2013) and, until now, was already commonly described in the soils of Arctic, Alpine, and temperate and Antarctic regions (Mercantini et al., 1989). In Antarctica, *Pseudogymnoascus* species were obtained from several substrates in soil (Mercantini et al., 1989; Arenz et al., 2006), mosses (Tosi et al., 2002), as the endophyte of *Colobanthus quitensis* (Rosa et al., 2010), as algicolous fungi of macroalgae (Loque et al., 2010; Godinho et al., 2013; Furbino et al., 2014), in freshwater lakes (Gonçalves et al., 2012), and in the lichenosphere (Santiago

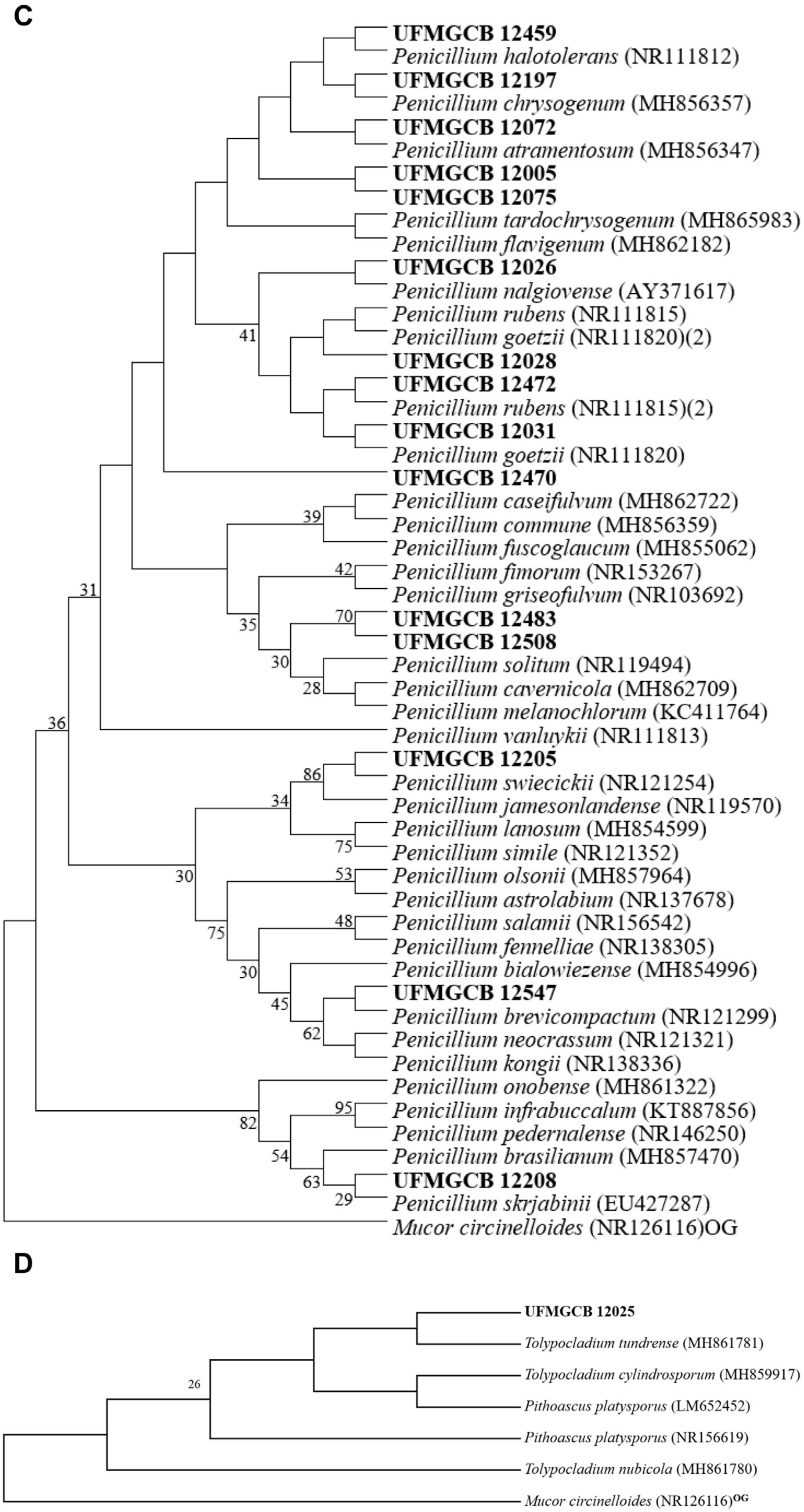

Fig. 1. (*continued*).

Table 3
Ecological indices of the fungal assemblages associated with marine animals from continental Antarctica.

Animal host	Ecological indices			
	Nº of fungal taxa	Fisher α	Margalef	Simpson's
Laevilacunaria antarctica	6	0.85	0.7	0.8
Antarctonemertes valida	12	1.7	1.5	0.9
Ascidia sp.	1	–	–	–
Halyclystus antarticus	2	0.3	0.2	0.5
Lumbricillus sp.	13	1.8	1.6	0.9
Magelonidae sp.	2	0.3	0.2	0.5
Nacella concinna	32	4.5	3.6	0.96
Ophiuroidea sp.	3	0.5	0.35	0.7
Tigriopus kingsejongensis	8	1	0.98	0.8
Trepaxonemata	4	0.6	0.5	0.7
General	**83**	**11.77**	**8.6**	**0.98**

– = means that the host displayed only one fungal taxa and for this reason it was not possible to calculate the diversity indices.

et al., 2015). *P. destructans* is characterised as a psychrophilic species able to decrease bat populations by the white-nose syndrome (WNS) in temperate regions (Lorch et al., 2011). Lorch et al. (2013) and Minnis and Lindner (2013) suggested that the diversity of *Pseudogymnoascus* species was greater than that previously reported.

The genus *Thelebolus* comprises 21 species and occurs mainly in cold habitats of the world (www.mycobank.org). *Thelebolus* species were previously reported as abundant in lakes and associated with skuas, petrels and other birds, and lichens in Antarctica (de Hoog et al., 2005, Brunati et al., 2009; Gonçalves et al. 2012; Santiago et al., 2015). *Glaciozyma* includes yeasts isolated from ice and found in cold environments (Turchetti et al., 2011). *G. martini* was reported by Turchetti et al. (2011) in soil close to the Lake Fletcher and Lichen Valley, Antarctica and in sediment sampled from Calderone Glacier, Apennines, Italy. *Tolypocladium* (*Ophiocordycipitaceae*) includes species with ecological profiles as parasites, entomopathogenic, soil-inhabiting (Quandt et al., 2014). *Tolypocladium* was described by Gams (1971) with 4 species. Later, the genus was revised by Bisset (1983) who proposed more species, including *Tolypocladium tundrese*. However, Arx (1986) proposed a new taxonomic combination for *T. tundrense* to *Beuveria tundrense*. According to MycoBank database (Robert et al., 2005), both names are recognized. *Tolypocladium* shelter species, *Tolypocladium inflatum*, produce the immunosuppressant drug cyclosporine A (Quandt et al., 2014). Martha et al. (1992) reported that conidia of *T. tundrense* displayed acute toxicity to *Aedes aegypti* larvae, probably due the production of cyclosporine A. *Penicillium* genera include several ubiquitous species with worldwide distribution. *Penicillium* species had already been detected in all substrates in Antarctica, including in macroalgae (Godinho et al., 2013), sponges (Henriquez et al., 2014), sediments (Gonçalves et al., 2013), and seawater (Gonçalves et al., 2017), indicating its high capability to survive in extreme conditions in the Antarctic ocean environments.

In contrast, we also detected that in the fungal community, most taxa represent singlets included as minority components associated with the marine animals predominately represented by cosmopolitan cold-adapted taxa, endemic species, and possible new species. Thirty-seven fungal taxa showed low molecular similarities or inconclusive information in comparison with known fungal ITS sequences deposited in the GenBank database. Among them, the taxa *Letendraea* sp., *Phoma* sp., *Geotrichum* sp., and *Mortierella* sp., which showed the lowest query coverage and identity percentages, may represent new species. These taxa warrant further analyses including detailed physiological, macro- and micromorphological characterization and molecular sequencing of other DNA regions, together with new phylogenetic analyses to be described as new species.

The fungal distribution in the different ecosystems of Antarctica correlates with the distribution of their respective hosts or substrates (Ruisi et al., 2007). However, how the marine fungi disperse and colonize their substrates in the Antarctic Ocean remains unknown. Godinho et al. (2013) and Furbino et al. (2014) proposed that thalli of Antarctic macroalgae, which shelter diverse fungal assemblages, might contribute to dispersal of fungi in the Antarctic Ocean. In our study, the Sorensen and Bray–Curtis indices showed the presence of some species (*A. psychrotrophicus*, *M. australis*, *P.* cf. *destructans*, and *P. verrucosus*) associated with different animals from distinct Antarctic sites. These similar results suggest that marine Antarctic invertebrates, such as macroalgae thalli, may contribute to the dispersal of Antarctic fungi across the Antarctic Ocean.

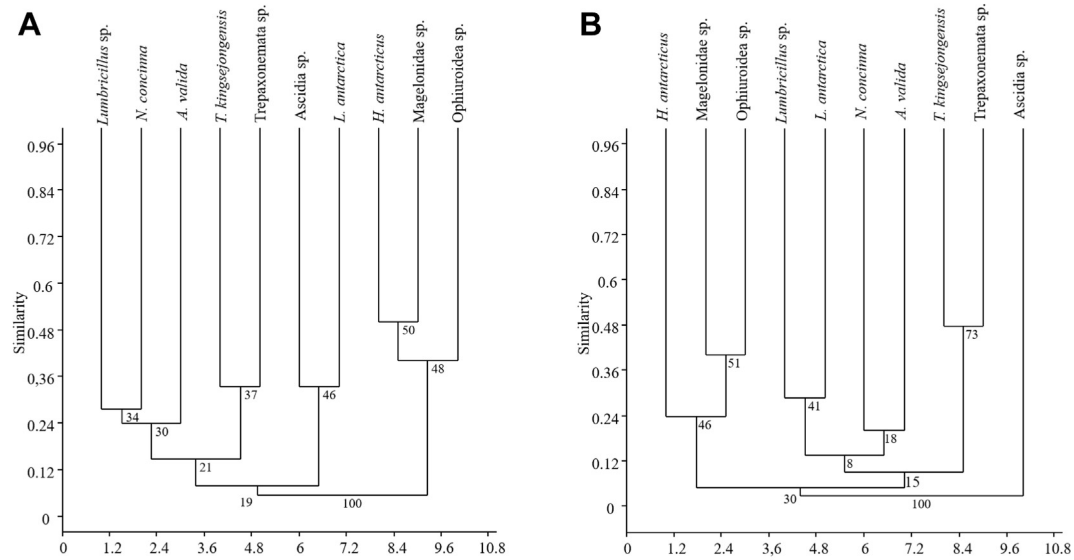

Fig. 2. Dendrogram showing the comparison of (A) Sorensen (B) Bray–Curtis similarities measures for the fungal assemblages isolated from marine animals of Antarctica.

5. Conclusion

According to Jones (2011), marine fungal diversity has been studied for the last 100 y; however, mycological studies of taxonomy, diversity, ecology, phylogeny, and biotechnological applications of this cryptic fungal group remain poorly known. Our results contribute to knowledge on the fungal diversity in the marine environment across the South Shetlands Islands and Antarctic Peninsula and their phylogenetic relationships with species that occur in other cold, temperate, and tropical regions of the World. Additionally, our results indicate that, despite the extreme living conditions, marine Antarctic animals shelter cryptic and complex fungal assemblages represented by endemic and cosmopolitan cold-adapted taxa, which may represent interesting models to study the different symbiotic associations between fungi and their animal hosts in extreme conditions of Antarctica.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2019.05.001.

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., et al., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Amend, A.S., Barshis, D.J., Oliver, T.A., 2012. Coral-associated marine fungi form novel lineages and heterogeneous assemblages. ISME J. 6, 1291–1301.

Arenz, B.E., Held, B.W., Jurgens, J.A., et al., 2006. Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. Soil Biol. Biochem. 38, 3057–3064.

Ausubel, J., Crist, D.T., Waggoner, P.E., 2010. First Census of Marine Life: Highlights of a Decade of Discovery. Census of Marine Life, Washington DC.

Bardou, P., Mariette, J., Escudié, F., et al., 2014. An interactive Venn diagram viewer. BMC Bioinf. 15, 293.

Bisset, J., 1983. Notes on *Tolypocladium* and related genera. Can. J. Bot. 61, 1311–1329.

Blackwell, M., 2011. The fungi: 1, 2, 3 ... 5.1 million species? Am. J. Bot. 98, 426–438.

Bridge, P.D., Spooner, B.M., 2012. Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem? Fungal Ecol. 5, 381–394.

Brunati, M., Rojas, J.L., Sponga, F., et al., 2009. Diversity and pharmaceutical screening of fungi from benthic mats of Antarctic lakes. Mar. Genom. 2, 43–50.

Brusca, R.C., Moore, W., Shuster, S.M., 2016. Invertebrates, 3^{a} Edition. Sinauer Associates, Massachusetts.

Christie, H., Norderhaug, K.M., Fredriksen, S., 2009. Macrophytes as habitat for fauna. Mar. Ecol. Prog. Ser. 396, 221–234.

Church, A.H., 1893. A marine fungus. Ann. Bot. Lond. 7, 399–400.

de Hoog, G.S., 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.

Donachie, S.P., Zdanowski, M.K., 1998. Potential digestive function of bacteria in krill, *Euphausia superba* stomach. Aquat. Microb. Ecol. 14, 129–136.

Ellis-Evans, J.C., 1996. Microbial diversity and function in Antarctic freshwater ecosystems. Biodivers. Conserv. 5, 1395–1431.

Ellis-Evans, J.C., 1985. Fungi from maritime Antarctic freshwater environments. Br. Antarct. Surv. Bull. 68, 37–45.

Fell, J.W., Hunter, I.L., 1968. Isolation of heterothallic yeast strains of *Metschnikowia* Kamienski and their mating reactions with *Chlamydozyma* Wickerham spp. Antonie Leeuwenhoek 34, 365–376.

Fell, J.W., Scorzetti, G., Connell, L., et al., 2006. Biodiversity of micro-eukaryotes in Antarctic Dry Valley soils with <5% soil moisture. Soil Biol. Biochem. 38, 3107–3119.

Furbino, L.E., Godinho, V.M., Santiago, I.F., 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.

Gams, W., 1971. *Tolypocladium*, eine Hyphomycetengattung mitgeschwollenen Phialiden. Persoonia 6, 185–191.

Godinho, V.M., Furbino, L.E., Santiago, I.F., et al., 2013. Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. ISME 7, 1434–1451.

Gonçalves, V.N., Campos, L.S., Melo, I.S., et al., 2013. *Penicillium solitum*: a mesophilic, psychrotolerant fungus present in marine sediments from Antarctica. Polar Biol. 36, 1823–1831.

Gonçalves, V.N., Carvalho, C.R., Johann, S., et al., 2015. Antibacterial, antifungal and antiprotozoal activities of fungal communities present in different substrates from Antarctica. Polar Biol. 38, 1143–1152.

Gonçalves, V.N., Vitoreli, G.A., de Menezes, G.C.A., Mendes, C.R.B., Secchi, E.R., Rosa, C.A., Rosa, L.H., 2017. Taxonomy, phylogeny and ecology of cultivable fungi present in seawater gradients across the Northern Antarctica Peninsula. Extremophiles 21, 1005–1015.

Gonçalves, V.N., Vaz, A.B.M., Rosa, C.A., et al., 2012. Diversity and distribution of fungal communities in lakes of Antarctica. FEMS Microbiol. Ecol. 82, 459–471.

Griffiths, H.J., 2010. Antarctic marine biodiversity – what do we know about the distribution of life in the southern ocean? PLoS One 5, e11683.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 9.

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

Jones, E.G.B., 2011. Fifty years of marine mycology. Fungal Divers. 50, 73–112.

Kirk, P.M., Cannon, P.F., Minter, D.W., et al., 2008. Dictionary of the Fungi, tenth ed. CAB International, Wallingford, UK.

Kochkina, G., Ivanushkina, N., Ozerskaya, S., Chigineva, N., Vasilenko, O., Firsov, S., Spirina, E., Gilichinsky, D., 2012. Ancient fungi in Antarctic permafrost environments. FEMS Microbiol. Ecol. 82, 501–509.

Kohlmeyer, J., Kohlmeyer, E., 1979. Marine Mycology. The Higher Fungi. Academic Press, New York.

Kurtzman, C.P., Fell, J.W., Boekhout, T., 2011. The Yeasts, a Taxonomic Study, fifth ed. Elsevier, Amsterdam, The Netherlands.

Lachance, M.A., Bowles, J.M., Starmer, W.T., et al., 1999. *Kodamaea kakaduensis* and *Candida tolerans*, two new ascomycetous yeast species from Australian hibiscus flowers. Can. J. Microbiol. 45, 172–177.

Loque, C.P., Medeiros, A.O., Pellizzari, F.M., et al., 2010. Fungal community associated with marine macroalgae from Antarctica. Polar Biol. 33, 641–648.

Lorch, J.M., Lindner, D.L., Gargas, A., et al., 2013. A culture-based survey of fungi in soil from bat hibernacula in the eastern United States and its implications for detection of *Geomyces destructans*, the causal agent of bat white-nose syndrome. Mycologia 105, 237–252.

Lorch, J.M., Meteyer, C.U., Behr, J.M., et al., 2011. Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. Nature 480, 376–378.

Martha, V., Jegorov, A., Weiser, J., et al., 1992. The mosquitocidal activity of conidia of *Tolypocladium tundrense* and *Tolypocladium terricola*. Cytobios 69, 163–170.

McGinn, A.P., 1999. In: Peterson, J.A. (Ed.), Safeguarding the Health of Oceans. Worldwatch Institute, Danvers, p. 87.

Mercantini, R., Marsella, R., Cervellati, M.C., 1989. Keratinophilic fungi isolated from Antarctic soil. Mycopathologia 106, 47–52.

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

Montemartini-Corte, A., 1991. Funghi di ambienti acquatici. Scienza e Coltura. In: Proceedings of the 1st Meeting on Biology in Antarctica, pp. 67–79.

Murray, G., 1893. Parasites on algae. Nat. Sci. 2, 120–123.

Quandt, C.A., Kepler, R.M., Gams, W., et al., 2014. Phylogenetic-based nomenclatural proposals for *Ophiocordycipitaceae* (*Hypocreales*) with new combinations in *Tolypocladium*. IMA Fungus 5, 121–134.

Robert, V., Stegehuis, G., Stalpers, J., 2005. The MycoBank Engine and Related Databases. http://www.mycobank.org. (Accessed 5 November 2018).

Rosa, L.H., Vaz, A.B.M., Caligiorne, R.B., et al., 2009. Endophytic fungi associated with the antarctic grass *Deschampsia antarctica* desv. (*Poaceae*). Polar Biol. 32, 161–167.

Rosa, L.H., Vieira, M.L.A., Santiago, I.F., et al., 2010. Endophytic fungi community associated with the dicotyledonous plant *Colobanthus quitensis* (Kunth) Bartl. (*Caryophyllaceae*) in Antarctica. FEMS Microbiol. Ecol. 73, 178–189.

Ruisi, S., Barreca, D., Selbmann, L., et al., 2007. Fungi in Antarctica. Rev. Environ. Sci. Biotechnol. 6, 127–141.

Santiago, I.F., Soares, M.A., Rosa, C.A., et al., 2015. Lichensfere: a protected natural microhabitat of the non-lichenised fungal communities living in extreme environments of Antarctica. Extremophiles 9, 1087–1097.

Tamura, K., Stecher, G., Peterson, D., et al., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Tosi, S., Casado, B., Gerdol, R., et al., 2002. Fungi isolated from Antarctic mosses. Polar Biol. 25, 262–268.

Turchetti, B., Thomas Hall, S.R., Connell, L.B., et al., 2011. Psychrophilic yeasts from Antarctica and European glaciers: description of *Glaciozyma* gen. nov., *Glaciozyma martinii* sp. nov. and *Glaciozyma watsonii* sp. nov. Extremophiles 15, 573–578.

Vaz, A.B.M., Rosa, L.H., Vieira, M.L.A., et al., 2011. The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. Braz. J. Microbiol. 42, 937–947.

von Arx, J.A., 1986. *Tolypocladium*, a synonym of Beauveria. Mycotaxon 25, 153–158.

White, T.J., Bruns, T.D., Lee, S.B., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, N.A., Gelfand, J., Sninsky, J., et al. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.

WoRMS Editorial Board, 2018. World Register of Marine Species. http://www.marinespecies.org.at.VLIZ. (Accessed 5 November 2018).

Yarden, O., 2014. Fungal association with sessile marine invertebrates. Front. Microbiol. 5, 228.