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Diversity and bioactivity of cultured aquatic fungi from the High Arctic region

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Abstract This study assessed the diversity and α-glycosidase inhibitory activity of cultured fungi isolated from four aquatic environments (stream, pond, glacial ice, and estuary) in the Ny-Ålesund region (Svalbard, Norway, High Arctic). A total of 134 fungal isolates were obtained from 13 water samples. Based on morphological characteristics and sequence analyses of the nuclear ribosomal DNA internal transcribed spacer region, these fungal isolates were identified as belonging to 47 species, with 26 belonging to the Ascomycota, 20 to the Basidiomycota, and one to the Zygomycota. The most frequently detected fungal species were *Vishniacozyma* sp. 2, *Cadophora* sp. 2, *Phenoliferia* sp. 1, *Dioszegia* sp. 2, and *Mortierella* sp.; these species occurred in 10, eight, seven, six, and five of the samples, respectively. Among the 134 fungal isolates, 17 isolates of 15 species displayed high α-glycosidase inhibitory activity in culture. The results suggest that diverse and distinct populations of cultured fungi are present in Arctic aquatic environments, and they include taxa that are potential sources of bioactive molecules that may be used as prototype drugs for medicinal proposals.

Keywords **aquatic habitats, bioactivity, diversity, environmental mycology, High Arctic**

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1 Introduction

Average Arctic temperatures have increased at almost twice the global average rate over the past 100 years (Solomon et al., 2007). Some resulting environmental changes are of great concern, such as retreating glaciers and the shrinkage and disappearance of lakes and ponds (Overpeck et al., 1997; Smith et al., 2005; Jepsen et al., 2013). Arctic aquatic environments (e.g., glaciers, lakes, and ponds) are unique habitats that may harbor many undiscovered and previously discovered species of microorganisms (e.g., fungi, algae, and bacteria), which may possess unique physiological characteristics. In particular, Arctic fungi have been described as a promising source of bioactive natural compounds (Frisvad, 2008; Chen et al., 2013). Hence, there is an urgent need to improve baseline information regarding the fungal diversity and bioactive substances in these habitats.

Currently, approximately 3000 fungal species have been reported from aquatic environments worldwide (Shearer et al., 2007), while only a few studies have been conducted on cultured aquatic fungal species in polar regions, including the Arctic (Kovayasi et al., 1967; Booth et al., 1971; Butinar et al., 2007; Gulis et al., 2012) and Antarctica (Ellis-Evans, 1985, 1996; Goncalves et al., 2012). Recently, the diversity and composition of aquatic fungal communities in the Arctic was revealed by high-throughput sequencing (Zhang et al., 2016), which can enhance the characterization of fungal diversity compared with culture methods. Thus, obtaining cultured fungal isolates may play

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a key role in the search for bioactive compounds. To the best of our knowledge, the bioactivity of cultured aquatic fungi in the Arctic has not been investigated previously.

Previous studies have proven that different bioactive fungal isolates found in polar regions represent a unique source of newly discovered prototype molecules that can be used in drug discovery studies (Santiago et al., 2012; Godinho et al., 2013). Additionally, fungi have been proven to be a source of α-glycosidase inhibitors (Kim et al., 2004; Kang et al., 2013; Kumar et al., 2014; Rivera-Chávez et al., 2013, 2015), which reduce the impact of carbohydrates on blood sugar and are regarded as effective anti-diabetic drugs used for the treatment of diabetes mellitus type 2 (adult-onset diabetes) (van de Laar et al., 2005), a very serious metabolic disorder that is characterized by high blood sugar. Therefore, the aims of this study were to clarify the diversity of cultured aquatic fungi in aquatic habitats near Ny-Ålesund and to assess their α-glycosidase inhibitory activity.

2 Materials and methods

2.1 Study sites and sample collection

The study area is located in the Ny-Ålesund region (78˚55′N, 11˚56′E), which is entirely within the High Arctic zone and has a high number of glaciers, lakes, ponds, and glacial streams. Sampling was performed in July 2013. Water samples were collected directly into Labplas TWIRL'EM sterile sampling bags (Labplas Inc., Sainte-Julie, QC, Canada). Then, the microbial samples were collected by filtering 1 L of the water samples. Microbial biomass was trapped onto 47-mm diameter, 0.2-μm pore size membrane filters (Pall Corporation, Port Washington, NY, USA). The membrane filters were placed in sterile centrifuge tubes at −20°C at the Chinese Yellow River Station and then flown to our laboratory in Beijing, China. The locations and environmental types of the 13 water samples are shown in Figure 1 and Table 1.

Figure 1 Detailed view of the four aquatic environments. **a**, stream; **b**, pond; **c**, glacial ice; **d**, estuary.

2.2 Isolation of aquatic fungi

The membrane filters were placed on solid medium in 9-cm diameter Petri dishes. The solid medium contained potato dextrose agar (PDA), tetracycline (50 $mg \cdot L^{-1}$), and streptomycin sulfate (50 $mg \cdot L^{-1}$). The Petri dishes were sealed by parafilm, incubated for 1 month at 12°C, and examined periodically. When fungal colonies developed, they were transferred to PDA slants. Subcultures were also incubated on PDA.

Table 1 Location and type of the sampled aquatic environments in the Ny-Ålesund region

Code	Coordinates	Altitude/m	Environment type
R1	78°55′18.25″N; 11°56′01.42″E	16	Stream
R2	78°54′46.23″N; 11°50′39.70″E	90	Stream
R3	78°57′47.21″N; 11°35′09.67″ E	25	Stream
R4	78°54′42.97″N; 11°58′30.76″E	37	Stream
R6	78°53′53.08″N; 12°09′36.08″E	43	Stream
R7	78°58′05.39″N; 11°34′15.17″E	9	Stream
L1	78°55′06.39″N; 11°56′36.11″E	18	Pond
L2	78°57′53.95″N; 11°35′05.65″E	18	Pond
L3	78°57′59.59″N; 12°04′06.91″E	45	Pond
S1	78°55′41.53″N; 11°54′23.05″E	0	Estuary
S2	78°54′14.35″N; 12°09′29.32″E	0	Estuary
X1	78°53′09.78″N; 12°10′31.12″E	171	Glacial ice
X2	78°54′00.48″N; 12°09′31.96″E	29	Glacial ice

2.3 DNA extraction, amplification, and sequencing

Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Cubero et al., 1999). The internal transcribed spacer (ITS) region between the small-subunit and large-subunit ribosomal DNA genes was polymerase chain reaction (PCR)-amplified with the primers ITS1F and ITS4 (White et al., 1990). The ITS region was amplified using the following PCR procedure: 95°C for 3 min, followed by 37 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 10 min. The PCR products were purified and sequenced with the same primers by Sangon Biotech Co., Ltd. (Beijing, China). The ITS sequence data in this study were deposited in GenBank under the accession nos. KR698797 to KR698930.

2.4 Identification and phylogeny

The isolated fungi were identified based on their phylogenetic position and sequence similarity. Sequence data were used to investigate phylogenetic relationships using Molecular Evolutionary Genetics Analysis (MEGA) software v. 6.0. Phylogenetic trees were constructed using the neighbor-joining algorithm with bootstrap values calculated from 1000 replicate runs. A maximum composite likelihood model was used to estimate evolutionary distance. For sequence similarity determination, nucleotide Basic Local Alignment Search Tool (BLASTn) searches were performed in the GenBank databases to find the most closely related fungal species. In addition, the identification of all the isolates was confirmed by their phenotypic characteristics, including colony aspect (texture and color) and growth rate.

2.5 Statistical analyses

To evaluate the similarity of the cultured fungal species from the four aquatic environments, a Venn diagram of shared and unique fungal species was made using Venny 2.0 (http://bioinfogp.cnb.csic.es/tools/venny/). The fungal diversity in the four aquatic environments was evaluated using the Shannon's diversity index (*H*′). Furthermore, a heatmap analysis was performed to visualize all the detected fungal species and to compare their abundances among the four aquatic habitats using R 3.1.1 statistical software.

2.6 Fungal cultivation and preparation of extracts for α-glycosidase activity assays

The fungal isolates were inoculated into 25 mL of liquid medium (comprising 0.5% soymeal, 1.0% corn steep liquor, 2.0% glucose, 1.0% dextrin, 0.5% of peptone, and 0.2% of $(NH_4)_2SO_4$) in 100-mL flasks. The flasks were incubated at 16°C for 15 d, and then the cultured materials and fermentation liquor from each flask were respectively transferred to 50-mL vials containing an equal volume of acetone. After a 24-h incubation at room temperature, the organic phase was decanted, and the solvent was removed using a vacuum centrifuge at 35°C. An aliquot of each dried extract was dissolved in dimethyl sulfoxide to prepare a 100 $mg \cdot mL^{-1}$ stock solution, which was stored at 4°C.

2.7 Assay for α-glycosidase inhibitory activity

The α-glycosidase inhibitory activity of the fungal isolates was determined according to the method described by Huang et al. (2004). α-glycosidase was extracted from the upper duodenum of female Kunming mice (22.0–25.0 g, 12 weeks old, specific-pathogen-free) (National Institutes for Food and Drug Control, Beijing, Certificate No. SCXK jing 2009-0017), and 4-nitrobenzene-α-D-glucopyranoside was used as the substrate to detect α-glycosidase activity. All animal procedures in this study were approved by the Animal Ethical Experimentation Committee of Beijing Union University according to the requirements of the National Act on the Use of Experimental Animals (China). The screening was based on measuring the absorbance of p-nitrophenol at 405 nm, which is generated by the reaction of 4-nitrobenzene-α-D-glucopyranoside and α-glycosidase. Acarbose, which is an oral α-glycosidase inhibitor, was used as a positive control. The formula used to calculate the inhibition rate is:

$$[(X-Z)/(Y-Z)]\times 100\%,$$

where *X* is the absorbance value in the presence of the sample; *Y* is the absorbance value in the presence of acarbose (control); and *Z* is the absorbance value in the absence of the sample and acarbose (blank). All assays were performed in triplicate.

3 Results

3.1 Isolation and identification of aquatic fungi

A total of 134 fungal isolates were obtained from the 13 water samples (Table S1). All the isolates were identified to the species level via a phylogenetic analysis (Figure 2) and by their sequence identity (Table 2). For example, fungal isolate I14F-01251 showed the highest sequence similarity (99%) with *Cadophora luteo-olivacea* (NR111149), and it clustered with *Cadophora luteo-olivacea* in one group with a high bootstrap value (100) in the phylogenetic tree (Figure 2); therefore, isolate I14F-01251 was identified as a *Cadophora* species.

Table 2 Molecular identification of 47 fungal species isolated from the aquatic environments in Ny-Ålesund: maximum identity was determined using BLASTn searches of the ITS region

No.	Representative isolate code	GenBank accession number	Close related species (GenBank accession number)	Number of bp (Maximum identity)	Identification
1	I14F-01218	KR698814	*Cadophora melinii* (JN689950)	462/484 (95%)	*Cadophora* sp. 1
2	I14F-01251	KR698847	*Cadophora luteo-olivacea* (NR111149)	540/543 (99%)	*Cadophora* sp. 2
3	I14F-01232	KR698828	*Cadophora viticola* (HQ661097)	503/506 (99%)	*Cadophora* sp. 3
4	I14F-01273	KR698869	*Cystobasidium laryngis* (KY103134)	530/534 (99%)	*Cystobasidium* sp. 1
5	I14F-01313	KR698909	*Cystobasidium psychroaquaticum* (KY103148)	533/535 (99%)	*Cystobasidium* sp. 2
6	I14F-01216	KR698812	*Dioszegia rishirensis* (KY103358)	425/432 (98%)	*Dioszegia* sp. 1
7	I14F-01299	KR698895	*Dioszegia fristingensis* (NR136970)	420/434 (97%)	*Dioszegia* sp. 2
8	I14F-01324	KR698920	*Dioszegia aurantiaca* (KY103346)	422/435 (97%)	*Dioszegia* sp. 3
9	I14F-01208	KR698804	*Glaciozyma martinii* (NR132821)	556/571 (99%)	*Glaciozyma* sp. 1
10	I14F-01331	KR698927	*Glaciozyma watsonii* (KY103477)	593/595 (99%)	*Glaciozyma* sp. 2
11	I14F-01284	KR698880	*Glarea lozoyensis* (NR137138)	432/447 (97%)	*Glarea* sp.
12	I14F-01322	KR698918	*Gremmeniella balsamea* (NR137589)	434/470 (92%)	Godroniaceae sp.
13	I14F-01263	KR698859	*Goffeauzyma gilvescens* (NR073228)	588/588 (100%)	*Goffeauzyma* sp.
14	I14F-01206	KR698802	*Glarea lozoyensis* (NR137138)	406/451 (90%)	Helotiales sp. 1
15	I14F-01242	KR698838	*Coleophoma paracylindrospora* (KU728491)	416/483 (86%)	Helotiales sp. 2
16	I14F-01249	KR698845	*Hymenoscyphus ohakune* (NR137109)	450/514 (88%)	Helotiales sp. 3
17	I14F-01303	KR698899	*Glarea lozoyensis* (NR137138)	418/460 (91%)	Helotiales sp. 4
18	I14F-01332	KR698928	*Variosporium delicatum* (KF730848)	450/478 (94%)	Helotiales sp. 5
19	I14F-01269	KR698865	*Holtermanniella festucosa* (KY102693)	516/517 (99%)	*Holtermanniella* sp.
20	I14F-01288	KR698884	*Acremonium persicinum* (NR131260)	472/538 (88%)	Hypocreales sp.
21	I14F-01217	KR698813	*Keissleriella trichophoricola* (KJ869113)	459/466 (98%)	*Keissleriella* sp.
22	I14F-01257	KR698853	*Mycosymbioces mycenaphila* (NR137807)	433/481 (90%)	Leotiomycetes sp.
23	I14F-01226	KR698822	*Leptosphaeria veronicae* (JF740255)	452/461 (98%)	*Leptosphaeria* sp. 1
24	I14F-01307	KR698903	*Leptosphaeria sclerotioides* (JF740193)	504/523 (99%)	*Leptosphaeria* sp. 2
25	I14F-01281	KR698877	*Leucosporidiella fragaria* (NR073287)	567/579 (98%)	*Leucosporidiella* sp. 1
26	I14F-01283	KR698879	*Leucosporidiella muscorum* (NR073286)	536/552 (97%)	*Leucosporidiella* sp. 2
27	I14F-01256	KR698852	*Mortierella antarctica* (NR111580)	588/593 (99%)	*Mortierella* sp.
28	I14F-01228	KR698824	*Mrakia frigida* (NR111044)	585/591 (99%)	*Mrakia* sp. 1
29	I14F-01259	KR698855	*Mrakia gelida* (KY104295)	556/557 (99%)	*Mrakia* sp. 2
30	I14F-01289	KR698885	*Mrakia fridida* (NR111044)	589/615 (96%)	*Mrakia* sp. 3
31	I14F-01312	KR698908	*Mycoarthris cf. coralline* (KC485428)	389/396 (98%)	*Mycoarthris* sp.
32	I14F-01219	KR698815	*Paraleptosphaeria macrospora* (JF740238)	479/484 (99%)	*Paraleptosphaeria* sp.
33	I14F-01261	KR698857	*Phenoliferia glacialis* (KY104503)	553/556 (99%)	*Phenoliferia* sp. 1
34	I14F-01287	KR698883	*Phenoliferia psychrophenolica* (KY104504)	538/562 (96%)	*Phenoliferia* sp. 2
35	I14F-01265	KR698861	*Phoma boeremae* (NR135962)	475/478 (99%)	*Phoma* sp.

Continued table

No.	Representative isolate code	GenBank accession number	Close related species (GenBank accession number)	Number of bp (Maximum identity)	Identification
36	I14F-01238	KR698834	*Darksidea delta* (NR137075)	410/475 (86%)	Pleosporales sp.
37	I14F-01227	KR698823	*Pseudeurotium bakeri* (NR145345)	494/499 (99%)	*Pseudeurotium* sp.
38	I14F-01233	KR698829	*Pseudogymnoascus destructans* (NR111838)	492/499 (99%)	*Pseudogymnoascus* sp.
39	I14F-01201	KR698797	*Taphrina carnea* (AF492083)	566/569 (99%)	*Taphrina* sp.
40	I14F-01220	KR698816	*Tetracladium* sp. (AJ890435)	494/514 (96%)	*Tetracladium* sp.
41	I14F-01244	KR698840	*Tolypocladium cylindrosporum* (AJ303055)	481/486 (99%)	*Tolypocladium* sp.
42	I14F-01280	KR698876	*Genolevuria amylolytica* (NR137810)	369/434 (85%)	Tremellales sp.
43	I14F-01234	KR698830	*Varicosporium delicatum* (DQ202516)	460/486 (95%)	*Varicosporium* sp.
44	I14F-01221	KR698817	*Venturia tremulae* (EU035438)	508/521(98%)	*Venturia* sp.
45	I14F-01237	KR698833	*Vishniacozyma tephrensis* (NR144812)	472/473 (99%)	*Vishniacozyma* sp. 1
46	I14F-01266	KR698862	*Vishniacozyma victoriae* (NR073260)	465/466 (99%)	*Vishniacozyma* sp. 2
47	I14F-01311	KR698907	*Vishniacozyma carnescens* (KY105817)	477/480 (99%)	*Vishniacozyma* sp. 3

Altogether, 134 fungal isolates were identified belonging to 47 species. These isolated strains belong to 27 genera from the phyla Ascomycota (17 genera), Basidiomycota (nine genera), and Zygomycota (one genus). The Ascomycota included five known orders: Helotiales (13 species), Hypocreales (two species), Pleosporales (six species), Taphrinales (one species), and Venturiales (one species). The Basidiomycota included four known orders: Cystobasidiales (two species), Cystofilobasidiales (three species), Sporidiobolales (four species), and Tremellales (nine species). The Zygomycota included only one known order: Mortierellales (one species). In addition, the most frequently detected species in the 13 water samples were *Vishniacozyma* sp. 2, *Cadophora* sp. 2, *Phenoliferia* sp. 1, *Dioszegia* sp. 2, and *Mortierella* sp., which were isolated from ten, eight, seven, six, and five of the samples, respectively (Table S1).

BLASTn searches in GenBank also showed that matching sequences with high similarity (⩾97%) were derived from fungi found in various habitats (Table S1), including plant roots, wood, soil, glacial sediment, glacial water, snow, streams, cryoconite holes, and lake sediment, within the Arctic (e.g., Svalbard), Antarctica, and alpine regions (e.g., Tibetan Plateau).

3.2 Dissimilarity of fungal diversity among the different aquatic habitats

Twenty-eight, 18, 12, and 16 fungal species were isolated from the stream, pond, glacial ice, and estuary, respectively (Table S2). The Shannon diversity indices were highest for the stream habitat (4.34), lowest for the ice habitat (3.22), and intermediate for the pond (3.92) and estuary (3.93) (Table S2). A Venn diagram demonstrated that the fungal species differed among the four aquatic habitats. The number of habitat-specific species ranged from three (the estuary) to 13 (the stream) (Figure 3a). Only three species were shared by the four aquatic habitats. The number of shared species among the different aquatic habitats was low; eight, five, and five species were shared between the stream and the pond, the pond and the glacial ice, and the estuary and the glacial ice, respectively.

To better understand the dissimilarity of fungal species among the different aquatic habitats, a heatmap analysis was also performed (Figure 3b). Only three fungal species coexisted in all four aquatic habitats, and these species were *Dioszegia* sp. 2, *Mrakia* sp. 2, and *Vishniacozyma* sp. 2. Thirteen species (e.g., *Venturia* sp. and *Taphrina* sp.) were found only in the stream. Nine species (e.g., Hypocreales sp. and *Glarea* sp.) were found only in the pond, and four species (i.e., Helotiales sp. 4, *Mycoarthris* sp., *Vishniacozyma* sp. 3, and *Mrakia* sp. 3) were found only in the glacial ice. Three species were found only in the estuary, including Helotiales sp. 5, *Dioszegia* sp. 3, and Godroniaceae sp. (Figure 3b). The dominant fungal species were *Cadophora* sp. 2 in the stream, *Vishniacozyma* sp. 2 in the pond, *Phenoliferia* sp. 1 in the glacial ice, and *Dioszegia* sp. 2, *Glaciozyma* sp. 2, and *Vishniacozyma* sp. 2 in the estuary (Figure 3b and Table S2).

3.3 Assay of α-glycosidase inhibitory activity

All the 134 aquatic fungal isolates were grown using liquid medium to obtain crude cell-free extracts, which were then screened for α-glycosidase inhibitory activity. Seventeen extracts of the fungal isolates (12.7%), which belonged to 15 species, displayed α-glycosidase inhibitory activity (Table 3). Among these 17 fungal isolates, Helotiales sp. 4 (I14F-01296), *Venturia* sp. (I14F-01221), *Dioszegia* sp. 2 (I14F-01229), *Mortierella* sp. (I14F-01243), and *Mrakia* sp. 2 (I14F-01250) had high levels of α-glycosidase inhibitory activity.

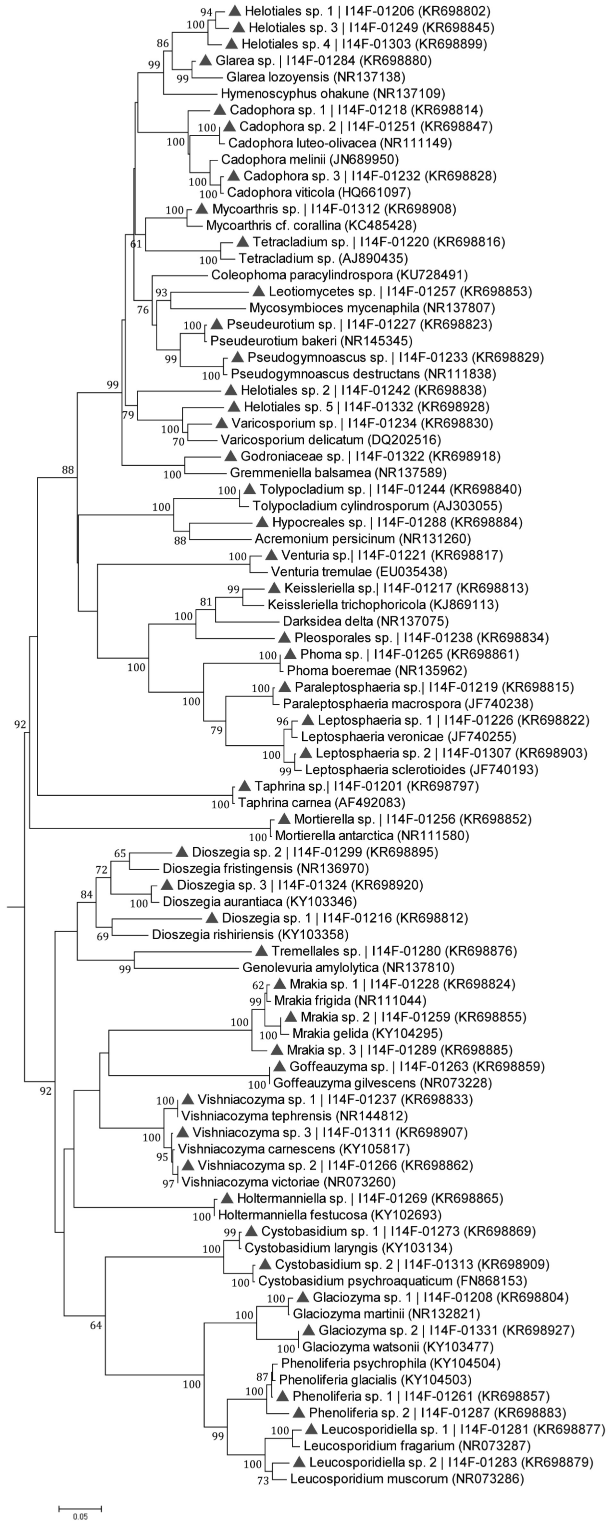

Figure 2 Phylogenetic tree of the 47 cultured fungal species (based on a representative isolate of each species) and other related fungal species based on the ITS (ITS1-5.8S-ITS2) region sequences of ribosomal DNA. The tree was constructed with the neighbor-joining method. Bootstrap support values are indicated for major nodes having values ≥60.

Table 3 The α-glycosidase inhibitory activity of 17 fungal isolates from the aquatic environments in the Ny-Ålesund region

Fungal species	Isolate no.	α-glycosidase inhibitory activity/%[#]
Cadophora sp. 1	I14F-01218	64.1 ± 0.02
Dioszegia sp. 2	I14F-01229	66.1 ± 0.01
Dioszegia sp. 2	I14F-01253	65.0 ± 0.01
Dioszegia sp. 2	I14F-01264	65.6 ± 0.02
Helotiales sp. 2	I14F-01242	64.4 ± 0.01
Helotiales sp. 3	I14F-01249	64.8 ± 0.01
Helotiales sp. 4	I14F-01296	67.1 ± 0.02
Hypocreales sp.	I14F-01288	63.1 ± 0.02
Keissleriella sp.	I14F-01217	57.4 ± 0.12
Leotiomycetes sp.	I14F-01257	62.8 ± 0.02
Leptosphaeria sp. 1	I14F-01226	65.5 ± 0.01
Tetracladium sp.	I14F-01220	63.7 ± 0.01
Mortierella sp.	I14F-01243	66.1 ± 0.01
Mrakia sp. 2	I14F-01250	66.1 ± 0.01
Phenoliferia sp. 1	I14F-01230	65.8 ± 0.01
Phenoliferia sp. 2	I14F-01287	63.7 ± 0.04
Venturia sp.	I14F-01221	66.1 ± 0.01

Notes: # Values are means of three replicate ± S.D.

4 Discussion

Among the 27 fungal genera detected in this study, many have been reported previously from aquatic habitats in polar regions, including *Vishniacozyma* and *Phenoliferia* in the Arctic (Butinar et al., 2007), and *Cadophora*, *Vishniacozyma*, *Mortierella*, *Phoma*, *Pseudeurotium*, and *Phenoliferia* in Antarctica (Ellis-Evans, 1985, 1996; Goncalves et al., 2012). Some of the fungal genera were found previously in non-aquatic habitats in the Arctic. For example, the genera *Acremonium*, *Morteriella*, *Tolypocladium*, and *Varicosporium* were also isolated from Arctic soil (Singh et al., 2012; Hafizah et al., 2013). In previous studies, a *Cadophora* sp. was isolated from a plant in Antarctica (Rosa et al., 2010), and a *Tolypocladium* sp. was isolated from Arctic soil in Svalbard (Hafizah et al., 2013), while a *Phoma* sp. was detected in Arctic plant roots (Botnen et al., 2013). Additionally, several fungal species, including species of *Cadophora*, *Vishniacozyma*, *Mrakia*, *Mortierella*, and *Phenoliferia* (as shown in Table S1), can even be found in Antarctica. These results suggest that some of the fungal species found in this study may originate from non-aquatic habitats in the Arctic ecosystem. It also suggests that the presence of specific psychrophilic and psychrotolerant fungi varies across different habitats within polar areas. However, the cultured fungal isolates obtained in this study represent a small portion of all the fungal species present in Arctic aquatic environments. In a previous study, we assessed the

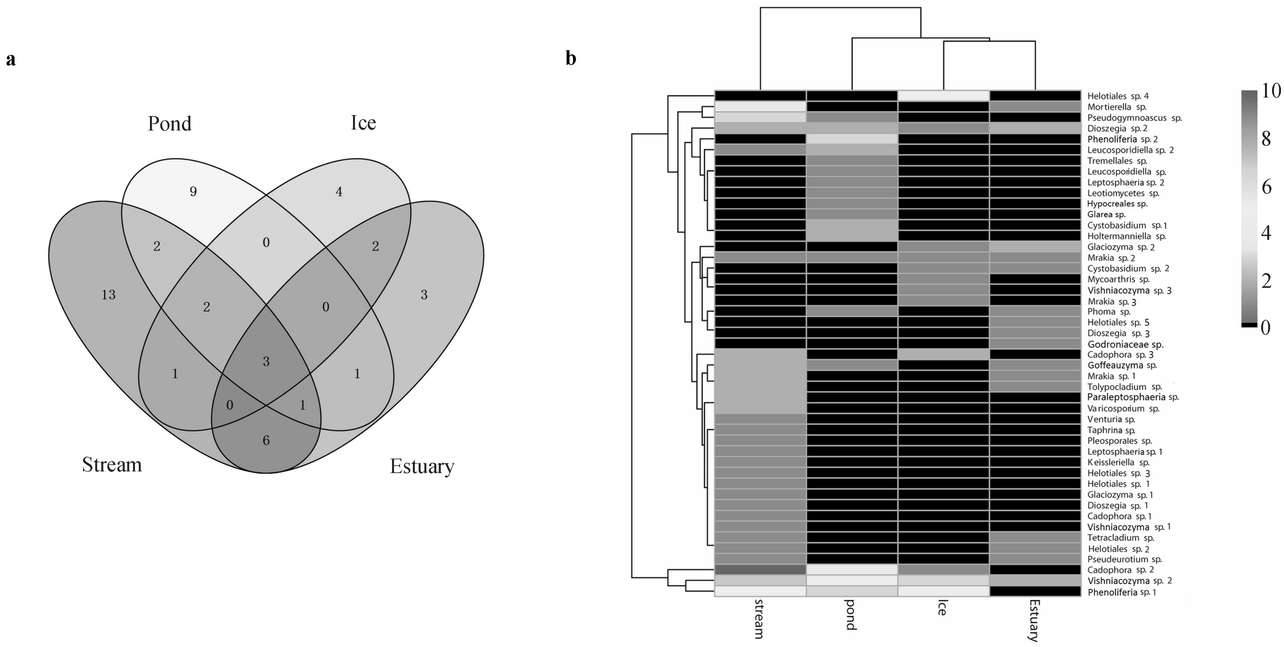

Figure 3 Venn diagram displaying the number of fungal species common among the different aquatic habitats of the Ny-Ålesund region (**a**); heatmap diagram showing the distribution and abundance of the 47 fungal species among the four aquatic habitats (**b**).

diversity of aquatic fungal communities in the same region using high-throughput pyrosequencing, and we detected 641 operational taxonomic units, which belonged to 90 known genera (Zhang et al., 2016).

The fungal communities from the different aquatic habitats (stream, pond, glacial ice, and estuary) in this High Arctic region differed in their composition, suggesting that aquatic fungal communities are selective in their habitat preference. Additionally, the highest level of fungal diversity was detected in the stream habitat, while the lowest level was detected in the glacial ice habitat, and intermediate levels were detected in the pond and estuary habitats. These data are consistent with previous findings regarding the variability of aquatic fungal communities from different habitats (stream, pond, glacial ice, and estuary), as revealed using high-throughput sequencing (Zhang et al., 2016). This is likely a result of the differences in the physicochemical properties of the respective habitats in this region, including temperature, salinity, and conductivity (Zhang et al., 2016).

The ability of Arctic and Antarctic fungi to survive in extreme environmental conditions suggests that they may possess unique metabolic pathways that allow them to generate specific or novel compounds with potential medicinal activity that could be used to develop new drugs (Santiago et al., 2012). Many fungal species isolated from Antarctic habitats have been shown to have potential medicinal activity and bioactive compounds (Santiago et al., 2012; Godinho et al., 2013). However, until now, the potential medicinal activity of Arctic fungi and their ability to produce bioactive compounds have been poorly understood.

Our results showed that the cell-free extracts of 15 fungal species have α-glycosidase inhibitory activity. This is the first time that Arctic fungi have been shown to have α-glycosidase inhibitory activity, suggesting that they produce bioactive secondary metabolites that are responsible for the observed α-glycosidase inhibitory activity. Some previous studies reported that only a few fungal species isolated from non-Arctic habitats are a source of α-glycosidase inhibitors (van de Laar F A et al., 2005; Kang et al., 2013; Kumar et al., 2014; Rivera-Chávez et al., 2013, 2015). Kang et al. (2013) found that extracellular α-glucosidase inhibitory activities were very low (2.1%–3.1%), or even undetectable, for 34 fungal supernatants, whereas higher intracellular α-glucosidase inhibitory activities were detected (20.6%–48.3%) in cell-free extracts. In particular, a new α-glucosidase inhibitory peptide was purified and identified from a cell-free extract of *Aspergillus oryzae*. Kim and Nho (2004) isolated an α-glucosidase inhibitor (SKG-3) from the fruiting bodies of *Ganoderma lucidum*, and they showed that SKG-3 is a highly specific and effective reversible inhibitor of α-glucosidase. The identification of 15 fungal species with α-glycosidase inhibitory activity in the present study substantially increases the potential number of bioactive secondary metabolites that might be used to develop new drugs.

In summary, this study showed that aquatic environments in the Arctic shelter rich, diverse, and complex fungal communities. Currently, many aspects of the taxonomy, ecology, and potential biotechnological applications of aquatic fungi from the Arctic remain poorly documented. Our results reinforce the need for further studies of aquatic fungal communities in the Arctic, as well as their phylogenetic relationships with species that occur in other cold regions (e.g., Antarctica or the Tibetan Plateau). In addition, we found that many aquatic fungal isolates had α-glycosidase inhibitory activity, which may provide new insights into the potential biotechnological applications of such fungi in Arctic aquatic environments

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Appendix Table 1 Molecular identification of 134 fungal strains isolated from aquatic habitats of the Ny-Ålseund region: maximum identification conducted using BLASTn searches of the ITS region

Sample code	Isolate code	Identification	GenBank accession number	Closest related species (GenBank accession number)	Number of bp (Maximum identify)	Reported habitat
R1	I14F-01201	*Taphrina* sp.	KR698797	*Taphrina carnea* (AF492083)	566/569 (99%)	Unreported
	I14F-01202	*Cadophora* sp. 2	KR698798	*Cadophora cf. luteo-olivacea* (KC180677)	497/504 (99%)	Hair root of plant in Argentina
	I14F-01203	*Cadophora* sp. 2	KR698799	*Cadophora cf. luteo-olivacea* (KC180677)	492/501 (98%)	Hair root of plant in Argentina
	I14F-01204	*Cadophora* sp. 2	KR698800	*Cadophora luteo-olivacea* (KF053558)	504/504 (100%)	Wood in Antarctica
	I14F-01205	*Cadophora* sp. 2	KR698801	*Cadophora cf. luteo-olivacea* (KC180677)	492/501 (98%)	Hair root of plant in Argentina
	I14F-01206	Helotiales sp. 1	KR698802	Helotiales sp. (JQ715700)	471/476(99%)	Soil in Antarctica
	I14F-01207	*Cadophora* sp. 2	KR698803	*Cadophora cf. luteo-olivacea* (KC180677)	497/504 (99%)	Hair root of plant in Argentina
R2	I14F-01208	*Glaciozyma* sp. 1	KR698804	*Glaciozyma martinii* (HQ432816)	556/557 (99%)	Sediment from glacier in Italy
	I14F-01209	*Cadophora* sp. 2	KR698805	*Cadophora luteo-olivacea* (KF053558)	504/504 (100%)	Wood in Antarctica
	I14F-01210	*Mortierella* sp.	KR698806	*Mortierella antarctica* (JX975843)	598/599 (99%)	Soil in North Victoria Land, Antarctica
	I14F-01211	*Vishniacozyma* sp. 2	KR698807	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
	I14F-01212	*Phenoliferia* sp. 1	KR698808	*Phenoliferia glacialis* (JQ857032)	541/542 (99%)	King George Island, Antarctica
	I14F-01213	*Vishniacozyma* sp. 2	KR698809	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
R3	I14F-0214	*Vishniacozyma* sp. 2	KR698810	*Vishniacozyma victoriae* (KP299253)	436/437 (99%)	Antarctic snow
	I14F-01215	*Vishniacozyma* sp. 2	KR698811	*Vishniacozyma victoriae* (KP299253)	436/437 (99%)	Antarctic snow
	I14F-01216	*Dioszegia* sp. 1	KR698812	*Dioszegia rishirensis* (AB545810)	418/424(99%)	Soil from island in Japan
	I14F-01217	*Keissleriella* sp.	KR698813	*Keissleriella trichophoricola* (KJ869113)	459/466 (98%)	Plant in Italy
	I14F-01218	*Cadophora* sp. 1	KR698814	*Cadophora melinii* (JN689950)	462/484 (95%)	Wood tissues of mechanical wounds in silver birch
	I14F-01219	*Paraleptosphaeria* sp.	KR698815	*Leptosphaeria* sp. (KJ542322)	492/492 (100%)	Root of alpine plant in China
	I14F-01220	*Tetracladium* sp.	KR698816	*Tetracladium* sp. (JX029123)	485/495 (98%)	Qinghai-Tibet Plateau
	I14F-01221	*Venturia* sp.	KR698817	*Venturia* sp. (AB916509)	499/502 (99%)	Bird feather in Norway
	I14F-01222	*Goffeauzyma* sp. 2	KR698818	*Goffeauzyma victoriae* (NR073228)	586/586 (100%)	Unreported
	I14F-01223	*Goffeauzyma* sp. 2	KR698819	*Goffeauzyma victoriae* (NR073228)	587/587 (100%)	Unreported
	I14F-01224	*Vishniacozyma* sp. 2	KR698820	*Vishniacozyma victoriae* (AY040654)	436/437 (99%)	Antarctic snow
	I14F-01225	*Mrakia* sp. 2	KR698821	*Mrakia gelida* (KM819098)	578/578 (100%)	Water from glacier in Antarctica
	I14F-01226	*Leptosphaeria* sp. 1	KR698822	*Leptosphaeria sclerotioides* (KM280063)	481/501 (96%)	Seed in Switzerland

Continued table

Sample code	Isolate code	Identification	GenBank accession number	Closest related species (GenBank accession number)	Number of bp (Maximum identify)	Reported habitat
R4	I14F-01227	*Pseudeurotium* sp.	KR698823	*Pseudeurotium bakeri* (KP055601)	509/512 (99%)	Crop field soil in South Korea
	I14F-01228	*Mrakia* sp. 1	KR698824	*Mrakia blollopis* (AB908178)	573/577 (99%)	Lake sediment in Antarctica
	I14F-01229	*Dioszegia* sp. 2	KR698825	*Dioszegia fristingensis* (EU070927)	425/432 (98%)	Phylloplane
	I14F-01230	*Phenoliferia* sp. 1	KR698826	*Phenoliferia glacialis* (JQ857037)	543/543 (100%)	King George Island, Antarctica
	I14F-01231	*Phenoliferia* sp. 1	KR698827	*Phenoliferia glacialis* (JQ857037)	542/543 (99%)	King George Island, Antarctica
	I14F-01232	*Cadophora* sp. 3	KR698828	*Cadophora viticola* (HQ661097)	503/506 (99%)	Grapevine nurseries in Spain
	I14F-01233	*Pseudogymnoascus* sp.	KR698829	*Pseudogymnoascus pannorum* (KP411562)	502/504 (99%)	Soil in caves, Canada
	I14F-01234	*Varicosporium* sp.	KR698830	*Varicosporium delicatum* (JQ412864)	455/478 (95%)	Streams in Alaska
	I14F-01235	*Cadophora* sp. 2	KR698831	*Cadophora cf. luteo-olivacea* (KC180677)	497/504 (99%)	Hair root of plant in Argentina
	I14F-01236	*Mortierella* sp.	KR698832	*Mortierella antarctica* (JX975843)	590/595 (99%)	Soil in North Victoria Land, Antarctica
	I14F-01237	*Vishniacozyma* sp. 1	KR698833	*Vishniacozyma tephrensis* (JX188132)	437/437 (100%)	Plant in USA
	I14F-01238	Pleosporales sp.	KR698834	Uncultured fungus clone (KF297131)	496/496 (100%)	Arctic soil in USA (78.78°N, 103.55°W)
	I14F-01309	*Pseudogymnoascus* sp.	KR698905	*Pseudogymnoascus pannorum* (KP411562)	499/504 (99%)	Soil in caves, Canada
R6	I14F-01239	*Phenoliferia* sp.	KR698835	*Phenoliferia glacialis* (JQ857037)	542/543 (99%)	King George Island, Antarctica
	I14F-01240	*Cadophora* sp. 2	KR698836	*Cadophora cf. luteo-olivacea* (KC180677)	496/504 (98%)	Hair root of plant in Argentina
	I14F-01241	*Paraleptosphaeria* sp.	KR698837	*Leptosphaeria* sp. (KJ542322)	479/486 (99%)	Root of alpine plant in China
	I14F-01242	Helotiales sp. 2	KR698838	Uncultured *Clathrosphaerina* clone (HQ212329)	479/587 (98%)	Arctic soil
	I14F-01243	*Mortierella* sp.	KR698839	*Mortierella antarctica* (JX975843)	590/595 (99%)	Soil in North Victoria Land, Antarctica
	I14F-01244	*Tolypocladium* sp.	KR698840	*Tolypocladium cylindrosporum* (FJ025173)	511/512 (99%)	Soil from alpine grassland
	I14F-01245	*Vishniacozyma* sp. 2	KR698841	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
	I14F-01301	*Mortierella* sp.	KR698897	*Mortierella antarctica* (JX975843)	590/595 (99%)	Soil in North Victoria Land, Antarctica
R7	I14F-01246	*Cadophora* sp. 2	KR698842	*Cadophora cf. luteo-olivacea* (KC180677)	490/501 (98%)	Hair root of plant in Argentina
	I14F-01247	*Phenoliferia* sp. 1	KR698843	*Phenoliferia glacialis* (JQ857037)	542/543 (99%)	King George Island, Antarctica
	I14F-01248	*Vishniacozyma* sp. 2	KR698844	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
	I14F-01249	Helotiales sp. 3	KR698845	Helotiales sp. (JX852359)	490/508 (96%)	Moss in Antarctica
	I14F-01250	*Mrakia* sp. 2	KR698846	*Mrakia gelida* (KM819098)	578/578 (100%)	Water from glacier in Antarctica

Continued table

Sample code	Isolate code	Identification	GenBank accession number	Closest related species (GenBank accession number)	Number of bp (Maximum identify)	Reported habitat
R7	I14F-01251	*Cadophora* sp. 2	KR698847	*Cadophora luteo-olivacea* (KF053558)	504/504 (100%)	Wood in Antarctica
	I14F-01252	*Cadophora* sp. 3	KR698848	*Cadophora viticola* (HQ661097)	504/506 (99%)	Grapevine nurseries in Spain
	I14F-01253	*Dioszegia* sp. 2	KR698849	*Dioszegia fristingensis* (EU070927)	426/433 (98%)	Phylloplane
	I14F-01254	*Phenoliferia* sp. 1	KR698850	*Phenoliferia glacialis* (JQ857037)	541/542 (99%)	King George Island, Antarctica
	I14F-01255	*Tolypocladium* sp.	KR698851	*Tolypocladium cylindrosporum* (FJ025173)	518/519 (99%)	Soils from alpine grassland
	I14F-01256	*Mortierella* sp.	KR698852	*Mortierella antarctica* (JX975843)	590/595 (99%)	Soil in North Victoria Land, Antarctica
	I14F-01302	*Vishniacozyma* sp. 2	KR698898	*Vishniacozyma victoriae* (KP299253)	432/432 (100%)	Antarctic snow
L1	I14F-01257	Leotiomycetes sp.	KR698853	Uncultured Leotiomycetes clone (JF449892)	426/466 (91%)	Leaf litter in Austria
	I14F-01258	*Cadophora* sp. 2	KR698854	*Cadophora cf. luteo-olivacea* (KC180677)	485/498 (97%)	Hair root of plant in Argentina
	I14F-01259	*Mrakia* sp. 2	KR698855	*Mrakia gelida* (KM819098)	578/578 (100%)	Water from glacier in Antarctica
	I14F-01260	*Cadophora* sp. 2	KR698856	*Cadophora luteo-olivacea* (KF053558)	504/504 (100%)	Wood in Antarctica
	I14F-01261	*Phenoliferia* sp. 1	KR698857	*Phenoliferia glacialis* (JQ857037)	542/543 (99%)	King George Island, Antarctica
	I14F-01262	*Vishniacozyma* sp. 2	KR698858	*Vishniacozyma victoriae* (KP299253)	437/437 (99%)	Antarctic snow
	I14F-01263	*Goffeauzyma* sp. 2	KR698859	*Goffeauzyma victoriae* (NR073228)	588/588 (100%)	Unreported
	I14F-01264	*Dioszegia* sp. 2	KR698860	*Dioszegia fristingensis* (EU070927)	425/432 (98%)	Phylloplane
	I14F-01265	*Phoma* sp.	KR698861	*Phoma herbarum* (KF889057)	485/486 (99%)	Unreported
	I14F-01266	*Vishniacozyma* sp. 2	KR698862	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
	I14F-01267	*Cadophora* sp. 2	KR698863	*Cadophora cf. luteo-olivacea* (KC180676)	497/507 (99%)	Hair root of plant in Argentina
L2	I14F-01268	*Cadophora* sp. 2	KR698864	*Cadophora cf. luteo-olivacea* (KC180677)	491/501 (98%)	Hair root of plant in Argentina
	I14F-01269	*Holtermanniella* sp.	KR698865	*Holtermanniella festucosa* (JX188167)	512/513 (99%)	Plant in USA
	I14F-01270	*Pseudogymnoascus* sp.	KR698866	*Pseudogymnoascus pannorum* (KP411562)	501/502 (99%)	Soil in caves, Canada
	I14F-01271	*Vishniacozyma* sp. 2	KR698867	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
	I14F-01272	*Holtermanniella* sp.	KR698868	*Holtermanniella festucosa* (JX188167)	512/513 (99%)	Plant in USA
	I14F-01273	*Cystobasidium* sp. 1	KR698869	*Cystobasidium laryngis* (AF190014)	518/521 (99%)	Unreported
	I14F-01274	*Vishniacozyma* sp. 2	KR698870	*Vishniacozyma victoriae* (KP299253)	436/437 (99%)	Antarctic snow
	I14F-01275	*Cystobasidium* sp. 1	KR698871	*Cystobasidium laryngis* (GQ911550)	519/521 (99%)	Glacier, Apennines, Italy

Continued table

Sample code	Isolate code	Identification	GenBank accession number	Closest related species (GenBank accession number)	Number of bp (Maximum identify)	Reported habitat
L3	I14F-01276	*Dioszegia* sp. 2	KR698872	*Dioszegia fristingensis* (EU070927)	427/433 (99%)	Phylloplane
	I14F-01277	*Phenoliferia* sp. 1	KR698873	*Phenoliferia glacialis* (JQ857037)	542/543 (99%)	King George Island, Antarctica
	I14F-01278	*Phenoliferia* sp. 1	KR698874	*Phenoliferia glacialis* (JQ857037)	542/543 (99%)	King George Island, Antarctica
	I14F-01279	*Phenoliferia* sp. 2	KR698875	*Phenoliferia psychrophenolica* (KC333169)	548/550 (99%)	Cryoconite holes on glaciers in Svalbard
	I14F-01307	*Leptosphaeria* sp. 2	KR698903	*Leptosphaeria sclerotioides* (EU265669)	495/501 (99%)	Plant seed in Switzerland
	I14F-1280	Tremellales sp.	KR698876	Uncultured Tremellales clone (FJ553848)	432/500 (86%)	Forest soil in Canada
X1	I14F-01281	*Leucosporidiella* sp.1	KR698877	*Leucosporidiella fragaria* (NR073287)	567/579 (98%)	Unreported
	I14F-01282	*Phenoliferia* sp. 2	KR698878	*Phenoliferia psychrophenolica* (KC333169)	543/553 (98%)	Cryoconite sediment in Svalbard
	I14F-01283	*Leucosporidiella* sp. 2	KR698879	*Leucosporidiella muscorum* (NR073286)	536/552 (97%)	Unreported
	I14F-01284	*Glarea* sp.	KR698880	*Glarea lozoyensis* (NR137138)	432/447 (97%)	Unreported
	I14F-01285	*Leucosporidiella* sp. 2	KR698881	*Leucosporidiella muscorum* (NR073286)	535/553 (97%)	Unreported
	I14F-01286	*Vishniacozyma* sp. 2	KR698882	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
	I14F-01287	*Phenoliferia* sp. 2	KR698883	*Phenoliferia psychrophenolica* (KC333169)	552/552 (100%)	Cryoconite sediment in Svalbard
	I14F-01288	Hypocreales sp.	KR698884	*Acremonium* sp. (JX171161)	525/528 (99%)	Antarctica
	I14F-01289	*Mrakia* sp. 3	KR698885	*Mrakiella cryoconiti* (JX188193)	569/570 (99%)	Superficial glacial melting water from Alps
	I14F-01290	Helotiales sp. 4	KR698886	Uncultured Helotiales clone (HQ212304)	498/499 (99%)	Arctic soil
	I14F-01291	*Phenoliferia* sp. 1	KR698887	*Phenoliferia glacialis* (JQ857032)	541/543 (99%)	King George Island, Antarctica
	I14F-01292	*Phenoliferia* sp. 1	KR698888	*Phenoliferia glacialis* (JQ857032)	541/543 (99%)	King George Island, Antarctica
	I14F-01293	*Phenoliferia* sp. 1	KR698889	*Phenoliferia glacialis* (JQ857032)	541/542 (99%)	King George Island, Antarctica
	I14F-01294	*Phenoliferia* sp. 1	KR698890	*Phenoliferia glacialis* (JQ857032)	541/543 (99%)	King George Island, Antarctica
	I14F-01295	Helotiales sp. 4	KR698891	Uncultured Helotiales clone (HQ212304)	487/488 (99%)	Arctic soil
	I14F-01296	Helotiales sp. 4	KR698892	Uncultured Helotiales clone (HQ212304)	479/479 (100%)	Arctic soil
	I14F-01297	*Vishniacozyma* sp. 2	KR698893	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
	I14F-01303	Helotiales sp. 4	KR698899	Uncultured Helotiales clone (HQ212304)	504/507 (99%)	Arctic soil
	I14F-01304	Helotiales sp. 4	KR698900	Uncultured Helotiales clone (HQ212304)	504/507 (99%)	Arctic soil
	I14F-01305	*Phenoliferia* sp. 1	KR698901	*Phenoliferia glacialis* (JQ857032)	541/543 (99%)	King George Island, Antarctica
	I14F-01306	*Cadophora* sp. 2	KR698902	*Cadophora luteo-olivacea* (KF053558)	504/504 (100%)	Wood in Antarctica

Continued table

Sample code	Isolate code	Identification	GenBank accession number	Closest related species (GenBank accession number)	Number of bp (Maximum identify)	Reported habitat
X2	I14F-01298	*Vishniacozyma* sp. 2	KR698894	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
	I14F-01299	*Dioszegia* sp. 2	KR698895	*Dioszegia fristingensis* (JQ857038)	434/436 (99%)	King George Island, Antarctica
	I14F-01300	*Glaciozyma* sp. 2	KR698896	*Glaciozyma watsonii* (EU149804)	580/581 (99%)	Soil from South Victoria land, Antarctica
	I14F-01308	*Mrakia* sp. 2	KR698904	*Mrakia gelida* (KM819098)	578/578 (100%)	Water from glacier in Antarctica
	I14F-01310	*Vishniacozyma* sp. 2	KR698906	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
	I14F-01311	*Vishniacozyma* sp. 3	KR698907	*Vishniacozyma carnescens* (KP131895)	436/437 (99%)	Cloacal swab, bird
	I14F-01312	*Mycoarthris* sp.	KR698908	Uncultured *Mycoarthris* clone (JF449666)	456/470 (97%)	Beech litter in Austria
	I14F-01313	*Cystobasidium* sp. 2	KR698909	*Cystobasidium psychroaquaticum* (JN572895)	518/521 (99%)	Springtail Sminthuridae feeding on fungus
	I14F-01314	*Vishniacozyma* sp. 2	KR698910	*Vishniacozyma victoriae* (KP299253)	435/436 (99%)	Antarctic snow
	I14F-01316	*Vishniacozyma* sp. 2	KR698912	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
S1	I14F-01315	*Dioszegia* sp. 2	KR698911	*Dioszegia fristingensis* (JQ857038)	434/435 (99%)	King George Island, Antarctica
	I14F-01317	*Vishniacozyma* sp. 2	KR698913	*Vishniacozyma victoriae* (KP299253)	435/437 (99%)	Antarctic snow
	I14F-01318	*Mrakia* sp. 1	KR698914	*Mrakia blollopis* (AB908178)	573/577 (99%)	Lake sediment in Antarctica
	I14F-01319	*Goffeauzyma* sp.	KR698915	*Goffeauzyma victoriae* (NR073228)	588/588 (100%)	Unreported
	I14F-01320	*Dioszegia* sp. 2	KR698916	*Dioszegia fristingensis* (EU070927)	426/433 (98%)	Phylloplane
	I14F-01321	*Vishniacozyma* sp. 2	KR698917	*Vishniacozyma victoriae* (KP299253)	437/437 (100%)	Antarctic snow
	I14F-01322	Godroniaceae sp.	KR698918	*Godronia cassandrae* (KC595271)	473/480 (99%)	Plant in Germany
	I14F-01323	*Tetracladium* sp.	KR698919	*Tetracladium* sp. (JX029123)	490/495 (99%)	Qinghai-Tibet Plateau
	I14F-01324	*Dioszegia* sp. 3	KR698920	*Dioszegia aurantiaca* (EU266497)	412/422 (98%)	Phylloplane
S2	I14F-01325	*Cystobasidium* sp. 2	KR698921	*Cystobasidium psychroaquaticum* (JN572895)	516/521 (99%)	Springtail Sminthuridae feeding on fungus
	I14F-01326	*Mrakia* sp. 2	KR698922	*Mrakia gelida* (KM819098)	578/578 (100%)	Water from glacier in Antarctica
	I14F-01327	*Pseudeurotium* sp.	KR698923	*Pseudeurotium bakeri* (KP055601)	502/505 (99%)	Crop field soil in South Korea
	I14F-01328	*Mortierella* sp.	KR698924	*Mortierella antarctica* (JX975843)	590/595 (99%)	Soil in North Victoria Land, Antarctica
	I14F-01329	*Tolypocladium* sp.	KR698925	*Tolypocladium cylindrosporum* (FJ025173)	512/515 (99%)	Soils from alpine grassland in Qilian
	I14F-01330	Helotiales sp. 2	KR698926	Uncultured *Clathrosphaerina* clone (HQ212329)	490/500 (98%)	Arctic soil
	I14F-01331	*Glaciozyma* sp. 2	KR698927	*Glaciozyma watsonii* (EU149804)	581/582 (99%)	Soil from South Victoria Land, Antarctica
	I14F-01332	Helotiales sp. 5	KR698928	*Variosporium delicatum* (KF730848)	450/478 (94%)	Aquatic environment
	I14F-01333	*Glaciozyma* sp. 2	KR698929	*Glaciozyma watsonii* (EU149804)	581/582 (99%)	Soil from South Victoria Land, Antarctica
	I14F-01334	*Phoma* sp.	KR698930	*Phoma herbarum* (KF889057)	471/474 (99%)	Unreported

Appendix Table 2 Distribution of fungal species in the four aquatic habitats from the Ny-Ålseund region: number of isolates in different aquatic habitats

	Stream	Pond	Ice	Estuary
Cadophora sp. 1	1	0	0	0
Cadophora sp. 2	10	4	1	0
Cadophora sp. 3	2	0	2	0
Cystobasidium sp. 1	0	2	0	0
Cystobasidium sp. 2	0	0	1	1
Dioszegia sp. 1	1	0	0	0
Dioszegia sp. 2	2	2	1	2
Dioszegia sp. 3	0	0	0	1
Glaciozyma sp. 1	1	0	0	0
Glaciozyma sp. 2	0	0	1	2
Glarea sp.	0	1	0	0
Godroniaceae sp.	0	0	0	1
Goffeauzyma sp.	2	1	0	1
Helotiales sp. 1	1	0	0	0
Helotiales sp. 2	1	0	0	1
Helotiales sp. 3	1	0	0	0
Helotiales sp. 4	0	0	5	0
Helotiales sp. 5	0	0	0	1
Holtermanniella sp.	0	2	0	0
Hypocreales sp.	0	1	0	0
Keissleriella sp.	1	0	0	0
Leotiomycetes sp.	0	1	0	0
Leptosphaeria sp. 1	1	0	0	0
Leptosphaeria sp. 2	0	1	0	0
Leucosporidiella sp. 1	0	1	0	0
Leucosporidiella sp. 2	1	2	0	0
Mortierella sp.	4	0	0	1
Mrakia sp. 1	2	0	0	1
Mrakia sp. 2	1	1	1	1
Mrakia sp. 3	0	0	1	0
Mycoarthris sp.	0	0	1	0
Paraleptosphaeria sp.	2	0	0	0
Phenoliferia sp. 1	5	3	5	0
Phenoliferia sp. 2	0	3	0	0
Phoma sp.	0	1	0	1
Pleosporales sp.	1	0	0	0
Pseudeurotium sp.	1	0	0	1
Pseudogymnoascus sp.	3	1	0	0
Taphrina sp.	1	0	0	0
Tetracladium sp.	1	0	0	1
Tolypocladium sp.	2	0	0	1
Tremellales sp.	0	1	0	0
Varicosporium sp.	2	0	0	0
Venturia sp.	1	0	0	0
Vishniacozyma sp. 1	1	0	0	0
Vishniacozyma sp. 2	7	5	3	2
Vishniacozyma sp. 3	0	0	1	0
Number of fungal isolates	59	33	23	19
Number of fungal taxa	28	18	12	16
Shannon index (*H′*)	4.34	3.92	3.22	3.93