Microb Ecol (2014) 67:775–787
DOI 10.1007/s00248-014-0374-9

FUNGAL MICROBIOLOGY

Diversity Patterns, Ecology and Biological Activities of Fungal Communities Associated with the Endemic Macroalgae Across the Antarctic Peninsula

Laura E. Furbino · Valéria M. Godinho · Iara F. Santiago · Franciane M. Pellizari · Tânia M. A. Alves · Carlos L. Zani · Policarpo A. S. Junior · Alvaro J. Romanha · Amanda G. O. Carvalho · Laura H. V. G. Gil · Carlos A. Rosa · Andrew M. Minnis · Luiz H. Rosa

Received: 1 November 2013 / Accepted: 20 January 2014 / Published online: 9 February 2014

Abstract We surveyed diversity patterns and engaged in bioprospecting for bioactive compounds of fungi associated with the endemic macroalgae, *Monostroma hariotii* and *Pyropia endiviifolia*, in Antarctica. A total of 239 fungal isolates were obtained, which were identified to represent 48 taxa and 18 genera using molecular methods. The fungal communities consisted of endemic, indigenous and cold-adapted cosmopolitan taxa, which displayed high diversity and richness, but low dominance indices. The extracts of endemic and cold-adapted fungi displayed biological activities and may represent sources of promising prototype molecules to develop drugs. Our results suggest that macroalgae along the marine Antarctic Peninsula provide additional niches where fungal taxa can survive and coexist with their host in the extreme conditions. We hypothesise that the dynamics of richness and dominance among endemic, indigenous and cold-adapted cosmopolitan fungal taxa might be used to understand and model the influence of climate change on the maritime Antarctic mycota.

L. E. Furbino · V. M. Godinho · I. F. Santiago · C. A. Rosa · L. H. Rosa (✉)
Departamento de Microbiologia, Universidade Federal de Minas Gerais, P. O. Box 486, CEP 31270-901 Belo Horizonte, MG, Brazil
e-mail: lhrosa@icb.ufmg.br

F. M. Pellizari
Laboratório de Ficologia e Qualidade de Água do Mar, Universidade Estadual do Paraná, Paranaguá, PR, Brazil

T. M. A. Alves · C. L. Zani
Laboratório de Química de Produtos Naturais, Centro de Pesquisas René Rachou, FIOCRUZ, Belo Horizonte, MG, Brazil

P. A. S. Junior · A. J. Romanha
Laboratório de Parasitologia Celular e Molecular, Centro de Pesquisas René Rachou, FIOCRUZ, Belo Horizonte, MG, Brazil

A. J. Romanha
Universidade Federal de Santa Catarina, Brazil, Santa Catarina, Brazil

A. G. O. Carvalho · L. H. V. G. Gil
Departamento de Virologia e Terapia Experimental, Centro de Pesquisas Aggeu Magalhães, FIOCRUZ, Recife, PE, Brazil

A. G. O. Carvalho
Departamento de Genética, Universidade Federal de Pernambuco, Recife, PE, Brazil

A. M. Minnis
Center for Forest Mycology Research, Northern Research Station, US Forest Service, Madison, WI, USA

Introduction

Antarctica is considered to be one of the harshest and most pristine ecosystems in the world where low temperature, different pH levels, low organic nutrient and water availability, strong winds and UV radiation are found. Antarctica is being influenced by strong and rapid climate change, mainly in the Peninsula region [20]. For these reasons, the Antarctic Peninsula offers unique habitat for studying the effects of climate change on taxonomy and diversity, ecology and evolution as well as for making biotechnological discoveries.

Different studies have been conducted on the Antarctic biota, including taxonomic and ecological studies on bacteria, protists, micro- and macroalgae, fungi, plants and animals [47]. Fungi are widely distributed in Antarctica, and their

occurrence has been recorded from a number of substrates including soil, wood, plants, invertebrates and vertebrates [6]. Marine fungi are an ecologically important group of microorganisms that play key roles in the energy flow of oceans [26, 31]. Fungi in marine environments are present on a variety of substrata such as decaying wood, leaves, macroalgae and seagrasses [28]. However, according to Bhadury et al. [4], the diversity and functions of fungi in marine systems have been underestimated. Among the mycological studies in Antarctica, a few works have addressed marine fungi, which were detected in water [14, 15], wood baits [21] and water and marine sediments [53].

Marine algae have an important role in organic matter mineral cycling, particularly in the littoral and infralittoral ecosystem in shallow waters of the Antarctic. Nedzarek and Rakusa-Suszczewski [40] suggest that algal beds cover about 30 % of the bottom surface in the Maritime Antarctic, with an estimate of 74,000 tons of wet biomass around only in Admiralty Bay, King George Island. According to Wiencke and Clayton [58], the Antarctic macroalgae composition is characterised by a high degree of endemism and the macroalgae play a fundamental role as primary producers, food for marine herbivores as well as in habitat structure [8]. Macroalgae also represent the majority of biomass in Antarctica [39] and shelter different Antarctic macro- and microorganisms. The endemic Antarctic macroalgae *Monostroma hariotii* Gain (Chlorophyta) and *Pyropia endiviifolia* (A. Gepp & E. Gepp) H. G. Choi & M. S. Hwang (Rhodophyta) are among the most abundant species across the different islands and, consequently, represent promising targets to recover associated marine fungi living in the extreme conditions of Antarctica.

Natural products are molecules of secondary metabolism derived from plants, animals or microorganisms, which can represent starting points as prototype compounds for drug discovery research. During recent years, the relevance of microorganisms in bioprospecting for drug discovery has been increasing, and, among microbial sources, the bioactive molecules produced by fungi represent a chemical reservoir for discovering new compounds with antibiotic, antioxidant, immunomodulating, anticancer and antiparasitic compounds. Marine fungi have been recognised as potential sources of novel natural products for pharmaceutical, agricultural and industrial applications, especially due to their ability to produce new secondary metabolites with different biological activities. The goals of this study were (1) to determine the distribution patterns and diversity dynamics (richness, dominance and similarity) of the endemic, cold-adapted and cosmopolitan taxa within the fungal communities associated with endemic Antarctic macroalgae, *M. hariotii* and *P. endiviifolia*, across the Antarctica Peninsula and (2) to evaluate the potential of these fungi to produce bioactive compounds.

Material and Methods

Sample Collection

Fragments of 180 fresh thalli of *Monostroma hariotii* Gain and 210 of *Penicillium endiviifolia* (A. Gepp & E. Gepp) H. G. Choi & M. S. Hwang were collected during December 2010 and January of 2011 in intertidal transects along a rocky coastline that becomes ice-free during the Antarctic summer. The macroalgal samples were collected along a 350-km transect through Elephant, King George and Deception Islands, on the Antarctic Peninsula (Fig. 1). Additionally, Table 1 shows the coordinates where the macroalgal samples were collected and the physical and chemical water parameters (temperature, salinity, conductivity and pH) that were recorded at each site using a multi-parameter probe Hexis TCS.

Macroalgae Identification

Complete and fertile samples of the macroalgae were sorted, washed and preserved in seawater with 4 % formalin in the NPo Almirante Maximiano H41 ship's laboratory for macro- and micromorphological analyses. The identification of the macroalgal specimens was based on the publications of Wiencke and Clayton [58] and Quartino et al. [43]. Nomenclatural updates followed Guiry and Guiry [22]. Voucher specimens were deposited in the herbarium of the Instituto de Botânica (SP) in 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 placed 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 $\mu g\ ml^{-1}$ for selective isolation of marine fungi. The plates were incubated for up to 60 days at 10 °C (a temperature chosen to isolate psychrophilic fungi that are capable of growth and reproduction in cold temperatures), and pure cultures of individual fungal colonies were transferred to new MA. 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 into the Culture Collection of Microorganisms and Cells of the Universidade Federal of Minas Gerais, Brazil (UFMGCB).

Fungal Identification

The protocol for DNA extraction from filamentous fungi followed Rosa et al. [45]. The internal transcribed spacer (ITS) region was amplified with the universal primers ITS1

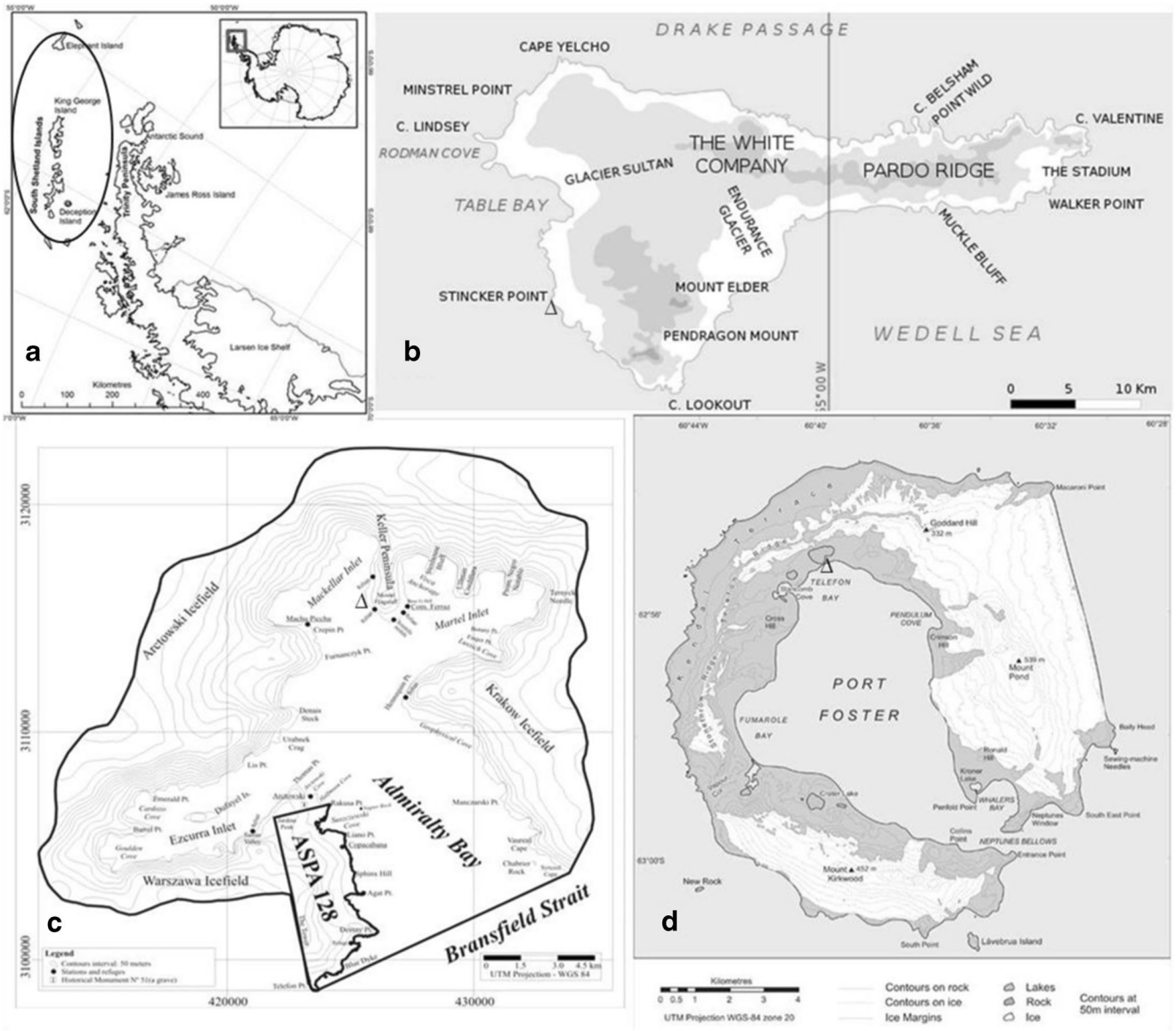

Fig. 1 Maps showing the positions of the islands sampled on the **a** Antarctic Peninsula across a transect of 350 km. Sampling sites Δ; **b**= Stinker Point (61°07.935′ S; 055°25.997′ W) at Elephant Island; **c** Keller Peninsula (62°05.163′ S; 058°24.784′ W) at Admiralty Bay at King George Island; **d** Telefon Bay (62°55.192′ S; 060°39.797′ W) at Deception Island

Table 1 Locations, number of macroalgae specimens collected and physical–chemical water measures where the *Pyropia endiviifolia* and *Monostroma hariotti* were obtained

Location/macroalgae species	Number of specimens	*Salinity* (ppt)	Conductivity (mS cm^{-1})	Temperature (°C)	pH
Elephant Island					
Pyropia endiviifolia	60	33.0	50.6	2.1	7.74
Monostroma hariotii	60	35.2	55.4	2.1	8.24
King George Island					
Pyropia endiviifolia	60	32.8	27.23	0.5	7.74
Monostroma hariotii	60	32.8	27.23	0.5	7.74
Deception Island					
Pyropia endiviifolia	90	32.1	49.89	3.7	7.49
Monostroma hariotii	60	32.1	49.89	3.7	7.49

and ITS4 [57]. Amplification of the ITS region was performed as described by Rosa et al. [45]. Amplification of the β-tubulin gene was performed with the Bt2a and Bt2b primers [18] according to protocols established by Godinho et al. [19].Yeasts were characterised via standard methods and the taxonomic keys of Kurtzman and Fell [32]. Yeast identities were confirmed by sequencing the D1–D2 variable domains of the large subunit ribosomal RNA gene using the primers NL1 and NL4 [33] and the ITS region using the primers ITS1 and ITS4 [57].

The obtained sequences were analysed with SeqMan P 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). Representative consensus sequences of algicolous fungal taxa were deposited into GenBank (Table 2). 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 [1]. Since sequences of the ITS region may be insufficient for recognising some fungal taxa [17], β-tubulin sequences, which are considered promising for a one-gene phylogeny [17], were used to elucidate the taxonomic positions of the taxa that could not be identified conclusively using ITS sequences. The following criteria suggested by Godinho et al. [19] were used to interpret BLAST results from the GenBank database. Taxa that displayed query coverage and identities ≤97 % or an inconclusive taxonomic position were subjected to phylogenetic ITS and β-tubulin-based analysis, with estimations conducted using MEGA Version 5.0 [51]. The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1,000 replicate runs. To complete the molecular identification, the sequences of known-type fungal strains or reference sequences found in GenBank obtained from fungal species deposited in international culture collections were added to improve the accuracy of the phylogenetic analysis. The information about fungal classification generally follows Kirk et al. [30], MycoBank (http://www.mycobank.org) and Index Fungorum (http://www.indexfungorum.org).

Diversity, Richness, Dominance and Distribution

To quantify species diversity, richness and evenness, we used the following indices: (1) Fisher's α, (2) Margalef's and (3) Simpson's, respectively. The similarities among fungal taxa from different areas were estimated using the Bray–Curtis measures. All diversity and similarity indices, rarefaction curves and the principal components analysis calculations were performed using the computer programme PAST, version 1.90 [23].

Fungal Cultivation and Preparation of Extracts for Biological Assays

All isolates of filamentous fungi were cultivated using solid state fermentation to produce crude extract according to protocols established by Santiago et al. [48]. We used 24-well microtiter plates to grow all yeasts and obtain their respective crude extracts. Each well of the plates was filled with 1.2 ml of sterile Marine Agar. After solidification, 10 μl at 1×10^4 yeast cells ml^{-1} from each purified yeast colony was transferred to the corresponding well of a microtiter plate. The plates were incubated at 10±2 °C for 15 days. After incubation, 1 ml of ethanol was added to each well, and the content was macerated, incubated at 10 °C for 48 h and filtrated using sterilised cotton pieces. The ethanol phase was transferred to 1.5-ml tubes and dried by evaporation in a vacuum centrifuge at 35 °C. An aliquot of each dried fungal extract was dissolved in dimethyl sulphoxide (DMSO, Merck, USA) 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. [7]. All crude extracts (dissolved in DMSO) were diluted to a final concentration of 250 μg ml^{-1} for use in the antimicrobial assay. The bacteria and yeast were grown at 37 °C in Mueller–Hinton (Difco, USA) and Sabouraud (Himedia, India) media, respectively. After 24 h under these conditions, microbial inocula were prepared by diluting the cell suspensions appropriately in Mueller–Hinton and RPMI1640 (supplemented with 2 % glucose) media for bacteria and yeast, respectively. Fifty microlitres of microbial inocula was added to each well in a 96-well plate and adjusted to $1–2\times10^8$ bacterial cells ml^{-1} and 1×10^6 yeast cells ml^{-1}. Twenty-five microlitres of extract and control solutions, as well as 25 μl of each medium, was added to attain the desired concentrations, and the plates were incubated at 37 °C for 24 h. As an indicator of microorganism growth, 10 μl of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT/Sigma-USA) (dissolved in sterile water at 5 mg ml^{-1}) were added to each well and incubated at 37 °C for 4 hormazan, a product of the reduction of MTT, was read at $\lambda_{570\ nm}$ in a microplate reader to measure the microbial growth. The results are expressed as percent inhibition in relation to the controls without drugs. Chloramphenicol (32 μg ml^{-1}) and amphotericin B (2 μg ml^{-1}) (both from Sigma/USA) were used as positive drug controls for the bacterial and yeast assays, respectively.

Table 2 Molecular identification of fungi associated with endemic Antarctic macroalgae *Monostroma hariotii* and *Pyropia endiviifolia*

Island/ macroalgae host	UFMGCB[a]	Number of isolates	Top BLAST search results [GenBank accession number]	Query cover (%)	Identity (%)	N° of bp analysed	Proposed taxa [GenBank accession number]
Elephant Island							
Pyropia endiviifolia	5966	20	*Cadophora malorum* (Kidd & Beaumont) W. Gams [JN585942]	100	100	593	*C. malorum* [KC811017[e]]
	5949[b,c]	20	*Penicillium chrysogenum* Thom [JQ781839]	100	99	587	*Penicillium* sp. 1 [KC811006[e]; KF578399[g]]
	5941[b]	8	*Pseudogymnoascus* sp. [HM589344]	100	99	487	*Pseudogymnoascus* sp. 1 [KC810978[e]]
	5936[b]	7	*Pseudogymnoascus* sp. [JX270556]	100	100	566	*Pseudogymnoascus* sp. 2 [KC810990[e]]
	6286[b,c]	3	*Penicillium steckii* K.M. Zalessky [HM469415]	100	99	414	*Penicillium* sp. 2 [KC81047[e]; KF578404[g]]
	5938[b]	2	*Penicillium* sp. [JX232275]	100	99	555	*Penicillium* sp. 3 [KC811044[e]]
	5977	2	*Thelebolus globosus* Brumm. & de Hoog [JX171196]	99	100	539	*T. globosus* [KF373539[e]]
	6305[b,c]	2	*Penicillium pusillum* G. Smith [EF626951]	99	98	432	*Penicillium* sp. 4 [KC811052[e]; KF578401[g]]
	5976[b]	1	*Aspergillus sydowii* (Bainier & Sartory) Thom & Church [HF546383]	100	99	541	*Aspergillus* sp. 1 [KC811058[e]]
	5973	1	*Antarctomyces psychrotrophicus* Stchigel & Guarro [JQ692162]	100	100	536	*A. psychrotrophicus* [KC811040[e]]
	5946[b]	1	*Cladosporium sphaerospermum* Penz. [JX171167]	100	100	643	*Cladosporium lignicola* Link [KC811048[e]]
	5940[b,c]	1	*Aspergillus austroafricanus* Jurjevic, S. W. Peterson & B. W. Horn [JQ301891]	100	100	570	*Aspergillus protuberus* Munt.-Cvetk. [KC811041[e]; BKF578413]
	6025[b]	1	*Mortierella* sp. [JQ670950]	100	99	634	*Mortierella antarctica* Linnem. [KC811043[e]]
	5937	1	*Oidiodendron truncatum* G.L. Barron [FJ914713]	100	100	557	*O. truncatum* [KC811038[e]]
	6297[b,c]	1	*Penicillium dipodomyicola* (Frisvad, Filtenborg & Wicklow) Frisvad [JX232278]	100	99	486	*Penicillium* sp. 5 [KF373541[e]; KF578403[g]]
Monostroma hariotii	6006[b,c]	16	*Penicillium steckii* [HM469415]	100	100	1456	*P. steckii* [KF419301[e]; KF623529[g]]
	5998[b,c]	6	*Penicillium chrysogenum* [JN561259]	100	99	587	*Penicillium* sp. 1 [KC811013[e]; KF578410[g]]
	6002[b,c]	2	*Aspergillus versicolor* (Vuill.) Tirab. [JQ781772]	100	98	530	*Aspergillus* sp. [KF373537[e]; KF578409[g]]
	6008[b]	1	*Cladosporium* sp. [DQ092512]	100	100	510	*Cladosporium* sp. [KC811049[e]]
	6020[b,c]	1	*Penicillium citrinum* Thom [EU645682]	100	99	519	*P. citrinum* [KC811054[e]; KF578408[g]]
	6024[b,c]	2	*Penicillium commune* Thom [HQ710533]	100	99	532	*P. crustosum* Thom [KC811051[e]; KF578400[g]]
	6023[b,c]	1	*Penicillium islandicum* Sopp [FJ872071]	98	97	564	*P. crustosum* [KF373538[e]; KF578407[g]]
King George Island							
P. endiviifolia	GP46L1	5	*Metschnikowia australis* (Fell & I.L. Hunter) Mendonça-Hagler, Hagler, Phaff & Tredick [U76526]	100	100	514	*M. australis* [KF419301[f]]
	5954[b]	4	*Cladosporium* sp. [HM999949]	99	100	485	*Cladosporium* sp. [KC810992[e]]
	5963[b]	3	*Pseudogymnoascus* sp. [JX270614]	99	100	434	*Pseudogymnoascus* sp. 1 [KC810996[e]]
	6033[b,c]	2	*Penicillium chrysogenum* [JQ781839]	100	100	587	*Penicillium* sp. [KC811004[e]; KF578405[g]]
	6031	1	*Dipodascus australiensis* Arx & J.S.F. Barker [HQ115737]	98	99	395	*D. australiensis* [KC811059[e]]
M. hariotii	MH47.1	35	*Metschnikowia australis* [U76526]	100	100	514	*M. australis* [KF373553[f]]
	MH33.1	22	*Guehomyces pullulans* (Lindner) Fell & Scorzetti [AF105394]	99	99	625	*G. pullulans* [KF373551[f]]

Table 2 (continued)

Island/ macroalgae host	UFMGCB[a]	Number of isolates	Top BLAST search results [GenBank accession number]	Query cover (%)	Identity (%)	N° of bp analysed	Proposed taxa [GenBank accession number]
	MH1.2	18	*Cryptococcus albidosimilis* Vishniac & Kurtzman [AF137601]	99	100	591	*Cr. albidosimilis* [KC811060[f]]
	MH38.2	7	*Rhodotorula laryngis* Reiersöl [AF189937]	100	99	615	*Rh. laryngis* [KF373552[f]]
	MH11.2	5	*Cryptococcus victoriae* M.J. Montes, Belloch, Galiana, M.D. García, C. Andrés, S. Ferrer, Torr.-Rodr. & J. Guinea [AF363647]	100	99	609	*Cr. victoriae* [KF373547[f]]
	5414[b]	5	*Pseudogymnoascus* sp. [JX845282]	100	99	428	*Pseudogymnoascus* sp. 2 [KC811056[e]]
	MH1.3	3	*Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison [AF070432]	99	99	588	*R. mucilaginosa* [KF373546[f]]
	5408[b]	3	*Phoma herbarum* Westend. [AY293800]	98	99	478	*Phoma* sp. [KF373545[e]]
	MH50.2	2	*Cystofilobasidium infirmominiatum* (Fell, I.L. Hunter & Tallman) Hamam., Sugiy. & Komag. [AF075505]	99	99	600	*C. infirmominiatum* [KF373554[f]]
	MH23.1	2	*Meyerozyma guilliermondii* (Wickerham) Kurtzman & M. Suzuki [U45709]	99	99	561	*M. guilliermondii* [KF373548[f]]
	MH3.3	1	*Cryptococcus adeliensis* Scorzetti, I. Petrescu, Yarrow & Fell [AF137603]	99	99	641	*Cr. adeliensis* [KF373550[f]]
	MH24.3	1	*Rhodotorula minuta* (Saito) F.C. Harrison [AF189945]	100	99	632	*Rh. minuta* [KF373549[e]]
Deception Island							
P.endiviifolia	5958[b,c]	4	*Penicillium dipodomyicola* [JX171186]	100	99	555	*Penicillium* sp. 1 [KF373535[e]; KF578412[g]]
	P172L1	3	*Meyerozyma guilliermondii* [U45709]	99	99	561	*M. guilliermondii* [KF373555[f]]
	5955[b]	2	*Pseudogymnoascus* sp. [HM589344]	100	99	536	*Pseudogymnoascus* sp. 1 [KC810974[e]]
	6304[b]	2	*Pseudogymnoascus* sp. [JX270343]	98	99	375	*Pseudogymnoascus* sp. 2 [KC810976[e]]
	6291[b]	2	*Verticillium leptobactrum* W. Gams [JQ782652]	100	98	484	*Verticillum* sp. [KC810999[e]]
	6030[b,c]	1	*Aspergillus* sp. [JN021552]	100	99	550	*Aspergillus* sp. 1 [KF373540[e]; KF578406[g]]
	6298[b]	1	*Penicillium chrysogenum* [JQ781839]	100	99	587	*Penicillium* sp. 2 [KC811001[e]]
	6300[b,c]	1	*Aspergillus versicolor* [FJ878625]	100	99	495	*Aspergillus* sp. 2 [KF373542[e]; KF578402[g]]
	6310[b]	1	*Aspergillus penicillioides* Speg. [FR727125]	100	100	499	*Aspergillus* sp. 3 [KF373543[e]]
	6311[b]	1	*Verticillium* sp. [JX270518]	99	95	466	*Lecanicillium* sp. [KF373544[e]]
M. hariotii	5994[b,c]	1	*Aspergillus niger* Tiegh. [FJ011542]	99	99	510	*Aspergillus tabacinus* Nakaz., Y. Takeda, Simo & A. Watan. [KF373536[e], KF578411[g]]
	5995[b]	1	*Pseudogymnoascus* sp. [HM589337]	99	99	517	*Pseudogymnoascus* sp. 1 [KC811057[e]]

Identification conducted using BLASTn searches of the ITS, β-tubulin and D1–D2 domains

[a] *UFMGCB* Culture of Microorganisms and Cells from the Federal Universidade of Minas Gerais

[b] Taxa subjected to phylogenetic analysis based on the [b] ITS1–5.8S-ITS2 region for elucidation of taxonomic positions

[c] Taxa subjected to phylogenetic analysis based on the [c] β-tubulin region for elucidation of taxonomic positions

[d] Taxonomic position suggested by the phylogenetic analyses

[e] D1/D2 variable domains sequence deposited

[f] ITS sequence deposited

[g] β-tubulin gene sequence deposited

The assessment of antifungal activity against filamentous fungi was performed with the target *C. sphaerospermum* CCT 1740. *Cladosporium sphaerospermum* is a cosmopolitan dematiaceous fungus that causes serious problems in patients with respiratory tract disease as well as subcutaneous phaeohyphomycosis [25]. Conidia were harvested from 7- to 10-day-old cultures by flooding plates with 5 ml of 0.85 % sterile saline, and the resulting suspensions were filtered using a qualitative paper filter to remove the mycelial fragments. The conidia concentrations were determined photometrically from a standard curve of absorbance at 620 nm, and the suspensions were adjusted with 0.85 % sterile saline to a concentration of 1.0×10^6 conidia ml^{-1} [11, 56]. A reference method [39] for the broth dilution antifungal susceptibility testing of yeast was adapted for the evaluation of antifungal activity against sporulating filamentous fungi [56]. Fifty microlitres of the conidial inocula were added to each well of a 96-well plate containing 25 μl of the extract (at 250 μg ml^{-1}) or the control solution. The plates were incubated at 25 °C for 48 h. Fungal growth was evaluated by measuring the absorbance of each well at 620 nm at 48 h, and the mean absorbance values and standard errors were used to evaluate fungal growth inhibition. The fungicide benomyl (1.16 μg ml^{-1}) (Sigma/USA) was used as a positive standard in all 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 Romanha et al. [44]. Each extract was tested in triplicate. Benznidazole at its IC_{50} (1 μl ml^{-1}) was used as positive control. The results were expressed as the percentage of growth inhibition. All assays were performed in triplicate.

Yellow Fever Virus Antiviral Assay

The antiviral assay was performed using a BHK-21 cell line expressing the yellow fever virus (YFV) bicistronic replicon (BHK-21-repYFV17D-LucNeoIres), which contains the firefly luciferase reporter gene and neomycin phosphotransferase gene. The BHK-21-repYFV17D-LucNeoIres cell line was maintained in minimum essential medium (MEM), supplemented with 10 % fetal bovine serum, 1 % (*v*/*v*) penicillin/streptomycin, and 500 μg ml^{-1} of Geneticin (Invitrogen, Carlsbad, CA, USA). YFV replicon cells were seeded in 96-well plates at a concentration of 2×10^4 cells per well in 100 μl of complete culture medium. After an interval of 24 h for cell adherence and subsequent observation of cell viability in each well, the culture medium was removed and then replaced with complete MEM containing 20 μg ml^{-1} of each fungal extract in a total volume of 100 μl. Pegylated interferon-alfa 2b (Heber Biotec S.A., Havana, Cuba) was used as positive control for inhibition of replicon replication at a concentration of 1,000 IU ml^{-1}. As negative controls, cells were treated with medium. Cell morphology and viability were evaluated by optical microscopy during the incubation period, in order to detect some possible cytotoxicity and avoid any misinterpretation of a possible inhibition of the replication of the reporter replicon due to cell death. After an incubation of 48 h, the cells were washed with phosphate-buffered saline and lysed in 20 μl of 1× lysis buffer (Promega, USA) for 5 min. The luciferase activity was determined from 20 μl of cell lysate using a commercial luciferase assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Luminescent signal was detected using Mithras LB 940 (Berthold Biotechnologies, Bad Wildbad, Germany). The assay was performed in duplicate, and the resulting values expressed in raw lights units. The values obtained from treatment with extracts were compared with that of the negative control by calculating the percentage inhibition value for each substrate relative to untreated cells (negative control).

Results

A total of 239 fungal isolates were recovered from 390 thalli (total of 1,950 tissue fragments) from the endemic Antarctic macroalgae, *M. hariotii* and *P. endiviifolia*. The numbers of fungal taxa and isolates differed between the two macroalgal species; 30 taxa and 104 isolates were recovered from *P. endiviifolia* and 20 taxa and 135 isolates from *M. hariotii*. The fungal isolates were identified by molecular sequencing of the ITS, β-tubulin and D1-D2 domains to represent 48 taxa and 18 different genera within the phyla Ascomycota (201 isolates), Basidiomycota (37 isolates) and Zygomycota (1 isolate) (Table 2).

Thirty-four fungal taxa presented low molecular similarities or inconclusive information in comparison with known fungal ITS sequences deposited in the GenBank database. The most frequent taxa associated with *M. hariotii* were *Metschnikowia australis*, *Guehomyces pullulans*, *Cryptococcus albidosimilis* and *Penicillium steckii*, with 35, 22, 18 and 16 isolates, respectively. *Cadophora malorum* (20), *Penicillium* sp. and *Pseudogymnoascus* species represented the most frequent fungi associated with *P. endiviifolia*. In general based on the isolate number, the most frequent species represented 56.5 % of the total fungal community; in contrast, 7.9 % of taxa were obtained as singletons and are rare components within the community. Two species of *Pseudogymnoascus* (*Pseudogymnoascus* sp. 1 and sp. 2) were found in the fungal community. *Pseudogymnoascus* sp. 1 and sp. 2 were recovered from *P. endiviifolia* and *Pseudogymnoascus* sp. 1 was recovered from *M. hariotii*.

Table 3 Diversity indices of fungal communities associated with the endemic Antarctic macroalgae *Pyropia endiviifolia* and *Monostroma hariotii* across the three Islands in Antarctica

Diversity index	Macroalgal species/Antarctic islands					
	Pyropia endiviifolia			*Monostroma hariotii*		
	Elephant	King George	Deception	Elephant	King George	Deception
Number of taxa	15	5	10	7	12	2
Number of isolates	71	15	18	29	104	2
Fisher-α	6.43	2.63	9.26	3.74	3.51	0
Margalef's	3.52	1.48	3.11	2.10	2.37	1.44
Simpson's	0.82	0.76	0.87	0.69	0.8	0.5

The diversity indices differed among the fungal communities of each macroalga across the three Antarctic Islands (Table 3). The fungal communities of *M. hariotii* collected in the Elephant and King George Islands showed higher diversity and richness indices in comparison with that of Deception Island. The fungal communities associated with *P. endiviifolia* collected in the Elephant and Deception Islands showed higher diversity and richness indices than King George Island.

The similarities among the fungal assemblages of the two macroalgae across the three Antarctic Islands were low. In addition, the fungal composition was variable among the macroalgal species collected in the three Islands. Figure 2a shows the Bray–Curtis values among the fungal assemblages of *M. hariotii* collected in the Elephant, King George and Deception Islands. According to Bray–Curtis values, the most similar fungal communities associated with *M. hariotii* were found in King George and Elephant Island. Regarding *P. endiviifolia*, the Bray–Curtis index showed that the fungal assemblages of King George and Deception Islands were more similar (Fig. 2b). Additionally, the similarities of the fungal assemblages were compared between the two endemic macroalgal species (Fig. 3). The Bray–Curtis showed that the fungal assemblage of *M. hariotii* from Deception Island and *P. endiviifolia* from Elephant Island were similar and the assemblages of *P. endiviifolia* from King George and *P. endiviifolia* from Deception Islands were similar as well.

The principal component analysis revealed that salinity and pH exhibited a positive correlation with the fungal assemblages of *M. hariotii* from Elephant and King George Island as well as *P. endiviifolia* from King George Island (Fig. 4). Conductivity and temperature showed a positive correlation

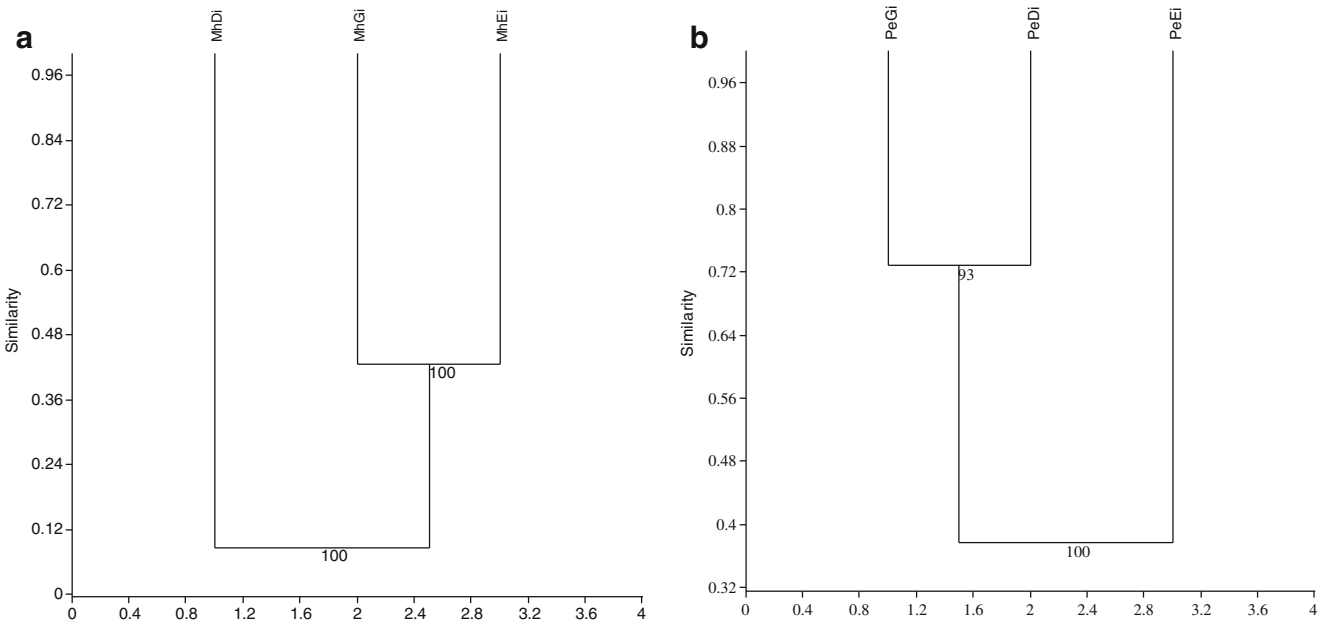

Fig. 2 Dendrograms showing the Bray–Curtis similarity measures for the fungal communities associated with the separated endemic Antarctic macroalgae *Monostroma hariotii* (**a**) and *Pyropia endiviifolia* (**b**). The results were obtained with 95 % confidence and bootstrap values calculated from 1,000 iterations. The sampled macroalgae were as follows: MhEi, *Monostroma hariotii* from Elephant Island; MhGi, *Monostroma hariotii* from King George Island; MhDi, *Monostroma hariotii* from Deception Island; PeEi, *Pyropia endiviifolia* from Elephant Island; PeGi, *Pyropia endiviifolia* from King George Island; PeDi, *Pyropia endiviifolia* from Deception Island

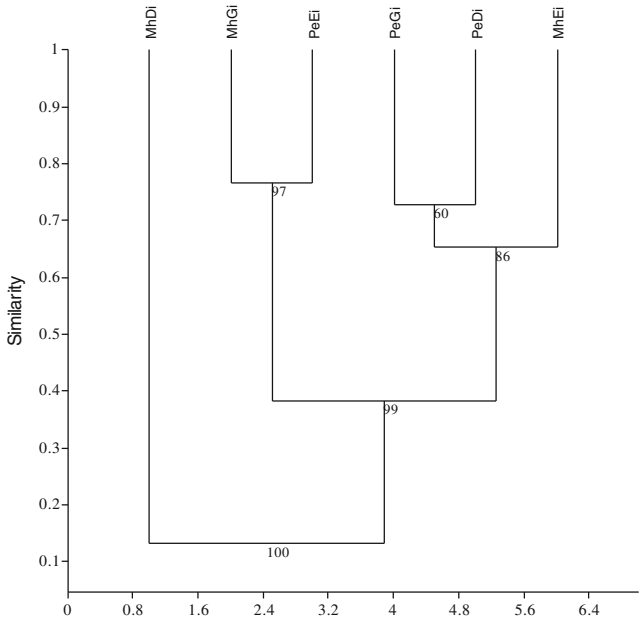

Fig. 3 Dendrogram showing the comparison of Bray–Curtis similarity measures for the fungal communities associated with the both endemic Antarctic macroalgae. The results were obtained with 95 % confidence and bootstrap values calculated from 1,000 iterations. The sampled macroalgae were as follows: PeEi, *Pyropia endiviifolia* from Elephant Island; MhEi, *Monostroma hariotii* from Elephant Island; PeGi, *Pyropia endiviifolia* from King George Island; MhGi, *Monostroma hariotii* from King George Island; PeDi, *Pyropia endiviifolia* from Deception Island; MhDi, *Monostroma hariotii* from Deception Island

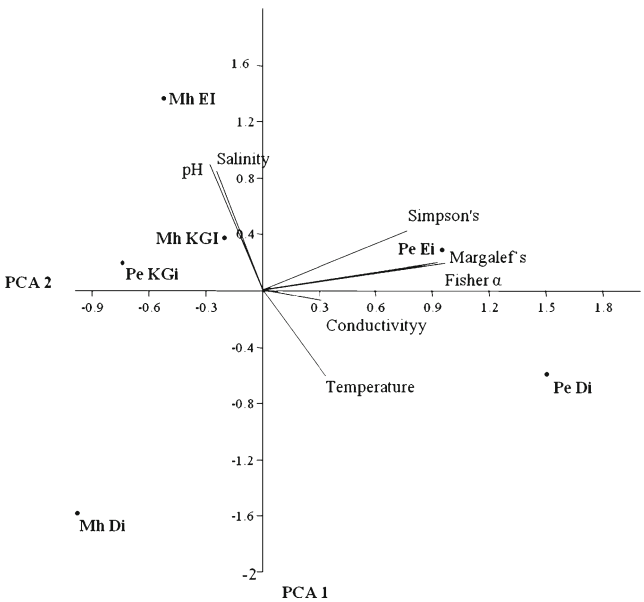

Fig. 4 Principal component analysis (PCA) plot calculated among the physic-chemical water parameters (temperature, salinity, pH and conductivity) obtained where the *Pyropia endiviifolia* (Pe) and *Monostroma hariotii* (Mh) were collected, as well as Fisher's (diversity), Margalef's (richness) and Simpson's (dominance) indices of the fungal assemblages associated with Antarctic macroalgae. *EI* Elephant Island, *KGI* King George Island, *DI* Deception Island

with Fisher's α, Margalef's and Simpson's indices for the fungal communities of *P. endiviifolia* from Elephant and Deception Islands.

A total of six algicolous fungal taxa were able to produce compounds with biological activities, which displayed values between 61 and 96 % of inhibition against the targets screened (Table 4). The extracts of *Pseudogymnoascus* species, *G. pullulans* and *M. australis* showed selective antifungal activities against *Candida albicans*, *C. krusei* and *Cladosporium sphaerosperum*. The extract of *Dipodascus australiensis* inhibited selectively the growth of *C. albicans*; *G. pullulans*, *M. australis* and *Pseudogymanoascus* sp. 1 were selective against *C. krusei*. Furthermore, *Pseudogymnnoascus* sp. 2 displayed 95 % antifungal activity against the target *C. sphaerospermum*, which was approximately the same value of the control drug benomyl (94.5 % of inhibition). Additionally, the extract of *Penicillium steckii* inhibited 96 % of yellow fever virus, which was better than the control interferon alpha (IFN-α) (68 %). No extract displayed antibacterial or trypanocidal activities.

Discussion

Macroalgae have been cited as important repositories of fungal diversity in oceans; however, only few taxonomic, ecological and biotechnological studies were published until recent years. Complementing the works of Loque et al. [35] and Godinho et al. [19], the present study represents a continuation of the first comprehensive analysis addressing the fungal communities associated with two endemic macroalgae along the Antarctic Peninsula. The fungal taxa associated with *M. hariotii* and *P. endiviifolia* were comprised of a few dominant fungal species and a high number of singletons, showing a presence of rich fungal communities under extreme conditions.

The methods of distinguishing and defining endemic, indigenous or cosmopolitan fungal species in Antarctica remain under development. According to Ruisi et al. [47], (1) endemic species are characterised as true psychrophilic fungi that are able to actively grow and reproduce only in Antarctica; (2) indigenous species are taxa well adapted and able to grow and reproduce even at low temperatures (psychrotolerant species) or fast sporulating forms that are able to finish their lifecycles in a very short time; (3) ecotypes of cosmopolitan species able to grow actively, at least under Antarctic summer conditions, showing mesophilic-psychrotolerant behaviour as an adaptation to the cold Antarctic climate; and (4) cosmopolitan species are fungal propagules that are transported to Antarctica but are unable to grow under Antarctic conditions. Based on these criteria, the fungal species *M. australis* and *A. psychrotrophicus* are considered endemic to Antarctica;

Table 4 Biological activities of the extracts of fungal species associated with Antarctic macroalgae

		Inhibition of the targets (%)							
		Bacteria			Fungi			Virus	Protozoa
Fungal species	UFMGCB	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Candida albicans*	*Candida krusei*	*Cladosporium sphaerosperum*	Yellow fever	*Trypanosoma cruzi*
Pseudogymnoascus sp. 1	5941	0±1	0±3	0±3	29±13	61±1	0±5	45±12	2.8±2
Pseudogymnoascus sp. 2	5936	0±2	0±2	0±5	58±20	29±2	95±11	0.4±7	0±5
Dipodascus australiensis	6031	0±5	0±5	0±4	71±19	41.5±5	0±3	44±3	10.4±2
Guehomyes pullulans	MH33.1	0±4	0±2	0±4	41.5±6	64±0	29±4	8±9	3±3
Metschnikowia australis	MH47.1.2	0±2	0±3	0±1	34.5±1	61±4	0±1	3±4	8±0.8
Penicillium steckii	6012	0±5	0±8	0±1	35±3	30±1	0±10	96±12	0±5
Control drugs	Chlo	100±5	98±12	83±6	–	–	–	–	–
	Amph B[3]	–	–	–	100±23	100±13	–	–	–
	IFN-α	–	–	–	–	–	–	68±12	–
	Benz	–	–	–	–		–	–	45±5.3
	Ben	–	–	–	–	–	94.5±10	–	–

UFMGCB Culture of Microorganisms and Cells of the Universidade Federal de Minas Gerais, *Chlo* chloramphenicol, *Amph B* amphotericin B, *IFN-α* interferon alpha, *Benz* benznidazole, *Ben* benomyl

C. malorum, *Cryptococcus victoriae*, *Cryptococcus adeliensis*, *Pseudogymnoascus* species and *Mortierella antarctica* represent indigenous taxa; and *C. albidosimilis*, *G. pullulans*, *Meyerozyma guilliermondii*, *Penicillium* species, *Phoma herbarum*, *Rhodotorula laryngis*, *Rhodotorula mucilaginosa* and *Rhodotorula minuta* represent cold-adapted cosmopolitan fungi with adaptations to survive in Antarctica. However, the biology and/or distributions of some species such as *D. australiensis*, *Oidiodendron truncatum* and several taxa identified only to generic rank remain more or less unknown.

Antarctomyces psychrotrophicus is not known outside of Antarctica and is an endemic, psychrophilic species of Thelebolales that has been isolated from soil of maritime and continental Antarctica [49], as an endophyte of *Deschampsia antarctica* [45], in association with the macroalga *Adenocystis utricularis* [35] and from lakes [20]. Hitherto now, *M. australis* was isolated only from Antarctic substrates including seawater [13], the stomach of the Antarctic krill species *Euphausia superba* [10], the algal thalli of *A. utricularis* at a high density [35], *Acrosiphonia arcta* and *Desmarestia menziesii* [19]. The discovery of *M. australis* in association with *M. hariotii* and *P. endiviifolia* from different areas of the Antarctic Peninsula reinforces the notion that this yeast may exhibit a specific ecological association with Antarctic macroalgae in the marine environment.

In Antarctica, *Cadophora* species were previously reported from a range of different substrates, namely mosses, a mummified seal carcass, skua feathers, soil, wood debris, *Colobanthus quitensis* as an endophyte and lakes [2, 20, 46, 52]. *Cadophora* (Helotiales) has a phialophora-like morphology, but this genus is phylogenetically distinct from *Phialophora* [24]. According to Blanchette et al. [5], *Cadophora* species appear to have a circumpolar distribution in the Antarctic as well as the Arctic suggesting an adaptation to the extreme polar environment. *Cadophora malorum* was found in other substrate in Antarctica; however, this study represents the first record of *C. malorum* in association with Antarctic macroalgae in the marine environment.

Pseudogymnoascus species (aff. Thelebolales, Leotiomycetes, Ascomycota) were abundant and associated with both species of endemic Antarctic macroalgae. Although *Pseudogymnoascus* and *Geomyces* have historically been considered to represent the sexual and asexual stages of the same genus under a two name per fungus system of classification, respectively, Minnis and Lindner [37] treated the two as closely related but distinct genera under a one name per fungus system of classification based on phylogenetic placement of the type species. *Pseudogymnoascus* species, typically and historically identified under the name *Geomyces*, have been recorded frequently in Antarctica with a ubiquitous distribution and from a number of substrates including in the soils of cold regions [37], mosses [52], leaves of *C. quitensis* [46], thalli of macroalgae [16, 28] and freshwater lakes [20]. According to Lorch et al. [36] and Minnis and Lindner [38], the diversity of *Geomyces* and allies including *Pseudogymnoascus* as revealed by DNA sequence data is

greater than previously recognised based on traditional taxonomic methods. We found 36 isolates identified as *Pseudogymnoascus* sp. in association with Antarctic macroalgae. The results suggest that these *Pseudogymnoascus* isolates represent two species, which are found in the northern hemisphere. Although a critical taxonomic reevaluation of the diversity of *Geomyces* and allies is not available, existing information suggests that all three species recovered in this study may represent undescribed species, and these will be subject to further studies to elucidate their taxonomic positions in the context of a biogeographic study comparing northern and southern hemisphere isolates.

Some *Cryptococcus* species have been found frequently in various Antarctic substrates and many of them are psychrophilic [54]. *Cryptococcus albidosimilis* has been identified in Antarctic arid soil [55], but its presence in association with Antarctic macroalgae is reported first here. *Guehomyces pullulans* occurs in diverse habitats and can be found in soil and plants [12]. *Guehomyces pullulans* has also been found in soil of Antarctica [54]. The present study represents the first reports of *Cr. albidosimilis* and *G. pullulans* in association with marine algae in Antarctica.

In Antarctica, species of *Penicillium* have been described from different substrates such as soil and wood [2], lakes [20] and macroalgae [19, 35]. *Penicillium chrysogenum*, an extremophile, was isolated as a dominant species from permafrost in Antarctica [60] and the thalli of the macroalgae, *Adenocystis arcta*, *M. hariotii*, *P. decipiens* and *Ulva intestinalis* [19]. *Penicillium steckii* has a worldwide distribution; however, even if this species (recorded as *Penicillium citrinum*) was found in the Victoria Land, Antarctica [41], to our knowledge, there are no other reports of *P. steckii* from Antarctica or other reports of *P. steckii* from Antarctica or reports of it a association with tropical, temperate or polar macroalgae.

Additionally, 26 taxa displayed low similarity with sequences of fungi deposited in GenBank database or high similarities with unidentified fungi. These taxa showed different levels of sequence similarity with taxa identified as members of genera *Aspergillus*, *Cladosporium*, *Penicillium*, *Phoma*, *Pseudogymnoascus* and *Verticillium*, which suggests that these fungi may represent new species. Further morpho-physiological, phylogenetic and taxonomic studies will be necessary to determine if these fungi are known taxa adapted to the cold conditions of Antarctica or new species.

Our results showed that the fungal communities associated with *M. hariotii* and *P. endiviifolia* display considerable richness when compared with the results of other studies on tropical [50] and temperate [59] macroalgal hosts. The Simpson's index showed that a few taxa have approximately the same occurrence in the community, which may be attributed to the extreme conditions of the Antarctic environment. One of these dominant taxa was the yeast *M. australis*, which has been isolated only in Antarctica and for this reason is considered an endemic species. In addition, a large number of single individuals (singletons) were recovered from the two endemic macroalgae. In Antarctica, the characterisation of fungal communities has shown that singletons are rare species that often represent more than half of the species within these communities [20]. The similarities analysis was low for the same and between the two macroalgal species across the three Antarctic Islands. Only the yeasts, *M. australis* and *M. guilliermondii*, occur in common in association with the *M. hariotii* and *P. endiviifolia*. However, we obtained only few isolates of these rare taxa and new studies using metagenomic techniques will be necessary to assist in understanding the relations among dominant and rare species within the fungal communities in associated with the endemic Antarctic macroalgae.

The symbiosis among fungi and marine macroalgae has been little studied, and the symbiotic relationships vary from parasitism to mutualism [28]. However, the specific ecological function of fungi associated with macroalgae is not well understood [28]. Some fungal genera found in association with endemic *M. hariotii* and *P. endiviifolia* have been recorded as parasites (*Aspergillus*, *Cryptococcus*, *Penicillium*, *Pseudogymnoascus*, *Rhodotorula* and *Verticillium*) and saprobes (*Cadophora*, *Cladosporium*, *Mortierella* and *Phoma*) [29] in marine ecosystems. However, the ecological role of the majority of genera such as *Antarctomyces*, *Cystofilobasidium*, *Dipodascus*, *Guehomyces*, *Metschnikowia*, *Meyerozyma* and *Oidiodendron* remains unknown to poorly known.

According to Santiago et al. [48], 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. *Penicillium steckii* isolated from *Monostroma hariotti* was able to produce antiviral compounds, which displayed better antiviral activity than the value of control drug IFN-α. Some *Penicillium* taxa associated with macroalgae have been described as producers of bioactive compounds. One *Penicillium* species isolated from the macroalga *U. intestinalis* was observed to produce cytotoxic metabolites including penochalasius A–C, penostatins A–I and penochalasius D–H [27]. The antibacterial and cytotoxic compounds Di(2-ethyl hexayl) phthalate and fungisterol were isolated from *P. brevicompactum*, which was isolated from the associated macroalga, *Pterocladia* sp. [3]. The extracts of *P. chrysogenum* recovered from the Antarctic endemic macroalga, *P. decipiens*, yielded extract with high and selective antifungal and/or trypanocidal activities [19]. Additionally, the extracts from *Pseudogymnoascus* sp. 1 UFMGCB 5941, *Pseudogymnoascus* sp. 2 UFMGCB 5936, *D. australiensis* UFMGC6031, *Guehomyes pullulans* MH33.1 and *M. australis* MH47.1.2 displayed selective antifungal activity. Li et al. [34] isolated the geomycins B and C

from *Geomyces* sp. obtained from soil in Antarctica, which displayed antifungal activity against *Aspergillus fumigatus* and antibacterial activity against *S. aureus*, *E. coli* and *Streptococcus peneumoniae*. A species identified under the catchall name, *Geomyces pannorum*, and isolated from leaf litter produces pannomycin, a *cis*-decalin secondary metabolite with potential antibacterial activity against *S. aureus* [42]. To our knowledge, there are no other reports that *D. australiensis*, *G. pullulans* and *M. australis* are able to produce bioactive metabolites with antimicrobial activities. All bioactive algicolous fungi found in association with *M. hariotii* and *P. endiviifolia* will be subjected to bioactivity-guided fractionation to isolate their bioactive compounds.

The Antarctic Peninsula is a pristine environment and represents one of the fastest changing regional climates on Earth, and its warming seems to have a profound influence on the marine environment [9]. Consequently, all biota living in the marine ecosystem of the Antarctic Peninsula are under the varying effects of climate change. We found fungal communities associated with endemic macroalgae with dominant and rare fungal species that are endemic, cold-adapted or cosmopolitan taxa, which displayed interesting dynamics (richness, dominance and similarities) between the hosts as well as along the Antarctic Peninsula. A decrease in endemic or cold-adapted fungal species associated with an increase of cosmopolitan taxa within the fungal communities associated with endemic macroalgae may reflect the influence of climate change in the maritime Antarctic Peninsula. For this reason, we hypothesise that observations on the balance and dynamics of richness, dominance and distribution among endemic, cold-adapted or cosmopolitan fungal taxa might be used to understand and model the influence of climate change on the maritime Antarctic microbiota. The extracts of fungi that displayed antimicrobial and antiviral activities may represent sources of promising prototype molecules to develop drugs, and this study highlights the need to discover and preserve this valuable fungal germplasm given that some cold-requiring species may not be able to persist in a warming environment.

Acknowledgements We acknowledge the financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Processes PROANTAR 557030/2009-9, Universal 471721/2013-1 and PROANTAR 407230/2013-0, Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG), Process Universal 0050-13 and the Financiadora de Estudos e Projetos (FINEP 2084/07), the Program for Technological Development of Tools for Health-PDTIS-FIOCRUZ. A.M. Minnis received financial support from the US Forest Service, Northern Research Station and the Inter-Agency Agreement F11RG00184 grant from the US Fish and Wildlife Service. The results of this study are part of the Master Science degree of LE Furbino within the Programa of Pós-Graduação in Biotecnologia of UFOP and the Pos-Doctoral of VM Godinho of CNPq under supervision of LH Rosa.

References

1. Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLASTand PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402
2. Arenz BE, Held BW, Jurgens JA, Farrell RL, Blanchette RA (2006) Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. Soil Bio Bioch 38:3057–3064
3. Atalla MM, Zeinab HK, Eman RH, Amani AY, Abeer AAEA (2011) Physiological studies on some biologically active secondary metabolites from marine-derived fungus *Penicillium brevicompactum*. www.Gate2Biotech.com, 1: 1–15
4. Bhadury P, Bik H, Lambshead JD, Austen MC, Smerdon GR, Rogers AD (2011) Molecular diversity of fungal phylotypes co-amplified alongside nematodes from coastal and deep-sea marine environments. PLoS ONE 6e26445:1–7
5. Blanchette RA, Held BW, Arenz BE, Jurgens JA, Baltes NJ et al (2010) An Antarctic hot spot for fungi at Shackleton's historic hut on Cape Royds. Microb Ecol 60:29–38
6. Bridge PD, Spooner BM (2012) Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem? Fungal Ecol 5:381–394
7. Carvalho CR, Gonçalves VN, Pereira CB, Johann S, Galliza IV et al (2012) The diversity, antimicrobial and anticancer activity of endophytic fungi associated with the medicinal plant *Stryphnodendron adstringens* (Mart.) Coville (*Fabaceae*) from the Brazilian savannah. Symbiosis 57:95–107
8. Charpy-Roubaud C, Sourina A (1990) The comparative estimation of phytoplanktonic, microphytobenthic and macrophytobenthic primary production in the oceans. Mar Microb Food Webs 4:31–57
9. Clarke A, Murphy EJ, Meredith MP, King JC, Peck LS et al (2007) Climate change and the marine ecosystem of the western Antarctic Peninsula. Phil Trans Royal Soc 362:149–166
10. Donachie SP, Zdanowski MK (1998) Potential digestive function of bacteria in krill, *Euphausia superba* stomach. Aquat Microb Ecol 14: 129–136
11. Espinel-Ingroff A, Kerkering TM (1991) Spectrophotometric method of inoculum preparation for the *in vitro* susceptibility testing of filamentous fungi. J Clin Microbiol 29:393–394
12. Fell JW, Guého-Kellermann E (2011) *Guehomyces* Fell & Scorzetti (2004). In: Kurtzman CP, Fell JW (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam
13. Fell JW, Hunter IL (1968) Isolation of heterothallic yeast strains of *Metschnikowia* Kamienski and their mating reactions with *Chlamydozyma* Wickerham spp. Anton Leeuw 34:365–376
14. Fell JW, Hunter IL, Tallman AS (1973) Marine basidiomycetous yeasts (*Rhodosporidium* spp. n.) with tetrapolar and multiple alletic bipolar mating systems. Can J Microbiol 19:643–657
15. Fell JW, Statzel AC (1971) *Sympodiomycetes* gen. n., a yeast-like organism from southern marine waters. Anton Leeuw 37:359–367
16. Frisvad JC, Samson RA (2004) Polyphasic taxonomy of *Penicillium* subgenus *Penicillium* a guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Stud Mycol 49:1–174
17. Gazis R, Rehner S, Chaverri P (2011) Species delimitation in fungal endophyte diversity studies and its implications in ecological and biogeographic inferences. Mol Ecol 20:3001–3013
18. Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330
19. Godinho VM, Furbino LE, Santiago IF, Pellizzari FM, Yokoya N et al (2013) Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. ISME 7:1434–1451

20. Gonçalves VN, Vaz ABM, Rosa CA, Rosa LH (2012) Diversity and distribution of fungal communities in lakes of Antarctica. FEMS Microbiol Ecol 82:459–471
21. Grasso S, Bruni V, Maio G (1997) Marine fungi in Terra Nova Bay (Ross Sea, Antarctica). Microbiologica 20:371–376
22. Guiry MD, Guiry GM (2012) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway.http://www.algaebase.org; searched on 11 October 2012
23. Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4:1–9
24. Harrington TC, McNew DL (2003) Phylogenetic analysis places the phialophora-like anamorph genus *Cadophora* in the *Helotiales*. Mycotaxon 87:141–151
25. Hoog GS de, Guarro J, Gené J, Figueras MA (2000) Atlas of clinical fungi, 2nd edn. Centraalbureau voor Schimmelcultures, Utrecht/ Universitat Rovira i Virgili, Reus
26. Hyde KD, Lee SY (1995) Ecology of mangrove fungi and their role in nutrient cycling. What gaps occur in our knowledge? Hydrobiologia 295:107–118
27. Iwamoto C, Yamada T, Ito Y, Minoura K, Numata A (2001) Cytotoxic cytochalasans from a *Penicillium* species separated from a marine alga. Tetrahedron 57:2997–3004
28. Jones EB (2011) Fifty years of marine mycology. Fungal Div 50:73–112
29. Jones EBG, Sakayarol J, Suetrong S, Smorithipol S, Pang KL (2009) Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. Fungal Div 35:1–187
30. Kirk PM, Cannon PF, Minter DW, Stalpers JA (2011) Dictionary of the Fungi, 10th edn. CAB International, Wallingford
31. Kohlmeyer J, Kohlmeyer E (1979) Marine mycology, the higher fungi. Academic Press.
32. Kurtzman CP, Fell JW (2011) The yeast, a taxonomic study, 5th edn. Amsterdam, Elsevier
33. Lachance MA, Bowles JM, Starmer WT, Barker JS (1999) *Kodamaea kakaduensis* and *Candida tolerans*, two new ascomycetous yeast species from Australian hibiscus flowers. Can J Microbiol 45:172–177
34. Li Y, Sun B, Liu S, Jiang L, Liu X, Zhang H, Che Y (2008) Bioactive asterric acid derivatives from the Antarctic ascomycete Fungus *Geomyces* sp. J Nat Prod 71:1643–1646
35. Loque CP, Medeiros AO, Pellizzari FM, Oliveira EC, Rosa CA, Rosa LH (2010) Fungal community associated with marine macroalgae from Antarctica. Polar Biol 33:641–648
36. Lorch JM, Lindner DL, Gargas A, Muller LK, Minnis AM, Blehert DS (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
37. Mercantini R, Marsella R, Cervellati MC (1989) Keratinophilic fungi isolated from Antarctic soil. Mycopathologia 106:47–52
38. Minnis AM, Lindner DL (2013) Phylogenetic evaluation of Geomyces and allies reveals no close relatives of Pseudogymnoascus destructans, comb. nov., in bat hibernacula of eastern North America. Fungal Biol 117:638–649
39. National Committee for Clinical Laboratory Standards (2003) Methods for dilution antimicrobial susceptibility testes for bacteria that grow aerobically. Approved standard. 5th ed. NCCLS document M11-A5. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA
40. Nedzarek A, Rakusa-Suszczewski S (2004) Decomposition of macroalgae and the release of nutrients in admiralty Bay, king George island, antarctica. Polar Biosci 17:16–35
41. Onofri S, Zucconi L, Tosi S (2007) Continental Antarctic fungi. IHW-Verlag, Eching bei München
42. Parish CA, Cruz M, Smith SK, Zink D, Baxter J et al (2009) Antisense-guided isolation and structure elucidation of pannomycin, a substituted *cis*-decalin from *Geomyces pannorum*. J Nat Prod 72: 59–62
43. Quartino ML, Zaixso HE, Boraso de Zaixso AL (2005) Biological and environmental characterization of marine macroalgal assemblages in Potter Cove, South Shetland Islands, Antarctica. Bot Mar 48:187–197
44. Romanha AJ, de Castro SL, Soeiro MNC, Lannes-Vieira J, Ribeiro I et al (2010) *In vitro* and *in vivo* experimental models for drug screening and development for Chagas disease. Mem Inst Oswaldo Cruz 105:233–238
45. Rosa LH, Vaz ABM, Caligiorne RB, Campolina S, Rosa CA (2009) Endophytic fungi associated with the Antarctic Grass *Deschampsia antarctica* Desv. (*Poaceae*). Polar Biol 32:161–167
46. Rosa LH, Vieira MLA, Santiago IF, Rosa CA (2010) Endophytic fungi community associated with the dicotyledonous plant *Colobanthus quitensis* (Kunth) Bartl. (*Caryophyllaceae*) in Antarctica. FEMS Microbiol Ecol 73:178–189
47. Ruisi S, Barreca D, Selbmann L, Zucconi L, Onofri S (2007) Fungi in Antarctica. Rev Environ Sci Biotechnol 6:127–141
48. Santiago IF, Alves TMA, Rabello A, Sales-Júnior PA, Romanha AJ et al (2012) Leishmanicidal and antitumoral activities of endophytic fungi associated with the Antartic angiosperms *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. Extremophiles 16:95–103
49. Stchigel AM, Cano J, MacCormack CW (2001) *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. Mycol Res 105:377–382
50. Suryanarayanan TS, Venkatachalam A, Thirunavukkarasu N, Ravishankar JP, Doble M, Geetha V (2010) Internal mycobiota of marine macroalgae from the Tamilnadu coast: distribution, diversity and biotechnological potential. Bot Mar 53:457–468
51. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739
52. Tosi S, Casado B, Gerdol R, Caretta G (2002) Fungi isolated from antarctic mosses. Polar Biol 25:262–268
53. Vaz ABM, Rosa LH, Vieira MLA, Garcia V, Teixeira LCRS et al (2011) The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in antarctica. Braz J Microbiol 42:937–947
54. Vishniac HS (2006) Yeast biodiversity in the Antarctic. In: Rosa CA, Péter G (eds) Biodiversity and ecophysiology of yeasts. Springer-Verlag, Heidelberg, pp 221–240
55. Vishniac HS, Kurtzman CP (1992) *Cryptococcus antarcticus* sp. nov, and *Cryptococcus albidosimilis* sp. nov, basidioblastomycetes from antarctic soils. Int J Syst Bacteriol 42:547–553
56. Wedge DE, Kuhajek JM (1998) A microbioassay for fungicide discovery. SAAS Bull Biochem Biotechnol 11:1–7
57. White TJ, Bruns TD, Lee SB (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis NA, Gelfand J, Sninsky J et al (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322
58. Wiencke C, Clayton MN (2002) Antarctic seaweeds. A.R.G. Gantner Verlag KG, Ruggell
59. Zuccaro A, Mitchell JI (2005) Fungal communities of seaweeds. In: Dighton J, White JFP, Oudemans P (eds) The fungal community: its organization and role in the ecosystem. CRC Press, /Taylor and Francis, Boca Raton, pp 533–579
60. Zucconi L, Selbmann L, Buzzini P, Turchetti B, Guglielmin M et al (2012) Searching for eukaryotic life preserved in Antarctic permafrost. Polar Biol 35:749–757