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EXPERIMENTAL ARTICLES

Fungal Diversity in the Antarctic Active Layer

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Abstract—Taxonomic diversity of fungi in the samples of the active layer of Antarctica was investigated using conventional microbiological techniques and metagenomic analysis of total DNA extracted from environmental samples. The list of Antarctic microscopic fungi was expanded, including detection of the species representing a portion of the fungal complex which is nonculturable or sterile on conventional nutrient media.

Keywords: mycelial fungi, Antarctica, active layer, biodiversity, ITS2, metagenomic analysis

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The problem of preservation of life at subzero temperatures in ice and permafrost rocks is of basic importance. Recent studies revealed the depths of permafrost sediments to be a natural reservoir of mycelial fungi. Viable microscopic Antarctic fungi have been revealed on both the surface [1–3] and in the depth of permafrost sediments [4–6]. It has been shown that occurrence of fungi in the surface horizons was associated with the presence of hosts, which, in the case of Antarctic fungi, are mainly birds, insects, invertebrates, nematodes, and plants—mosses and lichens [7]. Actively conducted studies resulted in a tenfold increase in the list of fungal genera found in Antarctica over the past 15 years: from 40 [8] to almost 400 (the British Antarctic Survey database http://www.antarctica.ac.uk//bas_research/data/access/fungi/). Even with regard to the presence of taxonomic synonyms in this database, which significantly increases the list of names, this is evidence of great interest in this region, which is explained not only by the desire to reveal rare or new microorganisms and to find new metabolites [9], but also to obtain information about the general reserve and biodiversity of microorganisms in Antarctic sediments.

In order to reveal Antarctic microorganisms, conventional microbiological methods are primarily used. However, there are works in which the metagenomic analysis with emphasis on prokaryotic diversity was carried out in parallel to cultural investigations [10, 11]. The complex of methods for investigation of mycelial fungi is not used very often [12].

It should be noted that a characteristic feature of all these studies is the fact that both groups of methods yield different, often not overlapping results [13]. For example, it was found that metagenomic analysis usually revealed more fungal species of higher taxa than conventional microbiological methods. The results of the diversity of anamorphic fungi are underestimated, in particular, at the expense of micromycetes with small spores. Thus, the study of Antarctic permafrost sediments by analysis of the total genomic DNA showed the absence of fungi of the genera *Penicillium, Cladosporium*, and others, which were invariably retrieved when the samples were analyzed by conventional microbiological methods [14].

The natural features of the Antarctic active layer determine the extremely irregular and microfocal distribution of the number and species diversity of microorganisms. Thus, it may be expected that mycological investigation of the samples from spatially remote areas of Antarctica carried out using a complex of methods may provide new and more complete information about the mycobiota of a given habitat.

This work represents the results of the study of fungal diversity in the samples of the active layer of Antarctic sediments taken from different, widely spaced points of the continent using both the conventional microbiological and molecular methods.

MATERIALS AND METHODS

The six active layer samples studied were obtained by the Russian Antarctic Expedition within the framework of the International Polar Year project "The Age of Antarctic Permafrost" in 2007–2009 in the ice-free oases along the continent's perimeter. The samples

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were obtained from the cores of four boreholes located at the stations Novolazarevskaya (Schirmacher Oasis), Druzhnaya (Sannefjord Bay), Leningradskaya (Wilson Hills), and the Mirny observatory (Oates Coast). All samples were taken from the surface and subsurface horizons (Table 1).

In order to reveal the most complete diversity of cultivated mycelial fungi, the previously developed methods for defrosting the samples of permafrost sediments were used [4]. Frozen samples were thawed out in water heated to 20, 37, and 52°C for 1 min on a water bath.

The standard Czapek synthetic medium with 2% sucrose (Cz) and malt extract agar organic medium (MEA) supplemented with lactic acid (0.4% vol/vol) to suppress bacterial growth were used. Petri dishes were incubated at 4 and 25°C for a month. The CFU number was determined in 1 g of the air-dry material, for which purpose the moisture content of the samples was measured gravimetrically. The enrichment method was used in parallel; weighed portions of the samples were added to a liquid nutrient medium (wort 3.5 B) and incubated at 4°C for 30–60 days with periodic visual observation.

The cultures were identified on the basis of the cultural and morphological characteristics determined on the recommended media according to the requirements of the present-day identification manuals [15, 16, etc.]. To identify the cultures of the subgenus *Penicillium*, we used the modern identification scheme based on the analysis of the macro- and micromorphological characteristics revealed at various combinations of nutrient media and cultivation temperatures [17].

The total genomic DNA was extracted from two samples of the Antarctic active layer obtained at the station Novolazarevskaya (Table 1, nos. 3, 4). The DNA was isolated using the UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, United States) according to the manufacturer's recommendations. The samples were ultrasonically pretreated to improve cell desorption from the surface of soil particles in order to reach the maximum DNA yield from the permafrost specimens.

Metagenomic analysis with emphasis on mycobiota biodiversity was carried out according to the following scheme: the region of the second internal transcribed ribosomal DNA spacer (ITS2) with the adjacent fragments of the genes encoding the 5.8S and 28S subunits was amplified with the conservative fungal-specific primers ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [18]. The heterogeneous amplicon was separated by molecular cloning in *E. coli* cells; the bacteria carrying the necessary inserts were selected, and the insert was retested by PCR amplification with the universal primers. The amplicons were directly sequenced using the automatic capillary device operating on the Sanger platform.

The PCR mixture contained the following: Tris-HCl, 50 mM, pH 9.0; KCl, 50 mM; $MgCl_2$, 1.5 mM; Triton X-100, 0.1%; glycerol, 10%; cresol red (Na), 0.05 mM; a mixture of nucleotides (Promega), 0.2 mM each; primers (Syntol), 0.05 μM each; TAQ-pol with inhibitory monoclonal antibodies (Syntol), 25 U/mL. The typical volume of the reaction mixture was 20 μL, of which the volume of the template DNA solution constituted up to 20%. The PCR program was as follows: primary denaturing, 7 min at 95°C followed by 35 cycles at 95°C for 30 s (denaturing), 54°C 45 s (annealing), and 72°C 60 s (elongation); 72°C 10 min (final elongation). A MyCycler Thermal Cycler 580BR 2261 amplifier (BioRad) was used. The reaction products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide, visualized, and documented with a Gel Doc XR System (BioRad).

Molecular cloning of the amplicon library based on the white-blue selection method was carried out by ligation into the pGEM type of vector (Promega pGEM®-T Vector System) using the competent cells of *E. coli* strain JM109 (Promega) according to the manufacturer's instruction. In order to verify the presence of the required nucleotide insertion and to produce a fragment for sequencing, amplification with the ITS3–ITS4 pair of primers was carried out (the composition and the parameters of the reaction were similar to those described above); a fragment of a single *E. coli* colony was added as template.

Direct sequencing of the DNA fragments was carried out by the method of fluorescently labeled terminators using a Quick Start Kit (Beckman Coulter) and a CEQ 8000 Genetic Analysis System (Beckman Coulter) according to the manufacturer's protocol using the ITS3 primer (4 pmol/reaction). The sequences were aligned and edited using the CEQ 8000 Software package (Beckman Coulter). Individual sequences were taken as operational taxonomic units (OTU); the sequences were compared using the Blastn software (http://www.insdc.org/) for rough taxonomic identification. UCHIME software package was used to exclude the possible chimera sequences [19].

The fungal sequences were submitted in the international DNA database (GenBank) under accession numbers HQ634119–HQ634136, JN653540. The remaining eukaryotic sequences were submitted under accession numbers JN653492–JN653539 and JN653541–JN653557.

RESULTS AND DISCUSSION

The numbers of fungi in the samples determined using the cultivation techniques varied considerably, depending on the sampling site and the sample character. In the sample from the station Druzhnaya, no fungi were revealed by plating. A very low number was found in the samples from the station Leningradskaya

Table 1. Mycelial fungi in the samples of Antarctic active layer

Sample no.	Location	Borehole	Geographical coordinates	Depth, cm	Maximal number, CFU/g	Taxa
1	Station Druzhnaya	LA55-Dr-02	69°44′58.2″ S, 73°42′25.0″ E	1–9	0	*Penicillium brevicompactum* Dierckx*
2	Station Novolazarevskaya	FDG-09-03	70°45′45.3″ S, 11°46′50.2″ E	0–0.5	13824.3	*Coprinellus micaceus* (Bull.) Vilgalys, Hopple et Jacq. Johnson**, *Geomyces pannorum* (Link) Sigler et J.W. Carmich., *Phoma herbarum* Cooke
3				0.5–2	2453.9	*Geomyces pannorum* (Link) Sigler et J.W. Carmich., *Penicillium chrysogenum* Thom., *P. crustosum* Thom., *Phoma herbarum* Cooke
4				2–6	1259.1	*Epicoccum nigrum* Link**, *Penicillium aurantiogriseum* Dierckx, *P. coprophilum* (Berk. et M.A. Curtis) Seifert et Samson, *P. crustosum* Thom., *P. griseofulvum* Dierckx, *Phoma herbarum* Cooke, *Pseudeurotium* sp.**
5	Observatory Mirny	LA55 Mr-01	66°33′11.4″ S, 93°00′29.5″ E	0–1	235.6	*Geomyces pannorum* (Link) Sigler et J.W. Carmich., *Leuconeurospora* sp.**, *Phoma herbarum* Cooke**, *Thelebolus microsporus* (Berk. et Broome) Kimbr.**
6	Station Leningradskaya	LA55 Ln-01	69°30′6.3″ S, 159°23′29.5″ E	0–1.5	7.4	*Acremonium strictum* W. Gams, *Geomyces pannorum* (Link) Sigler et J.W. Carmich., *Leuconeurospora* sp.**

* Revealed using the enrichment method.
** The strains were identified using the molecular biological method.

and the observatory Mirny. The samples of the station Novolazarevskaya situated in Schirmacher Oasis, where the fungi were relatively numerous (10^3–10^4 CFU/g) and their number decreased with sampling depth, were drastically different in this respect.

The micromycete species diversity was low; an average of 1–3 species were determined per sample. The fungal abundance and diversity were not directly interrelated. The application of enrichment techniques made it possible to reveal in sample no. 1 the fungi of the species *Penicillium brevicompactum*, whereas no fungi were revealed in this sample by plating.

In the uppermost horizon of the profile from the station Novolazarevskaya (Table 1, no. 2), which contained over 10^4 CFU/g consisted of sandy turfs of dead green moss, representatives of the species *Phoma herbarum* and *Geomyces pannorum*, which are well adapted to low-temperature conditions, predominated. Fungi of these two species virtually constituted the abundance of fungi in this sample. In the dishes were incubated at 4°C, a 10% increase in the number of fungi was observed due to growth of *Geomyces pannorum*, which did not develop at 25°C. The two samples taken from the lower horizons of the profile consisted of sand bound by dead moss rhizoids, with an admixture of the residues of aerial mossy parts. Here, the total number of fungi was slightly lower, although the species diversity increased. Members of different *Penicillium* species (five species in two horizons) were found; this is one of the most widespread genera in Antarctica [20] able to preserve viability at low temperatures in the presence of protectors, which are organic compounds of plant origin.

Most of the fungal species revealed belonged to cosmopolites and psychrotolerant species, although it is also necessary to note the presence of xerotolerant organisms, which is natural for Antarctic arid conditions. Thus, for example, of the six *Penicillium* species revealed, four belonged to xerotolerant species [21]: *P. aurantiogriseum, P. brevicompactum, P. chrysogenum*, and *P. crustosum*. Analyzing the species composition, it is possible to note the predominance of anamorphic fungi with small spores. The predominance of anamorphs is considered to be a characteristic feature of Antarctica, because shortening of the life cycle under extreme conditions decreases the metabolic expenditure of an organism [3]. However, the presence of sterile mycelium, which is on the whole characteristic of the Arctic and Antarctic habitats, also gives evidence of a possible presence of the teleomorphs incapable of forming fruit bodies in the culture.

The possibility to alter the cultural and morphological properties to some degree is inhered by many fungi adapted to metabolism in low-temperature habitats. Moreover, many psychrotolerant fungi have a higher growth rate at low temperatures and a lower growth rate at 26°C than the isolates of the same species from the temperate zone samples [22]. Together with the presence of a large number of sterile forms, this seriously interferes with the process of culture identification using the conventional mycological methods.

Therefore, in our studies, we used molecular biological analysis both to identify the sterile mycelium from the samples and to specify the diagnoses due to the obliterated diagnostic features some of the freshly isolated strains possessed in a culture.

For example, in the process of studying the samples, we succeeded in identifying the strain determined as the anamorph of *Ascomycetes* to the generic level of *Teberdinia* sp. (99% homology) (Table 2). The difficulties in the identification of this strain resulted from the absence of growth at above 15°C, whereas the species of this genus were described at 18°C [23].

The isolate of dark sterile mycelium from the subsurface sample had the cultural and morphological features similar to those of the representatives of the species *Epicoccum nigrum*; however, the absence of sporulation prevented its identification. Molecular biological analysis confirmed the isolate being identified with this species with 100% homology.

On the whole, three cultures out of the seven identified (Table 2) appeared to be teleomorphs—*Coprinellus micaceus* (this species was previously not reported to be found in Antarctica), *Pseudeurotium* sp., and *Thelebolus microsporus*.

The results of the study of the taxonomic diversity of fungi using analysis of the total DNA isolated by direct extraction from two samples of the Antarctic active layer are shown in Table 3.

When the amplification products were sequenced from these samples, 84 OTU of the ITS 2 region were obtained, of which only 19 OTU belonged to fungi. Despite the fact that we used the primers specific for the kingdom *Fungi*, a number of sequences identified with other eukaryotes were noted among the sequences obtained: mosses (*Plantae, Bryophyta, Bryopsida*, orders *Bryales* and *Dicranales*), about 12%; green algae (*Plantae, Chlorophyta*), about 12%; infusoria (*Chromalveolata, Alveolata, Ciliophora*), over 40%. Moreover, 10 sequences were obtained, for which no homologous ones were found in the GenBank database.

From the taxonomic point of view, all the fungal OTU obtained belonged to the division *Ascomycota*, subdivision *Pezizomycotina*. Most species belonged to the classes *Leotiomycetes* and *Dothideomycetes*.

In sample 3, five fungal sequences similar to those available in the GenBank database for three fungal species were revealed. Interestingly, one of these species was *Geomyces pannorum*, the species which plating also revealed as a dominant one in this sample. Both samples contained the psychrotolerant fungus *Herpotrichia juniperi*, a causal agent of brown felt blight of conifers, capable of growth at mild negative temperatures [24] and widespread in the Alps, and

Table 2. Molecular biological identification of strains from the Antarctic active layer samples

Strain number in the VKM	Diagnosis based on the cultural and morphological characteristics	GenBank data				Definitive strain name
		GenBank number	closely related species	homology, %	coverage, %	
FW-3194	Mycelia sterile	JN835200	*Coprinellus micaceus*	99	100	*Coprinellus micaceus*
FW-3198	*Ascomycetes* anamorph	JN835209	*Pseudeurotium* sp., *Teberdinia* sp.	99	97–99	*Teberdinia* sp.
FW-3200	Mycelia sterile	JN835210	*Epicoccum nigrum*	100	100	*Epicoccum nigrum*
FW-3201	*Ascomycetes*	JN835197	*Thelebolus globosus, Thelebolus microsporus*	99	98–100	*Thelebolus microsporus*
FW-3206	Mycelia sterile	JN835191	*Phoma herbarum*	100	100	*Phoma herbarum*
FW-3219	Yeast-like fungus	JN835211	*Leuconeurospora* sp.	99	99	*Leuconeurospora* sp.
FW-3220	Yeast-like fungus	JN835193	*Leuconeurospora* sp.	99	92	*Leuconeurospora* sp.

Tumularia aquatica, an aquatic anamorphic ascomycete also found in the cold waters of Alpine streams [25].

In sample 4, 14 fungal OTU were found, which were closely related to 10 taxa. They were represented by both ascomycetous anamorphs and teleomorphs. Only one of the species, *Phoma herbarum*, which is closely related to plants, was also revealed by the standard microbiological methods. Among the ascomycetous teleomorphs, two species of the family *Thelebolaceae* were isolated: *Thelebolus microsporus*, a psychrophilic fungus repeatedly revealed in the habitats of penguins and skua gulls in Antarctica, and the recently described Antarctic fungus *Antarctomyces psychrotrophicus* [26], from which a new antifreeze protein highly active under alkaline conditions was isolated in Japan [27].

The ascomycetes found—the soil-inhabiting *Pseudeurotium bakeri* and *Clathrosphaerina zalewskii* inhabiting plant residues, which have not been previously described in Antarctica, as well as the dark-colored anamorphic fungus *Phialophora alba*—are widespread in nature and are preserved relatively well at low temperatures in the presence of protectors, which may be organic substances. The anamorphic ascomycete *Tetracladium maxilliforme* (*Titaea maxilliformis*) occurs in running waters of Arctic ecotopes [28]. Moreover, it belongs to the genus, which was revealed recently by genomic DNA analysis in the course of Antarctic research [29]. The sequence close to *Capronia villosa* (Table 3) described as a finding in New Zealand was determined in the same sample. Interestingly, the fungi of this species have been recently found in the Russian Arctic, in Polar Ural [30]. It is essential that the molecular biological investigations failed to reveal small-spore ascomycetes of the genus *Penicillium* in sample 4, while they were determined by plating: *Penicillium aurantiogriseum, P. coprophilum, P. crustosum*, and *P. griseofulvum*.

The application of cultural and molecular approaches for investigation of Antarctic samples led to the acquisition of different lists of taxa of mycelial fungi, which only coincided in two dominant species—*Geomyces pannorum* and *Phoma herbarum*. However, complex investigations are extremely informative. The application of cultural methods, which make it possible to obtain pure cultures, is useful for the understanding of the physiological potential of the fungi inhabiting low-temperature ecotopes. The study of samples using the method of direct DNA extraction provides for a more complete picture of fungal diversity in the layer of Antarctic sediments and reveals new mycelial fungi previously unknown for this environment.

Table 3. Diversity of the fungal operational taxonomic units (OTU) revealed by molecular biological methods in the Antarctic active layer samples

Number of OTU types	OTU number in samples		NCBI match				The bp number	GenBank accession numbers	Occurrence of the species representatives
	3	4	closely related species	homology, %	coverage, %	E-value			
1		1	*Antarctomyces psychrotrophicus* Stchigel et Guarro	96	90	3.00E-126	311	HQ634130	Soil, Antarctica
2		1	*Capronia villosa* Samuels	86	96	2.00E-80	300	HQ634132	Lichen fungi, New Zealand
3		2	*Clathrosphaerina zalewskii* Beverw.	89	80–88	<1.00E-73	261–287	HQ634127, HQ634134	Deciduous foliage, Europe, New Zealand, Japan
4	3		*Geomyces pannorum* (Link) Sigler et J.W. Carmich.	97–99	99–100	<6.00E-79	176–278	HQ634120, HQ634121, HQ634123	Air, soil, wood, typical of Arctic permafrost sediments
5	1	1	*Herpotrichia juniperi* (Duby) Petr.	93–99	58–100	<5.00E-51	237–276	HQ634119, HQ634124	Snow mold, subalpine regions of the Alps
6		2	*Phialophora alba* J.F.H. Beyma	93–96	66–85	<2.00E-70	206–280	HQ634126, HQ634129	Soil, wood, occurs everywhere (ubiquitous)
7		1	*Phoma herbarum* Westend.	98	100	2.00E-142	290	HQ634128	Plants, occurs everywhere (ubiquitous)
8		2	*Pseudeurotium bakeri* C. Booth	97	98	4.00E-127	275	HQ634131 JN653540	Soil, decomposing plants, occurs everywhere (ubiquitous)
9		1	*Tetracladium maxilliforme* (Rostr.) Ingold	94	97	3.00E-113	274	HQ634133	Water, Europe, New Zealand
10		2	*Thelebolus microsporus* (Berk. et Broome) Kimbr.	97–98	95–99	<5.00E-126	260–291	HQ634125, HQ634135	Algal mats in lakes, soil, Antarctica
11	1	1	*Tumularia aquatica* (Ingold) Descals et Marvanová	89–92	81–89	<4.00E-74	285–286	HQ634122 HQ634136	Wood substrates, widespread on the British Isles, in Northern and Central Europe, on Mauritius, and in the SAR

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REFERENCES

1. Vishniak, H.S., The microbiology of Antarctic soils, in *Antarctic Microbiology*, Friedmann, E.I., Ed., New York: Willey-Liss, 1993.
2. Onofri, S., Selbmann, L., Zucconi, L., Tosi, S., Fenice, M., Barreca, D., and Ruisi, S., Studies on Antarctic fungi, *Polarnet Tech. Rep.*, 2005, vol. 1, pp. 49–52.
3. Ruisi, S., Berreca, D., Selbmann, L., Zucconi, L., and Onofri, S., Fungi in Antarctica, *Rev. Environ. Sci. Biotechnol.*, 2007, vol. 6, pp. 127–141.
4. Kochkina, G.A., Ivanushkina, N.E., Karasev, S.G., Gavrish, E.Yu., Gurina, L.V, Evtushenko, L.I., Spirina, E.V., Vorob'eva, E.A., Gilichinskii, D.A., and Ozerskaya, S.M., Survival of micromycetes and actinobacteria under conditions of long-term natural cryopreservation, *Microbiology* (Moscow), 2001, vol. 70, no. 3, pp. 356–364.
5. Gilichinsky, D.A., Wilson, G.S., Friedmann, E.I., McKay, C.P., Sletten, R.S., Rivkina, E.M., Vishnivetskaya, T.A., Erokhina, L.G., Ivanushkina, N.E., Kochkina, G.A., Shcherbakova, V.A., Soina, V.S., Spirina, E.V., Vorobyova, E.A., Fyodorov-Davydov, D.G., Halle, B., Ozerskaya, S.M., Sorokovikov, V.A., Laurinavichyus, K.S., Shatilovich, A.V., Chanton, J.P., Ostroumov, V.E., and Tiedje, J.M., Microbial populations in Antarctic permafrost: biodiversity, state, age and implication for astrobiology, *Astrobiology*, 2007, vol. 2, pp. 275–311.
6. Onofri, S., Zucconi, L., Selbmann, L., Ripa, C., and Frisvad, J.C., Searching for eukaryotic life preserved in Antarctic permafrost, in *Mat. 38th. COSPAR Sci. Ass.*, Bremen, 2010, p. 9.
7. Tosi, S., Casado, B., Gerdol, R., and Caretta, G., Fungi isolated from Antarctic mosses, *Polar Biol.*, 2002, vol. 25, pp. 262–268.
8. Vishniac, H.S., Biodiversity of yeasts and filamentous microfungi in terrestrial Antarctic ecosystems, *Biodiv. Conservation*, 1996, vol. 5, pp. 1365–1378.
9. Kozlovsky, A.G., Zhelifonova, V.P., Antipova, T.V., Adanin, V.M., Ozerskaya, S.M., Ivanushkina, N.E., Gollmick, F.A., and Gräfe, U., A new N-carboxylester derivative of 3,12-dihydroroquefortine, *Heterocycles*, 2003, vol. 60, no. 7, pp. 1639–1644.
10. Smith, J.J., Tow, L.A., Stafford, W., Cary, C., and Cowan, D.A., Bacterial diversity in three different Antarctic cold desert mineral soils, *Microb. Ecol.*, 2006, vol. 51, pp. 413–421.
11. Babalola, O.O., Kirby, B.M., Le Roes-Hill, M., Cook, A.E., Cary, S.C., Burton, S.G., and Cowan, D.A., Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils, *Environ. Microbiol.*, 2009, vol. 11, pp. 566–576.
12. Malosso, E., Waite, I.S., English, L., Hopkins, D.W., and O'Donnell, A.G., Fungal diversity in maritime Antarctic soils determined using a combination of culture isolation, molecular fingerprinting and cloning techniques, *Polar Biol.*, 2006, vol. 29, no. 7, pp. 552–561.
13. Margesin, R. and Miteva, V., Diversity and ecology of psychrophilic microorganisms, *Res. Microbiol.*, 2011, vol. 162, pp. 346–361.
14. Kochkina, G., Ivanushkina, N., Ozerskaya, S., Chigineva, N., Vasilenko, O., Firsov, S., Spirina, E., and Gilichinsky, D., Ancient fungi in Antarctic permafrost environments, *FEMS Microbiol. Ecol.,* 2012, vol. 82, no. 2, pp. 501–509.
15. Boerema, G.H., De Gruyer, J., Noordeloos, M.E., and Hamers, M.E.C., *Phoma Identification Manual*, CABI Publishing, 2004.
16. van Oorschot, C.A.N., A revision of *Chrysosporium* and allied genera, *Stud. Mycol.*, 1980, vol. 20, pp. 1–89.
17. Frisvad, J. and Samson, R.A., *Penicillium* subgenus *Penicillium*: new taxonomic schemes, mycotoxins and other extrolites, *Stud. Mycol.*, 2004, vol. 49, pp. 1–260.
18. White, T.J., Bruns, T., Lee, S., and Taylor, J., Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in *PCR Protocols: A Guide to Methods and Applications*, Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, T.J., Eds., San Diego: Academic, 1990, pp. 315–322.
19. Elgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R., UCHIME improves sensitivity and speed of chimera detection, *Bioinformatics*, 2011, vol. 27, no. 16, pp. 2194–2200.
20. Duncan, S.M., Fungal diversity and cellulytic activity in the Historic Huts, Ross Island, Antarctica, *PhD Thesis,* Univ. Waikato, 2007.
21. De Hoog, S., Zalar, P., van der Ende, B.G., and Gunde-Cimerman, N., Relation of halotolerance to human-pathogenicity in the fungal tree of life: an overview of ecology and evolution under stress, in *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*, Gunde-Cimerman, N., Oren, A., and Plementtas, A., Eds., Springer, 2005, pp. 371–395.
22. Ivanushkina, N.E., Kochkina, G.A., and Ozerskaya, S.M., Fungi in ancient permafrost sediments of the Arctic and Antarctic regions, in *Life in Ancient Ice*, Castello, J. and Rogers, S., Eds., Ch. 9, Princeton: Princ. Univ. Press, 2005, pp. 127–139.
23. Sogonov, M.V., Schroers, H.J., Gams, W., Dijksterhuis, J., and Summerbell, R.C., The hyphomycete *Teberdinia hygrophila* gen. nov., sp. nov. and related anamorphs of *Pseudeurotium* species, *Mycologia*, 2005, vol. 97, no. 3, pp. 695–709.
24. Schneider, M., Grunig, C.R., Holdenrieder, O., and Sieber, T.N., Cryptic speciation and community structure of *Herpotrichia juniperi*, the causal agent of brown felt blight of conifers, *Mycol. Res.*, 2009, vol. 113, no. 8, pp. 887–896.
25. Gessner, M.O. and Robinson, C.T., Aquatic hyphomycetes in alpine streams, in *Ecology of a Glacial Flood*

Plain, Ward, J.V. and Uehlinger, U., Eds., Kluwer: Dordrecht, 2003, pp. 123–137.

26. Stchigel, A.M., Cano, J., MacCormack, W., and Guarro, J., *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica, *Mycolog. Res.*, 2001, vol. 105, no. 3, pp. 377–382.
27. Xiao, N., Suzuki, K., Nishimiya, Y., Kondo, H., Miura, A., Tsuda, S., and Hoshino, T., Comparison of functional properties of two fungal antifreeze proteins from *Antarctomyces psychrotrophicus* and *Typhula ishikariensis*, *FEBS J.*, 2010, vol. 277, no. 2, pp. 394–403.
28. Engblom, E., Lingdell, P., Marvanova, L., and Muller-Haeckel, A., Foam spora in running waters of Southern Greenland, *Polar Res.*, 1986, vol. 4, no. 1, pp. 47–51.
29. Bridge, P.D. and Newsham, K.K., Soil fungal community composition at Mars Oasis, a southern maritime Antarctic site, assessed by PCR amplification and cloning, *Fungal Ecol.*, 2009, vol. 2, no. 2, pp. 66–74.
30. Zhurbenko, M.P., Lichenophilic fungi of Russian Arctics, *Extended Abstract of Doctoral (Biol.) Dissertation*, St.-Petersburg, Komarov Botanical Inst., Russ. Acad. Sci., 2010.

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