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Studies on diversity of soil microfungi in the Hornsund area, Spitsbergen

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Abstract: We assessed culturable soil microfungal diversity in various habitats around Hornsund, Spitsbergen in the High Arctic, using potato dextrose agar (PDA) medium. Thermal growth classification of the fungi obtained was determined by incubating them in 4°C and 25°C, permitting separation of those with psychrophilic, psychrotolerant and mesophilic characteristics. In total, 68 fungal isolates were obtained from 12 soil samples, and grouped into 38 mycelial morphotypes. Intergenic spacer regions of these morphotypes were sequenced, and they represented 25 distinct taxonomic units, of which 21 showed sufficient similarity with available sequence data in NCBI to be identified to species level. Soil under ornithogenic influence showed the highest species diversity, including sequences assigned to *Mortierella macrocystis, M. elongata, Mortierella* sp., *Cudoniella* sp., *Varicosporium elodeae*, *Beauveria bassiana*, *Geomyces pannorum*, *Penicillium* sp. and *Atradidymella muscivora*. Fourteen taxa were classified as psychrophilic, seven mesophilic, and four psychrotolerant.

Key words: Arctic, Svalbard, soil microbiology, microfungi.

Introduction

The environmental extremes typical of polar regions are thought to contribute to low soil microbial abundance and diversity (Arenz and Blanchette 2011), with

low temperature, dryness and exposure to UV-radiation being limiting factors for survival. The availability of typically low levels of organic matter also affects development of microbes in the soil. Animal faeces, carcasses and plants are important contributors to the nitrogen (N) and phosphorus (P) cycles in High Arctic soils (Stempniewicz *et al*. 2007; Zmudczyńska *et al.* 2012). These cycles are driven by the presence of vegetation and litter in the soil. The latter is thought to promote P mineralization but reduce N mineralization (Jonasson *et al*. 2006). Fungi are believed to play an important role in the phosphorus cycle in Arctic soil, increasing P availability and decreasing levels of inorganic N (Hart *et al*. 1993), in particular during the decomposition of litter.

The High Arctic is one of the coldest regions of the world. In soil environments such as the Arctic tundra, low organic content is associated with low primary production in the sparse and discontinuous plant cover. In such conditions soil microorganisms experience not only physical stress but also nutrient limitation due to the oligotrophic conditions (Bergero *et al*. 1999). However, fungi are commonly isolated from these environments, including vegetation (Tosi *et al*. 2002), snow (Abyzov 1993), cryoconite holes (Singh and Singh 2012), sea ice (Gunde-Cimerman *et al*. 2003) and soil (Alias and Suhaila 2007; Omar *et al*. 2009; Rao *et al*. 2012).

Microorganisms capable of surviving and growing at 0°C were first defined as psychrophiles by Sinclair and Stokes (1963). Later, Morita (1975) developed this classification, recognising that both psychrophilic and psychrotolerant taxa have the ability to grow at 0°C, but that the maximum growth temperature for psychrophilic taxa was at 20°C or below, whereas psychrotolerant taxa have a higher maximum growth temperature. Psychrophiles are usually adapted not only to low temperatures, but also to further environmental constraints, making them true extremophiles (Feller and Gerday 2003). Psychrophilic microorganisms are reported from various permanently cold environments, from the deep sea to high mountain and polar regions (D'Amico *et al*. 2006).

Mycological research in extreme locations can be traced to the early 19th century, with the description of the montane *Amanita nivalis* Grev. Most of the earlier studies focused on higher fungi (Watling 1987), including parasitic or saprophytic fungi found on or near plants and lignicolous fungi associated with wood. In the last three decades, the study of Arctic ecosystems and their microbiota has received increasing attention, and a range of microfungi have been described from various substrates on Svalbard (Robinson *et al*. 2004; Alias and Suhaila 2007; Kurek *et al*. 2007; Pang *et al*. 2009, 2011; Butinar *et al*. 2011; Gawas-Sakhalkar and Singh 2011; Singh and Singh 2012).

Studies on Svalbard initially concentrated on macrofungi, but until the early 1980s there were very few published reports. Since then, a considerable amount of mycological research has been carried out in this area. Despite Svalbard's geographic isolation and High Arctic climate, its macrofungal communities are sur-

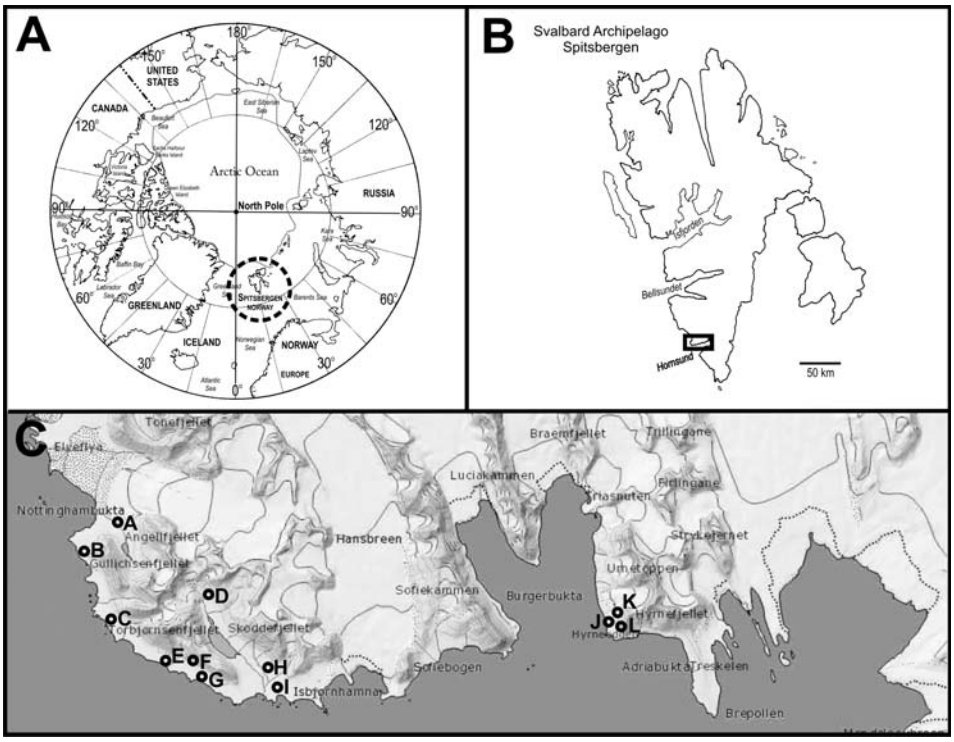

Fig. 1. Study area. **A**. Location of Spitsbergen. **B**. Location of Hornsund. **C**. Sampling locations.

prisingly diverse. The known flora of Svalbard macrofungi currently consists of *ca* 300 species. Many of these appear to have an Arctic-alpine distribution, while some also occur in Antarctic and sub-Antarctic areas. Some are also found in the "lowlands" of Europe, where they are limited mainly to "cold-climate" regions (Jalink and Nauta 2004). Recent mycological studies on Svalbard have focused on microfungi. Alias and Suhaila (2007) reported 89 microfungal taxa from the soil of Ny-Ålesund, while Pang *et al*. (2009, 2011) reported six marine fungi isolated from wood debris collected at Longyearbyen. Recently, yeasts and filamentous fungi from glacier cryoconite holes have been investigated by Singh and Singh (2012).

Hornsund is a large fjord in the south of Spitsbergen (Fig. 1). The area is typical for western Svalbard, including high mountains, large valleys, considerable snow and ice cover and deep marine fjords, giving large local climate variation (Migała *et al*. 2008), and including a range of non-glaciated and glaciated environments. The Polish Polar Station at Hornsund was established in 1957. Currently, data on the diversity of soil microfungi are lacking from the Hornsund area. This study aims to rectify this, assessing microfungal diversity in various habitats, and also providing basic ecophysiological information on the thermal growth characteristics of the microfungi obtained.

Materials and methods

Study area and sample collection. — Sampling took place during the 2010 boreal summer (August 2010) at Hornsund, Spitsbergen (77°00'04"N, 15°33'37"E) (Fig. 1). Twelve soil samples were collected, as described in Table 1. The sampling locations represented a range of habitats with a diversity of soil physical and chemical characteristics, and included soils from dry and bare fellfields, moist moss tundra, ornithogenic sites, a vertebrate-influenced pond shore and a glacier foreland. At each sampling location, 10 g of soil was obtained covering the surface to 10 cm depth using a sterile spatula. Soil samples were stored in sealed sterile Falcon tubes. The samples were then refrigerated at 4°C, and subsequently transported at this temperature, taking 6 d in transit to the National Antarctic Research Center, Kuala Lumpur, Malaysia, where they were stored at -20°C until further analysis.

Fungal isolation and thermal classification. — Isolation of soil microfungi was based on the soil plating method of Warcup (1950). Sterilized Potato Dextrose Agar (PDA) was used as the growth medium. Chloramphenicol 0.2 g/l (Krishnan *et al.* 2011) was added to the medium in order to suppress bacterial growth. Approximately 0.1 g of each soil sample was transferred using a sterilized spatula into individual sterile Petri dishes (n = 8 replicates from each of the 12 soil samples). Cooled sterilized PDA agar was then poured into each Petri dish and the plate was gently swirled to distribute the sample evenly. Four replicates from each soil sample were then incubated at each of 4°C or 25°C in order to determine their thermal growth classification. These plates were examined daily for fungal growth for 10–15 d. Active growing mycelia were then isolated and sub-cultured onto fresh PDA Petri dishes, with isolates obtained at 4°C and 25°C continuing to be incubated at these temperatures. Isolates that grew only at 4°C were classified as psychrophilic, those which grew only at 25°C as mesophilic and those growing at both 4°C and 25°C as psychrotolerant.

Molecular identification. — The axenic mycelia morphotypes were identified using a molecular approach. Mycelia of the 38 fungal morphotypes obtained were scraped from the surface of each plate and ground into fine powder in liquid nitrogen using a mortar and pestle. Genomic DNA was extracted using the DNeasy Plant DNA Extraction Kit (Qiagen) according to the manufacturer's instructions. The intergenic spacer regions of the nuclear rRNA genes were amplified using primer pairs ITS4/ITS5 (White *et al*. 1990). PCR reactions were performed in a 50 µL volume containing ca. 20 ng DNA, 0.2 µM of each primer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$ and 1.25 U of Taq Polymerase (Invitrogen). The amplification cycle consisted of an initial denaturation step of 95°C for 2 min followed by 35 cycles of (a) denaturation (95°C for 1 min), (b) annealing (54°C for 1 min) and (c) elongation (72°C for 1.5 min) and a final 10 min elongation step at 72°C. The PCR products were analysed by agarose gel electrophoresis and sent to Tri-I

Table 1

Details of sampling locations in the Hornsund area where soil samples were collected. NA – data not available.

Sampling sites	Location	Habitat	Average Temperature (°C)		pH	Altitude m a.s.l.	Latitude (N)	Longitude (E)
			Air	Soil	Water			
A	North-western slopes of Kvartsittknattane	Dry lichen tundra, rocky surface	6.35	7.10	–	118	77°03'52.80"	15°10'47.04"
B	Hyttevika (near the Hyttevika Hus)	Moist moss tundra	6.40	9.25	–	4	77°03'01.20"	15°08'41.64"
C	Skjerstranda at the foothill of Trulsenfjellet	Dried runnel	7.30	9.55	–	23	77°01'28.50"	15°12'52.74"
D	Revdalen (northern part), near Revvatnet lake and Revelva river	Birds colony, ornithogenic tundra	6.50	9.15	–	67	77°01'43.38"	15°22'15.90"
E	Ralstranda (southern part), at the foot of Rotjesfjellet, near Revelva river	Pond	5.85	8.50	5.24	16	77°00'24.12"	15°20'36.54"
F	Rotjesfjellet (south-eastern slope, at the peak)	Small birds colony, dry lichen and moss tundra, rocky surface	6.75	8.30	–	399	77°00'34.86"	15°23'01.26"
G	Rotjesfjellet (south-east slope, at the foothill)	Small birds colony, dry lichen and moss tundra, rocky surface	7.15	8.25	–	50	77°00'16.32"	15°24'01.92"
H	Ariekammen (slopes at the southern peak)	Dry lichen tundra, rocky surface	7.25	8.00	–	250	77°00'39.90"	15°30'25.50"
I	Fuglebergsletta, neighbourhood of the Hornsund Station	Vertebrate-influence pond	4.55	7.00	5.98	11	77°00'06.00"	15°31'54.60"
J	Hyrneodden point, Mariesletta	Glacier foreland	NA	NA	–	14	77°01'27.60"	16°03'15.48"
K	Hyrneodden point, Mariesletta	Glacier foreland	NA	NA	–	14	77°01'23.16"	16°03'21.48"
L	Hyrneodden point, Mariesletta	Glacier foreland	NA	NA	–	14	77°01'22.38"	16°03'11.46"

Biotech. Inc. (Taiwan) for sequencing. The sequences obtained were checked for ambiguity, assembled and submitted to the National Center for Biotechnology Information (NCBI) for a nucleotide BLAST search. A number of "nearest neighbour" sequence identities were generated, and an ITS (ITS1, 5.8S and ITS2) sequence difference of ≤ 4% was considered to indicate the same taxon in this study (following Smith *et al*. 2007); this also takes into account PCR and sequencing error rates and the reported 1.5% difference in ITS sequence between isolates of the same species in community studies (Izzo *et al*. 2005).

Data analysis. — Frequency of occurrence of particular isolates (38 morphotypes) was calculated by using the following formula:

$$Frequency\ of\ occurrence = \frac{Number\ of\ plates\ with\ occurrence}{Number\ of\ plates} \times 100$$

Species diversity and evenness were calculated using the Shannon-Wiener Diversity Index (*H'*) and Pielou Evenness Index (*J'*). The indices were calculated as:

$$H' = -\sum (p_i \times \ln p_i)$$

and

$$J' = \frac{H'}{\ln(S)}$$

where S is the total number of species in the community (species reaches) and p_i is the proportional abundance (*i.e.* proportion of individuals) of i^{th} species (Ludwig and Reynolds 1988).

Results

Microfungal diversity. — Sixty-eight isolates were cultured from the samples and grouped into 38 fungal morphotypes. Molecular analysis (Table 2) demonstrated that some had identical ITS sequences or differed by ≤ 4% and were therefore considered the same taxon, giving a total of 25 distinct taxa. A BLAST search in NCBI revealed that 21 of these taxa had ITS sequence similarity > 96% with the closest sequence match of a known taxon and are therefore treated here as that taxon. The other 4 taxa had a lower percentage similarity with the sequences in NCBI and could only be assigned at the genus level.

Table 3 lists the fungal taxa isolated and identified from the 12 soil samples, and their frequency of occurrence (from a maximum of 68 plates) at both incubation temperatures. The most frequently encountered taxon was *Phialocephala lagerbergii* (11.8% of plates); followed by *Mortierella macrocystis* (10.3%), while *Geomyces pannorum*, *Atradidymella muscivora* and *Beauveria bassiana* all occurred on > 7.0% of the plates. No microfungi were cultured from the soil of the dry and bare fellfields (sample A). Soil collected from a vertebrate-influenced pond gave the highest fungal diversity index (1.95, sample I) followed by that of ornithogenically-influenced soil collected near a bird colony (1.82, sample D) and that from soil under mosses (1.74, sample B) (Table 4). *Atradidymella muscivora, Beauveria bassiana*, *Mucor hiemalis*, *Herpotrichia juniperi*, and *Cosmospora villior* were found in sample I, while *Mortierella elongata, Varicosporium elodeae*, and *Geomyces pannorum*, were common species found in the ornithogenically-influenced sample D. Common species isolated from highly vegeta-

Table 2

Fungal strains used in the present study, based on morphological examination, along with their identification based on molecular techniques.

Strain number(s)	ITS sequence length	Closest sequence match			% Sequence coverage	% Match
		Identity	Genbank accession no.	Source		
HND9R1-1	484	*Atradidymella muscivora* M.L. Davey *et* Currah	EU817829	Mosses, Canada	100	100
HND9R2-1	484	*A. muscivora*	EU817829	Mosses, Canada	100	99
HND10R1-1	484	*A. muscivora*	EU817829	Mosses, Canada	100	100
HND10R2-1	484	*A. muscivora*	EU817829	Mosses, Canada	100	100
HND10R2-4	484	*A. muscivora*	EU817829	Mosses, Canada	100	100
HND10R1-3	561	*Bactrodesmium gabretae* Koukol *et* Kolářová	FN561756	Green needle on the ground, Czech Republic	35	93
HND11R2-1	513	*Beauveria bassiana* (Bals.-Criv.) Vuill.	JN379808	Poland	100	100
HND11R2-2	513	*B. bassiana*	JN379808	Poland	100	100
HND6R2-1	517	*Coniochaeta* sp.	HM595554	Conifers, Russia	100	94
HND12R4-1	517	*Coniochaeta* sp.	HM595554	Conifers, Russia	100	94
HND12R1-1	503	*Cosmospora vilior* (Starbäck) Rossman *et* Samuels	GU726751	Soil, cosmopolitan	100	98
HND5R5-3	503	*Cudoniella* sp.	AY789371	Soil, cosmopolitan	99	98
HND1R7-2	591	*Cystodendron* sp.	FM200645	Sweden	90	94
HND4R7-3a	528	*Exophiala heteromorpha* (Nannf.) de Hoog *et* Haase	AB190397	Wood pulp, Sweden	100	100
HND5R5-1	510	*Geomyces pannorum* (Link) Sigler *et* J.W. Carmich.	JF320819	Rotten "panno", Germany	100	100
HND5R6-2(2)	510	*G. pannorum*	JF320819	Rotten "panno", Germany	100	100
HND12R8-1	472	*Herpotrichia juniperi* (Duby) Petr.	FJ904463	Conifers, Switzerland	96	100
HND2R5-3	532	*Isaria farinosa* (Holmsk.) Fr.	HQ880828	Denmark	100	100
HND10R8-1	530	*Lecanicillium lecanii* (Zimm.) Zare *et* W. Gams	DQ007047	Soil, North America	91	99
HND2R2-1	612	*Mortierella alpina* Peyronel	FJ478130	Stem, China	100	99
HND7R1-1	585	*M. clonocystis* W. Gams	HQ630318	Germany	98	99
HND5R2-1	589	*M. elongata* Linnem.	AB542092	Soil, Japan	100	100
HND1R7-3	589	*M. macrocystis* W. Gams	AJ878782	Soil, European beech	100	96
HND5R6-2(1)	610	*Mortierella* sp.	DQ093725	Plant root, Lithuania	100	88
HND5R7-1	610	*Mortierella* sp.	DQ093725	Plant root, Lithuania	100	88
HND7R6-3	587	*Mucor hiemalis* Wehmer	HM037968	Freshwater, European	100	99
HND2R8-2(a)	488	*Neonectria ramulariae* Wollenw.	JF735314	Fruit, Portugal	100	100
HND2R5-2	526	*Penicillium* sp.	GU565124	China	100	99
HND2R5-4	526	*Penicillium* sp.	GU565124	China	100	99
HND11R8-1	528	*Penicillium* sp.	JF429675	Cave sediment, Australia	100	99

Table 2 – *continued*.

Strain number(s)	ITS sequence length	Closest sequence match			% Sequence coverage	% Match
		Identity	Genbank accession no.	Source		
HND4R5-1	529	*Phialocephala lagerbergii* (Melin *et* Nannf.) Grünig *et* T.N. Sieber	AB190400	Japan	100	99
HND4R7-1B	528	*P. lagerbergii*	AB190400	Japan	100	99
HND4R7-1	528	*P. lagerbergii*	AB190400	Japan	100	99
HND4R8-6	528	*P. lagerbergii*	AB190400	Japan	100	99
HND2R6-1a	506	*Tolypocladium cylindrosporum* W. Gams	FJ411410	New Zealand	100	100
HND1R4-2	502	*T. inflatum* W. Gams	GU354362	Soil, Greenland	100	100
HND5R5-4	496	*Varicosporium elodeae* W. Kegel	GQ152148	Portugal	100	98
HND12R8-2(2)	495	*V. elodeae*	GQ152148	Portugal	100	97

tion-influenced soil (sample B) included *Phialocephala lagerbergii*, *Tolypocladium inflatum,* and *Mortierella clonocystis*.

Thermal growth characteristics. — Fourteen of the taxa isolated were classified as psychrophilic, seven were mesophilic, and four were psychrotolerant. Although representatives of the Helotiales were the most frequently isolated overall

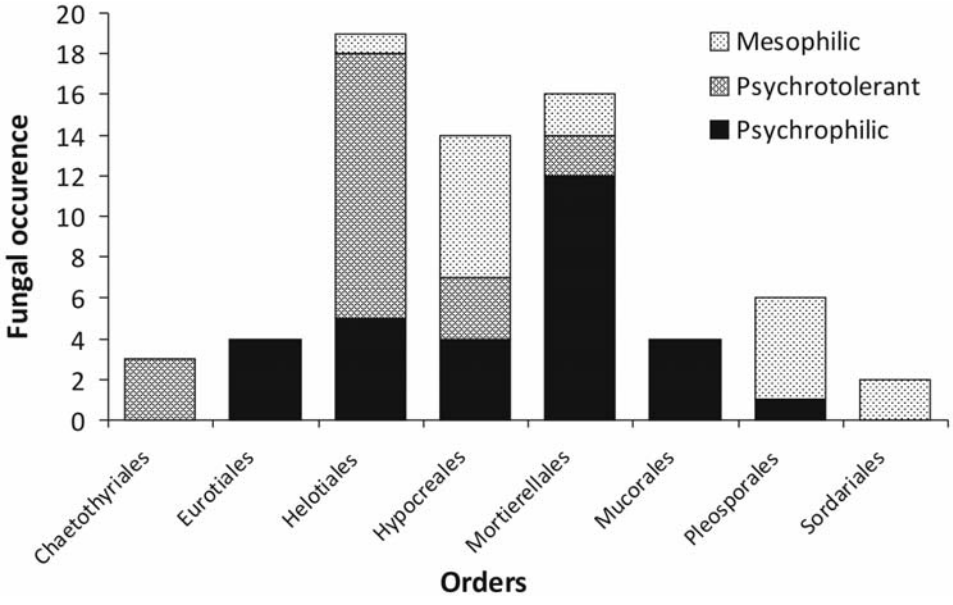

Fig. 2. Fungal occurrence (number of plates) across all sampling locations, indicating taxonomic order and thermal classification.

Table 3

Frequency of occurrence (number of plates) of soil microfungi at the two different culture temperatures (4°C, 25°C), identified by their closest ITS sequence match (see Table 2). (* ≥ 96% match with the ITS sequence)

Species	Order	No. of isolate(s)	Locality / Habitats	Number of occurrences	
				4°C	25°C
Atradidymella muscivora Davey *et* Currah*	Pleosporales	5	C, G, I	0	5
Bactrodesmium gabretae Koukol *et* Kolárová	Helotiales	1	C	0	1
Beauveria bassiana (Bals.-Criv.) Vuill.*	Hypocreales	4	D, F, I	0	4
Coniochaeta sp.*	Sordarialess	2	E, I	0	2
Cosmospora vilior (Starbäck) Rossman *et* Samuels*	Hypocreales	1	I	0	1
Cudoniella sp.*	Helotiales	2	D, F	2	0
Cystodendron sp.	Helotiales	1	B	1	0
Exophiala heteromorpha (Nannf.) de Hoog *et* Haase*	Chaetothyriales	3	J, K	2	1
Geomyces pannorum Sigler *et* J.W. Carmich.*	Helotiales	5	D, F, G	3	2
Herpotrichia juniperi (Duby) Petr.*	Pleosporales	1	I	1	0
Isaria farinosa (Dicks.) Fr.*	Hypocreales	1	K	1	0
Lecanicillium lecanii (Zimmerman) Zare *et* Gams*	Hypocreales	3	C, H	1	2
Mortierella alpina Peyronel*	Mortierellales	2	E, K	1	1
Mortierella clonocystis Gams*	Mortierellales	2	B	2	0
Mortierella elongata Linnem.*	Mortierellales	2	D	0	2
Mortierella macrocystis Gams	Mortierellales	7	B, D, H	7	0
Mortierella sp.	Mortierellales	3	D, G	3	0
Mucor hiemalis Wehmer*	Mucorales	4	E, H, I	4	0
Neonectria ramulariae Wollenw.*	Hypocreales	1	K	1	0
Penicillium sp. 1*	Eurotiales	2	L	2	0
Penicillium sp. 2*	Eurotiales	2	F, G	2	0
Phialocephala lagerbergii (Melin *et* Nannf.) Grünig *et* T.N. Sieber*	Helotiales	8	B, E, J, L	5	3
Tolypocladium cylindrosporum Gams*	Hypocreales	2	K	2	0
Tolypocladium inflatum Gams*	Hypocreales	2	B	0	2
Varicosporium elodeae W. Kegel*	Helotiales	2	D, I	2	0

Table 4

Diversity measures for soil microfungi cultured from each of the eleven sampling sites (see Table 1 for details).

Sites	A	B	C	D	E	F	G	H	I	J	K
Species richness (*S*)	0.00	9.00	5.00	12.00	5.00	5.00	5.00	4.00	7.00	6.00	6.00
Shannon-Wiener Diversity Index (*H'*)	0.00	1.74	1.06	1.82	1.33	1.33	1.33	1.04	1.95	0.64	1.56
Evenness Index (*J'*)	0.00	0.19	0.21	0.15	0.27	0.27	0.27	0.26	0.28	0.11	0.26

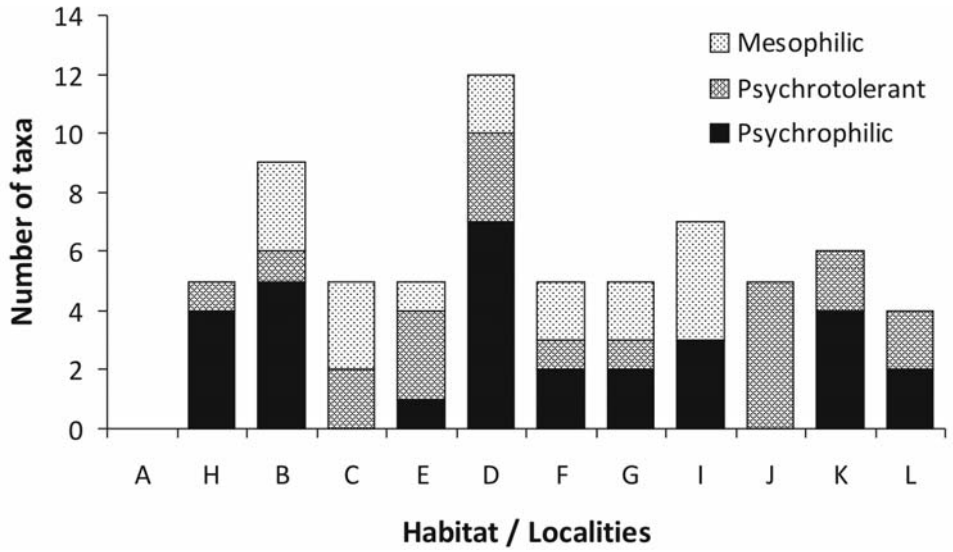

Fig. 3. Numbers of microfungal taxa obtained and their thermal classification at each sampling site (see Table 1 for site descriptions).

(Table 3), Mortierellales dominated the psychrophilic strains (Fig. 2). *Mortierella macrocystis* (Mortierellales) and *Mucor hiemalis* (Mucorales) were the most frequently isolated psychrophilic species while the most frequently isolated mesophilic species were *Atradidymella muscivora* and *Beauveria bassiana*. Members of the Helotiales were the most isolated psychrotolerant taxa, represented by *Geomyces pannorum* and *Phialocephala lagerbergii.* Although the numbers of psychrotolerant taxa obtained were low, they were present at most collection sites excepting samples A and I (Fig. 3). Mesophilic species were isolated from all locations except the glacier foreland area (Fig. 3).

Discussion

Species composition and diversity. — Ornithogenically-influenced soil collected near bird colony contained the highest diversity of fungi (8 taxa, sample D). Ornithogenic influences can affect fungal distribution and occurrence in the polar regions as shown both in the present and previous studies (Marshall 1998; Tosi *et al*. 2002). This influence is likely to act through two mechanisms: (1) nutrient enrichment from manure and carcasses (Arnebrant *et al*. 1990; Smykla *et al*. 2007; Hii *et al.* 2009; Zmudczyńska *et al*. 2012) and (2) the likely role played by birds as carriers of fungal propagules (Marshall 1998; Quinn 2008). Arenz and Blanchette (2011) studied the occurrence of microfungi in the Ross Sea, Victoria Land Dry Valleys and Antarctic Peninsula regions of Antarctica, reporting ascomycetes as

the most abundant group, as found in the current study. Zygomycetes such as *Mucor hiemalis* and *Mortierella* spp. were also present in the soils around Hornsund. Both groups have been reported in other studies in the Arctic and Antarctic regions (Bergero *et al*. 1999; Krishnan *et al*. 2011; Arenz and Blanchette 2011). The few identified species recorded previously in the Arctic region include *Neonectria ramulariae* in tundra (Fiedurek *et al*. 2003), *Cudoniella* sp. in an Arctic-alpine ecosystem (Medardi 2006) and *Exophiala heteromorpha* in permafrost (Ozerskaya *et al*. 2009). Species recorded previously in Antarctic studies include *Beauveria bassiana* (Laichmanova *et al*. 2009), *Isaria farinosa* (Laichmanova *et al*. 2009; Pearce *et al*. 2009), *Lecanicillium lecanii* (Tosi *et al*. 2002), *Cosmospora vilior* (Arenz *et al*. 2006) and *Coniochaeta* sp. (Adams *et al*. 2006). *C. vilior* (Samuels *et al*. 2006; Capdet and Romero 2010), *I. farinosa* (Zimmerman 2008) and *L. lecanii* (Pearce *et al*. 2009) are also reported to occur in temperate and tropical regions. The presence of *Atradidymella muscivora* in the current study is a new record for the Spitsbergen.

Studies from Franz Joseph Land, High Arctic (Bergero *et al*. 1999), Ny-Ålesund, Svalbard (Gawas-Sakhalkar and Singh 2011), and King George Island, maritime Antarctic (Krishnan *et al*. 2011) reported that filamentous fungi including the genera *Mortierella*, *Geomyces*, *Phialocephala*, *Penicillium*, and *Mucor* were the most frequently isolated taxa. Members of *Mortierella*, including *M. alpina*, *M. clonocystis*, and *M. elongata* were present in the current study. *Phialocephala lagerbergii* (= *Cadophora lagerbergii*, *Phialophora lagerbergii*) were the most frequently isolated taxa in the present study followed by *Geomyces pannorum*. *Phialocephala* has previously been recorded in Svalbard (Gawas-Sakhalkar and Singh 2011), while *Geomyces pannorum* is a commonly encountered fungus in cold regions of the world (Adams *et al*. 2006; Gawas-Sakhalkar and Singh 2011; Krishnan *et al*. 2011). Both taxa have been reported in studies of fungal diversity from Antarctic historic huts, suggesting a possible human influence (Arenz *et al*. 2006; Blanchette *et al*. 2010), and have also been associated with migrating birds (Marshall 1998; Del Frate and Caretta 1990). Marshall (1998) also reported *G. pannorum* being frequently isolated from Antarctic soils influenced by seals, birds and humans. These findings are consistent with the frequent occurrence of both species in vertebrate-influenced sites in the present study. *Geomyces pannorum* has been described as strongly keratinophilic (Mercantini *et al*. 1989). Thus, the presence of this species in Arctic soils may be linked with that of keratin-containing debris such as feathers.

Six of the 25 taxa identified here have previously been found from Arctic or Alpine ecosystems including *Mucor hiemalis, Cosmospora vilior*, *Varicosporium elodeae, Neonectria ramulariae*, *Cudoniella* sp. and *Tolypocladium inflatum.* Arctic, Alpine and Antarctic studies (Bergero *et al*. 1999; Gawas-Sakhalkar and Singh 2011; Arenz *et al*. 2011) have indicated *Mucor hiemalis* as a common soil fungus, and it is known to degrade cellulose, starch and pectin (Thorman *et al*.

2001). Capdet and Romero (2010) reported that *Cosmospora vilior* utilizes fallen twigs and grows on the stomata of plants in boreal ecosystems. *Varicosporium elodeae* is an endophyte of highland rushes with a temperate (Yuki *et al*. 2005) and boreal-Arctic-alpine distribution (Chlebicki 2009). *Neonectria ramulariae* has previously been isolated from Arctic tundra soil (Fiedurek *et al*. 2003), causing cankers in various plants (Chaverri *et al*. 2011). The biological relationships of helotialean fungi in ecosystems are diverse (Wang *et al*. 2006). An aquatic Helotiales fungus, *Cudoniella* sp., has been reported previously to occur in an Arctic-alpine ecosystem (Medardi 2006). The saprophytic (Kerry 1990) and pathogenic (Davey *et al*. 2009) abilities of these fungi suggest they may play a role in nutrient cycling in the Hornsund ecosystem. Thus, the presence of these species in the current study may be related to the nutrient-rich environment with high vegetation abundance. *Herpotrichia juniperi*, a new record to the Arctic, is known as a plant pathogen causing a conifer-needle disease (Hartig 1888; Bazzigher 1976); hence its role or activity in the Svalbard soil ecosystem is unclear.

Tolypocladium cylindrosporum, T. inflatum, Isaria farinosa, *Beauveria bassiana* and *Lecanicillium lecanii* have been described to display entomopathogenic properties. *Tolypocladium inflatum* is a well-known entomopathogen in Norwegian and Arctic soils (Weiser 1987; Gams 1971). *Isaria farinosa*, *Beauveria bassiana* (Laichmanova *et al*. 2009) and *Lecanicillium lecanii* (Miller *et al*. 2004) are representative of taxa that are known to be entomopathogenic elsewhere, including in Antarctic studies (Onofri *et al*. 2007; Laichmanova *et al*. 2009). Hence they may also play this role in these High Arctic ecosystems.

Thermo-classification of isolates. — Many of the fungal taxa identified in the current study (Table 3) have been reported in other studies of Arctic and Antarctic fungi. However, ecophysiological characterizations of these taxa in terms of thermal characteristics have rarely been attempted. Taxa that have previously been described as psychrophilic or psychrotolerant include *Mortierella alpina* and *Mucor hiemalis* (Bergero *et al*. 1999; Tosi *et al*. 2002; Gawas-Sakhalkar and Singh 2011), *P. lagerbergii* (Bergero *et al*. 1999), *Geomyces pannorum* (Mercantini 1989; Krishnan *et al*. 2011), and *Neonectria ramulariae* (Fiedurek *et al*. 2003; Chaverri *et al*. 2011). However, the classification of some taxa based on the current study differed from previous reports, including that of *Mortierella elongata* (cf. Magan 2007).

The sampling sites examined here experience a very large topoclimatic variation (Migała *et al*. 2008). Alias and Suhaila (2007) reported that mesophilic fungi were common in soils at Ny-Ålesund, Svalbard, contrasting with the more general finding of Robinson (2001) who concluded that psychrotolerant fungi generally dominate in the Arctic. The present study, rather, found that psychrophilic species were abundant and well represented around Hornsund. Based on a study in the similarly extreme coastal continental Antarctic, Singh *et al*. (2006) suggested that psychrophilic fungi are common in cold regions as they possess resilient resting

spores. However, in reality, studies have not addressed explicitly the existence and effectiveness of different survival mechanisms of fungi in these ecophysiological groups.

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