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ORIGINAL ARTICLE

Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica

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We surveyed the distribution and diversity of fungi associated with eight macroalgae from Antarctica and their capability to produce bioactive compounds. The collections yielded 148 fungal isolates, which were identified using molecular methods as belonging to 21 genera and 50 taxa. The most frequent taxa were *Geomyces* species (sp.), *Penicillium* sp. and *Metschnikowia australis*. Seven fungal isolates associated with the endemic Antarctic macroalgae *Monostroma hariotii (Chlorophyte)* displayed high internal transcribed spacer sequences similarities with the psychrophilic pathogenic fungus *Geomyces destructans*. Thirty-three fungal singletons (66%) were identified, representing rare components of the fungal communities. The fungal communities displayed high diversity, richness and dominance indices; however, rarefaction curves indicated that not all of the fungal diversity present was recovered. *Penicillium* sp. UFMGCB 6034 and *Penicillium* sp. UFMGCB 6120, recovered from the endemic species *Palmaria decipiens (Rhodophyte)* and *M. hariotii*, respectively, yielded extracts with high and selective antifungal and/or trypanocidal activities, in which a preliminary spectral analysis using proton nuclear magnetic resonance spectroscopy indicated the presence of highly functionalised aromatic compounds. These results suggest that the endemic and cold-adapted macroalgae of Antarctica shelter a rich, diversity and complex fungal communities consisting of a few dominant indigenous or mesophilic cold-adapted species, and a large number of rare and/or endemic taxa, which may provide an interesting model of algal–fungal interactions under extreme conditions as well as a potential source of bioactive compounds.
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Introduction

Antarctica represents one of the most pristine ecosystems in the world, characterised by short food chains dominated by microorganisms. The fungal mats in the Antarctic exhibit complex communities that are able to survive under extreme environmental conditions, including dehydration, freeze-thaw cycles, low nutrient concentrations, low temperatures, osmotic stress and ultraviolet radiation irradiation (Fell *et al.,* 2006; Gonçalves *et al.,* 2012). The Antarctic fungal communities include representatives of genera and species from the major fungal phyla *Ascomycota*, *Basidiomycota*, *Zygomycota*, *Chytridiomycota* and *Glomeromycota*, as well as *Oomycetes* (*Heterokontophyta*, *Oomycota*) and slime moulds (*Mycetozoa*) traditionally studied by mycologists (Bridge and Spooner, 2012). In recent decades, mycological studies in Antarctica have mainly focused on the fungi present in soil, ice and lakes, and their associations with plants. However, limited attention has thus far been devoted to the fungal diversity of the Antarctic marine environment. Few existing studies address marine Antarctic

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fungi, which have been detected in water (Fell and Statzel, 1971; Fell *et al.,* 1973), wood baits (Grasso *et al.,* 1997) and water and marine sediments (Vaz *et al.,* 2011). According to Bridge and Spooner (2012), only 42 fungal species have been described from the Antarctic marine ecosystem.

The known marine macroalgal communities from Antarctica are not very diverse in comparison with that of warmer regions. However, this flora is characterised by a high degree of endemism and the presence of cold-adapted species (Wiencke and Clayton, 2002; Oliveira *et al.,* 2009). In addition, macroalgae are important primary producers, producing ~74 000 tons of wet biomass and having a key role in organic carbon fluxes in Antarctica (Nedzarek and Rakusa-Suszczewski, 2004). Also, macroalgae may shelter large numbers of associated organisms, including microbial mats surviving under extreme conditions (Loque *et al.,* 2010).

According to Bugni and Ireland (2004), fungi recovered from macroalgae represent the second largest source of marine fungi and include parasites, saprobes or mutualistic species. A number of macroalgal species have been studied in detail worldwide with respect to their associated fungal communities, which include the genera *Ascophyllum*, *Ballia*, *Caulerpa*, *Ceramium*, *Ceratiodictyon*, *Cladophora*, *Chondrus*, *Dictyota*, *Dilsea*, *Egregia*, *Enteromorpha*, *Fucus*, *Gelidiella*, *Gracilaria*, *Grateloupia*, *Halimeda*, *Halymenia*, *Hypnea*, *Laminaria*, *Lobophora*, *Padina*, *Porphyra*, *Portieria*, *Saccorhiza*, *Sargassum*, *Stoechospermum*, *Turbinaria* and *Ulva* (Kohlmeyer and Volkmann-Kohlmeyer, 1991, Stanley, 1992; Zuccaro and Mitchell, 2005; Zuccaro *et al.,* 2008; Suryanarayanan *et al.,* 2010). To the best of our knowledge, except for an initial contribution by Loque *et al.* (2010), no other data are available regarding the species composition of fungal communities associated with Antarctic macroalgae. In this study, we acquire to present information on the diversity and distribution of fungal communities associated with endemic and cold-adapted macroalgae across latitudinal gradients along the Antarctic Peninsula and their capability to produce bioactive compounds.

Materials and methods

Macroalagae collection

Sixty fresh thalli from each selected macroalgal species (four *Phaeophyceae*, three *Chlorophyta* and one *Rhodophyta*) were collected during December 2010 and January 2011 in intertidal transects along a rocky coastline that becomes ice free during the Antarctic summer. Samples of *Adenocystis utricularis* (Bory de Saint-Vicent) Skottsberg, *Adenocystis* species (sp.), *Desmarestia menziesii* J Agardh, *Phaeurus antarcticus* Skottsberg, *Acrosiphonia arcta* (Dillwyn) Gain, *Monostroma hariotii* Gain, *Ulva intestinalis* Linnaeus and *Palmaria decipiens* RW Ricker (Figure 1) were collected onboard the Brazilian Navy Polar Ship Almirante Maximiano (H41) along a 350-km transect through Elephant, King George and Deception Islands, in the Antarctic Peninsula (Figure 2). Physical and chemical water parameters (temperature, salinity, conductivity, dissolved oxygen and pH) were also recorded at each site using a multiparameter probe Hexis TCS (Yellow Springs, OH, USA).

Macroalgae identification

Complete and fertile samples were sorted, washed and preserved in seawater formalin (4%) in the ship's laboratory, with the aim of performing macro- and micromorphological analyses. The identification of the macroalgal specimens was based on the publications of Papenfuss (1964), Ricker (1987), Wiencke and Clayton (2002), Quartino *et al.* (2005) and Amsler *et al.* (2009). Nomenclatural updates followed Guiry and Guiry (2012). Exsiccatae vouchers were manufactured for deposition in the SP Herbarium of the Jardim Botânico of São Paulo, Brazil.

Fungal isolation

Five discs 8 mm in diameter were cut from each macroalgal specimen and washed twice using sterile local seawater for 2 min. The discs were inoculated in Petri dishes containing marine agar (Difco, Franklin Lakes, NJ, USA) supplemented with 2% glucose and chloramphenicol (Sigma, St Louis, MO, USA) at a concentration of 200 $\mu g\,ml^{-1}$ for selective isolation of marine fungi. All of the inoculated Petri dishes were incubated for up to 60 days at 10 °C, and individual colonies of fungi were purified on marine agar. Long-term preservation of fungi was carried out at −80 °C using cryotubes with sterile 15% glycerol. All of 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.

Fungal identification

The protocol for DNA extraction from filamentous fungi followed Rosa *et al.* (2009). 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). Yeasts were characterised via standard methods (Yarrow, 1998), and their identification was carried out using the taxonomic keys of Kurtzman *et al.* (2011). Yeast identities were confirmed by sequencing the D1-D2 variable domains of the large subunit ribosomal RNA gene using the primers NL1 and NL4, as described by Lachance *et al.* (1999).

Amplification of the *β-tubulin* gene was performed with the Bt2a and Bt2b primers (Glass and Donaldson, 1995). PCR assays were conducted in

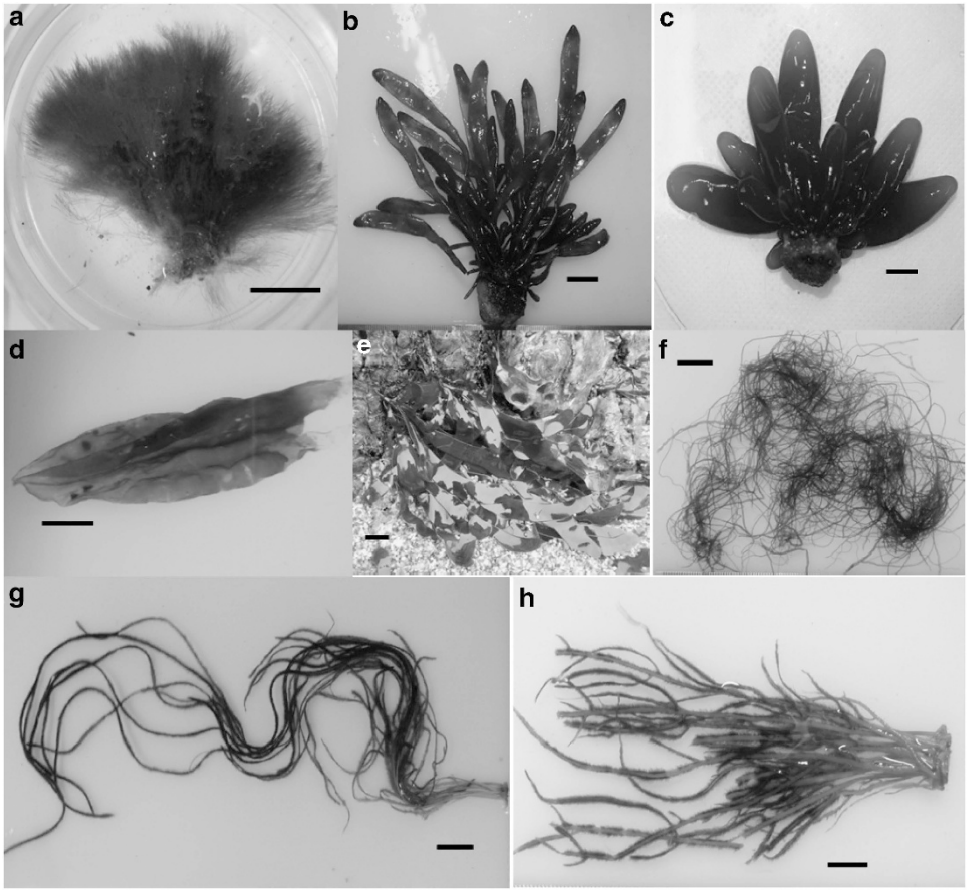

Figure 1 Macroalgae collected from the Antarctic Peninsula, with information on their distributions given in parenthesis. (**a**) *Acrosiphonia arcta* (Dillwyn) Gain (cosmopolitan); (**b**) *Adenocystis* sp. (most likely endemic); (**c**) *Adenocystis utricularis* (Bory de Saint-Vicent) Skottsberg (Australia, New Zealand and Antarctica); (**d**) *Monostroma hariotii* Gain (Antarctic and Subantartic islands); (**e**) *Palmaria decipiens* RW Ricker (Antarctic and Subantarctic islands); (**f**) *Ulva intestinalis* Linnaeus (cosmopolitan); (**g**) *Phaeurus antarcticus* Skottsberg (Antarctic and the Subantarctic islands); (**h**) *Desmarestia menziesii* J Agardh (Antarctic and the Subantarctic islands). Bars represent 1 cm.

50 μl reaction mixtures containing 1 μl of genomic DNA (10 ng μl^{-1}), 5 μl of PCR buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.8), 2 μl of dNTPs (10 mM) plus 3 μl of $MgCl_2$ (25 mM), 1 μl of each primer (50 pmol μl^{-1}), 1 μl of dimethyl sulfoxide (DMSO; Merck, Billerica, MA, USA), 2 μl betaine (5 M), 0.2 μl of Taq polymerase (5 U μl^{-1} DNA) and 33.8 μl of ultrapure sterile water. PCR amplifications were performed with the Mastercycler pro (Eppendorf, Hamburg, Germany), programmed for initial denaturation at 94 °C for 5 min, followed by 35 cycles of 1 min of denaturation at 94 °C, primer annealing for 1 min at 59 °C and extension for 1.30 min at 72 °C, with a final 7-min elongation step at 72 °C. After amplification of the *β-tubulin* template, excess primers and dNTPs were removed from the reaction mixture using a commercial GFX column with the PCR DNA Purification kit (Amersham Bioscience, Roosendaal, Netherlands). Purified PCR fragments were resuspended in 50 μl of Tris-EDTA buffer.

The amplified DNA was concentrated and purified using the Wizard Plus SV Miniprep DNA Purification System (Promega, Madison, WI, USA) and sequenced using an ET Dynamic Terminator Kit in a MegaBACE 1000/Automated 96 Capillary DNA sequencer (GE Healthcare, Piscataway, NJ, USA). The obtained sequences were analysed with SeqMan Π with Lasergene software (DNASTAR Inc., Madison, WI, USA), and a consensus sequence was obtained using Bioedit v. 7.0.5.3 software (Carlsbad, ON, Canada). 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). The consensus sequences of the algicolous fungi were deposited into GenBank (see the accession numbers in the Table 1). However, according to Gazis *et al.* (2011), sequencing of the ITS region may fail to recognise some fungal genera. For this reason, the *β-tubulin* sequences, which are considered promising for a one-gene phylogeny (Frisvad and Samson, 2004), were used to elucidate the taxonomic positions of the inconclusive taxa identified using ITS sequences. In addition, the followed criteria were used to interpret the sequences from the GenBank database: for query coverage and sequence identities $\geqslant$99%, the genus and species were accepted; for

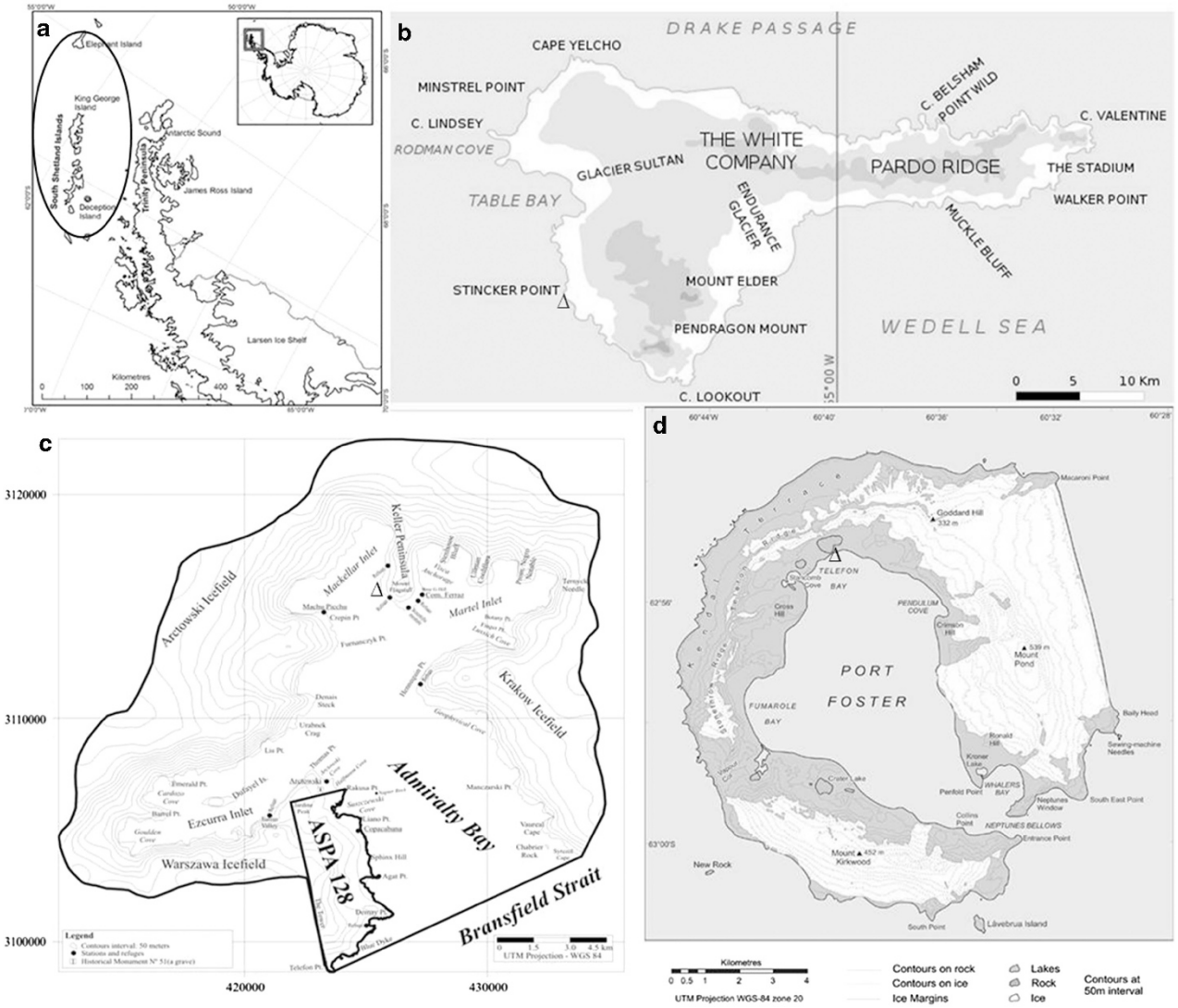

Figure 2 Maps showing the positions of the islands sampled on the (**a**) Antarctic Peninsula across a transect of 350 km. Sampling sites: Δ, *Ulva intestinalis*, *Palmaria decipiens* and *Phaeurus antarcticus* in (**b**) = Stinker Point (61°07.935'S; 055°25.997'W) at Elephant island; **Δ**, *Acrosiphonia arcta* and *D. menziesii* in (**c**), Keller Peninsula (62°05.163'S; 058°24.784'W) at Admiralty Bay at King George Island; Δ, *Adenocystis utricularis*, *Monostroma hariotii*, *Adenocystis* sp. in (**d**), Telefon Bay (62°55.192'S; 060°39.797'W) at Deception Island.

query coverage and sequence identities showing 98%, the genus and species were accepted, but term 'cf.' (Latin for confer = compares with), used to indicate that the specimen resembles, but has certain minor features not found in the reference species, was included; for query coverage and sequence identities between 95% and 97%, only the genus was accepted; and for query coverage and sequence identities $\leqslant$95%, the isolates were labelled with the order or family name or as 'unknown' fungi. However, taxa that displayed query coverage and identities $\leqslant$97% or an inconclusive taxonomic position were subjected to phylogenetic ITS and *β-tubulin* analysis, with estimations conducted using MEGA Version 5.0 (Tamura *et al.,* 2011). The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1000 replicate runs. To complete the molecular identification, the sequences of known-type fungal strains or reference sequences obtained from fungal species deposited in international culture collections found in GenBank were added to improve the accuracy of the phylogenetic analysis. The information about fungal taxonomic hierarchical follows the rules established by Kirk *et al.* (2011) and the MycoBank (http://www.mycobank.org), and Index Fungorum (http://www.indexfungorum.org) databases.

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. A rarefaction curve was calculated using the Mao Tao index (Colwell *et al.*, 2004). The similarities

Table 1 Molecular identification of fungi associated with Antarctic macroalgae: identification conducted using BLASTn searches of the of the internal transcribed spacer (ITS) region and *β-tubulin* gene

Macroalgae species	*UFMGCB*[a] *code*	*No. of isolates*	*Top BLAST search results (GenBank accession number)*	*Query coverage (%)*	*Identity (%)*	*No. of bp sequenced and analysed*	*Proposed species or taxonomic group (GenBank accession number)*
Adenocystis utricularis	D1N10.34.1	1	*Debaryomyces hansenii* (HE681104)	100	99	524	*Debaryomyces hansenii* (KC485456[b])
	D1N10.13.1	1	*Meyerozyma caribbica* (JQ686909)	100	99	547	*Meyerozyma caribbica* (KC485457[b])
Acrosiphonia arcta	6063[c]	19	*Geomyces pannorum* (DQ189229)	98	99	729	*Geomyces* sp. (KC333881[d])
	K2.13.1	6	*Metschnikowia australis* (U76526)	99	99	484	*Metschnikowia australis* (KC485458[b])
	6074[c,e]	2	*Penicillium chrysogenum* (HQ652873)	99	99	662	[f]*Penicillium* sp. (KC333882[d], KC823167[g])
	K2.6.1	1	*Candida sake* (EU326138)	100	99	588	*Candida sake* (KC485459[b])
	6361[c,e]	1	*Cladosporium perangustum* (JF499836)	100	97	486	[f]*Cladosporium* sp. (KC341715[d], KC823166[g])
	6079	1	*Cladosporium tenuissimum* (JQ246357)	100	99	476	*Cladosporium tenuissimum* (KC341716[d])
	K2.9.1	1	*Debaryomyces hansenii* (HQ860269)	100	99	546	*Debaryomyces hansenii* (KC485460[b])
	6077[c]	1	*Mortierella* sp. (JX270406)	90	97	562	[f]*Mortierella* sp. (KC341717[d])
	6083[c]	1	*Phoma* sp. (JQ388278)	99	99	468	[f]*Phoma* sp. (KC341718[d])
	6318	1	*Thelebolus microsporus* (GU004196)	99	99	495	*Thelebolus globosus* (KC341719[d])
Desmarestia menziesii	K1.17.1	1	*Metschnikowia australis* (FJ911872)	99	99	446	*Metschnikowia australis* (KC485461[b])
	6084[c,e]	1	*Penicillium chrysogenum* (JN021549)	100	98	486	[f]*Penicillium* sp. (KC341720[d], KC823168[g])
Ulva intestinalis	6101[c,e]	6	*Penicillium commune* (JN676122)	100	100	496	[f]*Penicillium* sp. (KC485461[d], KC823169[g])
	6092[c,e]	3	*Penicillium solitum* (JN642222)	98	99	534	[f]*Penicillium discolor* (KC485423[d], KC823170[g])
	6090	3	*Antarctomyces psychrotrophicus* (JN104511)	100	99	482	*Antarctomyces psychrotrophicus* (KC485424[d])
	CM.01.2	1	*Cryptococcus victoriae* [AY040653]	98	99	605	*Cryptococcus victoriae* [KC485462[b]]
	6096	1	*Engyodontium album* (HM214540)	90	99	566	*Engyodontium* sp. (KC485425[d])
	6097[c]	1	*Geomyces luteus* (AJ938164)	100	98	451	*Geomyces luteus* (KC485426[d])
	6328	1	*Helotiales* sp. (HQ533820)	90	99	477	*Helotiales* sp. (KC485427[d])
	6326	1	*Mycoarthris corallinus* (AF128440)	97	98	458	*Mycoarthris* cf. *corallines* (KC485428[d])
	6325[c,e]	1	*Penicillium chrysogenum* (KC009835)	98	98	507	[f]*Penicillium* sp. (KC485429[d], KC823171[g])
	6095	1	*Thelebolus microsporus* (GU004196)	100	100	485	*Thelebolus globosus* (KC485430[d])
Monostroma hariotii	6120[c,e]	12	*Penicillium chrysogenum* (KC009826)	100	99	512	[f]*Penicillium* sp. (KC485431[d], KC823175[g])
	6112[c]	7	*Geomyces pannorum* (JF311913)	100	99	439	*Geomyces* cf. *destructans* (KC485432[d])
	D1N18.31.1	4	*Meyerozyma guilliermondii* (JF766631)	100	99	537	*Meyerozyma guilliermondii* (KC485463[b])
	6330	1	*Cryptococcus laurentii* (FJ743631)	100	98	570	*Cryptococcus* cf. *laurentii* (KC485433[d])
	6130[c]	1	*Lecanicillium araneicola* (AB378506)	99	92	573	[f]*Cordycipitaceae* sp. (KC485434[d])
	6138[c]	1	*Paecilomyces anatarcticus* (AJ879113)	100	96	475	[f]*Helotiales* sp. (KC485435[d])
	6316[c]	1	*Pezizella discreta* (JF908571)	99	97	487	[f]*Hyaloscyphaceae* sp. (KC485436[d])
	D1N18.42.1	1	*Rhodotorula mucilaginosa* (JQ277252)	100	99	537	*Rhodotorula mucilaginosa* (KC485464[b])
Palmaria decipiens	6034[c,e]	11	*Penicillium chrysogenum* (JN032681)	100	99	538	[f]*Penicillium* sp. (JX976546[d], KC823172[g])
	6049[c]	8	*Geomyces pannorum* (DQ189229)	91	99	554	*Geomyces* sp. (KC485437[d])
	6056	2	*Acremonium strictum* (AY138844)	92	99	565	*Acremonium* sp. (KC485438[d])

Table 1 (Continued)

Macroalgae species	*UFMGCB*[a] *code*	*No. of isolates*	*Top BLAST search results (GenBank accession number)*	*Query coverage (%)*	*Identity (%)*	*No. of bp sequenced and analysed*	*Proposed species or taxonomic group (GenBank accession number)*
	6050	2	*Fusarium oxysporum* (HQ248198)	91	99	524	*Fusarium* sp. (KC485439[d])
	E2N5.42.1	2	*Yamadazyma mexicana* (FJ455104)	100	99	500	*Yamadazyma mexicana* (KC485465[b])
	6047	1	*Aspergillus candidus* (FJ441637)	93	99	548	*Aspergillus* sp. (KC485440[d])
	6053	1	*Chaetomium globosum* (JN689341)	66	99	751	*Chaetomium* sp. (KC485441[d])
	6140[c,e]	1	*Penicillium raistrickii* (FR670335)	78	97	665	[f]*Penicillium* sp.(KC485442[d], KC823173[g])
	6048[c,e]	1	*Penicillium spinulosum* (DQ132828)	74	99	697	[f]*Penicillium spinulosum* (KC485443[d], KC823174[g])
Phaeurus antarcticus	6141[c,e]	13	*Penicillium chrysogenum* (JQ665262)	100	100	482	[f]*Penicillium* sp. (KC485444[d], KC823176[g])
	6148[c]	8	*Geomyces pannorum* (DQ189229)	100	99	495	*Geomyces* sp. (KC485445[d])
	6153[c,e]	2	*Penicillium raistrickii* (FR670335)	81	98	631	[f]*Penicillium* sp. (KC485446[d], KC823178[g])
	6164[c,e]	1	*Aspergillus terreus* (JQ070071)	100	100	536	[f]*Aspergillus terreus* (KC485447[d], KC823163[g])
	6167	1	*Eurotium herbariorum* (JN942870)	100	99	429	*Eurotium herbariorum* (KC485448[d])
	6327	1	*Eurotium repens* (AY373890)	100	99	482	*Eurotium repens* (KC485449[d])
	6320[c,e]	1	*Penicillium steckii* (HM469415)	100	99	467	[f]*Penicillium steckii* (KC485450[d], KC846137[g])
Adenocystis sp.	6323[c,e]	3	*Penicillium chrysogenum* (JQ665262)	95	99	504	[f]*Penicillium* sp. (KC485451[d], KC823164[g])
	6106	1	*Aspergillus conicus* (HE578068)	99	99	426	*Aspergillus conicus* (KC485452[d])
	6110[c]	1	*Geomyces pannorum* (JF311913)	100	99	451	*Geomyces* sp. (KC485453[d])
	6109[c,e]	1	*Penicillium citrinum* (FJ765031)	93	100	507	[f]*Penicillium citrinum* (KC485454[d], KC823177[g])
	6322[c,e]	1	*Penicillium commune* (FJ499454)	100	98	542	[f]*Penicillium* sp. (KC485455[d], KC823165[g])

When the number of isolates was >1, 50% of the isolates were sequenced and the best sequence was deposited in the GenBank database.
[a]UFMGCB = Culture of Microorganisms and Cells from the Federal Universidade of Minas Gerais.
[b]D1/D2 sequences deposited.
[c]Taxa subjected to phylogenetic analysis based on the ITS region for elucidation of taxonomic positions.
[d]ITS sequences deposited.
[e]Taxa subjected to phylogenetic analysis based on the *β-tubulin* regions for elucidation of taxonomic positions.
[f]Taxonomic position suggested by the phylogenetic analyses.
[g]*β-tubulin* sequences deposited.

among fungal taxa from different areas were estimated using the Sorensen coefficient and Bray-Curtis measures. All of the results were obtained with 95% confidence, and bootstrap values were calculated from 1000 iterations. All diversity and similarity indices, rarefaction curves and the principal components analysis calculations were performed using the computer Program PAST, version 1.90 (Hammer *et al.,* 2001). Further information about these indices can be found in Magurran (2011).

Fungal cultivation and preparation of extracts for biological assays

All fungal isolates were cultivated using solid state fermentation. Five-millimetre-diameter plugs from each filamentous fungus were inoculated into the centres of Petri dishes (60 mm diameter, with 20 ml of marine agar). The plates were incubated at 10 ± 2 °C for 15 days, and the cultured materials from each Petri dish were then cut and transferred to 50-ml vials containing 35 ml of ethanol. After 48 h at room temperature, the organic phase was decanted, and the solvent was removed under a vacuum centrifuge at 35 °C (Santiago *et al.,* 2012). An aliquot of each dried extract was dissolved in DMSO (Merck) to prepare a 100-mg ml^{-1} stock solution, which was stored at -20 °C.

Assay for antimicrobial activity

Susceptibility testing against *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 12600, *Pseudomonas aeruginosa* ATCC 10145, *Candida*

albicans ATCC 18804, *Candida krusei* ATCC 6258 and *Cladosporium sphaerospermum* CCT 1740 was performed using a protocol established by Carvalho *et al.* (2012). All extracts (dissolved in DMSO) were diluted to a final concentration of 250 μg ml^{-1} for use in the antimicrobial assays. The results are expressed as the percent inhibition in relation to controls without drugs. All antimicrobial assays were performed in duplicate.

In vitro assays with intracellular amastigote forms of Trypanosoma cruzi

In vitro assays with amastigote forms of *T. cruzi* were performed according to protocols established by Buckner *et al.* (1996) with some modifications. *Trypanosoma cruzi* (Tulahuen strain) expressing the *E. coli β-galactosidase* gene were grown on a monolayer of mouse L929 fibroblasts. Cultures to be assayed for β-galactosidase activity were grown in Roswell Park Memorial Institute 1640 medium (pH 7.2-7.4) without phenol red (Gibco BRL, Cergy-Pontoise, France), plus 10% foetal bovine serum and glutamine. L929 fibroblasts were seeded into 96-well tissue culture microplates at a concentration of 4.0×10^3 per well in a volume of 80 μl and incubated overnight. β-galactosidase-expressing trypomastigotes were then added at a concentration of 4.0×10^4 per well in a volume of 20 μl. After 2 h, the medium containing trypomastigotes that did not penetrate into the cells was discarded and replaced with 200 μl of fresh medium. After 48 h, the medium was discarded again and replaced with 180 μl of fresh medium and 20 μl of the test extracts. Each extract was tested in triplicate. After 7 days of incubation, chlorophenol red β-D-galactopyranoside (100 μM final concentration) and Nonidet P-40 (Sigma-Aldrich, St. Louis, MO, USA) at final concentration of 0.1% were added to the plates, followed by incubation overnight at 37 °C, and the absorbance was measured at 570 nm in an automated microplate reader. Benznidazole at its 50% inhibitory concentration (IC_{50}; 1 μl ml^{-1}) was used as a positive control. The results were expressed as the percentage of growth inhibition. All assays were performed in triplicate.

Minimal inhibitory concentration and IC_{50}

The crude antimicrobial extracts were subjected to determination of a minimal inhibitory concentration (MIC) and the trypanocidal extracts were subjected to determination of a IC_{50}. MIC and IC_{50} represent the lowest concentrations of a crude extract (or compound) that inhibits the functions of a target (in this study, microorganisms and *T. cruzi*) by 100% and 50%, respectively. This quantitative measure indicates how much of a particular crude extract is needed to inhibit a given biological process by 50%. SoftMax Pro 5.3 (Sunnyvale, CA, USA) was used to calculate MIC values via nonlinear curve fitting of two or more independent experimental data sets to a four-parameter logistic dose–response model. No constraints were applied to the curve-fitting calculations. All assays were performed in duplicate.

Nuclear magnetic resonance spectroscopy

Bioactive extracts were analysed via nuclear magnetic resonance (NMR) spectroscopy in a Varian INOVA 600 MHz spectrometer (Varian Inc., Palo Alto, CA, USA). ^{1}H-NMR spectra were recorded in DMSO-d_6. The samples were prepared at a concentration of ~10 mg ml^{-1}, and the results were recorded in 3 mm NMR tubes using a standard ^{1}H pulse programme.

Results

Macroalgae collection and fungal identification

A total of 148 fungal isolates were recovered from 391 tissue fragments from the eight macroalgal species, which were identified by DNA sequences of the ITS region and *β-tubulin* gene in 21 different genera within of the phyla *Ascomycota*, *Basidiomycota* and subphylum *Mortierellomycotina* (*Zygomycota*; Table 1). All of the screened macroalgae harboured associated fungi, and 50 taxa were identified. However, the number of fungal taxa and diversity indices differed among the macroalgae. Among the phyla characterised, the most represented orders were *Eurotiales* (43.24%), *Helotiales* (32.43%), *Saccharomycetales* (11.48%), *Hypocreales* (6%) and *Thelebolales* (4.05%). In contrast, taxa of the orders *Tremellales*, *Capnodiales*, *Mortierellales*, *Pleosporales*, *Sordariales* and *Sporidiobolales* were found as minority components of the fungal communities with an abundance of ⩽1.35%.

Twenty-eight fungal taxa presented low molecular similarities or inconclusive information in comparison with known fungal ITS sequences deposited in the GenBank database. To identify these fungi, phylogenetic trees of the ITS (Figure 3) and/or *β-tubulin* (Figure 4) genes were constructed to illustrate their relationship with GenBank sequences. All ITS (Figure 3i) and *β-tubulin* (Figure 4c) sequences of the *Penicillium* isolates were analysed and the taxa *P. spinulosum*, *P. steckii*, *P. citrinum* and *P. chrysogenum* were identified. The other *Penicillium* taxa displayed inconclusive sequence analyses when compared with the sequences of the type species, and were identified as *Penicillium* sp.

Thirty-six fungal isolates displayed ITS sequence similarities with *Geomyces* sp. Among them, the seven isolates were identified as *G. destructans*, which showed 100% of query coverage and 98% identity; also these fungal isolates presented only 1.1% of sequence difference in comparison with the type species *G. destructans* (GenBank Access number EU884921; Table 1; Figure 3g). All other *Geomyces* isolates formed a separated cluster when

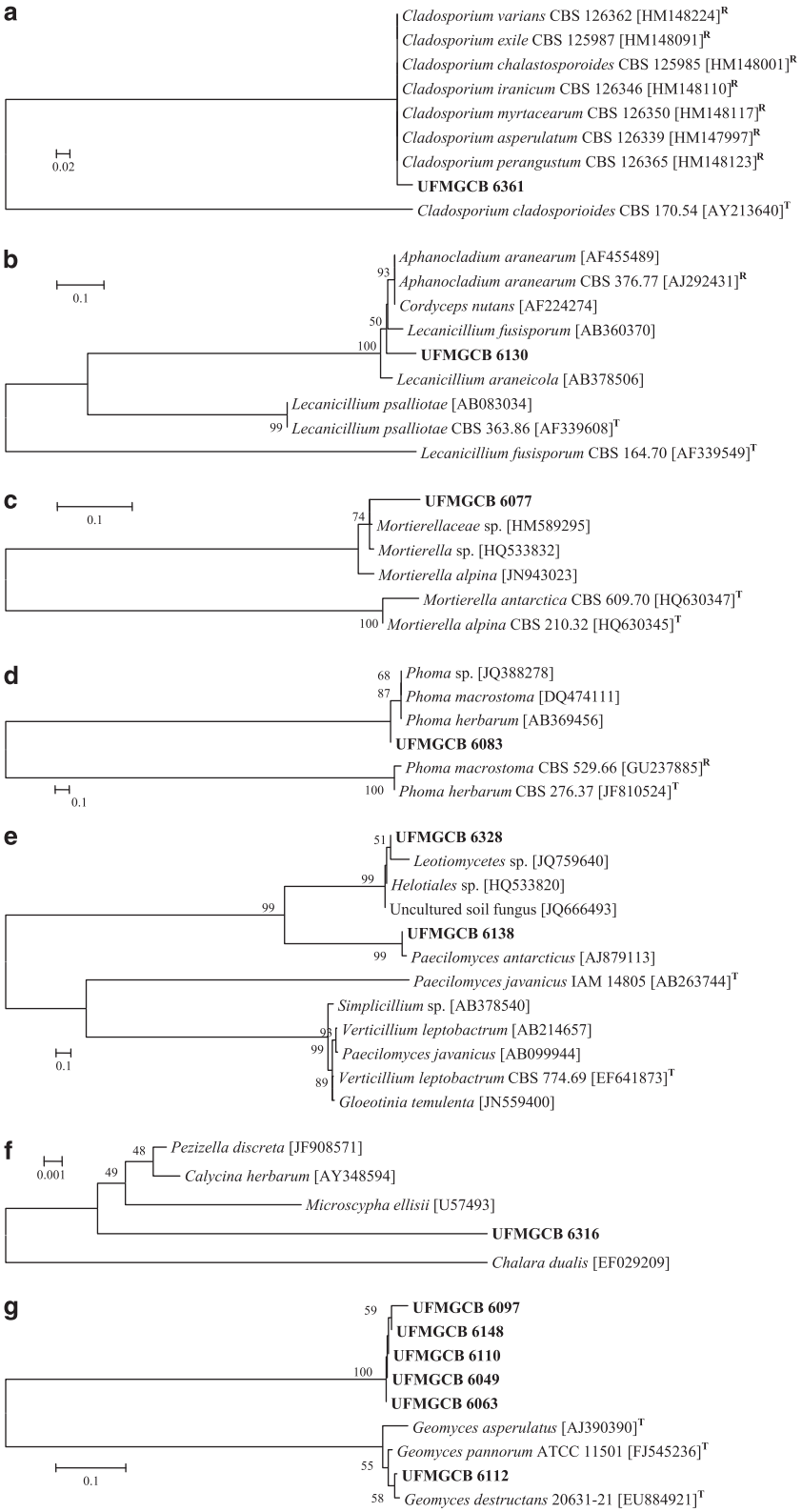

Figure 3 Phylogenetic analysis (**a–i**) of the sequences of fungi (in bold) associated with Antarctic macroalgae in comparison with type (**T**) or reference (**R**) sequences of the closest species, following BLAST analysis, deposited in the GenBank database. The trees were constructed based on the ITS region sequences using the maximum composite likelihood method.

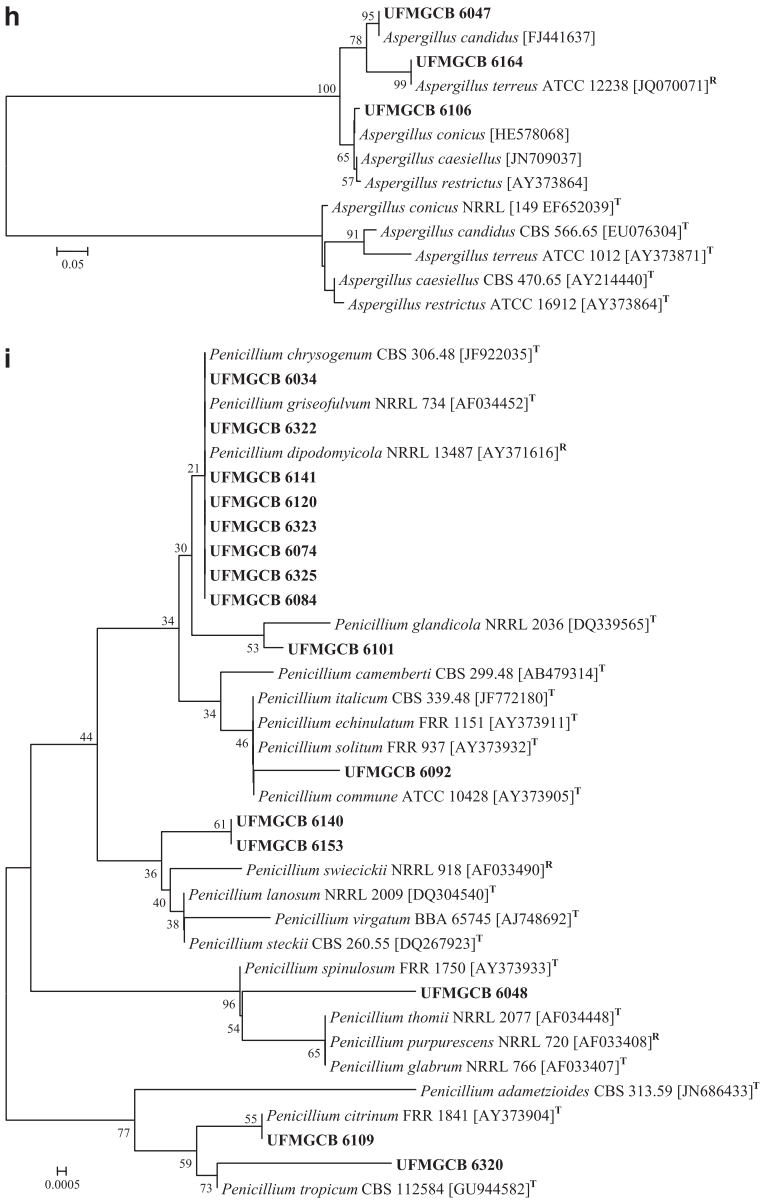

Figure 3 Continued.

compared with sequences of the type species of *G. pannorum* (GenBank Access number FJ545236) and *G. asperulatus* (GenBank Access number AJ390390; Figure 3g); these taxa were identified as *Geomyces* sp.

In addition, after ITS (Figure 3) and *β-tubulin* (Figure 4) sequence analysis, 27 fungal taxa were identified as belonging to the genera *Acremonium*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Engyodontium*, *Fusarium*, *Geomyces*, *Mortierella*, *Penicillium*, *Phoma* as well as the families *Cordycipitaceae*, *Hyaloscyphaceae* and order *Helotiales*, which displayed low sequence similarities when compared with fungal sequences deposited in the GenBank.

Diversity, richness, dominance and distribution

The most frequent fungal taxa identified were *Penicillium* sp. (35.8%), *Geomyces* sp. (24.32%) and *M. australis* (4.72%). However, in general, the fungal communities associated with the Antarctic macroalgae presented high values of the diversity (Fisher's $\alpha = 22.0$), richness (Margalef's = 10.41) and dominance (Simpson's = 0.94) indices. The phylum *Chlorophyta* displayed a Fisher's $\alpha = 5.67$ and 9.33 fungal taxa per macroalgal species, which was followed by *Rhodophyta* (Fisher's $\alpha = 4.47$ and 9 fungal taxa/macroalgal species) and *Phaeophyta* (Fisher's $\alpha = 2.7$ and 4 fungal taxa/macroalgal species). However, the values of the indices differed among the macroalgae (Table 2). The fungal

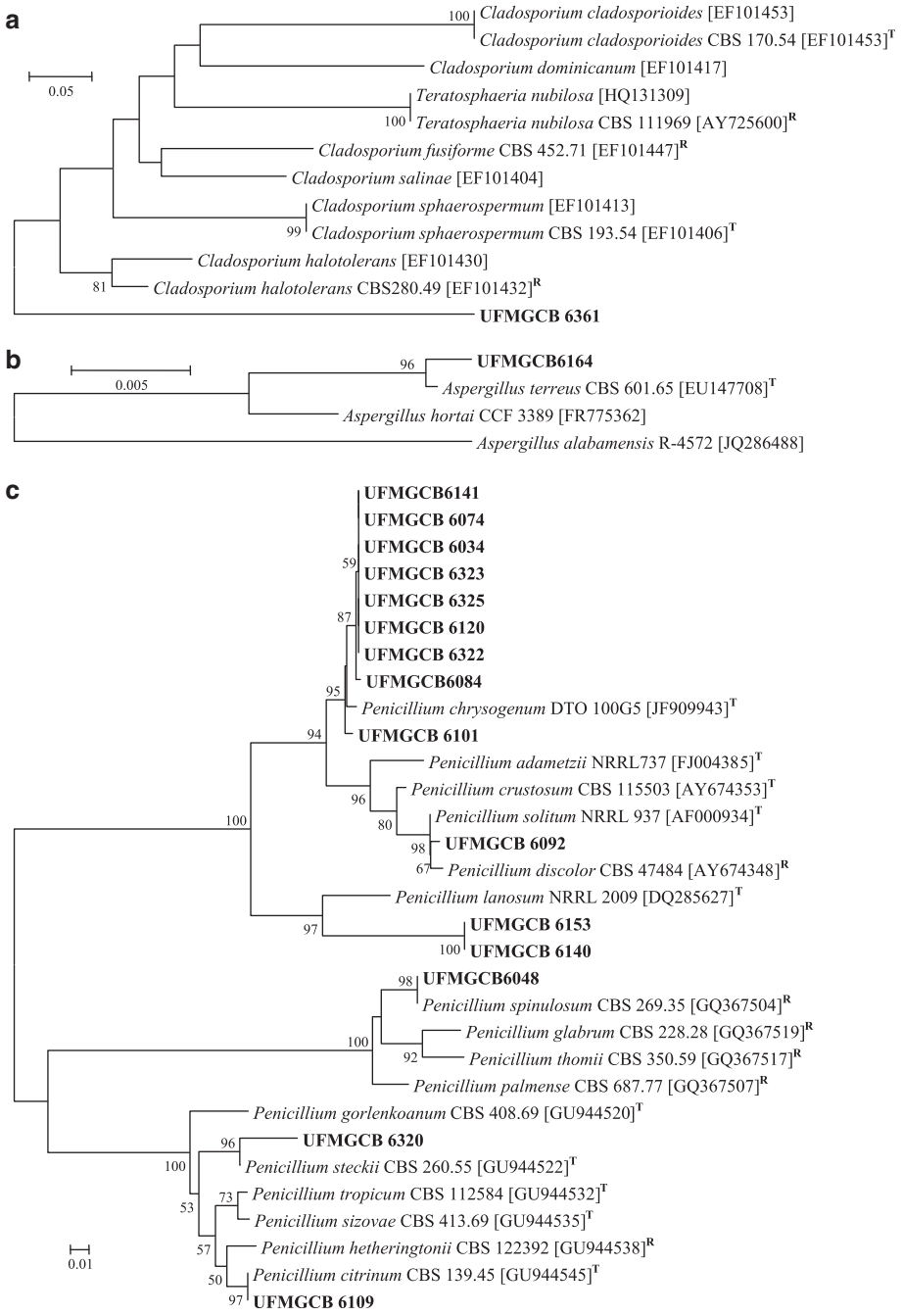

Figure 4 Phylogenetic analysis (**a–c**) of the *β-tubulin* sequences of the UFMGCB fungi (in bold) in comparison with type (**T**) or reference (**R**) sequences of the closest species, following BLAST analysis, deposited in the GenBank database. The trees were constructed based on the *β-tubulin* gene sequences using the maximum composite likelihood method.

communities associated with *U. intestinalis* and *Adenocystis* sp. showed high diversity and dominance values, whereas the communities corresponding to *A. utricularis* and *D. menziesii* displayed the lowest diversity, richness and dominance values. In addition, the *A. arcta* fungal community showed the highest richness, but moderate diversity and dominance. Only two fungal species were isolated from the macroalgae *A. utricularis* and *D. menziesii*, which exhibited the lowest Fisher's α, Margalef's and Simpson's indices. In addition to the high fungal diversity (Fisher's α values) found in the sampled macroalgae, the Mao Tao rarefaction curves continued to rise and did not reach an asymptote (Figure 5), indicating that not all fungal diversity had been recovered. Thirty-three fungal taxa (66%) occurred as singletons corresponding to a single macroalgal species, representing rare components of the fungal communities.

Table 2 Physico-chemical parameters of water and diversity indices of fungal communities associated with the Antarctic macroalgae

Parameters/ diversity indices	*Phyla of macroalgae host species*							
	Phaeophyta				*Chlorophyta*			*Rhodophyta*
	Adenocystis utricularis	*Adenocystis* sp.	*Desmarestia menziesii*	*Phaeurus antarcticus*	*Acrosiphonia arcta*	*Monostroma hariotii*	*Ulva intestinalis*	*Palmaria decipiens*
Temperature (°C)	3.7	3.7	0.5	2.1	0.5	3.7	2.1	2.1
Conductivity ($mS\,cm^{-1}$)	49.89	49.89	27.23	50.6	27.23	49.89	50.6	50.6
Salinity (p.p.t.)	32.1	32.1	32.8	33.0	32.8	32.1	33.0	33.0
pH	7.49	7.49	7.74	7.74	7.74	7.49	7.74	7.74
Optical Density ($mg\,l^{-1}$)	79.9	79.9	100	34.6	100	79.9	34.6	34.6
Number of fungal taxa	2	5	2	7	10	8	10	9
Fisher's α	0 (0—0.8)[a]	7.8 (0.93—7.82)	0 (0—0.8)	3 (0.86—3.1)	4.77 (1.61—4.0)	3.7 (1.27—3.74)	8.54 (2.21—8.54)	4.47 (1.74—4.47)
Margalef's	1.44 (0—1.44)	2 (0.5—2.1)	1.44 (0—1.44)	1.82 (0.6—1.82)	2.55 (1.13—2.26)	2.1 (0.9—2.1)	0.03 (1.35—3.05)	2.3 (1.18—2.37)
Simpson's	0.5 (0—0.5)	0.73 (0.2—0.77)	0.5 (0—0.5)	0.66 (0.5—0.75)	0.64 (0.46—0.75)	0.72 (0.55—0.8)	0.83 (0.65—0.86)	0.7 (0.6—0.82)

[a]The numbers in parentheses represent the lower and upper diversity values, respectively, with 95% of confidence and bootstrap values calculated from 1000 iterations.

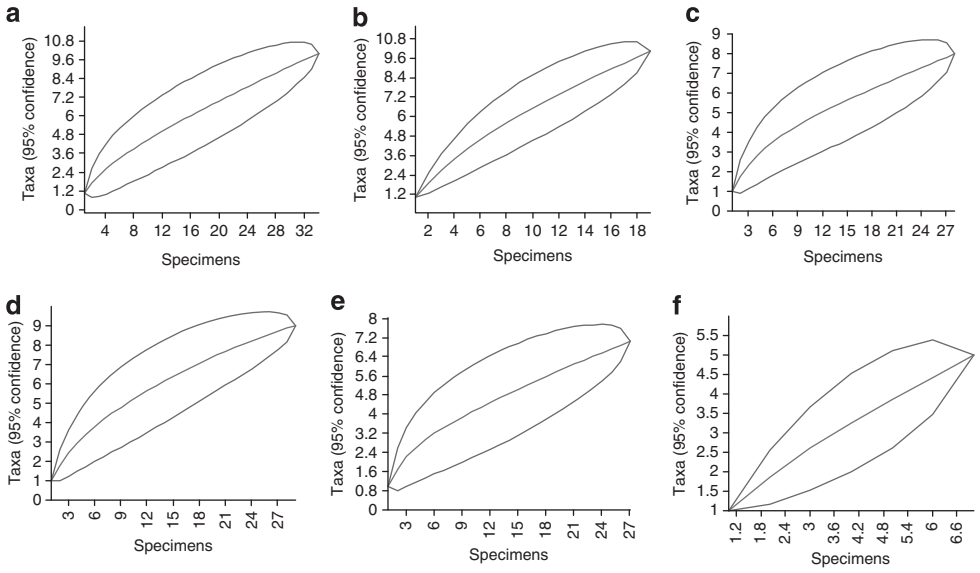

Figure 5 Species accumulation curves for the fungal communities associated with Antarctic macroalgae. *Acrosiphonia arcta* (**a**), *Ulva intestinalis* (**b**), *Monostroma hariotii* (**c**), *Palmaria decipiens* (**d**), *Phaeurus antarcticus* (**e**) and *Adenocystis* sp. (**f**).

The fungal composition was variable among the macroalgal species, which was confirmed by the values of the Sorensen and Bray-Curtis similarity indices (Figure 6). The Sorensen index showed that the most similar fungal communities were found in *P. antarcticus* and *Adenocystis* sp., both of which are brown macroalgal species, but with different morphologies. However, the Bray-Curtis similarity between the fungal communities associated with the endemic macroalgae *P. decipiens* and *M. hariotii* indicated the highest similarity with the presence and abundance of the *Geomyces* and *Penicillium* taxa associated with both macroalgae. In addition, *Penicillium* taxa occurred in association with seven other macroalgal species, followed by *Geomyces* (six macroalgae).

The principal components analysis of the physical and chemical parameters of the water where the macroalgae were sampled related to the fungal diversity indices (Figure 7) revealed that conductivity exhibited a positive correlation with the Fisher's α and Simpson's indices for the fungal assemblages

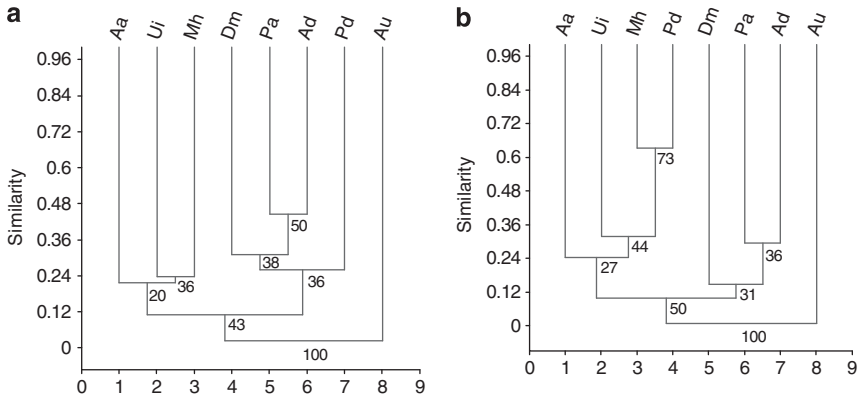

Figure 6 Dendrograms showing the Sorensen (**a**) and (**b**) Bray-Curtis similarity measures for the fungal communities associated with the Antarctic macroalgae. The results were obtained with 95% confidence and bootstrap values calculated from 1000 iterations. The sampled macroalgae were Aa, *Acrosiphonia arcta*; Ad, *Adenocystis* sp.; Au, *Adenocystis utricularis*; Dm, *Desmarestia menziesii*; Pa, *Phaeurus antarcticus*; Mh, *Monostroma hariotii*; Pd, *Palmaria decipiens*; and Ui, *Ulva intestinalis*.

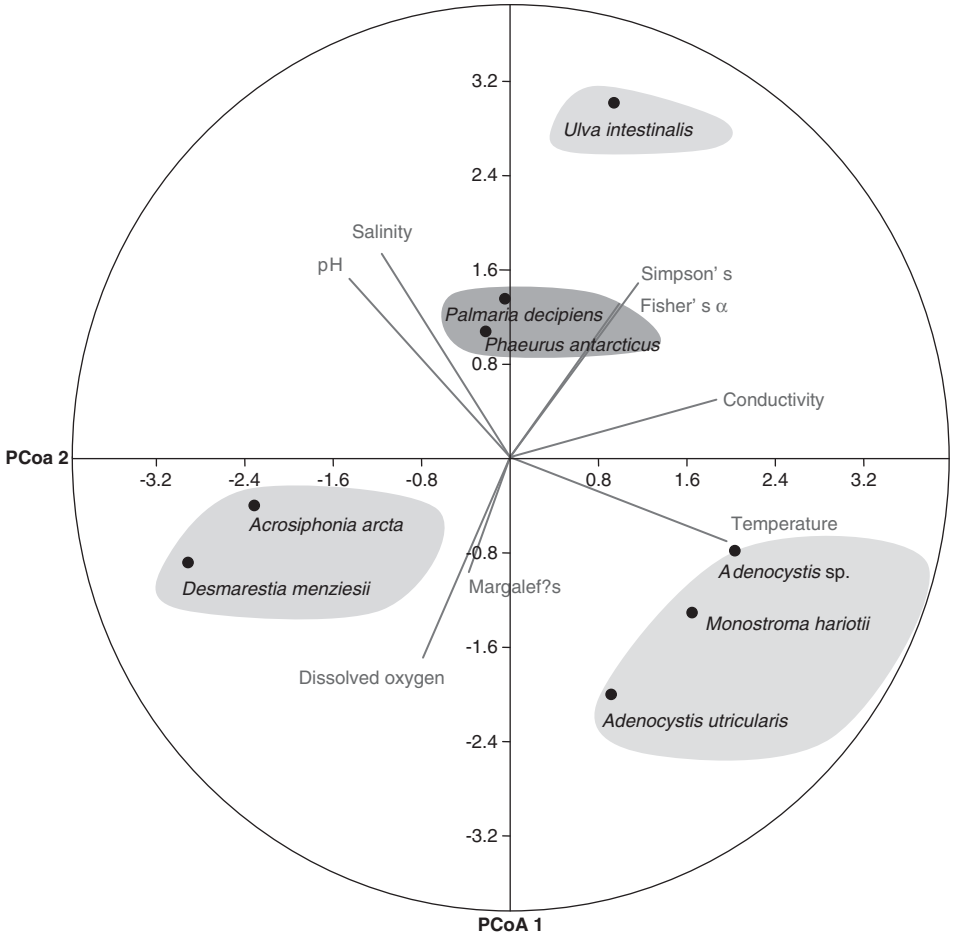

Figure 7 Principal component analysis plot calculated among the physicochemical water parameters (temperature, salinity, pH, dissolved oxygen and conductivity) obtained where the macroalgae were collected and Fisher's β (diversity), Margalef's (richness) and Simpson's (dominance) indices of the fungal communities associated with Antarctic macroalgae.

associated with *U. intestinalis*; dissolved oxygen with Margalef's index for *A. arcta* and *D. menziesii*; temperature with *Adenocystis* sp., *M. hariotii* and *A. utricularis*; and salinity and pH with *P. decipiens* and *P. antarcticus*.

Biological activities

All fungal isolates were grown using solid state fermentation techniques to obtain crude extracts, which were then screened against bacteria, fungi and parasite targets to detect bioactive compounds.

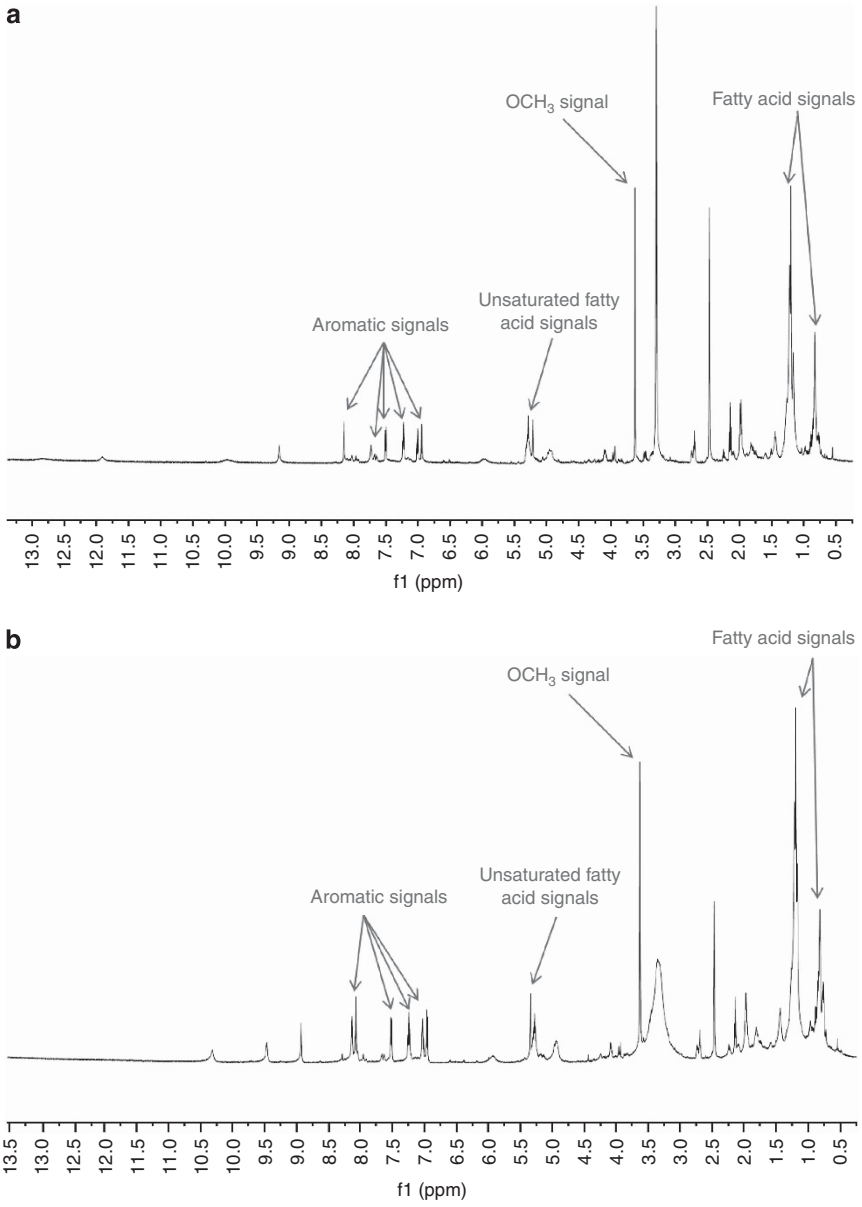

Figure 8 ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of bioactive ethanol extracts from the freeze-dried culture media of (**a**) *Penicillium* sp. UFMGCB 6034 and (**b**) *Penicillium* sp. UFMGCB 6120. Regions of interest are labelled above the corresponding signals.

The extracts of *Penicillium* sp. UFMGCB 6034 and *Penicillium* sp. UFMGCB 6120 recovered from the endemic macroalgae *P. decipiens* and *M. hariotii*, respectively, were able to produce bioactive extracts. *Penicillium* sp. UFMGCB 6120 displayed antifungal activity against the filamentous fungus *Cladosporium sphaerospermum*, producing 96% inhibition and an MIC value of 250 $\mu g\,ml^{-1}$. In addition, *Penicillium* sp. UFMGCB 6034 and *Penicillium* sp. UFMGCB 6120 exhibited 100% inhibition of trypomastigote forms of *T. cruzi*, with IC_{50} values of 1.28 and 0.45 $\mu g\,ml^{-1}$ being obtained, respectively, which were close to or lower than the IC_{50} (1 $\mu g\,ml^{-1}$) of the control drug (Benznidazole) used in the experiments. In addition, these two trypanocidal extracts showed low toxicity against normal fibroblast cells.

^{1}H NMR (600 MHz, DMSO-d_6) analyses of the ethanol extracts from the freeze-dried culture media of *Penicillium* sp. UFMGCB 6034 and *Penicillium* sp. UFMGCB 6120 were performed. Preliminary analysis indicated the presence of functional groups associated with aromatic protons, methoxy protons, unsaturated fatty-acid olefinic protons, and fatty-acid methylene and methyl protons, as indicated in Figure 8. Both *Penicillium* sp. UFMGCB 6034 and

Penicillium sp. UFMGCB 6120 clearly contain the same major components, as demonstrated by the similar ^{1}H NMR signals observed.

Discussion

Macroalgae collection and fungal identification

Marine algae are known to shelter fungal species, but few taxa have been studied in detail. The initial work of Loque *et al.* (2010) and the present study, which addresses the fungal communities associated with eight macroalgae sampled along a latitudinal gradient over 350 km on the Antarctica Peninsula, represent the first systematic analyses of this kind. Similar to other mycological studies conducted in the Antarctic that have characterised fungi in soil (Fell *et al.,* 2006), wood debris (Arenz *et al.,* 2006), lakes (Gonçalves *et al.,* 2012) and in associations with plants (Rosa *et al.,* 2009), the fungal taxa associated with the Antarctic macroalgae examined in this study comprised a few dominant taxa. Our results agree with those of Suryanarayanan *et al.* (2010), who found few dominant fungal species associated with 25 macroalgae occurring along the coast of southern India. However, algicolous fungi remain relatively unexplored as a group. The presence of *Geomyces* sp., *Penicillium* sp. and *M. australis* as dominant species associated with the investigated Antarctic macroalgae suggests that they may exhibit an interesting ecological relationship with their hosts.

Diversity, richness, dominance and distribution

Studies on fungal diversity in Antarctica show inconsistencies in the discrimination of endemic, indigenous and cosmopolitan species. Endemic species are characterised as true psychrophilic fungi that are able to actively grow and reproduce only in Antarctica (Ruisi *et al.,* 2007). According to Onofri *et al.* (2007), indigenicity is indicated by the observation of a large number of isolates over time as well as being commonly recorded in Antarctica from different sites and substrata. In addition, the majority of Antarctic fungi consist of ecotypes of cosmopolitan species that show mesophilic-psychrotolerant behaviour as an adaptation to the cold Antarctic climate (Zucconi *et al.,* 1996). On the basis of the above criteria, the fungal species *M. australis* is indigenous to Antarctica, and *Penicillium* is a cosmopolitan mesophilic-psychrotolerant group that shows adaptation to the cold Antarctic climate. In contrast, *Antarctomyces psychrotrophicus* (not found outside of Antarctica), which was part of the minority component associated with the macroalga *U. intestinalis*, has been reported to be endemic to Antarctica (Stchigel *et al.,* 2001).

Geomyces (*Helotiales*, *Ascomycota*), found as the most abundant taxa associated with Antarctic macroalgae, includes about 10 named species, which show a wide distribution (http://www.mycobank.org/). *Geomyces* sp. have been frequently recorded in Antarctica, are a celluloytic, keratinophilic, psychrophilic and halotolerant fungi with a ubiquitous distribution in the soils of cold regions that is able to colonise and to utilise different carbon sources (Mercantini *et al.,* 1989). In addition, *Geomyces* taxa were found on the thalli of the macroalgae *A. utricularis* and *D. anceps* (Loque *et al.,* 2010), leaves of *Colobanthus quitensis* (Rosa *et al.,* 2010), mosses (Tosi *et al.,* 2002) and in freshwater lakes (Gonçalves *et al.,* 2012). Seven fungal isolates found associated with the endemic macroalgae *M. hariotii* displayed high sequence similarity with *G. destructans*. *Geomyces destructans* is characterised as a psychrophilic pathogenic fungus able to decrease bats' population in temperate regions (Lorch *et al.,* 2011). According to Lorch *et al.* (2012), the diversity of *Geomyces* taxa, also classified as *Gymnostellatospora* and *Pseudogymnosascus*, is far greater than previously recognised based on traditional taxonomic methods, and can be underestimated. In addition, we found in association with Antarctic macroalgae 36 isolates identified as *Geomyces* sp., which may include new species and will be subject to further studies to elucidate their taxonomic positions.

The yeast *M. australis*, which has been reported to be endemic to Antarctica, has been isolated from Antarctic seawater (Fell and Hunter, 1968), the stomach of the Antarctic krill species *Euphausia superba* (Donachie and Zdanowski, 1998) and, recently, at a high abundance from the algal thalli of *A. utricularis* (Loque *et al.,* 2010). In addition, *M. australis* has been found in marine sediment and freshwater in Antarctica (Vaz *et al.,* 2011). The recovery of *M. australis* in association with the macroalgae *A. arcta* and *D. menziesii* reinforces the notion that this yeast may exhibit a specific association with Antarctic macroalgae.

The cosmopolitan genus *Penicillium* has been recovered from alpine and tundra soils as well as permafrost layers (Gunde-Cimerman *et al.,* 2003). In Antarctica, species of *Penicillium* have been described from the soil (Azmiá and Seppelt, 1998), lakes (Ellis-Evans, 1996), wood (Arenz *et al.,* 2006) and on the macroalga *A. utricularis* (Loque *et al.,* 2010). As an extremophile, *P. chrysogenum* has been isolated as a dominant species from Arctic subglacial ice (Gunde-Cimerman *et al.,* 2003, Sonjak *et al.,* 2005). In addition, according to Bugni and Ireland (2004) *Penicillium* represents one of the more common genera isolated from macroalgae. In the present study, we found different *Penicillium* taxa associated with the Antarctic macroalgae. According to Frisvad and Samson (2004), Scott *et al.* (2004) and Houbraken *et al.* (2011), the taxonomy of the group *Penicillium* has been regarded to be especially difficult and needs a polyphasic study including physiological, morphological, multilocus sequence typing and chemical

methods. For these reasons, the *Penicillium* sp. associated with the Antarctic macroalgae were identified using the term 'cf.' or in genus rank. These *Penicillium* taxa will be the subject of further taxonomic evaluations.

The non-asymptotic species accumulation curves (Mao Tao analysis) generated for the fungal communities found in associations with the examined Antarctic macroalgae showed that a large number of single individuals (singletons) were recovered, which are considered rare species and often represent more than half of the species within these communities. Similar results were described for the fungal communities of *A. utricularis*, *D. anceps* and *P. decipiens* collected at King George Island, Antarctica (Loque *et al.,* 2010).

Twenty-seven fungal taxa associated with the Antarctic macroalgae displayed a low molecular similarity with the sequences of known fungi deposited in GenBank, suggesting that they are candidates for new fungal species. However, not all described fungal species are deposited in the GenBank database, and further taxonomic analyses including detailed physiological and macro- and micromorphological characterisation and molecular sequencing of other DNA regions, together with new phylogenetic analyses, will be needed to describe these potentially new species. According to the recent checklist of Antarctic fungi published by Bridge and Spooner (2012), the specimens of *Meyerozyma caribbica*, *Mycoarthris coralline* and *Yamadazyma mexicana*, which were found as minor components within the fungal communities associated with Antarctic macroalgae, represent the first records of these species in Antarctica.

According to Ruisi *et al.* (2007), the distribution of fungi in Antarctica is related to the distribution of their hosts or substrates, such as bird feathers and dung, invertebrates, vegetation, soils, rocks and lichens. However, marine fungal dispersal remains unknown. In the present study, the calculated similarity indices (Sorensen and Bray-Curtis) revealed the presence of some species (*D. hansenii*, *Geomyces* sp., *Helotiales* sp., *M. australis*, *Penicillium* sp. and *T. globosus*) in association with different macroalgae and from distinct Antarctic islands. The high values of the Bray-Curtis similarity index (which has priority regarding the abundance of common species) between the fungal communities associated with endemic macroalgae *P. decipiens* and *M. hariotii* indicate that *Geomyces* and *Penicillium* sp. might show a specific association with macroalgae adapted to extreme conditions.

Fungi are one of the largest and most diverse kingdoms of eukaryotes and are important biological components of terrestrial ecosystems. According to Bridge and Spooner (2012), there are many records of marine fungi from Antarctica, although there is little available information about their ecological roles. In the present study, some of the fungal taxa found to be associated with Antarctic macroalgae were identified as species known to be mutualists, saprobes and parasites. Bridge and Spooner (2012) suggest that Antarctic fungi occupy many different ecological niches, but their significance in these niches remains poorly understood. Understanding how mutualistic, saprobic and pathogenic fungi achieve their lifestyles is crucial for understanding their ecological functions and their impact on Antarctic macroalgae under the extreme conditions of this continent. In addition, the abiotic seawater parameters in the areas where the macroalgae were collected revealed low temperatures and high salinities, demonstrating that the fungi found in association with the macroalgae may exhibit specific mechanisms that allow them to survive in extreme conditions.

Biological activities

In general, fungi have been reported to represent prolific sources of various compounds, including several bioactive molecules. However, the potential of extremophile fungi to produce bioactive compounds is poorly understood. According to Santiago *et al.* (2012), the ability of Antarctic fungi to survive in extreme conditions suggests that they may display unusual biochemical pathways that allow them to generate specific or new molecules that could be used to develop new drugs. Our bioprospecting results show that the extracts from *Penicillium* sp. UFMGCB 6034 and *Penicillium* sp. UFMGCB 6120, which were recovered as dominant species from endemic macroalgae, *P. decipiens* and *M. hariotii*, displayed high and selective antifungal and/or trypanocidal activities with low MIC and IC_{50} values. The combination of low MIC and IC_{50} values together with the ^{1}H NMR spectral data suggesting the presence of functionalised aromatic compounds warrants extensive bioassay-directed fractionation to specifically identify the constituents responsible for the observed biological activity.

Marine fungi have been described as a promising source of antimicrobial compounds. Marine *Penicillium* sp. are known to produce a large variety of compounds with a wide range of biological and pharmacological activities. *Penicillium* sp. isolated from the alga *U. intestinalis* were observed to produce cytotoxic metabolites including communesins (Numata *et al.,* 1993), penochalasius A-C (Numata *et al.,* 1995), penostatins A-I (Takahashi *et al.,* 1996, Iwamoto *et al.,* 1998) and penochalasius D-H (Iwamoto *et al.,* 2001). The antibacterial and cytotoxic compounds di(2-ethyl hexayl) phthalate and fungisterol were isolated from *P. brevicompactum*, which was isolated from the associated marine alga *Pterocladia* sp. (Atalla *et al.*, 2011). The extracts of *Penicillium* sp. UFMGCB 6034 and *Penicillium* sp. UFMGCB 6120 will be subjected to further systematic chemical bioactivity-guided fractionation to isolate their bioactive compounds.

Conclusion

Although marine fungi have been studied for the last 100 years (Jones, 2011), many aspects regarding their taxonomy, ecology and potential biotechnological applications remain poorly documented. Our results are consistent with a suggestion by Bridge and Spooner (2012) that the true diversity of Antarctic fungi may be far greater than is currently estimated. Our results also suggest that macroalgae can act as a substrate to shelter fungi in Antarctica and contribute to the knowledge of the cryptic and unknown algicolous Antarctic mycota. We showed that endemic and cold-adapted macroalgae living under the extreme conditions of Antarctica shelter rich, diverse and complex fungal communities consisting of few dominant indigenous or mesophilic cold-adapted species. In addition, we detected a large number of rare and/or endemic taxa, which may provide an interesting model of algal–fungal interactions in extreme conditions as well as a potential source of bioactive compounds. Our results reinforce the call for further studies of fungal communities present across the Antarctic marine environment as well as their phylogenetic relationships with species that occur in other cold regions. Finally, the present work suggests that examination of the fungi associated with macroalgae in Antarctica may provide new insights into the biological mechanisms underlying tolerance and adaptation to extreme marine polar conditions.

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